Instituto Juan March
de Estudios e Investigaciones

108 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Integration of Transcriptional Regulation and Chromatin Structure

Organized by

J. T. Kadonaga, J. Ausió and E. Palacián

J. Ausió
M. Beato
M. E. Bianchi
A. Bird
P. Chambon
B. M. Emerson
L. Franco
S. M. Gasser
F. Grosveld
W. Hörz
K. A. Jones
J. T. Kadonaga
U. K. Laemmli
M. Levine
E. Palacián
D. Reinberg
R. G. Roeder
F. Sauer
R. Tjian
C. Wu
K. Yamamoto

Instituto Juan March
de Estudios e Investigaciones

108 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Integration of Transcriptional Regulation and Chromatin Structure

Organized by

J. T. Kadonaga, J. Ausió and E. Palacián

J. Ausió
M. Beato
M. E. Bianchi
A. Bird
P. Chambon
B. M. Emerson
L. Franco
S. M. Gasser
F. Grosveld
W. Hörz
K. A. Jones

J. T. Kadonaga
U. K. Laemmli
M. Levine
E. Palacián
D. Reinberg
R. G. Roeder
F. Sauer
R. Tjian
C. Wu
K. Yamamoto

The lectures summarized in this publication were presented by their authors at a workshop held on the 10th through the 12th of April, 2000, at the Instituto Juan March.

Depósito legal: M-22.139/2000
Impresión: Ediciones Peninsular. Tomelloso, 27. 28026 Madrid.

INDEX

PAGE

PAGE

Introduction

J. T. Kadonaga, J. Ausió and E. Palacián

The regulation of gene transcription is critical for the proper growth and development of an organism. In eukaryotes, there are tens of thousands of protein-coding genes, each of which has its own unique program of transcription. Someday, we may perhaps be able to decipher the underlying code in the DNA that directs the proper extent of transcription of each gene at the appropriate time and place. This code, in some respects, might be thought of as the transcriptional component of a gene expression code, and would represent a significant achievement in biology.

How might we, however, move forward toward the solution of this gene expression code? One reasonable approach, which is the subject of this Juan March Workshop, is to investigate the basic molecular mechanisms by which transcription is regulated in eukaryotes. The current evidence indicates that the regulation of transcription of protein-coding genes by RNA polymerase II involves the basal transcription machinery, sequence-specific DNA-binding proteins that interact with cis-control elements, numerous co-regulatory factors, and the structure and constitution of the chromatin template. In this Workshop, we have sought to encompass and to integrate all of these factors.

The many stimulating and fascinating talks and discussions at the Workshop have brought forth many current concepts. First, all of the factors that participate in the transcription process play an active role in the regulation of gene expression. Indeed, even the basal transcription factors and the chromatin template participate in gene-selective transcription. Second, the processes by which transcription is regulated are of immense complexity. Factors can alternatively act as activators or as repressors, depending on their context. Moreover, reversible chemical modifications of chromatin such as by methylation (of DNA or histones), acetylation, or ubiquitination can also variably affect gene expression. There are a multitude of pathways and mechanisms by which genes can be activated or repressed. Clearly, we should minimize our expectations and remain completely open-minded with regard to how genes might be regulated. Third, there is the question of how many more regulatory factors remain to be discovered? Have we found most of the relevant factors, or are there many others yet to be identified? Of course, the answers to these questions are, at present, a matter of opinion. Fourth, we can see the emergence of new approaches and tools for the analysis of chromatin structure and transcriptional regulation. Such new assays and techniques will lead to future advances and revolutions in our understanding of gene expression.

The entirety of the Workshop cannot be summarized in a short statement. The Fundación Juan March provided the ideal setting for both talks and discussions. It is our hope that each participant was able to leave with at least a small handful of new knowledge and insight.

Jim Kadonaga, Juan Ausió and Enrique Palacián

Session 1: Fundamental aspects of chromatin and transcription
Chair: Susan M. Gasser

Studies of basal transcription and chromatin assembly

Alan Kutach, Jennifer Butler, Patricia Willy, Dmitry Fyodorov, and James T. Kadonaga

Department of Biology, 0347, University of California, San Diego,
La Jolla, CA 92093-0347 USA

Biochemical and genetic analysis of the DPE. In our studies of basal transcription by RNA polymerase II, we have been focussing on the characterization of a novel core promoter element termed the DPE (for downstream promoter element). The DPE functions cooperatively with the initiator (Inr) to bind to TFIID and to direct accurate and efficient initiation of transcription in TATA-less promoters. Interestingly, the addition of a DPE motif at a downstream position can compensate for the loss of transcription that occurs upon mutation of an upstream TATA box. In addition, photo-affinity cross-linking experiments suggested that dTAFII60 and dTAFII40 interact with the DPE. Thus, the DPE is functionally analogous to the TATA box, as both elements are recognition sites for the binding of TFIID and are functionally interchangeable for basal transcription activity. I will describe recent studies of the sequences that can function as a DPE as well as the range of promoters that use the DPE as a core promoter element. These studies have revealed, somewhat surprisingly, that the DPE sequence motif is as common as the TATA box in Drosophila core promoters. I will also describe a genetic analysis of enhancer-core promoter specificity in Drosophila.

Chromatin assembly by ACF and dNAP-1. In a reaction consisting entirely of purified components, ACF and Drosophila NAP-1 (dNAP-1, a core histone chaperone) can mediate the deposition and assembly of core histones into periodic nucleosome arrays in an ATP-dependent process. ACF consists of Acf1 and ISWI polypeptides. ISWI is an ATPase that is also present in the NURF and CHRAC chromatin remodeling complexes. Acf1 is a novel protein that contains two PHD fingers, one bromodomain, and two new conserved regions. The Acf1 and ISWI subunits act synergistically in the assembly of chromatin. Thus, the Acf1 polypeptide confers additional functionality to the general motor activity of ISWI. Recent studies of functionally important subregions of Acf1 will be described.

Structural characterization of acetylated chromatin

Juan Ausió, Marta Garcia-Ramirez, LeAnn Howe*, Maya Iskandar, Mario Laszczak, Kenna Miskelly, Susan C. Moore, Corinne Rocchini and Xiaoying Wang

Department of Biochemistry and Microbiology, University of Victoria, British Columbia, Canada, V8W 3P6. (*) Present address: Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, 16802, USA

Despite the renewed interest in histone acetylation that has followed the genetic studies in yeast (1) and the identification of histone acetyltransferases as integral components of the transcriptional eukaryotic complexes (2), the precise structural role of this important post-translational histone modification still remains elusive. While the initial hypothesis was that this modification is responsible for weakening the histone-DNA interactions in a way that would lead to a more "open" chromatin conformation, it does not appear to be that simple.

At the nucleosome level the acetylated particle adopts a more asymmetric structure (3) which is mainly the result of the DNA ends flanking this chromatin particle binding less tightly to the histones and adopting a stretched conformation (4, 5). The acetylated histone tails also exhibit a significant (ca.10%) increase in their α-helical content (6). As the ionic strength of the medium increases, these acetylated histone tails are more readily released from their nucleosomal DNA interaction(s) (7) than their non-acetylated counterparts, as expected from the charge neutralization resulting from acetylation. However, under physiological ionic conditions, the histone tails are persistently bound (7) to the nucleosome regardless of the extent of acetylation. Thus, not surprisingly, the evidence in support of histone acetylation facilitating the binding of transcription factors to nucleosomally organized DNA has been very controversial (8-10).

At the chromatin fiber level, in the absence of linker histones, histone acetylation induces an extended chromatin conformation (5) which is more amenable to transcription. However, when the full complement of histones is present, the extent of folding of the fiber does not appear to be greatly affected by this post-translational modification (11,12). Importantly, we have found that under physiological ionic strength conditions the inter-chromatin fiber association which is characteristic of native chromatin is abolished by histone acetylation and the acetylated chromatin fiber exhibits an enhanced solubility (6). This decrease in the inter-nucleosome and inter-fiber association may play a very important role in facilitating the processes of transcriptional initiation and elongation within the nucleus.

References:

1. Grunstein, M. (1997) *Nature* **389**, 349-352.
2. Mizzen, C.A., and Allis, C.D. (1998) *Cell. Life Sci.* **54**, 6-20
3. Ausió, J., and van Holde, K.E. (1986) *Biochemistry* **22**, 1421-1428.
4. Norton, V.G., Imai, B.S., Yau, P., and Bradbury, E.M. (1989) *Cell* **57**, 449-457.
5. Garcia-Ramirez, M., Rocchini, C., and Ausió, J. (1995) *J. Biol. Chem.* **270**, 17923-17928.
6. Wang, X., Moore, S.C., Laszckzack, M., and Ausió, J. (2000) *Mol. Cell. Biol.* (submitted for publication).
7. Mutskov, V., Gerber, D., Angelov, J., Ausió, J., Workman, J., and Dimitrov, S. (1998) *Mol. Cell. Biol.* **18**, 6293-6304.
8. Lee, D.Y., Hayes, J.J., Pruss, D., and Wolffe, A.P. (1993) *Cell* **72**, 73-84.
9 Howe, L., and Ausió, J. (1998) *Mol. Cell. Biol.* **18**, 1156-1162.
10. Panetta, G., Buttinelli, M., Flaus A., Richmond, T.J., and Rhodes, D. (1998) *J. Mol. Biol.* **282**, 683-697.
11. McGhee, J.D., Nickol, J.M., Felsenfeld, G., and Rau, D.C. (1983) *Nucleic Acids Res.* **12**, 4065-4075.
12. Dimitrov, S. Makarov, V., Apostolova, T., and Pashev, I. (1986) *FEBS Lett.* **197**, 217-220.

Molecular genetic dissection of *Saccharomyces cerevisiae* TAF25p, an integral subunit of both the general transcription factor TFIID and the chromatin modifying transcriptional co-activator SAGA

P. Anthony Weil, Ed Klebanow, Brian Esau and Steven Sanders

Department of Molecular Physiology & Biophysics, Vanderbilt University, School of Medicine, Nashville, TN 37232-0615, USA
(Email: tony.weil@mcmail.vanderbilt.edu,
Ph# 615-322-7007; FAX 615-322-7236)

We have purified, characterized and cloned the genes encoding the components of the yeast TFIID complex. Yeast TFIID is comprised of TBP complexed with 14 distinct TBP Associated Factors or TAFs ranging in size from M_r=150,000 to M_r=17,000. Several of these TAFs are present in two moles/mole of TFIID. Interestingly, all of the TAFs which are present in this supra-stoichiometric amount (ie. 2/1) are also integral subunits of the Spt-Ada-Gcn5 Acetylation or SAGA transcriptional co-activator complex. The SAGA complex contains the Gcn5p protein which is capable of catalyzing the acetylation of nucleosomal histones and consequently stimulating transcription. One such TFIID/SAGA shared subunit is TAF25p. TAF25p is encoded by *TAF25*, a single copy essential yeast gene. We decided to use this gene as a reagent with which to try and dissect out TFIID-specific from SAGA-specific functionalities. Accordingly we have embarked upon a genetic dissection of the structure-function relationships of *TAF25*. Sequence alignments of yeast TAF25p with the homologous TAFs from human (hTAF$_{II}$30), fission yeast (*S. pombe*), plant (*A. thaliana*), worm (*C. elegans*), mouse (mTAF$_{II}$31), and *Drosophila* (dTAF$_{II}$30a/ß) indicate that only two blocks of TAF25p sequence (aa's 74-141 and aa's 180 to 208) are conserved between these disparate organisms. A truncated *S. cerevisiae TAF25* gene termed *TAF25^{mini}* comprised of the gene sequences encoding just those amino acids conserved between the disparate eukaryotic species described above was able to support growth of yeast when the expressed mini-TAF25p was the only form of TAF25p in the cell. This result argues that both the TFIID-specific and SAGA-specific functionalities of the molecule are resident in just the residual 50% of the molecule.

We have begun systematic alanine scanning mutagenesis of the remaining conserved portion of *TAF25^{mini}* targeting the 19 amino acids therein which are absolutely conserved between species. Our intention in this experiment was twofold; first to prove that the conserved region was mutationally sensitive and thus truly important for TAF25p function as would be predicted and second, to generate reagents that ultimately will be useful for dissecting out TFIID-specific from SAGA-specific functions of TAF25p. Mutation of two of these 19 aa's to alanine proved lethal; mutation of 86P and 194E to Alanine had no effect upon growth and hence TAF25p function while separate mutation of the other conserved residues (88I, 89P, 90D, 97L, 101G, 106D, 108R, 118Q, 119K, 123D, 188L, 184T, 195Y, 196G or 202P) to Alanine all conferred varying extents of temperature sensitive yeast cell growth. We are in the process of performing additional experimentation in order to use these genetic reagents to identify and characterize TFIID from SAGA functionalities of TAF25p. We are conducting similar experiments upon TAF40p, a TAFp unique to the yeast TFIID complex. It is our hope that by applying biochemical and genetic approaches to this problem we will be able to gain further insights into both the molecular mechanisms of transcriptional regulation and chromatin structure in eukaryotes.

Role of the TRAP/SMCC coactivator complex in the function of diverse activators

Robert G. Roeder, Mitsuhiro Ito, Sohail Malik and Chao-Xing Yuan

Lab. of Biochemistry &Molecular Biology. The Rockefeller University. New York, NY, USA

Studies of the thyroid hormone receptor (TR) have shown a ligand-dependent association with a novel thyroid receptor-associated protein (TRAP) complex and consequent target promoter activation dependent upon RXR and general positive cofactor (PCs) but not TBP-associated factors (TAFs)(1,2). TRAP220 has been identified as the subunit responsible for ligand-dependent interactions with TR, and similar interactions with other receptors have suggested a broader role for the TRAP complex in nuclear receptor function (3). The function of the TR-TRAP complex on DNA templates, as well as the uniqueness of TRAPs relative to nuclear receptor cofactors associated with chromatin remodeling, has suggested a multistep model for promoter activation through different groups of cofactors (2,3). More recent studies have shown virtual identity between the earlier-described TRAP complex and an SRB- and MED-containing cofactor complex called SMCC (4). SMCC was isolated on the basis of resident yeast Mediator homologues and shown to mediate activation by p53 and VP16, apparently through interactions with a distinct (TRAP80) subunit (4,5). These and other studies indicate that the TRAP/SMCC complex, like the distantly related yeast Mediator, is involved in the function of diverse activators, whereas the ability of TR and VP16 (or p53) to interact simultaneously with the complex provides a mechanism for activator synergy. Activator (TR) interactions with the TRAP complex also appear to stabilize TRAP association with RNA polymerase II. A TRAP220 knockout study in mice has revealed gene-selective TRAP220 functions in early development, and provided genetic confirmation of the role of TRAP220 in TR and VDR function in vitro (6). In a further analysis of the USA fraction that originally was found to be essential for activator function in vitro (7), the derived PC2 has been identified as a TRAP/SMCC subcomplex (8). Consistent with earlier demonstrations of a functional synergy between TRAP/SMCC and USA-derived positive cofactors (1,2,4,5), PC2 now has been shown to act synergistically with PC3 and PC4 in a system reconstituted with essentially homogeneous factors (8). A more complete TRAP/SMCC subcomplex (apparently lacking mainly SRB10 and SRB11) also has been found in the USA fraction and shown to act synergistically with PC3/PC4 (8). Hence, the potent USA coactivator activity appears to result from the action both of Mediator-like complexes and of "architectural" cofactors like PC1/PARP, PC3/TopoI, PC4 and PC52. These studies reflect a pleasing convergence of three distinct coactivator studies (on TRAP, SMCC and USA-derived positive cofactors) in this laboratory, as well as other studies of the yeast mediator and more recently-described mammalian mediator complexes.

References:

(1) J.D. Fondell, H. Ge and R.G. Roeder. Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex. Proc.Natl.Acad.Sci.USA 93: 8329-8333, 1996.

(2) J.D. Fondell, M. Guermah, S. Malik and R.G. Roeder. Thyroid receptor-associated proteins and general positive cofactors mediate thyroid receptor function in the absence of the TATA box-binding protein-associated factors of TFIID. Proc.Natl.Acad.Sci.USA, 96: 1959-1964, 1999.

(3) C.-X. Yuan, M. Ito, J.D. Fondell, Z.-Y. Fu and R.G. Roeder. The TRAP220 component of a thyroid hormone receptor-associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion. Proc. Natl. Acad. Sci. USA 95: 7939-7944, 1998.

(4) M. Ito, C.-X. Yuan, S. Malik, W. Gu, J.D. Fondell, S. Yamamura, Z.-Y. Fu, X. Zhang, J. Qin and R.G. Roeder. Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. Molecular Cell, 3: 361-370, 1999.
(5) W. Gu, S. Malik, M. Ito, C.-X. Yuan, J. D. Fondell, X. Zhang, E. Martinez, J. Qin and R.G. Roeder. A novel human SRB/MED-containing cofactor complex (SMCC) involved in transcription regulation. Molecular Cell 3: 97-108, 1999.
(6) M. Ito, C.-X. Yuan, H.J. Okano, R.B. Darnell and R.G. Roeder. Involvement of the TRAP220 component of the TRAP/SMCC coactivator complex in embryonic development, thyroid hormone receptor function and regulation within the pituitary-thyroid axis. Molecular Cell, in press, 2000.
(7) M. Meisterernst, A.L. Roy, H.M. Lieu and R.G. Roeder. Activation of class II gene transcription by regulatory factors is potentiated by a novel activity. Cell 66: 981-993, 1991.
(8) S. Malik, W. Gu, W. Wu, J. Qin, and R.G. Roeder. The USA-derived transcriptional coactivator PC2 is a submodule of TRAP/SMCC and acts synergistically with other PCs. Molecular Cell, in press, 2000.

Composition and activities of transcriptional regulatory complexes

Robert Nissen, Michael Cronin and Keith Yamamoto

Department of Cellular and Molecular Pharmacology, University of California, San Francisco, California 94143-0450, USA (yamamoto@cgl.ucsf.edu)

Multiprotein regulatory complexes are structurally dynamic, their composition governed by response elements, availability of regulatory factors, and physiologic status. This "mixed assembly" model for combinatorial regulation implies that DNA binding regulators, and their various coactivators and corepressors, interact flexibly to enable assembly into multiple final complexes, yet also specifically, to ensure precise assembly into the appropriate complex. Such integration of different contexts by intracellular receptors (IRs) yields distinct regulatory complexes.

For example, the thyroid hormone receptor (TR) represses transcription in the absence of hormone at the 'simple' response element, DR4, whereas it represses in the presence of hormone at 'tethering' AP1 elements. Hence, both response element and hormonal contexts operate together as determinants of the composition of receptor-containing regulatory complexes, and the resulting direction of regulation. From both classes of response elements, TR-mediated repression involved histone deacetylation, with likely consequent effects on chromatin structure. However, genetic evidence showed that the NcoR/SMRT corepressor requirement differed between the two complexes.

Glucocorticoid receptor (GR) similarly represses at AP1 and NFkappaB sites in the presence of hormone, largely accounting for the antiinflammatory effects of glucocorticoids. However, in contrast to the findings with TR, GR-mediated repression appears to be independent of histone deacetylation. Indeed, GR had no inhibitory effect on NfkappaB binding; more importantly, RNA pol II recruitment was maintained under repressing conditions.

Thus, different types of complexes confer repression by TR and GR at different response elements. We conclude that IRs house multiple potential regulatory surfaces that form differentially in response to signals, and the resulting complexes effect different mechanisms of regulation, some resulting in alterations in chromatin structure and others altering directly the functions of the initiation complex. Formation of such alternative surfaces contributes to the capacity of one receptor to specify multiple regulatory patterns in different physiologic, cellular and gene contexts.

References:

Lefstin, J.A., Yamamoto, K.R. (1998) Allosteric effects of DNA on transcriptional regulators. Nature, 392, 885-888.

Darimont, B.D., Wagner, R.L., Apriletti, J.W., Stallcup, M.R., Kushner, P.J., Baxter, J.D., Fletterick, R.L., Yamamoto, K.R. (1998) Structure and specificity of nuclear receptor-coactivator interactions. Genes Dev. 12, 3343-3356.

Yamamoto, K.R., Darimont, B.D., Wagner, R.L., Iniguez-Lluhi, J.A. (1998) Building transcriptional regulatory complexes: Signals and surfaces. Cold Spring Harb. Symp. Quant. Biol. 63, 587-598.

Session 2: Regulation of gene expression

Chair: Beverly M. Emerson

Control of transcription by retinoic acid receptors

Dilworth, F.J., Fromental-Ramain, C., Yamamoto, K. and Chambon, P.

IGBMC, CNRS/INSERM/ ULP/Collège de France, BP 163, 67404 Illkirch Cedex, C.U. de Strasbourg, France.

All-trans and *9-cis* retinoic acid (RA) signals are transduced by RAR/RXR heterodimers that act as functional units controlling the transcription of RA responsive genes (1-3 and refs therein). With the aim of elucidating the underlying molecular mechanisms, we have developed an *in vitro* transcription system using a chromatin template made up of a minimal promoter and a DR5-based RA response element (RARE). RARα and RXRα were expressed in and purified from baculovirus-infected *Sf9* cells, and transcription by a HeLa cell nuclear extract was carried out on naked DNA or "crude" chromatin assembled in vitro using a Drosophila embryo extract (4). Transcription from naked DNA was not affected by the presence of RA and/or RAR/RXR heterodimers. In contrast, very little transcription occurred from these "crude" chromatin templates in the absence of RA or RAR/RXR heterodimers, while their addition resulted in a dosage-dependent stimulation of transcription that never exceeded that occurring on naked DNA templates. Most importantly, the addition of synthetic agonistic or antagonistic retinoids to the chromatin transcription system mimicked their stimulatory or inhibitory action *in vivo*, and activation by a RXR-specific retinoid was subordinated to the binding of an agonist ligand to the RAR partner. Moreover, the addition of the p300 coactivator generated a synergistic enhancement of transcription.

However, we failed to show that remodeling of the "crude" chromatin templates was required to relieve nucleosomal repression. In contrast, using "purified" chromatin templates, we have recently demonstrated (5) that, irrespective of the presence of histone H1, both ATP-driven chromatin remodeling activities (6) and histone acetyltransferase (HAT) activities of coactivators (p300 and TIF2) (7) recruited by liganded receptors, are required to achieve transcriptional activation. In vitro DNA footprinting and chromatin immunoprecipitation (ChIP) analysis, together with "order of addition" experiments, indicate that coactivator HAT activities and two ATP-driven remodeling activities are sequentially involved at distinct steps preceding initiation of transcription. Thus, both ATP-driven chromatin remodeling and HAT activities act in a temporally-ordered and interdependent manner to alleviate the repressive effects of nucleosomal histones on transcription by RARα/RXRα heterodimers. Dissection of this transcription system and its reconstruction from pure components should ultimately lead to the elucidation of the molecular mechanisms by which RAR/RXR heterodimers control transcription from cognate chromatin templates in a ligand-dependent manner.

References:

(1) Chambon, P. (1996). A decade of molecular biology of retinoic acid receptors. FASEB J *10*, 940-954.

(2) Kastner, P., Mark, M., and Chambon, P. (1995). Nonsteroid nuclear receptors: What are genetic studies telling us about their role in real life? Cell *83*, 859-869.

(3) B. Mascrez, M. Mark, A. Dierich, N. Ghyselinck, P. Kastner, and P. Chambon : The RXRα ligand-dependent activation function 2 (AF-2) is important for mouse development. Development (1998) 125. 4691-4707.

(4) Dilworth, F. J., Fromental-Ramain, C., Remboutsika, E., Benecke, A., and Chambon, P. (1999). Ligand-dependent activation of transcription *in vitro* by retinoic acid receptor α / retinoid X receptor α heterodimers that mimics transactivation by retinoids *in vivo*. Proc Natl Acad Sci U S A *96*, 1995-2000.

(5) Dilworth, F.J., Fromental-Ramain, C., Yamamoto, K., and Chambon P: ATP-driven chromatin remodeling activities and coactivator histone acetyltransferases act sequentially during transcriptional activation by RAR/RXR heterodimers in vitro. submitted.
(6) Brown, C. E., Lechner, I., Howe, I., and Workman, J. L. (2000). The many HATs of transcription coactivators. Trends Biochem Sci *25*, 15-19.
(7) Kingston, R. E., and Narlikar, G. J. (1999). ATP-dependent remodeling and acetylation as regulators of chromatin fluidity. Genes Dev *13*, 2339-2352.

Ancillary role of NF1 in activation of the MMTV promoter by steroid hormone receptors

R. Koop, L. Di Croce, F. Prado and M. Beato

I.M.T., Philipps-Universität, E.-Mannkopff-Str. 2,
D-35037 Marburg, Germany

Hormonal induction of the MMTV promoter is mediated by a regulatory unit including among other regulatory sequences five binding sites for steroid hormone receptors, upstream of a binding site for the transcription factor NF1. Induction requires the integrity of these *cis*-acting elements, but the corresponding factors do not synergize on free DNA. *In vivo* and in reconstituted mononucleosomes, the promoter is located on a phased nucleosome, which allows binding of the hormone receptors to only two of their five cognate sites and precludes binding of NF1. Hormone treatment results in rapid simultaneous occupancy of all five receptors binding sites and the NF1 site on the surface of a nucleosome-like particle. In *S. cerevisiae* and in *Drosophila* embryo extracts the functional synergism between hormone receptors and NF1 depends on positioned nucleosomes, but does not require the proline-rich transactivation functions of NF1. In a different promoter context, these transactivation functions synergize with hormone receptors in yeast. In *Drosophila* extracts promoter bound hormone receptors recruit the NURF complex, which remodels the MMTV chromatin and facilitates NF1 binding. Binding of NF1 stabilizes an open nucleosome conformation by precluding its folding back to the closed conformation. This favors full receptor binding and the appearance of a nuclease hypersensitive site in yeast chromatin. Our findings show that positioned nucleosomes account for constitutive repression and participate in hormonal induction by mediating the reciprocal synergism between receptors and NF1, in a process involving ATP-dependent nucleosome remodeling.

ATP-driven chromatin remodeling complexes

C.Wu

Lab. of Molecular Cell Biology, NIH, Bethesda, MD 20892

The packaging of eukaryotic DNA in nucleosomes and the condensation and folding of nucleosome arrays in chromatin create barriers that restrict access to the genome. Our laboratory investigates how chromatin is unraveled to allow entry by the enzymes that transcribe, replicate or repair DNA. We have purified and characterized a four-subunit protein complex from Drosophila designated Nucleosome Remodeling Factor (NURF), which acts in an Atp-dependent manner to facilitate the sliding of histone octamers. NURF consists of 4 subunits: ISWI, the ATP-hydrolyzing engine which is also found in two other Drosophila complexes ACF and CHRAC, NURF-55, a WD-repeat protein shared with several chromatin modifying enzymes, NURF-38 inorganic pyrophosphatase, and a very large, novel polypeptide of 301K. The contributions of each NURF subunit and the mechanism of chromatin remodeling will be discussed. We have also purified a multiprotein ATP-dependent chromatin remodeling complex from yeast, called the ARI1 complex (ATPase Related to ISWI), which is involved in both transcription and DNA processing. Genetic and biochemical studies of the ARI1 complex and its multiple subunits implicate this complex in DNA replication, recombination or repair.

References:

Martinez-Balbas, M.A., Tsukiyama, T., Gdula, D., and Wu, C. Drosophila NURF-55, a WD repeat protein involved in histone metabolism. *Proc. Natl. Acad. Sci. USA* 95: 132-137 (1998).

Gdula, D., Sandaltzopoulos, R. Tsukiyama, T., Ossipow, V., and Wu, C. Inorganic Pyrophosphatase is a component of the Drosophila Nucleosome Remodeling Factor Complex. *Genes Dev.* 12:3206-3216 (1998).

Wu, C., Tsukiyama, T., Gdula, D., Georgel, P., Martinez-Balbás, M., Mizuguchi, G., Ossipow, V., Sandaltzopoulos, R., Wang, H.-M. ATP-Dependent Remodeling of Chromatin for Transcription. *Cold Spring Harbor Symp. Quant. Biol.* 63:525-534 (1998).

Tsukiyama, T., Palmer, J., Landel, C.C., Shiloach, J., and Wu, C. Characterization of ISWI subfamily of ATP-dependent chromatin remodeling factors in *S. cerevisiae*. *Genes Dev.* 13: 686-697 (1999).

Hamiche, A., Sandaltzopoulos, R., Gdula, D.A., Wu, C. ATP-Dependent Histone Octamer Sliding Mediated by the Chromatin Remodeling Complex NURF. *Cell* 97: 833-842 (1999).

Deuring, R., Fanti, L., Armstrong, J.A., Sarte, M., Papoulas, O., Prestel, M., Daubresse, G., Verardo, M., Moseley, S., Berloco, M., Tsukiyama, T., Wu, C., Pimpinelli, .S., Tamkun, J.W. The ISWI chromatin remodeling protein is required for gene expression and the maintenance of higher order chromatin structure in vivo. *Molec. Cell* (in press).

Chromatin-specific Trans-Activation by the LEF-1:Beta-catenin Complex.

Anton Tutter, Christy J. Fryer, Glenn McAlpine, and Katherine A. Jones.
The Salk Institute for Biological Studies, 10010 North Torrey Pines Road
La Jolla, CA 92037 USA

Lymphoid enhancer-binding factor (LEF-1) and the closely related T-cell factor (TCF) proteins are high-mobility group (HMG) proteins that are expressed broadly at early stages in development but restricted to lymphoid cell lineages in adults. The ability of LEF-1 and the closely related T-cell factor (TCF) proteins to bend DNA strongly lead to their classification as architectural transcription factors. In T cells, the LEF-1/TCF proteins function as potent but very context-restricted activators of lymphoid-specific genes, and LEF-1 strongly activates the T cell receptor (alpha-chain) and HIV-1 enhancers. Although we and others originally cloned the LEF-1/TCF proteins as lymphoid-selective transcriptional activators, recent studies have shown that these factors play a more ubiquitous role in development as heterodimeric partners for beta-catenin upon activation of the Wnt/Wg signaling pathway. Wnt/Wg signaling specifies important cell-fate choices during embryonic development, including the establishment of segment polarity in *Drosophila* and dorsal-ventral axis patterning in *Xenopus*. In the absence of Wnt signaling, the LEF-1/TCF factors are either transcriptionally inactive or actively repress Wnt/*Wg*-responsive genes. Aberrant activation of LEF-1:β-catenin complex is also strongly implicated in the etiology of colon carcinoma, melanoma and skin tumors.

Our previous studies to examine the mechanisms of transcription activation by LEF-1 in vitro revealed an essential role for chromatin. LEF-1 is unable to stimulate transcription on its own, but can activate the HIV-1 or TCRα enhancers synergistically with other factors, including NF-κB, CREB, AML-1, and Ets-1. T-cell enhancer activation requires the context-dependent activation domain (CAD) of LEF-1 as well as the HMG domain, and LEF-1 was found to bind co-operatively with AML1:Ets-1 to the TCRα enhancer on chromatin, but not nonchromatin, templates. Recently, we extended these studies to analyze the mechanism of trans-activation by the LEF-1: β-catenin complex. Recombinant beta-catenin strongly enhances LEF-1 activation in a chromatin-dependent manner, and also regulates the binding of LEF-1 to chromatin templates. The N-terminus of LEF-1, which binds beta-catenin, and the HMG domain are necessary and sufficient for activation in vitro, and the CAD is not required. Our studies inidicate that P-300 is essential for beta-catenin activation, and we have identified a region of beta-catenin that behaves as a strong dominant negative inhibitor of trans-activation in vitro. Studies are underway to characterize co-activators that may interact directly with beta-catenin to mediate transcription activation in this chromatin system.

Chromatin, cell cycle and ß-globin gene transcription

Frank Grosveld

Dept. of Cell Biology and Genetics, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR, Rotterdam, The Netherlands.

The Locus Control Region (LCR) is required for the activation of all of the human β-like globin genes during development. The early embryonic developmental programme is executed in nucleated primitive red blood cells that express the ε and γ genes. The foetal/adult programme takes place in a definitive red blood cells that first express the γ and later the δ- and β genes. This difference in expression programme correlates with changes in the chromatin structure within the β globin locus. Repression of the early genes (ε and γ) in late cells is achieved by as yet unknown factors acting on sequences flanking these genes. Superimposed on this is a mechanism in which the early genes (ε and γ) suppress the late genes (δ and ß) by competition for the interaction with the LCR. In particular the latter mechanism has allowed a series of studies to examine the transcriptional process at the level of the single cell. These studies indicate that the LCR interacts with individual globin genes and that LCR/gene interactions are dynamic with complexes forming and dissociating continually. The levels of expression of each of the genes appear to depend on: (1) the frequency of interaction which is itself dependent on the distance of the gene to the LCR, (2) the affinity/stability of the LCR/gene complex which is dependent on the balance of transcription factors such as EKLF. When individual hypersensitive region in the LCR are deleted from a complete transgenic locus that is integrated in a pericentromeric region, the locus becomes sensitive to two types of position effects. One of these is classical Position Effect Variagation (PEV) while the other is a Timing Position Effect (TPE). The PEV can be modified by an increase in the concentration of EKLF which acts on the LCR and results in more cells expressing the locus, which is accompanied by a general increase in DNAse sensitivity throughout the locus. TPE is dependent on the cell cycle resulting in a limited period of expression in of all the red cells. Examination of single cells shows that both types of position effects involve the relocalisation of the transgenic locus in the nucleus. The implications of these experiments for the role of the LCR in the activation of the locus will be discussed.

An important difference between primitive and definitive erythroid cells is the number of cell divisions (definitive cells proliferate more). This difference appears to be related to the presence of GATA1 in definitive cells. This transcription factor (which is essential for definitive erythropoiesis) is known to be important for the activation of a number of erythroid genes including the β-globin locus. However overexpression of this factor in transgenic mice show it to be important in the regulation of the cell cycle and in particular the balance between the proliferation and differentiation of red cells. A putative mechanism of the multiple functions of GATA1 will be presented.

Session 3: Remodeling and analysis of chromatin structure
Chair: Enrique Palacián

Selective gene regulation by chromatin remodeling complexes *in vitro*

Shilpa Kadam, Rajesh Bagga, and Beverly M. Emerson

The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037 USA

Cellular specialization is controlled by the precise action of tissue- and stage-specific transcription factors. We are particularly focusing on the processes by which gene switching and long-range communication between DNA elements within a chromosomal locus are established. A critical aspect of this regulation is the ability of chromatin remodeling complexes or enzymatic machinery to facilitate the interaction of transcription factors with their target genes when packaged into nucleosomal structures. A variety of multi-subunit protein complexes have been described that disrupt chromatin structure and promote protein-DNA interaction, yet the basis for functional selectivity of particular remodeling complexes for specific genes is poorly understood.

Using chromatin-assembled human ß–globin genes, we have shown that promoter "opening" by nucleosomal disruption is dependent upon the interaction of an erythroid-specific DNA binding protein, Erythroid Krüppel-like factor (EKLF), with a CACC box at –90. This critical step, which is required for transcriptional activation, occurs only when EKLF acts in combination with a member of the mammalian SWI/SNF family of ATP-dependent chromatin remodeling complexes. Thus, developmentally regulated human ß-globin promoter remodeling and transcriptional activation is a SWI/SNF-dependent process in vitro. To analyze the functional specificity of mammalian SWI/SNF, we examined its ability to facilitate chromatin remodeling and transcriptional activation using a variety of DNA-binding transcription factors on nucleosome-assembled ß-globin and HIV-1 promoters. We find that SWI/SNF is highly selective for the class of transcription factor that it functions with. The basis of this functional selectivity has been examined. We find that mammalian SWI/SNF interacts directly with EKLF and the region of interaction has been mapped to the zinc finger DNA-binding domain. Other zinc finger-containing proteins, such as GATA-1 and Sp1, also interact directly with mammalian SWI/SNF and function in chromatin remodeling and transcriptional activation. By contrast, TFE-3 and NF-kB do not contain zinc finger domains and fail to interact with SWI/SNF. The DNA-binding domain alone is sufficient for SWI/SNF to function in targeted chromatin remodeling and the minimal recombinant SWI/SNF subunits required for this specificity have been defined.

References:

Armstrong, J. A. , Bieker, J. J. and Emerson, B. M. A SWI/SNF-related chromatin remodeling complex, E-RC1, is required for tissue-specific transcriptional regulation by EKLF in vitro. *Cell* 95, 93-104 (1998).

Armstrong, J. A. and Emerson, B. M. Transcription of chromatin: these are complex times. *Curr. Opin. Genet. Dev.* 8, 165-172 (1998).

The transglutaminase reaction as a probe for nucleosome structure and dynamics

Luis Franco, Concepción Abad, Manuel Boix-Chornet and Esteban Ballestar

Department of Biochemistry and Molecular Biology. University of Valencia. E-46100 BURJASSOT (Valencia), SPAIN

Transglutaminases (TGases; EC 2.3.2.13) constitute a family of enzymes that catalyse a calcium-dependent acyl transfer reaction from the (-carboxamide group of a peptide-bound glutamine to various primary amines, most commonly the ,-amino group of a lysine or one of the primary amino groups of a polyamine, although the enzyme can also use some non-physiological amines. In the former instance, the reaction yields dimers or oligomers produced by the cross-linking of the protein substrate(s) and this obviously requires a protein molecule acting as glutaminyl substrate and a second one acting as lysyl substrate. It is also possible to obtain cross-linked products in which both protein molecules act as glutaminyl substrates, if a polyamine acts as a bridge between them (Chen and Metha, 1999).

The possibility of using the TGase-catalysed reaction to analyse protein structure and organization was foreseen by Folk (1980), although he noted that a limitation of this approach would be the failure of many proteins to act as TGase substrates. We have found that core histones are good glutaminyl substrates and that out of the 16 glutamines of the four histones, 9 (namely Gln^{95} of H2B; Gln^{5}, Gln^{19} and Gln^{125} of H3; Gln^{27} and Gln^{93} of H4; and Gln^{24}, Gln^{104} and Gln^{112} of H2A) are the amine acceptors in free histones (Ballestar *et al.*, 1996). When native nucleosomes are used as TGase substrates with monodansylcadaverine (DNC) as amine donor, only Gln^{5}, Gln^{19} of H3, which are located in the N-terminal tail, and Gln^{22} of H2B are dansylated. The latter residue is not modified in the free histone (Ballestar *et al.*, 1996) and we have shown that the interaction of the adjacent lysines with DNA results in the reactivity of H2B Gln^{22} in core particles. Taking advantage of this fact, we have used the specificity of the TGase reaction to study the changes induced by increasing ionic strength in the interaction between the histone N-terminal tails and nucleosomal DNA (Ballestar & Franco, 1997). This topic was also investigated by a different approach. We prepared reconstituted nucleosome core particles containing either H2B modified with DNC by the TGase reaction at Gln^{22} or H3 modified with the same procedure at Gln^{5} and Gln^{19} The dissociation of the histone tails was then followed by the decrease of the fluorescence anisotropy of the probe. These methods allowed us to describe the ionic strength-dependent structural transitions of the histone tails (Ballestar & Franco, 1997).

We also studied the influence of ionic strength changes (in the 0.2-2.0 M range) on the TGase-catalysed modification of glutamine residues of the nucleosome cores, in an effort to cast some light on the nature of the salt-induced conformational transitions of the core particle. The partial unfolding that occurs in going to higher ionic strength values results in an increase in the number of reactive glutamines up to a maximum value of 16 per nucleosome. Labelling of some residues (*e. g.*, Gln^{104} and Gln^{112} of H2A) requires the unwinding of DNA and the dissociation of the H2A-H2B dimers. Gln^{76} of H3, which is not a substrate in the free histone, is labelled in the native structure of the tetramer only when the H2A-H2B dimers are dissociated. These results are easily interpreted in terms of the current data on the structure of nucleosomes (Luger

et al., 1997). The reactivity increase of Gln^{95} of H2B occurs as DNA unwinds, indicating that the presence of DNA constitutes the only obstacle to the reactivity of this residue. These data are discussed in the light of the current models for DNA unwinding (Ballestar *et al.*, 2000). Some of the above conclusions were also reached by using core particles assembled with histones dansylated at specific glutamines. The TGase reaction can therefore be used as a probe to study the dynamics of conformational changes in nucleosomes.

Finally, the possibility that core histones were TGase substrates *in vivo* is discussed taking into account their capability to act *in vitro* both as glutaminyl and lysyl substrates (Ballestar *et al.*, 1996; Ballestar & Franco, 2000), as well as in view of some other data found by several authors (Cooper *et al.*, 1999; Lesort *et al.*, 1998; Piredda *et al.*, 1999).

References:

Ballestar, E., Abad, C., & Franco, L. (1996) Core histones are glutaminyl substrates for tissue transglutaminase. *J. Biol. Chem.* **271**, 18817-18824.

Ballestar, E. & Franco, L. (1997) Use of the transglutaminase reaction to study the dissociation of histone N-terminal tails from DNA in nucleosome core particles. *Biochemistry* **36**, 5963-5969.

Ballestar, E., Boix-Chornet, M. & Franco, L. (2000) Study of the ionic strength-induced conformational changes in the nucleosome followed by the selective accessibility of histone glutamines in the transglutaminase reaction (manuscript submitted).

Chen, J. S. K. & Mehta, K. (1999). Tissue transglutaminase: an enzyme with a split personality. *Int. J. Biochem. Cell Biol.* **31**, 817-836.

Cooper, A. J. L., Sheu, K.-F. R., Burke, J. R., Strittmatter, W. J., Gentile, V., Peluso, G. & Blass, J. P. (1999) Pathogenesis of inclusion bodies in $(CAG)_n/Q_n$-expansion diseases with special reference to the role of tissue transglutaminase and to selective vulnerability. *J. Neurochem.* **72**, 889-899.

Folk, J.E. (1980) Transglutaminases. *Annu. Rev. Biochem.* **49**, 517-531.

Lesort, M., Attanavanich, K., Zhang, J. & Johnson, G. V. W. (1998) Distinct nuclear localization and activity of tissue transglutaminase. *J. Biol. Chem.* **273**, 11991-11994.

Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F., & Richmond, T. J. (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251-260.

Piredda, L., Farrace, M.G., Lo Bello, M., Malorni, W., Melino, G., Petruzzelli, R. & Piacentini, M. (1999) Identification of 'tissue' transglutaminase binding proteins in neural cells committed to apoptosis. *FASEB J.* **13**, 355-364.

Transcription and chromatin

George Orphanides, Gary LeRoy, Alejandra Loyola, Sangtaek Oh, Jae-Hyun Kim, Helen Cho and Danny Reinberg

Howard Hughes Medical Institute, Division of Nucleic Acid Enzymology, Department of Biochemistry, Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA

The machinery that transcribes protein coding genes in eukaryotic cells must contend with repressive chromatin structures in order to find its target DNA sequences. Mechanisms that decompact chromatin to facilitate access to DNA have been studied for transcription of protein-coding genes by RNA polymerase II (RNAP II), a process that requires rapid access to genes for the response to environmental signals and programmed cellular events, but apply to any process requiring interaction with DNA.

A diverse array of proteins modify the structure of chromatin at gene promoters to help transcriptional regulatory proteins access their DNA recognition sites. The way in which disruption of chromatin structure at a promoter is transmitted through an entire gene has not been defined. Recent studies suggest that the passage of an RNA polymerase II through a gene trigger mechanisms that propagate the breakdown of chromatin. Regions of the genome that are actively transcribed have more open and accessible chromatin structures than non-transcribed regions. Transcriptionally-active, accessible regions have been associated with a loss of structural proteins that are involved in the maintenance of higher-order chromatin structure. For example, histone H1, which binds to nucleosomes and promotes chromatin folding, is depleted in transcribed chromatin. A closer look at the chromatin of transcribed genes reveals alterations in histone proteins. Acetylation of lysine residues in the N-terminal "tails" of histones has long been correlated with transcriptional competence. A good example of this phenomenon is found at the transcriptionally-active β-globin locus of chicken erythrocytes, which contains 33 kilobases of accessible chromatin and is enriched in acetylated histones. Structurally, histone tail acetylation disrupts histone-DNA and inter-nucleosomal interactions by neutralizing positively-charged lysine residues. The enzymes that catalyze these covalent modifications are the histone acetyltransferases (HATs) and histone deacetylases (HDACs), which add or remove an acetyl group, respectively. The recent finding that many proteins that regulate transcription are, or can recruit, HATs and HDACs has reinforced the link between histone acetylation/deacetylation and gene activity.

Another class of factors that manipulate chromatin structure and have roles in transcriptional regulation are the chromatin remodeling complexes, which use energy from ATP hydrolysis to disrupt chromatin and make DNA more accessible. Chromatin remodeling enzymes have been purified from a variety of organisms, and most cells contain more than one type of complex. The different complexes contain structurally-related catalytic subunits, but differ in the way in which they manipulate chromatin. Most of these enzymes can alter the conformation of a nucleosome to increase DNA access, and one complex, RSC, can transfer an entire octamer of histone proteins from one region of DNA to another. It is likely that chromatin remodeling and HAT activities cooperate to overcome the transcriptional repression imposed by chromatin packaging.

Disruption of histone-DNA contacts by acetylation of histones or by chromatin remodeling allows DNA-binding proteins to compete with histones for DNA. Does this simple

paradigm also apply to elongation? Will weakening of histone-DNA contacts in a nucleosome allow its passage by an RNA polymerase? Theoretically, transcription elongation through a nucleosome is much more challenging than the binding of a DNA-binding protein to chromatin, as it requires transient disruption of histone-DNA contacts throughout the 147 bp of nucleosomal DNA, and not just over a short region. Initial experiments examining how an RNA polymerase copes with a nucleosome established that during elongation the entire octamer of histone proteins is transferred backwards on the DNA fragment through a transiently-formed DNA loop. The observation that the histone octamer does not leave the DNA throughout the elongation process is consistent with experiments suggesting that histones remain associated with the DNA of genes being transcribed in the cell. Although these studies have provided useful mechanistic insights, the templates used contained only a single nucleosome and, therefore, are not subject to repressive inter-nucleosome interactions that occur in natural chromatin.

RNAP II in a cell travels at a rate of 25 nucleotides/second, an elongation rate that can only be approached in vitro on free DNA templates. How does the polymerase achieve these rates in the seemingly repressive context of chromatin? This question has prompted researchers to seek conditions that will facilitate transcription elongation in a chromatin environment in vitro. Although different factors that can enhance transcription elongation through chromatin in vitro have been described, it is not clear which of these, if any, perform this role in a cell. If factors play a role in this process in a cell, how are they likely to be targeted to transcribed regions of the genome? It is well established that activities that modify chromatin structure can be recruited to promoter regions to facilitate transcription initiation through direct interactions with DNA-bound activator proteins, but how are they directed to downstream regions to facilitate elongation? One way in which these activities could gain access to the entire transcribed region is by hitching a ride on the polymerase as it travels on its journey through a gene. In this way, the chromatin modifier would gain access to, and promote the disruption of, the entire transcribed region.

For this targeting mechanism to be effective, the modifying activities must be able to specifically recognize and bind to polymerases that are in the act of elongation, and not to polymerases that are free in the nucleus or are at the promoter. It is likely that the "tag" that distinguishes an elongating polymerase is phosphorylation of its CTD tail. The first evidence that a chromatin remodeling activity can specifically recognize and bind to an elongating polymerase was the finding that the PCAF HAT, which acetylates histones H3 and H4 in a nucleosome, binds specifically to the phosphorylated, elongating form of the RNAP II. More recently, the Svejstrup laboratory has taken a biochemical approach to characterize the composition of the elongating S. cerevisiae RNAP II complex. This resulted in the isolation of a heterotrimeric complex, termed elongator, that associates only with the phosphorylated, elongating form of RNAP II and can also be found in a free form. The 60 kDa subunit of the elongator is the Elp3 protein that contains HAT activity towards all four histones. These characteristics of elongator suggest that it is recruited to elongating RNAP II in order to acetylate the histones of transcribed chromatin. Genetic experiments reveal that the elongator complex is dispensable for yeast survival, yet has an important function in the induction of certain genes.

The binding of a transcriptional activator protein to DNA can result in the ordered recruitment of chromatin remodeling and HAT complexes to a promoter to facilitate transcription initiation. Recent studies suggest that FACT, in addition to directly facilitating elongation through chromatin, may play a central role in the sequential recruitment of chromatin modifying activities during elongation. A hunt for proteins that can bind specifically to the largest subunit of FACT resulted in the identification of SAS3, the catalytic component of the yeast NuA3 HAT complex (R. Sternglanz and J Workman, pers. com.). This protein-protein

interaction suggests that FACT can recruit a further HAT activity to transcribed chromatin regions. Consistent with this proposal, NuA3 differs from other yeast HAT complexes in its inability to be recruited to chromatin by DNA-binding transcriptional activator proteins, suggesting that it is instead directed to chromatin by FACT. Via its SSRP1 subunit, FACT also interacts specifically with CHD1, a member of the SNF2 family of proteins that form the catalytic subunits of chromatin remodeling complexes. This observation raises the intriguing possibility that FACT recruits a CHD1-containing remodeling complex to facilitate elongation through chromatin. Through these interactions, FACT may play a pivotal role in a cascade of events that begins with the acetylation of histones by HATs that travel with RNAP II and culminates in the sequential recruitment of activities that unravel chromatin structure. Each event in the cascade would result in an increase in chromatin accessibility until the progress of RNAP II is completely unhindered.

The overall picture that emerges is one in which the modification of chromatin by HATs that track with RNAP II leads to derepression of an entire transcription unit. This model also implies a mechanism for the way in which active transcription can be turned off. Competition with histone deacetylases (HDACs) free in the nucleus means that maintaining histones in an acetylated state requires constant transcription, a proposal supported by experiments showing that the establishment of an unfolded chromatin domain in vivo requires transcription elongation and histone acetylation. The state of histone tail acetylation is a dynamic equilibrium determined by the activities of HATs bound to elongating RNAP II and HDACs. Once the RNAP II traffic along a gene is decreased - governed by signals at the promoter - the equilibrium shifts in favor of the HDACs. Loss of histone tail acetylation would then result in the rapid conversion of chromatin structure to a repressed conformation.

A plethora of evidence suggests that transcription elongation has a direct role in decompacting chromatin in transcriptionally active regions. However, the part played by elongation in the overall decompaction of the template is uncertain. Eukaryotic chromosomes are mosaics of accessible, transcriptionally-active domains and compacted, transcriptionally-silent regions. Two types of DNA sequences are involved in establishing these domains. Enhancer regions contain multiple binding sites for transcriptional activators and can promoter the general decompaction of chromatin over large regions encompassing several genes. Insulator regions, found at the boundaries of uncompacted and compacted chromatin domains, antagonize enhancer function by blocking the propagation of enhancer-driven chromatin disruption. It is likely that acetylation of large chromatin domains promoted by activators bound at enhancers is the first step in the decompaction of transcriptionally-active chromatin. This may result in partial chromatin disruption, leading to the initiation of transcription. Elongating RNAP II and its associated chromatin modifiers would then propagate this disruption.

Activities such as chromatin remodeling complexes, HATs and FACT, can disrupt chromatin structure at the level of the nucleosome to facilitate transcription elongation in vitro. However, the nucleosome is only the first level in chromatin compaction. The degree of DNA compaction faced by the elongating RNAP II in a cell is not known and, therefore, it is unclear whether these activities are sufficient for elongation through chromatin in vivo. Two scenarios, that differ in the extent of chromatin decompaction which occurs following the binding of activators to enhancers and promoters, are possible. In one scenario, the recruitment of chromatin-modifying activities by activators results in complete decompaction of chromatin surrounding the activator binding sites and only partial decompaction elsewhere in the gene. In this case, elongating RNAP II faces a compacted chromatin template. In an alternative model, activators promote decompaction of chromatin over the entire gene. In this case, the elongating RNAP II would find partially-decompacted nucleosomes in its path.

Functions of the TBP-related factor TRF-1

Robert Tjian, Michael Holmes and Shinako Takada

Howard Hughes Medical Institute, University of California, Berkeley. Molecular and Cell Biology Department, 401 Barker Hall. Berkeley, CA 94720-3204

The TATA-binding protein (TBP)-related factor 1 (TRF) is expressed in a tissue-restricted fashion during *Drosophila* embryogenesis and may serve as a promoter specific recognition factor that can replace TBP in regulating transcription. However, bona fide RNA polymerase II target promoters that would preferentially respond to TRF1, but not TBP, remained elusive. We recently employed several complementary methods, including polytene chromosome staining, chromatin immunopreciptitation, microarray gene expression profiling, and transient co-transfection assays, to identify the *Drosophila* gene tudor as containing a TRF1-responsive promoter. Reconstituted in vitro transcription reactions and Dnase I footprinting assays confirmed the ability of TRF1 to bind preferentially to a TC-box element and direct transcription of the tudor gene from an alternate promoter. These studies suggest that metazoans have evolved multiple gene selective and tissue specific components of the core transcription machinery to regulate gene expression.

Having obtained evidence for the role of TRF-1 in mediating RANA pol II transcription, we next turned to its potential involvement in RNA pol III transcription. Co-immunoprecipitation experiments revealed that *Drosophila* TRF1 can form a complex with a newly identified BRF molecule that is active in directing the transcription of tRNA, U6 RNA and 5S RNA by RNA polymerase III. Surprisingly, the bulk of BRF in Drosophila cells appears to be associated with TRF1 rather than TBP and these two transcription factors co-localize at multiple polytene chromosome sites containing RNA pol III genes. Depletion of either TRF1 or BRF from cell extracts severely impairs transcription whereas removal of TBP had no detectable effect on transcription of RNA pol III genes. Supplementing TRF1 or BRF depleted extracts with purified recombinant TRF1:BRF complex efficiently restores transcription. These data suggest that in Drosophila, the TRF1:BRF rather than a TBP:BRF complex plays a major role in regulating RNA pol III transcription. Thus, our studies provide evidence that TRF1, like TBP may be responsible for directing the transcription of both RNA Polymerase II and III promoters.

Session 4: Chromatin structure and transcriptional regulation
Chair: Juan Ausió

Functional studies of proteins that bind to methylated DNA

Brian Hendrich, Huck-Hui Ng, Jacky Guy and Adrian Bird

Institute of Cell and Molecular Biology, University of Edinburgh, The King's Buildings, Edinburgh EH9 3JR, U.K.

Proteins that bind to methylated DNA are likely mediators of the biological consequences of DNA methylation (1). Two activities of this kind, Methyl-CpG binding Proteins 1 and 2 (MeCP1 and MeCP2) were earlier implicated in gene silencing. MeCP2 is a relatively abundant chromosomal protein whose localisation in the nucleus is dependent on CpG methylation. Mutations in MeCP2 cause embryonic lethality in mice, but cells lacking the protein are viable. Recent work from others has established that Rett Syndrome, a common form of X-linked mental retardation in women, is caused by mutations in the MeCP2 gene (2). MeCP2 can recruit histone deacetylases and may therefore cause transcriptional silencing by linking DNA methylation with changes in chromatin structure. We identified a family of proteins that contain a motif related to the methyl-CpG binding domain of MeCP2: MBDs 1 - 4. One of these proteins, MBD2, is a key component of MeCP1 and also acts as a deacetylase-dependent transcriptional repressor in vivo. In the absence of MBD2, MeCP1 levels are greatly reduced and repression of methylated genes is compromised in vivo. We have been unable to obtain evidence supporting a recent report that MBD2 is a DNA demethylase. Another MBD protein, MBD1, also has the capability to repress transcription of methylated genes in vitro and in vivo. We have mapped the domain responsible for repression and find that histone deacetylases are again implicated (3). Thus three methyl-CpG binding proteins, MeCP2, MBD1 and MBD2 are now implicated in the silencing of methylated genes. The fourth related protein, MBD4, is implicated in DNA repair at sites of 5-methylcytosine deamination (4).

The data on DNA binding by MBD3 is presently less clearcut, as mammalian MBD3 has a comparatively weak preference for binding to methylated DNA in vitro and does not localise to heavily methylated foci in vivo. Work from other labs has established that MBD3 is an integral component of the i2/NuRD complex which contains both histone deacetylases and a putative ATP-dependent chromatin remodeling protein. Therefore there is considerable interest in the possibility that it might respond to the DNA methylation signal. MBD2 and MBD3 are in fact closely related proteins, and it is striking that they presently appear to be associated with distinct complexes. In a phylogenetic study, we identified genes encoding MBD2/3-related proteins in two insects: the fruitly *Drosophila* and the cricket *Acheta* (5). The two insects differ in that *Acheta* has 5-methylcytosine in its genome, whereas Drosophila does not. Correspondingly, the *Acheta* MBD2/3 binds methylated DNA efficiently, whereas *Drosophila* MBD2/3 does not bind DNA detectably and possesses a highly divergent DNA binding domain. These data argue that MBD2/3 in *Drosophila*, and perhaps also other organisms, serves a function that is independent of DNA methylation.

References:

1. Bird, A. & Wolffe, A. P. (1999) Cell 99, 451-454.
2. Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U. & Zoghbi, H. Y. (1999) Nature Genetics 23, 185-188.
3. Ng, H.-H., Jeppesen, P. & Bird, A. (2000) Mol Cell Biol 20, 1394-1406.
4. Hendrich, B., Hardeland, U., Ng, H.-H., Jiricny, J. & Bird, A. (1999) Nature 401, 301-304.
5. Tweedie, S., Ng, H.-H., Barlow, A. L., Turner, B. M., Hendrich, B. & Bird, A. (1999) Nature Genetics 23, 389-390.

Mice knockout for HMG1 and HMG2

Marco E. Bianchi, Lorenza Ronfani, Sabina Calogero, Tiziana Bonaldi, Jordi Bernués#, Paola Scaffidi, Susanne Müller, Giacoma Mazzola and Monica Beltrame*

DIBIT, Istituto Scientifico San Raffaele, Milano, Italy; #Centre d'Investigació i Desenvolupament-Consejo Superior de Investigaciones Científicas, Barcelona, Spain; and *Dipartimento di Biologia e Genetica dei Microrganismi, Università di Milano, Italy.
e-mail bianchi.marco@hsr.it

High Mobility Group (HMG) proteins are small chromatin components, that fall in three separate families.

HMG-14 and HMG-17 are directly associated to histones inside nucleosomal cores (1).

HMG-I(Y) and HMGI-C are characterized by AT-hook domains, short amino acid stretches that fit into the minor groove of AT-rich DNA. HMG-I(Y) facilitates the assembly of "enhanceosomes" (2).

HMG1, HMG2 and HMG4 each contain two domains called HMG boxes, and an acidic tail. The three proteins are more than 80% identical to each other, and all three have been extremely conserved in vertebrate evolution. Their biochemical activities are indistinguishable, but their pattern of expression is different. HMG1 is very abundant in essentially all cell nuclei, HMG2 is abundant only in specific tissues, and HMG4 is expressed only in embryos. Proteins of the HMG1 family bind with low affinity to linear DNA, and have no sequence specificity. However, they can be recruited to specific DNA sites by interactions with other proteins. HMG-boxes then bind to the minor groove of DNA, and bend it significantly (reviewed in 3).

HMG1 can interact with HOX and OCT gene products, steroid nuclear receptors, p53, RAG1 and TBP, plus assorted viral proteins. Weak interaction between HMG1 and its partners can be detected in the absence of DNA. In all cases investigated, the surface of interaction invariably involves either HMG box, and the DNA binding domain of the partner protein (with the exception of TBP). In vitro, HMG1 facilitates the binding of its partners to their cognate DNA sequences. In transfection assays, a transient increase in the nuclear concentration of HMG1 brings about an enhancement of transcription controlled by parter transcription factors, and an increase in the yield of RAG1-mediated V(D)J recombination.

Perhaps at odds with its designation as "chromatin protein", HMG1 does not bind tightly either to condensed metaphase chromosomes, or to interphase chromatin, although it can bind tightly to nucleosomes reconstituted in vitro (4). When cultured cells are permeabilized with detergents, HMG1 diffuses away from the nucleus, whereas histone H1 an HMGI-(Y) remain tightly bound. Overall, the properties of HMG1 are intermediate between those of structural components of chromatin, and transcription factors.

To get more insight into the role(s) of HMG1, we generated mice where the *Hmg1* gene is deleted (5). *Hmg1* –/– pups were born alive, but died within 24 hours due to hypoglycaemia. Surprisingly, glycogen reservoires in the liver were not utilized, as if the appropriate transcriptional and biochemical programs failed to be activated by low of glucose levels in the blood. HMG1-deficient mice survived if they were given glucose injections immediately after birth. However, they displayed pleiotropic defects (but no alteration in the immune repertoire), and eventually wasted away within 3 weeks.

Fibroblast cell lines lacking HMG1 grew normally, and showed no gross chromatin abnormality. However, in these cells the activation of gene expression by the glucocorticoid receptor (GR) was impaired. Moreover, in *Hmg1* -/- mice, thymocytes were partially resistant to dexamethasone-induced apoptosis, and the level of circulating corticosterone was elevated. These data confirm that HMG1 is indeed involved in transcriptional activation by GR and other nuclear hormone receptors.

We also generated HMG2-less mice. HMG2 is normally expressed in adult thymus, spleen and testis. Early embryonic expression is widespread, but becomes pregressively reduced. *Hmg2* -/- mice are apparently normal, save for a marked male infertility. Germ and Sertoli cells undergo significant apoptosis, and immobile or malformed spermatozoa are predominant. This correlates with a peak of expression of HMG2 in wildtype spermatocytes; no HMG2 is detectable in either spermatogonia or late spermatids. Possibly, HMG2 facilitates the transcription of specific genes in cells undergoing meiosis.

We are currently breeding HMG1 and HMG2 mutant mice.

References:

1. Bustin, M. (1999). Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. Mol. Cell. Biol. *19*, 5237-46.
2. Thanos, D., and Maniatis, T. (1995). Virus induction of INF- gene expression requires the assembly of an enhanceosome. Cell *83*, 1091-1100.
3. Bianchi, M. E., and Beltrame, M. (1998). Flexing DNA: HMG-box proteins and their partners. Am. J. Hum. Genet. *63*, 1573-1577.
4. Falciola, L., Spada, F., Calogero, S., Längst, G., Voit, R., Grummt, I., and Bianchi, M. E. (1997). High mobility group 1 (HMG1) protein is not stably associated with the chromosomes of somatic cells. J. Cell Biol. *137*, 19-26.
5. Calogero, S., Grassi, F., Aguzzi, A., Voigtländer, T., Ferrier, P., Ferrari, S., and Bianchi, M. E. (1999). The lack of chromosomal protein HMG1 does not disrupt cell growth, but causes lethal hypoglycaemia in newborn mice. Nature Genet. *22*, 276-280.

The GAGA factor of *Drosophila* acts as a transcriptional activator but it also interacts with SAP18, a component of the Sin3-HDAC corepressor complexes.

Maria Llülsa Espinás, Alejandro Vaquero, Sílvia Canudas, Laura Fanti*, Sergio Pimpinelli*, Jordi Bernués and Fernando Azorín.
Departament de Biologia Molecular i Cel.lular. Institut de Biologia Molecular de Barcelona-CSIC. Jordi Girona Salgado 18-26. 08034 Barcelona. Spain.
*Dipartimento di Genetica e Biologia Molecolare, Università di Roma "La Sapienza". 00185 Roma. Italy.

The GAGA factor is organised in several distinct domains: a central DNA binding domain (DBD), a C-terminal glutamine-rich domain (Q-domain), a N-terminal POZ/BTB-domain and a relatively long domain (140 aa) that links the POZ/BTB and DBD domains (linking-domain) (1). The Q-domain confers to GAGA transactivation activity *in vitro* (2). Its presents an internal modular structure, acts independently of the rest of GAGA and stimulates transcription by enhancing pre-initiation complex formation and re-initiation. In *Drosophila* SL2 cells, GAL4BD-Q fusions significantly activate transcription though GAGA itself shows only a moderate transactivation activity (that is dependent on the Q-domain). The N-terminal POZ/BTB-domain is a protein-protein interaction domain that mediates the formation of GAGA oligomers which bind DNA with high affinity and specificity (3,4). The POZ/BTB-domain also contributes to the interaction with other nuclear proteins and, through a yeast two-hybrid screen, SAP18 was identified as a GAGA-interacting protein (5). SAP18 is a component of the Sin3-HDAC corepressor complexes (6). dSAP18 is highly homologous to mSAP18 and cSAP18 (60% identity). *In vitro*, the C-terminal region of SAP18 contributes mostly to the interaction that requires also the linking-domain of GAGA. Immunostaining of polytene chromosomes reveals a significant overlapping of GAGA and SAP18 which co-localise at the bithorax complex, where GAGA is known to be bound at some repressed PREs (7). These results presents GAGA as a multifunctional factor that could participate both in transcriptional activation and chromatin-mediated repression processes.

1.- Granok *et al.* (1995) *Curr. Biol.* **5**, 238-241.
2.- Vaquero *et al.*, submitted.
3.- Espinás *et al.* (1999) *J. Biol. Chem.* **274**, 16461-16469.
4.- Katsani *et al.* (1999) *EMBO J.* **18**, 698-708.
5.- Espinás *et al.*, in preparation.
6.- Zhang *et al* (1997) *Cell* **89**, 357-364.
7.- Strutt *et al.* (1997) *EMBO J.* **16**, 3621-3632.

Structure-function relationships in oligonucleosomal transcription templates

Mayel Chirinos, Francisco Hernández, and Enrique Palacián

Centro de Biología Molecular "Severo Ochoa", CSIC-UAM, Madrid, Spain

To investigate basic structure-function relationships in chromatin, the transcription properties of oligonucleosomes of known composition were determined using a simple and efficient in vitro transcription system, which allows evaluation of RNA synthesis under a wide variety of salt conditions. Oligonucleosomes containing different core histone domains were assembled on three different DNA species with a promoter for T7 RNA polymerase: two circular (pGEMEX-1 and pT207-18) and one linear (T7-207-18). In pGEMEX-1, the region downstream from the promoter is devoid of nucleosome positioning sequences, while in pT207-18 and T7-207-18 it contains 18 tandem repeats of a 207-bp positioning sequence.

Two alterations of the core histone octamers were investigated: the absence of the tail domains (1) and that of the H2A·H2B dimers (2,3). In vitro RNA synthesis by bacteriophage T7 RNA polymerase was conducted under several salt conditions inducing in the templates different degrees of folding and self-association. Sedimentation equilibrium was used to evaluate the number of histone elements incorporated to T7-207-18, and sedimentation velocity to estimate the degree of folding. At different concentrations of KCl and $MgCl_2$, both changes in the histone octamer are accompanied by substantial increases in transcription efficiency after allowing for the aggregation observed under certain conditions. In the absence of KCl and at low $MgCl_2$ concentration, the presence of 2 mM spermidine causes substantial aggregation of the intact oligonucleosomes but has a much smaller effect on those deprived of the histone tails or with only $(H3{\cdot}H4)_2$. Unexpectedly, the folding of intact oligonucleosomes that takes place upon increasing the Mg^{2+} concentration was found to be accompanied by stimulated RNA synthesis.

Assembly with intact core histone octamers affects pGEMEX-1 transcription mainly at the initiation level, while T7-207-18 is almost exclusively inhibited at the level of elongation. Under conditions promoting a low transcription rate, with pGEMEX-1, the block to initiation due to the presence of core histone octamers is substantially relieved when $(H3{\cdot}H4)_2$ is substituted for the whole octamer, or this is deprived of the tail domains. With T7-207-18, under assay conditions allowing transcription of the whole coding region in the naked DNA, the size of the transcription products indicates that RNA elongation is facilitated in the absence of the histone tail domains as compared with the template containing intact histone octamers. A much larger facilitation is found with templates lacking the H2A·H2B dimers. In this case, the size distribution of the transcription products is entirely similar to that corresponding to the free DNA.

References:

1. Chirinos, M., Hernández, F., & Palacián, E. (1998) Biochemistry 37, 7251-7259.
2. Puerta, C., Hernández, F., Gutiérrez, C., Piñeiro, M., López-Alarcón, L., & Palacián, E. (1993) J. Biol. Chem 268, 26663-26667.
3. Chirinos, M., Hernández, F., & Palacián, E. (1999) Arch. Biochem. Biophys. 370, 222-230.

How chromatin is remodeled *in vivo*

Philip D. Gregory, S. Barbaric and Wolfram Hörz

Institut für Physiologische Chemie, Universität München, Schillerstr. 44, 80336 München, Germany

We are investigating the mechanism of chromatin remodeling that is characteristic of many regulated promoters during gene activation and are focusing on the *PHO5* promoter from yeast [1]. In the repressed state, the *PHO5* promoter is organized in an array of positioned nucleosomes that is only interrupted by a short hypersensitive site. Upon activating the gene by phosphate starvation, two nucleosomes upstream and two downstream of the hypersensitive site are disrupted, and the transcription factor Pho4 binds to two UAS elements.

The transcription factor Pho4 is strictly required for the chromatin transition, and we can show that not only the DNA binding domain but also the activation domain is needed. Heterologous activation domains, for example from the glucocorticoid receptor, are also capable of triggering the chromatin transition when fused to the Pho4 DNA binding domain.

When we bring the *PHO5* promoter under galactose control by replacing either or both UAS elements by Gal4 binding sites we find that one high affinity Gal4 binding site is sufficient for the chromatin transition and that again four nucleosomes are remodeled. The Gal4 DNA binding domain can bind to a nucleosomal site *in vivo*, generating a triple complex between histones, DNA and factor. This binding results in a local chromatin perturbation which is very different, however, from the four nucleosome transition usually seen.

Neither the SWI/SNF nucleosome remodeling machine nor the histone acetyl transferase Gcn5 are required for *PHO5* activation and chromatin opening. However, under submaximally inducing conditions, Gcn5 is required for activation. In its absence, a novel chromatin pattern at the promoter is observed consisting of randomized nucleosomes [2].

We have extended our studies to another structural gene of the *PHO* family, the *PHO8* gene which encodes a weakly expressed alkaline phosphatase. This gene is also under phosphate control, regulated through Pho4, and it undergoes a characteristic chromatin transition upon phosphate starvation [3]. For the activation of the *PHO8* promoter, SWI/SNF is essential even under maximally inducing conditions. In its absence, chromatin is frozen in the closed configuration. Gcn5 also makes an important contribution to activation and chromatin opening. Both activities act at a point subsequent to activator binding [4]. The basis for the differential SWI/SNF and Gcn5 requirement of *PHO5* and *PHO8* will be discussed.

References:

[1] Svaren, J. and Hörz W. Transcription factors vs nucleosomes: Regulation of the *PHO5* promoter in yeast. *Trends Biochem. Sci.* 22, 93-97 (1997).

[2] Gregory,P.D., Schmid,A., Zavari,M., Lui,L., Berger,S.L., and Hörz,W. (1998). Absence of Gcn5 HAT activity defines a novel state in the opening of chromatin at the *PHO5* promoter in yeast. *Mol. Cell* 1, 495-505.

[3] Barbaric,S., Fascher,K.D., and Hörz,W. (1992). Activation of the weakly regulated *PHO8* promoter in *S. cerevisiae*: Chromatin transition and binding sites for the positive regulator protein Pho4. *Nucl. Acids Res.* 20, 1031-1038.

[4] Gregory,P.D., Schmid,A., Zavari,M., Münsterkötter,M., and Hörz,W. (1999). Chromatin remodelling at the PHO8 promoter requires SWI-SNF and SAGA at a step subsequent to activator binding. *EMBO J.* 18, 6407-6414.

Session 5: Higher order chromatin structure and gene regulation
Chair: Katherine A. Jones

Localization of yeast Sir2p and its homologues, and the nucleolar response to MAP kinase activation in budding yeast

Severine Perrod[2], Elisa M. Stone[1], Patrick Heun[2], Moira Cockell[2], Lorraine Pillus[1] and Susan M. Gasser[2]

[1] Department of Biology, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0347; and [2] Swiss Institute for Experimental Cancer Research (ISREC), Chemin des Boveresses 155, CH-1066 Epalinges/Lausanne, Switzerland

Two different aspects of long-range chromatin and nuclear organization will be discussed. First, we have examined the structure, function and localization of several homologues of the *Saccharomyces cerevisiae* Silent information regulator, Sir2p[3]. In all eukaryotic species having multiple Sir2 family members, we find at least one homologue more closely related to yeast Hst2p, than to Sir2p itself. These proteins form a phylogenetic subfamily, characterized by a cytoplasmic, rather than nuclear, localization. Surprisingly, we find that elevated levels of Hst2p in the yeast cytoplasm can derepress subtelomeric silencing and improve repression in the rDNA. A mutated form of the human homologue of Hst2p, hsirT2^{182L}, has been identified as a melanoma antigen. Introduction of the equivalent point mutation into the yeast *SIR2* gene, abolishes its silencing function at rDNA and telomeres, producing a protein that disrupts both TPE and rDNA repression in presence of wild-type Sir2p. The dominant negative phenotypes of this inactive mutant and of the cytoplasmic Sir2p homologue Hst2p, can be best understood in terms of its recently reported deacetylase activity, which is conserved among all proteins containing the Sir2 core domain.

Along an independent line of research, we have observed a striking nuclear response to MAP kinase activation[4]. It is well-established that during the mating pheromone response in budding yeast, activation of a MAP kinase cascade results in well-characterized changes in cytoskeletal organization and gene expression. Although a reorganization of genes have been observed in mammalian cells, no information is available on the morphology of the yeast nucleus during the major transcriptional reprogramming that accompanies zygote formation. We find that budding yeast nuclei assume an unusual dumbbell shape, reflecting a spatial separation of chromosomal and nucleolar domains, in response to mating pheromone. Within the chromosomal domain, telomeric foci persist and maintain their associated complement of Sir factors. The nucleolus, on the other hand, assumes a novel cup-shaped morphology and is positioned distal to the mating projection tip. Neither microtubules nor actin polymerization are necessary for the observed changes. We find that activation of the pheromone-response MAP kinase pathway by ectopic expression of *STE4* or *STE11* leads to identical nuclear and nucleolar reorganization, even in the absence of pheromone. Mutation of downstream effectors Fus3p and Kss1p, or of the transcriptional regulator Ste12p, blocks these changes, while overexpression of Ste12p promotes dumbbell shaped nuclei. Thus, activation of the Ste12p transcription factor is necessary, and may be sufficient, for the nuclear remodeling that coincides with changes in gene expression elicited by the mating pheromone response in yeast.

References:

[3] Perrod, S., Cockell, M.M., Laroche, T., Renauld, H., Bonnard, C. and Gasser, S.M. A cytosolic homologue of Sir2p modulates rDNA and telomeric silencing in yeast, *submitted.*

[4] Stone, E.M., Heun, P., Laroche, T., Pillus, L. and Gasser, S.M. (2000) MAP kinase signaling induces nuclear reorgan-ization in budding yeast. **Curr. Biology,** *in press*

Specific gain or loss of function by satellite-specific DNA-binding drugs fed to developing *Drosophila melanogaster*

Sam Janssen*, Olivier Cuvier* and Ulrich K. Laemmli

Departments of Biochemistry and Molecular Biology, University of Geneva, 30, Quai Ernest-Ansermet, CH1211- Geneva 4, Switzerland.

Position effect variegation (PEV) is an epigenetic phenomenon that arises from a stochastic gene inactivation either in *cis* or *trans* as mediated by large blocks of satellite heterochromatin. Cis-acting DNA motifs involved in PEV are unknown. To address this issue, we synthesized two DNA minor groove binding polyamides that specifically target two different DNA satellites of *Drosophila melanogaster* with subnanomolar affinity. P9 is composed of *N*-methylpyrrole (Py) amino acids, it targets the numerous AT-tracts of satellite III and the AATAT repeats of satellite I. Compound P31 is composed of both *N*-methylimidazole and Py units, it specifically binds two repeats of the GAGAA satellite. Specificity of targeting was established by footprinting and staining of nuclei and polytene chromosomes using fluorescently tagged compounds. P9 and P31 were fed to developing *white-mottled* flies. Remarkably, we observed that P9 (not P31) resulted in a gain of function by suppressing the *white-mottled* eye phenotype. These drugs were also fed to developing *brown-dominant* flies. In case, P31 (not P9) led to a loss of function (homeotic transformations) which corresponds to the phenotypes of mutations in the *Trl* gene which encodes the GAGA factor. Suppression of PEV in *white-mottled* flies by P9 and induction of homeotic transformations in *brown-dominant* flies by P31, are molecularly explained by chromatin opening of the drug-targeted DNA satellites.

LOCAL CONCENTRATION OF DNA *versus* LINEAR PACKING RATIO IN CHROMATIN FIBERS AND CHROMOSOMES

Joan-Ramon Daban

Departament de Bioquímica i Biologia Molecular, Facultat de Ciències, Universitat Autònoma de Barcelona, 08193-Bellaterra, Barcelona, Spain

Since the length of the chromosomal DNA molecules exceeds the dimensions of the cellular structures in which they are contained, the linear packing ratio (defined as the ratio between the length of extended DNA and the length of the structure that contains it) is widely used to measure the degree of DNA compaction. Here I show that the local concentration of DNA (defined as the mass of DNA per unit volume of the biological structure that contains it) is more appropriate than the DNA packing ratio to validate structural models for chromatin condensation in metaphase chromosomes. The local concentration of DNA in metaphase chromosomes of different organisms has been determined in several laboratories. The average of these measurements is 0.17 g/mL. In the first level of chromosome condensation, DNA is wrapped around histones forming nucleosomes. This organization limits the DNA concentration in nucleosomes to 0.3-0.4 g/mL. Furthermore, in the structural models suggested in different laboratories for the 30-40 nm chromatin fiber, the estimated DNA concentration is significantly reduced; it ranges from 0.04 to 0.27 g/mL. The DNA concentration is further reduced when the fiber is folded into the successive higher order structures suggested in different models for metaphase chromosomes; the estimated minimum decrease of DNA concentration represents an additional 40%. These observations suggest that most of the models proposed for the 30-40 nm chromatin fiber are not dense enough for the construction of metaphase chromosomes. In contrast, it is well known that the linear packing ratio increases dramatically in each level of DNA folding in chromosomes. Thus, the consideration of the linear packing ratio is not enough for the study of chromatin condensation; the constraint resulting from the actual DNA concentration in metaphase chromosomes must be considered for the construction of models for condensed chromatin.

Supported in part by grants PR95-611 and PB98-858 (DGESIC), and 1998GR-72 (Generalitat de Catalunya)

Ubiquitin-conjugase activity of TAFII250 mediates activation of gene expression in *Drosophila.*

Anh-Dung Pham and Frank Sauer

Ubiquitination of histones has been correlated with transcriptional active chromatin structures. A biochemical approach identified the Drosophila coactivator TAFII250, the central subunit within the general transcription factor TFIID, as a histone-specific ubiquitin-conjugating enzyme (ubc).

TAFII250 mediates mono-ubiquitination of the linker histone H1 in vitro. A point mutation within the putative ubc-domain of TAFII250 significantly abolished H1-specific ubc-activity of the coactivator in vitro and, in the *Drosophila* embryo, significantly reduced the expression of genes targeted by the maternal activators. Thus, coactivator-mediated ubiquitination of histone H1 or other unidentified proteins within the trans-activation pathway may contribute to the processes directing activation of eukaryotic transcription.

Regulation of enhancer-promoter interactions in the *Drosophila* embryo

Jumin Zhou, Yutaka Nibu, Mattias Mannervik, Sumio Ohtsuki, and Michael Levine

Dept. Mol. Cell Biol. Division of Genetics and Development, University of California, Berkeley, CA 94720

Complex enhancers direct stripes, bands, and tissue specific patterns of gene expression in the early Drosophila embryo. These enhancers are typically 300 bp to 1 kb in length and contain clustered binding sites for both transcriptional activators and repressors. Multiple enhancers can work independently of one another to generate composite patterns of gene expression, such as multiple eve stripes. This enhancer autonomy is due to short-range repression. Repressors bound to one enhancer do not interfere with activators in the neighboring enhancer. Approximatley one-half of all repressors in the early embryo function only over short distances. They must bind within 100 bp of either upstream activators or the core promoter in order to mediate repression. At least three distinct short-range repressors, Snail, Kruppel, and Knirps, interact with a common corepressor protein, CtBP. The human homolog of CtBP binds to the carboxyl terminus of the adenovirus E1a protein, and attenuates E1a-mediated transcriptional activation. CtBP binds to a specific sequence motif in the E1a protein: P-DLS-K/R. This motif is conserved in Snail, Kruppel, and Knirps, and is essential for protein-protein interactions with the Drosophila CtBP corepressor protein. Whereas CtBP appears to mediate short-range repression, a second corepressor protein, Groucho, may be responsible for long-range silencing. Groucho-dependent repressors, such as Hairy, can work over distances of at least 1 kb. The early Drosophila embryo contains at least three different corepressor proteins: CtBP, Groucho, and a homolog of the yeast rpd3 histone deacetylase. However, mutations in rpd3 cause relatively mild segmentation defects, suggesting that CtBP and Groucho mediate the major pathways of repression in the early embryo. Mutations in other coregulatory proteins can also cause specific patterning defects. For example, mutations in the Drosophila homolog of the CBP histone acetyltransferase cause a specific loss of gene activation in the dorsal ectoderm. Since the dorsal ectoderm is patterned by TGF-beta signaling, this observation suggests that there may be relatively specific interactions between CBP and Smads in the early embryo.

Enhancer-promoter interactions are regulated by insulator DNAs and promoter competition. The latter mechanism was first described in the chicken globin locus. A shared enhancer can interact with multiple promoters, but preferentially interacts with just one. This preferred interaction sequesters the enhancer so that it is unable to activate other genes within a complex. This type of competition mechanism appears to account for the selective interaction of the shared AE1 enhancer with the ftz gene within the Antennapedia gene complex in *Drosophila*. AE1 prefers ftz over the equidistant Scr gene, in part, because the ftz promoter contains a TATA element while Scr does not. Many, but not all, enhancers exhibit a similar preference for TATA-containing vs. TATA-less promoters in the early embryo. Some of these enhancers contain one or more GAGA elements, which bind the ubiquitous zinc finger protein, Trithorax-like (Trl). The insertion of synthetic GAGA sites in the naïve rhomboid lateral stripe enhancer (NEE) changes its regulatory activity so that the modified enhancer now prefers TATA-containing promoters. The native enhancer lacks GAGA elements and promiscuously activates both TATA-containing and TATA-less promoters. While distal GAGA elements might influence promoter targeting, proximal GAGA sites

within the core promoter help mediate an insulator activity, whereby distal enhancers are unable to "jump over" the promoter and activate neighboring genes.

The Bithorax gene complex (BX-C) is over 300 kb in length and genetic studies suggest that most of this DNA corresponds to cis-regulatory information. Each of the three Hox genes contained within the BX-C is regulated by 60-80 kb of cis-DNA. In the case of the Abd-B gene most of this cis-DNA maps 3' of the transcription unit. Previous studies suggest that insulator DNAs organize this extended 3' cis-DNA into a series of separate chromatin loop domains. This organization poses a potential paradox: how do remote enhancers such as IAB5 overcome the blocking effects of intervening insulators and interact with the Abd-B promoter over a distance of ~60 kb? Evidence is presented for a novel type of cis-regulatory element, the PTS (promoter targeting sequence), which facilitates long-range enhancer-promoter interactions. In transgenic embryos the insertion of the PTS between an insulator and distal enhancer permits the enhancer to overcome the blocking activity of the insulator and activate a lacZ reporter gene.

References:

Ohtsuki, S., Levine, M. and Cai, H.N. (1998) Different core promoters possess distinct regulatory activities in the *Drosophila* embryo. **Genes Dev.** *12*, 547-556.

Nibu, Y., Zhang, H., and Levine, M. (1998). Interaction of short-range repressors with *Drosophila* CtBP in the embryo. **Science** *280*, 101-104.

Ohtsuki, S. and Levine, M. (1998). GAGA mediates the enhancer blocking activity of the *eve* promoter in the *Drosophila* embyro. **Genes Dev.**, *12*, 3325-3330.

Nibu, Y., Zhang, H., Bajor, E., Barolo, S., Small, S., and Levine, M. (1998). dCtBP mediates transcriptional repression by Knirps, Krüppel, and Snail in the *Drosophila* embryo. **EMBO J.**, *17*, 7009-7020.

Mannervik, M., Nibu, Y., Zhang, H., and Levine, M. (1999). Transcriptional coregulators in develoment. Science *284*, 606-609.

Zhou, J. and Levine, M. (1999). A novel cis-regulatory element, the PTS, mediates an anti-insulator activity in the Drosophila embryo. **Cell** *99*, 567-575.

POSTERS

TRANSCRIPTION AND DNA REPLICATION INITIATE AT THE SAME GENOMIC REGIONS IN EUKARYOTIC CHROMOSOMES

Francisco Antequera, Myriam Cuadrado, Sonia Delgado, María Gómez and Mónica Segurado

Instituto de Microbiología Bioquímica.CSIC/Universidad de Salamanca. Campus Miguel de Unamuno. 37007 Salamanca. Spain

DNA replication origins (ORI) have a modular structure integrated by a core element binding the origin recognition proteins and auxiliary elements some of which bind transcription factors. The correlation between transcription and replication in animal cells is based on the early replication of actively transcribed genes during S phase and on the localization of some ORIs close to gene promoters. We have asked whether gene promoter regions were asociated with replication origins in mammalian cells and in the fission yeast *Schizosaccharomyces pombe.*

Quantitative analysis by competitive PCR on replication intermediates at several mammalian genes whose promoters lie within CpG islands showed that these regions, but not their flanks, were present in a population of short DNA nascent strands suggesting their proximity to replication origins. This is supported by the fact that CpG island sequences were enriched in that population indicating that they constitute a significant fraction of mammalian ORIs.

We have also developed an approach to isolate chromosomal ORIs directly from replication intermediates and tested its efficiency in *S. pombe* by two-dimensional electrophoresis. Mapping of a number of the novel ORIs onto cosmids showed a strong preference to localize at intergenic regions overlapping gene promoters. Mapping of transcription and replication initiation sites at nucleotide resolution and deletion analysis indicate that regulatory elements for both processes lie in close proximity suggesting a possible relationship between transcription and DNA replication *in vivo.*

Ours results suggest a similar organization of transcription and replication regulators at promoter/ORI regions in mammalian cells and in *S. pombe.*

A role of NC2/(DR1/DRAP1) as positive cofactor in enhancer-dependent RNA polymerase II transcription

Enrique Castaño (1), Petra Gross (1), Zhengxin Wang (2), Robert G. Roeder (2), and Thomas Oelgeschläger (1)

(1) Eukaryotic Gene Regulation Laboratory
Marie Curie Research Institute
The Chart, Oxted, Surrey, RH8 OTL, United Kingdom

(2) Laboratory of Biochemistry and Molecular Biology
The Rockefeller University
1230 York Avenue, New York, USA

Regulated transcription by RNA polymerase II transcription requires in addition to general transcription factors activators, co-activators, repressors, and co-repressors. NC2 (DR1/DRAP1) has been characterised previously as a general repressor of class II transcription that inhibits productive preinitiation complex formation through interactions with TFIID.

Surprisingly, we find that immunodepletion of NC2 (DR1/DRAP1) from HeLa nuclear extracts dramatically decreases activator-dependent RNA polymerase II transcription in vitro while basal transcription levels remain unchanged.
Importantly, adding back recombinant NC2 strongly represses both activator-dependent transcription and basal levels of transcription in NC2-depleted extracts. However, we have identified chromatographic HeLa nuclear extract fractions that can selectively restore activator-dependent transcription levels in NC2-depleted nuclear extracts without affecting basal transcription levels. Our observations raise the possibility of coactivator activities associated with NC2 and point to an interplay of positive and negative cofactors in enhancer-dependent transcription regulation.

Transcriptional elongation and genome stability in *hpr1* and *tho2* mutants of *S. cerevisiae*.

S. Chávez, A. G. Rondón and A. Aguilera.
Departamento de Genética, Facultad de Biología, Universidad de Sevilla, 41012 Sevilla, Spain. e-mail: schavez@cica.es

Transcriptional activity can increase genome instability, as it has been shown for recombination between direct repeats in *S. cerevisiae*, inmunoblobin gene rearrangements and deletions in bacterial plasmids.

To envisage the molecular mechanisms underneath these phenomena we have studied mutants of *S. cerevisiae* apparently affected in both transcription and genome instability. The *HPR1* and *THO2* genes are good candidates for linking transcription and genome stability, as *hpr1* and *tho2* mutants show both high rates of deletion and chromosome loss, and severe transcriptional defects. Additional support for this view lies on the isolation of extragenic suppressors of the recombination phenotype of *hpr1* mutants, two of them, *SRB2 and HRS1*, being elements of the RNA PolII holoenzyme.

We have previously shown that *HPR1* and *THO2* do not play a significant role in promoter activation but on transcript elongation (1, 2). The relevance of this role is sequence specific, being essential for instance in the commonly used *lacZ* reporter-gene. In order to understand the roles of Hpr1p and Tho2p in transcription elongation and genome instability we have performed an *in vivo* analysis of this sequence dependence.

To define the whole range of phenotypes related to genome instability caused by these mutations we have determined their effect on inverted-repeat recombination.

We have also studied if mutants affected in other genes involved in RNA PolII elongation, also show the hyperrecombination phenotypes of *hpr1* and *tho2*.

Finally, to determine a possible physical interaction between Hpr1p and Tho2p, a tagged form of Tho2p has been expressed in yeast cells, and a new protein complex has been isolated.

1. Chávez S, Aguilera A (1997) Genes Dev 11: 3459-3470
2. Piruat JI, Aguilera A (1998) EMBO J 17: 4859-4872

Histone H3 phosphorylation in response to different stimuli: from mitogens to light.

Claudia Crosio, Nicolas Cermakian, David Allis, André Hanauer and Paolo Sassone-Corsi

IGBMC, B.P.163, 67404 Illkirch CU de Strasbourg, France

Remodelling of chromatin structure appears to have a primary role in transcriptional regulation, and posttranslational modifications of histones are thought to contribute to this process. Widespread phosphorylation of histones H1 and H3 correlates with mitosis in many cells. Additionally, rapid and transient phosphorylation of a subset of histone H3 molecules correlates with the activation of immediate-early genes.

We have recently identified the Rsk2 kinase as a candidate for mitogen-activated histone H3 kinase. Rsk2 is activated by the MAPK signalling pathway resulting in H3 phosphorylation; Rsk2 readily phosphorylates H3 *in vitro*. Furthermore, fibroblasts derived from a patient affected by the Coffin-Lowry syndrome (bearing an inactivating mutation in the Rsk2 gene) are defective in mitogen-activated phosphorylation of H3, a defect thet can be rescued upon ectopic expression of Rsk2. Interestingly, these cells are severely impaired in c-*fos* induced expression upon growth factor stimulation, strongly suggesting that H3 phopshorylation may be a step in the activation of immediate-early gene transcription.

Recently we focused our attention on H3 phosphorylation in response to physiological stimuli in animal model. In particular, prompted by recent findings indicating that the MAPK cascade is involved in circadian clock rhythmicity in the mouse, we have started to investigate the possibility of coupling light and circadian rhythmicity to chromatin remodelling, and in particular to histone H3 phosphorylation.

Studies of Histone deacetylase (HDAC) enzymes, SIN3 co-repressor proteins and CHD chromatin remodelling enzymes in fission yeast

Karl Ekwall Dept of Biosciences, Karolinska Institutet, NOVUM, Sweden, phone: +46 8 585 88713 (office) fax: +46 8 585 88510, email: karl.ekwall@cbt.ki.se
Lab. address: University College Södertörn, Box 4101, S-141 04 Huddinge, SWEDEN

(1) HDAC enzymes and co-repressor proteins

Histone proteins are modified by acetylation of lysine residues in the amino-terminal tails. Acetylation is regulated by two different enzymes: acetyl-transferases, which add acetyl groups and deacetylases (HDAC) which remove them. In general, heterochromatin (repressed chromosomal regions) are under-acetylated and gene rich (active) regions are hyper-acetylated. Aberrant histone acetylation is implicated both in developmental defects and cancer. Thus far, three fission yeast (*S. pombe*) HDAC genes have been identified: *hda1*$^+$ which is most closely related to *S. cerevisiae* HOS2 gene, *clr3*$^+$ related to *S. cerevisiae* HDA1 gene, and *clr6*$^+$ related to *S. cerevisiae* RPD3 gene. In addition, three SIN3 genes (pombe sin three) have been identified: *pst1*$^+$, *pst2*$^+$ and *pst3*$^+$. The *S. pombe* HDAC and SIN3 homologues are required for diverse chromosomal functions such as gene silencing (*clr3* and *clr6*), gene activation (*hda1*), function of retrotransposon elements (*pst1*) and chromosome segregation (*clr6 and pst1*). Interestingly, Clr6 and Clr3 show distinct punctuate patterns of nuclear localisation and are parts of physically distinct protein complexes. We are interested in understanding how sub-nuclear targeting of HDAC proteins is mediated in fission yeast to achieve various chromosomal functions. We are working with purification of HDAC combined with protein mass spectroscopy analysis to study the protein composition of the different complexes, and immunoflourescence microscopy to explore the specific interactions between SIN3, HDACs and their target sites in chromatin.

(2) Chromatin remodelling enzymes

The human Mi-2 protein belongs to the family of CHD (Chromo-Helicase domain/ATPase-DNA binding domain) chromatin-remodelling factors. Patients with dermatomyositis (DM) have autoantibodies against Mi-2, and a relative risk for cancer that is six times higher than the risk in the general population. The Mi-2 protein is physically and functionally associated with HDAC complexes, and with metastasis associated. Recently the *Drosophila* Mi-2 homologue was implicated in gene repression. We have identified two CHD proteins in fission yeast, Hrp1 and Hrp3. Overexpression of Hrp1 interferes with chromosome condensation and centromere function. *hrp1*$^+$ is not an essential gene but deletion of hrp1 results in defects in centromere function and gene silencing of marker genes inserted in centromeric and mating type regions. We are using genetic and biochemical analysis to explore the specific protein-protein interactions involved in interaction between fission yeast CHD protein family members (Hrp1p and Hrp3p) and other co-repressors to investigate the exact role of CHD proteins in gene repression and their relationship to HDAC's.

Core histone acetylation is regulated *in vivo* by linker histone stoichiometry in a variant-specific and cell cycle-dependent manner.

Akash Gunjan and David T. Brown.

We investigated the relationship between linker histone stoichiometry and the acetylation of core histones *in vivo*. Exponentially growing cell lines induced to overproduce either of two H1 variants, $H1^0$ or H1c, displayed significantly reduced rates of incorporation of ^{3}H-acetate into all four core histones. This effect was also observed in nuclei isolated from these cells upon labeling with ^{3}H-acetyl CoA. Analysis of the rates of core histone acetylation and deacetylation in these cell lines indicated that reduced histone acetyltransferase (HAT) activity in H1-overproducing cell lines was primarily responsible for these observations. Nuclear extracts prepared from control and H1-overexpressing cell lines displayed similar levels of HAT activity on chromatin templates prepared from control cells. In contrast, extracts prepared from control cells were significantly less active on chromatin templates prepared from $H1^0$ or H1c-overexpressing cells than on templates prepared from control cells. Hence, inhibition of core histone acetylation is not due to lower amounts of HATs in the H1-overexpressing cells, but due to the structure of the H1-containing chromatin. Further, this inhibition does not appear to depend on chromatin higher-order structure, as it persists even after digestion of the chromatin with DNase I. Thus, this effect is likely to be mediated by histone H1 primarily at the level of the nucleosome. In density arrested cells and their nuclei, core histone acetylation was inhibited upon overproduction of $H1^0$, but not upon overproduction of H1c. The results suggest that alterations in chromatin structure resulting from changes in linker histone stoichiometry may modulate the levels or rates of core histone acetylation *in vivo* in a H1 variant-specific and cell-cycle dependent manner.

The nuclear factor NFκB engages CBP/p300 and histone acetyltransferase activity for transcriptional activation of the Interleukin-6 gene promoter.

Wim Vanden Berghe, Karolien De Bosscher, Elke Boone, Stéphane Plaisance & Guy Haegeman

Department of Molecular Biology, University of Gent - VIB, Ledeganckstraat 35, 9000 Gent, Belgium;
Tel. ++329.264.5166, Fax. ++329.264.5304, e-mail : guy.haegeman@dmb.rug.ac.be

Expression of the pleiotropic cytokine interleukin-6 (IL6) can be stimulated by the pro-inflammatory cytokine tumor necrosis factor (TNF) and/or the microbial alkaloid staurosporine (STS). The transcriptional mechanisms of both type of inducers were thoroughly investigated. Whereas transcription factors binding to the activator protein-1-, cAMP-responsive element-, and CAAT enhancer-binding protein-responsive sequences are necessary for gene activation by STS, nuclear factor (NF) kB alone is responsible and sufficient for inducibility by TNF, which reveals distinct signaling pathways for both compounds. At the cofactor level, cAMP-responsive element-binding protein-binding protein (CBP) or p300 potentiate basal and induced IL6 promoter activation via multiple protein-protein interactions with all transcription factors bound to the promoter DNA. However, the strongest promoter activation relies on the p65 NFκB subunit, which specifically engages CBP/p300 for maximal transcriptional stimulation by its histone acetyl-transferaseactivity. Moreover, treatment of chromatin-integrated promoter constructions with the histone deacetylase inhibitor trichostatin A exclusively potentiates TNF-dependent (i.e. NFκB-mediated) gene activation, while basal or STS-stimulated IL6 promoter activity remains completely unchanged. Similar observations were recorded with other natural NFκB-driven promoters, namely IL8 and endothelial leukocyte adhesion molecule (ELAM). We conclude that, within an "enhanceosome-like" structure, NFκB is the central mediator of TNF-induced IL6 gene expression, involving CBP/p300 and requiring histone acetyltransferase activity.

Aniridia-associated translocations and transgenic analysis define a distant downstream regulatory region for PAX6

Dirk A. Kleinjan, Anne Seawright, Andreas Schedl, Roy A. Quinlan, Sarah Danes, Muriel Lee and Veronica van Heyningen

PAX6 is a member of the PAX gene family of developmental regulatory genes. It has been identified as the gene mutated in the human eye disease aniridia (iris hypoplasia) as well as in the mouse Small eye (Sey) phenotype. PAX6 shows a specific expression pattern during development with expression found in all structures of the developing eye, in specific regions of the brain, the neural tube, the olfactory epithelium and pancreas (Walther, 1991). Gene dosage of Pax6 is important for correct eye development as heterozygous Sey/+ mice have eye anomalies. Homozygous Sey/Sey mice die soon after birth with no eyes, no nasal structures and brain abnormalities.

In the human situation most aniridia patients have been shown to carry loss of function point mutations, leading to haploinsufficiency. However, we have identified five aniridia patients with an intact PAX6 gene, but carrying chromosomal rearrangements 30-120 kb downstream of the PAX6 polyA signal (Fantes, 1995 and unpublished). In those cases a position effect on the PAX6 gene has been hypothesised as the cause of the disease. We have been studying the nature of the position effect by means of YAC transgenesis, DNaseI hypersensitive site mapping and by interspecies comparison.

Correction of homozygous and heterozygous Small eye phenotypes has been achieved with a 420 kb human YAC containing the 25 kb PAX6 gene, and about 200 kb genomic flanking sequences on each side. The same YAC, truncated 7-10 kb downstream of the intact PAX6 gene failed to correct the homozygote lethality or eyeless phenotype, although the heterozygote phenotype was ameliorated (Schedl, 1996). We have generated transgenic mice with a shorter YAC, extending approximately 120 kb downstream of PAX6, roughly to the most distal aniridia breakpoint, and thereby about 80 kb shorter than the YAC which was shown to rescue the Smalleye phenotype. We show that this shorter YAC is unable to rescue the Smalleye phenotype in those transgenic mice, suggesting the presence of a regulatory control region in the 80 kb region between the shorter and the longer YACs. We have carried out a DNaseI hypersensitive sites analysis of that region using a PAX6 expressing lens cell line and a non-expressing cell line and have identified a number of hypersensitive sites spread out over a 25 kb region. We show that a fragment carrying a subset of these hypersensitive sites is able to direct the expression of a LacZ reporter gene in the eye and nasal epithelium of transgenic embryos. Through interspecies comparison we have identified another, conserved DNA element in this region which does not form a hypersensitive site in the lens cell line, but directs LacZ expression in the fore- and hindbrain of transgenic embryos. Taken together these results provide evidence for the presence of a complex regulatory control region at a large distance downstream of the PAX6 gene.

Walther et al. (1991) Development 3(4):1435-1449.
Fantes et al. (1995) Hum Mol Genet 4(3):415-422.
Schedl et al. (1996) Cell 86(1):71-82.

Retinoblastoma Tumor Suppressor Family Proteins Repress Transcription by Recruiting the mSIN3 Histone Deacetylase Complex via RBP1

Albert Lai

Retinoblastoma (RB) tumor suppressor family proteins block the S phase entrance of cell cycle by repressing transcription of E2F-regulated genes. One mechanism of which RB represses transcription involves both histone deacetylase (HDAC)-dependent and –independent repression activities being recruited to promoters via RBP1, a previously identified "pocket"-associated RB binding protein. RBP1 actively represses transcription via two distinct regions of the protein (R1 and R2). We showed previously that R2 associates with all known mammalian Rpd3-like histone deacetylases (HDAC1, 2 and 3). We have now identified the mSIN3 containing histone deacetylase complex as the HDAC activity being recruited by the R2 repression domain via a direct interaction with the Sap30 component of the complex. Interactions between RBP1 and Sap30 or RB and RBP1 are both required for the recruitment of HDAC activity to RB. In addition all identified components of the mSIN3 containing HDAC complex co-localize with RBP1, RB, p130 and E2F to discrete regions of the nucleus in quiescent primary human diploid fibroblasts. Upon entry to S phase, RB family members no longer co-localize with components of the mSIN3 complex including RBP1. We propose a model of which RB family members repress transcription of genes required for S phase entry by recruiting the mSIN3 containing HDAC complex via the association with RBP1.

The histone-fold CCAAT trimer NF-Y recruits RFX and TFIID on the MHC class II Ea promoter.

G. Caretti, M. Frontini, C. Imbriano, C. Sidoli, R. Mantovani,.
Dipartimento di Genetica e di Biologia dei Microrganismi, Via Celoria 26. 20143 Milano.
Tel: 39-02-26605239. Fax: 39-02-2664551. E-mail:mantor@mailserver.unimi.it

The MHC class II Ea promoter depends on the X-box trimer RFX, the CCAAT-box NF-Y and on the Initiator binding TFIID. We focused our attention in two directions.
(i) We produced and purified the RFX subunits, reconstituted DNA-binding and dissected the interactions with NF-Y. RFX and NF-Y do not interact in solution, but make cooperative interactions in EMSA: a minimal NF-Y, composed of the evolutionary conserved domains is sufficient and RFXAP N-terminal half is expendable. Altering the X-Y distance abolishes cooperativity, indicating that DNA imposes severe spatial constraints. When tested on a highly positioned nucleosome, RFX binds DNA well and NF-Y does not further increase its affinity. Transfections of NF-Y subunits, but not RFX, in class II negative cells improves basal transcription and coexpression of the two activators has a synergistic effect, while modestly increasing CIITA-mediated activation. These results show that interactions between the two trimers on DNA are key to MHC class II expression.
(ii) NF-Y and TFIID contains histone fold subunits: the H2B-H2A-like NF-YB-NF-YC and hTAFII20-hTAFII135, the H3-H4-like hTAFII31-hTAFII80, and hTAFII18-hTAFII28. Using Agarose-EMSA, we find that NF-Y recruits purified holo-TFIID on the Ea promoter. We dissected the interplay between NF-Y and isolated TAFIIs with histonic structures in EMSA, protein-protein interactions, DNase I footprinting and transfections. hTAFII20, hTAFII28, hTAFII18-hTAFII28, hTAFII80 and hTAFII31-hTAFII80, but not hTAFII18 and hTAFII135, bind to NF-Y histonic subunits in solution; only hTAFII31-hTAFII80 has intrinsic, but not sequence-specific Ea binding capacity and modifies NF-Y footprints, protecting the Ea -30/-5 region: this indicates thast they are important for NF-Y-mediated recruitment of TFIID on Ea. Expression of hTAFII28 and hTAFII18 in mouse cells significantly and specifically reduces NF-Y activation in GAL4-based fusion experiments. NF-Y can therefore bind multiple TAFIIs, potentially accomodating a vast array of diverse promoters.
Taken together, these data establish NF-Y as a pivotal protein in the recruitment of both "upstream" and "basal" factors on CCAAT-containing promoters.

A key role for the histone H4 N-terminus in nucleosome remodeling by ISWI and CHRAC.

Cedric R. Clapier [1, 2], Gernot Langst[1], Davide F.V. Corona[2], Peter B. Becker[1,4] & Karl P. Nightingale.[3]

Adolf Butenandt Institut, Molekularbiologie, Luwig-Maximilians-Universitat Munchen, Schillerstrasse 44, 80336 Munchen, Germany. [2] EMBL International PhD Programme. [3] Dept. of Biochemistry, University of Cambridge, 80 Tennis Court Rd, Cambridge, CB2 1GA. UK.

Chromatin accessibility complex (CHRAC) belongs to the family of chromatin remodeling complexes that contain the ATPase ISWI as their catalytic core. ISWI catalyzes a number of ATP-dependent chromatin remodeling reactions, reflecting its ability to induce nucleosome sliding, but the underlying mechanism(s) of chromatin remodelling remain mysterious. The ATPase activity of ISWI is stimulated by nucleosomes, but not by free histones and only poorly by free DNA. As a first step towards defining the interaction of a remodeling factor with its substrate we generated a set of "hybrid" nucleosomes from recombinant histones which lacked defined individual histone N-termini. We show that the histone H4 N-termini (in the context of a nucleosome) are both necessary and sufficient to stimulate ISWI-dependent ATPase activity. These domains are also essential for chromatin remodelling; both for isolated ISWI to generate nucleosome regularity and for CHRAC to induce nucleosome sliding.

Transcriptional control of pancreatic β-cells by nuclear factors causing autosomal dominant diabetes

M Parrizas, MA Maestro, A Peralvarez, S Fernandez, J Ferrer

H. Clínic Universitari. IDIBAPS. Barcelona, Spain

Recent human genetic studies have identified mutations in IPF1/IDX1/PDX1, HNF1α, HNF1β, and HNF4α as the cause of autosomal dominant diabetes (MODY). Patients with these defects show impaired insulin secretion in response to glucose, although the precise mechanisms underlying β-cell dysfunction remain unknown. The fact that numerous transcription factor defects result in a nearly identical phenotype suggests the existence of a common regulatory network involving both cell-specific and more ubiquitously expressed genes. Using HNF1α- nullizygous mice, we show that insulin-producing pancreatic cells are largely intact and normally arranged in islet structures in the absence of HNF1α, but they display abnormal gene expression patterns. We have thus identified GLUT-2 glucose transporter as a pancreatic β-cell specific target of HNF1α by immunohistochemistry and RT-PCR analyses. Transiently transfected minigenes containing 5' flanking mouse GLUT-2 sequences can be induced by both HNF1α and IPF1/IDX1, albeit at different cis elements, suggesting that the two MODY genes may act on a common target. Using chromatin immunoprecipitation experiments, we show that both HNF1α and IPF1/IDX1 directly interact with the mouse GLUT-2 promoter *in vivo* in a β-cell pancreatic line. These results provide a model to dissect common molecular mechanisms involved in the transcriptional control of pancreatic β-cell function by nuclear factors causing MODY.

GENETIC DISSECTION OF TRANSCRIPTIONAL ACTIVATION BY Rap1p. EVIDENCE FOR A MULTI-STEP PROCESS.

Fatima-Zahra IDRISSI, Natalia GARCÍA-REYERO, and Benjamin PIÑA.
Centre d'Investigació i Desenvolupament. Consejo Superior de Investigaciones Científicas. Jordi Girona 18, 08034 Barcelona, SPAIN
Tel. 343 4006157. FAX: 343 2045904. Email: bpcbmc@cid.csic.es

Rap1p binds to the DNA consensus sequence ACACCCAYACAYYY. We have shown that transcriptional activation by Rap1p were allosterically controlled by the DNA sequence it is bound to. When Y bases were Cs (telomeric sites), thrancriptional synergism between adjcent sites was lower than when these bases were Ts (UASrpg sites). In addition, only transcriptional synergism through UASrpgs was orientation dependent. We have dubbed "RPG effect" the increased, orientation-dependent activation through UASrpgs.

We performed a genetic analysis of transcriptional activation by Rap1p in different genetic backgrounds, using artificial reporters with various combinations of Rap1p DNA binding sites. Our results classify the different constructs into four transcriptional status: A- Repressed (no Rap1p DNA binding sites); B- De-repressed (a single site, regardless its sequence and orientation); C- Activated (two telomeric sites or two UASrpgs in the reverse orientation); and D- RPG effect (two UASrpgs in the forward orientation). These different classes of constructs were selectively affected by some mutations. Histone depletion abolished repression (class A), but affected very little the rest. Deletion of the Hrs1 gene potentiated de-repression (class B), but had milder or no effects in the rest. SWI mutants almost abolished the synergistic effect of two telomeric sequences (class C), affected much less de-repression (class B) and had no effect on the rest. Up to now, we have found no mutation affecting the RPG effect.

We interpret these results as indicative for a multi-step process for Rap1p-dependent transcriptional activation. In a first step, a single Rap1p molecule would alleviate chromatin repression with minimal interactions with the transcriptional machinery; this effect may facilitate binding of other factors to DNA. In a second step, two adjacent Rap1p molecules activate transcription by themselves, probably by increasing recruitment of the SWI/SNF and/or other chromatin-remodeling complexes. The third step, the RPG effect, seemed independent from chromatin modification and remodeling complexes (SWI/SNF, Ada, and SAGA), and unaffected by mutations on the mediator complex (*hrs1* and *gal11*). We propose it is mediated by the interaction of Rap1p/UASrpg complexes with a still unknown cofactor, whose characterization we are currently aiming to.

Role of the forkhead thyroid-specific transcription factor TTF-2 and the constitutive factor CTF/NF-1 in the control of the thyroperoxidase gene expression.

Lourdes Ortiz, Isabel Cuesta and Pilar Santisteban.
Instituto de Investigaciones Biomédicas *Alberto Sols* (CSIC), Arturo Duperier 4. E-28029, Madrid (Spain)

The mechanisms by which cells selectively activate the transcription of a specific gene are essential. Tissue-specific transcription factors are the main mediator of tissue-specific gene expression. It has become clear that transcriptional activation is defined not only by the activity of an individual factor, but rather, depends on combinatorial interactions between multiple proteins. The focus of our work has been to understand the regulatory mechanism underlying hormonal transcription of the thyroperoxidase (TPO), a tissue-specific gene expressed only in differentiated thyroid cells. To TPO promoter bind three thyroid-specific transcription factors: and an ubiquitous factor. The tissue-specific factors, TTF-1, Pax-8 and TTF-2 are members of the homeo-, paired-box and forkhead transcription factors respectively. Recently we have identified the ubiquitous factor as a member of the CTF/NF-1 family of constitutive transcription factors. Several ligands regulate the expression of the TPO gene mainly through the *cis* element where TTF-2 binds. We have demonstrated that TTF-2 is under hormonal control and that the TTF-2 binding site acts as a hormone response element. This function depends on multimerization and specific orientation suggesting that TTF-2 is part of a complex interaction network within the TPO promoter. Neighboring regulatory elements of TTF-2 bind TTF-1 and CTF/NF-1. By GST-pull down assay we have evidence that both TTF-2 and CTF/NF-1 interact physically. This interaction appears to be functional, since the TPO promoter activity is lost in transfection experiments in which the distance between CTF/NF-1 and TTF-2 binding site has been altered. Thus, the control of TPO transcription, which take place exclusively in thyroid-differentiated cells, depends on the correct stereospecific interaction of TTF-2 and CTF/NF1.

In these previous results, obtained *in vitro*, the influence of other factors such as the chromatin structure, the relative abundance of other nuclear proteins and the methylation state of the TPO promoter has not been studied. For this reason we have determined the genomic organization of the TPO promoter by *in vivo* footprinting. The most prominent protection in both DNA strands were found in the guanines located within the CTF/NF-1 and the TTF-2 binding-sites, confirming our *in vitro* results. The TTF-1 binding sites are more sensible to methylation. In the lower strand we have observed that the guanines located in the Pax-8 binding site are protected to methylation. Furthermore, the binding of this factor modulates the DNA conformation, leading to the more adjacent guanines to be more exposed and generating regions sensible to hypermethylation. Another important region of hypermethylation is located in the region adjacent to the TATA box, mainly due to the binding of the basic transcription machinery. Thus, we can conclude that the most active sites in the TPO transcription are the CTF/NF-1, TTF-2 and Pax-8 binding-sites.

The interaction observed between CFT/NF-1 and the forkhead factors could be a general mechanism of action of both families of transcription factors. A similar example has been reported in the control of albumin transcription by HNF3ß. The conserved interaction between these families of transcription factors might be explained by their specific properties. Thus, the CTF/NF-1 binding site is masked inside of the nucleosomal structure. The binding of the forkhead proteins to their cognate site desestabilizes this structure and makes the CTF/NF-1 site accessible to exert its transactivation effect. The ability of the forkhead domain to induce DNA bending would favor its contact with CTF/NF1 factors.

These results open a very interesting question in the thyroid cells context concerning how site-specific DNA binding factors regulate higher-order chromatin structure, and thereby control the transcriptional competence of target thyroid-specific genes. To this respect, TTF-2 would be a possible candidate playing a similar role as chromatin regulator as HNF-3ß, another member of the forkhead family structurally similar to linker histone proteins.

A helix-turn motif in the C-terminal domain of histoneH1.

Roger Vila[1], Imma Ponte[1], M. Angeles Jiménez[2], Manuel Rico[2] and Pedro Suau[1]

[1]Departamento de Bioquímica y Biología Molecular. Facultad de Ciencias Universidad Autónoma de Barcelona. 08193 Bellaterra. Barcelona. Spain.
[2]Instituto de Estructura de la Materia. CSIC. Serrano, 119. 28006 Madrid. Spain.

The structural study of peptides belonging to the terminal domains of histone H1 can be considered as a step towards the understanding of the function of H1 in chromatin. The conformational properties of the peptide Ac-EPKRSVAFKKTKKEVKKVATPKK (CH-1), that belongs to the C-terminal domain of histone H1° (residues 99-121) and is adjacent to the central globular domain of the protein, were examined by means of ^{1}H-NMR and CD. In aqueous solution CH-1 behaved as a mainly unstructured peptide, although turn-like conformations in rapid equilibrium with the unfolded state could be present. Addition of trifluoroethanol resulted in a substantial increase of the helical content. The helical limits, as indicated by (i, i+3) NOE cross-correlations and significant up-field conformational shifts of the $C^{\alpha}H$ protons, span from Pro100 to Val116, with Glu99 and Ala117 as N- and C-caps. A structure calculation performed on the basis of distance constraints derived from NOE cross-peaks in 90% trifluoroethanol confirmed the helical structure of this region. The helical region has a marked amphipathic character, due to the location of all positively charged residues on one face of the helix and all the hydrophobic residues on the opposite face. The peptide has a TPKK motif at the C-terminus, following the α-helical region. The observed NOE connectivities suggest that the TPKK sequence adopts a type (I) β-turn conformation, a σ-turn conformation or a combination of both, in fast equilibrium with unfolded states. Sequences of the kind (S/T)P(K/R)(K/R) have been proposed as DNA binding motifs. The CH-1 peptide, thus, combines a positively charged amphipathic helix and a turn as potential DNA-binding motifs.

PML is associated with histone deacetylase complex and regulates Rb-mediated transcriptional repression of E2F.

Sadeq Vallian1*, Wen-Shu Wu2, Sharon Roth2, Wen-Ming Yang3, Edward Seto3, and Kun-Sang Chang2

1Division of Bilogy, Faculty of Sciences, Isfahan University, Iran; 2Department of Laboratory Medicine, 3Department of Biochemistry and Molecular Biology, University of Texas MD Anderson Cancer Center; and 4H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, USA..

*The presenter
Email: svallian@genetics.nrcgeb.ac.ir Tel/Fax: 0098-31-684669

The promyelocytic leukemia protein, PML, was originally identified in the breackpint region of the t(15;17) chromosomal translocation in acute promyelocytic leukemia (APL). PML is a nuclear phosphoprotein that functions as a growth suppressor. Our previous study showed that PML can function as a transcriptional repressor and was found to be able to specifically repress the Sp1-mediated transcriptional activity of the epidermal growth factor receptor (EGFR) promoter. Here, our data demonstrate that PML represses transcription by association with histone deacetylase (HDAC). PML coimmunoprecipitated HDAC activity in an Rb-independent manner and colocalized with HDAC *in vivo*. All three known isoforms of HDACs were found to interact physically with PML *in vitro*. Transient expression of PML significantly reduced histone-3 acetylation of its target site in vivo. Furthermore, expression of PML derepressed Rb-mediated transcriptional repression of E2F target gene. This data support a role for PML in chromatin structure and assembly which its disruption in APL cells may conribute to the development of leukemia.

A glimpse at subtelomeric order

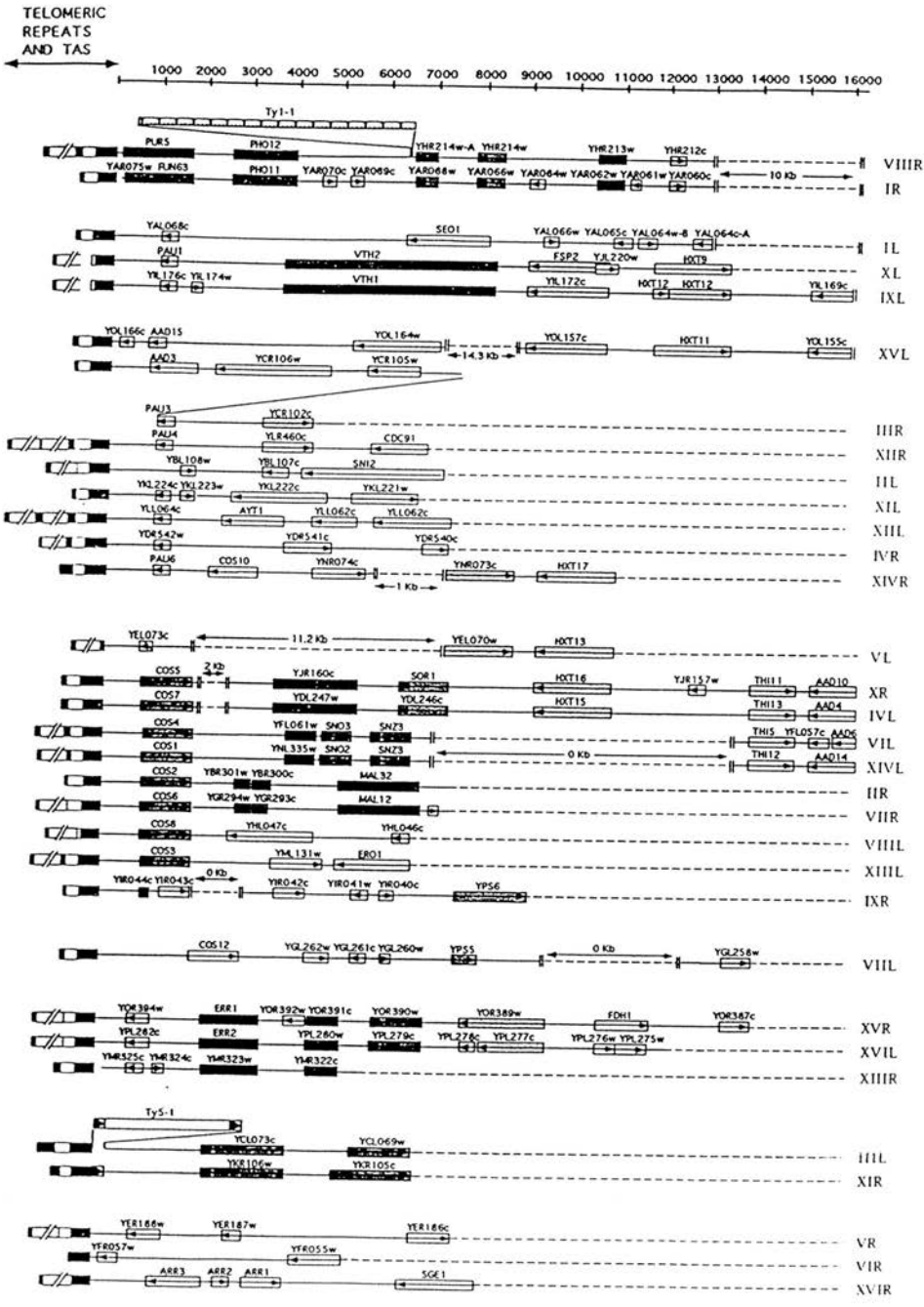
Miguel A. Vega-Palas
Instituto de Bioquímica Vegetal y Fotosíntesis, Centro de Investigaciones
Isla de la Cartuja, c/ Américo Vespucio s/n, 41092 Seville, Spain.

Telomeres play an essential role in cell biology stabilizing chromosomes and facilitating complete replication of chromosomal termini. Telomeric DNA usually contains tandem repetitions of a short motif flanked by middle repetitive elements. These elements are known as TAS, from Telomere Asociated Sequences, and can be followed by unique or by repetitive DNA. Therefore, telomeric regions have a complex organization[1-3]. In *Saccharomyces cerevisiae,* telomeric sequences are composed of about 350 bp of tandem $(C_{1-3}A)_n$ repeats. These repeats are followed by two main TAS: the Y' and the X elements[4]. Y' elements are found only in a subset of the telomeres. X elements are present in all telomeres and exist in two main forms: a complete form containing the X core and the STR-A,B,C,D elements, or a short form containing essentially the X core or part of it[4-6]. When both TAS are present at the same region the X elements are always located after the Y' elements, at their centromeric side. They can be separated by interstitial $(C_{1-3}A)_n$ repeats.

This report shows a map of all the telomeric regions from *Saccharomyces cerevisiae*. It is the first telomeric map described for an eukaryotic organism. It includes all the TAS and many subtelomeric genes. In addition, this map also displays several subtelomeric groups of homology identified in this study. The identification of these groups of homology allows to propose the formation of multifiber heterohcromatin complexes that correspond with specific telomeric agroupations. The structural and functional significance of these groups of homology will be discussed.

References

1. Blackburn, E. *Nature* **350**, 569-573 (1991).
2. Greider, C. *Annu. Rev. Biochem.* **65**, 337-365 (1996).
3. Zakian, V. *Annu. Rev. Genet.* **23**, 579-604 (1989).
4. Louis, E. *Yeast* **11**, 1553-1573 (1995).
5. Chan, C. & Tye, B. *Cell* **33**, 563-573(1983).
6. Walmsley, R., Chan, C., Tye, B. & Petes, T. *Nature* **310**,157-160 (1984).



Loss- and gain-of-function mutations show a Polycomb group gene function for Ring1A in mice.

Camelia Marcos-Rodríguez[1], María del Mar Lorente[1], Claudia PŽrez[1,2], Jon Schoorlemmer[1], Angel Ramírez[3], Thomas Magin[4] and Miguel Vidal[1]

[1]Developmental and Cell Biology, Centro de Investigaciones Biológicas, Velázquez 144, 28006 Madrid, Spain.

[2]Department of Animal Pathology: Animal Medicine, Faculty of Veterinary Science, University of León, 24071 León, Spain.

[3]Cell and Molecular Biology, CIEMAT, Avda. Complutense, 28040 Madrid, Spain.

[4]Department of Molecular Biology, Institut for Genetics, University of Bonn, Ršmerstrasse 164, Bonn 53117, Germany.

The products of the Polycomb group (PcG) of genes act as transcriptional repressors involved in the maintenance of homeotic gene expression patterns throughout development, from flies to mice. Biochemical and molecular evidence suggests that the mouse *Ring1A* gene is a member of the PcG of genes. However, genetic evidence is needed to establish PcG function for Ring1A, since contrary to all other murine PcG genes, there is no known *Drosophila* PcG gene encoding a homolog of the Ring1A protein. To study*Ring1A* function we have generated a mouse line lacking *Ring1A*, and mouse lines overexpressing *Ring1A*. Homozygous *Ring1A* $^{-/-}$ mice show anterior transformations and other abnormalities of the axial skeleton. Ectopic expression of *Ring1A* also results in dose-dependent anterior transformations of vertebral identity. We demonstrate that *Ring1A* is involved in the repression of *Hox* genes, as indicated by the rostral and caudal shifts of the anterior boundaries of a subset of *Hox* genes in *Ring1A* $^{-/-}$ and *Ring1A* overexpressing mice, respectively. Taken together, these results provide genetic evidence for a PcG function of the mouse *Ring1A* gene, although its contribution to segmental identity can differ from that of other PcG genes.

LIST OF INVITED SPEAKERS

Juan Ausió	Dept. of Biochemistry and Microbiology, University of Victoria, British Columbia V8W 3P6 (Canada). Tel.: 1 250 721 8863. Fax: 1 250 721 8855. E-mail: jausio@uvic.ca
Miguel Beato	I.M.T., Philipps-Universität. E.Mannkopff-Str. 2, D-35037 Marburg (Germany). Tel.: 49 6421 286 6286. Fax: 49 6421 286 5398. E-mail: BEATO@IMT.Uni-Marburg.DE
Marco E. Bianchi	DIBIT, Istituto Scientifico San Raffaele. via Olgettina 58, 4 piano A1, I-20132 Milano (Italy). Tel.: 39 02 26434 780. Fax: 39 02 26434 861. E-mail: bianchi.marco@hsr.it
Adrian Bird	Institute of Cell and Molecular Biology, University of Edinburgh, The King's Buildings, EH9 3JR Edinburgh (UK). Tel.: 44 131 650 5670. Fax: 44 131 650 5379. E-mail: A. Bird@ed.ac.uk
Pierre Chambon	IGBMC, CNRS/INSERM/ULP/Collège de France. BP 163, 67404 Illkirch Cedex, C.U. De Strasbourgh (France). Tel.: 33 3 88 6532 13. Fax: 33 3 88 65 3203. E-mail: chambon@ titus.u-strasbg.fr
Beverly M. Emerson	The Salk Institute for Biological Studies. 10010 North Torrey Pines Road, La Jolla, CA.92037 (USA). Tel.: 1 858 453 6560. Fax: 1 858 535 8194. E-mail: emerson@salk.edu
Luis Franco	Dept. of Biochemistry and Molecular Biology, University of Valencia. Dr. Moliner 50, 46100 Burjassot, Valencia (Spain). Tel.: 34 96 386 43 85. Fax: 34 96 386 46 35. E-mail: luis.franco@uv.es
Susan M. Gasser	Swiss Institute for Experimental Cancer Research (ISREC). Chemin des Boveresses 155, CH-1066 Epalinges/Lausanne (Switzerland). Tel.: 41 21 692 5886. Fax: 41 21 652 6933. E-mail: sgasser@eliot.unil.ch
Frank Grosveld	Dept. of Cell Biology and Genetics, Erasmus University Rotterdam. P.O. Box 1738, 3000 DR Rotterdam (The Netherlands). Tel.: 31 10 408 71 69. Fax: 31 10 408 9468
Wolfram Hörz	Institut für Physiologische Chemie, Universität München. Schillerstr. 44, 80336 München (Germany). Tel.: 49 89 5996 420. Fax: 49 89 5996 440. E-mail: hoerz@bio.med.uni-muenchen.de
Katherine A. Jones	The Salk Institute for Biological Studies. 10010 North Torrey Pines Road, La Jolla, CA.92037 (USA). Tel.: 1 858 453 4100. Fax: 1 858 535 8194. E-mail: kathy_jones@qm. salk.Edu

James T. Kadonaga	Dept. of Biology, 0347, University of California, San Diego. 9500 Gilman Drive, La Jolla, CA.92093-0347 (USA). Tel.: 1 858 534 4608. Fax: 1 858 534 0555. E-mail: jkadonaga@ucsd.edu
Ulrich Laemmli	Depts. of Biochemistry and Molecular Biology, Geneva University, Sciences II. 30 Quai Ernest-Anserment, CH-1211 Geneva 4 (Switzerland). Tel.: 41 22 702 6122. Fax: 41 22 702 6868. E-mail: Ulrich.Laemmli@molbio.unige.ch
Michael Levine	Dept. Mol. Cell. Biol. Division of Genetics and Development, University of California. (USA). Present address: Zürich University (Sabbatical year), Zürich (Switzerland). Fax: 41 1 635 6820
Enrique Palacián	Centro de Biología Molecular "Severo Ochoa", CSIC-UAM, 28049 Madrid (Spain). Fax: 34 91 397 47 99. E-mail: epalacian@trasto.cbm.uam.es
Danny Reinberg	Howard Hughes Medical Institute, Div. of Nucleic Acid Enzymology, Dept. of Biochemistry, Robert Wood Johnson Medical School, Piscataway, NJ.08854 (USA). Tel.: 1 732 235 4195. Fax: 1 732 235 5294. E-mail: reinbedf@UMDNJ.EDU
Robert G. Roeder	Lab. of Biochemistry & Molecular Biology. The Rockefeller University. 1230 York Avenue, New York. NY.10021 (USA). Tel.: 1 212 327 7000. Fax: 1 212 327 7949. E-mail: roeder@rockvax.rockefeller.edu
Frank Sauer	ZMBH. Im Neuenheimer Feld 282, 69120 Heidelberg (Germany). Tel.: 49 6221 546 858. Fax: 49 6221 545 894. E-mail: f.sauer@mail.zmbh.uni-heidelberg.de
Robert Tjian	Howard Hughes Medical Institute, University of California, Berkeley, Molecular and Cell Biology Department. 401 Barker Hall, Berkeley, CA.94720-3204 (USA). Tel.: 1 510 642 4442. Fax: 1 510 643 9547. E-mail: jmlim@uclink4.Berkeley.edu
Carl Wu	Lab. of Molecular Cell Biology, NIH. 37 Convent Drive, Bethesda, MD.20892 (USA). Tel.: 1 301 435 3700. Fax: 1 301 435 3697. E-mail: carlwu@helix.nih.gov
Keith Yamamoto	Dept. of Cellular and Molecular Pharmacology, University of California, San Francisco CA.94143-0450 (USA). Tel.: 1 415 476 8445. Fax: 1 415 476 6129. E-mail: yamamoto@cgl.ucsf.edu

LIST OF PARTICIPANTS

Francisco Antequera	Instituto de Microbiología Bioquímica. CSIC/Universidad de Salamanca. Campus Miguel de Unamuno, 37007 Salamanca (Spain). Tel.: 34 923 29 44 62. Fax: 34 923 22 48 76
Fernando Azorín	Dept. de Biologia Molecular i Cel.lular. Institut de Biologia Molecular de Barcelona-CSIC. Jordi Girona Salgado 18-26, 08034 Barcelona (Spain). Tel.: 34 93 400 61 37. Fax: 34 93 204 59 04. E-mail: fambmc@cid.csic.es
Enrique Castaño	Eukaryotic Gene Regulation Laboratory, Marie Curie Research Institute, RH8 0TL The Chart, Oxted (UK). Tel.: 44 1883 722 306. Fax: 44 1883 714 375. E-mail: e.castano@mcri.ac.uk
Margarita Cervera	Instituto de Investigaciones Biomédicas, CSIC. Arturo Duperier 4, 28029 Madrid (Spain). Tel.: 34 91 397 54 02. Fax: 34 91 585 45 87. E-mail: margarita.cervera@uam.es
Sebastián Chávez	Dpto. de Genética, Facultad de Biología, Universidad de Sevilla, 41012 Sevilla (Spain). Tel.: 34 95 455 09 20. Fax: 34 95 455 71 04. E-mail: schavez@cica.es
Claudia Crosio	IGBMC. B.P. 163, 67404 Illkirch CU de Strasbourg (France). Tel.: 33 388 653 406. Fax: 33 388 65 32 46. E-mail: claudia@titus. u-strasbg.fr
Joan Ramon Daban	Departament de Bioquimica i Biologia Molecular, Facultat de Ciéncies, Universitat Autónoma de Barcelona, 08193 Bellaterra, Barcelona (Spain). Tel.: 34 93 581 12 64. Fax: 34 93 581 12 64. E-mail: JoanRamon.Daban@uab.es
Dale Dorsett	Memorial Sloan-Kettering Cancer Center, Molecular Biology Program. 1275 York Ave, New York, NY.10021 (USA). Tel.: 1 212 639 8498. Fax: 1 212 717 3623. E-mail: d-dorsett@ski.mskcc.org
Karl Ekwall	University College Södertörn. Box 4101, S- 141 04 Huddinge (Sweden). Tel.: 46 5858 887 19. Fax: 46 8 585 885 10. E-mail: karl.ekwall@cbt.ki.se
Patrick E. Fields	Yale University School of Medicine, HHMI. Section of Immunology. 310 Cedar Street, New Haven, CT.06510 (USA). Tel.: 1 203 785 5383. Fax: 1 203 737 1764. E-mail: pef2@midway.uchicago.edu
Judith Glaven	Cell Press. 1050 Massachusetts Avenue, Cambridge, MA. (USA). Tel.: 1 617 661 7057. Fax: 1 617 661 7061. E-mail: jglaven@cell.com

Terri Grodzicker	Genes & Development, Cold Spring Harbor Laboratory Press. 10 Skyline Drive, Plainview, NY.11803 (USA). Fax: 1 516 367 8532. E-mail: grodzick@cshl.org
Akash Gunjan	Imperial Cancer Research Fund, Clare Hall Laboratories, Blanche Lane. South Mimms, EN6 3LD Potters Bar, Herts (U.K.). Tel.: 44 171 269 3842. Fax: 44 171 269 3801. E-mail: akash_gunjan@hotmail.com
Guy Haegeman	Dept. of Molecular Biology. University of Gent - VIB. Ledeganckstraat 35, 9000 Gent (Belgium). Tel.: 32 9 264 5166. Fax: 32 9 264 5304. E-mail: guy.haegeman@dmb.rug.ac.be
Dirk A. Kleinjan	MRC Human Genetics Unit. Crewe Road, EH4 2XU Edinburgh (UK). Tel.: 44 131 332 2471. Fax: 44 131 343 2620
Albert Lai	McGill University, McIntyre Medical Building, Dept. of Biochemistry. 3655 Promenade Sir William Osler, Rm 822, H3G 1Y6 Montreal, PQ. (Canada). Tel.: 1 514 398 72 68. Fax: 1 514 398 73 84. E-mail: lai@med.mcgill.ca
Roberto Mantovani	Dipt. di Genetica e di Biologia dei Microorganismi. Via Celoria 26, 20143 Milano (Italy). Tel.: 39 02 266 05 239. Fax: 39 02 266 45 51. E-mail: mantor@mailserver.unimi.it
Karl P. Nightingale	Dept. of Biochemistry. University of Cambridge. 80 Tennis Court Rd, Cambridge CB2 1GA (UK). Tel.: 44 1223 333 669. Fax: 44 1223 333 743. E-mail: knpn22@mole.bio.cam.ac.uk
Marcelina Parrizas	Hospital Clínic Universitari, IDIBAPS. c/Villarroel 170, 08036 Barcelona (Spain). Tel.: 34 93 227 54 00. Fax: 34 93 451 52 72. E-mail: parrizas@clinic.ub.es
Benjamín Piña	Centre d'Investigació i Desenvulupament, CSIC. Jordi Girona 18, 08034 Barcelona (Spain). Tel.: 34 93 400 61 57. Fax: 34 93 204 59 04. E-mail: bpcbmc@cid.csic.es
Guy Riddihough	Science. 1200 New York Avenue, 20005 NW Washington, DC. (U.S.A.). Tel.: 1 202 326 7097. Fax: 1 202 789 4669. E-mail: griddiho@aaas.org
Pilar Santisteban	Instituto de Investigaciones Biomédicas Alberto Sols, (CSIC). Arturo Duperier 4, 28029 Madrid (Spain). Tel.: 34 91 585 46 44. Fax: 34 91 585 45 87. E-mail: psantisteban@iib.uam.es
Michael H. Sieweke	Centre d'Immunologie Marseille Luminy, Parc Scientifique de Luminy. Case 906, 13288 Marseille Cedex (France). Tel.: 33 4 91 26 94 94. Fax: 33 4 91 26 94 30. E-mail: sieweke@ciml.univ-mrs.fr

Pedro Suau	Dpto. de Bioquímica y Biología Molecular, Facultad de Ciencias, Univ. Autónoma de Barcelona, 08193 Bellaterra, Barcelona (Spain). Tel.: 34 93 581 1391. Fax: 34 93 581 1264. E-mail: Pere.Suau@uab.es
Didier Trouche	UPR 9006 CNRS. 118 route de Narbonne, 31 062 Toulouse Cedex (France). Tel.: 33 5 61 33 59 15. Fax: 33 5 61 33 58 86. E-mail: trouche@ibcg.biotoul.fr
Sadeq Vallian	Division of Biology, Faculty of Sciences, Isfahan University, Isfahan (Iran). Fax: 98 31 684 669. E-mail: svallian@genetics.nrcgeb.ac.ir
Miguel A. Vega Palas	Instituto de Bioquímica Vegetal y Fotosíntesis, Centro de Investigaciones Isla de la Cartuja. Américo Vespucio s/n, 41092 Sevilla (Spain). Tel.: 34 95 448 95 01. Fax: 34 95 446 00 65. E-mail: palas@cica.es
Miguel Vidal	Developmental and Cell Biology, Centro de Investigaciones Biológicas. Velázquez 144, 28006 Madrid (Spain). Tel.: 34 91 561 18 00. Fax: 34 91 562 75 18. E-mail: mvidal@cib.csic.es
P. Anthony Weil	Dept. of Molecular Physiology & Biophysics, Vanderbilt University, School of Medicine, Nashville, TN.37232-0615 (USA). Tel.: 1 615 322 7007. Fax: 1 615 322 7236. E-mail: tony.weil@mcmail.vanderbilt.edu
Kerstin Weiss	Institute for Molecular Biology and Biophysics. ETH. Honggerberg HPK F6, Zürich (Switzerland). Tel.: 41 1 633 2476. Fax: 41 1 6331282. E-mail: weiss@mol.biol. ethz.ch

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.

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.

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.

*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**

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.

83 **Workshop on Bacterial Transcription Factors Involved in Global Regulation.**
Organizers: A. Ishihama, R. Kolter and M. Vicente.

84 **Workshop on Nitric Oxide: From Discovery to the Clinic.**
Organizers: S. Moncada and S. Lamas.

85 **Workshop on Chromatin and DNA Modification: Plant Gene Expression and Silencing.**
Organizers: T. C. Hall, A. P. Wolffe, R. J. Ferl and M. A. Vega-Palas.

86 **Workshop on Transcription Factors in Lymphocyte Development and Function.**
Organizers: J. M. Redondo, P. Matthias and S. Pettersson.

87 **Workshop on Novel Approaches to Study Plant Growth Factors.**
Organizers: J. Schell and A. F. Tiburcio.

88 **Workshop on Structure and Mechanisms of Ion Channels.**
Organizers: J. Lerma, N. Unwin and R. MacKinnon.

89 **Workshop on Protein Folding.**
Organizers: A. R. Fersht, M. Rico and L. Serrano.

90 **1998 Annual Report.**

91 **Workshop on Eukaryotic Antibiotic Peptides.**
Organizers: J. A. Hoffmann, F. García-Olmedo and L. Rivas.

92 **Workshop on Regulation of Protein Synthesis in Eukaryotes.**
Organizers: M. W. Hentze, N. Sonenberg and C. de Haro.

93 **Workshop on Cell Cycle Regulation and Cytoskeleton in Plants.**
Organizers: N.-H. Chua and C. Gutiérrez.

94 **Workshop on Mechanisms of Homologous Recombination and Genetic Rearrangements.**
Organizers: J. C. Alonso, J. Casadesús, S. Kowalczykowski and S. C. West.

95 **Workshop on Neutrophil Development and Function.**
Organizers: F. Mollinedo and L. A. Boxer.

96 **Workshop on Molecular Clocks.**
Organizers: P. Sassone-Corsi and J. R. Naranjo.

97 **Workshop on Molecular Nature of the Gastrula Organizing Center: 75 years after Spemann and Mangold.**
Organizers: E. M. De Robertis and J. Aréchaga.

98 **Workshop on Telomeres and Telomerase: Cancer, Aging and Genetic Instability.**
Organizer: M. A. Blasco.

99 **Workshop on Specificity in Ras and Rho-Mediated Signalling Events.**
Organizers: J. L. Bos, J. C. Lacal and A. Hall.

100 **Workshop on the Interface Between Transcription and DNA Repair, Recombination and Chromatin Remodelling.**
Organizers: A. Aguilera and J. H. J. Hoeijmakers.

101 **Workshop on Dynamics of the Plant Extracellular Matrix.**
Organizers: K. Roberts and P. Vera.

102 **Workshop on Helicases as Molecular Motors in Nucleic Acid Strand Separation.**
Organizers: E. Lanka and J. M. Carazo.

103 **Workshop on the Neural Mechanisms of Addiction.**
Organizers: R. C. Malenka, E. J. Nestler and F. Rodríguez de Fonseca.

104 **1999 Annual Report.**

105 **Workshop on the Molecules of Pain: Molecular Approaches to Pain Research.**
Organizers: F. Cervero and S. P. Hunt.

106 **Workshop on Control of Signalling by Protein Phosphorylation.**
Organizers: J. Schlessinger, G. Thomas, F. de Pablo and J. Moscat.

107 **Workshop on Biochemistry and Molecular Biology of Gibberellins.**
Organizers: P. Hedden and J. L. García-Martínez.

---

*: Out of Stock.

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 1999, a
total of 136 meetings and 11
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 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/biology

The lectures summarized in this publication were presented by their authors at a workshop held on the 10th through the 12th of April, 2000, at the Instituto Juan March.

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

There is a limited edition of 450 copies of this volume, available free of charge.