Instituto Juan March de Estudios e Investigaciones

83

CENTRO DE REUNIONES INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Bacterial Transcription Factors Involved in Global Regulation

Organized by

A. Ishihama, R. Kolter and M. Vicente

Y. V. Brun
R. R. Burgess
S. Busby
K. F. Chater
J. Errington
R. E. Glass
R. L. Gourse
R. Hengge-Aronis
C. F. Higgins
A. Ishihama
R. C. Johnson
R. Kolter
V. de Lorenzo
T. Nyström
J. S. Parkinson
S. Pedersen
M. Salas
D. Siegele
M. Vicente

IJM
83
Wor

IJM-83-Wor

Instituto Juan March de Estudios e Investigaciones

83

CENTRO DE REUNIONES INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Bacterial Transcription Factors Involved in Global Regulation

Organized by

A. Ishihama, R. Kolter and M. Vicente

Y. V. Brun
R. R. Burgess
S. Busby
K. F. Chater
J. Errington
R. E. Glass
R. L. Gourse
R. Hengge-Aronis
C. F. Higgins
A. Ishihama
R. C. Johnson
R. Kolter
V. de Lorenzo
T. Nyström
J. S. Parkinson
S. Pedersen
M. Salas
D. Siegele
M. Vicente

Instituto Juan March (Madrid)

The lectures summarized in this publication were presented by their authors at a workshop held on the 11th through the 13th of June, 1998, at the Instituto Juan March.

Depósito legal: M. 28.284/1998
Impresión: Ediciones Peninsular. Tomelloso, 27. 28026 Madrid.

Instituto Juan March (Madrid)

PAGE

INDEX

Introduction: M.Vicente, R. Kolter and A. Ishihama 9

Transcriptional machinery: components and choices
Chairs: Jeff Errington and Steen Pedersen................. 13

Akira Ishihama: Control of activity and specificity of RNA polymerase.................................... 15

Robert E. Glass: Genetic and immunological dissection of the β subunit of the multimeric RNA polymerase.. 16

Richard R. Burgess: The interaction of *E. coli* sigma subunits with core RNA polymerase............... 17

Short talks:
Annie Kolb: Selectivity of RNA polymerases containing the σ^{70} and σ^{S} factors................ 19

Miki Jishage: Anti-sigma factor for the major sigma subunit of *Escherichia coli* RNA polymerase.................................... 20

Víctor de Lorenzo: IHF in sigma-54 promoters: DNA bending and more.. 21

Short talk:
Eduardo Santero: Construction of a bacterial promoter region with an enhancer bearing a multiple response element....................... 22

Monitoring the global physiological state of the cell
Chairs: Margarita Salas and Richard Burgess.............. 23

Reid C. Johnson: Regulation of recombination and transcription by the Fis protein..................... 25

Christopher F.Higgins: H-NS, chromatin structure and gene expression................................... 26

Instituto Juan March (Madrid)

PAGE

Steve Busby: CRP and FNR: master craftsmen, but also oddjobmen.. 27

Steen Pedersen: Subsaturation: A general regulatory mechanism for translation and transcription?.......... 29

Short talk:
Juan Luis Ramos: Activation and repression of transcription at the double tandem divergent promoters for *xylR* and *xylS* genes of the TOL plasmid of *Pseudomonas putida*.................... 31

John S. Parkinson: Dissecting the three-protein brain of *E. coli*....................................... 32

Short talks:
Evelyne Richet: Synergistic activation of the *malEp* promoter by MalT and CRP: two roles for CRP.. 34

Bernt Eric Uhlin: H-NS and StpA proteins stimulate expression of the maltose regulon in *Escherichia coli*............................. 35

Directing transcription to regulate cell growth and survival.
Chairs: Keith F. Chater, Robert E. Glass, John S. Parkinson and Steve Busby.................. 37

Roberto Kolter: Population dynamics during stationary phase.................................... 39

Richard L. Gourse: Lessons from rRNA promoters about bacterial transcription and its regulation...... 40

Regine Hengge-Aronis: Regulation of cellular levels and activity of σ^S in *Escherichia coli*......... 42

Deborah Siegele: Entering and exiting stationary phase in *E. coli*.................................... 43

Thomas Nyström: The *Escherichia coli* defense against aging: global regulation aimed at avoiding oxidative damage.................................... 45

Instituto Juan March (Madrid)

PAGE

Short talk:
Cecília María Arraiano: The relation between transcriptional and post-transcriptional factors in the regulation of cell growth and survival.. 47

Margarita Salas: Regulation of bacteriophage ϕ29 DNA transcription.................................... 48

Short talks:
Francisca Reyes-Ramírez: Molecular signalling between the redox sensor protein NIFL and the transcriptional activator NIFA.................. 50

Claude Gutierrez: Interplay between global regulators of *Escherichia coli*: effect of RpoS, Lrp and H-NS on transcription of the gene *osmC*.................................... 51

José E. González-Pastor: cAMP-CRP, H-NS and IHF are involved in the regulation of the microcin C7 production genes, which transcription is stationary phase induced and σ^S-dependent................................ 52

Claudio O. Gualerzi: Antagonistic transcriptional control by Fis and H-NS and growth-phase-dependent changes in mRNA stability modulate the expression of cold-shock protein CspA at 37°C........................... 53

Fernando Rojo: Expression of the *PalkB* promoter from the *Pseudomonas oleovorans* alkane degradation pathway is subject to carbon source- and growth phase-dependent regulation.. 54

Belén Calles: Mapping of the residues contacted by phage ϕ29 regulatory protein p4 at the RNA polymerase α-subunit.................... 56

Instituto Juan March (Madrid)

PAGE

Regulation of transcription leading to cell division and differentiation
Chairs: Deborah Siegele and Christopher F. Higgins 57

Miguel Vicente: Transcriptional regulation of essential cell division genes........................ 59

Yves V. Brun: Regulation of cell division during the cell cycle of *Caulobacter crescentus*............. 61

Short talk:
Richard D'Ari: Cell division and ppGpp in *Escherichia coli*................................ 62

Keith F. Chater: New uses for old transcription factors, and other novelties in the control of *Streptomyces* differentiation 63

Jeff Errington: Regulation of prespore-specific transcription in *Bacillus subtilis*.................... 64

Short talks:
Andrea Feucht: Compartment-specific activation of σ^F in *Bacillus subtilis*: analysis of SpoIIE activity and localisation in cell division mutants................................ 66

Gabriella H. Kelemen: The role of sigma factors in controlling differentiation in *Streptomyces coelicolor* A(3)2............................... 67

POSTERS... 69

José A. Aínsa: A response regulator-like protein controlling sporulation in *Streptomyces coelicolor*... 71

Manuel Ballesteros: Transcription requirements for Gearbox promoters................................ 72

Ildefonso Cases: Mutational analysis of the *rpoN* gene cluster in *Pseudomonas putida*.................... 73

Mª Trinidad Gallegos: Sequences in σ^N that determine holoenzyme formation and properties.................... 74

Instituto Juan March (Madrid)

PAGE

Johannes Geiselmann: Transcriptional activation by bending of DNA.. 75

Claudio O. Gualerzi: Thermoregulation of *Shigella* and *Escherichia coli* EIEC pathogenicity. A temperature-dependent structural transition of DNA modulates accessibility of *vir*F promoter to transcriptional repressor H-NS........................ 76

Paolo Landini: Mechanism of transcription activation by the *E. Coli* Ada protein............................ 77

Concepción Nieto: The *mal* regulon of *Streptococcus pneumoniae*.. 78

Xavier Perret: High resolution transcriptional analysis of the 536 kb symbiotic replicon of *Rhizobium* sp. NGR234................................. 80

Pablo Rodríguez-Palenzuela: Mechanisms of bacterial resistance to host defense proteins.................... 81

Antonio Tormo: GASP phenotype in enterobacteria........ 82

Ariane Toussaint: *Ralstonia eutropha* RpoN operon and the expression of the byphenyl degradation genes of the Tn*4371* transposon................................ 83

José A. Vázquez-Boland: Control of virulence gene expression in pathogenic *Listeria* by the transcription factor PrfA.............................. 84

Magda Villarroya: Stationary-phase induction of *dnaN*, *dnaN** and *recF*, three genes of *Escherichia coli* involved in DNA replication and repair................. 85

LIST OF INVITED SPEAKERS.................................. 86

LIST OF PARTICIPANTS...................................... 88

Instituto Juan March (Madrid)

Introduction

M. Vicente, R. Kolter and A. Ishihama

Instituto Juan March (Madrid)

Despite their constrained dimensions, microbes are very successful life forms, able to colonise the most extreme environments. Microbial cells, when compared to the eukaryotic ones, contain very simple structures. Some microbes (*Mycoplasma*) may cope with life with just 1% the quantity of genes that a human cell needs to carry. As the structure of the bacterial genes is relatively simple - without introns or other adornments - the economy in genetic material is even more remarkable: one human cell contains more DNA than one thousand *Escherichia coli* cells. Their limited amount of genetic information is nevertheless managed by microbes in an exquisite manner, exploiting all imaginable devices both at the molecular, physiological, and cellular levels to attain their primary goal: survival and proliferation. The basic mechanisms used by microbial cells to manage their small and compact genetic information involve, nevertheless, similar principles as those found in their eukaryotic counterparts.

Although *E. coli* contains 4288 potential genes (some 30% with unassigned function), the number of RNA polymerase molecules per cell is calculated to be limited to 2000. This paucity in the amount of transcriptional enzyme is managed by the regulatory networks of the bacterial cell in a very efficient manner. Bacteria can respond to a diversity of stimuli and stresses by wisely regulating the molecular mechanisms involved in transcription. At least one third of the molecules in the polymerase core enzyme population are not engaged in transcription, forming a pool that can be called into action in response to environmental changes. Free core RNA polymerase molecules can be directed to transcribe specific regulons by association to an assortment of alternative sigma factors (at least seven in *E. coli*) which are themselves expressed under specific circumstances. If required, the cell can specifically inactivate some of its sigma factors by complementary anti-sigmas.

A set of nearly one hundred transcription factors can modify the expression of specific genes by establishing intermolecular contacts with DNA and the different subunits of RNA polymerase. In addition to the dedicated transcription factors, members of the class of DNA-binding proteins (IHF, Fis, HU, HNS...) are known to contribute to transcription regulation by modifying the architecture of many regulatory regions, bringing together in three dimensional space DNA sequences that may be not so near each other.

The expression of genes controlling functions essential for cell proliferation involves a high complexity, beginning with the number of

Instituto Juan March (Madrid)

promoters implicated. Even bacteriophages use to their advantage many of the molecular subtleties present in bacterial cells to fine tune the expression of relevant genes to the specific needs of the different stages in their lytic cycle. Their case can be considered as a rudimentary stage of differentiation, which in those bacteria that undergo differentiation (*e.g. Caulobacter crescentus*) is achieved, as it is in eukaryotic cells, by sequentially turning on and off some genes, and by compartmentalization.

Survival is one of the goals of bacteria, and it involves processes in which they have been peculiarly inventive. Under adverse circumstances *Bacillus subtilis* cells undergo sporulation. The production of a spore is the result of different sigma factors being selectively activated and sequestered into different compartments, what results in the activation of specific genes. Even cells of *E. coli*, which do not sporulate, are not dull in their demise. Entrance into stationary phase triggers the expression of specific genes directed by the appearance of a specific sigma factor, σ^S, and the inhibition of σ^D, the "housekeeper" sigma which transcribes most of the promoters during exponential growth, by an anti-sigma.

It is within this perspective that bacterial gene expression is viewed in recent times as a rather elaborate web of interactions, comprising various regulatory circuits controlled by an assortment of molecules. The relative simplicity of the *lac* operon served in the past as a model to stimulate research in Molecular Genetics, but it may no longer be considered as the paradigm of the bacterial expression unit. When studied at the global level, the complexity of bacterial regulatory mechanisms may serve to stress that the complexity of eukaryotic cells is not unique in the phylogenetic scale.

The description of the molecules involved in the initiation of transcription, in modifying the architecture of DNA, and monitoring the global state of the cell comprised the first part of the Workshop on "*Bacterial Transcription Factors Involved in Global Regulation*". How these mechanisms operate at the cellular level during the growth and survival of bacterial populations was discussed in the second part. The workshop finished with descriptions of the regulatory circuits operating during cell division, differentiation and sporulation.

June 1998
Miguel Vicente
Roberto Kolter
Akira Ishihama

Instituto Juan March (Madrid)

Transcriptional Machinery: components and choices

Chairs: Jeff Errington
Steen Pedersen

Instituto Juan March (Madrid)

Control of Activity and Specificity of RNA Polymerase

Akira ISHIHAMA
National Institute of Genetics, Mishima, Shizuoka 411, Japan

Bacterial RNA polymerase is composed of core enzyme with the subunit structure $\alpha_2\beta\beta'$ and one of multiple molecular species of σ subunit with promoter recognition activity, both together forming holoenzyme. In *Escherichia coli*, for instance, seven different species of σ subunit have been identified, each recognizing a specific set of promoters. We found that the total number of core enzyme in *E. coli* stays constant at a level characteristic of the rate of cell growth, which is less than the total number of genes on the *E. coli* chromosome, while the number of each σ molecule varies in response to cell growth conditions. In addition to the level control, the activity of each σ subunit is subject to control by cytoplasmic solutes and nucleoid structures. Furthermore unused σ subunits are sometimes stored as complexes with anti-σ subunits (or regulators of sigma factors).

Promoters for the genes constitutively expressed in growing cells are recognized by holoenzyme $E\sigma^{70}$ but transcription of the promoters for inducible genes requires in addition one of more of transcription factors. *E. coli* contains more than 100 species of transcription factors, which can be classified into four groups based on the contact site on RNA polymerase. A systematic effort is being made for genetic mapping of the contact sites for class-I factors on the C-terminal domain of α subunit. Several lines of chemical and physical evidence have supported direct protein-protein contact between RNA polymerase subunits and transcription factors. In certain cases, more than two factors simultaneously interact with a single molecule of the RNA polymerase.

The molecular composition of "transcription apparatus" should therefore be different between genes, each being composed of RNA polymerase core enzyme, one of seven different σ subunits, and one or two (or more than two for a limited number of promoters) of more than 100 species of transcription factors. Global pattern of gene transcription is thus determined by the differentiation state of a fixed number of RNA polymerase core enzyme.

Instituto Juan March (Madrid)

Genetic And Immunological Dissection Of The β Subunit Of The Multimeric RNA Polymerase

Robert E. Glass, Talat Malik, Khalid Ahmad & Nihal Buyukuslu
Division of Genetics, School of Clinical Laboratory Sciences, Queens Medical Centre, Nottingham University, Clifton Boulevard, Nottingham NG7 2UH UK

The complex, multimeric DNA-dependent RNA polymerases are highly conserved throughout nature, suggesting a common evolutionary origin. RNA polymerase of *Escherichia coli* is the most well characterised, comprising a holoenzyme with subunit stoichiometry of $\alpha_2\beta\beta'\sigma$ ($M_r \sim 450{,}000$), with which other non-essential components may be naturally associated. As a means of further elucidating the role of the extreme C-terminal region of the β subunit, we have carried out a mutational analysis involving random, PCR-mediated mutagenesis of this 3' region of *E. coli rpoB*, followed by the isolation and characterisation of *trans*-dominant-negative mutations. A number of substitutions of conserved residues in this region were obtained that exhibited different degrees of growth inhibition in a host expressing the chromosomally-encoded wild-type form of the β subunit. These substitutions pinpoint an important, highly conserved motif in the β subunit. The properties of the purified holoenzymes carrying the most extreme *trans* dominant-negative mutations in this motif and the mutant characteristics *in vivo* were similar to those of certain previously identified active site mutants, suggesting that the altered RNA polymerases were capable of promoter binding and RNA chain initiation but were deficient in the subsequent transcriptional stage. Starting with three of these primary lethal mutations, we have selected for intragenic suppressors, located within the same 3'-region, that prevent expression of the *trans*-dominant phenotype. The majority of the second-site substitutions pinpoint highly conserved residues and were allele-specific. One particular missense substitution that acts on all three primary site mutations whilst not appreciably affecting assembly proficiency has been investigated in greater detail. The importance and functional co-operativity of the protein segments within the C-terminal region defined by the primary mutations is supported by the isolation of suppressors of three different primary mutations in the same three clusters.

This work was supported by grants from the Medical Research Council (UK).

Instituto Juan March (Madrid)

Juan March Workshop - Bacterial Transcription Factors Involved in Global Regulation

THE INTERACTION OF *E. COLI* SIGMA SUBUNITS WITH CORE RNA POLYMERASE

Richard R. Burgess, Terrance M. Arthur, Bradley C. Pietz, Lin Rao, Debra B. Jensen, and Scott McMahan McArdle Laboratory for Cancer Research, 1400 University Avenue, University of Wisconsin-Madison, Madison WI 53706. burgess@oncology.wisc.edu

Competition between the various sigma factors for binding to the *E. coli* RNA polymerase core enzyme plays an integral role in global gene regulation. Whereas the first sigma factor was discovered 30 years ago (2), the precise nature of the sigma-core interaction is not yet understood and is a major research focus of this laboratory. Evidence has been presented suggesting that multiple regions of the sigma subunit may be involved in binding site contacts with core, in particular, region 2.1 (3). Little is known about the complementary sites on the core enzyme, although, deletion studies have implicated a region of beta' in sigma binding (1).

We have used chemical cleavage of the beta and beta' subunits of *E. coli* RNA polymerase and far-western analysis as a primary tool to map a major interaction domain on core RNA polymerase for sigma70. Beta and beta' over-expression vectors (T7-promoter-based) containing N- or C-terminal hexahistidine tags were constructed. Washed inclusion bodies were cleaved with various chemical agents: hydroxylamine, iodosobenzoic acid, and 2-nitro-5-thiocyanobenzoic acid. A nested set of cleavage products was purified on a nickel column under denaturing conditions, separated by SDS-PAGE to form an ordered fragment ladder (12), and probed with 32-P-labeled sigma70 Using this method we have localized a strong binding site for sigma70 near the N-terminus of beta' (19). We have confirmed this result by utilizing *in vitro* co-immobilization assays and have narrowed the location to less than 100 amino acids of beta' (19). We are now in the process of defining the region of the sigma70 subunit that makes contacts with this binding site (20). These methods will allow us to characterize the interaction domains of other core/holoenzyme binding factors such as alternate sigmas, sigma-like factors, or transcription regulators.

This "fragment ladder far-western" method (12,19,20) represents a major advance in mapping protein-protein interaction domains that should prove very useful to many researchers studying multisubunit protein complexes.

We have also prepared monoclonal antibodies to all the *E. coli* sigmas and all the core polymerase subunits. Using His-tag ladders of beta and beta', we have mapped several MAb's (12) to beta and beta' by essentially the method described above. We have used immunoaffinity chromatography with a MAb to beta', NT73 (4,5), to isolate core and all forms of holoenzyme from cells grown under different growth conditions. We have probed changes in sigma70 conformation by changes in accessibility to a number of proteases of free sigma, sigma bound to core or sigma in an open promoter complex (18).

Finally, we have analyzed the function of sigma70 region 2.1 by site-directed mutagenesis. Mutations in amino acid positions 383, 385, and 386 were characterized in vivo and in vitro. Results indicate that, while these mutations do not have a large effect on sigma binding to core polymerase under the conditions tested, they result in severe defects in productive transcription (17).

References

1) Luo, J. Sharif, K. Jin, R. Fujika, N. Ishihama, A. and Krakow, J. Molecular anatomy of the beta prime subunit of the E. coli RNA polymerase: identification of regions involved in polymerase assembly, Genes to Cells, 1: 819-827, 1996.

Selected and recent references to bacterial RNA polymerase from the Burgess Lab

2) Burgess, R.R., Travers, A.A., Dunn, J.J., and Bautz, E.K.F. Factor Stimulating Transcription by RNA Polymerase. Nature, 221: 43-46, 1969.

Instituto Juan March (Madrid)

3) Lesley, S.A., and Burgess, R.R. Characterization of the *Escherichia coli* Transcription Factor Sigma-70: Localization of a Region Involved in the Interaction with Core RNA Polymerase. Biochemistry, 28: 7728-7734, 1989.
4) Hager, D., Jin, D.J., and Burgess, R.R. Use of Mono Q High Resolution Ion Exchange Chromatography to Obtain Highly Pure and Active *E. coli* RNA Polymerase. Biochemistry, 29: 7890-7894, 1990.
5) Thompson, N.E., Hager, D.A., and Burgess, R.R. Isolation and Characterization of a Polyol-responsive Monoclonal Antibody Useful for Gentle Purification of *E. coli* RNA Polymerase. Biochemistry, 31: 7003-7008, 1992.
6) Nguyen, L.H., Jensen, D.B., Thompson, N.E., Gentry, D.R., and Burgess, R.R. In Vitro Functional Characterization of Overproduced *E. coli* katF/rpoS Gene Product. Biochemistry, 32: 11112-11117, 1993.
7) Gentry, D.R. Hernandez, V.J., Nguyen, L.H., Jensen, D.B., and Cashel, M. Synthesis of the stationary-phase sigma factor, sigma-S, is positively regulated by ppGpp. J. Bacteriol. 175: 7982-7989, 1993.
8) McMahan, S.A. and Burgess, R.R. Use of Aryl azide crosslinkers to investigate protein-protein interactions: An optimization of important conditions as applied to *E. coli* RNA polymerase and localization of a sigma70-alpha crosslink to the C-terminal region of alpha. Biochemistry, 33: 12092-12099, 1994.
9) Nguyen, L. H. and Burgess, R. R. Overproduction and purification of sigma-S, the *E. coli* stationary phase specific sigma transcription factor. Protein Expression Purif. 8: 17-22, 1996.
10) Burgess, R.R. Purification of Overproduced *E. coli* RNA polymerase sigma factors by solubilizing inclusion bodies and refolding from Sarkosyl, Meth. in Enzymol. 273: 145-149, 1996.
11) Landini, P., Hajec, L., Nguyen, L.H., Burgess, R.R., and Volkert, M. The leucine-responsive regulatory protein (Lrp) acts as a specific repressor for sigmaS-dependent transcription an the *E. coli aidB* gene. Molec. Microbiol. 20: 947-955, 1996.
12) Rao, L., Jones, D. P., Nguyen, L. H., McMahan, S. A., and Burgess, R.R. Epitope mapping using histidine-tagged protein fragments: application to *E. coli* RNA polymerase sigma-70. Anal. Biochem. 241: 173-179, 1996.
13) Nguyen, L. H. and Burgess, R. R. Comparative analysis of the interactions of *E. coli* sigma-S and sigma-70 RNA polymerase holoenzyme with the stationary-phase-specific *bolAp1* promoter. Biochemistry, 36: 1748-1754, 1997.
14) Breyer, M.J., Thompson, N.E., and Burgess, R.R., Identification of the epitope for a highly cross-reactive monoclonal antibody on the major sigma factor of bacterial RNA polymerase, J. Bacteriol., 179: 1404-1408, 1997.
15) Rockabrand, D., Livers, K., Austin, T., Kaiser, R., Jensen, D., Burgess, R. and Blum, P. Role of DnaK and RpoS in starvation-induced thermotolerance of *E. coli*. J. Bacteriol., 180: 846-854, 1998.
16) Jensen, D. B. and Burgess, R. R. Changes in the levels of sigma factors bound to *E. coli* core DNA-dependent RNA polymerase in cells grown under different conditions as measured by quantitative direct fluorescence western blot analysis, in preparation.
17) Rao, L. and Burgess, R.R. Mutational analysis of *E. coli* sigma70 region 2.1, in preparation.
18) McMahan, S. A. and Burgess, R.R. Mapping protease susceptibility sites on the *E. coli* transcription factor sigma70, in preparation.
19) Arthur, T.M. and Burgess, R.R. Localization of a sigma70 binding site on the N-terminus of the *E. coli* RNA polymerase beta prime subunit, in preparation.
20) Pietz, B.C. and Burgess, R.R. Localization of a beta prime binding site on the *E. coli* RNA polymerase - sigma70 subunit, in preparation.

Instituto Juan March (Madrid)

Selectivity of RNA polymerases containing the σ^{70} and σ^{S} factors

A.Kolb, F.Colland, M. Barth and R. Hengge-Aronis

The sigma subunit of RNA polymerase plays an essential role in promoter recognition. The stationary phase sigma factor of *E.coli* σ^{S} is homologous to σ^{70} the major sigma factor especially in the domains involved in the recognition of the -10 and -35 regions of the promoter .Thus a number of promoters can be recognized *in vitro* by both σ^{S} and σ^{70} RNA polymerase holoenzymes although with different affinities and kinetics. Some parameters like supercoiling or ionic strength are able to affect differently promoter recognition by the respective holoenzymes .The *osmY* promoter is repressed *in vivo* and *in vitro* by a number of repressors like IHF, the cAMP-CRP complex, Lrp and also to a lesser extent by HNS. The location of the DNA binding sites of these repressors have been characterised : they are centred from positions -105 to the -10 region of the promoter. In every case, we find that RNA polymerase containing the sigmaS factor ($E\sigma^{S}$) is more competent than $E\sigma^{70}$ in relieving repression by IHF, CRP, HNS and Lrp.These data suggest a novel role for IHF, CRP, HNS and Lrp as modulators of sigma selectivity.

Instituto Juan March (Madrid)

Anti-Sigma Factor for the Major Sigma Subunit of *Escherichia coli* RNA Polymerase

Miki JISHAGE and Akira ISHIHAMA
Department of Molecular Genetics, National Institute of Genetics,
Mishima, Shizuoka 411, Japan

Switching of the transcription pattern in *Escherichia coli* during the growth transition from exponential to stationary phase is accompanied by the replacement of the RNA polymerase-associated σ^{70} subunit (σ^{D}) with σ^{38} (σ^{S}). Some of the σ^{70} subunit in stationary-phase cell extracts was found to exist as a complex with a novel protein, designated Rsd (Regulator of Sigma D). The intracellular level of Rsd starts to increase during the transition from growing to stationary phase. The *rsd* gene was identified at 90 min on the *E. coli* chromosome. Over-expressed and purified Rsd protein formed complexes *in vitro* with σ^{70} but not with other σ subunits, σ^{N}, σ^{S}, σ^{H}, σ^{F} and σ^{E}. Analysis of proteolytic fragments of σ^{70} indicated that Rsd binds at or downstream of region 4, the promoter -35 recognition domain. The isolated Rsd inhibited transcription *in vitro* to various extents depending on the promoters used. We propose that Rsd is an anti-σ^{70} factor and plays a role in storing unused σ^{70} in an inactive form in stationary-phase cells for reutilization upon release from stationary phase.

Instituto Juan March (Madrid)

IHF in sigma-54 promoters : DNA bending and more

*Bertoni, G., Carmona, M., Pérez-Martín, J. and de Lorenzo, V.**
Centro Nacional de Biotecnología-CSIC, Campus de Cantoblanco, Madrid 28049

Prokaryotic promoters dependent on the alternative sigma factor σ^{54} are one of the very few examples of transcriptional control at a distance in bacteria. Also, unlike most positively regulated promoters, the cognate activators (generically known as members of the NtrC family), act on the σ^{54} polymerase bound to a downstream -12/-24 sequence motif, i.e., activate at a post-recruitment step. The σ^{54} -dependent *Pu* promoter of *Pseudomonas putida* is activated by the XyR protein by virtue of a mechanism that requires the contacts between the regulator of the system (the XylR protein) oligomerized at the upstream activating sequences (UAS) and the σ^{54} -RNAP bound downstream [1]. Such an interaction between proteins attached to distant sites (> 120 bp) implies the looping out of the intervening DNA sequence. This makes the region between the UAS and the σ^{54}-RNAP a target for additional co-regulation devices which allow transcription only when the geometry of the entire region adopts a particular configuration [2].

The intervening DNA sequence between the UAS and the -12/-24 sequence in *Pu* is intrinsically curved and contains a functional site for the Integration Host Factor (IHF protein). This site can be functionally replaced by intrinsically bent DNA. IHF function can also be substituted to some extent by the HU protein *in vitro* and *in vivo* and by mammalian HMG1 *in vitro* [3]. These data indicate the outstanding role of IHF as an architectural element in σ^{54} promoters.

When the *Pu* promoter is assayed *in vivo* in an IHF-minus strain of *Pseudomonas*, its activity is abolished completely [4], in sharp contrast with the behaviour of the same promoter in *E. coli*. This effect can be traced to the role of IHF in recruiting the σ^{54} -RNAP to the -12/-24 motif, so that in the absence of the factor, the lack of binding of the polymerase to the promoter hinders any other subsequent activation of the system by XylR. We have studied the mechanism of such recruitment, which is fully independent of XylR and, involves, instead, the binding of the αCTD of the RNAP to DNA placed upstream but adjacent to (or overlapping) the IHF site. This mechanism is confirmed by results obtained with *in vitro* transcription experiments with XylR on DNA templates bearing the -12/-24 sequences and the IHF binding site but lacking the UAS. By varying the occupancy of -12/-24 by σ^{54}-RNAP it is possible to either inhibit transcription by XylR from solution by adding IHF (through the structural effect called *restriction* [5]) or to activate the promoter with the same factor or HU. These data substantiate the co-activator function of IHF in (at least some) σ^{54} promoters, which may occlude its architectural role in the cases when the σ^{54}-RNAP does not have much affinity for its target sequences. These observations add an unexpected level of complexity to the regulation of σ^{54} promoters, since it was generally believed that σ^{54}-RNAP was permanently bound to -12/-24 forming an stable closed complex.

[1] Pérez-Martín, J. and de Lorenzo, V. 1996. ATP binding to the σ^{54}-dependent activator XylR triggers a protein multimerization cycle catalysed by UAS DNA. *Cell* 86, 331-339

[2] Pérez-Martín, J. and de Lorenzo, V. 1997. Clues and consequences of DNA bending in transcription. *Ann. Rev. Microbiol.* 51 : 593-628

[3] Pérez-Martín, J. and V. de Lorenzo. 1997. Co-activation *in vitro* of the σ^{54} dependent promoter *Pu* of the TOL plasmid of *Pseudomonas putida* by the prokaryotic histone HU and the mammalian HMG-1 protein. *J. Bacteriol.* 179: 2757-2760

[4] Calb, R. et al. 1996. Structure and function of the *Pseudomonas putida* integration host factor. *J. Bacteriology* 178 : 6319-6326

[5] Pérez-Martín, J. and V. de Lorenzo. 1995. Integration Host Factor (IHF) supresses promiscuous activation of the σ^{54}-dependent promoter *Pu* of *Pseudomonas putida*. *Proc. Natl. Acad. Sci. USA* 92 : 7277-7281

Instituto Juan March (Madrid)

Construction of a Bacterial Promoter Region with an Enhancer Bearing a Multiple Response Element

J. A. Molina-López and E. Santero. Departamento de Genética. Facultad de Biología. Universidad de Sevilla. Ap. 1095- 41080-Sevilla. Spain

Unlike the major form of RNA polymerase holoenzyme containing σ^{70}, the alternate form of holoenzyme containing σ^N (σ^{54}) allows transcriptional activation at a distance. Activators of σ^N-dependent systems bind to sites located more than 100 pb upstream of the transcription start site and contact RNA polymerase bound to the promoter via formation of a DNA loop of the intervening DNA. At some promoters which have a strong binding site for RNA polymerase such as the *glnAp2*, binding sites for the activator may be moved away and still are functional, thus showing characteristics of eukaryotic enhancers. This feature could potencially allow that many different activators regulate transcription from the same promoter, thus significantly increasing versatility and complexity of the regulation.

We have synthesized a DNA fragment, which we call multiple response element, bearing binding sites for 5 different σ^N-dependent activators, and cloned it in different positions relative to the RNA polymerase binding site found in the *glnAp2* promoter. Based on comparison of transcriptional activation by each of the 5 activators, the following conclusions will be discussed:

i) From a mechanistic point of view, some promoter regulatory regions may allow regulation of a bacterial transcriptional machinery to be very versatile and potentially complex.

ii) Capacity of activation in the absence of binding sites is different among activators.

iii) Transcriptional activation is relatively insensitive to the position of the activator binding sites.

iv) Active transcription of the intervening DNA between RNA polymerase and activator binding sites does not limit efficiency of activation.

v) Transcriptional activation from binding sites located within the transcriptional unit is not efficient because binding of the activator may interfere with subsequent steps of transcription.

vi) Some forms of activator may be very sensitive to changes in the position of the activator binding sites. Deficiency in oligomer formation may explain this sensitivity.

vii) Neither the activator nor the position from which it activates appears to affect selection of the transcription start site.

Instituto Juan March (Madrid)

Monitoring the global physiological state of the cell

Chairs: Margarita Salas
Richard R. Burgess

Instituto Juan March (Madrid)

Regulation of recombination and transcription by the Fis protein

Reid C. Johnson, Stacy K. Merickel, Sarah McLeod, Leah Corselli, and Hanna S. Yuan*

Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA USA 90095-1737
*Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan.

The *E. coli* Fis protein is a general nucleoid associated protein that regulates a variety of DNA transactions. Fis cellular levels vary greatly under different growth conditions. Fis levels are very low in stationary phase but increase enormously upon a nutrient upshift to a maximum of about 40,000 dimers/cell. In early-mid exponential growth in rich media batch cultures, Fis is one of the most abundant DNA binding proteins in the cell. Under steady state growth conditions, Fis levels vary with respect to the growth rate. The talk will focus on the contrasting mechanisms by which Fis activates site-specific DNA inversion and transcription.

Fis controls the frequency and directionality of Hin-catalyzed site-specific recombination by binding to two precisely spaced sites in a recombinational enhancer segment. The two Hin-bound recombination sites are captured by the enhancer at the base of a DNA branch to form an invertasome structure. Fis coordinately activates the catalytic activity of the four Hin subunits using a mobile β-hairpin motif that protrudes over 20 Å from the core. Three amino acids near the tip of only one of the two β-arms within each Fis dimer are of primary importance and probably contact the Hin recombinase at its dimer interface to induce a quaternary change in the recombinase structure.

Fis together with σ^S are required for transcription of the *proP P2* promoter and the combined growth phase expression patterns of these two proteins result in a transient period of transcription of the proline and glycine betaine transporter in late exponential phase. Fis activates the σ^S-dependent *proP P2* promoter when bound to a site located at -41 using a region located close to the DNA binding domain but removed from the β-arms. This is the same region that contacts the α-ctd to activate *rrnB P1* transcription (R. Gourse and co-workers) and is also identical to the region that mediates cooperative binding with Xis to stimulate phage λ excision. A crystal structure of an activation-defective mutant within this region suggests the importance of a cleft within Fis where intimate contacts with various other proteins occurs. CRP also activates *proP P2* when bound to a site located at -121. CRP activation requires Fis but does not require the Fis transcriptional activation region.

References:

Ball, C.A., Osuna, R., Ferguson, K.C., and Johnson, R.C. (1992) Dramatic changes in Fis levels upon nutrient upshift in *Escherichia coli*. *J. Bacteriol.*, 174: 8043-8056.

Safo, M.K., Yang, W.-Z., Corselli, L., Cramton, S. E., Yuan, H. S., and Johnson, R. C. (1997) The transactivation region of the Fis protein that controls site-specific DNA inversion contains extended mobile β-hairpin arms. *EMBO J.*, 16: 6860-6873.

Xu, J. and Johnson, R.C. (1997) Activation of RpoS-dependent *proP* P2 transcription by the Fis protein in vitro., *J. Mol. Biol.*, 270: 346-359.

Bokal, A.J., Ross, W., Gaal, T., Johnson, R.C., and Gourse, R.L. (1997) Molecular anatomy of a transcriptional activation patch: Fis-RNA polymerase interactions at the *Escherichia coli rrnB* P1 promoter., *EMBO J.*, 16: 154-162.

Instituto Juan March (Madrid)

H-NS, chromatin structure and gene expression

C.F. Higgins, MRC Clinical Sciences Centre, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Rd., London W12 0NN.

H-NS is one of the most abundant nucleoid-associated proteins. hns mutations confer a variety of phenotypes, influencing recombination, DNA rearrangements, and the expression of a varied subset of genes. Many properties of H-NS distinguish its mode of action from that of classical transcription factors, particularly its lack of DNA binding specificity, its physical properties, and the effects of H-NS on DNA topology. The role of H-NS in maintaining chromatin structure, and possible mechanisms by which H-NS might influence gene expression will be discussed.

Instituto Juan March (Madrid)

Abstract for Juan March Symposium: Bacterial Transcription Factors involved in Global Regulation

CRP and FNR: master craftsmen, but also oddjobmen

Steve Busby

School of Biochemistry, The University of Birmingham, Birmingham B15 2TT, U.K.

CRP and FNR are two related bacterial global transcription regulatory proteins. The activity of CRP is triggered by increases in intracellular cyclic AMP levels, whilst the activity of FNR is triggered by anaerobiosis. The CRP regulon includes over 100 genes whose promoters are controlled by CRP: the majority of these target promoters are activated by CRP and control genes involved in catabolic processes. The FNR regulon includes nearly 50 genes whose promoters are controlled by FNR: the majority of these target promoters are activated by FNR and control genes involved in adaptation to the absence of oxygen in the environment.

The organisation of CRP- and FNR-dependent promoters will be briefly reviewed. Although there is great diversity in the way the CRP- and FNR-dependent promoters are organised, in most cases, CRP or FNR activate transcription by making direct contact with RNA polymerase. In order to activate transcription at promoters of different architectures, CRP and FNR use different activating regions to interact with RNA polymerase at different promoters. These activating regions are small surface-exposed patches that can interact with different targets in RNA polymerase. Presumably, these different activating regions have evolved to allow CRP and FNR to function at a diverse range of promoters: this is likely to be a prerequisite for a successful global regulator.

Another prerequisite for a global regulator is that it should be able to function synergically with other transcription factors. Studies of the organisation of many promoters that are co-dependent on CRP or FNR, and another transcription activator suggest that, in most of these cases, co-dependence on two transcription activators is due to the necessity for both activators to make independent contacts with RNA polymerase. It can be argued that this mechanism for co-dependence gives the cell the maximum flexibility to "mix and match" different transcription factors. Study of the *E. coli nir* promoter, which is co-dependent on FNR and the nitrite/nitrate-triggered activators, NarP and NarL, have revealed an alternative mechanism by which a bacterial promoter can be co-dependent on two activators.

Bibliography

Bell, A, Gaston, K, Cole, J & Busby, S (1989) Cloning of binding sequences for the *Escherichia coli* transcription activators, FNR and CRP: location of bases involved in discrimination between FNR and CRP. *Nucl.Acids. Res.* 17 3865-3874

Spiro, S, Bell, A, Gaston, K, Roberts, R, Busby, S & Guest, J (1990) Interconversion of the DNA-binding specificities of two related transcription activators, CRP and FNR. *Mol. Microbiol.* 4 1831-1838

Williams, R, Bell, A, Sims G & Busby, S (1991) The role of two surface exposed loops in transcription activation by the *Escherichia coli* CRP and FNR proteins. *Nucl. Acids Res.* 19 6705-6712

Kolb, A, Busby, S, Buc, H, Garges, S & Adhya, S (1993) Transcriptional regulation by cyclic AMP and its receptor protein. *Ann. Rev. Biochem.* 62 749-795

Instituto Juan March (Madrid)

Tyson, K, Cole, J, & Busby, S (1994) Nitrite and nitrate regulation at the promoters of two *Escherichia coli* operons encoding nitrite reductase. *Mol. Microbiol.* **13** 1045-1046

Busby S, West, D, Lawes, M, Webster C, Ishihama, A, & Kolb A (1994) Transcription activation by the *Escherichia coli* cyclic AMP receptor protein: receptors bound in tandem at promoters can interact synergystically. *J. Mol. Biol.* **241** 341-352

Busby, S & Ebright, R (1994) Promoter structure, promoter recognition and transcription activation in prokaryotes. *Cell* 79 743-746

Busby, S, Kolb, A & Buc, H (1995) The *E. coli* cyclic AMP receptor protein. In *Nucleic Acids and Molecular Biology* (Lilley, D. & Eckstein, F., eds) 9 177-191, Springer-Verlag

Wing H, Williams S & Busby S (1995) Spacing requirements for transcription regulation by *Escherichia coli* FNR protein. *J. Bacteriol.* 177 6704-6710

Busby, S & Kolb, A (1996) The CAP Modulon. In *Regulation of Gene Expression in E. coli* (Lin, E & Lynch, S, eds) chapter 12, pp 255-279, R.G. Landes, New York

Savery, N, Belyaeva, T, Wing, H & Busby S (1996) Regulation of promoters by two transcription activators: evidence for a "simultaneous touching" model. *Biochemical Society Transactions* **24** 351-353

Rhodius, V, West, D, Webster, C, Busby, S & Savery, N (1997) Transcription activation at Class II CRP-dependent promoters: the role of different activating regions. *Nucl. Acids Res.* 25 326-333

Busby, S & Ebright, R (1997) Transcription activation at Class II CAP-dependent promoters. *Molecular Microbiology* **23** 853-859

Darwin, A, Tyson, K, Busby, S & Stewart, V (1997) Differential regulation by the homologous response regulators NarL and NarP of *Escherichia coli* K12 depends on DNA binding site arrangement. *Molecular Microbiology* 25 583-595

Murakami, K, Owens, J, Belyaeva, T, Meares, C, Busby, S & Ishihama, A (1997) Positioning of two alpha subunit carboxy-terminal domains of RNA polymerase along promoter DNA by two transcription factors. *Proc. Natl. Acad.Sci. (USA)* **94** 11274-11278

Williams, S, Savery, N, Busby, S & Wing, H (1997) Transcription activation at Class I FNR-dependent promoters: identification of the activating surface of FNR and the corresponding contact site in the C-terminal domain of the RNA polymerase α subunit. *Nucl. Acids Res.* 25 4028-4034

Wu, H-C, Tyson, K, Cole, J & Busby, S (1998) Regulation of the *E. coli nir operon* by two transcription factors: a new mechanism to account for co-dependence on two activators. *Mol. Microbiol* **27** 493-505

Belyaeva, T, Rhodius, V, Webster, C & Busby, S (1998) Transcription activation at promoters carrying tandem DNA sites for the *Escherichia coli* cyclic AMP receptor protein: organisation of the RNA polymerase α subunits. *J. Mol. Biol* in press

Li, B, Wing, H, Lee, D, Wu H-C, & Busby S (1998) Transcription activation by *Escherichia coli* FNR protein: similarities to, and differences from, the CRP paradigm. *Nucl. Acids Res.* in press

Instituto Juan March (Madrid)

Subsaturation: A general regulatory mechanism for translation and transcription?

Steen Pedersen, Institute of Molecular Biology, University of Copenhagen, Denmark.

Several years ago Kaj Frank Jensen from our institute and I presented a model for how global regulation might be achieved in *Escherichia coli.* (Jensen & Pedersen, 1990). The model was formulated broadly to encompass the synthesis of all macromolecules, and, in contrast to previous models for global regulation, e.g. by Maaloe, it had a number of predictions which could be tested experimentally.

The model had the premise that the processes involved in macromolecular synthesis were unsaturated with their various substrates thereby creating a competition in the cell which enable the cell to regulate its activities. Also, if the polymerisation rates of the chain elongation reactions were variable this could at least in part determine the concentration of initiation competent DNA and RNA polymerases, ribosomes etc and thereby affect regulation (Jensen & Pedersen, 1990).

We have in the following years tested some of the predictions from the model:

In a strain deleted for the *cmk* gene, encoding cytidine monophosphate kinase, the cellular concentration of CDP is reduced. Because the reduction to deoxyribonucleosides takes place at the diphosphate level this lead to reduced pools of both dCTP and dTTP. The rate of replication fork movement in this strain was reduced to approximately 50% and this rate became normal immediately after induction of *cmk* expression (Fricke *et al.*, 1995). Thus the rate of DNA replication is limited by the concentration of its pyrimidine substrates.

For the transcription process, I expect from Thomas Nyström's title that he will tell you that the various sigma factors compete for a limited concentration of RNA polymerase. Therefore, the promoters seem to be unsaturated with RNA polymerase, at least under some conditions.

The RNA chain elongation rate varies by approximately 40% at different growth rates (Vogel & Jensen, 1994b) and is specifically influenced by the concentration of the nucleotide ppGpp (Vogel *et al.*, 1992; Sorensen *et al.*, 1994). In addition to the effect of ppGpp, the reduction in the RNA chain elongation rate is almost certainly brought about by limiting concentrations of the some of the nucleoside triphosphates because the *pyrE* gene is regulated by an attenuation mechanism (Poulsen & Jensen, 1987). However, a large fraction of the transcription in the cell, that of the stable RNA species, takes place with a much faster rate than mRNA transcription and this rate is independent of the growth rate (Vogel & Jensen, 1994a; Vogel & Jensen, 1995).

When the concentration of initiation competent ribosomes was reduced by inducing large amounts of *lacZ* mRNA, an immediate reduction of the expression of most proteins was found. To a large extent, the reduction correlated with the calculated affinity between the mRNA and 3' end of 16S rRNA (Vind *et al.*, 1993). This indicated, that all except the very strongest ribosome binding sites are subsaturated with ribosomes in vivo.

Also, the peptide polymerisation rate varies with the cellular growth rate (Pedersen, 1984). This has to do with the use of the specific codons in the mRNA, whereas mRNA structure seem not to be a rate barrier for the ribosome (Sorensen *et al.*, 1989). Also, mutants defective in modification of tRNA translate slower (Diaz *et al.*, 1987) [Krÿger et al., submitted for publication]. Therefore, most likely the concentration of the cognate charged tRNAs determines the peptide elongation rate. That this must be the case can of course

Instituto Juan March (Madrid)

also be deduced from the existence of the attenuator regulation of several amino acid biosynthesis operons. These would only work if the amino acid concentration influenced the peptide elongation rate.

We determined the codon specific translation rate for four codons (Sorensen & Pedersen, 1991). Two of these, the two glutamic acid codons GAA and GAG, are read by the same tRNA but with a four-fold difference in rate. We envisage this result to be caused by a different affinity between the codon and the anticodon. Because the slower of the two codons predominantly are found in the 5' end of mRNAs (Bulmer, 1988) this enabeles the cell to have a substrate dependent regulation of the initiation frequency and at the same time a maximal utilisation of the expensive translational machinery on other parts of the mRNA.

The global regulation model (Jensen & Pedersen, 1990) therefore seems qualitatively to describe many aspects of the replication, transcription and translation in the living cell. The important question is of course if the model also in quantitative terms provides an adequate description of the cell.

Literature cited:

Bulmer, M. (1988). J. Theor. Biol. 133, 67-71.
Diaz, I., Pedersen, S. & Kurland, C. (1987). Mol. Gen. Genet. 208, 373-376.
Fricke, J., Neuhard, J., Kelln, R. A. & Pedersen, S. (1995). J. Bacteriol. 177, 517-523.
Jensen, K. F. & Pedersen, S. (1990). Microbiol. Rev. 54, 89-100.
Pedersen, S. (1984). EMBO J. 3, 2895-2898.
Poulsen, P. & Jensen, K. F. (1987). Mol. Gen. Genet. 208, 152-158.
Sorensen, M. A., Jensen, K. F. & Pedersen, S. (1994). J. Mol. Biol. 236, 441-454.
Sorensen, M. A., Kurland, C. G. & Pedersen, S. (1989). J. Mol. Biol. 207, 365-377.
Sorensen, M. A. & Pedersen, S. (1991). J. Mol. Biol. 222, 265-280.
Vind, J., Sorensen, M. A., Rasmussen, M. D & Pedersen, S. (1993).J. Mol. Biol. 231, 678-688.
Vogel, U. & Jensen, K. F. (1994a).J. Biol. Chem. 269, 16236-16241.
Vogel, U. & Jensen, K. F. (1994b). J. Bacteriol. 176, 2807-2813.
Vogel, U. & Jensen, K. F. (1995). J. Biol. Chem. 18335-18340.
Vogel, U., Sorensen, M. A., Pedersen, S., Jensen, K. F. & Kilstrup, M. (1992). Mol. Microbiol. 6, 2191-2200.

Instituto Juan March (Madrid)

Activation and Repression of Transcription at the Double Tandem Divergent Promoters for *xylR* and *xylS* Genes of the TOL Plasmid of *Pseudomonas putida*

Silvia Marqués, María-Trinidad Gallegos, Maximino Manzanera and Juan L. Ramos

Consejo Superior de Investigaciones Científicas, Estación Experimental del Zaidín, Department of Biochemistry, E-18008 Granada, Spain

The *xylR* and *xylS* genes are divergent and control transcription of the TOL plasmid catabolic pathways for toluene metabolism. Four promoters are found in the 300 bp intergenic region: Pr1 and Pr2 are constitutive σ^{70}-dependent tandem promoters that drive expression of *xylR*, while the expression of the *xylS* gene is driven from Ps2, a constitutive σ^{70}-dependent promoter, and by the regulatable σ^{54} class Ps1 promoter. In Ps1 the XylR targets (UASs) overlap the Pr promoters, and two sites for IHF binding are located at the -2/-30 region and the -137/ -156 region, the latter overlapping the Pr promoters. When the XylR protein binds to the UASs in the absence of effector, it represses expression from Pr promoters. In the XylR-plus background and in the absence of an effector the level of expression from Ps1 is low, although detectable, whereas Ps2 is active. In this background and in the presence of an effector, XylR increases autorepression. In a σ^{54}-deficient *Pseudomonas putida* background, no expression occurred from Ps1 regardless of the presence of an effector. However, in the presence of an effector the amount of RNA produced from Pr promoters was almost undetectable. This suggests that when no transcription occurred at the Ps1 promoter, clearance of XylR from the UASs was almost negligible. In this background, expression from Ps2 was very high regardless of the presence of an effector; this suggests that RNA-polymerase/σ^{54} modulates expression from the downstream Ps2 σ^{70}-dependent promoter. In a *P. putida* IHF-minus background and in the presence of effector, Ps1 expression was the highest found; in contrast, the basal levels of this promoter were the lowest observed. This suggests that IHF acts *in vivo* as a repressor of the σ^{54}-dependent Ps1 promoter. In an IHF-deficient host background, expression from Ps2 in the presence of effector was negligible. Thus binding of RNA-polymerase/σ^{54} at the upstream promoter may modulate expression from the Ps2 promoter.

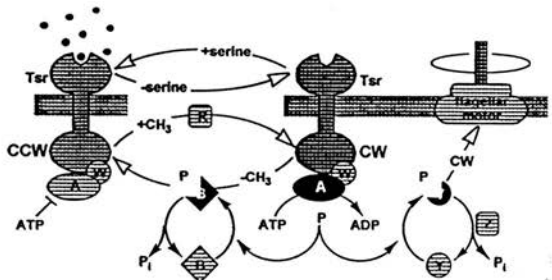
Instituto Juan March (Madrid)

Dissecting the Three-Protein Brain of *E. coli*

John S. Parkinson
Biology Department
University of Utah
Salt Lake City, UT 84112 USA

E. coli and other motile bacteria are attracted or repelled by many chemicals in their environment. Although the readout of the chemotaxis sensory system is locomotion rather than transcriptional control, this signaling pathway is an excellent model for exploring general mechanisms of stimulus detection and signal processing at the molecular level. *E. coli* cells detect attractant and repellent gradients as they swim about by comparing current chemoeffector levels with those averaged over the past few seconds. Concentration differences of less than 1% can elicit net movements in favorable directions by triggering changes in the rotational behavior of the flagellar motors. The cells' are sensitive to such small stimuli over a nanomolar to millimolar concentration range, a feat that is still only poorly understood.

Most chemoeffectors are sensed by "methyl-accepting chemotaxis proteins" (MCPs), which are transmembrane molecules with a periplasmic ligand-binding domain and a cytoplasmic signaling domain (see figure below). My group works with Tsr, the serine chemoreceptor, which, like other MCPs, communicates with the flagellar motors through a phosphorelay of cytoplasmic signaling proteins. The phosphorylated form of CheY, the response effector, interacts with switch proteins at the base of the flagellar motors to initiate clockwise (CW) rotational episodes. CheY obtains its phosphoryl groups from CheA, a histidine autokinase. CheA is stably associated with MCP signaling domains through a coupling protein, CheW. In this ternary signaling complex, CheA's autophosphorylation activity is modulated allosterically by changes in receptor ligand occupancy.



Receptor molecules have two signaling states, a CW mode that stimulates CheA autophosphorylation ~200-fold, and a CCW mode that suppresses CheA activity. The cell's swimming behavior reflects the proportions of signaling complexes in the CCW and CW states. These proportions shift upon changes in ligand occupancy, triggering increases or decreases in phospho-CheY level and resultant flagellar responses.

Covalent modification of the receptor signaling domain (reversible carboxymethylation at specific glutamic acid residues) also shifts the CCW/CW equilibrium and represents the cell's memory store. In isotropic environments, the receptor's methylation state is directly proportional to its current extent of ligand occupancy. In effect, the receptor signaling complexes function as comparators that alter CheA activity whenever ligand occupancy and methylation state are out of balance. Two opposing enzymes control MCP methylation state, CheR, a methyltransferase, and CheB, a methylesterase. CheR activity is unregulated, whereas CheB activity is feedback regulated upon phosphorylation by receptor signaling complexes. Thus, changes in ligand occupancy modulate CheA activity to alter the phosphorylation states of CheY and CheB, triggering a fast motor response and a slower sensory adaptation, respectively.

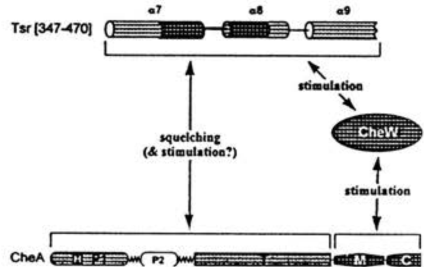
Instituto Juan March (Madrid)

My lab is analyzing structure-function relationships in Tsr, CheW, and CheA to investigate the mechanism of CheA control in receptor signaling complexes. Much of our recent work has focused on characterizing and exploiting soluble fragments of the Tsr signaling domain that produce CW or CCW signals *in vivo* and *in vitro*. Our principal findings are as follows:

- CW-signaling Tsr fragments bind CheW with a 2 μM affinity and stimulate CheA autophosphorylation rate about 200-fold *in vitro*. Stimulation requires the CheW coupling factor and is maximal at equimolar ratios of CheW and CheA. Tsr fragments stimulate CheA in highly cooperative fashion, with a Hill coefficient over 5 and optimal stoichiometries of more than 8:1:1 (Tsr:CheW:CheA). Assembly of functional stimulatory complexes is slow, requiring at least an hour at room temperature. These properties suggest that CheA stimulation occurs in higher order receptor complexes, which could account for *in vivo* observations of receptor clustering.

- CW-signaling Tsr fragments can be converted to CCW-signaling ones by introducing any of several single amino acid replacements that *in vivo* lock full-length receptors in a CCW-signaling mode. CCW-signaling fragments bind CheW with 2 μM affinity and inhibit or "squelch" CheA autophosphorylation activity ~5-fold *in vitro*. Tsr-CCW squelching of CheA is non-cooperative, rapid, reversible, and CheW-independent, implying that Tsr-CCW fragments inhibit CheA through direct contacts. This interaction is evidently weak, because attempts to demonstrate direct binding between Tsr-CCW and CheA have thus far been unsuccessful.

- The probable regions of contact between Tsr, CheW, and CheA molecules are summarized in the figure on the right. The smallest active Tsr signaling fragments are ~120 residues in length and encompass a highly-conserved segment (indicated by dark shading in the figure) that most likely contains the critical contact sites for CheA and CheW. Most mutational changes that affect the ability of Tsr fragments to bind CheW or to squelch CheA fall within this segment of the Tsr signaling domain. In CheA, the C-terminal M/C region is essential for stimulatory receptor control, but not for squelching control. The M/C region of CheA is also essential for binding to CheW (affinity ~25 μM), which presumably accounts for its role in stimulatory receptor coupling. The Tsr-CheA contacts involved in squelching must lie outside the M/C segment, most likely in the catalytic and/or substrate domains of CheA (designated P1 and T in the figure). Mutations that specifically abolish the ability of Tsr-CCW to squelch CheA also abrogate the ability of Tsr-CW to stimulate CheA, suggesting that Tsr-CheA contacts involved in squelching control are also important in stimulatory control.



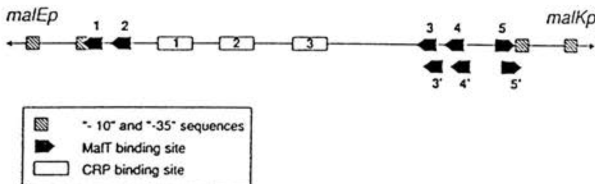
- Structural studies indicate that the segment of the Tsr signaling domain shown above may have a helical hairpin configuration. This suggests a simple model for allosteric control of CheA in which the CheA substrate and catalytic domains are bound to opposing arms of the hairpin. In this way receptor shifts between the CW and CCW signaling conformations could regulate CheA activity simply by manipulating the access of the substrate histidine to the catalytic center. Some ramifications of this model will be discussed at the meeting, including the idea that receptor signaling complexes are functionally networked to achieve very high detection sensitivity.

Instituto Juan March (Madrid)

Synergistic activation of the *malEp* promoter by MalT and CRP: two roles for CRP.

Evelyne RICHET, Unité de Génétique Moléculaire, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France.

Activation of the *malEp* promoter from *E. coli* depends on the synergistic action of MalT, the activator of the maltose regulon, and CRP, a global regulator. Previous studies have shown that *malEp* activation depends on the formation of a higher-order structure involving cooperative binding of MalT onto sites 1, 2 and sites 3', 4', 5', and bending of the intervening DNA by CRP bound to the three CRP binding sites.



Analysis of the mechanism whereby this higher-order structure triggers initiation of transcription at *malEp* has shown that MalT is the primary activator, that it acts from the MalT sites 1 and 2 and that one function of the nucleoprotein structure is to stabilize MalT onto these sites. Recent *in vivo* and *in vitro* studies have also revealed that besides its architectural role in the assembly of the nucleoprotein complex, the molecule of CRP bound to the CRP site 1 (centered at - 76.5) plays a specific role in the activation process by directly contacting either MalT or the RNA polymerase. In particular, we have identified at the surface of CRP amino-acids whose side chains are important for *malEp* activation. These amino acids are located in the AR1 region, a surface element of CRP known to contact the C-terminal domain of the α subunit of the RNA polymerase at class I CRP-dependent promoters.

Instituto Juan March (Madrid)

H-NS and StpA proteins stimulate expression of the maltose regulon in *Escherichia coli.*

Jörgen Johansson and Bernt Eric Uhlin
Department of Microbiology
Umeå University
S-90187 Umeå, Sweden

The nucleoid-associated protein H-NS is a major component of the chromosome-protein complex, and it is known to influence the regulation of different genes in *Escherichia coli*. Its role in gene regulation is manifested by the increased expression of several gene products in *hns* mutant strains. We have found that H-NS, and the largely homologous protein StpA, play a positive role in the expression of genes in the maltose regulon (1). In studies with *hns* mutant strains and derivatives deficient also in the *stpA* gene, we found that expression of the LamB porin was decreased. Our results showed that both the amount of LamB protein and *lamB* mRNA were greatly reduced in *hns* and *hns, stpA* mutant strains. The same result was obtained when we monitored the amount of transcription from the *malEFG* operon. The *lamB* gene is situated in the *malKlamBmalM* operon, which forms a divergent operon complex together with the *malEFG* operon. The activation of these genes depends on the action of the maltose regulon activator MalT and the global activator CRP. Our results suggest that the H-NS and StpA proteins directly stimulate the expression, and hence have a positive role in the control, of the maltose-regulon.

1) Johansson, J., Dagberg, B., Richet, E., and Uhlin, B.E. 1998. H-NS and StpA proteins stimulate expression of the maltose-regulon in *Escherichia coli*. Manuscript submitted for publication.

Instituto Juan March (Madrid)

Directing transcription to regulate cell growth and survival

Chairs: Keith F. Chater
Robert E. Glass
John S. Parkinson
Steve Busby

Instituto Juan March (Madrid)

Population Dynamics During Stationary Phase

Erik Zinser and Roberto Kolter
Department of Microbiology and Molecular Genetics
Harvard Medical School
Boston, MA 02115

Non-sporulating bacteria such as *Escherichia coli* must continue to metabolize to survive extended periods of starvation. Populations of *E. coli* grown in rich media lose 90-99% of viable cells during the first few days of starvation, but the surviving 1-10% population experiences only minor net death thereafter. Zambrano et al. (1993) demonstrated that this surviving population of *E. coli* cells undergoes dramatic shifts in population composition, indicating that intense selection occurs when nutrients become scarce. That the survivors were selected because they had beneficial mutations was verified by identifying in one survivor a mutation that, when transferred to the wild-type parent by P1 transduction, conferred the same Growth Advantage in Stationary Phase (GASP) phenotype that the survivor had. This mutation was identified as an allele of *rpoS* (*rpoS819*), whose product, σ^{S819}, is very unstable and thus leads to a general attenuation of expression of the σ^S regulon in stationary phase. Analysis of many starved cultures of *E. coli* demonstrates that there is a very strong selection for mutations in *rpoS* : virtually all survivors of two-week-long incubations have an attenuated *rpoS* allele. Interestingly, the nature of the mutations in *rpoS* mutations is quite variable. Laboratory strains (Jishage and Ishihama, 1997) , as well as natural isolates of *E. coli* from the soil and human intestine (Gupta, 1997) , likewise show dramatic allelic variation of *rpoS*, suggesting that strong selection pressures are exerted upon the *rpoS* global regulator both in the laboratory and in the natural setting.

Multiple rounds of population takeover occur during prolonged starvation. Aged mutants of the *rpoS819* strain can outcompete the *rpoS819* parent strain. This "second round" GASP phenotype is referred to as $GASP_{II}$, to differentiate from the "first round" GASP phenotype of *rpoS819*, denoted as $GASP_I$. One $GASP_{II}$ strain has been analyzed in detail. This strain has at least three GASP-conferring mutations in addition to the one in *rpoS*. All three $GASP_{II}$ alleles confer a partial GASP phenotype relative to the triple mutant. One mutation is in the gene *lrp*, encoding the global regulator Lrp. The majority of Lrp-controlled genes are involved in amino acid metabolism. That both of the GASP mutations identified thus far are in global transcription regulators suggests that there is intense pressure for starved cells to make multiple changes in cell composition and metabolism, resulting in global shifts in the metabolic and physiologic state of the cell. We are working to identify the other two $GASP_{II}$ mutations.

We have begun to address the physiological basis for GASP. All three $GASP_{II}$ mutations and the $GASP_I$ mutation, *rpoS819*, confer enhanced growth on amino acids as sole carbon and energy sources. Hence, we propose these mutations are selected for because they enhance the ability of the starved cells to take up and metabolize the amino acids released by the dying cells. Consistent with this model, we have observed by bioassay that dying cells release serine, and GASP mutants consume it as they grow and take over the population.

Gupta, S. (1977). Microbial Survival: Mutations that Confer a Competitive Advantage During Starvation. M.Sc. Thesis. Harvard University.

Jishage, M. and Ishihama, A. (1997). Variation in RNA polymerase sigma subunit composition within different stocks of *Escherichia coli* W3110. J. Bacteriol. 179, 959-963.

Zambrano, M.M., Siegele, D.A., Almirón, M., Tormo, A. and Kolter, R. (1993). Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures Science 259,1757-1760.

Instituto Juan March (Madrid)

Lessons from rRNA Promoters about Bacterial Transcription and its Regulation

Tamas Gaal, Wilma Ross, Sarah E. Aiyar, J. Alex Appleman, Melanie M. Barker, Christine Hirvonen, and Richard L. Gourse. Department of Bacteriology, Unversity of Wisconsin, 1550 Linden Drive, Madison, WI 53706, USA

Ribosomal RNA synthesis is extremely efficient, accounting for 60-70% of all transcription when *E. coli* cells are growing rapidly, yet rRNA transcription is carefully regulated to keep ribosome synthesis at the appropriate level for the growth rate. Our studies on the mechanisms responsible for the high activity and the regulation of rRNA transcription have provided information important not only to an understanding of ribosome synthesis but also to understanding bacterial transcription in general.[1]

We showed a few years ago that rRNA promoters contain a third RNAP recognition element, the UP element, that interacts not with the sigma subunit of RNAP like the -10 and -35 hexamers (the other two RNAP recognition elements), but with the C-terminal domain of the alpha subunit (αCTD). We have shown more recently that UP elements are widespread in bacterial promoters; although the UP elements present in rrn P1 promoters are more active than most, many or even most promoters derive some contribution to their overall activities from interactions between upstream sequences and the αCTD. The residues in α responsible for interacting with DNA form a surface-exposed patch on the αCTD that is conserved throughout eubacteria. Therefore, the sequences on DNA to which α binds should also be conserved. We have recently determined the "consensus" UP element sequence using an in vitro selection from a pool of about 10^{12} promoters with random upstream sequences. The best UP elements increase transcription about 325-fold, far better than even the UP elements found in rRNA promoters.[2-5]

We have also characterized the interaction between RNAP and a transcription factor, FIS, responsible for activation of rRNA transcription. Together, FIS and UP elements account for the unusual strength of *rrn* P1 promoters. Studies on the role of α interactions with activators like FIS and CRP suggest a common architecture in bacterial transcription complexes: protein-protein interactions between surface exposed residues on transcription factors and αCTD adjacent to their respective DNA binding surfaces lead to transcription activation.[6]

However, many of the regulatory properties of rRNA transcription (growth rate-dependent regulation and stringent control) are explained not by these features of *rrn* P1 promoters, but at least in part by the intrinsic instability of the complexes that *rrn* P1 promoters form with RNAP. The intrinsic instability of the initiation complex leads to the dependence of *rrn* P1 promoters on the concentration of the initiating NTP for activity. NTP pools vary with growth rate, resulting in regulation of rRNA transcription and a homeostatic control mechanism for ribosome synthesis.[7]

Multiple mechanisms affect rRNA transcription simultaneously. As a result, "NTP-sensing," FIS (whose concentration also changes with growth conditions), and other control systems work together to determine the final rRNA synthesis rate. Our studies on rRNA transcription illustrate the necessity for dissecting promoter regions into their component parts in order to

Instituto Juan March (Madrid)

identify the individual contributors that lead to gene expression. The challenge now is to determine how the mechanisms work together and which mechanisms contribute to the final transcription rate during specific nutritional and environmental conditions.[8-9]

Supported by NIH grant GM37048.

Selected references:

1. Gourse et al. (1996) Annu. Rev. Microbiol. 50, 645-677.
2. Ross et al. (1993) Science 262, 1407-1413
3. Blatter et al. (1994) Cell 78, 889-896.
4. Gaal et al. (1996) Genes Dev. 10, 16-26.
5. Estrem et al. (1998) Manuscript submitted.
6. Bokal et al. (1997) EMBO J. 16, 154-162.
7. Gaal et al. (1997) Science 278, 2092-2097.
8. Appleman et al. (1998) J. Bacteriol. 180, 1525-1532.
9. Bartlett et al. (1998) J. Mol. Biol., manuscript in press.

Instituto Juan March (Madrid)

REGULATION OF CELLULAR LEVELS AND ACTIVITY OF σ^S IN ESCHERICHIA COLI

Gisela Becker, Eberhard Klauck, and Regine Hengge-Aronis
Dept. of Biology, University of Konstanz, 78457 Konstanz, Germany

σ^S or RpoS is a sigma subunit of RNA polymerase, that acts as a master regulator in the general stress response in *E.coli* [1]. σ^S is a very close relative of the vegetative sigma factor σ^{70} and recognizes similar promotor sequences. Whereas growing cells not exposed to any particular stress contain hardly detectable σ^S levels, various stress conditions such as starvation or hyperosmotic shift result in strongly elevated cellular levels of σ^S. This regulation is mainly post-transcriptional and involves translational control as well as control of the turnover of σ^S which in non-stressed cells is a highly unstable protein [2]. Evidence has been presented that σ^S contains a "turnover element", i.e. a not further defined region that may function as a recognition element for the proteolytic machinery [4, 6]. The protease involved is the ClpXP protease [6]. In addition, the response regulator RssB is required for σ^S turnover [3, 5].

In an attempt to pinpoint the turnover element in σ^S, we isolated mutants with alterations in specific amino acids of σ^S. Previous work [4, 6] had roughly located the turnover element somewhere in the middle of the σ^S sequence, perhaps not far downstream from region 2.4., which is involved in recognition of the -10 region of promoters. Whereas the sequences of σ^S and σ^{70} are very similar in their respective 2.4. regions, sequences diverge in a region shortly downstream of 2.4. As σ^{70} is not subject to proteolysis, we hypothesized that one or several amino acids in this part of σ^S might be crucial for its degradation, and therefore exchanged the respective codons in *rpoS* for those present in *rpoD*. A *rpoS* mutant with a single amino acid exchange was obtained which resulted in elevated σ^S levels during log phase growth since it eliminated σ^S proteolysis completely. Mutations in adjacent amino acids had similar but much less pronounced effects. These mutations precisely localize the turnover element in σ^S.

Moreover, in vivo activities of wildtype and mutant σ^S was determined by assaying the expression of various σ^S-dependent genes in wildtype, *rssB* and *clpP* backgrounds. The results of these experiments are the following: (1) RssB not only promotes σ^S proteolysis, but also interferes with its activity; this effect is not promoter-specific, and is not observed in the stable σ^S mutant; (2) in the absence of RssB (i.e. where RssB cannot interfere with σ^S activity), the stable σ^S variant exhibits reduced activity for the expression of several but not all σ^S-dependent genes, i.e. the mutated amino acid may also be involved in promoter recognition of σ^S-controlled genes.

These data suggest that the turnover element in σ^S may be a binding site for RssB (which is supported by preliminary in vitro experimental evidence), and that RssB not only promotes σ^S proteolysis but also interferes with σ^S activity. Thus, RssB might be (have been?) an anti-sigma factor that has been recruited to serve as a recognition factor by the proteolytic machinery.

1. Hengge-Aronis, R. 1996. *In* F. C. Neidhardt (ed.), E.coli and S.typhimurium: Cellular and Molecular Biology. American Society for Microbiology. Washington D.C.
2. Lange, R., and R. Hengge-Aronis. 1994. Genes Dev. 8: 1600-1612.
3. Muffler, A., D. Fischer, S. Altuvia, G. Storz, and R. Hengge-Aronis. 1996. EMBO J. 15: 1333-1339.
4. Muffler, A., D. D. Traulsen, R. Lange, and R. Hengge-Aronis. 1996. J. Bacteriol. 178: 1607-1613.
5. Pratt, L. A., and T. J. Silhavy. 1996. Proc. Natl. Acad. Sci. USA 93: 2488-2492.
6. Schweder, T., K.-H. Lee, O. Lomovskaya, and A. Matin. 1996. J. Bacteriol. 178: 470-476.

Instituto Juan March (Madrid)

ENTERING AND EXITING STATIONARY PHASE IN *E. COLI*.

Deborah Siegele, Dept. of Biology, Texas A&M University, College Station, TX 77843

In many environments bacteria must adapt to fluctuations in the nutrient supply. Many bacteria have evolved starvation-survival programs that help to maximize cell survival during prolonged periods of starvation, while maintaining the ability to respond rapidly and resume growth when nutrients become available. The transitions between exponential growth and stationary phase involve striking changes in the pattern of gene expression. Understanding how these different physiological programs are initiated will involve determining how gene expression is regulated during each of these growth transitions.

Entering stationary phase. To study how stationary phase-induced gene expression is regulated, we have focused on how expression of the *mcb* operon is regulated in *E. coli*. The *mcb* operon contains the genes required for production of and immunity to the peptide antibiotic, microcin B17 (7). Expression of the *mcb* operon is induced when *E. coli* cells are starved for carbon, ammonia, or phosphate, and also when they enter stationary phase in rich medium (2). Although the *mcb* operon was originally found on a conjugative plasmid, the regulation of *mcb* expression reflects the activities of host factors involved in sensing and responding to starvation. No plasmid-encoded products are need to regulate the activity of the major operon promoter, P*mcb*.

Stationary-phase induction of P*mcb* transcription does not require σ^S, a key regulator of the *E. coli* starvation response (5). Stationary-phase induction is also independent of cAMP and ppGpp (2, 12), two factors involved in regulating the expression of other starvation-induced genes. The activity of P*mcb* is known to be affected by three *E. coli* regulatory proteins: OmpR (6), MprA (a.k.a. EmrR) (3), and IHF (10). However, none of these factors is essential for stationary-phase induction of P*mcb* expression.

We have done further mutational analysis of P*mcb*, using a combination of deletion analysis and random mutagenesis to identify regulatory elements in the promoter (8). Our results indicate that a P*mcb* expression is regulated by at least four different factors, acting at four different sites in the promoter. We have localized sequences needed for regulation by OmpR, MprA, and IHF. We have also found evidence for regulation by one or more unidentified factors that act upstream of position -164 and on the core promoter.

Exiting stationary phase. When nutrients again become available to starved cells, many of the changes that occurred to cope with starvation must be reversed before growth can resume. Little is known about how these changes are controlled. The ability to respond quickly to nutrients will clearly be advantageous to an organisms competing for limited resources. Work from several laboratories indicates that like entry into stationary phase, recovery from starvation is also a regulated, temporally ordered process, which helps to ensure rapid reentry into the growth cycle.

Proteins synthesized specifically during outgrowth from stationary phase have been observed in *Vibrio* sp. S14 (1, 9), *Pseudomonas putida* KT 2442 (4), and during outgrowth of *Bacillus subtilis* spores (13). Synthesis of these outgrowth-specific proteins is not detected in starved or

Instituto Juan March (Madrid)

exponentially growing cells. Expression of these proteins is likely to be controlled by factors involved in regulating the outgrowth response, so identifying the genes encoding outgrowth-specific proteins will provide important tools for studying the outgrowth phase of the bacterial life cycle.

My laboratory has identified nine outgrowth-specific proteins in *E. coli*, which are synthesized transiently during the first hour of recovery from starvation (11). These proteins were identified by two-dimensional gel electrophoresis of proteins pulse-labeled during recovery from starvation. The outgrowth-specific proteins appeared in two kinetic classes: seven outgrowth-specific proteins were first detected 2 to 5 min after the addition of nutrients, while the remaining two outgrowth-specific proteins were first detected 15 to 17 min after the addition of nutrients. We are currently using a reverse genetics approach to identify the genes encoding these proteins.

1. Albertson, N. H., T. Nyström and S. Kjelleberg (1990) Macromolecular synthesis during recovery of the marine *Vibrio* sp. S14 from starvation. J. Gen. Microbiol. **136**: 2201-2207.
2. Connell, N., Z. Han, F. Moreno and R. Kolter (1987) An *E. coli* promoter induced by the cessation of growth. Mol. Microbiol. **1**: 195-201.
3. del Castillo, I., J. E. González-Pastor, J. L. San Millán and F. Moreno (1991) Nucleotide sequence of the *Escherichia coli* regulatory gene *mprA* and construction and characterization of *mprA*-deficient mutants. J. Bacteriol. **173**: 3924-3929.
4. Givskov, M., L. Eberl and S. Molin (1994) Responses to nutrient starvation in *Pseudomonas putida* KT2442: two-dimensional electrophoretic analysis of starvation- and stress-induced proteins. J. Bacteriol. **176**: 4816-4824.
5. Hengge-Aronis, R. (1996) Regulation of gene expression during entry into stationary phase, p. 1497-1512. *In* F. C. Neidhardt et al. (ed.), *Escherichia coli and Salmonella: cellular and molecular biology*. American Society for Microbiology, Washington, D.C.
6. Hernández-Chico, C., J. L. San Millán, R. Kolter and F. Moreno (1986) Growth phase and OmpR regulation of transcription of microcin B17 genes. J. Bacteriol. **167**: 1058-1065.
7. Kolter, R. and F. Moreno (1992) Genetics of ribosomally synthesized peptide antibiotics. Annu. Rev. Microbiol. **46**: 141-163.
8. Mao, W. and D. A. Siegele Genetic analysis of the stationary-phase-induced *mcb* operon promoter in *Escherichia coli*. Mol. Microbiol. **27**: 415-424.
9. Marouga, R. and S. Kjelleberg (1996) Synthesis of immediate upshift (Iup) proteins during recovery of marine *Vibrio* sp. strain S14 subjected to long-term carbon starvation. J. Bacteriol. **178**: 817-822.
10. Moreno, F., J. L. San Millán, I. del Castillo, J. M. Gómez, M. C. Rodríguez-Sáinz, J. E. González-Pastor and L. Díaz-Guerra (1992) *Escherichia coli* genes regulating the production of microcins MccB17 and MccC7, p. *In* R. James, C. Lazdunski and F. Pattus (ed.), *Bacteriocins, Microcins, and Lantibiotics*. Springer-Verlag, Berlin Heidelberg.
11. Siegele, D. A. and L. J. Guynn (1996) *Escherichia coli* proteins synthesized during recovery from starvation. J. Bacteriol. **178**: 6352-6356.
12. Siegele, D. A. and H. Khandpur. Unpublished results. .
13. Wachlin, G. and M. Hecker (1982) Activation of protein biosynthesis in outgrowing spores of *Bacillus subtilis*. Zeit. Allgemeine Mikrobiol. 22: 495-502.

Instituto Juan March (Madrid)

THE *ESCHERICHIA COLI* DEFENSE AGAINST AGING: GLOBAL REGULATON AIMED AT AVOIDING OXIDATIVE DAMAGE.

Thomas Nyström, Department of Microbiology, Lund University, Sweden

During self-replication of bacteria such as *Escherichia coli* old, damaged and potentially non-functional proteins are distributed equally to both daughter cells. In addition, old proteins are rapidly diluted with new ones as long as the environment supports translation and growth. Thus, in times of prosperity, prokaryotic cells like *E. coli* appear to lack an aging process and are essentially immortal. In contrast, prokaryotes do die if circumstances arrest multiplication; cells of a non-differentiating bacterial population entering stationary phase due to the depletion of an essential nutrient become moribund and the viable cell counts drop stochastically. However, it has been recognized that *E. coli*, like the differentiating bacteria *Bacillus* and *Myxococcus*, possess specific genetic programs that have evolved to fight external physiochemical forces and prolong stasis survival. The physiological functions of several survival-related proteins suggests that denaturation and spontaneous aging of proteins and their component amino acids is a problem during stasis. Consistant with this notion, disufide bridge formation of a cytoplasmic alkaline phosphatase increases significantly during growth arrest. Normally, such disufide bridge formation should not and does not occur in the cytoplasm due to its reducing environment. In addition, the ArcA-dependent reduced production of respiratory substrates and components of the aerobic respiratory apparatus during stasis may be an additional defence mechanism mustered by the cell to reduce superoxide production and to protect itself against oxidative damage of proteins and other macromolecules.

The genes encoding stasis-defence proteins belong to several global regulatory networks, including the FadR, ArcA, SoxS, OxyR and the stringent control regulon, and at least three different sigma factors, σ^{70}, σ^{32} and σ^{38}, are required for the expression of these genes. Interestingly, a mutation in *rpoS*, encoding σ^{38}, not only abolishes transcription of some 35 stationary phase genes, but also causes elevated induction of some σ^{70}- and σ^{54}-dependent genes. We have quantitated this phenomenon and shown that the effect for the σ^{70}-dependent genes analyzed, *uspA* and *fadD*, is at the transcriptional level and that the effect can be mimicked by overproduction of σ^{70}. In addition, overproduction of σ^{70} markedly reduces stationary phase induction of σ^{38}-dependent genes. Our data suggest that, at least during stasis, sigma factors compete for a limiting amount of core RNA polymerase and that pertubations in the level of one sigma factor can significantly alter the level of expression of stasis-induced genes dependent on the other sigma factors. In addition, some σ^{70}-dependent genes that are normally repressed during stasis fail to be so in *rpoS* mutants.

Instituto Juan March (Madrid)

Taken together, the data indicate that sigma factor competition is an important mechanism of gene regulation during entry of cells into stationary phase.

Nyström, T. and Neidhardt, F.C. (1994). Expression and role of the universal stress protein, UspA, of Escherichia coli during growth arrest. **Mol. Microbiol.** 11: 537-544.

Nyström, T. (1994) The glucose starvation stimulon of Escherichia coli: induced and repressed synthesis of enzymes of central metabolic pathways and the role of acetyl phosphate in gene expression and starvation survival. **Mol. Microbiol.** 12: 833-843.

Nyström, T. (1995). The trials and tribulations of growth arrest. **Trends in Microbiology, TIM.** 3: 131-136.

Nyström, T. (1995) The glucose-starvation stimulon of Escherichia coli: Role of Integration host factor in starvation survival and growth-phase-dependent protein synthesis. **J. Bacteriol.** 177:5707-5710

Nyström, T, Larsson, C, and Gustafsson L. (1996) Bacterial defense against aging: Role of the Escherichia coli ArcA regulator in gene expression, readjusted energy flux, and survival during stasis. **EMBO J** 15:3219-3228.

Farewell A, Diez, A. A., DiRusso, C., and Nyström, T. (1996) Role of the Escherichia coli FadR regulator in stasis survival and growth phase-dependent expression of the uspA, fad and fab genes. **J. Bacteriol.** 178: 6443-6450.

Instituto Juan March (Madrid)

The relation between transcriptional and post-transcriptional factors in the regulation of cell growth and survival

J. M. Santos[1], Miguel Vicente[2], and C. M. Arraiano[1]

[1]Instituto de Tecnologia Química e Biológica, Univ. Nova de Lisboa, Apt. 127, 2780 Oeiras, Portugal.
[2]Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, 28006 Madrid, Spain.

Transcriptional and post-transcriptional factors are involved in the control of gene expression. We have been studying bacterial systems where transcription and mRNA decay are involved in the rapid response to different environmental conditions. We have looked at the gene *bolA* as an example of a gene involved in cell division that can be induced during the transition to stationary phase. The gearbox promoter P1 is responsible for the late-phase induction and this promoter is recognised by the sigma factor S (σ^S) encoded by *rpoS* (KatF).

We have analysed the levels of *bolA* mRNA not only in stationary phase but also during exponential phase with or without different stress conditions. we have observed that *bolA* was also induced under sudden stress. The response to carbon source depletion was one of the examples where the mRNA levels were even higher that the levels obtained in late stationary phase. Unexpectedly, this response was independent of σ^S. The promoter seems to be recognised by another sigma factor. Northern blot analysis of the inductory behaviour of *bolA* P1 along the growth curve and in response to sudden nutritional stress and reversion (re-addition of 0,4% Glucose) suggest an additional mode of expression regulation at the mRNA level. There seems to be a specific cleavage site on the transcript (which we suspect is made by RNaseE) leading to fast decay. A very stable degradation product μ results from this cleavage. When *bolA* is induced this cut seems to be inhibited thus increasing mRNA stability. In the reversion experiments μ reappears strongly after readdition of glucose to the medium at the same time *bolA* P1 full-transcript resumes back down to constitutive levels.

Furthermore, in a reversion experiment, we stressed an *E. coli* strain deficient in σ^S during early logarithmic phase (OD 0,3) and observed induction still occurs, as well as reversion to basal levels after glucose readdition. In this second phase of the experiment, the appearance of m was even more important than with the wild type. This further suggests an alternative regulation process at the level of mRNA stability which enables the cell to respond faster to the need of turning on or off genes which suddenly become respectively necessary or unnecessary.

This is an example where transcriptional and post-transcriptional controls work in consonance to achieve the global regulation of the expression of this cell division gene.

Instituto Juan March (Madrid)

REGULATION OF BACTERIOPHAGE Ø29 DNA TRANSCRIPTION. Margarita Salas, María Monsalve, Mario Mencía, Belén Calles, Montserrat Elías, Ana Camacho, José A. Horcajadas and Fernando Rojo[1]. Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), and [1]Centro Nacional de Biotecnología (CSIC), Universidad Autónoma. Canto Blanco. 28049 Madrid. Spain.

Bacteriophage ø29 early promoters are recognized by the *Bacillus subtilis* RNA polymerase (RNAP) containing the major σ^A subunit, while transcription from the late promoter, PA3, requires, in addition to the σ^A RNA polymerase, the product of the viral gene 4, p4. Protein p4 binds to a region centered at position −82 relative to the PA3 transcription start site, recognizing an 8 bp long inverted repeat. This DNA region has a sequence-directed curvature that greatly increases by protein p4 binding. Protein p4 activates late transcription by stabilizing the binding of the RNAP to the promoter as a closed complex (1). Indeed, protein p4 can induce the binding of purified *B. subtilis* RNA polymerase α subunit to the late promoter. This binding does not occur with p4 mutants at residue Arg 120, or when a deletion mutant at the α subunit, lacking the 15 C-terminal amino acids, are used (2, 3). The use of reconstituted RNAPs with deletion-containing α subunits indicated that the last 15 residues of the latter are required for p4-dependent transcription activation. In addition, by site-directed mutagenesis at the C-end of p4, several basic residues have been identified, including Arg 120, that contribute to maintain the DNA bending. Since p4 is a dimer in solution and binds to DNA as a tetramer we propose a model in which two of the p4 subunits would interact with the RNAP while the other two would be used for DNA binding and bending (4).

The regulatory protein p4 also represses transcription from the early promoters A2b and A2c, that are divergently transcribed from the late A3 promoter. Binding of p4 displaces the RNAP from the A2b promoter, both by steric hindrance and by the bending induced upon binding (5). Protein p4

Instituto Juan March (Madrid)

represses the A2c promoter by binding to a DNA site centered at position –71, immediately upstream from RNAP, in a way that does not hinder RNAP binding. In the presence of p4, RNAP can form an initiated complex at PA2c that generates short abortive transcripts, but cannot leave the promoter (6). Mutation of p4 residue Arg 120, which relieves the contact between the two proteins, leads to a loss of repression. In addition, deletion of the 15 C-terminal amino acids of the RNAP α subunit also leads to a loss of repression (7). Therefore, the contact of p4 and RNAP through the same regions can activate or repress transcription depending on the promoter. By construction of mutant promoters we showed that the position of protein p4 binding site relative to that of RNAP does not dictate the outcome of the interaction. Rather, it is the absence or presence of a –35 consensus box for σ^{A}-RNAP what determines activation or repression, respectively (8).

The role of the "extended –10 motif" in ø29 promoters and that of the viral protein p6 in the control of transcription will be also discussed.

References

1. B. Nuez, F. Rojo and M. Salas (1992). Proc. Natl. Acad Sci. USA, 89, 11401-11405.
2. M. Mencía, M. Salas and F. Rojo (1993). J. Mol. Biol., 233, 695-704.
3. M. Mencía, M. Monsalve, F. Rojo and M. Salas (1996). Proc. Natl. Acad. Sci. USA, 93, 6616-6620.
4. M. Mencía, M. Monsalve, M. Salas and F. Rojo (1996). Mol. Microbiol. 20, 273-282.
5. F. Rojo and M. Salas (1991). EMBO J., 10, 3429-3438.
6. M. Monsalve, M. Mencía, F. Rojo and M. Salas (1996). EMBO J. 15, 101-109.
7. M. Monsalve, M. Mencía, M. Salas and F. Rojo (1996). Proc. Natl. Acad. Sci. USA, 93, 8913-8919.
8. M. Monsalve, B. Calles, M. Mencía, M. Salas and F. Rojo (1997). Molecular Cell, 1, 1-9.

Instituto Juan March (Madrid)

Molecular signalling between the redox sensor protein NIFL and the transcriptional activator NIFA.

Francisca Reyes-Ramirez, Andre Sobzcyk , Jason A. Barrett and Ray Dixon

Nitrogen Fixation Laboratory
John Innes Centre, Norwich NR4 7UH, UK

The prokaryotic enhancer-binding protein, NIFA, activates transcription of the nitrogen fixation (*nif*) genes by promoting formation of open promoter complexes by the σ^{54}-holoenzyme form of RNA polymerase. Stimulation of open promoter complex by NIFA requires nucleotide triphosphate hydrolysis catalysed by the activator. NIFA has the characteristic three-domain structure of σ^{54} (σ^{N})-dependent transcriptional activators, comprising (i) an amino-terminal domain with potential regulatory properties, which is not homologous to the canonical phosphoacceptor domain of the response regulator family, (ii) a central domain possessing nucleoside triphosphatase activity, which interacts with the σ^{N}-RNA polymerase holoenzyme, and (iii) a C-terminal domain which recognises specific DNA target sites. In both *Azotobacter vinelandii* and *Klebsiella pneumoniae* the ability of NIFA to activate transcription is modulated by the anti-activator protein NIFL in response to environmental oxygen and fixed nitrogen. Biochemical analysis of purified NIFL indicates that it is a flavoprotein with FAD as prosthetic group. Oxidation and reduction of the flavin acts as a molecular switch to control the activity of NIFA, indicating that NIFL is a redox responsive regulatory protein. In addition to its ability to act as a redox sensor, the activity of NIFL is also responsive to adenosine nucleotides, particularly ADP. This response overrides the redox switch suggesting that NIFL also senses the energy status *in vivo*. NIFL is comprised of two domains; the amino terminal domain binds FAD and is primarily involved in sensing the redox status, whereas the C-terminal domain binds adenosine nucleotides and may interact with NIFA.

The mechanism(s) by which NIFL prevents transcriptional activation by NIFA is not clear but appears to be mediated by protein-protein interactions. Analysis of a truncated form of NIFA lacking the amino-terminal domain indicate that this domain has a regulatory function in modulating the catalytic activity of NIFA in response to NIFL. In contrast to native NIFA, the truncated protein is not responsive to inhibition by the oxidised form of NIFL and nucleotide triphosphatase activity of the truncated form is apparently unregulated. However, ΔN_{191}- NIFA is still responsive to high concentrations of the ADP- bound form of NIFL even though catalytic activity is not inhibited. These data suggest that NIFL has the ability to inhibit NIFA by two different mechanisms; one requiring the amino-terminal domain, involving inhibition of the catalytic activity of the central domain, and the second mechanism which acts at a stage in the activation pathway prior to catalysis of the open promoter complex. Since the second mechanism does not apparently require the DNA binding function of NIFA and does not inhibit catalytic activity it is possible that the ADP bound form of NIFL prevents the activator from forming productive contacts with the polymerase. We have isolated mutant forms of NIFA which are resistant to the inhibitory form(s) of NIFL. Whereas some of these mutations give rise to constitutively active forms of NIFA, other mutations only resist the negative function of NIFL under certain environmental conditions. It therefore appears that NIFA is able to integrate environmental signals as a consequence of its interaction with different inhibitory conformers of NIFL.

Instituto Juan March (Madrid)

Interplay between global regulators of *Escherichia coli* : effect of RpoS, Lrp and H-NS on transcription of the gene *osmC*.

Jean Bouvier[1], Sylvie Gordia[1], Gabriele Kampmann[2], Roland Lange[2],

Regine Hengge-Aronis[2] and Claude Gutierrez[1*]

[1] Laboratoire de Microbiologie et Génétique Moléculaire,

UPR 9007 du CNRS, 118 Route de Narbonne,F-31062, Toulouse Cedex, France.

[2] Universität Konstanz, Fakultät für Biologie, Fach M 606

D-78457 Konstanz, Germany

Transcription of the *osmC* gene of *Escherichia coli* is regulated as a function of the phase of growth. It is induced during the decelerating phase, before entry into stationary phase. *osmC* expression is directed by two overlapping promoters, $osmC_{p1}$ and $osmC_{p2}$. $osmC_{p2}$ is mainly transcribed by E-σ^s, the RNA polymerase using the σ^s (RpoS) sigma factor, and is responsible for the growth-phase regulation. Transcription from $osmC_{p1}$ is independent of σ^s. The leucine-responsive protein (Lrp) binds the *osmC* promoter region in band shift experiments. In vivo analysis using *osmC-lacZ* transcriptional fusions demonstrated that Lrp affects expression of both promoters. It represses transcription of $osmC_{p1}$ and activates transcription of $osmC_{p2}$ by E-σ^s. Absence of Lrp results in an increase in the amount of RpoS during exponential growth in minimal medium. The nucleoid associated protein H-NS also represses *osmC* transcription from both promoters. The effect on $osmC_{p2}$ is probably mediated by the increase in σ^s concentration in the cytoplasm of *hns*⁻ mutants, while the effect on $osmC_{p1}$ is independent of σ^s. No binding of H-NS to the promoter region DNA could be detected, indicating that the effect on $osmC_{p1}$ could also be indirect.

Instituto Juan March (Madrid)

cAMP-CRP, H-NS and IHF are involved in the regulation of the microcin C7 production genes, which transcription is stationary phase induced and σ^S-dependent.

José Eduardo GONZÁLEZ-PASTOR[1], Annic KOLB[2], José Luis SAN MILLÁN[1], y Felipe MORENO[1].

[1]Unidad de Genética Molecular, Hospital Ramón y Cajal. Carretera de Colmenar, km 9.1. 28034 Madrid, Spain.
[2]Unité de Physicochimie des Macromolécules Biologiques (URA 1149 du CNRS), Institut Pasteur. Rue du Dr. Roux, 25. 75724 Paris Cedex 15, France.

Microcin C7 (MccC7) is a modified heptapeptide (1.177±1 dalton), ribosomally synthesized, that inhibits protein synthesis. MccC7 is produced by *Escherichia coli* cells that harbour the conjugative plasmid pMccC7 (43 kb). This plasmid contains six genes involved in MccC7 production and immunity (*mcc*).

Like other microcins, MccC7 production is induced when cells enter into stationary phase. It results from the activation of the main promoter, *mccp*, responsible of transcription of the five genes involved in production, which constitute an operon. The cAMP-CRP complex is absolutely required for this activation, and the RNA polymerase σ^S subunit is required for optimal induction. DNAse I footprinting analysis have shown that cAMP-CRP complex binds to the *mccp* region, at position -59.5 from the transcriptional start site. Transcription *in vitro* from *mccp* can be initiated by RNA polymerase reconstituted with σ^S, and also with σ^{70}, the vegetative sigma factor, and in both cases the transcription is enhanced by the cAMP-CRP complex. We conclude that the transcription of the *mcc* genes in stationary phase is, essentially, due to the binding of the cAMP-CRP complex and the σ^S-containing RNA polymerase to *mccp* promoter.

On the other hand, the histone-like proteins H-NS and IHF inhibit transcription from *mccp* promoter. DNAse I footprinting analysis have shown that binding sites of H-NS and IHF overlap the *mccp* promoter and cAMP-CRP site, which explains the inhibitor effect of these proteins. Furthermore, H-NS repress transcription *in vitro* from *mccp*. Taken these results together, we conclude that these histone-like proteins are directly involved in repression of *mcc* genes transcription.

There are not many stationary-phase induced and σ^S-dependent promoters studied in detail at molecular level. Our results could contribute to a better understanding of the regulatory mechanims in stationary phase.

Instituto Juan March (Madrid)

Antagonistic transcriptional control by Fis and H-NS and growth-phase-dependent changes in mRNA stability modulate the expression of cold-shock protein CspA at 37°C

Anna Brandi, Maurizio Falconi, Roberto Spurio, Cynthia L. Pon and Claudio O. Gualerzi
Laboratory of Genetics, Dept. of Biology, University of Camerino, I-62032 Camerino (MC), Italy

When the temperature falls below 20°C, *Escherichia coli* cells respond with the activation of over a dozen genes. According to the current model, the single stranded nucleic acid binding protein CspA (product of *cspA*), which is the transcriptional activator of at least two other cold shock genes (*hns* and *gyrA*), mediates this global response. Cold shock was reported to trigger de novo synthesis of CspA whose intracellular level increases from a negligible amount to mM concentration during the initial stage of the cold shock growth lag. We demonstrate, however, that *cspA* is transcribed and translated very efficiently also at 37°C, but only during early exponential growth. With increasing cell density, both CspA mRNA and its product disappear rapidly and become virtually undetectable already in mid-log phase. Accordingly, the extent of the cold shock induction of *cspA* was found to be inversely proportional to the intracellular level of CspA and varies as a function of the growth phase in a way suggesting that this protein is capable of feedback autorepression. Dilution with fresh medium of stationary phase cells in which CspA is barely detectable brings about a burst (followed by a renewed decline) of *cspA* expression. Both transcriptional and post-transcriptional controls are responsible for this behavior; transcription of *cspA*, like that of *hns*, was found to be under the antagonistic control of DNA-binding proteins Fis and H-NS both *in vivo* and *in vitro* and sequence homologies detected in the promoter regions of these genes and contiguous or partially overlapping binding sites for these proteins likely are the physical basis for this similarity. Finally, a ribonuclease whose activity requires the intact 5'untranslated leader of *cspA* mRNA and which is inhibited by the cold-shock DeaD box RNA helicase (CsdA) drastically reduces the half-life of CspA mRNA and contributes to its rapid disappearance with increasing cell density.

Instituto Juan March (Madrid)

Expression of the *PalkB* promoter from the *Pseudomonas oleovorans* alkane degradation pathway is subject to carbon source- and growth phase-dependent regulation

Luis Yuste, Inés Canosa and Fernando Rojo
Centro Nacional de Biotecnología, CSIC, Campus de la Univesidad Autónoma, 28049-Madrid, Spain

Pseudomonas oleovorans GPo1 contains a set of enzymes that allow it to grow at the expense of medium-chain-length alkanes. The genes coding for these enzymes are clustered in two operons; one includes a regulatory gene, *alkS*, and one subunit of one of the enzymes of the pathway, while the genes corresponding to the rest of the enzymes are grouped on a second operon that is transcribed for a promoter named *PalkB*. Transcription form *PalkB* requires the presence of an inducer such as octane and the activator protein AlkS. Expression of the first enzyme of the pathway, alkane hydroxylase, is known to be influenced by the carbon source present in the growth medium, suggesting that there might be an additional overimposed level of regulation connecting the expression of the *alk* genes with the metabolic status of the cell. We have constructed reporter strains to analyze the influence of the carbon source and of the growth phase on the expression of the *PalkB* promoter. Expression from *PalkB* was most efficient when cell grew at the expense of citrate or glucose, but decreased significantly when lactate, acetate or succinate were used as carbon sources. These organic acids are known to be preferred carbon sources in Pseudomonads. Interestingly, when cells were grown in a rich medium, induction of *PalkB* promoter was very low during the exponential phase, but rapidly increased when cells approached to stationary phase. *PalkB* promoter was shown to be independent from the alternative sigma factor σ^{54}, involved in the expression of genes related to carbon and nitrogen metabolism, as well as from σ^{S}, which directs RNA polymerase to stationary phase promoters. Exponential silencing of *PalkB* was partially relieved when the AlkS activator was overexpressed, or when the promoter was moved to an unrelated genetic background such as *Escherichia coli*.

Instituto Juan March (Madrid)

Mutants have been isolated in which exponential silencing was absent. Our results suggest that *PalkB* expression might be modulated by at least two proteins, the AlkS activator, and an as yet unidentified repressor which is expressed or activated when cells grow in a rich medium. A number of indirect evidences suggest that catabolic repression by lactate or succinate occurs through a mechanism different from that responsible of the exponential silencing observed in rich medium. Progress in the elucidation of the global regulatory network controlling *PalkB* expression will be discussed.

Instituto Juan March (Madrid)

Mapping of the residues contacted by phage Φ29 regulatory protein p4 at the RNA polymerase α-subunit

Belen Calles[1], María Monsalve[1], Fernando Rojo[2] and Margarita Salas[1]
[1]Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Universidad Autónoma, Canto Blanco, 28049-Madrid, Spain; [2]Centro Nacional de Biotecnología (CSIC), Campus de la Universidad Autónoma de Madrid, Canto Blanco, 28049-Madrid, Spain.

Protein p4 from *Bacillus subtilis* phage Φ29 activates transcription at the viral late A3 promoter (PA3) and represses transcription at the early A2c promoter (PA2c), located 219 bp apart. In both cases, protein p4 binds upstream from RNA polymerase (RNAP) and interacts with the C-terminal domain of the RNAP α subunit (α-CTD). At PA3, the interaction stabilizes RNAP at the promoter, overcoming the rate limiting step of this promoter (closed complex formation). At the early A2c promoter, the interaction between protein p4 and RNAP holds RNAP at the promoter as an initial transcribing complex that can make short abortive transcripts but cannot clear the promoter. A remarkable characteristic of protein p4 is that both activation of PA3 and repression of PA2c occur by an interaction that involves the same surface of protein p4 and the same domain of RNAP, namely the α-CTD. It is the affinity of RNAP for the promoter what determines which will be the final effect of the p4-RNAP interaction: if RNAP binds weakly to the promoter, its interaction with p4 will facilitate transcription initiation, but if RNAP binds stably to the promoter, an additional interaction with protein p4 will impede promoter clearance and repression will occur. The surface of protein p4 which interacts with RNAP is well characterized; it is known that residue Arg-120 is critical both to interact with the RNAP α-CTD and to activate or repress transcription. Nevertheless, the residues of the RNAP α-CTD which interact with protein p4 are not known, although it has been shown that deletion of the last 15 residues of the C-terminal domain of the *B. subtilis* RNAP α subunit impairs the interaction between the two proteins. To get a deeper insight into the protein p4-RNAP interaction, several acidic residues of the RNAP α-CTD which were plausible targets for p4 residue Arg-120 were stepwise changed into alanine. In addition, a truncated α-subunit lacking the last 4 residues, two of which are acidic, was also obtained. The mutant α-subunits were purified and reconstituted into RNAP holoenzyme *in vitro*. Reconstituted RNAPs were active at recognizing p4-independent promoters. Their ability to interact with and to be regulated by protein p4 is currently under study; the results obtained will be presented.

Instituto Juan March (Madrid)

Regulation of transcription leading to cell division and differentiation

Chairs: Deborah Siegele
Christopher F. Higgins

Instituto Juan March (Madrid)

Transcriptional Regulation of Essential Cell Division Genes

Miguel Vicente, Klas Flärdh, Ana Martínez-Hortigüela, and Manuel Ballesteros. Departamento de Biología Celular y del Desarrollo. CIB. CSIC. Madrid, Spain.

The correct timing of *ftsZ* expression, and the correct FtsZ concentration, are required for division to occur at normal sizes. Cells in which *ftsZ* is expressed continuously (yielding 80 % of the wild type FtsZ levels), divide with the same division time as the wild type, but at the expense of becoming 1.5 times larger. The *ftsZ* cell division gene is part of a gene cluster that has been named the *dcw* cluster (Vicente *et al.*, 1998).

Many bacterial species contain this cluster of cell division genes which, in those bacteria possesing a cell wall, also contains genes coding for enzymes that participate in its synthesis. In several bacteria the genes in the cluster are arranged in close proximity to each other and are transcribed in the same direction, which in *E. coli* is coincident with the progression of the replication fork. It has been postulated, but not directly proven, that these genes may be transcribed as a polycistronic mRNA initiated at the upstream promoter (*mraZ1p*) (Hara, and Park, 1993). It remains to be proven, as well, if this mRNA is subsequently processed to attain the differential expression of the different genes. The transcriptional organization of the 16 genes involved in the cluster has only been described in detail for the *ftsQAZ*, and the *mraZWR-ftsI* regions (Flärdh *et al.*, 1997; Hara *et al.*, 1997).

The *ftsZ* gene is transcribed from at least six promoters found within the coding regions of the upstream *ddlB*, *ftsQ*, and *ftsA* genes. The contribution of each one to the final yield of *ftsZ* transcription has been estimated using transcriptional *lacZ* fusions. A comparison with results from chromosomal fusions able to report the transcriptional activity from all the promoters in the cluster, both upstream and downstream the *ddlB* gene, suggests that over 60% of the transcripts reaching the *ftsZ* structural gene are originated upstream from *ddlB*.

Among the promoters involved in *ftsZ* expression, *ftsQ1p* belongs to a distinct class of *E. coli* promoters, which have been named Gearbox promoters because their final yield is constant per cell and cell cycle independently of the growth rate of the population. Together with another gearbox promoter, *bolA1p*, *ftsQ1p* is recognised by both RNA polymerase sigma factors σ^D and σ^S, and depends on the latter for growth rate regulation. Analysis of mutations introduced at *bolA1p* suggest

Instituto Juan March (Madrid)

that the postulated -10 sequence CGGCTAGTAT (Vicente *et al.*, 1991) is important in maintaining the potency of the promoter and its dependency on growth rate.

Flärdh, K., Garrido, T., and Vicente, M. 1997. Contribution of individual promoters in the *ddlB-ftsZ* region to the transcription of the essential cell divison gene *ftsZ* in *Escherichia coli*. *Mol. Microbiol.*, 24: 927-936

Hara, H., and Park, J. T. 1993. A far upstream region is required for expression of the *ftsI* gene coding for penicillin-binding protein 3 of *Escherichia coli*. In de Pedro, M. A., Höltje, J. V., and Löffelhardt, W. (eds) *Bacterial growth and lysis: metabolism and structure of the bacterial sacculus*.. p 303-308. Plenum Publishing Co. London

Hara, H., Yasuda, S., Horiuchi, K., and Park, J. T. 1997. A promoter for the first nine genes of the *Escherichia coli mra* cluster of cell division and cell envelope biosynthesis genes, including *ftsI* and *ftsW*. *J. Bacteriol.* 179: 5802-5811

Vicente, M., Kushner, S. R., Garrido, T., and Aldea, M. 1991. The role of the 'gearbox' in the transcription of essential genes. Mol. Microbiol. 5: 2085-2091

Vicente, M., Gómez, M. J., and Ayala, J. A. 1998.Regulation of transcription of cell division genes in the *Escherichia coli dcw* cluster. Cell. Mol. Life Sci. 54: 317-324

Instituto Juan March (Madrid)

Regulation of cell division during the cell cycle of *Caulobacter crescentus*. Yves V. Brun,
Department of Biology, Indiana University, Bloomington, Indiana 47405, USA

In the differentiating bacterium *Caulobacter crescentus*, the developmental process is tightly linked to progression through the cell cycle. The sequential stages of the differentiation pathway are readily distinguished by specific morphological changes that provide a convenient indication of the stage of the cell cycle. Each cell division produces two different cell types: a flagellated swarmer cell unable to initiate DNA replication and a sessile stalked cell that initiates DNA replication immediately after cell division. The swarmer cell differentiates into a stalked cell following a fixed period after which DNA replication can be initiated. The stalked cell elongates, initiates cell division, and synthesizes a flagellum at the pole opposite the stalk to produce an asymmetric predivisional cell. The concentration of FtsZ varies dramatically during the *Caulobacter* cell cycle. After cell division, FtsZ is present only in the progeny stalked cell. *ftsZ* is not transcribed in swarmer cells where it is repressed by the cell cycle response regulator CtrA. CtrA is also a repressor of DNA replication in swarmer cells. Transcription of *ftsZ* and DNA replication initiate during differentiation when CtrA is degraded. FtsZ starts accumulating at the time of *ftsZ* transcription initiation, is relatively stable, and begind localizing at specific subcellular sites. The maximum concentration of FtsZ is reached in the middle of the cell cycle. At the end of the replication period and coincident with the initiation of cell division, transcription of *ftsZ* decreases. Proteolysis of FtsZ increases, especially in the swarmer pole of the predivisional cell, causing a rapid drop in FtsZ concentration and preventing FtsZ inheritance in swarmer cells. The *ftsQ* and *ftsA* genes are co-transcribed by a promoter found in the 3'-end of the *ddl* gene and their transcription is terminated between *ftsA* and *ftsZ* by a rho-independent terminator. P_{QA}, is transcribed at the end of the DNA replication period, following *ftsZ* transcription. Transcription from P_{QA} is inhibited by an inhibition of DNA replication whereas transcription of *ftsZ* is not. The burst of *ftsQA* transcription following the completion of DNA replication may represent one way to coordinate the beginning of cytokinesis with the end of the replication period. The completion of late steps in cell division (DIVp) is required for the activation of flagellar rotation and for stalk synthesis at the new pole of the cell. *ftsA* is one of the cell division genes required for the DIVp step and it may be that the temporal control of *ftsA* (and perhaps *ftsQ*) transcription helps to coordinate developmental events and the cell division cycle as well.

Instituto Juan March (Madrid)

Cell Division and ppGpp in *Escherichia coli*

Danièle Joseleau-Petit and Richard D'Ari
Institut Jacques Monod (CNRS, Université Paris 6, Université Paris 7)
2, place Jussieu, F-75251 Paris Cedex 05, France

Escherichia coli is a rod-shaped Gram negative bacterium. Inhibition of PBP2 (penicillin-binding protein 2), either genetically or with the specific ß-lactam mecillinam, causes cells to become cocci and in rich medium these spheres stop dividing. Division can be restored either by amplification of the cell division proteins FtsQ, FtsA and FtsZ (all three are must be overexpressed) or by a doubling of the ppGpp pool. Since the *ftsQ*, *ftsA* and *ftsZ* genes form an operon and since the nucleotide ppGpp is a known transcriptional regulator of many operons, we speculated that it may be a positive effector of *ftsQAZ* transcription. This also fit with the observation that at least one promoter of the *ftsQAZ* operon has higher expression at lower growth rates, when the ppGpp pool is higher. We therefore carried out quantitative S1 mapping of *ftsQAZ* mRNA 5'-ends in cells with different ppGpp concentrations. All five promoters of the *ftsQAZ* operon are unaffected during the stringent response (elicited by isoleucine starvation of wild type cells), when the ppGpp pool is increased tenfold. Since isoleucine starvation blocks net protein synthesis, this result did not rule out an indirect regulation of *ftsQAZ* transcription by ppGpp, for example, via RpoS. However, the *ftsQAZ* promoters are also unaffected during exponential growth in the *argS201* mutant, in which the ppGpp pool is doubled compared to wild type. To see whether ppGpp might have a post-transcriptional effect on the amount of cell division proteins, we measured the concentration of FtsZ protein in exponentially growing wild type cells and in mecillinam resistant *argS201* cells, using quantitative immunoblotting; there was no difference (±15%). These results show that the *ftsQAZ* operon is not the ppGpp target responsible for mecillinam resistance. Nevertheless, a mere doubling of the ppGpp pool permits *E. coli* to carry out cell division as a coccus. We are currently trying to identify the ppGpp targets which, at intermediate ppGpp levels, allow cells to divide as spheres in the absence of PBP2.

Instituto Juan March (Madrid)

New uses for old transcription factors, and other novelties in the control of *Streptomyces* differentiation

K. F. Chater, J. A. Aínsa, C. J. Bruton, M. J. Buttner, K. Flärdh, G. H. Kelemen, N. J. Ryding, J. A. Soliveri, C. A. Whatling. John Innes Centre, Norwich, NR4 7UH, UK.

The mycelial organism *Streptomyces coelicolor* A3(2) produces unigenomic spores after the controlled multiple cell division of multigenomic aerial hyphae. Six genes (the early *whi* genes) have been implicated in the regulation of this process of sporulation septation[1]. One of them, *whiG*, encodes a σ factor similar to motility sigma factors of enteric bacteria (σ^F) and *Bacillus* spp. (σ^D). RNA polymerase containing σ^{WhiG} is responsible for transcribing *whiH* and *whiI*, two of the other sporulation regulatory genes. The emerging consensus sequence for σ^{WhiG}-dependent promoters is similar to that of σ^F- and σ^D-dependent promoters. WhiH and WhiI are members of well-known families of transcriptional regulators. The closest known relatives of WhiH are regulators (mostly repressors) of genes involved in carbon metabolism, including FadR (fatty acid metabolism), LctR (lactate metabolism) and PdhR (pyruvate dehydrogenase). *whiH* appears to be (directly or indirectly) autoregulatory. WhiI resembles response regulators, but has an atypical phosphorylation pocket, and the *whiI* gene is not located near a gene for a sensor kinase. The other three early *whi* genes are members of smaller gene families encoding proteins that are functionally and structurally uncharacterised. Two are so far confined to the Actinomycetales branch of bacterial phylogeny. Members of the *whiJ* family have all been found in searches for genes that influence morphological or physiological differentiation in streptomycetes; while *whiB*-like (*wbl*) genes have been found by DNA-based searches, with at least six such genes in *S. coelicolor*, most of which appear to have homologues in most actinomycetes. It seems likely that the progenitor of all high G+C Gram-positives already possessed a set of *wbl* genes. Homologues of the sixth early *whi* gene, *whiA*, have so far been found in both low and high G+C Gram-positives. A mutation in *whiA* prevents transcription of *whiH* in a *whiH*$^+$ background, but not in a *whiH* mutant, implying an interaction between WhiA and WhiH. In the absence of any of the six early *whi* genes, expression of the late sporulation σ factor gene *sigF* is greatly reduced or undetectable[2].

References.

[1] Chater, K. F. (1993). Genetics of differentiation in *Streptomyces*. Ann. Rev. Microbiol. 47, 685-713

[2] Kelemen, G. H., Brown, G. L., Kormanec, J., Potuckova, L., Chater, K. F., and Buttner, M. J. (1996). The positions of the sigma factor genes, *whiG* and *sigF*, in the hierarchy controlling the development of spore chains in the aerial hyphae of *Streptomyces coelicolor* A3(2). Mol. Microbiol. **21**, 593-603.

Instituto Juan March (Madrid)

Regulation of prespore-specific transcription in *Bacillus subtilis*

Jeff Errington*, Richard A. Daniel, Andrea Feucht, Peter J. Lewis and Ling Juan Wu

Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, England, U.K.

Early in the process of spore formation in *Bacillus subtilis*, asymmetric cell division produces a large mother cell and a much smaller prespore. Differentiation of the prespore is initiated by activation of an RNA polymerase sigma factor, σ^F, specifically in that cell. σ^F is negatively regulated by an anti-sigma factor, SpoIIAB, which is in turn regulated by an anti-anti-sigma factor, SpoIIAA[1,2,3,4]. The interaction between SpoIIAB and SpoIIAA is complex because SpoIIAB is also a protein kinase that inactivates SpoIIAA by phosphorylating it on a specific serine residue[2,4]. The release of σ^F activity in the prespore is driven by the action of a specific serine phosphatase, SpoIIE, which converts SpoIIAA-P back to its active unphosphorylated form[5,6,7,8]

An important feature of the mechanism that regulates σ^F activity is its dependence on formation of the division septum[9,10,11]. SpoIIE protein plays an important role in coupling transcription and morphogenesis because it has a second activity required for correct formation of the asymmetric division septum[6,12]. Indeed, SpoIIE protein is targeted to a ring-like structure at the division site before septation begins, probably by interacting with one or more division proteins, possibly including the tubulin-like protein, FtsZ[13,14,15,16,17]

Recent results suggest that SpoIIE is active and dephosphorylates SpoIIAA-P at a significant rate in the predivisional cell, even though σ^F remains dormant[17]. Studies with a fully active SpoIIE-GFP fusion show that after asymmetric division, the phosphatase protein from the ring structure is sequestered onto the prespore face of the septum[16]. Sequestration of SpoIIE into the prespore could drive activation of σ^F by overwhelming the competing kinase reaction, thus explaining both the cell-specificity of σ^F activation and its dependence on septation.

References

1. Duncan, L. & Losick, R. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2325-2329.
2. Min, K. -T., Hilditch, C. M., Diederich, B., Errington, J. & Yudkin, M. D. (1993) *Cell* **74**, 735-742.
3. Alper, S., Duncan, L. & Losick, R. (1994) *Cell* **77**, 195-205.
4. Diederich, B., Wilkinson, J. F., Magnin, T., Najafi, S. M. A., Errington, J. & Yudkin, M. D. (1994) *Genes Devel.* **8**, 2653-2663.
5. Duncan, L., Alper, S., Arigoni, F., Losick, R. & Stragier, P. (1995) *Science* **270**, 641-644.
6. Feucht, A., Magnin, T., Yudkin, M. D. & Errington, J. (1996) *Genes Devel.* **10**, 794-803.
7. Arigoni, F., Duncan, L., Alper, S., Losick, R. & Stragier, P. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 3238-3242.
8. Lewis, P. J., Magnin, T. & Errington, J. (1996) *Genes to Cells* **1**, 881-894.
9. Beall, B. & Lutkenhaus, J. (1991) *Genes Devel.* **5**, 447-455.
10. Levin, P. A. & Losick, R. (1994) *J. Bacteriol.* **176**, 1451-1459.
11. Daniel, R. A., Harry, E. J., Katis, V. L., Wake, R. G. & Errington, J. (1998) *Mol. Microbiol.* (in press).

Instituto Juan March (Madrid)

12. Barak, I. & Youngman, P. (1996) *J. Bacteriol.* **178**, 4984-4989.
13. Arigoni, F., Pogliano, K., Webb, C. D., Stragier, P. & Losick, R. (1995) *Science* 270, 637-640.
14. Barak, I., Behari, J., Olmedo, G., et al. (1996) *Mol. Microbiol.* **19**, 1047-1060.
15. Levin, P. A., Losick, R., Stragier, P. & Arigoni, F. (1997) *Mol. Microbiol.* 25, 839-846.
16. Wu, L. J., Feucht, A. & Errington, J. (1998) *Genes Devel.* **12**, (in press).
17. Feucht, A., Daniel, R. A. & Errington, J. (1998) (in preparation).

Instituto Juan March (Madrid)

Compartment-specific activation of σ^F in *Bacillus subtilis*: analysis of SpoIIE activity and localisation in cell division mutants

Andrea Feucht, Richard A. Daniel and Jeffery Errington
Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, U.K.

During sporulation in *Bacillus subtilis* an asymmetric cell division creates two unequal-sized compartments with distinct fates. Differential gene expression in the two compartments, called mother cell and prespore, is governed by newly synthesised transcription factors (sigma factors). The activation of the first compartment-specific sigma factor, σ^F, is regulated by three other proteins, SpoIIE, SpoIIAA and SpoIIAB. SpoIIE is a membrane-bound phosphatase initially localised at the two potential sites of septation (1). During septation, SpoIIE is sequestered to the prespore side of the asymmetric septum (2). SpoIIE dephosphorylates the anti-anti-sigma factor SpoIIAA-P. SpoIIAA counteracts the inhibitory effect of the anti-sigma factor SpoIIAB, resulting in the release of σ^F in the prespore (3, 4).

Interestingly, the activation of σ^F is dependent on the formation of the asymmetric septum (5). To further understand this effect, we engineered a strain in which expression of the essential cell division gene *ftsL* is under control of an inducible promoter. Cells that sporulate in the absence of the inducer do not form a septum and fail to activate σ^F. Subsequent addition of inducer leads to immediate septation and activation of σ^F in the prespore.

Depletion of FtsL did not affect the ability of SpoIIE to localise to potential division sites. Surprisingly, it also had little effect on the dephosphorylation of SpoIIAA, suggesting that SpoIIE activity does not require the formation of the asymmetric septum.

A previously isolated *spoIIE* mutant bypasses the dependence of σ^F activation on formation of the asymmetric septum. The mutant protein is partially delocalised and produces increased levels of SpoIIAA.

On the basis of these and previously published results, we suggest that SpoIIE activity is modulated by two spatial effects. Early in sporulation the protein is targeted to tight zones at the potential division sites. This could restrict its effective activity on the dispersed cytoplasmic SpoIIAA-P. After septation, sequestration of most of the cellular SpoIIE into the small prespore compartment could provide a sufficently high concentration of phosphatase to overwhelm the competing kinase reaction and release σ^F activity in that compartment.

(1) Arigoni et al. (1995). *Science* **270**: 637-640
(2) Wu et al. (1998). *Genes Dev.*, in press
(3) Errington (1996). *TIG* **12**: 31-34
(4) Stragier and Losick (1996). *Annu Rev Genet* **30**: 297-341
(5) Levin and Losick (1994). *J. Bacteriol.* **176**: 1451-1459

Instituto Juan March (Madrid)

The role of sigma factors in controlling differentiation in *Streptomyces coelicolor* A(3)2.

Gabriella H. Kelemen and Mark J. Buttner
John Innes Centre, Norwich, NR4 7UH, United Kingdom.

Two alternative sigma factors, encoded by *whiG* and *sigF*, play important roles in the transformation of the multigenomic aerial mycelium into chains of unigenomic spores in *Streptomyces coelicolor*. The initiation of sporulation is dependent on *whiG*, while *sigF* is involved in spore maturation after sporulation septa have been laid down. Transcription studies performed on both these genes in wild type and in several *whi* mutants showed *whiG* transcription to be independent of known sporulation genes, while *sigF* transcription is dependent on all six known early sporulation (*whi*) genes. However, *sigF* is not directly dependent on *whiG*, suggesting an involvement of other sigma factors in sporulation.

In a search for suitable candidates, a new group of genes homologous to *sigF* has been identified and three of them have been sequenced. The genes encode alternative sigma factors that belong to the group of sporulation specific and stress response sigma factors of *Bacillus subtilis*. Two of the new sigma factor genes are potentially part of polycistronic operons and they are preceded by sequences coding for putative anti-sigma factors. The biological functions of these new sigma factors and their activation are currently being investigated and their potential involvement in differentiation will be discussed.

Instituto Juan March (Madrid)

POSTERS

Instituto Juan March (Madrid)

A RESPONSE REGULATOR-LIKE PROTEIN CONTROLLING SPORULATION IN *Streptomyces coelicolor*.

J. A. Aínsa, H. D. Parry, G. H. Kelemen, M. J. Buttner and K. F. Chater.
John Innes Centre, Norwich, NR4 7UH, UK.

In *Streptomyces* spp. sporulation takes place in an aerial mycelium. During this process, the long multigenomic apical compartments of aerial hyphae become subdivided by specialized sporulation septa into unigenomic prespore compartments. Six different regulatory loci (the "early" *whi* genes) are needed for normal sporulation septation[1]. Here we describe the molecular analysis of one of these genes, *whiI*. Previous studies have indicated that the *whiI* gene may act at an stage immediately before sporulation septation. Like the other early *whi* genes, it is necessary for the expression of certain late sporulation genes *whiE* and *sigF*[2]. Cosmids capable of complementing *whiI* mutants were identified from the *S. coelicolor* ordered cosmid library[3]. Further subcloning and the upcoming sequence data from the *S. coelicolor* genome project[4] allowed us to identify the *whiI* gene. It encodes a member of the response-regulator family of proteins. However, unlike many proteins of this kind, *whiI* is not adjacent to a gene for a histidine protein kinase, and it lacks two of the four highly conserved residues that make up a typical phosphorylation pocket in these proteins, raising questions about whether it is regulated by something other that phosphorylation. Among other known proteins, one of the most similar to WhiI is RedZ, a transcriptional activator of *redD*, which in turn activates undecylprodigiosin antibiotic biosynthesis in *S. coelicolor*[5]. Interestingly, RedZ also lacks a typical phosphorylation pocket. The determination of the transcription start site of *whiI* has identified a promoter whose sequence matches promoters that depend on the sporulation-specific sigma factor encoded by another early *whi* gene, *whiG*. This has been further confirmed by *in vitro* transcription analysis using purified σ^{WhiG}.

References: 1) Chater, 1993. Ann. Rev. Microbiol. 47: 685-713. 2) Kelemen et al, 1996. Mol. Microbiol. 21: 593-603. 3) Redenbach et al, 1996. Mol. Microb. 21: 77-96. 4) The Sanger Centre, http://www.sanger.ac.uk/. 5) Gunthrie et al, 1998. Microbiology, in press.

Instituto Juan March (Madrid)

Transcription requirements for Gearbox promoters.

Manuel Ballesteros[1], Shuichi Kusano[2], Akira Ishihama[2] and Migel Vicente[1].

1:Departamento de Biología Celular y del Desarrollo, Centro de Investigaciones Biológicas (CSIC). Madrid. SPAIN.

2: Department of Molecular Genetics. National Institute of Genetics. Mishima. JAPAN

Gearbox promoters have been defined as those which yield a constant amount of gene product per cell and per cell cycle at any growth rate, so that their activity is inversely growth-rate dependent. Peculiar -10 and -35 regions has been proposed to be present in these promoters. In principle several promoters may conform to the definition of Gearbox promoters: *ftsQ1p, bolA1p, mcbAp, sspAp* or *rmfp*. Among these, only *ftsQ1p* and *bolA1p* are RpoS-dependent promoters.

bolA1p, which has been up to now the typical example of a Gearbox promoter, participates in the control of expression of the *bolA* morphogene and it is efficiently transcribed *in vitro* by reconstituted RNA polymerase holoenzyme containing RpoS (σ^S) as well RpoD (σ^D) sigma factors.

ftsQ1p is localised within the *ddlB* gene, immediately downstream from the Housekeeper *ftsQ2p* promoter, and participates in the transcription of the essential cell-division genes *ftsQ, A* and Z.

The most potent promoters in the *ddlB-ftsA* region have been analysed for σ^S-dependent transcription. Only *ftsQ1p* was found to be transcribed *in vitro* by RNA polymerase holoenzyme coupled to σ^S ($E\sigma^S$). This dependency on σ^S was also found *in vivo* when single copy fusions to a reporter gene were analysed in *rpoS* and *rpoS*$^+$ backgrounds. Although *ftsQ1p* can be transcribed by RNA polymerase containing either σ^D or σ^S there is a preference for $E\sigma^S$ when the assay conditions included potassium glutamate and supercoiled templates, a property shared with the *bolA1p* Gearbox promoter. The rest of the promoters assayed, *ftsQ2p* and *ftsZ2p3p4p*, similarly to the control *bolA2p* promoter, were preferentially transcribed by $E\sigma^D$, the housekeeper polymerase. The *ftsQ1p* and the *bolA1p* promoters also share the presence of A-T rich sequences upstream the -35 region, and the requirement for an intact wild type α subunit for a proficient transcription.

We conclude that *ftsQ1p* and *bolA1p* share important regulatory properties with a common molecular background and we propose to restrict the clan of Gearbox promoters to those that may behave in a similar fashion.

Instituto Juan March (Madrid)

Mutational analysis of the *rpoN* gene cluster in *Pseudomonas putida*

Ildefonso Cases José Pérez-Martín and Victor de Lorenzo. Centro Nacional de Biotecnología CSIC, Campus de Cantoblanco 28049 Madrid, Spain

The DNA sequence of the genomic region downstream the *rpoN* gene of *Pseudomonas putida*, which codes for the alternate sigma factor σ^{54}, has revealed the existence of four open reading frames. The sequence and arrangement of these four ORFs is conserved in other gram negative bacteria, such as *E. coli*, *K. pneumoniae*, and others. Mutations in these genes have been reported to affect the activity of some σ^{54} dependent promoters or more indirectly, some σ^{54} related functions. We were interested in determining the possible role of these genes in the activity of the σ^{54}-dependent promoter *Pu* , which drives the transcription of the genes required for the catabolism of toluene in *Pseudomonas putida*. This promoter is tightly regulated by culture conditions; it is silenced during exponential growth in rich medium and further repressed by some additional carbon sources as glucose. Non-polar insertion mutants in these four genes were engineered and the phenotype regarding *Pu* expression was studied. None of the insertions affected exponential silencing, and two of them rendered no apparent phenotype in any of the condition studied. However, insertions in the *ptsN* and *ptsO* genes, modified the expression of *Pu* in response to additional carbon sources. While the ptsN minus strain had lost repression by glucose, the insertion in the *ptsO* gene rendered low levels of *Pu* expression both in repressive or not repressive conditions, in a phenotype that seemed symmetrical to that produced by the *ptsN* knockout. These results suggest a new mechanism of carbon repression in Pseudomonads, that, given the similarity of *ptsN* and *ptsO* with different enzymes of the phosphoenolpyruvate: sugar phosphotransferase system (PTS), could involve a phosphotransfer chain.

Instituto Juan March (Madrid)

Mª Trinidad Gallegos
Department of Biology, Imperial College of Science
Technology and Medicine, Prince Consort Road
London SW7 2BB (U.K.).
Tel.: 44 171 589 51 11. Fax: 44 171 584 20 56
E-mail: mtrini@ic.ac.uk

SEQUENCES IN σ^N THAT DETERMINE HOLOENZYME FORMATION AND PROPERTIES.

Sigma subunits of bacterial RNA polymerases are closely involved in many steps of promoter specific transcription initiation. The specialised sigma-N (σ^N) protein is needed for enhancer-dependent transcription, and the σ^N holoenzyme binds rare promoters in a transcriptionally inactive form. To begin to define the σ^N interface with core RNA polymerase, sequences of σ^N important for binding core RNAP have been determined. Purified partial sequences of σ^N were prepared and assayed for retention of core binding, and two discrete fragments of σ^N which bind the core but differ significantly in affinity for it, were identified. The low affinity segment of σ^N included Region I sequences, an amino terminal part of σ^N which mediates gene activation, and the higher affinity site is within a 95 residue fragment of Region III. It appears that the high affinity determinant allows the lower affinity containing sequence Region I to make effective contact with core RNAP, and we propose that the core to Region I contact mediates the transcription inactivity of the σ^N holoenzyme closed complex.

Heparin is a recognised inhibitor of closed complex formation at promoters binding the σ^N holoenzyme. The heparin sensitivity of the σ^N holoenzyme and associated closed promoter complexes is shown to result from dissociation of σ^N and core, a reaction occuring independently of promoter DNA. The heparin resistance of open promoter complexes is suggested to result from an altered σ^N-core-DNA interaction.

Instituto Juan March (Madrid)

Transcriptional activation by bending of DNA

Sylvie Déthiollaz, Patrick Eichenberger, Manuel Engelhorn & Johannes Geiselmann
Laboratoire du Contrôle de l'Expression Génique; Université Joseph Fourier; 460, rue de la Piscine, CERMO; BP 53 X; F-38041 Grenoble Cedex 9; France

The cAMP receptor protein (CRP) of *Escherichia coli* activates the transcription of many genes by binding to a specific site upstream of the promoter. We have studied a series of derivatives of the *malT* promoter where the CRP binding site is placed at variable distances upstream. When CRP is close to the promoter (centered at about -60) activation of transcription is brought about by direct contacts with RNA polymerase. Fine adjustments of the position around -60 allow either the downstream or upstream activating region I (ARI) of CRP to interact with RNA polymerase. The mechanism of transcriptional activation relies mostly on indirect interactions between the DNA upstream of the CRP binding site and the back of RNA polymerase when CRP is bound further upstream. These contacts are favored by the DNA bend that accompanies the binding of CRP to DNA. We show by kinetic UV-laser footprinting that these far-upstream contacts serve mainly to accelerate promoter escape.
A heterologous protein, IHF, which bends DNA to a similar degree as does CRP, activates transcription at our model promoters solely by DNA bending. We have isolated and characterized several mutants of IHF possessing an up to ten-fold increased activation potential. All of them possess a reduced DNA-bending angle compared to wild type IHF. We conclude that transcriptional activation by IHF can be maximized simply by optimizing the DNA bend induced by this protein.

Instituto Juan March (Madrid)

Thermoregulation of *Shigella* and *Escherichia coli* EIEC pathogenicity. A temperature-dependent structural transition of DNA modulates accessibility of *vir*F promoter to transcriptional repressor H-NS.

Maurizio Falconi[1], Bianca Colonna[2], Gianni Prosseda[2], Gioacchino Micheli[3], and Claudio O. Gualerzi[1]

[1]Laboratorio di Genetica, Dipartimento di Biologia MCA, Università di Camerino, Camerino (MC), 62032 Italy, [2]Dipartimento di Biologia Cellulare e Sviluppo, Università di Roma La Sapienza, and [3]Centro Acidi Nucleici C.N.R., Roma, 00185 Italy

Shigella and enteroinvasive *Escherichia coli* (EIEC) cause disease in humans by a similar complex mechanism of pathogenicity requiring the environmentally-controlled expression of genes located on the chromosome as well as on a large virulence plasmid (pINV). The expression of plasmid-borne *vir*F, which encodes a transcriptional regulator belonging to the AraC family, is required to initiate a cascade of events eventually resulting in the activation of several operons coding for invasion functions. In this article, we demonstrate that H-NS, one of the main proteins associated with the nucleoid of enterobactericeae, controls the temperature-dependent expression of the virulence genes by repressing the *in vivo* transcription of *vir*F only below a critical temperature (approximately 32°C). This temperature-dependent transcriptional regulation has been reproduced *in vitro* and the targets of H-NS on the *vir*F promoter have been identified, by *in vitro* and *in vivo* footprinting experiments, as two sites centered around -256 and -1 from the transcriptional start and separated by an intrinsic DNA curvature. H-NS was found to bind cooperatively to these two sites below 32°C, but not at 37°C and DNA supercoiling within the *vir*F promoter region did not influence H-NS binding. DNA supercoiling was found to be necessary, however, for the H-NS-mediated transcriptional repression. Electrophoretic analysis of the intrinsic curvature of DNA fragments derived from the *vir*F promoter between 4°C and 60°C showed that the fragment comprising the two H-NS binding sites undergoes a specific and temperature-dependent conformational transition at approximately 32°C. Our results suggest that this modification of the DNA target may modulate a cooperative interaction between H-NS molecules bound at two distant sites in the *vir*F promoter region and thus represent the physical basis for the H-NS-dependent thermoregulation of virulence gene expression.

Instituto Juan March (Madrid)

PAOLO LANDINI
School of Biochemistry, University of Birmingham
84 Jacoby Place, Priory Road
Edgbaston, Birmingham B5 7UW (U.K.)
Tel.: 44 121 414 5434. Fax: 44 121 414 7366. E-mail: p.landini@bham. ac.uk

Mechanism of transcription activation by the *E. coli* Ada protein.

The Ada protein (^{me}Ada) activates transcription from three *Escherichia coli* promoters: *ada*, *aidB* and *alkA*. At the *ada* and *aidB* promoters, ^{me}Ada binds between 60 and 40 base pairs upstream from the transcription start. This region is also a binding site for the C-terminal domain of the α subunit of RNA polymerase (αCTD); deletion of αCTD severely decreases ^{me}Ada-independent binding of RNA polymerase to *ada* and *aidB* and affects transcription initiation at these promoters. However, ^{me}Ada activates transcription by protein/protein interaction with negatively charged residues in the the C-terminal domain of RNA polymerase σ^{70} subunit (σ^{70}CTD). The target of Ada activation appears to be different at the *alkA* promoter, where Ada is able to recruit αCTD to the promoter and to interact with positively charged amino acids in σ^{70}CTD. These differences in the mechanism of Ada activation are consistent with different architectures displayed at the Ada-dependent promoters.

Instituto Juan March (Madrid)

The *mal* regulon of *Streptocccccus pneumoniae*

C.Nieto, A.Puyet* and M.Espinosa

*Centro de Investigaciones Biológicas,C.S.I.C. , Velázquez 144,E-28006 Madrid and *Departamento de Bioquímica y Biología Molecular IV,Facultad de Veterinaria , Universidad Complutense de Madrid, 28040 Madrid,Spain.*

The *Streptococcus pneumoniae* MalR protein regulates the transcription of two divergent promoters, *malXCD* and *malMP*, involved in maltosaccharide uptake and utilization, respectively. MalR belongs to the LacI-GalR family of transcriptional repressors (1).
The similarities between MalR and the other members of this family spans mainly through a helix-turn-helix motif, involved in DNA binding.
MalR has been purified and its function as a transcriptional repressor has been analyzed . The protein binds specifically to two operator sequences in the intergenic region between *malXCD* and *malMP* operons. The affinity of MalR for the *malMP* binding sequence is higher than for the *malXCD* site .
The binding consensus sequence in *malXCD* and *malMP* regions has been also determined (2). The operator recognition sequences is very similar in both operons. Only a weak difference is detected at the 3´end. This slight difference could explain the MalR differential affinity for both operators. These MalR recognition sequences are located, in both operons, very close to the start site of transcription.
The binding consensus sequence for MalR is a sequence closely related to that recognized by others members of the LacI-GalR family (3). This homology is more obvious for PurR, suggesting a common mechanism for DNA interaction. In that sense, a MalR molecular modeling by replacement of PurR crystal structure have been performed. The model showed a good fit between both repressors specially in the DNA binding domain.
Like others members of the LacI-GalR family , the binding to its recognition sites should be abolished when an inductor molecule is present (4) . The effect of different sugars on MalR DNA binding to its targets was analyzed. In the presence of maltose the MalR DNA binding is dramatically reduced. Under these conditions, MalR could change from active to inactive form, by interaction with the inductor molecule and the expression of the structural genes is switched-on.
The transcriptional repressor activity of MalR has also been identified in *E.coli* cells using transcriptional fusions of *malXCD* and *malMP* operators regions with reporters genes (2).
Besides, we hope to get aditional and complementary information about MalR protein by the characterization of the available MalR mutants, R^c(constitutive mutants) and R^n (non inducible mutants). In the former the expression of *malM* is constitutive whereas in the latter the expression of *malM* is very low in any condition of growth (5).

Instituto Juan March (Madrid)

We are also trying to investigate in more detail the mechanism of regulation by MalR protein. Preliminary results suggest a competition between RNA polymerase and MalR for its targets in the DNA and/or by the induction of a complex srtucture in the DNA which could prevent the binding of the RNA polymerase to its target.

(1) Puyet A., Ibañez M. and M.Espinosa (1993) J. Biol. Chem. **268**, 25402-25408.
(2) Nieto C. , Espinosa M. and Puyet A. (1997)J. Biol. Chem. **272**, 30860-30865.
(3) Schumacher M.A., Choi K.Y., Zalkin H. and Brennan R.G. (1994) Science **266**, 763-770.
(4) Lewis M., Chang G., Horton N.C., Kercher M.A., Pace H.C., Schumacher M.A., Brennan R.G. and Lu P. (1996) Science **271**, 1247-1254.
(5) Lacks S.A. Genetics (1968) **60**, 685-706.

Instituto Juan March (Madrid)

High resolution transcriptional analysis of the 536 kb symbiotic replicon of *Rhizobium* sp. NGR234

X. Perret[1], C. Freiberg[2], A. Rosenthal[2], W. J. Broughton[1] and R. Fellay[1].

[1] Laboratoire de Biologie Moléculaire des Plantes supérieures, University of Geneva, 1 chemin de l'Impératrice, 1292 Chambésy, Geneva, Switzerland.
[2] Institut für Molekulare Biotechnologie, Abteilung Genomanalysis, Beutenbergstrasse 11, 07745 Jena, Germany.

Under nitrogen-limiting conditions, soil bacteria of the genera *Azorhizobium*, *Bradyrhizobium*, *Rhizobium* and *Sinorhizobium* (collectively called rhizobia) form symbiotic associations with leguminous plants. As a result, infectious rhizobia penetrate the root-cortex and invade specialised root structures called nodules. Within the nodule cells, the bacteria enlarge and differentiate into nitrogen-fixing bacteroids. Symbiotic specificity varies from rhizobia which are devoted to one or a few legumes to *Rhizobium* sp. NGR234 which nodulates more than 110 genera of legumes (S.G. Pueppke and W.J. Broughton, unpublished). In NGR234, most of the symbiotic determinants are carried by pNGR234*a*, a plasmid of 536 kb (1). Its complete nucleotide sequence revealed 416 open reading frames (orf's) of which, 139 have unknown functions (2). As befit a curable extrachromosomal replicon, no genes essential to transcription, translation or primary metabolism were found. Most loci involved in nitrogen fixation (*nif*, and *fix*) are clustered between nucleotides 433,000 to 489,000, whereas nodulation genes (*nod*, *nol* and *noe*) are found in several regions dispersed throughout pNGR234*a*. Although the mechanisms behind regulation of *nod*-gene expression are highly variable, they all require the *nodD*-gene(s) (3). NodD proteins belong to the LysR family of transcriptional activators, and bind to specific sequences (*nod*-boxes) in the promoter regions of flavonoid-inducible nodulation genes. Sequence analysis revealed 17 *nod*-boxes, as well as 15 NifA-σ^{54} consensus sequences involved in the regulation of *nif*, and *fix* genes. In order to analyse the expression of all the predicted orf's, the complete nucleotide sequence of pNGR234*a* was divided into 441 PCR products designed to represent coding as well as intergenic regions. These templates were probed with radioactively labelled-RNA prepared from bacterial cultures grown under various experimental conditions, as well as from bacteroids found in determinate and indeterminate nodules. These results which represent the first high resolution transcription map of a large rhizobial replicon, are summarised in a colour coded figure. Generally, time of expression correlates well with the presence of known regulatory sequences. Interestingly, many genes of insertion (IS) and transposon (Tn) like sequences are strongly expressed in all conditions tested, and many loci other than known *nod*, *nif*, and *fix* are shown to be transcribed under symbiotic conditions.

References:

1. Broughton WJ, Heycke N, Meyer z A H and Pankhurst CE (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3093.
2. Freiberg C, Fellay R, Bairoch A, Broughton WJ, Rosenthal A and Perret X (1997) *Nature* **387**, 394.
3. Fellay R, Rochepeau P, Relić B and Broughton WJ (1995) in *Pathogenesis and Host Specificity in Plant diseases* (U.S. Singh, R.P. Singh, and K. Kohmoto, eds; Pergamon, Oxford) pp.199-200.

Instituto Juan March (Madrid)

MECHANISMS OF BACTERIAL RESISTANCE TO HOST DEFENSE PROTEINS

LÓPEZ-SOLANILLA, E., GARCÍA-OLMEDO, F. AND RODRÍGUEZ-PALENZUELA, P.

Dep. Biotechnology.
E.T.S.Ingenieros Agrónomos, UPM. Av.Complutense
28040 Madrid, Spain.

Background and objectives
An effective defense mechanism against invading pathogens, shared by plants and animals, is mediated by small cationic proteins, as cecropins, magainins and defensins from animals, and thionins and snakins from plants. These proteins exert a toxic action against bacteria, possibly through the alteration of membrane permeability. The role of antimicrobial peptides in pathogenesis has been highlighted by the observation of increased susceptibility to infection in *Drosophila* mutants affected in their synthesis, but no such evidence is available with respect to plant peptides because appropriate plant mutants with decreased peptide levels have not yet been obtained. An alternative line of evidence about the role of antimicrobial peptides is based in the production of peptide-sensitive mutants of the pathogen. The hypothesis that the peptides are involved in defence would be supported by a decrease of virulence in this type of mutants. Indeed, in the animal pathogen *Salmonella typhimurium*, both rough lipopolysaccharide (LPS) mutants and *sapA-F* mutants, which show increased sensitivity to antimicrobial peptides (*sap* stands for sensitive to antimicrobial peptides), have reduced virulence, suggesting that resistance to host peptides has a direct role in *Salmonella* pathogenesis [1]. Similarly, we have previously found that thionin- and LTP-sensitive mutants of *Ralstonia (Pseudomonas) solanacearum* were both altered in their LPS structure and are avirulent in tobacco [2].
Erwinia chrysanthemi, is an economically important phytopathogenic bacterium that causes soft-rot diseases in a wide range of crops. Little is known about the mechanisms that enable *E. chrysanthemi* to resist the action of antimicrobial agents from the plant host. Although *S.typhimurium* and *E.chrysanthemi* have very different pathogenic behaviour, the fact that they are phylogenetically related and that antimicrobial peptides occur in their respective animal and plant hosts lead us to investigate the possible role of the Sap system in plant-pathogen interactions.

Results and conclusions
The *sapA-F* (sensitive to antimicrobial peptides) operon from the pathogenic bacterium *Erwinia chrysanthemi* has been characterized. It has five ORFs that are closely related (71% overall amino acid identity) and are in the same order as those of the *sapA-F* operon from *Salmonella typhimurium*. An *E. chrysanthemi sap* mutant strain, BT105, was obtained by marker-exchange. Mutant BT105 was more sensitive than the wild type to wheat α-thionin and to snakin-1, the most abundant antimicrobial peptide from potato tubers. Mutant BT105 was less virulent than the wild type in potato tubers: lesion area was 37% of control and growth rate was two orders of magnitude lower. The magnitude of the effect of *sapA-F* inactivation on virulence in potato tubers and chicory leaves was greater than the effects of mutations affecting the *pel* operon and the Hrp system in the same pathogen.
These results indicate that the interaction of antimicrobial peptides from the host with the *sapA-F* operon from the pathogen plays a similar role in animal and in plant bacterial pathogenesis. They also show that this operon is important for the pathogenicity of *E. chrysanthemi*.

References

[1] Parra-Lopez C, Baer MT, Groisman EA, (1993). The EMBO Journal 12, 4053-4062.
[2] Titarenko E, López-Solanilla E, García-Olmedo F, Rodríguez-Palenzuela P, (1997). Journal of Bacterioloy 179, 6699-6704.

Instituto Juan March (Madrid)

GASP PHENOTYPE IN ENTEROBACTERIA

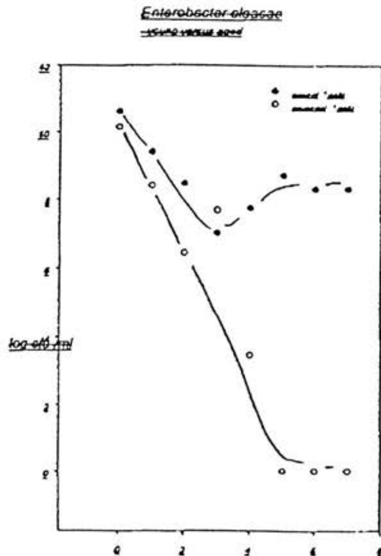
Esteban Martínez, Ignacio Barquero and Antonio Tormo

We have studied the GASP (Growth Advantage in Stationary Phase) phenotype in several enterobacteria. Aged cells in stationary phase can displace young cells from the culture. Aging involves genetic changes. The *rpoS* gene has been described to be involved in this process. In fact, certain down mutation in this gene give the cell a GASP phenotype.

Our results show that several genus of bacteria also present this phenotype. We have studied three. Two of them, *Enterobacter cloacae* and *Shigella dysenteriae*, present the *rpoS* gene and the GASP phenotype. We have isolated and sequenced the *rpoS* gene from young (wild) and aged *E.cloacae*. They differ in one point mutation that changes glutamic acid in position 215 in the wild RpoS protein into a lysine in the aged *E.cloacae*.

We have also studied the presence of the GASP phenotype in *rpoS*-less bacteria such as *Providentia stuartii*. This bacteria also presents this phenotype indicating that there can be several pathways leading to this phenotype.

It is interesting to point out the implications that this behaviour can have. Mutations in this gene can make bacteria to be able to displace other strains from the medium in which they coexist. Besides, pathogenity has been related, in several bacteria, to a wild *rpoS* gene. Also this gene make the cells stress resistant during stationary phase. It is therefore possible to construct GASP strains that would also be non pathogenic and stress sensitive. This strains could be used as a bacterial tool in controlling the presence and displacement of other bacteria.



Instituto Juan March (Madrid)

Ralstonia eutropha RpoN operon and the expression of the biphenyl degradation genes of the Tn*4371* transposon. Sébastien MOUZ(1), Christophe MERLIN(1¶) and Ariane TOUSSAINT(1,2).(1) Laboratoire de Microbiologie, Université Joseph Fourier, France. (2) Laboratoire de Génétique des Procaryotes, Université Libre de Bruxelles, Belgium.; (¶) Present address: Rome Laboratory, Michigan State University, East Lansing, Michigan, USA).

Tn*4371* is a 55 kbp long catabolic transposon which encodes enzymes that catalyse the complete degradation of biphenyl and 4-chlorobiphenyl (BP/4CBP). It provides *Alcaligenes eutrophus/Ralstonia eutropha* strains (e.g. H16 and CH34) with the ability to use biphenyl as a carbon and energy source. Expression of the *bph* genes is activated 2 fold by biphenyl and was found to be blocked in an H16 strain which carries an *rpoN* mutation. The CH34 *rpoN* gene was cloned and found not to complement some of this H16*rpoN* defects. Sequencing of the CH34 *rpoN* operon revealed the presence of genes which differ from those present around the *rpoN* gene in other strains.
Characterisation of the main Tn*4371* *bph* promoter, which drives the synthesis of an 11 KB mRNA, showed that it has the characteristics for recognition by σ70. Nevertheless CH34*rpoN*/Tn*4371* does not grow normally with biphenyl as the sole carbon and energy source suggesting that σ54 control over *bph* gene expression is indirect. Experiments are in progress to determine which genes involved in biphenyl degradation are transcribed by σ54-RNA polymerase.

Instituto Juan March (Madrid)

Control of virulence gene expression in pathogenic *Listeria* by the transcription factor PrfA.

Y. Vega [1], M.T. Ripio [1], F. Gómez-Gallego [2], C. Dickneite [3], R. Böckmann [3], G. Domínguez-Bernal [1], B. González-Zorn [1], M. Suárez [1], J.M. Bautista [2], W. Goebel [3], J. Kreft [3], J.A. Vázquez-Boland [1]*

Grupo de Patogénesis Molecular Bacteriana, Unidad de Microbiología e Inmunología[1], and Dpto. de Bioquímica y Biología Molecular IV[2], Facultad de Veterinaria, Universidad Complutense de Madrid (Spain); Lehrstuhl für Mikrobiologie, Biozentrum, Universität Würzburg (Germany)[3]

The expression of virulence genes in the Gram-positive, facultative intracellular pathogen *Listeria monocytogenes* is subject to positive control mediated by the pleiotropic regulator protein PrfA, encoded by *prfA*, a gene of the *hly* pathogenicity island. PrfA binds via a helix-turn-helix (HTH) motif located in its C-terminal region to target DNA sequences of dyad symmetry called PrfA-boxes. These PrfA-boxes are 14 bp-long and centred at position -41 relative to the transcription start site in PrfA-dependent promoters. *prfA* is autoregulated by positive feedback mediated by a PrfA-dependent promoter.

Transcriptional activation of *prfA* requires a temperature of 37°C, similar to that of the mammalian host. However, this is not sufficient for full expression of *prfA* and PrfA-dependent virulence determinants, which also requires that *L. monocytogenes* senses at the same time an adequate composition of the extracellular medium. This probably represents a fail-safe mechanism devised by *L. monocytogenes* to prevent the expression of virulence genes when these are not required, for example when the bacteria are outside an appropriate host niche.

PrfA shares substantial sequence similarity with CRP (CAP) from *Escherichia coli*, suggesting it might be functionally related to the members of the CRP/FNR superfamily of bacterial transcription factors. In our laboratory in Madrid we have recently identified *prfA** mutants from *L. monocytogenes*, providing for the first time a clear hint on the possible functional relatedness between PrfA and CRP. *Mutatis mutandi*, these *prfA** strains were analogous to *crp** mutants in that they overexpressed PrfA-dependent genes, including *prfA* itself, under culture conditions where the PrfA regulon is normally downregulated. These *prfA** mutants carried a Gly145Ser substitution that dramatically increased the binding affinity of PrfA for its target DNA site. Interestingly, the mutation was in a PrfA stretch of eleven aminoacids showing a high degree of sequence similarity (70%) with the D α-helix of CRP. In this region, several *crp** mutations map in *E. coli* that allow CRP to function in the absence of cAMP, the cofactor required for its allosteric activation. A well characterized CRP* mutation that presumably mimics the conformational change induced by the cofactor, Ala144Thr, locates in the aligned proteins at the very same position of our PrfA mutation.

Based on these observations, we hypothesize that PrfA might function through a cofactor-mediated allosteric transition mechanism similar to that of CRP, and that the Gly145Ser mutation represents a cofactor-independent PrfA* form that is "freezed" in an active conformation. Two typical features of CRP are particularly well conserved in PrfA. One is the HTH motif in the C-terminal region, for which the functional homology between the two proteins has been already documented. The other is a series of short antiparllel β-strands separated by glycine residues, which may form a β-roll structure spanning a significant portion of the N-terminal half of the protein. The prediction of such a structure in PrfA is very intriguing since in CRP it forms the pocket where the activatory cofactor cAMP is buried in the N-terminal domain of the protein, but cAMP is not present in detectable amounts, and is not known to function as an effector molecule in low G+C Gram-positive bacteria. In fact, most residues that are important in CRP for cAMP binding are not conserved in PrfA and addition of exogenous cAMP did not result in PrfA regulon activation. However, conformational studies performed with the extrinsic fluorescence probe 8-anilino-1-naphtalenesulfonic acid (ANS) revealed that addition of cAMP to purified PrfA produced a fluorescence quenching equivalent to that characteristically shown by CRP. This suggests that the putative activatory cofactor of PrfA might be a cyclic nucleotide similar to cAMP. We are actively working in the identification of this hypothetic PrfA cofactor and in the genetic characterization of the signal transduction system that connects the PrfA transcriptional system with the extracellular environment.

Instituto Juan March (Madrid)

Stationary-phase induction of *dnaN*, *dnaN** and *recF*, three genes of *Escherichia coli* involved in DNA replication and repair

Magda Villarroya, Ignacio Pérez-Roger, Fernando Macián and M. Eugenia Armengod

The β subunit of DNA polymerase III holoenzyme, the *E. Coli* chromosomal replicase, is a sliding DNA clamp responsible for tethering the polymerase to DNA and endowing it with high processivity. The gene encoding for β, *dnaN*, maps between *dnaA* and *recF*, which are involved in initiation of DNA replication at *oriC* and resumption of DNA replication at disrupted replication forks, respectively. Moreover, *dnaN* contains an internal in frame gene, *dnaN**, which may be involved in a DNA recovery process. In exponentially growing cells, *dnaN* and *recF* are predominantly expressed from the *dnaA* promoters. However, we have found that stationary-phase induction of internal promoters drastically changes the expression pattern of the *dnaA* operon genes. The induction of the *dnaN* and *dnaN** promoters, but not that of the *recF* promoters, is dependent on the stationary phase σ factor, RpoS. Interestingly, activity of terminators controlling transcription initiated at the *recF* promoter increases concomitantly to the growth curve. Consequently, *recF* expression is mediated by the *dnaN* promoters in starved cells. In contrast, *dnaN** expression depends largely on its own promoter due to a probable occlusion of its ribosome binding site on *dnaN* transcripts. Studies with *lacZ* translational fusions indicate that the stationary-phase-induced levels of β* and RecF are much lower than those of β because of extensive translational regulation. Our results suggest that *E. Coli* cells have evolved stationary-phase-dependent mechanisms in order to coordinate expression of *dnaN*, *recF* and *dnaN** independently of the *dnaA* regulatory region. These mechanisms might be a part of a developmental program aimed to maintain DNA integrity under stress conditions.

Instituto Juan March (Madrid)

LIST OF INVITED SPEAKERS

Yves V. Brun Department of Biology, Jordan Hall 142, Indiana University, Bloomington, IN. 47405-6801 (USA). Tel.: 1 812 855 7239. Fax: 1 812 855 6705. E-mail: ybrun@bio.indiana.edu

Richard R. Burgess McArdle Laboratory for Cancer Research, 1400 University Avenue, University of Wisconsin-Madison, Madison, WI. 53706 (USA). Tel.: 1 608 262 2177. Fax: 1 608 262 2824. E-mail: burgess@oncology.wisc. edu

Steve Busby School of Biochemistry, The University of Birmingham, Birmingham B15 2TT (U.K.). Fax: 44 121 414 39 82

Keith F. Chater John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH (U.K.). Tel.: 44 1603 452 571. Fax: 44 1603 456 844. E-mail: chater@bbsrc.ac.uk

Jeff Errington Sir William Dunn School of Pathology, University of Oxford, South Park Road, Oxford OX1 3RE (U.K.). Tel.: 44 1865 27 55 61. Fax: 44 1865 27 55 56. E-mail: erring@molbiol.ox.ac.uk

Robert E. Glass Division of Genetics, School of Clinical Laboratory Sciences, Queens Medical Centre, Nottingham University, Clifton Boulevard, Nottingham NG7 2UH (U.K.). Tel.: 44 115 970 9226. Fax: 44 115 9709 906. E-mail: Robert.Glass@nottingham.ac.uk

Richard L. Gourse Department of Bacteriology, University of Wisconsin, 1550 Linden Drive, Madison, WI. 53706 (USA). Tel.: 1 608 262 9813. Fax: 1 608 262 9865. E-mail: rgourse@bact.wisc.edu

Regine Hengge-Aronis Department of Biology, University of Konstanz, 78457 Konstanz (Germany). Fax: 49 7531 88 3356

Christopher F. Higgins MRC Clinical Sciences Centre, Imperial College School of Medicine, The Hammersmith Hospital, Du Cane Road, London W12 ONN (U.K.). Tel.: 44 181 383 8249. Fax: 44 181 383 8337. E-mail: chiggins@rpms.ac.uk

Akira Ishihama Dept. Molecular Genetics, National Institute of Genetics, Mishima Shizuoka 411, (Japan). Tel.: 81 559 81 6741. Fax: 81 559 81 6746. E-mail: aishiham@lab.nig.ac.jp

Reid C. Johnson Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA. 90095-1737 (USA). Fax: 1 310 206 5272. E-mail: RJOHNSON@biochem.medsch.ucla.edu

Instituto Juan March (Madrid)

Roberto Kolter	Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA. 02115 (USA). Tel.: 1 617 432 1776. Fax: 1 617 638 7664. E-mail: kolter@mbcrr.harvard.edu
Víctor de Lorenzo	Centro Nacional de Biotecnología, CSIC, Campus de Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 585 45 73. Fax: 34 91 585 45 06. E-mail: vdlorenzo@cnb.uam.es
Thomas Nyström	Department of Microbiology, Lund University, Solvegatan 12, 223 62 Lund (Sweden). Tel.: 46 46 222 04 63. Fax: 46 46 157 839. E-mail: Thomas.Nystrom@Mikrbiol.lu.se
John S. Parkinson	Biology Department, University of Utah, Salt Lake City, UT. 84112 (USA). Tel.: 1 801 581 6307. Fax: 1 801 581 4668. E-mail: Parkinson@Biology.Utah.edu
Steen Pedersen	Institute of Molecular Biology, University of Copenhagen, Oster Farimagsgade 2A, Copenhagen (Denmark). Tel.: 45 35 32 20 96. Fax: 45 35 32 20 96. Fax: 45 33 93 52 20. E-mail: ateenp@ biobase.dk
Margarita Salas	Centro de Biología Molecular "Severo Ochoa", CSIC, Fac. de Ciencias, Univ. Autónoma, Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 397 50 70. Fax: 34 91 397 47 99. E-mail: Msalas@ cbm.uam.es
Deborah Siegele	Department of Biology, Texas A&M University, College Station, TX. 77843 (USA). Tel.: 1 409 862 4022. Fax: 1 409 845 2891. E-mail: siegele@bio.tamu.edu
Miguel Vicente	Departamento de Biología Celular y del Desarrollo, CIB, CSIC, 28006 Madrid (Spain). Tel.: 34 91 561 18 00. Fax: 34 91 562 75 18. E-mail: cibmv5j@fresno.csic.es

Instituto Juan March (Madrid)

LIST OF PARTICIPANTS

José A. Aínsa	Department of Genetics, John Innes Centre, Colney Lane, Norwich, NR4 7UH (U.K.). Tel.: 44 1603 452 571. Fax: 44 1603 456 844.
Cecília M. Arraiano	Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Apt. 127, 2780 Oeiras (Portugal). Tel.: 351 1 446 9548. Fax: 351 1 441 1277. E-mail: Cecilia@itqb.unl.PT
Manuel Ballesteros	Departamento de Biologia Celular y del Desarrollo, Centro de Investigaciones Biológicas, (CSIC), c/Velázquez 144, 28006 Madrid (Spain). Tel.: 34 91 564 45 62. Fax: 34 91 562 75 18. E-mail: cibb110@fresno.csic.es
Belén Calles	Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM) Univ. Autónoma, Cantoblanco 28049 Madrid (Spain). Tel.: 34 91 397 84 35. Fax: 34 91 397 84 90. E-mail: bcalles@Trasto.cbm.uam.es
Ildefonso Cases	Centro Nacional de Biotecnología, CSIC, Campus de Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 585 45 73. Fax: 34 91 585 45 06. E-mail: Icases@cnb.uam.es
Richard D'Ari	Institut Jacques Monod, CNRS, Université Paris 6, Université Paris 7, 2 Place Jussieu, F-75251 Paris Cedex 05 (France). Fax: 33 1 44 27 57 16.
Andrea Feucht	Sir William Dunn School of Pathology, University of Oxford, South Park Road, Oxford OX1 3RE (U.K.). Tel.: 44 1865 27 55 62. Fax: 44 1865 27 55 56. E-mail: feucht@molbiol.ox.ac.uk
Mª Trinidad Gallegos	Department of Biology, Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BB (U.K.). Tel.: 44 171 589 51 11. Fax: 44 171 584 20 56. E-mail: mtrini@ic.ac.uk
Johannes Geiselmann	Laboratoire du Contrôle de l'Expression Génique, Université Joseph Fourier, 460 rue de la Piscine, CERMO, BP 53 X, F-38041 Grenoble Cedex 9 (France). Tel.: 33 4 76 63 56 62. Fax: 33 4 76 51 43 36. E-mail: Hans.Geiselmann@ujf.grenoble.fr
José E. González-Pastor	Unidad de Genética Molecular, Hospital Ramón y Cajal, Crta. Colmenar Km 9,1, 28034 Madrid (Spain). Tel.: 34 91 336 85 42. Fax: 34 91 336 90 16. E-mail: eduardo.gonzalez@hrc.es
Claudio O. Gualerzi	Laboratory of Genetics, Dept. of Biology, University of Camerino, I-62032 Camerino (MC) (Italy). Tel.: 39 737 40 32 40. Fax: 39 737 40 32 43. E-mail: Gualerzi@cambio.unicam.it

Instituto Juan March (Madrid)

Claude Gutierrez	Laboratoire de Microbiologie et Génétique Moléculaire, UPR 9007 du CNRS, 118 Route de Narbonne, F-31062 Toulouse Cedex (France). Tel.: 33 561 33 58 72. Fax: 33 561 33 58 86. E-mail: clg @ibcg.biotoul.fr
Miki Jishage	Department of Molecular Genetics, National Institute of Genetics, Mishima, Shizuoka 411, (Japan). Tel.: 81 559 81 6742. Fax: 81 559 81 67 46.
Gabriella H. Kelemen	John Innes Centre, Norwich Research Park, Norwich, NR4 7UH (U.K.). Tel.: 44 1603 45 25 71. Fax: 44 1603 45 49 70. E-mail: kelemen@bbsrc.a.uk
Annie Kolb	Unité de Physicochimie des Macromolécules Biologiques, Institut Pasteur, (CNRS URA 1773), F-75724 Paris Cedex 15 (France). Tel.: 33 1 4568 8644. Fax: 33 1 4061 3060. E-mail: akolb@ pasteur.fr
Paolo Landini	School of Biochemistry, University of Birmingham, 84 Jacoby Place, Priory Road, Edgbaston, Birmingham B5 7UW (U.K.). Tel.: 44 121 414 5434. Fax: 44 121 414 7366. E-mail: p-landini@bham. ac.uk
Concepción Nieto	Centro de Investigaciones Biológicas, CSIC, Velázquez 144, 28006 Madrid (Spain). Tel.: 34 91 561 18 00. Fax: 34 91 562 75 18.
Xavier Perret	Laboratoire de Biologie Moléculaire des Plantes Supérieures, University of Geneva, 1 chemin de l'Impératrice, 1292 Chambésy, Geneva (Switzerland). Tel.: 41 22 906 17 40. Fax: 41 22 906 17 41. E-mail: perret@sc2a.unige.ch
Juan L. Ramos	Department of Biochemistry, Estación Experimental del Zaidín, CSIC, 18008 Granada (Spain). Tel.: 34 958 12 10 11. Fax: 34 958 12 96 00.
Francisca Reyes-Ramírez	Nitrogen Fixation Laboratory, John Innes Centre, Norwich NR4 7UH (U.K.). 44 1603 45 69 00. Fax: 44 1603 45 49 70. E-mail: reyes@bbsrc.ac.uk
Evelyne Richet	Unité de Génétique Moléculaire, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15 (France). Tel.: 33 1 456 88 494. Fax: 33 1 456 88 960. E-mail: erichet@pasteur. fr
Pablo Rodríguez-Palenzuela	Departamento de Biotecnología, E.T.S. Ingenieros Agrónomos, UPM, Av. Complutense, 28040 Madrid (Spain). Tel.: 34 91 336 57 05. Fax: 34 91 336 57 57. E-mail: pablo@bit.etsia.upm.es

Instituto Juan March (Madrid)

Fernando Rojo Centro Nacional de Biotecnología, CSIC, Campus de la Universidad Autónoma, Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 585 45 39. Fax: 34 91 585 45 06. E-mail: frojo@cnb.uam.es

Eduardo Santero Departamento de Genética. Facultad de Biología, Universidad de Sevilla, Ap. 1095, 41080 Sevilla (Spain). Tel.: 34 95 455 71 06. Fax: 34 95 455 71 04. E-mail: esantero@cica.es

Antonio Tormo Departamento de Bioquímica, Fac. de Ciencias Biológicas, Ciudad universitaria, 28040 Madrid (Spain). Tel.: 34 91 394 41 56. Fax: 34 91 394 46 72. E-mail: tormo@solea.quim.ucm.es

Ariane Toussaint Laboratoire de Microbiologie, Université Joseph Fourier, 38041 Grenoble Cedex 9 (France). Tel.: 33 4 76 51 43 46. Fax: 33 4 76 51 43 36. E-mail: ariane@alize.ulb.ac.be

Bernt Eric Uhlin Department of Microbiology, Umeå University, S-90187 Umeå (Sweden). Tel.: 46 90 785 6731. Fax: 46 90 77 26 30. E-mail: bernt.eric.uhlin@micro.umu.se

José A. Vázquez-Boland Grupo de Patogenésis Molecular Bacteriana, Unidad de Microbiología e Inmunología, Fac. de Veterinaria, Universidad Complutense, 28040 Madrid (Spain). Tel.: 34 91 394 37 04. Fax: 34 91 394 39 08. E-mail: vazquez@eucmax.sim.ucm.es

Francisca Vicente Merck Sharp & Dohme de Esapaña, S.A., Centro de Investigación Básica, c/Josefa Valcárcel 38, 28027 Madrid (Spain).

Magdalena Villarroya Instituto de Investigaciones Citológicas de la FVIB, Amadeo de Saboya 4, 46010 Valencia (Spain). Tel.: 34 96 339 12 50. Fax: 34 96 360 14 53. E-mail: villar@ochoa.fib.es

Instituto Juan March (Madrid)

Texts published in the
SERIE UNIVERSITARIA
by the
FUNDACIÓN JUAN MARCH
concerning workshops and courses organized within the
Plan for International Meetings on Biology (1989-1991)

---

*: Out of stock.

*246 **Workshop on Tolerance: Mechanisms and Implications.**
Organizers: P. Marrack and C. Martínez-A.

*247 **Workshop on Pathogenesis-related Proteins in Plants.**
Organizers: V. Conejero and L. C. Van Loon.

*248 **Course on DNA - Protein Interaction.**
M. Beato.

*249 **Workshop on Molecular Diagnosis of Cancer.**
Organizers: M. Perucho and P. García Barreno.

*251 **Lecture Course on Approaches to Plant Development.**
Organizers: P. Puigdomènech and T. Nelson.

*252 **Curso Experimental de Electroforesis Bidimensional de Alta Resolución.**
Organizer: Juan F. Santarén.

253 **Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses.**
Organizers: F. García-Arenal and P. Palukaitis.

254 **Advanced Course on Biochemistry and Genetics of Yeast.**
Organizers: C. Gancedo, J. M. Gancedo, M. A. Delgado and I. L. Calderón.

*255 **Workshop on the Reference Points in Evolution.**
Organizers: P. Alberch and G. A. Dover.

*256 **Workshop on Chromatin Structure and Gene Expression.**
Organizers: F. Azorín, M. Beato and A. A. Travers.

257 **Lecture Course on Polyamines as Modulators of Plant Development.**
Organizers: A. W. Galston and A. F. Tiburcio.

*258 **Workshop on Flower Development.**
Organizers: H. Saedler, J. P. Beltrán and J. Paz-Ares.

*259 **Workshop on Transcription and Replication of Negative Strand RNA Viruses.**
Organizers: D. Kolakofsky and J. Ortín.

*260 **Lecture Course on Molecular Biology of the Rhizobium-Legume Symbiosis.**
Organizer: T. Ruiz-Argüeso.

261 **Workshop on Regulation of Translation in Animal Virus-Infected Cells.**
Organizers: N. Sonenberg and L. Carrasco.

*263 **Lecture Course on the Polymerase Chain Reaction.**
Organizers: M. Perucho and E. Martínez-Salas.

*264 **Workshop on Yeast Transport and Energetics.**
Organizers: A. Rodríguez-Navarro and R. Lagunas.

265 **Workshop on Adhesion Receptors in the Immune System.**
Organizers: T. A. Springer and F. Sánchez-Madrid.

*266 **Workshop on Innovations in Proteases and Their Inhibitors: Fundamental and Applied Aspects.**
Organizer: F. X. Avilés.

Instituto Juan March (Madrid)

267 **Workshop on Role of Glycosyl-Phosphatidylinositol in Cell Signalling.**
Organizers: J. M. Mato and J. Larner.

268 **Workshop on Salt Tolerance in Microorganisms and Plants: Physiological and Molecular Aspects.**
Organizers: R. Serrano and J. A. Pintor-Toro.

269 **Workshop on Neural Control of Movement in Vertebrates.**
Organizers: R. Baker and J. M. Delgado-García.

Texts published by the
CENTRE FOR INTERNATIONAL MEETINGS ON BIOLOGY

1 **Workshop on What do Nociceptors Tell the Brain?**
Organizers: C. Belmonte and F. Cerveró.

*2 **Workshop on DNA Structure and Protein Recognition.**
Organizers: A. Klug and J. A. Subirana.

*3 **Lecture Course on Palaeobiology: Preparing for the Twenty-First Century.**
Organizers: F. Álvarez and S. Conway Morris.

*4 **Workshop on the Past and the Future of Zea Mays.**
Organizers: B. Burr, L. Herrera-Estrella and P. Puigdomènech.

*5 **Workshop on Structure of the Major Histocompatibility Complex.**
Organizers: A. Arnaiz-Villena and P. Parham.

*6 **Workshop on Behavioural Mechanisms in Evolutionary Perspective.**
Organizers: P. Bateson and M. Gomendio.

*7 **Workshop on Transcription Initiation in Prokaryotes**
Organizers: M. Salas and L. B. Rothman-Denes.

*8 **Workshop on the Diversity of the Immunoglobulin Superfamily.**
Organizers: A. N. Barclay and J. Vives.

9 **Workshop on Control of Gene Expression in Yeast.**
Organizers: C. Gancedo and J. M. Gancedo.

*10 **Workshop on Engineering Plants Against Pests and Pathogens.**
Organizers: G. Bruening, F. García-Olmedo and F. Ponz.

11 **Lecture Course on Conservation and Use of Genetic Resources.**
Organizers: N. Jouve and M. Pérez de la Vega.

12 **Workshop on Reverse Genetics of Negative Stranded RNA Viruses.**
Organizers: G. W. Wertz and J. A. Melero.

*13 **Workshop on Approaches to Plant Hormone Action**
Organizers: J. Carbonell and R. L. Jones.

*14 **Workshop on Frontiers of Alzheimer Disease.**
Organizers: B. Frangione and J. Ávila.

*15 **Workshop on Signal Transduction by Growth Factor Receptors with Tyrosine Kinase Activity.**
Organizers: J. M. Mato and A. Ullrich.

16 **Workshop on Intra- and Extra-Cellular Signalling in Hematopoiesis.**
Organizers: E. Donnall Thomas and A. Grañena.

*17 **Workshop on Cell Recognition During Neuronal Development.**
Organizers: C. S. Goodman and F. Jiménez.

Instituto Juan March (Madrid)

18 **Workshop on Molecular Mechanisms of Macrophage Activation.**
Organizers: C. Nathan and A. Celada.

19 **Workshop on Viral Evasion of Host Defense Mechanisms.**
Organizers: M. B. Mathews and M. Esteban.

*20 **Workshop on Genomic Fingerprinting.**
Organizers: M. McClelland and X. Estivill.

21 **Workshop on DNA-Drug Interactions.**
Organizers: K. R. Fox and J. Portugal.

*22 **Workshop on Molecular Bases of Ion Channel Function.**
Organizers: R. W. Aldrich and J. López-Barneo.

*23 **Workshop on Molecular Biology and Ecology of Gene Transfer and Propagation Promoted by Plasmids.**
Organizers: C. M. Thomas, E. M. H. Willington, M. Espinosa and R. Díaz Orejas.

*24 **Workshop on Deterioration, Stability and Regeneration of the Brain During Normal Aging.**
Organizers: P. D. Coleman, F. Mora and M. Nieto-Sampedro.

25 **Workshop on Genetic Recombination and Defective Interfering Particles in RNA Viruses.**
Organizers: J. J. Bujarski, S. Schlesinger and J. Romero.

26 **Workshop on Cellular Interactions in the Early Development of the Nervous System of Drosophila.**
Organizers: J. Modolell and P. Simpson.

*27 **Workshop on Ras, Differentiation and Development.**
Organizers: J. Downward, E. Santos and D. Martín-Zanca.

28 **Workshop on Human and Experimental Skin Carcinogenesis.**
Organizers: A. J. P. Klein-Szanto and M. Quintanilla.

*29 **Workshop on the Biochemistry and Regulation of Programmed Cell Death.**
Organizers: J. A. Cidlowski, R. H. Horvitz, A. López-Rivas and C. Martínez-A.

*30 **Workshop on Resistance to Viral Infection.**
Organizers: L. Enjuanes and M. M. C. Lai.

31 **Workshop on Roles of Growth and Cell Survival Factors in Vertebrate Development.**
Organizers: M. C. Raff and F. de Pablo.

32 **Workshop on Chromatin Structure and Gene Expression.**
Organizers: F. Azorín, M. Beato and A. P. Wolffe.

33 **Workshop on Molecular Mechanisms of Synaptic Function.**
Organizers: J. Lerma and P. H. Seeburg.

34 **Workshop on Computational Approaches in the Analysis and Engineering of Proteins.**
Organizers: F. S. Avilés, M. Billeter and E. Querol.

35 **Workshop on Signal Transduction Pathways Essential for Yeast Morphogenesis and Cell Integrity.**
Organizers: M. Snyder and C. Nombela.

36 **Workshop on Flower Development.**
Organizers: E. Coen, Zs. Schwarz-Sommer and J. P. Beltrán.

37 **Workshop on Cellular and Molecular Mechanism in Behaviour.**
Organizers: M. Heisenberg and A. Ferrús.

38 **Workshop on Immunodeficiencies of Genetic Origin.**
Organizers: A. Fischer and A. Arnaiz-Villena.

39 **Workshop on Molecular Basis for Biodegradation of Pollutants.**
Organizers: K. N. Timmis and J. L. Ramos.

40 **Workshop on Nuclear Oncogenes and Transcription Factors in Hematopoietic Cells.**
Organizers: J. León and R. Eisenman.

Instituto Juan March (Madrid)

41 **Workshop on Three-Dimensional Structure of Biological Macromolecules.**
Organizers: T. L Blundell, M. Martínez-Ripoll, M. Rico and J. M. Mato.

42 **Workshop on Structure, Function and Controls in Microbial Division.**
Organizers: M. Vicente, L. Rothfield and J. A. Ayala.

43 **Workshop on Molecular Biology and Pathophysiology of Nitric Oxide.**
Organizers: S. Lamas and T. Michel.

44 **Workshop on Selective Gene Activation by Cell Type Specific Transcription Factors.**
Organizers: M. Karin, R. Di Lauro, P. Santisteban and J. L. Castrillo.

45 **Workshop on NK Cell Receptors and Recognition of the Major Histocompatibility Complex Antigens.**
Organizers: J. Strominger, L. Moretta and M. López-Botet.

46 **Workshop on Molecular Mechanisms Involved in Epithelial Cell Differentiation.**
Organizers: H. Beug, A. Zweibaum and F. X. Real.

47 **Workshop on Switching Transcription in Development.**
Organizers: B. Lewin, M. Beato and J. Modolell.

48 **Workshop on G-Proteins: Structural Features and Their Involvement in the Regulation of Cell Growth.**
Organizers: B. F. C. Clark and J. C. Lacal.

49 **Workshop on Transcriptional Regulation at a Distance.**
Organizers: W. Schaffner, V. de Lorenzo and J. Pérez-Martín.

50 **Workshop on From Transcript to Protein: mRNA Processing, Transport and Translation.**
Organizers: I. W. Mattaj, J. Ortín and J. Valcárcel.

51 **Workshop on Mechanisms of Expression and Function of MHC Class II Molecules.**
Organizers: B. Mach and A. Celada.

52 **Workshop on Enzymology of DNA-Strand Transfer Mechanisms.**
Organizers: E. Lanka and F. de la Cruz.

53 **Workshop on Vascular Endothelium and Regulation of Leukocyte Traffic.**
Organizers: T. A. Springer and M. O. de Landázuri.

54 **Workshop on Cytokines in Infectious Diseases.**
Organizers: A. Sher, M. Fresno and L. Rivas.

55 **Workshop on Molecular Biology of Skin and Skin Diseases.**
Organizers: D. R. Roop and J. L. Jorcano.

56 **Workshop on Programmed Cell Death in the Developing Nervous System.**
Organizers: R. W. Oppenheim, E. M. Johnson and J. X. Comella.

57 **Workshop on NF-κB/IκB Proteins. Their Role in Cell Growth, Differentiation and Development.**
Organizers: R. Bravo and P. S. Lazo.

58 **Workshop on Chromosome Behaviour: The Structure and Function of Telomeres and Centromeres.**
Organizers: B. J. Trask, C. Tyler-Smith, F. Azorín and A. Villasante.

59 **Workshop on RNA Viral Quasispecies.**
Organizers: S. Wain-Hobson, E. Domingo and C. López Galíndez.

60 **Workshop on Abscisic Acid Signal Transduction in Plants.**
Organizers: R. S. Quatrano and M. Pagès.

61 **Workshop on Oxygen Regulation of Ion Channels and Gene Expression.**
Organizers: E. K. Weir and J. López-Barneo.

62 **1996 Annual Report**

Instituto Juan March (Madrid)

63 **Workshop on TGF-β Signalling in Development and Cell Cycle Control.**
Organizers: J. Massagué and C. Bernabéu.

64 **Workshop on Novel Biocatalysts.**
Organizers: S. J. Benkovic and A. Ballesteros.

65 **Workshop on Signal Transduction in Neuronal Development and Recognition.**
Organizers: M. Barbacid and D. Pulido.

66 **Workshop on 100th Meeting: Biology at the Edge of the Next Century.**
Organizer: Centre for International Meetings on Biology, Madrid.

67 **Workshop on Membrane Fusion.**
Organizers: V. Malhotra and A. Velasco.

68 **Workshop on DNA Repair and Genome Instability.**
Organizers: T. Lindahl and C. Pueyo.

69 **Advanced course on Biochemistry and Molecular Biology of Non-Conventional Yeasts.**
Organizers: C. Gancedo, J. M. Siverio and J. M. Cregg.

70 **Workshop on Principles of Neural Integration.**
Organizers: C. D. Gilbert, G. Gasic and C. Acuña.

71 **Workshop on Programmed Gene Rearrangement: Site-Specific Recombination.**
Organizers: J. C. Alonso and N. D. F. Grindley.

72 **Workshop on Plant Morphogenesis.**
Organizers: M. Van Montagu and J. L. Micol.

73 **Workshop on Development and Evolution.**
Organizers: G. Morata and W. J. Gehring.

74 **Workshop on Plant Viroids and Viroid-Like Satellite RNAs from Plants, Animals and Fungi.**
Organizers: R. Flores and H. L. Sänger.

75 **1997 Annual Report.**

76 **Workshop on Initiation of Replication in Prokaryotic Extrachromosomal Elements.**
Organizers: M. Espinosa, R. Díaz-Orejas, D. K. Chattoraj and E. G. H. Wagner.

77 **Workshop on Mechanisms Involved in Visual Perception.**
Organizers: J. Cudeiro and A. M. Sillito.

78 **Workshop on Notch/Lin-12 Signalling.**
Organizers: A. Martínez Arias, J. Modolell and S. Campuzano.

79 **Workshop on Membrane Protein Insertion, Folding and Dynamics.**
Organizers: J. L. R. Arrondo, F. M. Goñi, B. De Kruijff and B. A. Wallace.

80 **Workshop on Plasmodesmata and Transport of Plant Viruses and Plant Macromolecules.**
Organizers: F. García-Arenal, K. J. Oparka and P.Palukaitis.

81 **Workshop on Cellular Regulatory Mechanisms: Choices, Time and Space.**
Organizers: P. Nurse and S. Moreno.

82 **Workshop on Wiring the Brain: Mechanisms that Control the Generation of Neural Specificity.**
Organizers: C. S. Goodman and R. Gallego.

---

*: Out of Stock.

Instituto Juan March (Madrid)

The Centre for International Meetings on Biology
was created within the
Instituto Juan March de Estudios e Investigaciones,
a private foundation specialized in scientific activities
which complements the cultural work
of the *Fundación Juan March*.

The Centre endeavours to actively and
sistematically promote cooperation among Spanish
and foreign scientists working in the field of Biology,
through the organization of Workshops, Lecture
and Experimental Courses, Seminars,
Symposia and the Juan March Lectures on Biology.

From 1989 through 1997, a
total of 109 meetings and 9
Juan March Lecture Cycles, all
dealing with a wide range of
subjects of biological interest,
were organized within the
scope of the Centre.

Instituto Juan March (Madrid)

Instituto Juan March de Estudios e Investigaciones
Castelló, 77 • 28006 Madrid (España)
Tel. 34 91 435 42 40 • Fax 34 91 576 34 20 • http://www.march.es

The lectures summarized in this publication were presented by their authors at a workshop held on the 11th through the 13th of June, 1998, at the Instituto Juan March.

All published articles are exact reproduction of author's text.

There is a limited edition of 400 copies of this volume, available free of charge.