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Fundación Juan March

Workshop on

Chromatin Structure and Gene Expression

Organized by

F. Azorín, M. Beato and A. A. Travers

F. Azorín
M. Beato
H. Cedar
R. Chalkley
M. E. A. Churchill
D. Clark
C. Crane-Robinson
J. R. Dabán
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PROGRAMME

Monday 24th September

1st Lecture Session: "Nucleosome structure"

Chairman: J. A. Subirana

9.00 - A.A. TRAVERS "DNA determinants of nucleosome positioning".

9,40 - F. AZORIN "Structural polymorphism of homopurine-homopyrimidine DNA sequences".

10,20 - T.J. RICHMOND "Structural studies on chromatin".

11,00 - Coffee break

11,30 - M.E.A. CHURCHILL "Protein motifs that recognize structural features of DNA".

15,00 - POSTER SESSION I

2nd Lecture Session: "Higher order structure".

Chairman: P. Puigdomenech

16,00 - A. RUIZ-CARRILLO "Regulation and role of H5 expression in erythroid cell differentiation".

16,40 - S.C.R. ELGIN "Chromatin structure and gene expression in drosophila".

17,20 - A.E. SIPPEL "Structural and functional organization of genomic chromatin domains".

18,00 - Coffee break

18,30 - H. CEDAR "The mapping of DNA replication units in animal cells".

19,10 - U.K. LAEMMLI "Topoisomerase II is required for chromosome condensation".

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Tuesday 25th September

3rd Lecture Session:	"Chromatin assembly and protein binding".
9,00 - T. KOLLER	"Nucleosome assembly in cell extracts".
9,20 - J.M. SOGO	"Nucleolar chromatin structure and its relation to rRNA synthesis".
10,00 - D. RHODES	"Differential nucleosome positioning on Xenopus oocyte and somatic 5S RNA genes".
10,20 - G.L. HAGER	"Access of transcription factors to regulatory sequences is modulated by chromatin structure".
11,00 - Coffee break	
11,30 - M. BEATO	"Nucleosome phasing and transcription of the MMTV promoter".
12,10 - Ö. WRANGE	"Derepression of the MMTV promoter by interference of chromatin assembly".
16,00 POSTER SESSION II	
4th Lecture Session:	(Selected Posters)
Chairman:	L. Cornudella
17,00 - R. CHALKLEY	"Role of an upstream element in the developmental regulation of the PEPCK gene chromatin structure".
17,30 - C. CRANE-ROBINSON	"Core histone acetylation in chromatin".

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18,00 - J.R. DABAN — "Reactivity of nucleosomes complexed with excess histones. Different mechanisms for the self-assembly of nucleosome core particles at physiological ionic strength".

18,30 - Cofee break

19,00 - E. DI MAURO — "DNA topology, eukaryotic DNA topoisomerase I, histone octamers".

19,30 - D. RHODES — "The solution structure of the DNA-binding domain of the oestrogen receptor - a 'double loop-zinc-helix' motif".

Wednesday 26th September

5th Lecture Session: "Chromatin structure and transcription. I".

Chairmam: P. Suau

9,00 - F. THOMA — "On the formation of chromatin structures on yeast minichromosomes".

9,40 - R.T. SIMPSON — "Chromatin organization and repression of transcription."

10,20 - W. HÖRZ — "Participation of nucleosomes in gene regulation in yeast".

11,00 - Cofee break

11,30 - D. CLARK — "Nucleosome structure, superhelical stress and transcription".

12,10 - C. WU — "Heat shock regulated transcription of a chromatin template assembled in vitro".

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<u>6th Lecture Session</u>:	"Chromatin structure and transcription. II".
Chairman:	J.R. Dabán
16,30 - J. WORKMAN	"Regulation of class II promoter function during in vitro nucleosome assembly".
17,10 - M. GRUNSTEIN	"Regulation of transcription by histones in the yeast, Saccharomyces cerevisiae".

18,00 - Cofee break

18,30 - ROUND TABLE DISCUSSION

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PREFACE

M. BEATO
Institut für Molekularbiologie
und Tumorforschung
Philipps - Universität
Marburg (Germany)

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Preface

The idea of organizing a Workshop on "Chromatin Structure and Gene Expression" in Madrid occurred to me one year ago as the consequence of three independent developments.

The work of my own group on the hormonal regulation of gene expression had led us to consider an otherwise unexpected role of chromatin structure on transcriptional repression. Much to our surprise, we found in 1989 that the regulatory region of the mouse mammary tumour virus (MMTV) has intrinsic information to wrap itself around the histone octamer in a precise way. Similar findings have been reported by Thomas Pellmann and Örjan Wrange from the Karolinska Institute in Stockholm. The observed rotational phasing of the DNA double helix around the histone octamer is such that the binding site for the transcription factor NF1 is inaccessible for binding, whereas the hormone receptors are perfectly able to recognize their binding sequences in the MMTV-nucleosome. These observations are in agreement with previous *in vivo* findings from Hélène Richard-Foy and Gordon Hager, from the N.I.H., and clearly offer a possibility to explain the inactivity of the MMTV promoter in the absence of hormones on the basis of chromatin structure. The hypothesis of a repression mediated by precise nucleosomal positioning was put forward.

Following completely independent lines of research, work on yeast and Drosophila during the past two years has also provided biochemical and genetic evidence for a role of chromatin structure in gene expression. Sarah Elgin has found that the regulatory region of the hsp26 gene of Drosophila appears to be organized precisely in chromatin in a way that sets the stage for activation by the heat shock factor. Wolfram Hörz from Munich described a similar situation for induction of the PHO5 promoter in yeast. Here, one binding site for the regulatory protein PHO4 is located in the linker region between two positioned nucleosomes. Upon binding of PHO4, the flanking nucleosomes are displaced and two other sets of binding sites for PHO4 and PHO2 are unmasked, leading to activation of

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transcription. Even more convincing arguments for a role of chromatin structure on gene expression derive from genetic evidence obtained in yeast. Michael Grunstein and his colleagues have constructed yeast strains that, when grown on glucose, are depleted of core histones and have a lower nucleosome loading of the DNA *in vivo*. Under these circumstances, most inducible genes become activated to a considerable extent even if their upstream activating sequences (UAS) are deleted. Small mutations in the amino- or carboxy-terminal tails of individual histones, in particular histone H4, resulted in alterations in the inducibility of individual genes. All of these results, in conjunction with the development of new methods for the precise structural analysis of chromatin *in vivo* and *in vitro*, provided a new and more solid basis for considering the role of chromatin structure in gene expression.

Finally, through my previous knowledge of the Fundación Juan March, I was aware of their program **"International Meetings on Biology"** intended to support the organization of small scientific meetings on "state of the art" topics in the field of biological research. When I shared these ideas with Andrés González of the Fundación Juan March, he enthusiastically supported my plans and asked me to formulate a proposal including some twenty invited speakers and a similar number of additional participants.

Being a newcomer to the chromatin field, I needed the help of more experienced persons to put together a meaningful program. I decided to ask Andrew Travers from the MRC in Cambridge, the mecca of chromatin research, who has made essential contributions to our understanding of DNA bending in nucleosomes. Andrew immediately accepted my offer to be an organizer and since then has been indispensable selecting topics and speakers for the Workshop. We also needed a third organizer who, in addition to helping with the general program, was aware of the developments in the chromatin field in Spain, to actively incorporate all relevant Spanish scientists. These two tasks have been accomplished very successfully by Fernando Azorin, from Barcelona, another traditional center of chromatin research. Together, we finally managed to formulate a coherent program proposal that was generously approved

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by the scientific advisory board of the Fundación Juan March. The final Workshop included some 28 oral presentations and 23 posters covering the most relevant areas of research in chromatin structure and function. Of the 53 participants, 16 came from Spain, emphasizing the national interest in this promising field of research.

My motivation in organizing the Workshop was not totally altruistic: I wanted to learn as much as possible on chromatin structure and function in a short time. Now, after the conclusion of the three days meeting, it may be said that this goal was fulfilled to my complete satisfaction. I want to thank my fellow organizers and all the colleagues who accepted our invitation to come to Madrid for making this excellent Workshop possible. The intensive exchange of information and the lively discussions proved that all participants benefitted, and that in spite of its specialization the Workshop was also useful to young scientists and students.

The Workshop was not only a scientific event of very high quality, but was also a great cultural pleasure. The site of the conference at the Fundación Juan March was excellent and the professional care given to every organizational matter was impressive. The quality of the food and wine lett nothing to be desired. In one of the short breaks we had a chance to visit a unique art exhibition by Andy Warhol in the building of the Fundación, and listened to revealing words on the modern version of social success. These experiences will remain in the memories of the participants as an example of an ideal combination of science, art and culture, which after all makes the intellectual sense of our lifes. For this, in the name of all the participants, I would like to thank the Fundación Juan March.

Marburg, October 1, 1990

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ORAL PRESENTATIONS

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DNA determinants of nucleosome positioning.

A. A. Travers, S. C. Satchwell and W.G. Turnell, MRC Laboratory of Molecular Biology, Hills Road, Cambridge.

Although the histone octamer can associate with an immense variety of DNA sequences these proteins can adopt well-defined, even precise locations, with respect to the primary DNA sequence. Such defined nucleosome positions can be described in terms of two parameters: a translation , marking where the histone octamer is placed along the DNA, and a rotation which defines the local orientation of the DNA relative to the protein surface, or more strictly to the direction of curvature of the DNA.

What are the molecular interactions that determine this precise positioning? In the nucleosome core particle the DNA is tightly wrapped with an average radius of curvature of 43Å. To accommodate this bending the local conformation of the DNA must vary such that the grooves must narrow on the inside of the DNA supercoil and widen on the outside. Sequence analysis of histone octamer binding sites revealed a pronounced periodic occurrence of certain short sequence elements such that AT-rich sequences preferentially occur where the minor groove of DNA faces in towards the histone octamer and GC-rich sequences adopt the opposite orientation. This sequence organisation allows the DNA to bend smoothly in a preferred configuration and thereby specifies the rotational orientation of the DNA in the nucleosome core particle.

By themselves such regular periodic signals are insufficient to specify translational positions. We would however expect that local variations in both the magnitude and direction of bending could select for the preferential location of sequences in a non-periodic manner. Sequence analysis of DNA from both nucleosome core particles and nucleosomes containing histone H5 reveals that a particular class of sequences - 'flexi-DNA' - are associated with a region close to but asymmetrically situated with respect to the sequence dyad. In other protein binding sites, notably those for the E.coli RNA polymerase and the FIS protein, these same sequences are preferentially located at sites where the bending of DNA can only be accommodated by unstacking of adjacent base-pairs - i.e. the DNA is kinked. The presence of these sequences in nucleosome core DNA correlates with the sharp bends observed at certain positions in the crystal structure of core particles (Richmond et. al, 1984). In addition in this same region the local sequence periodicity differs significantly from the average.

To attempt to predict the preferred binding sites for histone octamers we have developed an algorithm which takes account of these local variations in sequence periodicity to generate defined

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translational positions. Using the histone octamer binding site on the Xenopus borealis 5S RNA gene as a template we can accurately derive the positions of nucleosomes bound to the MMTV LTR promoter.

Finally, we note that although the physicochemical properties of DNA can act as determinants of nucleosome positioning they may not by themselves be sufficient to specify precisely chromatin organisation in vivo.

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Structural polymorphism of homopurine-homopyrimidine DNA sequences.

F.Azorin.
Grupo de Química Macromolecular. Centro de Investigación y Desarrollo CSIC. Diagonal 647. Barcelona 08028. Spain.

DNA in solution is structurally polymorphic. Studies of the conformational behaviour depictted by DNA sequences with a simple mono- or dinucleotide repeat have revealed the high degree of structural flexibility of the DNA molecule. In particular, homopurine-homopyrimidine sequences are known to adopt several conformations which deviate significantly from the cannonical B-form. $d(A/T)_n$ adopt a "B-like" conformation which appear to be associated with the regular bending of DNA. Furthermore, $d(G/G)_n$ and $d(GA/CT)_n$ sequences are known to undergo transition to triple-stranded conformations in response to changing environmental conditions. At low pH these sequences exist as a homopyrimidine-homopurine-homopyrimidine triplex containing CGC^+ and, in the case of $d(GA/CT)_n$ sequences, TAT base triads. At neutral pH in the presence of magnesium, $d(G/C)_n$ sequences form a GGC triplex instead of the CGC^+ triplex found at low pH. In addition to triple-stranded helices, $d(GA/CT)_n$ sequences can also adopt other non-B conformations and at least six acid-induced conformations have been identified in linear poly(GA/CT). Recently, we reported a zinc-induced structural transition of a $d(GA/CT)_n$ sequence contained into negatively supercoiled DNA. This novel structure, called *H-DNA, is characterized by a peculiar chemical reactivity pattern. In *H-DNA, most of the polypyrimidine strand is sensitive to osmium tetroxide modification while only the central part of the polypurine strand is reactive with diethylpyrocarbonate. Formation of a homopurine-homopyrimidine-homopurine triplex migth account for these results. *H-DNA is induced at neutral pH by moderate concentrations of zinc and it requires negative superhelicity. Stabilization of *H-DNA appears to be specific of nucleotide sequence and metal-ion. From several different divalent cations investigated only zinc, cadmium, manganese and cobalt were shown to be efficient in the stabilization of *H-DNA (zinc › cadmium » manganese, cobalt). Similarly, from all the different homopolymeric sequences tested, only $d(GA/CT)_n$ showed the zinc-induced transition to *H-DNA. Zinc ions play an important role as cofactors on many biological processes. In particular, a class of transcriptional regulators in eukaryotic cells contain zinc as a coordinated metal-ion. Upon DNA binding, the zinc ions contained within these trans-acting factors might influence the conformation of the regulatory cis-element.

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STRUCTURAL STUDIES ON CHROMATIN

T. J. Richmond, M. Struck-Donatz, D. Sargent, M. M. Struck, and T. Rechsteiner Institut für Molekularbiologie und Biophysik
ETH-Hönggerberg, CH-8093 Zürich, Switzerland

Nucleosome core particles containing DNA of a specific sequence, that from the regulatory region of the 5S RNA gene of *L. variegatus*, have been assembled *in vitro* from the histone octamer and crystallized. These crystals are monoclinic, $P2_1$, a=107.5 Å, b=182 Å, c=110.5 Å, γ=90°, and contain two particles per asymmetric unit (412 kd/asu). In order to conserve crystals, the X-ray structure determination is proceeding to 4.5 Å using data collected at -180° C on the image plate at the EMBL Outstation (DESY). Further data, extending on average to 3.5 Å, have been collected at 4° C where each crystal yields 1 to 2 exposures. Results from the data collection at -180° C will be presented.

The structure of the core particle containing mixed sequence DNA has been redetermine at 9Å resolution under conditions which avoid the use of alcohol. The original map at 7Å was determined from crystals to which 25±2% 1,6-hexanediol was added, in part, to improve the quality of the X-ray diffraction. Comparison of the two structures shows that the particles have the same overall size and shape, but that the last turn of the double helix at one end of the DNA superhelix makes substantially different interactions with histone H2A.

The histones of the H2A/H2B dimer have been expressed in *E. coli*, isolated, and combined with chicken erythrocyte H3/H4 tetramers and a 146 bp defined sequence DNA. The crystals of nucleosome core particles obtained with the bacterially expressed histones permit structural studies using histone dimers having sequence alterations. The first mutant constructed converts lysine119 in histone H2A to a cysteine. In chromatin, this lysine is frequently found conjugated via an isopeptide bond to the protein ubiquitin. The cysteine mutant will be used to map the site of ubiquitination via labeling with a multi-heavy atom group.

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Protein Motifs that Recognize Structural Features of DNA

Mair E.A. Churchill
MRC Laboratory of Molecular Biology, Cambridge ENGLAND CB2 2QH

Unlike proteins such as transcription factors which bind to DNA sequence specifically, chromosomal proteins and nucleases bind to many different DNA sequences. A few of these 'non-sequence-specific' DNA binding proteins, DNase I and HU type proteins, are known to bind to DNA primarily in the minor groove. In contrast to many sequence-specific DNA binding proteins, whose structures are understood or for which structural units, such as the helix-turn-helix, zinc-finger, or others have been described, the types of putative structural units that a protein might use for minor groove binding are relatively undefined. However, there are well characterised drugs, such as Hoechst 33258, that are potential models for 'non-sequence-specific' DNA recognition since they exhibit relatively low sequence specificity and bind to DNA in the minor groove.

On the basis of sequence analysis a few protein motifs for minor groove recognition have been proposed. These include an alpha-helix for protamine and for a decapeptide sequence found in the globular domain of histone H1, an anti-parallel ß-ribbon, and a special type of ß-turn structure for the sequence motifs SPK(R)K(R) ('SPKK') and 'SPXX'. However, these protein motifs are relatively poorly understood both structurally and functionally.

The termini of histone H1 and sea urchin spermatogenous H1 and H2B, which are essential for correct chromatin condensation, often contain repeats of 'SPKK'. Suzuki isolated a fragment of the 'SPKK'-rich N-terminus of sea urchin spermatogenous H1 containing six 'SPKK' repeats (S6 peptide) from proteolytic digests of H1 and showed that it binds to DNA and competes with the minor groove binding drug Hoechst 33258. He proposed a special type of ß-turn structural unit for 'SPKK' and suggested that the motif binds in the minor groove of DNA using a similar mode of interaction as the drug Hoechst.

In collaboration with Suzuki, the sequence selectivity of Hoechst, S6, and SPRKSPRK (S2) peptides has been determined using quantitative hydroxyl radical footprinting. Both S2 and S6 peptides protect the minor groove of DNA from hydroxyl radical cleavage preferentially at A/T-rich sites. These results are consistent with the earlier proposal of Suzuki, but whether the mode of binding of the peptides is similar to that of Hoechst has not yet been determined. The structure of A/T-rich DNA is different from random sequence DNA, because of the distinctly narrow minor groove. Therefore it appears that peptides, of only 8 amino acids, can adopt a structure that allows recognition of sequence dependent features of the local architecture of DNA such as the shape of the minor groove. The implications of sequence specificity of the 'SPKK' and other basic protein motifs will be discussed.

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Regulation and role of H5 expression in erythroid cell differentiation

S. Rousseau, J.M. Sun, J. Boix, J. Renaud and Adolfo Ruiz-Carrillo
Cancer Research Center of Laval University, Quebec, Canada

The linker histones (H1, avian H5, and mammalian H1°) are involved in the condensation/maintenance of chromatin into the 30 nm fiber, a structure that correlates with chromatin inactivity. Expression of H1 isotypes is linked to cell growth, and that of H5 and H1°, in some degree, to the state of differentiation/loss of proliferation potential, H5 being erythroid-specific. We have studied the effects of H5 on proliferation and differentiation by using cells transfected with an inducible H5 gene, and lines of transformed pre-erythroblasts. While H5 induces arrest of proliferation in a dose dependent manner, the differentiation potential of the cells is, between certain limits, independent of H5 content. Hence, H5 (or H1° in mouse erythroleukemia cells) does not appear to play a major role in the early stages of differentiation but, on the contrary, differentiation increases the rate of H5 gene transcription.

We have analyzed the expression of several H5 gene constructs to understand the mechanisms leading to the increased H5 content of the maturing erythrocyte. We have found that the basal H5 expression is increased several-fold during differentiation due to the activation of at least two stage-specific enhancers flanking the gene. The two enhancers do not have additive effect on transcription but have comparable strength. We have also isolated and characterized a novel protein factor that represses transcription of the H5 gene in cell-free extracts. The repressor is present in inactive mature erythrocytes but not in active precursor cells which contain a presumably different factor that recognizes a subset of the same sequence element.

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Chromatin Structure and Gene Expression in Drosophila

Sarah CR Elgin, BD Allan, RF Clark, C Craig, Q Lu (Washington University), RL Glaser, JT Lis (Cornell), TC James (Wesleyan) and JC Eissenberg (St. Louis University)

Regulation of gene expression can be affected by chromatin structure at several levels. While the bulk of the DNA is packaged with the histones into a nucleosome array, the promoter and enhancer elements of many active and inducible genes are found in DNase 1 hypersensitive sites (DH sites) in eukaryotic chromatin. These nucleosome-free regions are thought to facilitate the interaction of the transcriptional machinery with the DNA. We have recently identified the DNA sequences required for formation of the DH sites of the Drosophila hsp26 gene by analysis of germline transformants carrying in vitro-modified hsp26- and hsp26/hsp70 - lacZ fusion genes. Three regions of the hsp26 gene, region A (-351 to -135), region $(CT)_n$ (-135 to -85) and region D (+11 to +632) contribute to the formation of the proximal and distal DH sites; they appear to act in a redundant manner. Any pairwise combination of these regions is sufficient to generate the proximal DH site when fused to the HSE-promoter region P (-85 to +11), but none can act effectively alone. The $(CT)_n$ element, which binds a nuclear protein, is a positive effector of gene transcription at this locus, independent of its role in chromatin structure. The results suggest that some abundant transcription factors may contribute to the formation of DH sites through their interaction with the RNA polymerase complex. Those constructs that fail to generate DH sites are also those that fail to be induced by heat shock, even when the TATA box and the HSE's are intact.

Packaging of larger "domains" can also dictate the accessibility of a gene to expression, as demonstrated by position effect variegation. We have previously reported the identification of a protein, HP-1, shown to be preferentially associated with the β-heterochromatin (extending into regions 41 and 80), region 31, some telomeres and in a banded pattern throughout the fourth chromosome. Analysis of a fourth chromosome insertional translocation *T(3;4)f/In(3L)P* and of the rearrangement *lt*^{x13}, broken proximal to *light*, confirms an autonomous interaction with chromosome 4 and β-heterochromatic material, respectively. *In situ* hybridization of the cloned gene indicated that HP-1 is encoded at 29A, the region containing *Su(var)205*, a dominant suppressor of position-effect variegation which is a homozygous lethal. Cloning and sequencing of this allele has shown the mutation to be a G-to-A transition at the first nucleotide of the last intron, which causes missplicing of HP-1 mRNA. This suggests that heterochromatin-specific proteins play a central role in the gene suppression associated with heterochromatic position effects. The protein sequence has a region of homology with *polycomb*, a known down-regulator of the homeotic genes; two-thirds of the 37 amino acids in this region are identical between HP-1 and *polycomb*, suggesting the presence of an inactivating "packaging" motif. Immunofluorescent staining of early embryos indicates that HP-1 does not enter the nuclei until approximately nuclear division state 10, implying that heterochromatin formation is a regulated, late event in Drosophila embryogenesis.

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STRUCTURAL AND FUNCTIONAL ORGANIZATION OF GENOMIC CHROMATIN DOMAINS

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Nuclear DNA is organized in topologically constrained loop domains defining basic units of higher-order chromatin structure. In order to investigate the relevance of loop domains for the control of gene activity, we mapped a specific eukaryotic gene locus in respect to its chromatin organization. The active chicken lysozyme gene is located within a 20 kb chromatin domain of elevated DNase sensitivity of DNA. Various sets of up to 9 DNase I hypersensitive sites (DHSs) within this chromatin region correlate with the different regulatory transcriptional states of the gene. DNA transfection studies show that DHSs mark the positions of multifactorial regulatory elements (enhancers, silencer, steroid response element), each responsible for a specific sub-aspect of the total control of lysozyme gene activity. The domain of general DNase sensitivity terminates at both ends in specific DNA elements. The border elements are attached to nuclear matrix/scaffold material (MARs/SARs) and have a dual cis-activity (A-elements) for the function of genes when they are inserted into the genome. First, A-elements significantly elevate reporter gene transcription in the presence of enhancers and secondly, they buffer inserted "mini-domains" constructs from the regulatory influence of random neighbouring genomic regions (1). DNA transfer of entire genomic domains into cells in culture or into transgenic animals mediate position independent and tissue specific high-level transcriptional activity of transgene constructs (2). The results support the conclusion that eukaryotic genomes are organized in successive chromatin loop domains confining regulatory units for the independent control of gene loci.

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THE MAPPING OF DNA REPLICATION UNITS IN ANIMAL CELLS

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We have developed a new technique for assaying the direction of replication of any DNA fragment in animal cells and this can be used to detect origins of replication as well as terminators. This method has now been applied to the human β globin domain and has enabled the localization of an origin at the 5' end of the β gene. Cells containing deletions in this region show an altered pattern of replication which confirms the existence of this origin. It has been previously shown that this globin domain replicates early in K562 cells, but late in the cell cycle in non-expressing cell types and this has now been demonstrated with a new and poweful technique which takes advantage of *in situ* hybridization. Despite these differences the globin origin remains fixed to the same locus, in several cell types regardless of their expression pattern. We have also used the replication direction assay to pinpoint DNA synthesis termination sites in several specific genomic regions and the preliminary results suggest that these loci may correspond to the boundaries of chromatin domains.

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Topoisomerase II is required for chromosome condensation

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Topoisomerase II is the major protein component of the metaphase scaffold and is thought to be structurally involved in the chromatin loops. We have studied the role of topoisomerase II in chromosome condensation in a cell-free mitotic extract derived from Xenopus eggs by specific immuno-depletion. HeLa nuclei,which have a high complement of endogenous topoisomerase II, are converted to mitotic chromosomes in the topo II-depleted extract equally well as in the control. Chicken erythrocyte nuclei, however, which have a very low content of topoisomerase II, do not convert to condensed chromosomes in the depleted extract, although their condensation is normal in the control. Addition of purified topoisomerase II from S. cerevisiae restores the potential to assemble the chicken nuclei into mitotic chromosomes in the deficient extract. Dosage experiments support the possible notion of a structural involvement of topoisomerase II in chromosome condensation.

In the topo II-depleted extract the erythrocyte nuclei progress to precondensation chromosomes which appear to consist of a cluster of swollen chromatids. These structures are free of the nuclear membrane and lamina. Late addition of yeast topoisomerase II rescues these structures, enabling their conversion to mitotic chromosomes.

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Nucleosome assembly in cell extracts[1]

Psoralen-crosslinking of DNA in chromatin is a powerful method to study chromatin structure, i.e. the distribution of nucleosomes (2) and transcription elongation by DNA-RNA crosslinking (3). Psoralen intercalates and crosslinks the inter- or non-nucleosomal DNA, but the DNA wrapped around the nucleosome core is not crosslinked (1). This technique is very useful to study chromatin assembly.

As expected based on published work (4) nucleosome assembly in Xenopus laevis oocyte extracts is very efficent. On the other hand using mammalian cell extracts (from monkey kidney cells or human 293 cells supplemented with SV40 large T-antigen) only 50-80 % of the number of nucleosomes in vivo could be assembled in vitro. We show, opposite to published suggestions (5), that in these extracts the extent of nucleosome assembly is not dependent upon DNA replication, but DNA replication appears to facilitate the precise folding of DNA in the nucleosome.

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Nucleolar chromatin structure and its relation to rRNA synthesis.

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By measuring the crosslinkability with psoralen and u.v. light in intact cells or purified nuclei or nucleoli, we mapped the chromatin structures of the ribosomal RNA genes in different organisms. A summary of the data is shown in Fig. 1.

In Xenopus, mouse and rat cells we found that two distinct types of ribosomal chromatin coexist. The coding region of one type contains nucleosomes and represents the inactive copies whereas the other type lacks a nucleosomal packing and corresponds to the transcribed genes. The relative amounts of the two types of chromatin are similar in interphase and metaphase, suggesting that the two states of chromatin are stably propagated through the cell cycle (1,2). Since the run-on transcriptional activities (3) of interphase nuclei and metaphase plates differ markedly (1), we investigated the ribosomal chromatin in rat liver before and after partial hepatectomy. Although the run-on transcriptional activities increased twofold in regenerating liver cells, the proportions of active and inactive ribosomal gene copies remained constant. On the other hand when nuclei from various tissues of the adult mouse were compared, significant differences in the proportions of active and inactive copies were found. The relative amounts of the active rDNA copies correlated with the differences of the run-on activities of the corresponding nuclei. Changes in the proportions of active and inactive ribosomal gene copies were also found during oocyte maturation in Xenopus laevis.

Our data suggest that not the loading of the genes with RNA polymerase I, but the cell type, i.e. cell differentiation, determines the proportion of active ribosomal gene copies, and this in turn determines the maximal possible polymerase loading in a given cell. Therefore we propose that, for the ribosomal RNA genes of a given cell type, the chromatin structure determines the maximal possible rate of rRNA synthesis.

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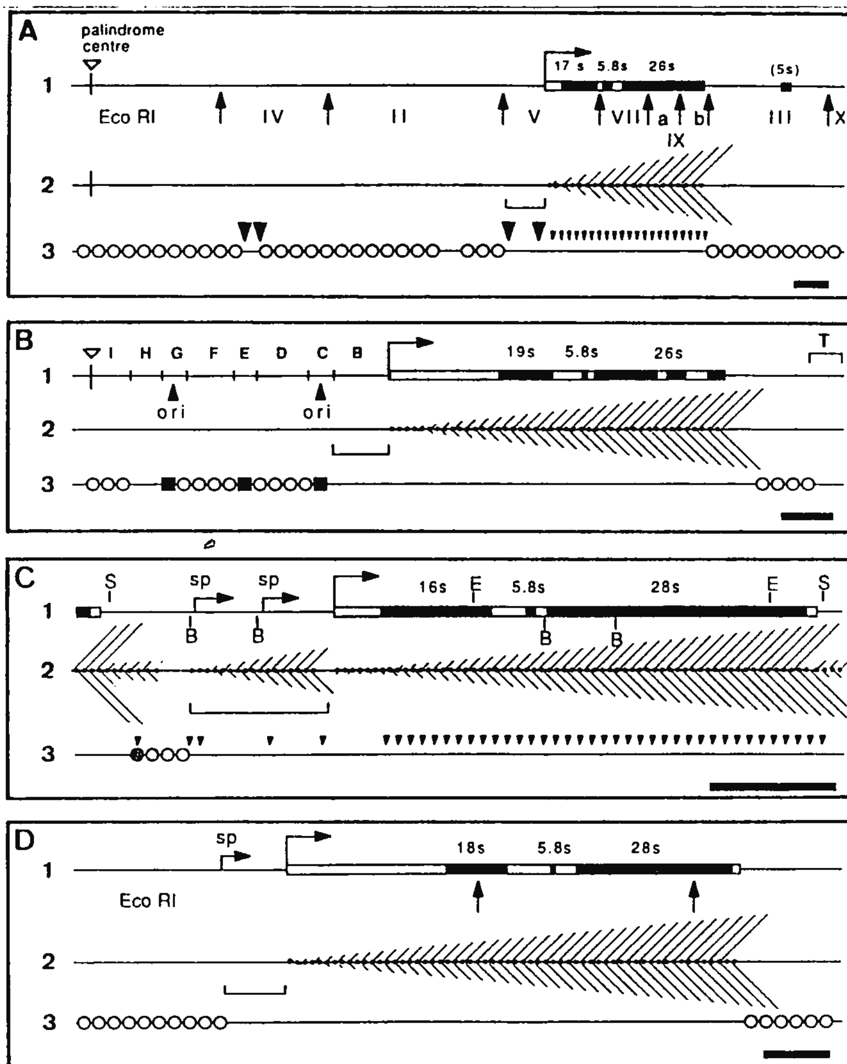
Figure 1:

Structural organization of ribosomal RNA genes of Dictyostelium discoideum (A), Physarum polycephalum (B), Xenopus laevis (C) and mouse (D).

Lanes 1: rDNA maps showing the positions of the coding regions.

Lanes 2: Representations of the transcription units.

Lanes 3: Chromatin structure. Regions that are not packaged in nucleosomes are represented as continuous lines whereas nucleosomal regions are indicated as rows of circles. Taken from Sogo and Lucchini (2).



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Differential nucleosome positioning on Xenopus oocyte and somatic 5S RNA genes

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The two families of Xenopus 5S RNA genes, oocyte and somatic appear to require the same transcription factors for activation of transcription. However, since transcription of these genes is developmentally regulated in that only somatic genes are transcribed in somatic cells, it is of interest to ask whether the assembly of chromatin structure is somehow involved in the activation or repression of these genes. Some years ago, using in vitro reconstitution, we showed that a histone octamer occupies an unique position on the somatic 5S RNA gene and that this position permits efficient binding of transcription factor IIIA. I will discuss recent results which show that the preference for histone positioning on the two genes is different.

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Access of Transcription Factors to Regulatory Sequences is Modulated by Chromatin Structure. Gordon L. Hager, Emery Bresnick, Charles Rories, Sam John and Trevor Archer. Natl Cancer Inst, NIH, Bethesda, MD, USA.

The 1300 bp MMTV LTR is organized *in vivo* in an array of six phased nucleosomes, designated A-F (Richard-Foy & Hager, EMBO J. 6, 2321 '87). Transcription activation of the hormone-inducible promoter leads to the selective displacement of the B nucleosome from this array, and the assembly of a transcription initiation complex, located partially in the A-B linker region, and partially on the A nucleosome (Cordingley et al., Cell 48, 261 '87). These observations suggest that the presence of histone octamer cores is in some cases compatible with factor-DNA interactions, but under some conditions must be removed to allow access to the template.

We are extending our analysis of this system to permit a detailed understanding of the constraints placed on DNA-transcription factor interactions by a positioned nucleosome array. PCR-amplified high resolution mapping of micrococcal nuclease accessibility indicates that the A, B and C nucleosomes are highly positioned. The detection of DNaseI 10 bp ladders also indicates that nucleosomes in this array are positioned at single base pair resolution.

Hyperacetylation of histones *in vivo* selectively inhibits hormone activation of the promoter (Bresnick et al., PNAS 87, 3977 '90). Studies *in vivo* indicate that general features of nucleoprotein organization for the phased array, such as octamer core positioning, are unchanged in hyperacetylated chromatin, but hormone-dependent disruption of the B nucleosome is prevented. No differences can be detected in the overall stability of B nucleosomes reconstituted *in vitro* with normal or hyperacetylated histones, or in the detailed positioning of the B core. These results suggest that hyperacetylation of octamer cores results in a relatively subtle change that can block successful interaction of the steroid receptor with its recognition sequences.

An A-B fragment of the MMTV promoter can be reconstituted from pure histones into a dinucleosome with uniquely positioned octamer cores. Core boundaries for the *in vitro* assembled dinucleosome correspond to the observed *in vivo* phasing pattern for LTR nucleosomes A and B. Nuclear factor one (NF1), a constituent of the MMTV transcription initiation complex, is excluded from the assembled dinucleosome while the glucocorticoid receptor is able to bind.

In summary, these results indicate that the detailed positioning of octamer core structures in regions of chromatin where nucleosomes are phased can have significant impact on the binding of transcription factors to the template, and that subtle alterations to the histone component of the nucleoprotein complex can also modulate factor-DNA interactions.

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"*Nucleosome phasing and transcription of the MMTV promoter*"

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Expression of the MMTV promoter in cells carrying genes driven by MMTV-LTR is strictly dependent on glucocorticoids or porgestins [1]. In contrast, the MMTV promoter is highly active when assayed as free DNA template under cell-free conditions in the absence of steroid hormone receptors [2]. One possible explanation for this apparent discrepancy could reside in the organization of the MMTV-LTR in chromatin. In nucleosome reconstitution experiments, the regulatory region of the MMTV promoter positions itself precisely on the surface of a histone octamer that covers the region between -45 and -190 encompassing the hormone responsive elements and the binding site for the essential transcription factor Nuclear Factor I (NFI) [3]. In the reconstituted nuclesome the binding site for NFI is located with the major groove pointing inwards and is therefore unaccessible, whereas two of the four binding sites for the hormone receptors are easily recognized by the receptors *in vitro* [3]. The exact positioning of the DNA double helix on the surface of the histone octamer is an intrinsic property of the nucleotide sequence of the MMTV, that is relatively unaffected by flanking sequences or free DNA ends. It reflects the preferred bendability of this particular DNA segment that is observed in minicircles in the absence of histones. We postulate that the silent state of the unstimulated MMTV promoter *in vivo*, is due to the nucleosome-mediated occlusion of the NFI binding site, and that one of the consequences of hormone treatment is to relieve this repression.

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DEREPRESSION OF THE MMTV PROMOTER BY INTERFERENCE OF CHROMATIN ASSEMBLY.

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The glucocorticoid receptor (GR) is a DNA binding transcription factor. It greatly induces transcription from the mouse mammary tumor virus (MMTV) promoter. This promoter is virtually silent in the absence of glucocorticoid hormone. The GR mediated induction is dependent on a glucocorticoid response element (GRE) which is localised within the -192/-76 segment of the promoter and which specifically binds GR *in vitro*. Upon hormone induction a nucleosome, specifically positioned over the GRE, appears to be removed or structurally altered[1]. Furthermore, a binding site for Nuclear Factor 1 (NF1), located within the same nucleosome, is occupied only in the presence of hormone[2] suggesting that induction increases the accessibility of the promoter, possibly by local alterations in the chromatin.
In vitro assembly of histone octamers on an MMTV promoter fragment results in the formation of a specifically positioned nucleosome at the -219/-76 segment and thus encompasses the GRE. Purified GR will bind to the entire GRE also when organised within such a nucleosome[3], this results in local structural changes of the GRE but does not interfere with the gross structure of the nucleosome[3]. In our hands the naked MMTV promoter is actively transcribed *in vitro* but the transcription is unaffected by addition of purified GR. The transcription is inhibited by preassembly of nucleosomes on the template but it remains GR insensitive.
Xenopus laevis oocyte injections were performed in the aim of testing the role of chromatin in the MMTV induction process. Glucocorticoid- as well as receptor-dependent induction of MMTV transcription was established in the oocytes. As shown previously, chromatin was assembled on the plasmid DNA which was injected into oocyte nuclei. The nucleosome density on injected plasmid DNA could be reduced by coinjection of unspecific competitor DNA. This resulted in a parallel increase of constitutive MMTV transcription, i e in the absence of GR and hormone while a reference Adeno major late promoter was unaffected or inhibited. Our results support the notion that an intact chromatin structure is required for maintaining a repressed MMTV promoter.

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Role of an Upstream Element in the Developmental Regulation of the PEPCK Gene Chromatin Structure. Roger Chalkley, Tony Ip and Steve Faber. Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN. 37212, USA.
We are studying the developmental and hormonal control of the PEPCK gene. Developmental control involves the accessibility of multiple ubiquitous DNA binding factors to the promoter of the gene. The role of upstream tissue-specific DNA-binding factors in generating accessibility is under investigation. We have identified three such regions upstream of the PEPCK gene at -4800, -6500 and -9500 bp. The -4800 bp region binds a liver-specific protein, activates transcription in a tissue-specific manner in transient transfection assays and demonstrates some of the properties of a DCR when assayed in transgenic mice. The specific protein which binds to this region is absent in extinguished, hybrid cell lines. In addition, we have performed *in vivo* footprinting to analyse protein binding to the PEPCK gene promoter in expressing and non-expressing cell lines. The effects of a range of hormones (which increase or decrease transcription levels) have been studied in terms of an assessment of the effect of hormonal induction on specific protein binding to the promoter DNA. We will discuss the role of co-operative DNA-protein interactions in establishing the active chromatin structure of the PEPCK gene.

Fundación Juan March (Madrid)

CORE HISTONE ACETYLATION IN CHROMATIN

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A) ACTIVE CHROMATIN

An affinity-purified antibody has been developed that recognises, principally, the epitope ε-acetyl lysine. Chromatin fragments prepared by micrococcal nuclease have been fractionated using this antibody and the protein and DNA sequence composition of this active chromatin fraction investigated. The chromatin used was prepared from erythrocytes of 15 day chicken embryos and also from HL60 cells (a human promyelocytic leukemia line).

Proteins

1. As expected, enrichment in acetylated core histones is observed, noticeably in histones H3 and H4 but also, rather strikingly, in H2B.
2. There is no obvious enrichment in ubiquitinated H2A or H2B.

Active Gene Sequences

1. Enrichment of adult α and β globin gene sequences of 10 to 30 fold is observed (expressed genes in these erythrocytes), whilst ovalbumin sequences are not enriched.
2. Enrichment of embryonic β_ρ sequences is also observed (~ x 10), although this gene is no longer active at 15 days.
3. Histone H5 gene sequences are somewhat enriched (~ x 4) in 15 day erythrocytes.
4. In HL60 cells, sequences of the amplified c-myc gene are enriched in the antibody-bound chromatin relative to the total chromatin.

B) ACETYL SITE DISTRIBUTION

We have observed the distribution of acetyl groups amongst the possible modification sites in partially acetylated H4, H3 and H2B by direct sequencing. The histones were extracted from pig thymus and HeLa cells (both with and without added butyrate).
We conclude:

1. For a given degree of acetylation, e.g. di-acetyl H3, site occupancy is highly (but not absolutely) specific, i.e. the acetyl groups are not randomly spread over all possible sites.
2. The specificity of site occupancy is not markedly dependent on the source of the histone or the presence/absence of butyrate (HeLa cells). Chromatin from butyrate treated HeLa cells is therefore a not unreasonable model for active chromatin.

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REACTIVITY OF NUCLEOSOMES COMPLEXED WITH EXCESS HISTONES. DIFFERENT MECHANISMS FOR THE SELF-ASSEMBLY OF NUCLEOSOME CORE PARTICLES AT PHYSIOLOGICAL IONIC STRENGTH

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The stability of nucleosome core particles and the interaction of different histone oligomers with nucleosomes have been investigated using non-denaturing gel electrophoresis, electron microscopy, solubility and supercoiling assays. Our results indicate that, in the presence of 0.2 M NaCl, H2A,H2B, H3,H4 and core histones can migrate spontaneously from aggregated nucleosomes containing excess histones to free DNA (146 base-pairs or circular). The systematic study of the reactivity of nucleosome cores and oligonucleosomes containing excess histones allow us to conclude that nucleosome core particles can be formed spontaneously in 0.2 M NaCl by the following mechanisms: (1) transfer of excess core histones from oligonucleosomes to free DNA; (2) transfer of excess H2A,H2B and H3,H4 associated separately with oligonucleosomes to free DNA; (3) transfer of excess H2A,H2B initially associated with oligonucleosomes to DNA, followed by the reaction of the resulting DNA-(H2A,H2B) complex with oligonucleosomes containing excess H3,H4; and (4) a two-step transfer reaction similar to that indicated in (3), in which excess H3,H4 are transferred to DNA before the reaction with oligonucleosomes containing excess H2A,H2B. These results together with our previous *in vitro* studies [P. Diaz & J.R. Daban, *Biochemistry* (1986) 25, 7736-7744; A.M. Aragay, P. Diaz & J.R. Daban, *J. Mol. Biol.* (1988) 204, 141-154] allow us to suggest a dynamic model for the self-assembly of nucleosome core particles at physiological ionic strength.

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DNA topology, eukaryotic DNA topoisomerase I, histone octamers.

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According to Aristoteles, the shape is the function. One can reach the same conclusion looking not at shells and skulls, but at DNA. This old sentence also answers to the question asked recently and very directly: "Why bend DNA?" (1). We are studying the relationship between DNA topology and protein interaction. Given that the conformation of a DNA molecule is determined by its nucleotide sequence (i), by the overall topology (ii) of the molecule and by its effects at local level (iii), we use protein sistems in which one has reason to predict that the three parameters are all equally relevant. These are: RNA polymerase, DNA topoisomerase I, nucleosomes. The presentation will deal with: 1) the topology dependence of DNA topoisomerase I reaction. We have analysed the Kcs, the binding, the nicking and the topisomerization steps of several eukaryotic DNA topoisomerases I, and have defined a relatively clear picture of the relationship between variation of DNA shape and topoactivation (2-5). 2) DNA topology and nucleosome deposition. As for DNA topoisomerase I, also in this system bending and linking reduction exert on the protein a clear attractive and phasing force (6,7). The similarity of the answer of the two protein systems to the same set of variations of the topology of DNA is trying to tell us something.

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Fundación Juan March (Madrid)

The solution structure of the DNA-binding domain of the oestrogen receptor - a 'double loop-zinc-helix' motif.

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Steroid hormone receptors control gene expression through binding, as dimers, to short palindromic response elements located upstream of the genes they regulate. An independently folded domain of approximately 70 amino acids directs this sequence specific DNA-binding and is highly conserved between different receptor proteins and related transcription factors This domain contains two zinc-binding Cys_2-Cys_2 sequence motifs, which loosely resemble the 'zinc finger' motifs found in TFIIIA. I will describe the structure of the DNA-binding domain from the oestrogen receptor, determined by 2D ^1H NMR techniques. The two 'zinc finger'-like motifs fold to form a single structural domain and are thus quite distinct from the independently folded units of the TFIIIA-type 'zinc fingers'. A model for the protein-DNA interaction will be presented.

Fundación Juan March (Madrid)

On the formation of chromatin structures on yeast minichromosomes.

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Due to the protection of DNA by histone proteins, the position of nucleosomes with respect to the underlaying DNA sequence might play a decisive role in regulation of DNA dependent processes. We use *in vitro* reconstitution experiments and construction of artificial minichromosomes in yeast *S. cerevisiae* to study chromatin structure and function. The following topics will be addressed:

Chromatinstructure of a constitutive promoter: It was suggested that poly(dT)·poly(dT) tracts serve as an element of the constitutive DED1 promoter by DNA-dependent exclusion of nucleosome formation (Struhl (1985) PNAS 82, 8419). We show that the naturally occurring poly(dT)·poly(dT)-rich sequence can be folded in nucleosomes with a similar efficiency as immediately flanking sequences. The nuclease sensitivity of the DED1 promoter region observed *in vivo* is therefore unlikely to be created by the poly(dT)·poly(dT)-rich sequence (Losa, Omari and Thoma(1990) Nuc. Acids Res. 18, 3495).

Mechanisms of nucleosome positioning: Multiple mechanisms are established: histone-DNA interactions, flanking structures, and chromatin folding. A pentamer of the sequence ($T^{CGG}TG_{TTA}GA^{GCC}TG_{TAA}C$) was reported to be the strongest nucleosome positioning sequence *in vitro* (Shrader and Crothers (1989) PNAS 86,7418). A pentamer and an octamer of this sequence was inserted into the TRP1 gene of a minichromosome in *S. cerevisiae* and the chromatinstructures were determined by mapping the accessibility of the DNA to m. nuclease. The artificial sequences did not form precisely positioned nucleosomes *in vivo,* nor did they act as a boundary and shift nucleosome positions on TRP1 (Tanaka & Zatchej).

DNA-repair at the nucleosome level: A circular minichromosome (YRpTRURAP) which contains the URA3 gene inserted in the TRP1ARS1 circle shows precisely positioned nucleosomes and four nuclease sensitive regions. YRpTRURAP is an ideal substrate to study repair of UV-induced pyrimine-dimers (PD) relative to the chromatin structures. The template strand of the URA3 was repaired at a fivefold higher rate than the nontranscribed strand, suggesting that the repair enzymes might recognize an 'opened-up' chromatin structure when the RNA-polymerase is stalled at the PD site. (Smerdon et al., (1990) Nuc. Acids Res. 18, 2045; Smerdon and Thoma (1990) Cell 61, 675-684; Smerdon, Washington State University, Pullman, WA 99164, USA).

Fundación Juan March (Madrid)

CHROMATIN ORGANIZATION AND REPRESSION OF TRANSCRIPTION

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Repression of transcription is likely to be as important in eukaryotic gene regulation as activation. In contrast to the burgeoning data concerning possible mechanisms of activation, there has been little information about how eukaryotic repressors might function. For nearly forty years, histones have been postulated to be general repressors of transcription. Recently, developing evidence suggesting positioning of nucleosomes about key *cis*-acting elements has fueled that speculation.

A fundamental question regarding nucleosome positioning has been whether the location of a *cis*-acting DNA element relative to the nucleosome has any effect on its function. We recently have shown that the answer to this question is yes, at least for one such element. Placing the 11 bp yeast ARS1 sequence within 40 bp of the pseudodyad of a core particle severely limited its function as a replication origin. Our studies of changes in chromatin structure induced by the yeast α2 repressor indicate that transcriptional elements might also be regulated by nucleosome positions. The yeast α2 operator was flanked by positioned nucleosomes in α-cells, where it represses transcription, but not in **a**-cells, where it does not. We suggested that these positioned nucleosomes might place critical transcriptional *cis*-acting elements in an inaccessible location leading to cell-type specific repression of transcription. High resolution mapping of the location of the nucleosomes in yeast minichromosomes and the yeast genome has shown that they are positioned extremely precisely and that the TATA box for the repressed **a**-cell specific genes is located very near the pseudodyad of the nucleosome in α-cells. Positioned nucleosomes extend from the operator well into the repressed genes.

The location of these nucleosomes is much more precise than predicted by stochastic mechanisms and totally different DNA sequences are present in the different positioned nucleosomes, suggesting an active organization of chromatin by the α2 repressor. Introduction of plasmids containing the α2 operator into the Grunstein H4 amino terminal deletion strains suggests that interactions of α2 with H4 are important for chromatin organization. Thus, deletions of residues 4-14 of H4 allows positioning while deletions of 4-19 or more abolish positioning. Studies of the effects of these deletions on expression of reporter genes coupled to **a**-cell specific promoters are underway.

In addition to α2, the MCM1 gene product, a cell type independent protein, binds to the α2 operator. *In vivo* footprints of the operator region using micrococcal nuclease are very similar for minichromosomes in **a**- and α-cells and the genomic **a**-cell specific genes in α-cells. UV photofootprints are also similar for the operator in minichromosomes and in the genome in the two cell types. In contrast, for the genomic genes, the operator has one G residue whose reactivity to dimethyl sulfate is reduced in α-cells. This feature is similar to the *in vitro* footprint of purified α2 protein and DNA; other aspects of the maps suggest differences in the interactions of MCM1 and α2 with DNA between the *in vivo* and *in vitro* situations.

The α2 and **a**1 genes are necessary for repression of haploid specific genes in **a**/α diploid yeast cells. Using *in vitro* synthesized proteins, we have shown that α2 and **a**1 proteins bind to a haploid specific operator sequence as a heterodimer. Methylation interference experiments indicate a different mode of interaction of this α2 complex from that for the homodimer/MCM1 complex. This is the first example of regulation by heterodimer formation for proteins containing homeodomains. We are currently analyzing the chromatin structure of haploid specific genes in haploid and diploid cells.

We hope to be able to understand how one protein, α2, in combination with either of two other proteins, MCM1 or **a**1, leads to repression of expression of two different sets of genes and how, or if, changes in chromatin structure are involved in such repression.

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PARTICIPATION OF NUCLEOSOMES IN GENE REGULATION IN YEAST

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PHO5 is one of three repressible acid phosphatase genes in yeast, which is turned off in cells growing in high phosphate medium and is derepressed upon phosphate starvation. It was previously shown that under repressed conditions a short DNaseI hypersensitive site (HSS) at position -370 of the PHO5 promoter is flanked by precisely positioned nucleosomes. Derepression of the gene leads to a loss of four nucleosomes at the promoter, two on each side of the HSS. It is known that *PHO5* is under the control of the two positive regulatory proteins PHO2 and PHO4 and the negative regulatory protein PHO80.

Results will be presented addressing the mechanism underlying the transition of the chromatin structure from a closed to an open configuration. To that end we have analyzed the influence of *PHO2* and *PHO4* on the nucleosome structure of the *PHO5* promoter. It is shown that removal of the nucleosomes during gene activation is strictly dependent on the presence of *PHO2* and *PHO4*. From overexpressing *PHO2* or *PHO4* in wild type and mutant strains (*pho2* and *pho4*) we could show that the two regulatory proteins are acting at different levels. PHO4 initiates the removal of nucleosomes during gene activation but that under physiological conditions PHO2 is also required.

To address the kinetics and more general requirements of the chromatin transition, we have inserted a point mutation into the chromosomal *PHO80* gene that confers a temperature sensitive phenotype. This makes it possible to derepress the regulated phosphatase genes by shifting cells from 24° to 37° rather than starving them for phosphate. Results obtained with these strains and implications for the mechanism of nucleosome removal will be discussed.

Fundación Juan March (Madrid)

Nucleosome structure, superhelical stress and transcription

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We assembled nucleosomes from purified core histones and a plasmid carrying a *Xenopus borealis* somatic 5S RNA gene, using the method of salt/urea dialysis. A nucleosome incorporating the 5S RNA gene abolishes transcription by RNA polymerase III in an extract made from *Xenopus laevis* oocyte nuclei. We tested the effect of supercoiling on transcription of the 5S RNA gene *in vitro*. We prepared relaxed, moderately negatively supercoiled and extremely negatively supercoiled plasmid, and assembled nucleosomes. These reconstitutes contain linker DNA which is positively supercoiled, relaxed, or negatively supercoiled, respectively, assuming that "classical" nucleosomes are formed. We found no evidence for a conformational change in the nucleosome in response to superhelical stress. We measured rates of transcription in the presence of camptothecin, a drug which prevents relaxation of the plasmid. The rate of transcription of the 5S RNA gene is independent of the degree of supercoiling of the plasmid, in the presence or absence of nucleosomes. Thus, nucleosome-mediated repression of 5S RNA gene transcription is *not* affected by the degree of supercoiling of the plasmid. We conclude that high superhelical stress alone is not sufficient to activate transcription of a 5S RNA gene assembled into a nucleosome.

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Heat Shock Regulated Transcription of a Chromatin Template Assembled in vitro

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In order to analyze the contribution of chromatin structure to the transcriptional control of gene expression, we have studied the transcriptional activity of a minimal heat shock promoter derived from the Drosophila hsp70 gene after reconstitution in chromatin. Chromatin was assembled at 26°C from supercoiled plasmid DNA using Xenopus oocyte S-150 extracts (Shimamura et al. MCB 9: 5573-5584, 1989). Transcription of the assembled template was assayed using unfractionated transcription extracts prepared from Drosophila embryos. With increasing deposition of nucleosomes, transcription of the assembled template was repressed when assayed with transcription extracts from unshocked embryos. The repression was slightly alleviated by prebinding of the general transcription factor TFIID (recombinant yeast TFIID) prior to chromatin assembly. Transcription of the assembled template was also repressed when assayed with transcription extracts from heat shocked embryos. However, prebinding of TFIID (but not other general transcription factors) resulted in 5 to 15-fold stimulation of transcription of the assembled template in a heat shock transcription extract. This stimulation is dependent on the order of addition of TFIID; no stimulation was observed when TFIID was added subsequent to chromatin assembly. The stimulation is also dependent on the transcriptional activator of heat shock genes, heat shock factor (HSF), since transcription was abolished by the deletion of heat shock elements (HSEs) in cis, or by the addition of competing HSEs in trans. Our data are consistent with a model in which prebinding of TFIID to the heat shock gene TATA box creates a nucleosome-free or nuclease hypersensitive heat shock promoter that has the potential for responding to the presence of an activated heat shock factor (C. Wu, Nature 309: 229-234, 1984).

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Regulation of Class II Promoter Function during In Vitro Nucleosome Assembly.

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We have used an in vitro nucleosome assembly/transcription protocol to investigate the effect of nucleosome assembly on the transcriptional control of class II genes. Concurrent nucleosome assembly effectively prevents the ability of basal transcription factors to form stable preinitiation complexes on class II promoters. However the presence of an upstream factor, USF, facilitated the formation of preinitiation complexes such that complex formation effectively competed with nucleosome assembly for occupancy of promoter sequences. This effect resulted in the relative fold of transcriptional stimulation by USF increasing as an increasing number of nucleosomes were assembled on template DNA. In contrast, the human heat shock factor did not alleviate promoter repression during nucleosome assembly and the stimulatory activity of HSF was not enhanced when template DNA was assembled into nucleosomes. These observations suggest that USF and HSF stimulate transcription by distinct mechanisms. DNase I footprinting and mobility shift assays indicated that HSF binding to the heat-shock element was blocked when the promoter was assembled into nucleosomes. However, binding of TFIID to the TATA box during nucleosome assembly was able to partially preserve subsequent HSF binding. Furthermore, HSF stimulated transcription from preexisting transcription complexes in nucleosome-assembled templates.

We have also examined the effect of derivatives of the S. cerevisiae transcription factor Gal4 during nucleosome assembly. These derivatives include Gal4(1-94) which contains the DNA binding and dimerization domains of the protein, Gal4-AH which also contains a moderate activation domain, and Gal4-VP16 which contains the potent activation domain of VP16. DNase I protection and mobility-shift studies illustrated that all of these derivatives stably bound to a promoter bearing 5 promoter-proximal binding elements either during or subsequent to nucleosome assembly. However basal transcription was effectively repressed during nucleosome assembly from promoters occupied by Gal4(1-94). This observation indicates that stably bound Gal4 derivatives cannot activate transcription solely by excluding nucleosomes from the promoter. In contrast the fold stimulation of transcription observed in the presence of Gal4-AH or Gal4-VP16 increased when template DNA was assembled into nucleosomes. Thus promoter function became more dependent on the presence of an activation domain when an increasing number of nucleosomes were assembled onto the template. By suppressing basal levels of transcription, the presence of nucleosomes on a class II promoter increased the magnitude of transcriptional regulation achieved by these acidic activation domains. These observations imply that the function of acidic activation domains intimately involves overcoming nucleosome-mediated repression of promoter activity.

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Regulation of transcription by histones in the yeast, *Saccharomyces cerevisiae.*
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Data will be presented showing that the nucleosome is a multi-functional chromosomal element.

(i) Our experiments argue that the core particle acts to repress transcription initiation in vivo. Nucleosome loss, caused by glucose repression of a *GAL1* promoter-histone H4 gene fusion, leads to the activation of all regulated genes examined to date. In most cases, the partial activation observed suggests that there are two stages involved in gene induction: a) a chromatin dependent stage in which the activator directly or indirectly mediates the loss of a nucleosome from the TATA promoter allowing a partially active preinitiation complex to initiate transcription and b) a chromatin-independent stage in which the activator communicates with the preinitiation complex to allow fully induced transcription.

(ii) We have also found in the virtually invariant histone H4 N-terminus two distinct functional domains. One (R) acts to repress transcription specific to the silent mating loci. Our evidence suggests that repression occurs through an interaction between R and Sir3, a repressor protein involved in silencing. Data will be presented showing that a second domain (A) is required for the activation of GAL1. Both these domains are in a region which has previously been shown to be dispensable for cell viability.

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CHROMATIN ASSEMBLY COUPLED TO DNA REPLICATION AND GENE TRANSCRIPTION IN *XENOPUS* EGG EXTRACTS.

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Xenopus eggs after fertilization undergo rapid cycles of division relying on material accumulated during oogenesis. We have derived *in vitro* systems from eggs to study nuclear events such as replication, chromatin assembly and nuclear formation and the relationships with gene activity. Two types of systems can be obtained, either a low-speed extract or a high speed extract (1). The low speed extract allows to follow nuclear formation and replication of double-stranded DNA molecules. The DNA synthesis event can be cyclic in these extracts. We have analysed conditions that allow multiple rounds of initiations in the system and are currently studying DNA organization at the chromatin level.

The high speed extract lacking membranous components does not replicate double stranded DNA although this template can be assembled into chromatin (2). However complementary DNA strand synthesis occurs on a single-stranded DNA molecule with enzymatic events which mimic those at the eukaryotic replication fork *in vivo*, including RNA priming. Chromatin assembly progresses coincidentally with DNA synthesis at a rate comparable with chromosomal replication *in vivo*. We have compared the chromatin assembly process on the two types of templates. We have also shown that with both templates ATP/Mg^{++} is essential to the physiological spacing of nucleosomes, but is not required for nucleosome deposition and the DNA supercoiling generated by chromatin assembly (3). In this system the assembly of transcription complexes has been analyzed on class III genes. A differential transcription of 5S and satellite I DNA gene was observed under conditions of chromatin assembly coupled to DNA replication. The 5S DNA gene is repressed whereas the satellite I DNA gene is transcribed (4). Not only replication of the template is required to observed this selective repression but also the arrangement of nucleosomes that generate a physiological spacing. Thus transition in chromatin structure may be an important variable in gene activation.

The mechanism which might regulate maintenance of local order and disorder in chromatin will be discussed.

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CHROMATIN STRUCTURE OF METHYLATED AND NONMETHYLATED CpG ISLANDS

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The mammalian genome is not packaged within the nucleus as a uniform structure. Instead, it seems to be organized in domains showing different properties in aspects like repair rate, histone modification, sensitivity to nucleases or DNA methylation. How these parameters correlate with one another is unclear, mainly due to the difficulty in defining precisely the domains themselves.

In terms of DNA methylation, the genome can be divided in nonmethylated CpG islands and methylated bulk DNA. CpG islands are regions about 1 to 2 Kb long, very rich in G+C content, that surround the 5' end of all housekeeping genes and some tissue specific ones [Bird, (1987) TIGS, 3, 342]. We have found that the differential characteristics of CpG islands in terms of methylation and base composition are paralleled by a chromatin conformation strikingly distinctive from the rest of the genome. Our results show that methylated CpGs are inaccesible to nucleases in mammalian nuclei compared to nonmethylated ones. This situation affects most, if not all, methylated CpGs and is specific for those dinucleotides, since restriction endonucleases not containing CpG in their recognition sequence digest chromatin extensively [Antequera et al. (1989) Cell, 58, 509]. This nuclease protection is probably mediated by proteins that bind methylated DNA in a sequence independent manner [Meehan et al. (1989) Cell, 58, 499].

By taking advantage of their differential sensitivity to nucleases, it is possible to isolate island and non island chromatin. Results of those studies have shown that CpG islands contain normally spaced nucleosomes, but have a nucleosome-free region corresponding with the area of highest G+C content. Histones H3 and H4 are highly acetylated and H1 is present in very low amounts compared to bulk chromatin [Tazi and Bird (1990) Cell, 60, 909].

A consistent feature of CpG islands is that they are never methylated in tissues of the organism (except in the inactive X chromosome) even when the associated gene is transcriptionally silent. In contrast to this, we have found a high proportion of methylated islands in permanent cell lines. Several studies have shown that methylation of an island invariably leads to inactivation of the associated gene. The *de novo* methylated islands adopt a nuclease resistent conformation in the nucleus compared to their nonmethylated counterparts [Antequera et al. (1990) Cell, 62, in press]. These results may explain the widely reported loss of specific functions in cultured cells.

Fundación Juan March (Madrid)

NUCLEOSOME POSITIONING ON MMTV PROMOTER *IN VITRO*

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Nucleosomes are precisely phased over MMTV-LTR *in vivo* (1). A highly specific role for nucleosome phasing on transcriptional control has been proposed for MMTV, since nucleosome positioning modulates binding of relevant transcriptional factors to their target sequences *in vivo* and *in vitro* (2,3,4).

In order to determine the factors involved in nucleosome positioning, we have performed *in vitro* chromatin reconstitution experiments with linear and circular DNA fragments containing MMTV promoter sequences able to accommodate one or two nucleosome cores.

The results obtained support the idea that both rotational and translational phasing are dictated by different features of the DNA sequence (5). Rotational phasing seems to be determined by the bendability of DNA. DNA is bent around the histone octamer in a configuration in which the orientation of the different base pairs is most energetically favoured. This results in a defined orientation of the DNA molecule relative to the center of curvature. The feature(s) of DNA sequence responsible for translational phasing are not yet clear, but we propose that A/T-rich sequences are at least partially responsible for the translational setting observed.

Based on statistical analysis of rotational preferences of different dinucleotides (6), we have developed an algorithm that is able to predict the most favorable rotational setting of a given DNA sequence over the histone octamer. The predicted rotational setting obtained over MMTV promoter and *Xenopus borealis* 5S RNA gene sequences, are in agreement with the experimental data obtained from the analysis of *in vitro* reconstituted nucleosome cores (4,7).

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ARE NUCLEOSOMES FORMED ON POSITIVELY SUPERCOILED DNA ?

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The nucleosome has been shown to repress transcription of certain genes when bound to the promoter. However, some RNA polymerases are able to negotiate nucleosomes on a gene once transcription has been initiated, by an unknown mechanism. It has been suggested that as RNA polymerase transcribes it may positively supercoil the DNA in front of it and negatively supercoil the DNA behind it (Liu & Wang, 1987). An attractive possibility is that nucleosomes may be disrupted or displaced by the positive supercoils generated in front of the polymerase. We have therefore asked whether "classical" nucleosomes can form on positively supercoiled DNA, or whether the core histones bind to form a different complex, such as an "open" nucleosome.

The formation of a classical nucleosome on a closed circular DNA requires a linking number change of -1, and therefore the linker DNA must absorb one positive superhelical turn per nucleosome. Thus, the formation of nucleosomes on a _negatively_ supercoiled plasmid _relaxes_ the linker DNA by one turn per nucleosome, but classical nucleosomes formed on a _positively_ supercoiled plasmid would _positively supercoil_ the linker DNA by one turn per nucleosome. Consequently, the formation of a classical nucleosome on a positively supercoiled plasmid is expected to be highly unfavourable.

We prepared moderately positively supercoiled plasmid and attempted to reconstitute nucleosomes by salt/urea dialysis. We used a wide variety of techniques to probe the structure of the complex formed: micrococcal nuclease digestion, relaxation with topoisomerase I, electron microscopy, circular dichroism, chemical cross-linking and modification, and DNAase I digestion. Surprisingly, we have found no significant differences between reconstitutes containing positively or negatively supercoiled plasmid. Our results indicate that nucleosomes are formed on positively supercoiled DNA, even when nucleosomes are packed quite closely, conditions under which very little linker DNA apparently must absorb an enormous positive superhelical stress. However, we cannot rule out the possibility that nucleosomes on positively supercoiled DNA are in a more open conformation, becoming classical nucleosomes only when the superhelical stress is relaxed (by topoisomerase I or by the first nick on digestion with micrococcal nuclease). This possibility is currently under investigation. The implications of our results will be discussed.

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ALTERED CHROMATIN STRUCTURE FORMS ON THE CpG ISLAND OF THE HMG-17 GENE DURING ERYTHROPOIESIS. Massimo P. Crippa[1], Joanne M. Nickol[2] and Michael Bustin[1]. LMC, NCI[1] and LMB, NIDDK[2], NIH, Bethesda, MD 20895 USA.

The HMG-14/-17 chromosomal proteins are among the most abundant, ubiquitous and evolutionary conserved nonhistones found in the nuclei of higher eucaryotes. These nucleosomal binding proteins may confer distinct properties to the chromatin structure of transcriptionally active genes. Here we study the expression and chromatin structure of the HMG genes during erythropoiesis, a process associated with major changes in the structure and transcriptional activity of the nucleus.

In chicken erythrocytes HMG-14a is the most prominent member of the HMG-14/-17 protein family however, HMG-14b is the true homologue of the mammalian HMG-14. During development the rate of synthesis of the various HMGs changes: between 7 and 9 days embryos, at a time when primitive erythrocytes are replaced by cells of the definitive lineage in the circulation, the ratio of HMG-14a to HMG-14b, HMG-17 and HMG-1 decreases 100, 90 and 60 fold respectively. Northern analysis of Percoll gradient-fractionated erythrocytes from 6 day embryos and nuclear run offs on erythrocytes from 5 day and 9 day embryos suggests that the synthesis of the mRNAs occurs in precursors cells, prior to their release in the circulation. HMG-14a is synthesized in primitive cells while HMG-14b is synthesized in definitive cells. The mRNAs for HMG-14a, -14b, -17 and -1 are down-regulated throughout development. Erythroid cells from 5 day embryos contain 2.5-10 time more RNA than cells from 14 day embryos; adult cells are devoid of HMG mRNA.

The chromatin structure of the gene for HMG-17 changes during erythropoiesis. The 5' region of the gene contains both constitutive and developmental-specific DNase I hypersensitive sites. A total of 5 DH sites were detected, 4 of these map within the first intron. Concomitant with the developmentally-associated transcriptional down-regulation, the gene acquires a nucleosomal repeat. The approximate position of 9 nucleosomes in the regulatory region of the gene is mapped. Two nucleosomes , with a 200bp repeat are positioned immediately down-stream from the start of transcription. Down- and up-stream from these the phasing changes to 75 bp which may be indicative of a an altered nucleosomal conformation, due to the underlying G+C rich DNA sequence, or to a non nucleosomal protein-DNA interactions. The 800 bp CpG island in the 5' region of the gene is unmethylated, regardless of the transcriptional state of the gene.

CONCLUSIONS:

1) A switch in the synthesis of HMG-14a/-14b occurs at a time during erythropoiesis when definitive cell lineage erythrocytes substitute for primitive erythrocytes in the circulation.
2) Both HMG mRNAs and proteins are synthesized in precursors cells, prior to their release in the circulation.
3) Sequences in the first intron of the HMG-17 gene may be important for the regulation of gene expression.
4) Changes in the chromatin structure of the TATA box region may be related to the transcriptional inactivation of the gene.
5) The CpG island of the gene appears to be unmethylated regardless of the transcriptional state of the gene, as expected for such DNA sequences in "housekeeping" genes.
6) Since the structure of the genes for the HMG-14/-17 protein family is very conserved throughout evolution, the results may be relevant to the chromatin structure of the whole gene family.

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HISTONE VARIANTS H2AvD AND H3.3 OF DROSOPHILA MELANOGASTER.
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We have cloned and characterized the sequences for two Drosophila histone variants, H2AvD and H3.3 Such variants are expressed at a basal level (rather than being S-phase regulated) as polyA+ mRNAs. Screening of cDNA libraries with homologous probes has identified the appropriate cDNA clones; genomic clones have been recovered for H2AvD. The gene encoding H2AvD is a single copy gene located in the interval 97D1-5, on a different chromosome from the repetitious histone gene cluster at 39D2-E2. The protein obtained by conceptual translation is 98% homologous (excluding the C-terminal tail) to the H2A.Z variant of mammals, while only 59% homologous to the Drosophila H2A protein encoded at 39D2-E2. An analysis of six H2A variants and eleven H2A S-phase regulated genes using maximum parsimony indicates that the variant gene was established prior to the separation of yeasts and Tetrahymena from other eukaryotes. H2AvD is expressed at high levels maternally and in the early embryo. An antibody specific to this protein shows widespread, but not general, staining of polytene chromosomes. An EMS-induced lethal mutation in the region 97 D1-5 contains a 311 bp deletion which removes the second exon of H2AvD. P-element mediated transformation using a 4.5 kb fragment containing H2AvD rescues this mutant, demonstrating that the H2A variant is an essential component of the Drosophila chromatin. The cDNA sequence of H3.3 gene also shows high conservation of the variant. The amino acid sequence derived differs in five amino acids from the H3 protein encoded at 39D2-E2, but is identical to the mammalian H3.3. In contrast, comparison of the DNA sequences of the two types of H3 genes of Drosophila shows only 77% homology; numerous neutral substitutions are observed. The high degree of conservation of the variant histones suggests that they play an essential role, perhaps generating alternative nucleosome structures pertinent to biological function.

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ELECTRON MICROSCOPY STUDY OF Φ_0-DNA COMPLEXES

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The chromatin of spermatozoa from the sea cucumber, *Holothuria tubulosa*, has the normal complement of histones accompanied by a small amount (about 4%) of a strongly basic low molecular weigth protein (8640 Da) called Φ_0(1). This protein is 77 amino acids long and is very similar to the C-terminal region of sea urchin sperm histone H1. This protein is most likely involved in the salt-dependent folding of chromatin into higher-order structures. The study of its complexes with DNA may be used to obtain information about the mechanism of folding of chromatin.

Some time ago we analyzed the interaction of Φ_0 with chicken erythrocyte chromatin particles of different complexity, from core to polynucleosomes (2). Addition of protein Φ_0 results in chromatin insolubilization and the interaction appears to be cooperative at any salt concentration within the range 0-0.15M ClNa. In order to obtain additional information about Φ_0 we have studied by electron microscopy the morphology of Φ_0 /DNA complexes. We observe different shapes (cables, doughnuts, rods) as a function of salt concentration and protein/DNA ratio. For the sake of comparation we have studied in parallel complexes with lysine-rich histones (H1 sea urchin sperm, H5) and protamines (salmine, clupeine). The morphology of the complexes depends on the type of DNA and protein used and on the salt concentration. We will present phase-like diagrams showing the behaviour of each complex.

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A 3-D view of chromatin and its organisation as seen by ultra-high resolution scanning electron microscopy

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The scanning electron microscope (SEM) has not traditionally been used to study the structure of macromolecular complexes such as chromatin. This has been due to the relatively low resolution of available instruments. Recent developements in field emission sources and "in-lens" facilities have resulted in SEMs with the ability to resolve down to 0.5-1.0nm, similar to the resolution of most TEMs. The SEM has the additional advantages of a 3 dimensional (3D) surface view of samples and limitless specimen thickness. In recent years the science of high resolution specimen preparation for SEM has progressed and promises to yield new information on the 3D structure and organisation within the cell. Initial studies on the 3D morphology of chromosomes(1) has lead to detailed studies of chromatin organisation in chromosomes and nuclei. Metaphase chromosomes have been isolated under near-physiological conditions and individual, undisrupted chromatin fibres have been examined in 3D. We have also used cell-free extracts of *Xenopus* eggs(2) to study the process of nuclear formation to mimick chromosome decondensation and nucleus reformation during the cell cycle. This has given us insight into how peripheral chromatin becomes organised in the nucleus and how this might interact with the nuclear pores. We postulate that the nuclear pore complex forms a functional unit with potentially active chromatin in a similar way to that sugested in the "Gene Gating" hypothesis(3).

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Fundación Juan March (Madrid)

CHROMOSOMAL REGION 4-75C_2 OF *D.HYDEI* CONTAINS A CLONED Z-DNA FORMING SEQUENCE AND IT IS IN A TRANSIENT B- TO Z-DNA FLUX IN VIVO DURING DEVELOPMENT

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We have studied the existence in vivo of Z-DNA epitopes in the developmentally regulated subregion 4-75C of the polytene chromosomes of *Drosophila hydei*. This subregion contains the sequence cloned in pF17, a plasmid selected from a *Drosophila* library by its ability to bind Anti-Z-DNA antibodies (1). We have observed that Z-DNA epitopes were only detected, at antibody concentrations lower than that shown to induce B- to Z- transitions (2), in subdivision C_2 during late third instar when the transcriptional activity of the locus was high. Accumulation of non histone chromosomal proteins in that locus was also detected during late third instar at the time of the Z-DNA formation. Northern blot data and nucleotide sequence analysis indicated that the Z-DNA forming sequence from 4-75C_2 locates in between two transcription units whose expression is regulated during third instar. Our results suggest that in subdivision 4-74C_2 a B- to Z- DNA flux occurs at a specific time during late third instar and that this flux may play a negative as well as a positive role in gene expression.

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Fundación Juan March (Madrid)

In vitro reconstitution of nucleosomes on a plasmid carrying mouse α-globin gene and its transcription by SP6 and T7 RNA polymerases.

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Different conditions have been investigated for the reconstitution of the nucleosomal structure by salt dyalysis method, on a plasmid carrying mouse α-globin gene and promoters for SP6 and T7 RNA polymerases. Random nucleosome positioning obtained in the absence of divalent cations changed to a more definied structure in the presence of Mg^{2+} as shown by sucrose gradient centrifugation and nuclease footprint of the end-labelled reconstituted plasmids.

A competition assay between T7 RNA polymerase and nucleosomal histones has been performed in order to find a limited positioning of nucleosomes around T7 RNA polymerase promotor.
In vitro transcription experiments on reconstituted templates showed that in the conditions used RNA polymerases can transcribe sequences involved in the nucleosomes without displacing them.

Fundación Juan March (Madrid)

CHROMATIN-ASSOCIATED SIGNALS AND THE INDUCTION AND SUPERINDUCTION OF PROTO-ONCOGENES.

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The proto-oncogene c-fos is rapidly and transiently activated by a variety of agents including polypeptide growth factors such as EGF, FGF, PDGF and Bombesin, as well as tumor promoters such as phorbol esters and okadaic acid. In the presence of protein synthesis inhibitors, the activation is stronger and more sustained, a phenomenon termed superinduction. Concomitant with induction and superinduction, the conformation of the c-fos gene undergoes transient alteration, as shown by nucleosome subfractionation (Chen and Allfrey, 1987) and DNAse hypersensitivity (Li and Villeponteau, 1990) studies. It is unclear whether it is the initiation of transcription that causes the conformational change, or if it is the conformational change that, by increasing accessibility, allows transcriptional initiation to occur.

We have shown (Mahadevan et al. 1988, 1989, 1990) that the earliest common chromatin-associated signalling responses that occur when cells are stimulated with the diverse agents mentioned above are the rapid phosphorylation of two proteins, pp33 and pp15. pp33 exists in two forms, complexed and chromatin-associated, that are resolved by a sequential extraction procedure developed in our laboratory. The phosphorylation of both forms of this protein is extremely sensitive to inhibition with 2-aminopurine, a procedure which specifically blocks the induction of the c-fos gene. The clearest indication that these early responses may be involved in producing a conformational change in chromatin comes from microsequencing analysis of these proteins, which has revealed the identity of pp15 as Histone H3. pp33 and Histone H3 phosphorylation are demonstrable in the presence of α-amanitin or actinomycin D, showing that they are not merely the result of transcriptional activation. Histone H3 has been shown to undergo several post-translational modifications such as acetylation, methylation and phosphorylation, and has been implicated in the relaxation of the c-fos gene nucleosome conformation associated with it's induction by serum growth factors (Chen and Allfrey, 1987). We thus have a potential mechanism by which receptor-ligand interaction at the cell surface may be transduced to transcriptional responses in the nucleus by a change in chromatin configuration brought about by the rapid activation of a nuclear kinase that phosphorylates Histone H3. The specific sites of phosphorylation on Histone H3, and it's possible significance to the regulation of nucleosome conformation and gene expression will be discussed.

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Fundación Juan March (Madrid)

STRUCTURAL CHANGES OF NUCLEOSOMAL PARTICLES AFFECTING TRANSCRIPTION

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Using an *in vitro* system containing isolated mononucleosomal particles as transcription templates and RNA polymerase II, an attempt has been made to evaluate the effects on transcription of different structural features of the nucleosomal particle. The loss of one H2A.H2B dimer from the nucleosomal core increases its efficiency as a template, allowing transcription of the entire DNA present in the particle. In contrast, the nucleosomal cores lacking the amino-terminal tails of core histones show transcriptional properties similar to those of the whole nucleosomal cores, with synthesis of short RNA chains (40 nucleotides or less). Acetylation of the amino-terminal ends of core histones, which causes moderate structural changes in the particle, seems to be correlated with a substantial increase in transcriptional efficiency. In contrast, modification of the globular regions of histones, which is accompanied by a large structural relaxation, appears to produce little additional effect on transcription. Our results show that either the loss of one H2A.H2B dimer or acetylation of the amino-terminal tails of histones causes a substantial increase in the transcriptional efficiency of nucleosomal cores. From these results, we speculate that, during elongation of RNA chains *in vivo*, acetylation of the amino-terminal domains of core histones induces a structural change of the nucleosomal particle upon interaction with the transcriptional machinery, which is accompanied by release of one H2A.H2B dimer and transcription of nucleosomal DNA.

Fundación Juan March (Madrid)

EXPLORING CHROMATIN STRUCTURE IN A SUBTELOMERIC REGION OF YEAST CHROMOSOME IX

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Previous studies in our laboratory on the organization of yeast SUC2 chromatin and its regulation by glucose led to the identification of a 3' flank region whose chromatin structure, surprisingly, was apparently related to the transcriptional activity of SUC2 (1). We have also found that this region is affected by the same regulatory genes as SUC2 (2). By means of deletion mutants constructed by us in multicopy plasmids we have discarded the existence in that region of SUC2 regulatory elements and also that the changes found in it were a consequence of SUC2 transcription.

These results suggested the existence of a previously unmapped, glucose regulated gene downstream SUC2 in the subtelomeric region of chromosome IX. We have sequenced a 3 kbp fragment contiguous to SUC2 and we have found two ORFs of 705 bp and 1251 bp. The first one is adjacent to SUC2 and transcribed from the same strand. It has a high content of charged aminoacids but not clear homology with any known protein. It is transcribed to a 0.85 kb mRNA in a constitutive form. In its promoter region there is a sequence element homologous to the ARS consensus sequence which has no ARS activity in plasmids. The second ORF is transcribed from the opposite strand to a 1.6 kb mRNA (3). The protein derived from it is highly homologous to animal peroxisomal 3-oxoacyl CoA thiolases. The transcription of this gene (POT1) is glucose repressible, as it was anticipated, and also it is fatty acid inducible. The chromatin region whose structure changes upon glucose derepression is located in the coding region of this gene, but also important changes are seen in the promoter of POT1 related with its transcriptional activity. This is the first case that a new gene is predicted and described as a consequence of chromatin studies.

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Fundación Juan March (Madrid)

The gene encoding protein ϕ. from the echinoderm *Holothuria tubulosa*: Preliminary analysis and structural correlations with histone H1

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Chromatin from germ cells of the sea cucumber *Holothuria tubulosa* contains the 5 somatic-type histones in normal relative amounts (1) and retains a nucleosomal organization with a constant spacing throughout spermatogenesis (2). During sperm maturation there is no bulk replacement of the histone complement, chromatin transitions being restricted to the addition of a specific arginine-rich H1 subtype (3) which coexists with the somatic species, plus the appearance at the onset of spermiogenesis of a highly basic protein called ϕ., which accumulates in the maturing sperm (1). Protein ϕ. has an average M_r of 8640 Da (4), represents about 4% of the histone mass in ripe sperm and has an amino acid sequence similar to that of the C-terminal part of the sea urchin sperm H1. Indeed half of the molecule has helicogenic residues which show amphipatic regions. This protein may be instrumental in stabilizing the highly regular chromatin fibers from sea cucumber spermatozoa.

In order to determine the structure of the gene coding for this protein, a gonad-specific λgt11 expression library has been recently constructed using polyA$^+$-enriched RNA from sea cucumber gonads as template for cDNA synthesis and screened with polyclonal anti-ϕ. antibodies (5). Several positive recombinant clones have been characterized and a full-length *Eco*RI cDNA insert sequenced on both strands (6). This cDNA sequence spans an open reading frame for a basic protein of 77 residues (8550 Da) flanked by leader and trailer tracts. The deduced protein sequence is mostly consistent with the partially established amino acid sequence of protein ϕ. although an Arg residue on the penultimate position of the C-terminus is missing.

We report here the construction of genomic libraries in λCharon 35 vectors after partial restriction of total sperm DNA and their probing with the cloned cDNA for the isolation of the ϕ. gene. A 17 kb *Sma*I-insert encompassing the gene has been isolated and subjected to restriction enzyme cleavage mapping by a combination of indirect methods based on the analysis of products of single and double complete digestions and direct approaches involving asymmetric end-labelling and partial cleavages.

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Fundación Juan March (Madrid)

Rapidly reversible changes of chromatin structures upstream of the TAT-Gene parallel transcriptional activity of the gene and the binding of the glucocorticoid receptor

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We are working on chromatin changes in the upstream region of the rat tyrosine aminotransferase gene. It has been shown in the past that upon induction of this gene by glucocorticoid hormones, glucocorticoid receptor binds to sequences located upstream at -2500 (Becker et al., Nature 324,686-688,1986 and Jantzen et al., Cell 49, 29-38,1987). We have shown that the process of hypersensitive site formation occurs rapidly and is readily reversible upon withdrawal of the hormone. Formation and disappearance of the upstream hypersensitive site parallel the transcriptional activity of the TAT gene as measured by run-on analysis. Using micrococcal nuclease we have demonstrated that the region of interest is nucleosomal before induction and after removal of the hormone but not in the induced state. With the use of Taq-Polymerase in a modified genomic footprinting procedure we have monitored receptor binding in the course of the induction and deinduction process. At present we try to develop a protocol that should make it possible to use native genomic acrylamide gels for a fine analysis of nucleosome positions.

Fundación Juan March (Madrid)

A New Method to Objectively Determine the Positions of Nucleosome Cores.

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ABSTRACT
Fourier transforms of DNase I digestion patterns allow an objective and accurate assessment of the position and mobility of the nucleosome cores. This method involves determining the amplitude of the 10.3 bp periodicity of the DNase I cutting probabilities in a 145 bp window moved in 1 bp steps along the DNA sequence. A maxima in the amplitude of the 10.3 bp periodicity indicates a preferred core position.

The validity of this model was tested on a random data set containing a sine wave of 145 points and a frequency of 10.3, simulating a positioned core. This proved the validity of the model and was able to determine the position of the sine wave with an accuracy better than 10 data points, corresponding to 10 bp experimentally.

Experimentally nucleosome cores were assembled on a 1,603 bp trout dynein heavy chain cDNA that contains an invariant 21 bp direct tandem repeat with occasional base substitutions and triplet insertions(Garber,A.T., Retief,J.D. and Dixon,G.H. (1989) EMBO J. 8, 1727-1734). This naturally occurring repeat provides a substrate containing structural information that is relatively easy to interpret. Nucleosomes assembly was carried out by the well established salt step method.

In the first 600 bp of the fragment(24 repeats), DNase I digestion reveals a 10.3 bp footprint, while two preferred positions can be determined by the Fourier windows method. From the shapes of the peaks and by changing the window size, it can be deduced that the first core is accurately positioned, covering an area of approximately 135 bp. The core at the second position has some freedom to slide, covering an area of approximately 185 bp. From the areas under these peaks it can be estimated that the preference for the second position is four times that of the first.

These experimentally determined positions correlate well with the areas of optimal nucleosomal fit as calculated from the DNA sequence and three-dimensional modelling of the DNA structure.

Fundación Juan March (Madrid)

Tissue Specific Variants of Topoisomerase I in Xenopus laevis.

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Topoisomerases are enzymes that catalyze changes in DNA topology. They have proven to be useful targets for chemotherapeutic agents. DNA topoisomerase I (topo I) has not been studied in a well characterized developmental system such as *Xenopus laevis*. Recently, we characterized an ovarian topo I from this organism with an unusually high molecular weight (MW), 165,000 (Richard and Bogenhagen, **J. Biol. Chem.** 264: No.8, pp.4704-4709, 1989). In addition to standard chromatographic methods, the purification protocol employed denaturing gel filtration followed by renaturation of the enzyme activity. The 165 kDa polypeptide was radiolabeled by a specific active site tagging procedure. The 165 kDa polypeptide was shown to possess topo I activity following elution from PAGE-SDS and renaturation. The MW of 165,000 is considerably larger than the MW of ~100,000 reported for most type I topoisomerases. Therefore we began experiments to investigate whether this ovarian enzyme was representative of the form found in other tissues. An antibody was raised against the 165 kDa polypeptide. The antibody failed to crossreact with or to immunoprecipitate topo I from a liver extract enriched for topo I activity. Upon purification of the liver enzyme to homogeneity, a protein with a more standard MW of 110,000 was identified. Antibodies raised against this enzyme failed to crossreact with the ovarian enzyme on western blots and immunoprecipitations of activity. The two forms appear to be antigenically distinct. *X. laevis* tissue culture cells have been used as another source of somatic topo I. These cells possess the 110 kDa form.

We have observed that upon oocyte maturation, the 165 kDa form of the enzyme is no longer present. Both progesterone treated oocytes and eggs have lost the 165 kDa form. We propose that there are two forms of topo I in *X. laevis*, a 165 kDa oocyte-specific form and a 110 kDa somatic form.

Fundación Juan March (Madrid)

ASSEMBLY OF CORRECTLY SPACED CHROMATIN IN A NUCLEAR EXTRACT FROM *XENOPUS LAEVIS* OOCYTES.

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Assembly of nucleosomes on relaxed, covalently closed DNA has been studied in a nuclear extract of *Xenopus laevis* oocytes (1). In the presence of ATP and Mg(II), correctly spaced nucleosomes containing the four histones H3, H4, H2A and H2B but lacking histone H1 are readily assembled on the DNA.

The rate of nucleosome assembly as monitored by DNA linking number reduction is unaffected by ATP and Mg(II). The pattern of micrococcal nuclease digestion shows that the nucleosomes assembled in the absence of ATP and Mg(II) are closely packed, with a periodicity of 150 bp. In contrast, in the presence of ATP and Mg(II) the spacing of nucleosomes is 180 bp, similar to that observed for nucleosomes assembled on DNA microinjected into oocyte nuclei (2).

The ATP and Mg(II) requirements for the assembly of correctly spaced nucleosomes are unrelated to the activity of the ATP and Mg(II) dependent topoisomerase II in the extract; addition of specific inhibitors of eukaryotic DNA topoisomerase II has no effect on assembly or spacing of the reconstituted nucleosomes.

To understand the role of ATP in nucleosome spacing we analyzed the effects of non-hydrolyzable ATP analogues, AMP-P-(NH)-P and γ-S-ATP. The pattern of micrococcal nuclease digestion of chromatin assembled in the presence of AMP-P-(NH)-P and Mg(II) shows that the nucleosomes are closely packed with a periodicity of 150 bp. Chromatin assembled in the presence of γ-S-ATP and Mg(II), on the other hand, displays correctly spaced nucleosomes. The results indicate that γ-S-ATP but not AMP-P-(NH)-P can substitute for ATP in the process.

We suggest that a "spacing factor" is involved in the assembly of correctly spaced nucleosomes. It is conceivable that in the absence of ATP the "spacing factor" is inactive and this could cause the observed tight packing of nucleosomes. In the presence of ATP the activation of the factor (protein phosphorilation, conformational change of the protein) could generate the correct spacing of the nucleosomes (3). Experiments are in progress to identify the factor involved in nucleosome spacing.

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Fundación Juan March (Madrid)

HISTONES ASSOCIATED WITH TRANSCRIBED RIBOSOMAL GENES ARE NOT HYPERACETYLATED

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The presence of histones upon transcription of the ribosomal genes has been studied by the following experimental strategy: a) crosslinking of histones to DNA in isolated nuclei either chemically (treated with formaldehyde) or by irradiation with picosecond UV laser; b) isolation of the covalently linked protein-DNA complexes; c) immunoprecipitation of the complexes by antibodies to the different histone species to select DNA fragments carrying a given histone and d) identification of selected DNA sequences in the immunoprecipitated DNA by hybridization to specific probes.

It was found that the highly transcribed ribosomal genes from *X. laevis* tadpoles were associated with histones as were the silent genes from erythrocytes. The acetylation state of histones bound to ribosomal genes in Guerin ascites tumor cells, grown in the presence of butyrate was studied by immunoprecipitation of crosslinked histone-DNA complexes by an antibody that specifically recognizes -N-acetyl lysine. The hybridization to specific ^{32}P-labeled DNA probes failed to detect ribosomal DNA sequences in DNA, precipitated by this antibody, i.e.the ribosomal genes, including transcribed ones, are not associated with hyperacetylated histones.

Fundación Juan March (Madrid)

SUPERSTRUCTURAL FEATURES OF THE REGULATIVE REGIONS OF THE FIVE GENES ENCODING THE SMALL SUBUNIT OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE FROM *Pisum sativum* AND THEIR POSSIBLE RELEVANCE IN CHROMATIN ORGANIZATION

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Superstructural features, deriving from the curvature of the axis of B DNA double helix, of the regulative regions of the five genes (*rbcS*) encoding the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase from *Pisum sativum*, have been analyzed adopting a theoretical method, developed by our research group, to predict DNA curvature from sequence (1,2). Theoretical curvature profiles show a good correlation with the decreased gel electrophoretic mobilities of multimers of oligonucleotides mimicking the superstructures of specific DNA regions.

From these studies, DNA sequences, interacting with regulatory factors, characterized by Chua and coworkers on the basis of biochemical and genetic analyses, show defined superstructural features (3) well correlated with their respective different expression (4).

On the basis of recent findings which correlate DNA superstructure with nucleosomes (5), we have tried the theoretical method recently developed by De Santis et al. (6) to obtain the virtual nucleosome positioning along the *rbcS*-3A and E9 regulative regions (-400, +10). The two genes show a largely different nucleosomes probability function, characterized by well defined maxima only in the case of *rbcS*-3A. This suggests that in the case of *rbcS*-3A gene the nucleosomes could have a more ordered array along the regulative region, favouring the interaction between LRE sequence and the regulative factor GT1.

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Fundación Juan March (Madrid)

The curvature vector in nucleosomal DNAs and theoretical prediction of nucleosome positioning.

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Using our model for predicting DNA superstructures from the sequence, [1-6] the average distribution of the phases of curvature along the sequences of the set of the 177 nucleosomal DNAs investigated by Satchwell et al. was calculated. The diagram obtained shows very significant features which allow the visualization of the intrinsic nucleosomal superstructure characterized by two quasi parallel tracts of a flat left handed superhelical turn connected by a left handed inflection in a perpendicular direction; such a superstructure appears to be closely related to the Klug and Travers nucleosome model. Therefore DNA contains a piece of superstructural information for the phasing of histone octamer DNA association complex in chromatin [7].
The nucleosomal curvature phase diagram was then adopted as a sensitive determinant for the nucleosome virtual positioning in DNAs via correlation function, obtaining a good agreement with the very recent experimental mapping of SV40 regulatory region as well as with the experimental positioning of nucleosomes in TRP1ARS1 yeast plasmid.
This analysis shows also the presence of a constant phase relationship between the virtual nucleosome positions which suggests its possible implication in the nucleosome condensation in chromatin.

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__The globular domain of histone H5 promotes DNA renaturation__

We propose results on the action of histone H5 and its isolated globular domain (GH5) to promote oligonucleotide DNA renaturation. A palindromic dodecamer (5' CGGCGATCGCCG 3') capable of forming both a duplex and a hairpin structure was used throughout the study. Under the conditions chosen (the oligonucleotide was heated at 90 °C for 1 minute followed by a rapid cooling on ice) the DNA was completely in the hairpin-form. At temperatures up to 20°C and at the low concentration of DNA, there was no detectable renaturation to the duplex-form, even after more than 1 hour. However, in the presence of equimolar amounts of H5 or GH5 this reaction was speeded up enormously.
A kinetic study at 25°C revealed that an intact GH5 molecule was necessary for this phenomenon. The presence of SDS completely inhibited the catalytic effect. As mentioned above at least equimolar amounts of GH5 were needed to observe a complete renaturation, and adding a large excess of GH5 did not influence the reaction. From the kinetic data it followed that the rate limiting step was not a first order reaction (as observed with the TFIIIA protein) but followed a second order mechanism.
The GH5-effect was heat-stable; one could heat a mixture of DNA and protein at 90°C for several minutes without loss of its ability to promote renaturation.
The fact that the hairpin-form (containing a stem of 4 bp) does not contain enough secondary structure to be stabilized by GH5, means that the latter recognizes longer DNA structures. It is further shown that GH5 preferes to bind the 12 bp duplex in stead of the "4 bp" hairpin. This gives us already an idea about the minimum site needed for binding of GH5. It worth noting that in order to promote the renaturation GH5 must denature first the hairpin in order to present this extended strand to the others. So GH5 does not only stabilize its end-product (the duplex), thats why you need stoichiometric amounts to overcome this product-inhibition, but also it deforms the substrate quite dramatically, converting a hairpin into an extended single strand.

Fundación Juan March (Madrid)

TRANSACTIVATOR DEPENDENT ASSOCIATION OF PROMOTER SEQUENCES WITH THE YEAST NUCLEOSKELETON.

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We have investigated the role of sub-nuclear structure in the control of transcription in the yeast Saccharomyces cerevisiae. Using near-physiological conditions we have demonstrated association of chromosomal transcription with a nucleoskeleton. Furthermore, we have extrapolated this analysis to autonomously replicating plasmids by studying the effect of introducing the PGK UAS, or its sub-components into a UAS assay plasmid. In the absence of any UAS components the inactive promoter was unattached whereas insertion of the active PGK UAS or any of its cis-active sequences led to nucleoskeleton attachment of the promoter. These data were confirmed by the demonstration that expression of the bovine papillomavirus transactivator E2 mediates attachment of its target sequence to the nucleoskeleton. Association with the nucleoskeleton is not a consequence of transcription as attachment was also observed in circumstances where transcription was not occurring. We propose that transcription occurs on the nucleoskeleton which functions to localize all of the components of the transcriptional machinery and the promoter sequences prior to polymerase initiation.

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ANIONIC DEPENDENCE OF THE STATE OF CRHOMATIN

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The mechanisms of chromatin condensation and dispersion are poorly understood. Small variations in the divalent cation concentrations, most notably Mg^{2+} ions, can dramatically affect the state of chromatin while changes in monovalent cation concentrations are much less effective. Some of these effects, on the stability of the double-helical form of DNA, can be explained by the counterion condensation theory on the screening of the negative phosphate charges.

We have examined Mg^{2+}-induced changes on the chromatin state in the presence of different anionic solutions. The experiments were performed in isolated nuclei from rat hepatocytes by using both the forward and side scattering measurements from flow cytometry and laser scanning microscopy either in the transmitted (brightfield; LSM) or confocal (CLSM) mode. 3-Dimensional reconstructions of condensed and unraveled chromatin were obtained using CLSM. The data show that the effect of Mg^{2+} on the state of chromatin was dependent on the anion present in the solution. Both the scattering measurements and the images obtained by LSM indicated that the unravelling of chromatin in 0 Mg^{2+} was much less efficient in the presence of glutamate, as compared to Cl ions. Even after the chromatin was fully dispersed in 0 Mg^{2+}, 140 KCl solutions, substitution of Cl by glutamate was sufficient to induce partial recondensation. Additionally, we found that the state of chromatin was more condensed in 2 mM Mg^{2+}, 140 mM Kglutamate when compared to 2 mM Mg^{2+}, 140 mM KCl. The addition of anion transport inhibitors, both 100 μM DIDS (4,4'diisothiocyanotostilbene 2,2'disulfonic acid) and 150 μM Niflumic acid, appeared to further enhance the unraveling of chromatin, with DIDS being the most potent of the two. Finally, we observed that 5 mM ATP caused the most dramatic condensation of chromatin, regardless of the presence of low Mg^{2+}. We are currently investigating the basis of these differential anionic requirements.

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SUMMARY

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Summary

A practical conundrum confronting any living cell is how to package an immense length of DNA in a form which both minimises the formation of Gordian knots and, at the same time, allows regulatory proteins and the machinery required for the repair, replication, transcription and recombination of the genome to access selected regions. The elegant and economical solution adopted by the eukaryotic line is to wrap short lengths of DNA around a core of extremely conserved histone proteins to form a fundamental building block which can then further assemble into a highly compacted structure. This complex of DNA with histones and multifarious other proteins is identified under the generic title of 'chromatin' and constitutes the natural template for the regulation of gene expression at the transcriptional level.

In prokaryotic organisms it has become clear that in any regulated process nothing important is left to chance. In eukaryotes a similar situation is likely to prevail. In the context of chromatin this means that the structures which are necessary to optimise the control of gene expression will be actively set in place. Thus structural flexibility of both DNA and protein molecules will be manipulated so that a required local conformation is utilised and stabilised by the regulatory process - for example, a natural tendency of DNA to bend will, in a functional context, be facilitated by a specific protein. Or again, if a nucleosome requires precise positioning, it is likely that this positioning will be specified by interactions additional to the histone-DNA contacts.

The predominant theme of the workshop was how the structure and organisation of chromatin can determine its genetic activity. At its simplest level we need to understand the properties of individual components - DNA and histones - and how these influence the structure of the nucleosome core particle and higher order structures. In the core particle some aspects of the intrinsic flexibility of DNA - notably its bendability - are utilised while others, such as its potential to adopt alternative non-B structures, are suppressed. Contained within the histones are particular peptide motifs, such as AK helices and 'SPKK', that contribute to this process and are believed to stabilise both the configuration of the double helix and also the condensation into higher order chromatin structures. It should however be noted that such short motifs, for example 'SPKK', can occur in different sequence contexts within an individual histone molecule. It does not necessarily follow that the particular biological roles and functional contacts of the isolated motifs are maintained in alternative contexts. In particular the ability of 'SPKK' to be phosphorylated in vivo suggests the possibility that a major selective pressure for this sequence is to maintain a functional duality with a DNA binding capability

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combined with a capacity to be covalently modified and thus potentially regulated. Another gap in our understanding of the structure of the nucleosome relates to the flexibility of the particle - our current images are essentially static. Could it be that the extreme conservation of the primary structures of histones relates not primarily to the ability to bind DNA but to conserve potential structural realignments necessary for condensation? Finally on this topic, it is perhaps worth noting that our view of detailed nucleosome structure might be unduly influenced by the abundance of studies that use, as their experimental material, histones from essentially inactive nuclei.

The preferred sequence organisation of nucleosomal DNA, as deduced form statistical studies, is sufficient by itself to specify precise positioning in vitro and is comprised of two principal features - periodically repeated rotational signals which determine the preferred configuration of the bound DNA and aperiodic preferences located close to the dyad which have been implicated as translational determinants. Are these same rules sufficient in vivo? A designer positioning sequence with a relatively high in vitro affinity for the histone octamer fails to position a nucleosome in vivo when inserted into a yeast minichromosome. Conversely an array of nucleosomes can be precisely positioned by an organising protein bound at a specific site. One such protein is the yeast α2 repressor whose biological function is to repress the activity of particular genes involved in mating type control. Either in synthetic constructs or in natural loci the α2 protein precisely locates an array up to at least four nucleosomes. In one locus, STE6, this array covers the TATA box and would presumably block access of the TFIID transcription factor. In this biological context it is the nucleosome, rather than the α2 protein that acts as the direct repressor and thus acts in its classically envisaged role. With this logic it would be expected that nucleosome loss should result in the activation of regulated genes. Indeed this is precisely what is observed in yeast when histone H4 production is artificially limited.

If arrays of nucleosomes can be generated by an organising protein, how is the signal transmitted? One clue is provided by the properties of nucleosomes assembled in vivo with mutant histones. Deletions of the highly conserved N-terminus of histone H4 in yeast result in the depression of the silent mating type loci. Point mutations in this region can be suppressed by second-site mutations in the Sir3 gene, which is known to be required for transcriptional silencing. These same deletions also abolish the precise arrays set up by the α2 protein. One compelling inference from these observations is that there should be direct contacts not only between nucleosomes themselves but also between nucleosomes and other protein components of chromatin. Similarly contacts between nucleosomes and the somewhat

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neglected HMG proteins might also be of regulatory significance. The precise nature of such putative contacts remains to be established and should constitute an important further line of study.

A further question raised by the organisation of nucleosome arrays is whether such arrays possess polarity. In other words, can adjacent nucleosome core particles talk to each other, and, if so, can a consequent polarity be stabilised by histone H1 or its homologues? The question is particularly relevant to Xenopus oocytes, where H1 appears to be absent, and also possibly to yeast, where the existence of a bona fide H1 histone remains a controversial issue. The observation that the sequence signals directing nucleosome positioning differ on the Xenopus somatic and ooycte 5S RNA genes raises the possibility, again, that the differential expression of these genes throughout development may be related, at least in part, to differences in accessibility to H1, and thus to a different propensity to form a condensed chromatin structure. Such an abiliity of H1 to act as a repressor of transcriptional activity may also be a property of other proteins, such as Polycomb, which have a peptide homologous to the HP-1 protein constituent of Drosophila heterochromatin.

How is chromatin rendered competent for transcriptional activity? Two principal mechanisms have been observed. In one case regulatory sites within the promoter region are free of nucleosomes and are immediately available to the cognate transcription factors or components of the transcription machinery. Such sites have been classically characterised as hypersensitive to DNase I cleavage. Again however the establishment of such nucleosome free regions requires the active participation of particular proteins. For example the promoter regions of the Drosophila heat shock genes can include islands of alternating CT sequences that appear to act in conjunction with TATA boxes to restrict nucleosomal binding in vivo. Similarly in vitro the establishment of assembled chromatin that is both transcriptionally competent and is appropriately regulated requires at least the initial association of the transcription factor TFIID with the TATA box. An alternative mode of attaining transcriptional competence that has been observed in both yeast and mammalian systems is the removal of nucleosomes as an integral part of the activation process. The induction of the MMTV LTR promoter by the glucocorticoid receptor requires the initial binding of at least one receptor molecule to the DNA exposed on the surface of a precisely positioned nucleosome. In vivo this interaction is followed by a destabilisation of the structure of this nucleosome uncovering a previously cryptic binding site for the activating protein, NF-1. However, in vitro binding of the receptor to the nucleosome does not obviously alter the structure of the latter. The precise mechanism of this disassembly remains to be elucidated. Nevertheless there is ample precedent for the alteration of core particle structure by

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the binding of ligands to the nucleosomal DNA. A case in point is a change in the rotational setting induced by distamycin. It is plausible that the primary binding of the glucocorticoid receptor could effect a similar rotation, perhaps assisted by negative superhelicity or an additional protein component.

The act of transcription or replication of a region of DNA can have significant structural and topological consequences on neighbouring sequences. To preserve the autonomy and precise regulation of individual genetic units separating insulators are required which would thus define a chromatin domain. There is now substantial evidence that the occurrence of sequences identified as scaffold or matrix attached regions correlates well with the boundaries of both DNA replication units where SARs/MARs are in close proximity to replication termination sites and also of units of transcriptional regulation. An outstanding question again is that of the dynamic status of these regions. Do they act as permanent markers of domains or is it possible to establish alternative domains, for example in different tissues or at different times?

So, is there a future for chromatin? From the studies presented at the workshop it is clear that the integration of the structural studies on histones and the functional studies on transcription factors is an essential step in understanding the true complexities of the control of gene expression in eukaryotic organisms. It is a prospect that will occupy us for some time to come - doubtless there will be several surprises and many interesting findings on the way. Perhaps in a few years time when again the intellectual challenges flung at us by the cell require resolution, we may once more enjoy the superb organisation and memorable hospitality of the Fundación Juan March.

Andrew Travers, Cambridge

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Workshop on
CHROMATIN STRUCTURE AND GENE EXPRESSION
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Estudio del mecanismo secretor de hormona juvenil en oncopeltus fasciatus.

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171 García Jiménez, J.:
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173 Fernández Aláez, C.:
Análisis estructural en sabinares de la provincia de León.

174 Furio Egea, J.:
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180 Moreno Rodríguez, J. M.:
Estudios ecológicos en jarales (cistion laurofilii): Variación anual de algunos factores del entorno y manifestaciones fenológicas.

182 Pons Vallés, M.:
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183 Herrero Ruiz de Loizaga, V. J.:
Estudio de reacciones químicas por haces moleculares. Aplicación a la reacción $C_2H_5Br + K$ Brk + C_2H_5.

193 Martín García, V. S.:
Utilización sintética en química orgánica de metales pesados como catalizadores. Oxidación asimétrica.

195 Badía Sancho, A.:
Receptores presinápticos en el conducto deferente de rata.

196 Estévez Toranzo, A.:
Supervivencia de patógenos bacterianos y virales de peces en sistemas de cultivo.

197 Lizarbe Iracheta, M.ª A.:
Caracterización molecular de las estructuras de colágeno.

203 López Calderón, I.:
Clonación de genes de «Saccharomyces cerevisiae» implicados en la reparación y la recombinación.

211 Ayala Serrano, J. A.:
Mecanismo de expresión de la PBP-3 de «E. coli»: Obtención de una cepa hiperproductora de la proteína.

240 **Genetic Strategies in Development.**
Symposium in honour of Antonio García Bellido. Lectures by S. Ochoa, S. Brenner, G. S. Stent, E. B. Lewis, D. S. Hogness, E. H. Davidson, J. B. Gurdon and F. Jacob.

244 **Course on Genome Evolution.**
Organized by E. Viñuelas. Lectures by R. F. Doolittle, A. M. Weiner/N. Maizels, G. A. Dover, J. A. Lake, J. E. Walker, J. J. Beintema, A. J. Gibbs, W. M. Fitch, P. Palese, G. Bernardi and J. M. Lowenstein.

246 **Workshop on Tolerance: Mechanisms and implications.**
Organized by P. Marrack and C. Martínez-A. Lectures by H. von Boehmer, J. W. Kappler, C. Martínez-A., H. Waldmann, N. Le Douarin, J. Sprent, P. Matzinger, R. H. Schwartz, M. Weigert, A. Coutinho, C. C. Goodnow, A. L. DeFranco and P. Marrack.

247 **Workshop on Pathogenesis-related Proteins in Plants.**
Organized by V. Conejero and L. C. Van Loon. Lectures by L. C. Van Loon, R. Fraser, J. F. Antoniw, M. Legrand, Y. Ohashi, F. Meins, T. Boller, V. Conejero, C. A. Ryan, D. F. Klessig, J. F. Bol, A. Leyva and F. García-Olmedo.

248 Beato, M.:
Course on DNA - Protein Interaction.

249 **Workshop on Molecular Diagnosis of Cancer.**
Organized by M. Perucho and P. García Barreno. Lectures by F. McCormick, A. Pellicer, J. L. Bos, M. Perucho, R. A. Weinberg, E. Harlow, E. R. Fearon, M. Schwab, F. W. Alt, R. Dalla Favera, P. E. Reddy, E. M. de Villiers, D. Slamon, I. B. Roninson, J. Groffen and M. Barbacid.

251 **Lecture Course on Approaches to Plant Development.**
Organized by P. Puigdoménech and T. Nelson. Lectures by I. Sussex, R. S. Poethig, M. Delseny, M. Freeling, S. C. de Vries, J. H. Rothman, J. Modolell, F. Salamini, M. A. Estelle, J. M. Martínez Zapater, A. Spena, P. J. J. Hooykaas, T. Nelson, P. Puigdoménech and M. Pagès.

252 **Curso Experimental de Electroforesis Bidimensional de Alta Resolución.**
Organizado por Juan F. Santarén. Seminarios por Julio E. Celis, James I. Garrels, Joël Vandekerckhove, Juan F. Santarén y Rosa Assiego.

253 **Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses.**
Organized by F. García-Arenal and P. Palukaitis. Lectures by D. Baulcome, R. N. Beachy, G. Boccardo, J. Bol, G. Bruening,

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J. Burgyan, J. R. Díaz Ruiz, W. G. Dougherty, F. García-Arenal, W. L. Gerlach, A. L. Haenni, E. M. J. Jaspars, D. L. Nuss, P. Palukaitis, Y. Watanabe and M. Zaitlin.

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

255 **Workshop on The Reference Points in Evolution.**
Organized by P. Alberch and G. A. Dover. Lectures by P. Alberch, P. Bateson, R. J. Britten, B. C. Clarke, S. Conway Morris, G. A. Dover, G. M. Edelman, R. Flavell, A. Fontdevila, A. García-Bellido, G. L. G. Miklos, C. Milstein, A. Moya, G. B. Müller, G. Oster, M. De Renzi, A. Seilacher, S. Stearns, E. S. Vrba, G. P. Wagner, D. B. Wake and A. Wilson.

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