Instituto Juan March
de Estudios e Investigaciones

85

CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Chromatin and DNA Modification: Plant Gene Expression and Silencing

Organized by

T. C. Hall, A. P. Wolffe, R. J. Ferl and M. A. Vega-Palas

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Introduction

Timothy C. Hall

Allan Wolffe likes to quote from a review which claims "chromatin is the last refuge of scoundrels". From this workshop, it appears that the number of scoundrels is increasing and that they are no longer seeking refuge!

Although it has long been recognized that transcriptionally active domains of chromatin are relatively highly acetylated, the identification of multiple histone acetyl transferases (HATs) and histone deacetylases (HDs) capable of modifying specific lysine residues in specific genes is very recent. Acetylation of lysine residues in the N-tails of core histones is now seen as loosening the chromatin structure thereby permitting access by transcription factors and potentiating a gene sequence for transcriptional activation. Conversely, methylation of both DNA and histones is now recognized to permit recruitment of proteins such as MeCP2 that condense chromatin, leading to transcriptionally inactive domains. The latter interactions are highly parallel to the inactivation of transgenes, specially in plants, that falls under the general term "gene silencing."

Fundamental insight to nucleosome packing and transcriptional activation has been gained through studies in animal models such as MMTV, and information concerning the relationship of chromatin modification and gene function is now growing for plants, especially through the study of trangenes. This workshop provided an unprecedented opportunity for the exchange of information between animal and plant scientists who are focusing on the relationship of chromatin to transcriptional activation and inactivation, and the outcome will be stimulating to research in both fields.

The presentations, summarized in this booklet, first explored chemical modifications of chromatin such as acetylation, methylation and phosphorylation. From the work discussed, it became apparent that parallel situations exist for animal, lower eukaryote and plant systems, with regulatory systems balancing HAT and HD activities for specific genes during development. Methylation of chromatin generally represses expression, and probably reflects genome defense systems that may be ancient in origin and retained to suppress the activity of retroviral elements that are now known to be ubiquitous and abundant in eukaryotes from maize to man. In addition to their original functions, these active defense mechanisms appear to be effective in silencing transgene sequences that are recognized as intrusive to the genome. Methylation may be signaled by sequence duplication and can probably act through various pathways to achieve transcriptional inactivation.

Examples of two step processes in gene function - potentiation and transcriptional activation - were provided for both animal and plant systems that are known to have precisely positioned nucleosomes. While acetylation is clearly a powerful force in potentiating a chromatin domain for activation, alternative or interactive systems such as the ATP-dependent remodeling of nucleosomes by SWI/SNF complexes is now well established and exciting information describing the interaction of such remodeling machines with nucleosome arrays was presented. It seems that for several gene systems we are drawing close to an understanding of the mechanisms that trigger the cascade of events involved in transcriptional activation.

New work predicting a highly compacted, interdigitated helical model for chromatin and contemplation of the potential role of DNA topology in setting chromatin structure suggested that much remains to be learned in the area of chromatin. It was also evident that features of higher order chromatin structure, such as the matrix attachment regions (MARs) need further evaluation as to their potential role for diminishing the effect of chromosomal location on activity. Especially important work was described relating transgene position, located by fluorescent in situ hybridization, with function. At an even higher level of organization, the existence of ordered chromosomal arrays in cells and even of mobile RNA arrays (coiled bodies) within the nucleus reminded us that many new treasures are likely to be found in the Pandora's Box that we know as the eukaryotic cell.

Session 1: Chromatin modifications

Chair: Robert J. Ferl

PHOSPHORYLATION, ACETYLATION AND TRANSCRIPTIONAL ACTIVITY

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Abundant biochemical and genetic data support long-standing correlations between histone modifications, notably acetylation and phosphorylation, and gene regulation. We favor the view that covalent changes in histone termini influence their interactions with DNA and/or other proteins to enhance transcription and other DNA-templated processes. The recent identification of histone-modifying enzymes as components of the transcription apparatus has provided critical evidence linking chromatin structure to transcriptional activity. Other studies have shown that histone phosphorylation also plays important roles in chromosome condensation during mitosis as well as an immediate-early response to mitogens. The kinases responsible for these phosphorylation events have remained elusive. Evidence will be presented that Rsk-2, a member of the $p90^{rsk}$ family of mitogen-activated kinases implicated in cell proliferation, is the kinase responsible for mitogen-stimulated histone H3 phosphorylation in human cells. Mutations in the *RSK-2* gene are causally linked to the Coffin-Lowry syndrome (CLS), an X-linked disorder, in humans. Cells derived from CLS-afflicted individuals (i. e. Rsk-2 deficient) fail to exhibit H3 phosphorylation after mitogen stimulation even though mitosis-related H3 phosphorylation is normal. Our results provide the first evidence that a core histone is a physiological substrate of a MAPK effector, and provide new insights into a poorly appreciated mechanism that links histone H3 phosphorylation to activation by mitogens. We suspect that a chromatin remodeling step involving H3 phosphorylation, possibly in concert with histone acetylation of mitogen-stimulated genes, is a critical part of a signaling pathway whose misregulation is intimately linked to a human disease. These data provide strong support for the concept that variegation of chromatin structure, through covalent modification of histones, plays an important role in gene regulation with significant implications for human biology and disease.

Histone acetylation: new facts - old questions

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ε-amino groups of specific lysine residues within the N-terminal extensions of core histones are posttranslationally modified by acetylation. The recent discovery that numerous transcriptional regulators exert histone acetyltransferase (HAT) or histone deacetylase (HD) activity stimulated the attention on the role of chromatin structure in gene regulation (1). It has been demonstrated that HATs and HDs can interact with specific DNA-binding activator or repressor proteins, thereby modulating transcriptional activity of specific promoters by locally changing chromatin structure. It is still unclear whether a distinct acetylation pattern directly alters nucleosomal structure or acts as a signal, much like protein phosphorylation, that triggers chromosomal events by changing protein-histone interactions; this view was conceptually supported by the recent findings that non-histone proteins, like p53 or general transcription factors are acetylated by HATs *in vivo* and *in vitro* (2, 3).

In fungi and plants, multiple enzyme forms of HATs and HDs exist. In *Physarum polycephalum* we could characterize 6 nuclear A-type HATs apart from the cytoplasmic B-enzyme (4) which is highly substrate and site specific, only acetylating H4 on lysines 12 and 5 in a sequential mode (5). In contrast to mammalian cells, where so far only HDs of the *RPD3*-type have been identified (6), in plants, there are at least 3 distinct families of histone deacetylases; in maize embryos, 2 *RPD3*-type HDs could be identified corresponding to the HD1-B enzyme activity. Besides, a nucleolar phosphoprotein (HD2) has been identified as a deacetylase (7) which shares sequence homology with a class of peptidyl-prolyl cis-trans isomerases (8, 9), and a third deacetylase, HD1-A, which is unrelated to *RPD3* and nucleolar HD2. The members of these HD families differ considerably in substrate and lysine site specificity, interaction with other regulatory proteins, and the expression pattern during the cell cycle and

differentiation pathways. Moreover, phosphorylation changes enzymatic properties and substrate/site specificities of HD1-A and HD2.

The facts that histone acetylation enzymes exert their functions as protein complexes of different composition, are associated with protein phosphorylation pathways, and have a very pronounced specificity for a distinct acetylation pattern suggest a highly complex role in the modulation of gene activity, rather than a unique structural effect on nucleosomes by simple changes of histone octamer charge. The multiplicity of HATs and HDs and the complex regulation suggest that histone acetylation has additional, yet unidentified functions in nuclear processes, apart from its well-established role in transcriptional activation and repression.

1. Struhl, K. *Genes Dev.* **12,** 599-606 (1998).
2. Gu, W. & Roeder, R. G. *Cell* **90,** 595-606 (1997).
3. Imhof, A., Yang, X-J., Ogryzko, V. V., Nakatani, Y., Wolffe, A. P. & Ge, H. *Curr. Biol.* **7,** 689-692 (1997).
4. Lusser, A., Brosch, G., López-Rodas, G. & Loidl, P. *Eur. J. Cell Biol.* **74,** 102-110 (1997).
5. Kölle, D., Sarg, B., Lindner, H. & Loidl, P. *FEBS Lett.* **421,** 109-114 (1998).
6. Taunton, J., Hassig, C. A. & Schreiber, S. L. *Science* **272,** 408-411 (1996).
7. Lusser, A., Brosch, G., Loidl, A., Haas, H. & Loidl, P. *Science* **277,** 88-91 (1997).
8. Aravind, L. & Koonin, E. V. *Science* **280,** 1167 (1998).
9. Dangl, M., Lusser, A., Brosch, G., Loidl, A., Haas, H. & Loidl, P. *Science* **280,** 1167 (1998).

CYTOSINE METHYLATION IN MAMMALIAN DEVELOPMENT AND GENOME DEFENSE

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Retroviral DNA represents 1 to 2 percent of the mammalian genome (exons of cellular genes represent only ~3 percent), but the constitutive promoters in most proviruses are paradoxically silent. The most abundant and active endogenous retrovirus in the mouse genome is the intracisternal A particle (IAP) element. We show here that cytosine methylation is rate-limiting for transcription of IAP proviruses, and that IAP transcript levels increase 50–100 fold in mouse embryos deficient in Dnmt1 (DNA methyltransferase-1). IAP proviral DNA is heavily methylated in all cell types except for the premeiotic oocyte and for a brief period in the development of the primordial germ cell, where retroviral DNA is not expressed at high levels. Cytosine methylation therefore suppresses IAP transcription for the large majority of the life of germ cells. De novo methylation of IAP proviral DNA is not coupled to DNA replication but is triggered in nondividing germ cells during gametogenesis; this occurs prior to meiosis in male germ cells but only after the pachytene stage of female meiosis I. The fact that restrictions on future transcriptional potential are simultaneously imposed on endoge-nous imprinted genes and on IAP retroviral DNA in nondividing germ cells suggests that similar mechanisms may regulate genomic imprinting and the silencing of parasitic sequences. The pre-mRNA for Dnmt1, the predominant de novo and maintenance DNA methyltransferase of mammals, undergoes complex alternative splicing of 5' exons which controls the production and localization of active Dnmt1 enzyme. There is also evidence of additional DNA methyltransferases of unusual character that may be involved in the de novo methylation of imprinted loci and parasitic sequences during gametogenesis and preimplantation development.

SUPPRESSION OF HOMOLOGOUS RECOMBINATION BY DNA METHYLATION

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Homologous recombination between dispersed DNA repeats creates chromosomal rearrangements that are deleterious to the genome. The methylation associated with DNA repeats in many eukaryotes might serve to inhibit homologous recombination and play a role in preserving genome integrity. This hypothesis has now been directly tested in the fungus *Ascobolus immersus*, by making use of the natural process of Methylation Induced Premeiotically (MIP) to methylate a discrete DNA region *in vivo*. It was shown that the frequency of crossing-over between flanking markers is reduced 300-fold when the region between the markers is methylated on both chromosomes and 50-fold when it is methylated on one of the two recombining chromosomes (1). The latter effect indicates that methylation does not act by simply blocking the initiation of recombination on the methylated homolog. Based on this and on the observation that transfer of methylation frequently occurs between alleles during recombination (1, 2), we have conducted experiments aimed at defining the extent to which many step(s) of the recombination process are affected by methylation. Results indicate that the formation of heteroduplex DNA, a central intermediate of the recombination reaction, is little affected by methylation when it contains mismatches that are poorly recognized by the mismatch repair machinery. These data strongly argue in favor of an inhibitory effect of methylation on crossing-over taking place late, possibly during maturation of the recombination intermediates into crossover products.

1.L. Maloisel, J. L. Rossignol, *Genes Dev* **12**, 1381-9 (1998).
2.V. Colot, L. Maloisel, J. L. Rossignol, *Cell* **86**, 855-64 (1996).

Session 2: Chromatin organization and gene expression

Chair: Timothy C. Hall

TRANSCRIPTIONAL CONTROL AND ACETYLATION

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Transcriptional control relies on the recruitment of macromolecular machines that modify both the transcriptional machinery itself and the chromatin environment in which it functions (1). Diverse sequence specific transcription factors such as steroid and nuclear hormone receptors, AP-1, E2F, c-Myb/v-Myb, MyoD, and CREB utilize a limited number of coactivator complexes to activate transcription. These include p300/CBP, PCAF and SRC-1, all of which are acetyltransferases (2). Conversely diverse transcriptional repressors including steroid and nuclear receptors, YY1, Mad/Max, Rb and MeCP2 utilize corepressor complexes containing deacetylases (3). Acetyltransferases and deacetylases represent novel drug targets for pharmacological intervention with the transcription process. A broad range of deacetylase inhibitors are currently available that are known to influence differentiation and development.

Acetylation is intimately connected to transcriptional regulation. Acetylation of the core histone amino (N-) terminal tails provides a means by which transcription factors can gain access to their recognition elements within nucleosomes and facilitates transcription from nucleosomal arrays (4, 5). The histone acetyltransferase activity of coactivator complexes is required for transcription activation and histone deacetylase activity is required for transcriptional repression (6, 7). Histones are locally modified on target promoters and specific lysines in particular histones are functional targets for acetyltransferases and deacetylases. Histone acetylation states are dynamic, with acetylated lysines in hyperacetylated histones turning over rapidly in transcriptionally active chromatin, but much less rapidly in the hypoacetylated histones of transcriptionally silent regions. The dynamics of histone acetylation provide a mechanistic foundation for the reversible activation and repression of transcription (8, 9).

The modification of histones in vitro and in vivo does not prove that these abundant structural proteins are the only targets for regulatory activity in vivo. Other components of the transcriptional machinery such as p53, TFIIE and TFIIF can be acetylated in vitro (10, 11). Modification of these more limiting factors in the eukaryotic nucleus might potentially have the dominant control function for transcription. Likewise coactivators or corepressors can influence the recruitment or function of the basal transcriptional machinery by mechanisms independent of the acetylation status of either the histones or any other proteins.

In order to understand the contribution that protein acetylation status makes to the transcription process it is important 1) to define the functional consequences of modifying particular proteins to known extents; 2) to identify the determinants of specificity in the modification process and 3) to understand the biological context in which particular acetyltransferases or deacetylases function.

I discuss how chromatin structural transitions dependent on acetylation facilitate both access of the transcriptional machinery to DNA and the processivity of DNA polymerase through a nucleosomal array. Although transcription factors can also be acetylated, the functional consequences currently described are small compared to modification of the histones. Histone deacetylases associate with a family of WD repeat proteins, RbAp48/p46 that have specificity for association with the histones. In particular we can identify a segment of histone H4 that is

exposed on the nucleosome surface as a target for Rbp48. RPD3, the catalytic subunit of histone deacetylase, associates with Rbp48 and we propose that RbAp48 targets the deacetylase activity to the histone tail domains. Overexpression of RPD3 can direct the dominant silencing of transcription within a chromatin template. The deacetylase does not interfere with transcription when nucleosome assembly is incomplete (6). Taken together we conclude that histone deacetylation mediated by the Rbp48/RPD3 complex can dominantly repress transcription. We have characterized two novel deacetylase complexes from Xenopus oocytes and eggs. The methyl CpG-binding transcriptional repressor MeCP2 and the Mi-2 chromodomain are associated with distinct deacetylases. The presence of MeCP2 in a histone deacetylase complex links DNA methylation to the directed modification of chromatin structure and function (12). The presence of the Mi-2 chromodomain potentially links both long range chromosomal organization to histone modification (13). These biological connections should provide new insight into the molecular mechanisms by which histone acetylation contributes to chromosomal function and into the compartmentalization of the genome into active and inactive domains that can be stably maintained throughout the development of an organism.

1. Wolffe, A. (1998) Chromatin: structure and function. Academic Press, London
2. Wade, P.A. and Wolffe, A.P. (1997) Curr. Biol. 7:82-84
3. Taunton, J. et al., (1996) Science 272:408-411
4. Lee, D.Y. et al., (1993) Cell 72:73-84
5. Ura, K. et al., (1997) EMBO J. 16:2096-2107
6. Wong, J. et al., (1998) EMBO J. 17:520-534
7. Mizzen, C.A. and Allis, C.D. (1998) Cell Mol. Life Sci. 54:6-20
8. Grunstein, M. (1997) Nature 389:340-352
9. Wolffe, A.P. (1997) Nature 387:16-17
10. Gu, W. and Roeder, R.G. (1997) Cell 90:595-606
11. Imhof, A. et al., (1997) Curr. Biol. 7:689-692
12. Jones, P.L. et al., (1998) Nature Genetics 19:187-191
13. Wade, P.A. et al., (1998) Curr. Biol. 8:(in press)

Characteristics of plant MARs that influence affinity for the nuclear matrix and enhancement of stable transgene expression.

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Based on preliminary evidence, we have hypothesized that there is a direct correlation between the affinity of plant MARs for the nuclear matrix and the magnitude of their enhancement of stable transgene expression (Allen et al, 1996, PLANT CELL 8, 899-913). In order to test this hypothesis, we have created a library of MARs from tobacco NT-1 cells. The library was created by cloning DNA fragments that are MARs by operational definition, that is, DNA fragments that remain bound to purified nuclear matricies. From this library we have selected MARs that bind strongly, moderately and weakly to the plant nuclear matrix. We have searched for sequence motifs in the various MARs that would allow us to predict if a DNA fragment will be a MAR and with what affinity it will bind to the nuclear matrix. Scatchard plots and cross competition experiments have allowed us to determine the approximate number of binding sites in the nuclear matrix for each of the categories of MARs and to determine if extent of binding is due to numbers of specific binding sites or to affinity for binding sites that are common to all MARs. We have further selected representative strong, medium and weak MARs to assess their ability to enhance expression of transgenes in plant cells in culture. We will present results of the gene expression studies, the biding studies and their possible correlations.

The organization of transcription and transcript processing

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Transcription occurs at a discrete number of sites within the nucleus and nucleolus in eukaryotic cells. We have used a combination of 3-D confocal microscopy following in situ hybridization, or incorporation of labelled precursors into nascent RNA, or both, to analyse the distribution of transcription sites within plant nuclei and nucleoli.

The detailed location of RNA polymerase I transcription sites in the nucleolus has been examined by incorporation of BrUTP into nascent transcripts followed by fluorescent antibody detection, showing that the transcription sites are small foci distributed through the dense fibrillar component of the nucleoli. Direct comparison of the localization of the transcription sites with in situ probes to the coding regions and intergenic spacer regions respectively of the rDNA suggests that most sites represent a single gene copy. We have further mapped different stages of transcript processing, both by examining the location of different pre-rRNA spacer regions (ETS and ITS1), and by the localization of several snoRNAs and proteins known to be involved in different stages of pre-rRNA processing (U3, U14, 7-2/ MRP, fibrillarin, SSB1). This has led to a model with successive stages of processing occurring in a highly organized, vectorial way, the transcription sites being enveloped by several concentric layers representing subsequent processing steps.

In order to examine nuclear (pol II and pol III) transcription sites in relation to chromosome domain structure, we made use of wheat addition and substitution lines. Chromosome domains were visualized by genomic in situ hybridization using total rye genomic probe in wheat lines containing a translocation of a rye chromosome arm or the addition of a pair of rye chromosomes. The chromosomes appeared as elongated domains and showed a clear centromere-telomere polarization, with the two visualised chromosomes lying approximately parallel to one another across the nucleus. Labelling with probes to telomeres and centromeres confirmed a striking Rabl configuration in all cells, with a clear clustering of the centromeres, and cell files often maintained a common polarity through several division cycles. Transcription sites were detected by BrUTP incorporation in unfixed tissue sections and revealed a pattern of numerous foci uniformly distributed throughout the nucleoplasm, as well as more intensely labelled foci in the nucleoli. The two techniques were combined to determine the relationship between the arrangement of chromosomes, centromeres and telomeres and the distribution of transcription sites. The gene rich regions in wheat chromosomes are clustered towards the telomeres. However, there was no indication of a difference in concentration of transcription sites between telomere and centromere poles of the nucleus. Neither could we detect any evidence that the transcription sites were preferentially localized with respect to the chromosome domain boundaries.

It has been suggested that transcriptionally active genes are located on loop domains which are anchored by specific nuclear matrix attachment regions (MARs). Thompson, Spiker and colleagues have isolated a number of such sequences and used them to flank introduced transgenes. Flanking with MARs increased the level of transcription of the transgenes. We have used fluorescence in situ hybridization to localize the transgenes in several of these transgenic tobacco lines, both in fixed nuclei and in nuclear haloes. We find significant differences in the

location of MAR-flanked genes in nuclear halo preparations compared to lines containing the same transgenes unflanked by MARs.

Processing of both pre-rRNA and pre-mRNA transcripts requires a number of small nuclear RNAs and small nucleolar RNAs (snRNAs and snoRNAs). Many of these small RNAs are concentrated in intranuclear structures called coiled bodies, along with various nuclear and nucleolar proteins. The function of coiled bodies is still poorly understood, but we have recently shown that they are involved in processing or transport of snoRNA precursors in plants. We have made stably transformed plant lines expressing a fusion of the spliceosomal protein U2B" with Green Fluorescent Protein (GFP). In these lines, prominent coiled bodies can be seen in living cells by fluorescence microscopy. Our initial analysis shows that the coiled bodies are dynamic, moving within the nucleoplasm, and sometimes moving from the nuclear periphery to the nucleolus, and even into the nucleolus. On occasions we have observed the coiled bodies fusing together. These results again suggest a role for coiled bodies in nuclear transport.

References:

Shaw, P. J., Highett, M. I., Beven, A. F., & Jordan, E. G. (1995) The nucleolar architecture of polymerase I transcription and processing. EMBO J. 14, 2896-2906

Beven, A. F., Lee, R., Razaz, M., Leader, D. J., Brown, J. W. S., & Shaw, P. J. (1996) The organization of ribosomal RNA processing correlates with the distribution of nucleolar snRNAs. J. Cell Sci. 109, 1241-1251.

Thompson, W. F., Beven, A. F., Wells, B., & Shaw, P. J. (1997) Sites of rDNA transcription are widely dispersed through the nucleolus in Pisum sativum and can comprise single genes. Plant J. 12, 571-582

Shaw, P. J., Beven, A. F., Leader, D. J., & Brown, J. W. S. (1998) Localization and processing from a polycistronic precursor of novel snoRNAs in maize. J. Cell Sci. 111, in press

ENGINEERING TRANSGENES TO MODIFY CHROMATIN STRUCTURE

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Recently we (Allen et al., Plant Cell 8, 899, 1996) and others (Mlynárová et al., Plant Cell 8, 1589, 1996) have used DNA sequences containing matrix attachment regions (MARs; otherwise known as scaffold attachment regions, or SARs) to modify expression of transgenes in plants and plant cells. The theory behind both sets of experiments is that MARs should modify chromatin structure, for example by mediating attachment to the proteinaceous nuclear matrix or by facilitating 'chromatin opening' to increase access by polymerases and transcription factors. Mlynárová et al. report that their MAR constructs show only slightly elevated expression, but that variability among independent transformants is greatly reduced. In contrast, our experiments show greatly increased average expression with little or no effect on variability. These and other experiments will be discussed with reference to a model in which the primary effect of MARs is to reduce the probability of transgene silencing. According to this model, the seemingly different MAR effects may reflect differences in the susceptibility to gene silencing of transgenes introduced by different transformation techniques as well as differences in the ability of MARs to cause chromatin opening in different types of cells.

Work from my laboratory has been supported by research grants from USDA-CRGO as well as by training grants from the McKnight Foundation and from a NSF/USDA/DOE consortium.

COMPACT INTERDIGITATED HELICAL MODEL FOR CHROMATIN

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Our previous electron microscopy and electrophoretic studies (1-3) of small chromatin fragments from chicken erythrocytes have shown that, in presence of 1.7 mM Mg^{2+}, these fragments form helical fibers that are more compact than normal solenoids or other models proposed for chicken erythrocyte chromatin. These studies have also shown that fiber folding is initiated from a basic element with 5-7 nucleosomes. In this work, using computer-generated models, we have found that a structural solution that allows the formation of compact fibers consists in the interdigitation of the successive helical turns of simple helices with few nucleosomes per turn. With this folding pattern we have constructed a family of models containing 13-14 nucleosomes per 11 nm. We show that interdigitation of the primary helix or solenoid formed by consecutive nucleosomes gives rise to secondary helices, in which nucleosomes from successive turns of the primary helix interact through their faces. Stacking of nucleosomes in secondary helices is probably related with early findings of several laboratories showing that the protein core of nucleosomes has a high tendency to associate forming helices. Tilt angles of nucleosomes with respect to the fiber axis of our family of models are 20, 29, 40, and 52°, for structures with 2.8, 3.8, 4.7, and 5.8 nucleosomes per turn in the primary helix, and 3, 4, 5, and 6 secondary helices, respectively; the diameter of all models is 36 nm. We have included linker DNA in the model containing 4.7 nucleosomes per turn and we show that there is enough space in the central region for 60 bp of linker DNA and a hole of 7 nm in diameter. The calculated cross-sectional radius of gyration of this model (12 nm) is in agreement with the values obtained for folded chicken erythrocyte chromatin in several laboratories using neutron and X-ray scattering. Additional details of the model can be found in (4).

(1) Bartolomé, S., Bermúdez, A. & Daban, J.-R. (1994). *J. Cell Sci.* **107**, 2983-2992.
(2) Bartolomé, S., Bermúdez, A. & Daban, J.-R. (1995). *J. Biol. Chem.* **270**, 22514-22521.
(3) Bermúdez, A., Bartolomé, S. & Daban, J.-R. (1998). *J. Cell Sci.* **111**, 1707-1715.
(4) Daban, J.-R. & Bermúdez, A. (1998). *Biochemistry* **37**, 4299-4304.
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Session 3: Nucleosomes positioning and gene expression

Chair: Alan Wolffe

Remodeling chromatin architecture of the *phas* promoter gives new room for expression.

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We have recently published collaborative *in vivo* and *in vitro* footprinting studies showing that a rotationally- and translationally-positioned nucleosome is present over the TATA region of the phaseolin (*phas*) promoter in vegetative tissues (Li et al., 1998). This, together with the presence of three phased TATA boxes, appears to participate in the very tight spatial regulation of this gene. Whereas very high levels of expression occur during embryogenesis, fusion constructs with diphtheria toxin A-chain have shown that the *phas* promoter is totally silent in vegetative tissues (van der Geest et al., 1995). Clearly, this provides a very discrete system for investigation of transcriptional activation processes in plants.

Although PvAlf, a VP1-like factor from bean (*Phaseolus vulgaris*) has been reported to participate in transient transcriptional activation from the *phas* promoter (Bobb et al., 1995), initial experiments in which *35S-PvAlf* and *phas-gus* constructs were stably integrated into tobacco showed no expression of the GUS reporter. However, treatment of young seedlings or mature leaves of plants transgenic for both constructs with ABA resulted in high levels of expression. This suggests that replication is not required for remodeling the *phas* promoter and that expression of PvAlf may initiate remodeling, permitting access by other transcription factors known from transient studies (Frisch et al., 1995) to be capable of supporting expression from the *phas* promoter in vegetative tissues. The possible involvement of histone acetylases in remodeling the *phas* promoter in association with activation in the presence of PvAlf is under investigation.

Selected references

Bobb, A. J., Eiben, H. G., and Bustos, M. M. (1995). PvAlf, an embryo-specific acidic transcriptional activator enhances gene expression from phaseolin and phytohemagglutinin promoters. The Plant Journal *8*, 331-343.

Frisch, D. A., van der Geest, A. H. M., Dias, K., and Hall, T. C. (1995). Chromosomal integration is required for spatial regulation of expression from the -ph a s eolin promoter. Plant J. *7*, 503-512.

Li, G., Chandler, S. P., Wolffe, A. P., and Hall, T. C. (1998). Architectural specificity in chromatin structure at the TATA box in vivo: Nucleosome displacement upon - phaseolin gene activation. Proc Natl Acad Sci U S A *95*, 4772-7.

van der Geest, A. H. M., Frisch, D. A., Kemp, J. D., and Hall, T. C. (1995). Cell ablation reveals that expression from the phaseolin promoter is confined to embryogenesis and microsporogenesis. Plant J. *109*, 1151-1158.

ROLE OF POSITIONED NUCLEOSOMES IN TRANSCRIPTIONAL CONTROL OF MMTV

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Gene expression in eukaryotes is regulated by the combinatorial interaction among sequence-specific and general transcription factors. The plan for these interactions is specified by the array of *cis* elements in the promoter and enhancer regions of genes, but its implementation involves the action of adaptors or co-activators and is modulated by the nucleosomal organization of DNA. We study these processes in the context of gene induction by steroid hormones .

Steroid hormone receptors are ligand modulated transactivators which bind to their genomic recognition sites as homodimers and recruit co-activators, the general transcription machinery and, very likely, chromatin remodeling activities (Beato et al., 1995) . To approach the molecular mechanisms involved we have studied the regulated transcription the Mouse Mammary Tumor Virus (MMTV), which is silent in the absence of hormones but highly active in response to glucocorticoids or progestins. Hormonal induction of the MMTV promoter is mediated by a complex hormone responsive unit (HRU) composed of five hormone receptor binding sites, upstream of a binding site for the transcription factor NF1, two octamer motifs, and the TATA box. Optimal induction requires the integrity of all these *cis*-acting elements, but the corresponding factors cannot bind to free DNA simultaneously. NF1 competes for binding of hormone receptors and OTF1 to their respective sites on the naked promoter DNA. In cells carrying chromosomal MMTV sequences, the HRU is located on a phased nucleosome in a way which allows binding of the hormone receptors to two of their five cognate sites, while precluding access to the NF1 site and to the octamer motifs (Truss et al., 1995) . In reconstituted nucleosomes, receptor binding is determined by the rotational orientation of the relevant major grooves, but the NF1 site is unaccessible no matter its helical orientation, as long as it is included within a positioned nucleosome (Eisfeld et al., 1997) .

Hormone treatment leads to a rapid alteration in chromatin structure and to a simultaneous occupancy of all five receptors binding sites, the NF1, and the octamer motifs, while a nucleosome-like particle remains in place, suggesting that it may facilitate full loading of the promoter with transcription factors (Truss et al., 1995) . This notion is supported by studies on the influence of nucleosome depletion on basal and induced

expression of MMTV promoter in yeast. The MMTV promoter exhibits a preferentially positioned nucleosome in *S. cerevisiae*, with similar location as in metazoan cells (Chávez et al., 1995) . Hormonal induction of MMTV transcription in yeast depends on a functional synergism between HR and NF1. Following depletion of nucleosomes, independent transactivation by NF1 or by GR, as well as binding of the individual proteins to the promoter, are enhanced, whereas the NF1-dependent hormone induction of the promoter and simultaneous binding of receptor and NF1 are compromised (Chávez and Beato, 1997) . Thus, positioned nucleosomes do not only account for constitutive repression but participate in induction by mediating cooperative binding and functional synergism between GR and NF1.

How the nucleosomal organization is remodelled following hormone induction remains an open question. Experiments with the MMTV promoter in reconstituted nucleosomes containing either an octamer of all four core histones or a tetramer of histones H3 and H4, show that NF1 and OTF1 can gain access to their respective sites on the tetramer but not on the octamer (Spangenberg et al., 1998) . Therefore remodelling may involve a removal of histone H2A/H2B dimers, which could be catalyzed by one of the recently discovered ATP-dependent chromatin remodeling activities, such as the SWI/SNF complex. Incubation of octamer particles with partially purified SWI/SNF complex induces ATP-dependent changes in their DNase I cleavage pattern, which becomes similar to that found with the tetramer particles (Spangenberg et al., 1997) . Such changes could be facilitated by posttranslational modifications of the N-terminal histone tails, since moderate hyperacetylation enhances MMTV promoter activity, even in the absence of hormone (Bartsch et al., 1996) . Several steroid hormones co-activators, including SRC-1/TIF-2 and p300/CBP, exhibit histone acetyltransferase (HAT) activity, and/or interact with other HATs/GCN5. Collectively these findings suggest an important role of chromatin in transcriptional control by steroid hormones.

We are approaching these issues genetically and biochemically. The possibility of reproducing some of the crucial events in yeast allows a genetic dissection of the factors involved in hormone induced nucleosome remodelling. The phenotype of mutations in the core histones as well as in HATS, histone deacetylases, and the components of the remodeling machinery may provide mechanistic hints. Biochemically, we study this process using *Drosophila* embryo extracts able to assemble minichromosomes. In this system a nucleosome is preferentially positioned over the MMTV promoter and precludes binding of NF1 even in the presence of ATP (Venditti et al., 1998). These minichromosomes can be transcriptionally activated by recombinant progesterone receptor added to the cell-free system prior or after chromatin assembly. Moreover, on

these minichromosomes templates we can reproduced *in vitro* the functional synergism between hormone receptors and NF1 which is not observed on naked DNA templates (Di Croce et al., 1998). Surprisingly in these MMTV minichromosomes the NF1 DNA binding domain is sufficient to synergize with the hormone receptor and to facilitate binding of the receptor to the HREs, likely by stabilizing an "open" conformation of the nucleosome. The combination of this assay with genetically manipulated chromatin components is a powerful tool for defining the underlying molecular mechanisms.

References:

-Bartsch, J., Truss, M., Bode, J. and Beato, M. (1996). Moderate increase in histone acetylation activates the mouse mammary tumor virus promoter and remodels its nucleosome structure. Proc. Natl. Acad. Sci. USA *93*, 10741-10746.
-Beato, M., Herrlich, P. and Schütz, G. (1995). Steroid hormone receptors: Many actors in search of a plot. Cell *83*, 851-857.
-Chávez, S. and Beato, M. (1997). Nucleosome-mediated synergism between transcription factors on the Mouse Mammary Tumor Virus promoter. Proc. Natl. Acad. Sci. USA *94*, 2885-2890.
-Chávez, S., Candau, R., Truss, M. and Beato, M. (1995). Constitutive repression and nuclear factor I-dependent hormone activation of the Mouse Mammary Tumor Virus promoter in yeast. Mol. Cell. Biol. *15*, 6987-6998.
-Di Croce, L., Koop, R., Venditti, P., Westphal, H. M., Nightingale, K., Becker, P., and Beato, M. (1998). Two-steps synegism between progesterone receptor and NF1 on MMTV minichromosomes. Submitted.
-Eisfeld, K., Candau, R., Truss, M. and Beato, M. (1997). Binding of NF1 to the MMTV promoter in nucleosomes: Influence of rotational phasing, translational positioning and histone H1. Nucleic Acids Res. *25*, 3733-3742.
-Spangenberg, C., Eisfeld, K., Luger, K., Richmonds, T. J., Truss, M. and Beato, M. (1998). The MMTV promoter positioned on a tetramer of histones H3 and H4 binds nuclear factor 1 and OTF1^. J. Mol. Biol. *278*, 725-739.
-Truss, M., Bartsch, J., Schelbert, A., Haché, R. J. G. and Beato, M. (1995). Hormone induces binding of receptors and transcription factors to a rearranged nucleosome on the MMTV promoter *in vivo*. EMBO J. *14*, 1737-1751.
-Venditti, P., Di Croce, L., Kauer, M., Blank, T., Becker, P. B., and Beato, M. (1998). "Assembly of the MMTV promoter in minichromosomes with positioned nucleosomes precludes NF1 binding but not restriction enzyme cleavage." Nucleic Acids Res. *27*, in press.

REGULATION OF TRANSCRIPTION BY CHROMATIN REMODELING MACHINES

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We have recently described a novel nucleosome array assay that couples the activity of a nucleosome remodeling activity to restriction endonuclease activity (Logie and Peterson, 1997). In our initial studies this assay was used to determine the kinetic parameters of ATP-dependent nucleosome disruption by the yeast SWI/SNF complex. In this assay SWI/SNF and ATP leads to a 100-fold increase in nucleosomal DNA accessibility, and initial rate measurements indicate that the complex can remodel one nucleosome every 4.5 minutes on an 11-mer nucleosome array. We have now extended these studies to encompass a quantitative analysis of a large number of ATP-dependent chromatin remodeling complexes, including the yeast RSC, Drosophila brm, CHRAC and NURF, and human SWI/SNF complexes. In the case of SWI/SNF and RSC, we have quantitated their activities using nucleosomal arrays reconstituted with hypoacetylated, hyperacetylated, or partially trypsinized histones (Logie et al., submitted). We find that rates of ATP-dependent nucleosome remodeling are not affected by the absence or hyperacetylation of the core histone N-termini. However, SWI/SNF and RSC are unable to catalytically remodel multiple nucleosomal arrays in the absence of the histone termini, and this catalytic activity of SWI/SNF was decreased by hyperacetylation. Furthermore, GST-histone tail fusions differentially affect the nucleosome remodeling and ATPase activities of SWI/SNF and RSC. These results suggest that the histone termini influence a late step of the remodeling reaction, subsequent to remodeling, but prior to intermolecular transfer of the remodelers to new arrays.

In vivo the SWI/SNF complex appears to function together with the GCN5 histone acetyltransferase (Pollard and Peterson, 1997; Pollard and Peterson, 1998). To determine the functional relationship between ATP-dependent nucleosome remodeling and histone acetylation, we have investigated the targeting of SWI/SNF and GCN5 to the yeast *HO* gene, using a chromatin immunoprecipitation assay. *HO* is a cell cycle regulated gene that is only expressed in haploid mother cells in a small window in late G1. The regulation of *HO* requires about 1800 bp of cis-acting upstream regulatory sequences, two different sequence-specific DNA binding transcriptional activators (SWI5, SWI4/SWI6), the SWI/SNF complex, and ADA/GCN5 histone acetyltransferase complexes. We have synchronized cells with nocodazole and then used a chromatin immunoprecipitation assay to monitor the targeting of GCN5 acetyltransferase activity to the *HO* promoter in early G1. We find that GCN5 acetylatransferase activity is targeted to the *HO* locus in mid-G1 prior to the onset of *HO* transcription, and that the domain of GCN5-dependent histone H3 acetylation is restricted to 1 kb of upstream regulatory sequences. Identical cell cycle studies have been performed in a battery of *swi* strains to determine the trans-acting requirements for GCN5 targeting. We find that GCN5-dependent acetylation of *HO* nucleosomes requires the SWI5 activator and the SWI/SNF complex. In contrast, the SWI4/SWI6 activator is not required to recruit GCN5 activity. These data suggest a model whereby the SWI/SNF complex functions at an early step to facilitate the subsequent targeting of GCN5-containing histone acetyltransferase complexes to the *HO* promoter.

<u>References Cited:</u>

Pollard, K. J. and Peterson, C. L. (1997). Role for ADA/GCN5 products in antagonizing chromatin-mediated transcriptional repression. Mol. Cell. Biol., 17, 6212-6222.

Logie, C. and Peterson, C. L. (1997). Catalytic nucleosome remodeling by the yeast SWI/SNF complex on nucleosome arrays. EMBO J., 16, 6772-6782.

Pollard, K. J. and Peterson, C. L. (1998). Chromatin remodeling: A marriage of two families? Bioessays, Solicited and In Press.

Logie, C., Tse, C., Hansen, J., and Peterson, C.L. The core histone N-terminal domains are required for catalytic chromatin remodeling by the SWI/SNF and RSC complexes. Submitted.

CHANGES IN PEA CHROMATIN DURING THE EMBRYOGENESIS-GERMINATION EVENTS.

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Chromatin is a highly dynamic entity and, apart from the remodelling required for the access of the transcriptional machinery to selected genes and for DNA replication, several physiological situations probably involve major structural changes. Among these situations the cycle embryogenesis-germination presents characteristics that are entirely its own, thus deserving a detailed analysis. We have studied several properties of the chromatin and of their associated enzymes in either ungerminated seeds or seedlings from pea (*Pisum sativum*). Changes in the nucleosomal repeat length (Ull and Franco, 1986), in the histone deacetylase isozymes (Sendra et al. 1988) and in the protein complement (Rodrigo and Franco, 1990; Ull et al. 1991) were found. To concentrate in the latter changes, a polypeptide of M_r = 16000, further referred to as P16, is present in the chromatin of embryonic axes from ungerminated pea seeds, but it rapidly disappears after the onset of germination to be not further found in adult plants.

P16 was purified to homogeneity, antibodies were raised and the latter were used for the screening of a λ-ZAP cDNA library representative of the mRNA of embryonic axes of pea seeds reared 30 days after flowering. Several positive clones were found, which contained an ORF coding for a polypeptide of 483 amino acids and M_r = 54500, designated as P54. Genomic Southern analysis showed that P54 gene is present as a single copy gene in pea genome.

The analysis of the P54 sequence revealed a 60% identity with the precursor of a 524 amino acids sucrose-binding soybean protein (SBP) (Grimes et al., 1992) and, to a lesser extent (31-34%), with some vicilins from cotton, pea and bean. The entire sequence of P16 is contained in the C-terminal third of P54. The latter must be, therefore, processed in a still unknown manner to yield P16.

As P16 is found in dry pea seeds but not in adult plants, the 1.4 kb P54 mRNA ought to be transcribed sometime during seed formation. We next analysed, by Northern blotting, the appearance of P54 mRNA during embryogenesis to find that 15 days after flowering it is still absent, but its level is very high only 5 days later. Accumulation of P54 mRNA and also of mature P16 takes place during the seed desiccation. A similar analysis was carried out along germination. Six hours after seed imbibition, P54 mRNA is no longer detectable in embryonic axes. The level of mature P16 is maintained in embryonic axes some more time, but it sharply decreases between 12 and 24 h after imbibition. The temporal correlation between the transcription of P54 gene and the desiccation/rehydration processes during embryogenesis and germination suggests that P54 gene is induced under hydric stress and we checked that this actually occurs even in adult plant tissues.

Several lines of evidence suggest that P54 is not a storage protein nor a LEA (late embryogenesis abundant) protein, although it shares some properties with them. On the other hand, mature P16 was first isolated from chromatin and the next experiments show that it actually is a chromatin component in dry seeds. First, immunolocalization experiments showed that, although P16 is also present in protein bodies, it definitely is a nuclear component. Biochemical data indicate that DNase I treatment causes the releasing of P16 from nuclei only after an extensive digestion, suggesting that P16, either directly or indirectly, interacts with DNA. Gel shift assays have demonstrated the existence of a non-specific interaction between DNA and P16 *in vitro*. Moreover, P16 co-migrates with nucleosomes in a native gel assay, and the possibility of an interaction between the protein and purified nucleosomes *in vitro* has also been ascertained. On the other hand, nucleosomes from pea embryonic axes were treated with formaldehyde under conditions in

which only histones and not weakly bound proteins become cross-linked (Louters and Chalkley, 1984). In these experiments P16 was recovered cross-linked to histones, indicating that P16 may be in close vicinity to them.

To further examine the *in vivo* relevance of this interaction, we isolated total acid-soluble proteins from purified nuclei under a set of conditions in which any *in vivo*-existing inter-protein disulphide bridge would be preserved. In this manner we detected the presence of H3 homodimers, but P16-H3 heterodimers were also clearly present. Therefore, P16 actually is a chromatin component, which interacts *in vivo* with nucleosomes.

Taking into account the above mentioned properties of P16, it is tempting to speculate that it may play a role in stabilizing chromatin structure during the desiccation of seeds.

REFERENCES

Grimes, H.D., Overvoorde, P.J., Ripp, K., Franceschi, V. and Hitz, W.D. (1992) A 62-kD sucrose binding protein is expressed and localized in tissues actively engaged in sucrose transport. *Plant Cell* **4**, 1561-1574.

Louters, L. And Chalkley, R (1984) In vitro exchange of nucleosomalhistones H2a and H2b, *Biochemistry* **23**, 547-552.

Rodrigo, M. I. and Franco, L. (1990) Histone variants from pea (*Pisum sativum*): Their differential presence in fractions obtained by DNase I digestion of nuclei, *Physiol. Plantarum* **78**, 602-608.

Sendra, R., Rodrigo, I., Salvador, M. L., and Franco. L. (1988) Pea histone deacetylases, *Plant Mol. Biol.* **11**, 857-866

Ull, M. A, and Franco, L. (1986) The nucleosomal repeat length of pea (*Pisum sativum*) chromatin changes during germination, *Plant Mol. Biol.* **7**, 25-31.

Ull, M. A., Herrero, M. E. and Franco, L. (1991) Putative HMG non-histone chromosomal proteins from pea (*Pisum sativum*), *Plant Sci.* 75, 55-62.

"*In Vivo*" Mapping of Replicating Chromatin Reveals Positioned Nucleosomes at the Replication Fork

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By the use of psoralen-crosslinking and primer extension, a method has been developed which allows the analysis of chromatin structure "*in vivo*". The fidelity of the technique was initially tested by mapping nucleosome positions in the well known yeast minichromosome TRURAP and in the intergenic spacers of yeast rRNA genes.

Previous work showed that the advance of the replication machinery transiently destabilises the nucleosomal organisation of the chromatin fiber. The first nucleosome on daughter strands is detected at a distance of about 300 nucleotides from the elongation point. We now questioned how fast newly formed nucleosomes are positioned. Analysis of sites of psoralen intercalation (which coincide with linkers and non-nucleosomal DNA) in the "*in vivo*" crosslinked rRNA intergenic spacers excised from the replicated branches of replication intermediates purified from preparative two-dimensional gels, reveals that: nucleosomes between the 5S gene and the 5' end of the rDNA transcription unit are positioned immediately after passage of the replication machinery. Possible mechanisms that may effect the immediate positioning of nucleosomes in this regulatory region include the action of specific factors, the DNA sequence, boundary effects, chromatin folding and histone modifications.

Our finding implicates a putative interaction between replication and gene expression. We speculate that in regulatory regions such as promoters fast repositioning of nucleosomes may transiently exclude the binding of factors involved in gene expression.

Session 4: Gene silencing

Chair: Timothy H. Bestor

Telomeric silencing and heterochromatin structure in *Saccharomyces cerevisiae.*

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Telomeres play an essential role in cell biology stabilizing chromosomes and facilitating complete replication of chromosomal termini. In addition, telomeres are known to silence the expression of subtelomeric reporter genes. Telomeric DNA usually contains tandem repetitions of a short motif flanked by subtelomeric, middle repetitive sequences (1). In yeast, telomeric sequences are composed of about 350 bp containing the $(C_{1\text{-}3}A)_n$ repeats and are followed by two main subtelomeric sequences: the Y' and X elements (2). Y's are highly conserved and are found in about 70% of the telomeres (2-5). X elements are present in all telomeres and can exist in two main forms: a complete form containing the X core and the STR-A,B,C,D elements, or a short form containing essentially the X core or part of it (2, 4-6). The complete X is found in about 80% of the telomeres while uncomplete forms are found in the remaining 20%. Previous reports have referred to the chromatin structure of yeast telomeres as heterochromatin. This denomination is based on structural and functional similarities that yeast telomeres share with *Drosophila* heterochromatin (7-9).

We have recently shown that telomeric silencing occurs in a natural telomeric region of *Saccharomyces cerevisiae* (10). More specifically, we have observed silencing of a retrotransposon (Ty5-1) that is localized in the left telomeric region of chromosome III (LIII). The chromatin structure of LIII have been analyzed in detail (11). In addition, mutations in telomeric structural components that release the repressive heterochromatin structures at LIII and affect its primary sequence have been identified.

References

1. Zakian. V. (1989) *Annu. Rev. Genet.* **23**, 579-604.
2. Louis, E. (1995) *Yeast* **11**, 1553-1573.
3. Walmsley, R., Chan, C., Tye, B. & Petes, T. (1984) *Nature* **310**, 157-160.
4. Chan, C. & Tye, B. (1983) *J. Mol. Biol.* **168**, 505-523.
5. Chan, C. & Tye, B.-K. (1983) *Cell* **33**, 563-573.
6. Louis, E., Naumova, E., Lee, A., Naumov, G. & Haber, J. (1994) *Genetics* **136**, 789-802.
7. Laurenson, P. and Rine, J. (1992) *Microbiol. Rev.* **56**, 543-560.
8. Weiler, K. & Wakimoto, B. (1995) *Annu. Rev. Genet.* **29**, 577-605.
9. Henikoff, S.(1990) *Trend in Genetics* **6**, 422-426.
10. Vega-Palas, M., Venditti, S. & Di Mauro, E. (1997) *Nature Genetics* **15**, 232-233.
11. Vega-Palas, M., Venditti, S. & Di Mauro, E. (1998) *J. Biol. Chem.* **273**, 9388-9392.

Gene silencing mutants in *Arabidopsis thaliana*

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Gene silencing in plants refers to the complete or partial inactivation of transgenes and homologous endogenous genes and is a frequently reported phenomenon in transgenic plants. Beside of the importance to gain control over this process during foreign gene transfer for agriculture, gene silencing appears to be essential for changes of gene expression patterns in the course of differentiation and evolution. This is especially relevant in the case of transcriptional gene silencing which is usually transmitted meiotically to subsequent generations. The stability of these epigenetic changes suggests the involvement of controlling trans-acting factors. In order to identify genetic components regulating transcriptional gene silencing in plants, we screened for mutants in the model plant *Arabidopsis thaliana* that are affected in the maintenance of gene silencing. A strain transgenic for a transcriptionally silenced hygromycin resistance marker gene was mutagenized by EMS or fast neutron radiation treatment. Putative mutants were screened for hygromycin resistance due to reactivation of the transgene and for transmission of the expressed state to their progeny. Eight mutants (*som1-3* generated by EMS and *som 4-8* obtained after irradiation) were shown to reactivate the silent *hpt* test locus *in trans*. The level of DNA methylation at the *hpt* locus and at centromeric repeats was found to be reduced in the *som* mutants. Complementation crosses indicated complex epigenetic interactions among the *som* mutant alleles and with the previously described *ddm1* allele which also elicits DNA hypomethylation. *Som* mutants can be classified into three groups: A) allelic or interacting with *ddm1* and with each other (*som 1, 4, 5 and 8*), B) non-allelic with *ddm1* and *som* mutants of group A (*som2*), and C) mutants with slow re-silencing after outcrosses which hinders their classification (*som 3, 6 and 7*). To characterize the mutants further, we addressed the question, whether their action is limited to transgenes of specific sequences, structures or at specific genomic positions. We performed crosses between the *soms* and a

transcriptionally silenced *gus* gene (provided by Hervé Vaucheret). All *som* alleles were able to reactivate this reporter gene, indicating that the release of silencing by the *som* mutations is not restricted to the *hpt* gene used for the screen. The latest progress in the mutant screen and in the analysis of the mutants will be discussed.

References

Mittelsten Scheid, O., Afsar, K. and Paszkowski, J. (1998) Release of epigenetic silencing by *trans*-acting mutations in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **95**: 532-637

Paszkowski, J. and Mittelsten Scheid, O. (1998) Plant genes: The genetics of epigenetics. Curr. Biol. **8**: R206-208

Dehio, C. and Schell, J. (1994) Identification of plant genetic loci involved in a post-transcriptional mechanism for meiotically reversible transgene silencing. Proc. Natl. Acad. Sci. USA **91**, 5538-5542

Furner I. J., Sheikh M. A. and Collett C. E. (1998) Gene silencing and homology-dependent gene silencing in Arabidopsis: genetic modifiers and DNA methylation. Genetics **149**: 651-662

Elmayan, T., Balzergue, S., Beon, F., Bourdon, V., Daubremet J., Guenet, Y., Mourrain, P., Palauqui J.-C., Vernhettes, S., Vialle, T., Wostrikoff, K. and Vaucheret, H.: Arabidopsis mutants impaired in cosuppression. Plant Cell, in press

Homology-dependent *trans*-silencing and methylation of unlinked transgenes

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Homology-dependent gene silencing in transgenic plants can occur when two homologous transgene loci, or a transgene with homology to an endogenous gene, are present in the same genome (Matzke and Matzke, 1998). Homology-dependent gene silencing is thought to involve two distinct mechanisms that operate at either the transcriptional or post-transcriptional level, respectively. Transcriptional gene silencing occurs when interacting genes share homology in promoter regions, whereas post-transcriptional gene silencing requires sequence homology in protein coding regions. Both types of homology-dependent gene silencing have been associated with changes in DNA methylation, which is generally concentrated in promoters in the case of transcriptional silencing and the 3' ends of genes in post-transcriptional gene silencing. One variant of transcriptional gene silencing is *trans*-silencing involves a silencing locus that is able to induce methylation and transcriptional inactivation of a target locus with which it shares DNA sequence identity in promoter regions. Silencing loci autonomously acquire stable methylation in promoter regions, which is somehow imposed on a sensitive target locus. *Trans*-silencing and methylation of a target locus in the presence of a stably methylated silencing locus presumably rely on a promoter sequence-specific signal that originates at the silencing locus and directs do novo methylation of homologous promoters at the target locus. A sequence-specific *trans*-acting methylation signal could act through DNA-DNA pairing or an RNA-DNA interaction. Current information suggests that different *trans*-silencing loci can operate through both types of mechanism.

Matzke, M.A. and Matzke, A.J.M. (1998) Cell Mol. Life Sci. 54: 94-103.

CHROMATIN MODIFICATION AND GENE SILENCING IN PLANTS

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Studying transgene silencing in petunia, we had obtained several indications for a role of chromatin modification in transcriptional silencing. Silenced states of a transgene were less accessible to endonucleases that active states (1), silenced alleles showed hypermethylation of cytosine residues at non-symmetrical positions (2), and a silenced allele could trans-inactivate an allelic homologue (3). Based on these observations we assumed that transcriptional silencing was based on local chromatin condensation and that trans-silencing was mediated by an exchange of repressive chromatin complexes among homologous copies. This assumption implies that plants have chromatin modifications systems that can affect the expression of transgenes but that are most likely primarily involved in the regulation of endogenous genes.

To examine the role of chromatin complexes in plant gene silencing, we characterised a genomic *de novo* methylation sequence that causes PEV like destabilisation of adjacent marker genes (4). In a South-Western screen, we identified and cloned a plant protein (RPS-BP1) that binds to the *de novo* methylation sequence. RPS-BP1 shows characteristic features that are found in proteins that form multiple-protein aggregates, and might act as an inducer of repressive chromatin complexes.

To address the potential role of chromatin remodelling in regulating the expression of endogenous genes, we tested whether proteins that were involved in chromatin remodelling in other systems, had a similar function in plants. We expressed the chromodomains of *Drosophila* Polycomb (Pc) and Heterochromatin Protein 1 (HP1) in tobacco. The two domains localise to multiple, but at least partly different regions in interphase nuclei. While expression of the HP1 chromodomain does not affect plant development, plants expressing the Pc chromodomain show pleiotropic mutant phenotypes and altered expression of at least one homeodomain gene. Our result support the assumption that the heterologous chromodomains exert related functions in *Drosophila* and in plants, and that chromatin modification mechanisms are involved in the regulation of certain plant genes, similar to chromatin-mediated gene regulation in *Drosophila*.

(1) Van Blokland, R., Ten Lohuis, M. and Meyer P. (1997) Condensation of chromatin in transcriptional regions of an inactivated plant transgene: an indication for an active role of transcription in gene silencing. **Mol. Gen. Genet. , 257: 1-13**

(2) Meyer, P., Niedenhof, I. and ten Lohuis, M. (1994). Evidence for cytosine methylation of non symmetrical sequences in plants. **The EMBO Journal 13: 2084-2088**

(3) Meyer, P., Heidmann, I. and Niedenhof, I. (1993) Difference in DNA-methylation are associated with a paramutation phenomenon in transgenic petunia. **The Plant Journal 4: 89-100**

(4) Ten Lohuis, M., Mueller, A., Heidmann, I., Niedenhof, I. And Meyer, P. (1995) A repetitive DNA-fragment carrying a hot spot for de novo DNA methylation enhances expression variegation in tobacco and petunia. **The Plant Journal 8: 919-932**

S1 SINE retroposons are methylated by a sequence specific process in *Brassica napus*:

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DNA methylation has been often proposed to operate as a genome defence system against parasitic mobile elements. To test this possibility, the methylation status of a class of plant mobile elements, the S1 SINEs, was analyzed in detail using the bisulfite modification method. We observed that S1 SINE retroposons are methylated at symmetrical and non-symmetrical positions. Methylated cytosines are not limited to transcriptionally important regions but are well distributed along the sequence. S1 SINE retroposons are two times more methylated then the average methylation level of the *Brassica napus* nuclear DNA. By *in situ* hybridization, we showed that this high level of methylation does not result from the association of S1 elements to genomic regions known to be highly methylated. We also show by sequence analysis that genomic region flanking S1 elements are not methylated. These results suggest that $S1_{Bn}$ elements were methylated by a sequence-specific process. A detailed analysis of the methylation context showed that S1 cytosines in symmetrical CpG and CpNpG sites are methylated at a level of 87% and 44% respectively. We observed that 5.3% of S1 cytosines in non-symmetrical positions were also methylated. 57% of this non-symmetrical methylation occurred at a precise motif (Cp(A/T)pA) that only represented 12% of the non-symmetrical sites in S1 sequences suggesting that it represents a preferred non-symmetrical methylation site. This motif is methylated in S1elements at only half the level observed for the Cp(A/T)pG sites. We show that non-S1 CpTpA sites can also be methylated in DNA from *B. napus* and from other plant species. Finally several evidence linking methylation to transcriptional inhibition will be presented.

Session 5: Nuclear factors interaction, gene expression and DNA structure

Chair: Miguel A. Vega-Palas

CHROMATIN STRUCTURE AND TRANSCRIPTION FACTOR VERACITY IN THE REGULATION ADH GENES IN ARABIDOPSIS AND MAIZE

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The overall goal of our research with the *Adh* genes from maize and arabidopsis is to gain further insight into the relationships between the regulation of gene transcription and chromatin structure in plants. We work on the premise that many of the necessary components that characterize regulated gene activity are contained in structural features that may not be revealed by simple sequence inspection and in vitro binding assays. Thus, we endeavor to include experiments that characterize gene structures in as close to the native, in vivo, state as possible in our investigations, and we seek to integrate data derived from several levels of chromatin structure into comprehensive models of regulated gene expression.

Multiple approaches have been applied our study of the *Adh* genes. In vivo DMS footprinting of the gene promoters as they enter and maintain active transcription provided an initial window into living cells, and indicate the binding sites of transcription related proteins (Ferl and Laughner, 1989; Ferl and Nick, 1987; Paul and Ferl, 1991). These experiments, combined with studies on chromatin structure (Ashraf et al., 1987; Paul et al., 1987; Vega-Palas and Ferl, 1995), and the molecular dissection of promoters (McKendree and Ferl, 1992; McKendree et al., 1990) have lead to a detailed description of the molecular state of *Adh* genes in a transcriptionally active mode. Some of the protein factors involved in regulating the expression of *Adh* genes have been cloned, and an understanding of the possible associations that exist among gene regulatory proteins and diverse cell signaling pathways is emerging (de Vetten and Ferl, 1995; de Vetten and Ferl, 1994; Lu et al., 1994; Lu et al., 1992; Lu et al., 1996). Recently, some of the in vitro data gathered from assays with cloned arabidopsis transcription factor has been integrated with data from an in an in vivo context developed with chromatin experiments in permeabilized protoplasts. Experiments using arabidopsis nuclei showed that the 5' region of the *Adh* gene contains a region of intense DNase I sensitivity from the TATA box out to approximately -450 (Vega-Palas and Ferl, 1995). New data from permeabilized arabidopsis protoplasts has expanded this view to show that there are four distinct sites of hypersensitivity centered around positions -425, -325, -200 and -60 in the *Adh* promoter (Paul and Ferl, 1998). The hypersensitive site at -200 coincides with the in vitro hypersensitive site created by purified transcription factors bound to the G-box element. The G-box is a functional cis element that plays a role in the signal transduction of hypoxia and other stresses in *Adh* (Lu et al., 1996), and the element is also found in a number of other inducible plant genes (e.g. Niu et al., 1996). It now seems possible that G-box related elements may also play a role in defining chromatin structure, and it is imperative that a clear understanding of in vivo G-box occupancy be developed by critical evaluation of in vivo and in vitro footprinting signatures.

Finally, a comprehensive understanding of the chromatin context of *Adh* expression is emerging from studies at several levels of chromatin organization. The distribution and position of nucleosomes and their relationship to DNase I hypersensitivity suggests a highly ordered chromatin context for *Adh* genes (Vega-Palas and Ferl, 1995, Torres et al., 1997). Nearby Matrix Attachment Regions in these genes may influence the localized chromatin structures (Paul and Ferl, 1993). However, the MAR-like structures that serve to organize the genomes of (at least) maize and arabidopsis into large structural loops do not seem to play a role in gene regulation. Rather, these Loop-Basement Attachment Regions (LBARs) serve to package the genome into large loops of discrete and defined length, creating a non-random organization of the genome, even at the level of bulk packaging (Paul and Ferl, 1998).

Ashraf M, Vasil V, Vasil I, Ferl RJ (1987) Chromatin structure of the 5' promoter region of the maize *Adh2* gene and its role in gene regulation. Molecular and General Genetics **208**: 185-190

de Vetten NC, Ferl RJ (1995) Characterization of a maize G-box binding factor that is induced by hypoxia. Plant J 7: 589-601

de Vetten NC, Ferl RJ (1994) Two genes encoding GF14 (14-3-3) proteins in Zea mays. Structure, expression, and potential regulation by the G-box binding complex. Plant Physiol **106**: 1593-1604

Ferl RJ, Laughner BH (1989) In vivo detection of regulatory factor binding sites of Arabidopsis thaliana Adh. Plant Molecular Biology 12: 257-266

Ferl RJ, Nick HS (1987) In vivo detection of regulatory factor binding sites in the 5' flanking region of maize Adh1. J Biol Chem **262**: 7947-7950

Lu G, de Vetten NC, Sehnke PC, Isobe T, Ichimura T, Fu H, van Heusden GP, Ferl RJ (1994) A single Arabidopsis GF14 isoform possesses biochemical characteristics of diverse 14-3-3 homologues. Plant Mol Biol 25: 659-67

Lu G, DeLisle AJ, de Vetten NC, Ferl RJ (1992) Brain proteins in plants: an Arabidopsis homolog to neurotransmitter pathway activators is part of a DNA binding complex. ProcNatlAcadSci USA 89:11490-4

Lu G, Paul AL, McCarty DR, Ferl RJ (1996) Transcription factor veracity: is GBF3 responsible for ABA-regulated expression of Arabidopsis Adh? Plant Cell 8: 847-57

McKendree WL, Jr., Ferl RJ (1992) Functional elements of the Arabidopsis Adh promoter include the G-box. Plant Mol Biol 19: 859-862

McKendree WL, Paul AL, DeLisle AJ, Ferl RJ (1990) In vivo and in vitro characterization of protein interactions with the dyad G-box of the Arabidopsis Adh gene. Plant Cell 2: 207-214

Niu X, Adams CC, Workman JL, Guiltinan MJ (1996) Binding of the wheat basic leucine zipper protein EmBP-1 to nucleosomal binding sites is modulated by nucleosome positioning. Plant Cell 8: 1569-1587

Paul AL, Ferl RJ (1991) In vivo footprinting reveals unique cis-elements and different modes of hypoxic induction in maize Adh1 and Adh2. Plant Cell 3: 159-168

Paul AL, Ferl RJ (1993) Osmium tetroxide footprinting of a scaffold attachment region in the maize Adh1 promoter. Plant Mol Biol 22: 1145-1151

Paul A-L, Ferl RJ (1998) Higher order chromatin structures in maize and arabidopsis. The Plant Cell **10**: (in press)

Paul A-L, Ferl RJ (1998) Pemiabilized arabidopsis protoplasts provide new insight into the chromatin structure of three plant alcohol dehydrogenase genes. Developmental Genetics 22: 7-16

Paul A-L, Vasil V, Vasil IK, Ferl RJ (1987) Constitutive and anaerobically induced DNase I hypersensitive sites in the 5' region of the maize *Adh1* gene. Proc. Natl. Acad. Sci. USA 84: 799-903

Torres L, Garrido B, Rodriguez J. Franco L (1997) Chromatin structure of the Arabidopsis thaliana promoter: analysis of nucleosomal positioning. Plant Science 129: 69-79.

Vega-Palas MA, Ferl RJ (1995) The Arabidopsis Adh gene exhibits diverse nucleosome arrangements within a small DNase I-sensitive domain. Plant Cell 7: 1923-1932

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Binding of the wheat G-box binding protein EmBP-1 to plant nuclosomes.

The bZIP proteins are a major group of eukaryotic transcription factors, many of which have been isolated from plant species [2]. The majority of plant bZIP proteins have been shown to bind the sequence containing CACGTG (G-box core) with high affinity [6]. Sequences with a G-box core have been shown to be functionally important in numerous promoters of plant genes including those regulated by light (the ribulose bisphosphate carboxylase small subunit gene *rbcS-1A* gene), abscisic acid (ABA; *Em*), UV light (the chalcone synthase gene), and an anaerobically regulated gene (alcohol dehydrogenase [*Adh*]) (reviewed by [8], [1]). The commonalty of the G-box in promoters of such diverse regulatory properties has led to the hypothesis that multiple protein interactions lead to response specificity. Interactions with nucleosomes must be taken into account in such a model to address the *in vivo* DNA-protein configuration. To understand how bZIP proteins function on nucleosome templates, we have examined nucleosome binding by the bZIP transcription factor EmBP-1.

EmBP-1 is a plant transcription factor implicated in ABA-induced gene expression in wheat [3, 11, 12]. Its DNA binding specificity and DNA binding and dimerization domains have been characterized, indicating that EmBP-1 is a typical bZIP transcription factor that shares many common properties with the well characterized yeast transcription factor GCN4 [4, 9]. EmBP-1 binds to the G-box with the highest known specificity of any plant bZIP protein [6]. EmBP-1 may also interact with VIVIPAROUS1, a maize regulatory protein which is involved in the response to ABA during maize embryo development [7, 13]. VIVIPAROUS1 interaction enhances EmBP-1s ability to bind an ABA response element (ABRE), supporting a multiple protein interaction model [5].

To investigate interactions of the basic leucine zipper transcription factor EmBP-1 with its recognition sites in nucleosomal DNA, an abscisic acid-response element and a high-affinity binding site for EmBP-1 were reconstituted into human and wheat nucleosome cores *in vitro* [10]. DNA

binding studies demonstrated that nucleosomal elements can be bound by EmBP-1 at reduced affinities relative to naked DNA. EmBP-1 affinity was lowest when the recognition sites were positioned near the center of the nucleosome. Binding was achieved with a truncated DNA binding domain; however, binding of full-length EmBP-1 caused additional strong DNase I hypersensitivity flanking the binding sites. Similar results were observed with nucleosomes reconstituted with either human or wheat histones, demonstrating a conserved mechanism of transcription factor-nucleosome interactions. We conclude that nucleosome positioning may play an important role in regulating interactions of EmBP-1 with its target sites in plant cells.

1. de Vetten N, Ferl RJ: Transcriptional regulation of environmentally inducible genes in plants by an evolutionary conserved family of G-box binding factors. Int. J. Biochem. 26: 1055-1068 (1994).
2. Foster R, Izawa T, Chua NH: Plant bZIP proteins gather at ACGT elements. FASEB J. 8: 192-200 (1994).
3. Guiltinan MJ, Marcotte WRJ, Quatrano RS: A plant leucine zipper protein that recognizes an abscisic acid response element. Science 250: 267-271 (1990).
4. Guiltinan MJ, Miller L: Molecular characterization of the DNA binding and dimerization domains of the bZIP transcription factor, EmBP-1. Plant Mol. Biol. 26: 1041-1053 (1994).
5. Hill A, Nantel A, Rock CD, Quatrano RS: A conserved domain of the viviparous-1 gene product enhances the DNA binding activity of the bZIP protein EmBP-1 and other transcription factors. J. Biol. Chem. 271: 3366-3374 (1996).
6. Izawa T, Foster R, Chua N-H: Plant bZIP protein DNA binding specificity. J. Mol. Biol. 230: 1131-1144 (1993).
7. Kao CY, Cocciolone SM, Vasil IK, McCarty DR: Localization and interaction of the cis-acting elements for abscisic acid, VIVIPAROUS1, and light activation of the C1 gene of maize. The Plant Cell 8: 1171-1179 (1996).
8. Menkens AE, Schindler U, Cashmore AR: The G-box: A ubiquitous regulatory DNA element in plants bound by the GBF family of bZIP proteins. Trends Biochem Sci 20: 506-510 (1995).
9. Niu X, Guiltinan MJ: DNA binding specificity of the wheat bZIP protein EmBP-1. Nucleic Acid Res. 22: 4969-4978 (1994).
10. Niu XP, Adams CC, Workman JL, Guiltinan MJ: Binding of the wheat basic leucine zipper protein EmBP-1 to nucleosomal binding sites is modulated by nucleosome positioning. Plant Cell 8: 1569-1587 (1996).
11. Quatrano RS, Bartels D, Ho THD, Pages M: New insights into ABA-mediated processes. Plant Cell 9: 470-475 (1997).
12. Quatrano RS, Guiltinan MJ, Marcotte WR: Regulation of gene expression by abscisic acid. : in Control of Plant Gene Expression, pp. 69-90, CRC press, Boca Raton, FL (1992).
13. Vasil V, Marcotte WR, Jr., Rosenkrans L, Cocciolone SM, Vasil IK, Quatrano RS, McCarty DR: Overlap of Viviparous1 (VP1) and abscisic acid response elements in the Em promoter: G-box elements are sufficient but not necessary for VP1 transactivation. Plant Cell 7: 1511-1518 (1995).

The Role of DNA Topology in Chromatin Organization: Transcriptional State of a Mouse Mammary Tumor Virus Promoter-Driven Gene Can Affect Topological Domain Size in Vivo

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Many individual genes in *Drosophila*, mouse, and human cells are organized with unrestrained negative DNA supercoiling, while, on average, the majority of the chromosome is torsionally relaxed, presumably due to the restraint of supercoils through the organization of DNA into nucleosomes. In *Drosophila*, while the hsp70 genes are organized with torsional tension before and after transcription, DNA immediately outside the functional hsp70 domain at locus 87A *Drosophila* is completely relaxed. This organization is possible through the organization of the chromosome into independent topological domains. Neither the nature of the structures that define topological domains in cells, nor the biological roles of topological domains have been unequivocally identified. Moreover, the relationships between transcription or transcriptional activation, and unrestrained supercoiling remain to be clearly established.

The number of topological domains within a 1.8 mb chromosomal region consisting of about 200 tandem repeats containing the Ha-v-ras gene driven by the mouse mammary tumor virus (MMTV) promoter was assayed *in vivo* by analyzing the relaxation of negative supercoils after introduction of a defined number of nicks in the DNA. The DNA within these tandem repeats contained a moderately high level of supercoiling and the DNA was organized into approximately 55 independent topological domains. Following the addition of the glucocorticoid dexamethasone, about 25% of the MMTV promoters were activated and supercoiling throughout the region was completely relaxed. However, following gene activation by dexamethasone, and after the addition of ✂-amanitin to block RNA polymerase elongation, supercoiling was again present and approximately 110 topological domains were detected. Furthermore, if transcription proceeded for an hour after dexamethasone treatment and then transcription elongation was blocked by ✂-amanitin approximately 215 topological domains were detected within the tandem array. These results demonstrate that an increase in the number of domain boundaries occurred upon gene activation and a further increase occurred upon transcription elongation. These results are consistent with the hypothesis that some topological domain boundaries in a living mouse cell are functional in nature, being established by the formation of activated and elongating transcription complexes.

Centromeric heterochromatin: a role for altered DNA structures?

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The centromere is a specialised chromosomal structure which is essential for the accurate segregation of chromosomes during mitosis and meiosis (for a review see Choo, 1997). The centromere nucleates the formation of the kinetochore and is also involved in sister chromatid cohesion and separation. In all the species studied to date, with the exception of the budding yeast *Saccharomyces cerevisiae*, centromeric DNAs are characteristically enriched in highly repetitive satellite DNA sequences. In *S.cerevisiae*, all the information required for proper chromosomal segregation is contained within a short 125 bp long A+T-rich sequence. On the other hand, in the fission yeast *Schizosaccharomyces pombe*, the centromeres are much larger, from 40 to 110 kb, and contain several types of complex centromere-specific repetitive elements flanking a central nonrepetitive A+T-rich sequence. The very high content in satellite DNA constitutes an important limitation for the molecular analysis of higher eukaryotic centromeres and, to date, no single-copy complex DNA sequence has yet been identified at these chromosomal regions. Though the presence of nonrepetitive DNA sequences at the centromere of higher eukaryotes cannot be completely ruled out, recent results strongly suggest that satellite DNAs are essential for proper centromere functioning both in humans and in *Drosophila*. In any case, the presence of satellite DNA at the centromere raises the question of its contribution to centromere organisation and function.

A remarkable feature of centromeric satellite DNAs is their apparent lack of evolutionary conservation. In general, centromeric satellites show little similarity among related species and, even in the same species, different chromosomes contain different arrays of satellite DNA sequences at the centromere. For instance, the *Drosophila* AAGAG satellite, which is an essential component of the centromere of the *Dp1187* minichromosome, does not seem to be universal to all *Drosophila* centromeres. Furthermore, centromeric satellites can also be found at other chromosomal locations showing no detectable centromere function and, *viceversa*, DNA sequences located outside of the centromere might acquire centromere activity acting as neo-centromeres. These observations suggest that the primary nucleotide sequence might not be the only determinant of centromere formation and function. Unlike in *S.cerevisiae*, centromeres of higher eukaryotes are embedded within large blocks of heterochromatin and the formation of a centromere-specific high order chromatin structure appears to be essential for

centromere function. Centromeric satellites are likely to play a crucial role in the formation of this high order structure. At this respect, it is interesting to note that, although showing no significant homology at the level of their nucleotide sequences, many centromeric DNAs share common structural properties. For instance, A+T-rich satellites are frequently found at the centromere of higher eukaryotes and similar A+T-rich sequences have also been found at the centromere of the yeasts *S.cerevisiae* and *S.pombe*. Several of these A+T-rich centromeric satellites were shown to be intrinsically curved reflecting common bendability properties (Martínez-Balbás *et al.*, 1990). "Telomere-like" satellites constitute a second class of repeated DNAs which are often found at the centromeres of many vertebrates, insects and plants. As found in most telomeric DNAs, these centromeric satellites show a marked pu/py strand-asymmetry resulting in one strand being G-rich in comparison with its complementary. Actually, many of them contain G-tracts similar to those found in telomeric DNA. Several of these "telomere-like" centromeric satellites were shown to be capable of forming altered DNA structures in which the G-rich strand forms very stable intramolecular hairpins. These observations suggest that, perhaps, what centromeric satellites share in common are the structural properties associated with their otherwise divergent nucleotide sequences.

The *Drosophila* dodeca-satellite (GTACGGGACCGA) is a member of this family of "telomere-like" centromeric satellites. The dodeca-satellite is found at the centromere of chromosome 3 in *D.melagonaster* and of several other chromosomes of different *Drosophila* species (Abad *et al.*, 1992; Carmena *et al.*, 1993). It was shown earlier that the dodeca-satellite G-strand forms very stable fold-back structures, stabilised by the formation of tandem G·A pairs, while the complementary C-strand remains basically unstructured (Ferrer *et al.*, 1995; Ortiz-Lombardía *et al.*, 1998). In this paper, we describe the purification and characterisation of DDP1, a single-stranded DNA binding protein of high molecular mass which binds the unstructured dodeca-satellite C-strand with high affinity and specificity. In polytene chromosomes, DDP1 localises to the chromocenter but its distribution is not constrained to the regions containing dodeca-satellite sequences. DDP1 is also found at the chromosome arms mostly co-localising with HP1. During embryo development, DDP1 becomes nuclear only after cellularization, at the same time that HP1, and is also found associated with the condensed mitotic chromosomes. These results are discussed in the context of the possible contribution of the structural properties of centromeric satellites to centromere formation and function.

Abad, J.P., Carmena, M., Baars, S., Saunders, R.D.C., Glover, D.M., Ludeña, P. Sentis, C., Tyler-Smith, C. and Villasante, A. (1992) Dodeca-satellite: a conserved G+C-rich satellite from centromeric heterochromatin of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **89**, 4663-4667.

Carmena, M., Abad, J.P., Villasante, A., and González, C. (1993) The *Drosophila melanogaster* dodecasatellite sequenec is closely linked to the centromere and

can form connections between sister chromatids during mitosis. *J. Cell Sci.* **105**, 41-50.

Choo, K.H. (1997) The Centromere. Oxford University Press.

Ferrer, N., Azorín, F., Villasante, A., Gutiérrez, C. and Abad, J.P. (1995) Centromeric dodeca-satellite DNA sequences form fold-back structures. *J. Mol. Biol.* **245**, 8-21.

Martínez-Balbás, A., Rodríguez-Campos, A., García-Remírez, M., Sainz, J., Carrera, P., Aymamí, J., and Azorín, F. (1990) Satellite DNAs contain sequences that induce curvature. *Biochemistry* **29**, 2342-2348.

Ortiz- Lombardía, M., Cortés, A., Huertas, D., Eritja, R., and Azorín, F. (1998) Tandem 5'-GA:GA-3' mismatches account for the high stability of the fold-back structures formed by the centromeric *Drosophila* dodeca-satellite. *J. Mol. Biol.* **277**, 757-762.

Differential activation of small heat-shock gene promoters during plant embryogenesis: involvement of heat-shock elements and chromatin structure?

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Results of nuclear run-on analyses in sunflower zygotic embryos showed that, despite the presence of functional Heat Shock *cis* Elements (HSEs) in the promoters of *Ha hsp17.7 G4* and *Ha hsp18.6 G2*, only the former was transcriptionally activated during embryogenenesis; whereas both promoters responded to heat stress in vegetative tissue (Carranco *et al.*, 1997). That would be similar to observations in Arabidopsis, where only a subset of the homologous class I small Heat Shock Proteins (sHSPs) are expressed in developing seeds, despite the efficient heat-induction of all proteins from this class (Wehmeyer *et al.*, 1996). We showed recently that expression from the *Ha hsp17.7 G4* promoter during late embryogenesis (in desiccating seeds) depends on its HSEs. However, differences between the developmental regulation and heat-shock response mediated by these HSEs allowed us to separate both by site-directed mutagenesis (Almoguera *et al.*, 1998). A crucial question remains unanswered: what prevents the activation of all sHSP gene promoters in desiccating seeds? To further investigate this point, we have set conditions for the analysis of the sunflower promoters, in the homologous system, by transient expression. Preliminary results showed that, in bombarded sunflower embryos, the three class I sHSP promoters: *Ha hsp17.6 G1*, *Ha hsp18.6 G2*, and *Ha hsp17.7 G4*, showed activities dependent on the integrity and complexity of their HSEs. Furthermore, these promoters could be activated in *trans* by different cloned HSFs from tomato, and this *trans*-activation depended also on the HSEs. These results differ from the *in nucleo* promoter activity, and suggest that factors not properly conserved during transient expression, such as the DNA methylation status or the chromatin structure, might control the differential activity of sHSP promoters in plant embryos.

References:

Almoguera *et al.* (1998) Plant J. 13: 437-446.
Carranco *et al.* (1997) J. Biol. Chem 272: 27470-27475.
Wehmeyer *et al.* (1996) Plant Physiol. 112: 747-757.

POSTERS

H4 acetylation, XIST RNA and replication timing are coincident and define X;autosome boundaries in two human chromosomes.

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Dosage compensation in mammals requires the stable genetic silencing during early embryogenesis, of one X chromosome in each female cell. This process has an absolute requirement for the presence, *in cis*, of a region defined genetically as the X inactivation centre (Xic). Establishment of the inactive X requires the relatively rapid (ie. 1-2 cell generations) dissemination of the silencing signal across an entire chromosome, a process that is often described as spreading and seen as analogous to position effect variegation (PEV) in *Drosophila*. The inactive X (Xi) differs from its active homologue (Xa) in a number of ways, including increased methylation of CpG islands, replication late in S-phase, underacetylation of histone H4 and association with *XIST* RNA, the latter being the non-coding transcript of a gene within the Xic that is, uniquely, expressed exclusively from Xi. Global changes in DNA methylation occur relatively late in development but the other properties all change during or shortly after the establishment of Xi and may play a role in the spreading mechanism. We describe the use of a cytogenetic approach to define the distribution of *XIST* RNA, deacetylated H4 and the late replicating DNA across two human X:autosome compound chromosomes. The results show complete coincidence of these parameters with all three being excluded from the autosomal component of the derived X chromosome. This suggests that there is little or no spreading of the inactive state onto the autosomal component of the derived X chromosome. DNA elements which are absent or sparse in the autosomal segments we have studied, may be required for such spreading to occur.

COMPACT INTERDIGITATED HELICAL MODEL FOR CHROMATIN

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Our previous electron microscopy and electrophoretic studies (1-3) of small chromatin fragments from chicken erythrocytes have shown that, in presence of 1.7 mM Mg^{2+}, these fragments form helical fibers that are more compact than normal solenoids or other models proposed for chicken erythrocyte chromatin. These studies have also shown that fiber folding is initiated from a basic element with 5-7 nucleosomes. In this work, using computer-generated models, we have found that a structural solution that allows the formation of compact fibers consists in the interdigitation of the successive helical turns of simple helices with few nucleosomes per turn. With this folding pattern we have constructed a family of models containing 13-14 nucleosomes per 11 nm. We show that interdigitation of the primary helix or solenoid formed by consecutive nucleosomes gives rise to secondary helices, in which nucleosomes from successive turns of the primary helix interact through their faces. Stacking of nucleosomes in secondary helices is probably related with early findings of several laboratories showing that the protein core of nucleosomes has a high tendency to associate forming helices. Tilt angles of nucleosomes with respect to the fiber axis of our family of models are 20, 29, 40, and 52°, for structures with 2.8, 3.8, 4.7, and 5.8 nucleosomes per turn in the primary helix, and 3, 4, 5, and 6 secondary helices, respectively; the diameter of all models is 36 nm. We have included linker DNA in the model containing 4.7 nucleosomes per turn and we show that there is enough space in the central region for 60 bp of linker DNA and a hole of 7 nm in diameter. The calculated cross-sectional radius of gyration of this model (12 nm) is in agreement with the values obtained for folded chicken erythrocyte chromatin in several laboratories using neutron and X-ray scattering. Additional details of the model can be found in (4).

(1) Bartolomé, S., Bermúdez, A. & Daban, J.-R. (1994). *J. Cell Sci.* **107**, 2983-2992.
(2) Bartolomé, S., Bermúdez, A. & Daban, J.-R. (1995). *J. Biol. Chem.* **270**, 22514-22521.
(3) Bermúdez, A., Bartolomé, S. & Daban, J.-R. (1998). *J. Cell Sci.* **111**, 1707-1715.
(4) Daban, J.-R. & Bermúdez, A. (1998). *Biochemistry* **37**, 4299-4304.
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Chromatin Structure of Abscisic Acid Responsive Elements in Maize.

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The phytohormone abscisic acid (ABA) regulates several events during seed development, and the response to environmental stresses such as desiccation, salt and cold. An important part of the physiological response to ABA is induction of transcription of genes that are believed to increase the desiccation tolerance of the embryo. The ABA responsive element (ABRE) plays a crucial role for transcription of many ABA inducible genes. The ABRE is an octameric sequence belonging to the ACGT-elements and is identical to elements (e.g. the G-box) that are inducible by other stimuli (reviewed by Busk and Pagès, 1998).
A large fraction of the ACGT-element binding proteins are present in the cytosol of dark-grown cells and light-dependent phosphorylation induces transport to the nucleus (Harter et al., 1994; Terzaghi et al., 1997). Also defense related genes are regulated by phosphorylation-induced binding to the ACGT-element/G-box (Dröge-Laser et al., 1997; Lois et al., 1989; Lawton et al., 1990).
We have previously shown that the seed-specific transcriptional activator VP1 induces transcription through the ABREs of the ABA-inducible *rab28* gene although protein binding on the element *in vivo* is independent of VP1 (Busk and Pagès, 1997). This suggests that VP1 acts as a transcriptional coactivator that binds to a preformed complex between the ABRE and an ABRE binding factor.
In the present study, we have performed *in vivo* footprinting of the *rab28* promoter at different developmental stages. Protein binding was observed on the ABREs in the rab28 promoter in 16 dap embryos before developmental induction of the gene. The binding was unaltered by exogenous ABA indicating that the DNA binding activity of the ABF is regulated by other cues than ABA. This indicates that the ABRE mediates transcription by a different mechanism than other ACGT-elements in plants.
Proteins from nuclear extracts formed different complexes with the ABRE depending on the developmental stage. Exogenous ABA shifted the complex formed in 16 dap embryos to the same mobility as the complex formed in 24 dap embryos where rab28 is expressed. These results suggest that ABA activates transcription via the ABRE by posttranslational modification of the ABF.
Expression studies in transgenic plants and in transient transformation suggested that one function of the constitutive protein binding to the ABRE could be to maintain an open chromatin structure on ABA-inducible promoters. In support of this, putative ABFs can invade chromatin *in vitro* (Niu et al., 1996).

References.
Busk P.K. and Pagès M. (1998). Plant Mol. Biol. **37**, 425-35. Busk P.K. and Pagès M. (1997). Plant Cell **9**, 2261-70. Dröge-Laser et al. (1997). EMBO J. **16**, 726-38. Harter et al. (1994). Plant Cell **6**, 545-59. Lawton et al. (1990). Plant Cell Rep. **8**, 504-7. Lois et al. (1989). EMBO J. **8**, 1641-8. Niu et al. (1996). Plant Cell **8**, 1569-87. Terzaghi et al. (1997). Plant J. **11**, 967-82.

MOLECULAR AND GENETIC CHARACTERIZATION OF A TRANSGENIC POPULATION OF CITRUS

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In vegetatively propagated and long-lived perennial fruit crops, an important requisite for evaluating the validity of genetic transformation techniques is the stability of transgene integration and expression over long periods of time. However, little is known about these features in woody fruit crops. Apomictic reproduction of citrus make them excellent woody plants for this type of studies, since it assures that transformation occurred on the same genotype.

A collection of 100 transgenic citrange plants (*Citrus sinensis* L. Osbeck x *Poncirus trifoliata* L. Raf.) for *uidA* and *nptII* genes have been maintained under screenhouse conditions over a period of 1-4 years. Only four morphologically off-type transgenic plants were detected among the population. On a ploidy analysis, these plants were characterized as tetraploids. No other phenotypical alterations were observed. Histochemical GUS assays, quantitation of both *uidA* and *nptII* gene expression and Southern analyses were performed to study integration and expression patterns. Transgene loci number varied from 1 to 6, and a significant negative correlation between insert number and GUS activities could be demonstrated. Methylation analyses are being currently performed for several transgenic plants with silenced *uidA* expression. No significant variance in number of transgene inserts were found among plants obtained using different transformation conditions. Stability of the integrated transgenes during long time periods and under natural environmental conditions was confirmed in all the transformants.

EFFECTS OF CHROMODOMAIN-PROTEINS ON TOBACCO DEVELOPMENT

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Our aim is to elucidate the role played by chromodomain proteins in plants. For this purpose, we started to introduce the chromodomains of two *Drosophila* proteins, Heterochromatin-associated protein 1 (HP1) and *Polycomb* (Pc) into tobacco nuclei, to test the nuclear localisation of the two domains and their effect on plant development. HP1, a nonhistone chromosomal protein preferentially associated with b-heterochromatin, and Pc, a repressor of homeotic genes in *Drosophila*, share a well-conserved, although not identical, chromodomain, which might play a common role in chromatin repression. The analysis of transgenic plants expressing each of the two chromodomains, linked to the green fluorescent protein, suggest that both chromodomains have specific and distinct target regions in the plant genome. Only expression of the Pc chromodomain interfered with plant development, showing homeotic mutations and a change in the number of organ in flowers and leaves. In order to see whether the expression of genes involved in development might be altered in phenotypic organs of Pc-chromodomain plants, we studied by RT-PCR the expression pattern of MADS box genes, and homeobox genes. In phenotypic shoot buds, we showed that the transcript level of one particular gene is modified, displaying an increase in the Polycomb-chromodomain-transformed plant in comparison to the SR1 control plant.

These data resemble the effect of the HP1 and Pc in *Drosophila*, where both chromodomains have been shown to have distinct chromosome binding activity, and where Pc is involved in the regulation of development-specific genes.

In parallel, we try to identify endogenous tobacco genes containing a chromodomain, using a PCR strategy. Preliminary results in this concern will be presented and discussed.

Molecular and genetic dissection of transgene-induced gene silencing in *Neurospora crassa*.

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The introduction of a transgene can lead to gene silencing or quelling of the homologous resident gene. Using the carotenoid biosynthetic gene albino-1 as a visual reporter for gene silencing, several general characteristics of quelling have been defined such as: reduction of the steady state level of the duplicated gene mRNA is due to a post-transcriptional effect on its accumulation; transgenes containing transcribed regions are able to induce gene silencing, while the promoter regions are ineffective; quelling is dominant in heterokaryotic strains indicating the involvement of a diffusible trans-acting molecule.
A saturating genetic dissection of quelling defective (*qde*) mutants has identified three complementation groups. This indicates that the affected genes may encode three separate components involved in the mechanism of gene silencing in *Neurospora*. The identification of the *qde* mutants constitutes the first necessary step in the identification of factors required for quelling.
Moreover, by analysing the *qde* mutants a complex scenario is emerging, in which several mechanisms of transgene-induced gene silencing are present in *Neurospora*.

Peloria, a natural epigenetic mutant of the *Linaria cycloidea* gene

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According to their symmetry flowers can be radially symmetrical or asymmetrical. In *Antirrhinum* (Scrophulariaceae) a species with asymmetrical flowers, the generation of this asymmetry is beginning to be understood. Two genes, *cycloidea* (*cyc*) and *dichotoma* (*dich*) known to play a key role in generating floral asymmetry have been cloned and analysed in our lab. Now we are investigating whether other *cyc/dich*-like genes are responsible for establishing dorsoventral asymmetry in other species with asymmetrical flowers: *Linaria*, *Sainpaulia*, *Schyzanthus*, petunia and *Senecio*.

We have found that in *Linaria vulgaris* the orthologue of *cycloidea* (*Lcyc*) is also involved in generating the floral asymmetry. This gene is expressed in the dorsal part of the floral meristem from very early stages of development. A mutation called Peloria, first described by Linnaeus in the XVIII century, in which the flower is radially symmetrical has shown complete linkage to *Lcyc*.

Although when we looked for lesions in *Lcyc* of Peloria we found none, we have discovered that the *Lcyc* locus of the mutant is heavily methylated and silenced, modification not found for other genes of the mutant. Moreover, in unstable mutants in which the wild-type flower phenotype is partially or completely recovered there is a parallel demethylation of *Lcyc*, accompanied by an increased in the transcription of the gene. Peloria is therefore and epigenetic mutant as well as one of the first described plant natural variants for which the molecular basis are starting to be understood.

Evidence for gene silencing in the fungal plant pathogen *Fusarium oxysporum* by repeat-induced point mutation in the absence of a sexual cycle

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Gene silencing in filamentous fungi has generally been associated with two phenomena at different stages of their life cycles: post-transcriptional inactivation of gene expression, termed quelling, occurs during the vegetative phase whereas repeat-induced point mutation (RIP) takes place premeiotically and thus requires passage of the organism through a sexual phase. The fungal plant pathogen *Fusarium oxysporum* belongs to the Deuteromycetes whose members do not possess a known sexual cycle.

F. oxysporum f.sp. *lycopersici* was transformed with three different plasmid constructs containing genes encoding a pectate lyase, a xylanase or a polygalacturonase whose coding regions were interrupted or partially replaced by a hygromycin resistance cassette. Ectopic integration of the three vectors was detected in all the transformants analyzed. Southern analysis of the transformants with the isoschizomers *MspI*/*Hpa*II and *Nde*II/*Sau*3A gave banding patterns consistent with the generation of new restriction sites within the duplicated regions, possibly through point mutations, that were not present in the untransformed strains. Northern analysis showed that accumulation of transcripts from the duplicated genes was strongly reduced in the transformants whereas genes encoding other extracellular enzymes were expressed at normal levels. Moreover, extracellular pectate lyase activity was 10 to 20fold reduced in the transformants harbouring duplicated sequences of the corresponding gene. The results indicate that RIP occurs in *F. oxysporum* in the absence of a sexual cycle. Isolation and sequence analysis of the affected genomic regions is currently underway in order to determine the nature and the extent of the induced mutations.

Genetic interactions between Chromatin and DNA-Recombination genes.

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We try to understand the DNA-structural requeriments for homologous recombination.

The S. *cerevisiae* SPT6p and SPT4p, along with SPT5p, form a protein complex (1). SPT6p interacts directly with the histone H3 globular-domain and *spt6* mutants show a general effect on chromatin structure that resembles the *HTA1-HTB1* deletion(2). Previous results from our lab have shown a hyper-recombination phenotype for *spt6* and *spt4* mutants (3).

Rad51p is a eukaryotic RecA homologous. The involvement of Rad51p in homologous recombination has been shown to be dependent on chromatin structure in the silenced *loci HMR* and *HML*(4).

In this report we present data that suggest a genetic interaction between Spt6p and Rad51p. We have extended this study to other mutants in other genes related to either chromatin structure, as the HMG homolog spt2/sin1, or homologous recombination, as *rad54*.

Our results will be discused on the basis of the functional relationship betwen homologous recombination and chromatin structure.

(1)Swanson MS and Winston F.1992. *SPT4,SPT5* and *SPT6* interactions: effects on transcription and viabiltity in *Saccharomyces cerevisiae*. Genetics 132:325-336.

(2)Bortvin A and Winston F.1996. Evidence that Spt6p controls chromatin structure by a direct interaction with histones. Science 272(5267):1473-1476.

(3)Malagón F. and Aguilera A. 1996. Differential intrachromosomal hyper-recomtination phenothype of *spt4* and *spt6* mutants of *S. cerevisiae*. Current Genetics,30, 101-106.

(4)Sugawara N, Ivanov EL, Fishman-Lobell J, Ray BL, Wu X, Haber JE (1995) DNA structure dependent requirements for yeast *RAD* genes in gene conversion. Letters to Nature 373:84-86.

The histone acetyltransferase activity of CBP stimulates transcription

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The CBP co-activator protein possesses intrinsic histone acetyltransferase (HAT) activity, and in addition, associates with two other histone acetyltransferases, P/CAF and SRC1. We set out to establish if the intrinsic HAT activity of CBP contributes to transcriptional activation. We show that a region of CBP which encompasses the HAT domain can stimulate transcription when tethered to the promoter. The stimulatory effect of this activation domain shows some promoter preference and is dependent on HAT activity. Analysis of fourteen point mutations reveals a direct correlation between HAT activity and transcriptional activation. We also find that the HAT domains of CBP and P/CAF share sequence similarity. Four conserved motifs are identified three of which are analogous to motifs A, B and D, found in other N-acetyltransferases. The fourth motif, termed E, is unique to CBP and P/CAF. Mutagenesis shows that all four motifs contribute to CBPHAT activity and transactivation. These results demonstrate that the HAT activity of CBP is directly involved in stimulating transcription.

GENE EXPRESSION STUDIES AND SEQUENCE VARIABILITY ANALYSIS OF *GRANDE1* RETROTRANSPOSON IN MAIZE AND RELATED SPECIES

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Grande1 elements constitute a new family of Ty3/*gypsy* type of retrotransposons present in the *Zea* genus in more than one thousand copies in *Zea diploperennis* and maize. This retrotransposon is remarkable for their high size of around 14-kb, that is consequence of a very large 3' region of more than 7 KB. Atypical entities of this region are two arrays of tandem-unrelated repeats with potential stable stem-loop structures. A big portion of that region is occupied by ORFs. However, only ORF23 is transcribed (in antisense orientation to the reverse transcriptase ORF), giving rise to a transcript of 900 b which is found in all the maize tissues explored. In the same way, gene 23 putative promoter from *Grande1*-7 is able to drive GUS activity in transient expression experiment in all the tissues examined. This is despite the high level of methylation of the DNA of *Grande1*, which genomic RNA is not detected in any tissue or situation, probably reflecting a non-functional retrotransposon. The P23 protein is being produced in *E. coli* for antibody production and function determination. The putative role of p23 protein, which is rich in glycine and acid amino acids, will be discussed.

We have analyzed the variation of the sequence of a 450-bp segment of LTRs (U3 and R regions) from *Grande1* retrotransposon in the *Zea* genus. Sequences were aligned and the phylogenetic relationships established. No identical sequences were found even from DNA samples from a particular line, subspecies or species, indicating a high sequence heterogeneity in the LTR amplified fragments. This high sequence variability is in agreement with non-functional *Grande1* elements nowadays, which could accumulate mutations since the time of inactivation. The finding of some relatively well conserved motifs, as the TATA box and others, between lines and species is contradictory with the last argument and will be discussed. The global phylogenetic tree shows a certain grouping of *Grande1* LTR sequences, but not by lines or species. Phylogenetic trees for each line reproduce well the global tree topology. This suggests that a subset of *Grande1* retrotransposable elements followed in each line a similar evolutionary history until the current situation. Similar analysis performed with reverse transcriptase (RT) sequences agrees with the traditional phylogeny, but only at the genus level. The analyses of LTR and RT sequences for *Zea* species is contradictory with the phylogeny of the *Zea* genus, indicating that the retrotransposon sequences are not good phylogenetic markers for reconstructing the phylogeny of *Zea* species. traditional phylogeny, but only at the genus level. The analysis of RT sequences for *Zea* species is contradictory with the phylogeny of the *Zea* genus, indicating that the retrotransposon sequences are not good phylogenetic markers for reconstructing the phylogeny of *Zea* species.

Large Scale Chromatin Domain Organization of Plant Genes.

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Experiments designed to directly investigate the large scale organization of the genomes of maize and Arabidopsis suggest that these genomes are organized into large structural loops, the median sizes of which vary between the two plant species. Further, hybridization analyses with specific gene probes reveal that individual genes occupy discrete domains within the distribution of organizational loops.

Limited digestion of nuclei with DNase I results in the conversion of the greater than 800 kb genomic DNA to an accumulation of fragments representing a collection of individual looped domains. The median size of the domain loops is about 45 kb in maize and about 25 kb in Arabidopsis. Hybridization analyses with specific gene probes reveal that individual genes occupy a discrete loop within the distribution created by DNase I. The maize Adh1 gene occupies a 90 kb loop and the maize GRF1 gene occupies a loop of 100 kb in length. Arabidopsis Adh is found within two distinct loops at 8.3 kb and 6.1 kb, whereas an Arabidopsis GRF gene occupies a single loop of 27 kb. The loops created by topoisomerase II mediated cleavage are identical in size to those created by DNase I in Arabidopsis. These results imply that chromatin packaging is not a random process of condensation, but rather that there are discrete structures in the higher order packaging of the genome that maintain gene organization.

The organization of the genome by these large-scale structures does not preclude further organization on a smaller scale. We (and many others in a variety of systems) have shown that smaller "units" of organization occur within the larger domain loops. For example, in maize Adh1, a 5' nuclear matrix attachment region (MAR) is retained on the matrix in nuclear preparations, and OsO4 footprinting indicates the MAR sequence is under torsional stress both in vivo and in vitro. However, this MAR does not participate in the generation of the large domain loop containing Adh1. The maize Adh1 promoter MAR defines the 5' edge of a region of DNase I hypersensitivity that is influenced by the transcriptional state of the gene, thus this MAR may play a role in creating structures necessary for transcriptional activation. Further, the 5' MAR contains three sets of a sequence very similar to recognition motif for the nuclear matrix binding protein SATB1. Confocal microscopy of maize cells incubated with SATB1 antibodies (kindly provided by T. Kohwi-Shigematsu) suggests that a SATB1 homolog is present in maize, and band-shift analyses with maize extracts and the SATB1 consensus sequence indicate that a complex is formed with a maize DNA binding protein and the SATB1 antibody in nuclei where Adh1 is transcriptionally active.

Thus, it appears that there are at least two levels of higher order chromatin that organize the genome into manageable units: the large scale structural loops that function in the overall condensation, and sub-sets of these large loops that probably organize the genome into functional units that contribute to gene regulation. It has long been known that the smaller, "functional" units of organization are maintained as discrete structures through specific attachments to the nuclear matrix. However, the implication that the global packaging of the eukaryotic genome is not through random condensation and that genes occupy a loop of discrete and defined length within the genome, is limited to these plant systems.

Phenotypic switching and gene silencing in the yeast *Candida albicans.*

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Most strains of the pathogenic yeast *Candida albicans* are capable of spontaneous switching between several heritable phenotypes distinguishable by colony morphology. This switching occurs at low frequency (around 10^{-4}). The mechanism regulating this switch is unknown, and it has been proposed to involve either a reversible DNA reorganization event or heritable changes in chromatin structure affecting gene expression. We have cloned a *C. albicans* gene that encodes a protein with sequence similarity to *Saccharomyces cerevisiae* Sir2, a protein involved in transcriptional silencing at several places in the *S. cerevisiae* genome. *C. albicans* strains without a functional *SIR2* gene show a high frequency of switching that can be suppressed by expression of this gene under the control of a heterologous promoter. These results indicate that switching in *C. albicans* involves factors similar to those controling gene silencing in *S. cerevisiae*.

STRUCTURAL AND FUNCTIONAL HETEROGENEITY OF Rap1p COMPLEXES WITH TELOMERIC AND UASrpg-LIKE DNA SEQUENCES

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Rap1p binds to a variety of related DNA sequences. We studied complexes of Rap1p and of its DNA binding domain with two of these sequences, the UASrpg sequence (5'-A C A C C C A T A C A T T T-3', RPG) and the S. cerevisiae telomeric consensus sequence (5'-A C A C C C A C A C A C C C-3', TEL). When cloned in front of a minimal CYC1 promoter, the two sequences differed in their transcriptional potential. Whereas RPG and TEL binding sites activated transcription with approximately the same strength, adjacent RPG sequences showed higher synergistic activity and orientation-dependence than TEL sequences. We also found different sequence requirements for Rap1p binding in vitro to both sequences, since a single base-pair that severely reduced binding of Rap1p to the RPG sequences, had very little effect on the TEL sequence. Both KMnO4 hypersensitivity assay and the hydroxyl radical foot printing analysis showed Rap1p binding domain distorted differently DNA molecules encompassing both sequences. We propose that Rap1p is able to build structural and functionally different complexes, depending on the type of DNA sequence the complex is build on.

The genetic analysis revealed a second aspect of the functional heterogeneity of the different Rap1p binding sites. Two types of mutations affected our constructs: histone depletion resulted on a overactivation of both constructs, with much greater effects in constructs bearing a single RPG and TEL sequeces, gal11- mutations recduced transcription similarly in all cases. Our conclusion is that Rap1p activates transcription by a dual mechanism of action: by overcoming histone repression and by direct or indirect interaction with components of the RNA polymerase holoenzyme. Our results suggest the relative significance of these two ways may change from one promoter to another depending on the base sequence of the Rap1p binding site. The relevance of this functional and structural heterogeneity for the multiple functions Rap1p binding sites appear to have in vivo will be discussed.

LOOKING FOR THE MECHANISMS RESPONSIBLE FOR SPECIFIC GENE EXPRESSION IN MAIZE ENDOSPERM TRANSFER CELLS LAYER. ISOLATION OF PROMOTERS ACTIVE IN THAT TISSUE.

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The maize endosperm transfer layer is a specialized group of cells placed at the basal portion of the seed, between the maternal phloem and the filial endosperm storage tissue. These cells have an extensive system of cell wall ingrowths that increase its surface area up to 20-fold and this feature and its location allow them to control the flow of nutrients coming to the grain from the rest of the plant. Consequently, they are an attractive target for improvement of grain filling.

As a first step towards biotechnological manipulation of the endosperm transfer layer function we are interested in understanding the mechanisms regulating specific gene expression in its cells. Consequently, we have isolated by several differential screening techniques a number of genes that are only active in that tissue and obtained the promoter regions of some of them. Comparison of the sequences of these promoters coming from genes plausibly involved in very different physiological processes and analysis of their binding patterns to nuclear proteins will allow us to delimitate the elements responsible for their exclusive activity.

Yeast Recombinant GCN5p Acetylates Sequentially Multiple Lysine Residues in Histone H3

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ABSTRACT

Recent discoveries that several transcriptional regulatory proteins are histone acetyltransferases or deacetylases have highlighted the role of histone acetylation in gene expression. Although the molecular mechanism(s) remains to be elucidated, the distinct specificities of histone acetyltransferases (and deacetylases) suggest differential functions in the transcriptional regulation.

Yeast Gcn5p, initially identified as a transcriptional co-activator is a histone acetyltransferase required for activation of several unrelated genes[1,2]. Previous works[3,4] have described that yeast recombinant Gcn5p acetylates only lysine 14 in histone H3. Contrary to these reports, our results demonstrate that yeast rGcn5 alone modifies sequentially several lysine residues in the N-terminal tail of H3 in the absence of any escort protein. Microsequencing analyses indicate that rGcn5p acetylates key lysine residues in the following order: Lys 14, 9 and 18.

Multiacetylation by yeast rGcn5p was observed using chicken and yeast core histones, isolated H3, and H3(1-50) peptide. Interestingly, when a synthetic peptide corresponding to amino acids 1-26 of H3 was used as a substrate, polyacetylation did not occur.

We propose three explanations why multiple acetylation has not been detected in previous studies. Our observation that rGcn5p acetylates sequentially imply a cooperative effect; acetylation of one lysine facilitates the acetylation of another. An explanation for this effect may be structural changes within the histone tails occur upon acetylation, allowing the recognition of another lysine residue.

REFERENCES

1- Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y. & Allis, C.D. *Cell* **84**, 843-851 (1996)
2- Kuo, M-H., Zhou, J., Jambeck, P., Churchhill, M. E. A. & Allis, D. C. *Genes Dev.* **12**, 627-639 (1998)
3- Kuo, M.-H., Brownell, J. E., Sobel, R. E., Ranalli, T. A., Cook, R. G., Edmondson, D. G., Roth, S. Y. & Allis, C.D. *Nature* **383**, 269-272 (1996)
4- Zhang, W., Bone, J. R., Edmondson, D. G., Turner, B. M. & Roth, S. Y. *EMBO J.* **17**, 3155-3167 (1998)

Identification of a plant protein motif with similarity to the mammalian methyl-CpG binding domain.

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DNA methylation is associated with transcriptional repression in both plant and animal genomes. Methylation is essential for mouse development and mutant *Arabidopsis* with reduced levels of methylation show pleiotropic developmental defects. To what extent are the functions of methylation and the mechanism of its action conserved between plants and animals? To address this question, we are particularly interested in the proteins that bind to methylated sequences since these are, to a large extent, the mediators of methylation dependent transcriptional silencing. MeCP2 was the first mammalian methyl-CpG binding protein for which the gene was cloned and it was shown to be a chromosomal protein capable of binding to a single methylated CpG pair. It is now clear that there is a family of such proteins in mammals with distinct binding properties but sharing a related domain that is crucial for methylated DNA binding. Both MeCP2 and the second family member, MBD1, are transcriptional repressors and recently MeCP2 was shown to interact with a histone deacetlyase complex suggesting that the processes of deacetylation and methylation co-operate to silence transcription.

We have identified a set of cDNAs from *Arabidopsis, Antirrhinum,* maize and rice that encode proteins with homology to the methylated DNA binding domains of MeCP2 and MBD1. Homology, both between the four plant proteins and with the mammalian proteins, is confined to this domain. Although identity is quite low the matches look significant. For example, comparing the same 55 amino acids, we find that MBD1 is as similar to the maize methyl-CpG binding domain-like sequence (53%) as it is to MeCP2. Overall, the four plant protein sequences share a similar bipartite structure with the conserved basic methyl-CpG binding-like region at the N-terminus and an species specific acidic C-terminal region.

We are in the process of testing these proteins for functional conservation by assessing any DNA binding specificity. Expression of the *Antirrhinum* gene (*AmMBD*) is confined to meristematic cells and emerging lateral organs with no expression in terminally differentiated cells, a pattern consistent with some of the abnormalities observed in methylation deficient *Arabidopsis*. However, expression of mouse *MeCP2* and *Mbd1* is negligible in undifferentiated stem cells (ES cells) but is up-regulated on differentiation and the expression patterns appear to be essentially that of "housekeeping" genes since the genes are on in all cell types. While we cannot make direct comparisons between plant and animal development, the contrasting expression patterns point to different roles for the plant and animal proteins.

We are currently investigating the function of the plant proteins by over-expression of sense and anti-sense MBD constructs in *Arabidopsis* and screening for transposon insertion mutants in *Antirrhinum*.

Seed specific expression: the role of bZIP and DOF factors in the activation of prolamin gene promoters

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Prolamins are the major seed storage proteins of cereals. They are specifically expressed in the developing endosperm and regulated in a temporal fashion. Extensive characterization of prolamin genes revealed conserved motives in their promoters, which are bound by nuclear proteins specific to the seed. One of such motives is the endosperm box, a bipartite element that confers tissue specificity and contains binding sites for bZIP factors in close vecinity to a highly conserved sequence called the prolamin box. We have cloned and characterized a prolamin box binding factor that belongs to the recently described DOF family of zinc-fingers, and is specifically expressed in the seed. Results will be presented on the protein-protein and protein-DNA interactions on the endosperm box and their relevance in the activation of prolamin genes

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Clonality of variegated transgene expression in the mouse mammary gland.

Beta-lactoglobulin (BLG) is the main whey protein in ruminant milk. Mice carrying ovine BLG transgenes are expressed in the mammary gland and secrete BLG protein into their milk (Simons *et al.*, 1987). Furthermore, this promoter can direct high-level expression of heterologous proteins in transgenic livestock (Wright *et al.*, 1992; Wilmut and Whitelaw, 1994). Genomic BLG transgenes are expressed efficiently in transgenic mice, with expression observed in all lines generated at a level related to transgene copy-number (Whitelaw *et al.*, 1992). Nevertheless, in some lines variable expression patterns was observed between siblings. On further analysis this variablity was shown to occur at the cellular level, with some cells expressing the transgene and others not. Endogenous mouse milk protien genes, e.g. ß-casein, are homogeneously expressed in the lactating gland. This variegated expression profile was not due to transgene rearrangements in these lines nor was it due to genetic background, as shown by back-cross experiments but was more prevalnt in high transgene copy-number integrants. Interestingly, in two lines which displayed varigated transgene expression the transgene was shown to integrated near to a centromere, allowing parallels with position-effect variation (PEV) in *Drosophila* to be drawn. From this initial analysis it was concluded that the mosiac expression pattern appears to correlate with chromosmal site of integration and/or copy-number (Dobie *et al.*, 1996). As part of our on going analysis of this phenomenom we wish to know if mosaic patterns of transgene expression reflect clonal expansion from committed progenitors. Although the clustering of expressing and non-expressing cells within the mammary gland is most suggestive of clonal expansion, we can not rule out other possibilities. For instance, cell-cell interactions could plausibly regulate transgene expression. In an attempt to determine the relationship with clonal expansion, we are generating transgenic mice harbouring an X-linked LacZ transgene and a variegating BLG transgene. Analysis of co-incidence of ß-gal and BLG expression in these mice will hopefully shed some light on the clonality of variegated transgene expression in the mouse mammary gland.

Dobie, K.W., Lee, M., Fantes, J.A., Graham, E., Clark, A.J., Springbett, S., Lathe, R., and McClenaghan, M. (1996) *Proc. Natl. Acad. Sci. USA* 93, 6659-6664.

Simons, J.P., McClenaghan, M., and Clark, A.J. (1987) Nature 328, 530-532.

Whitelaw, C.B.A., Garris, S., McClenagahn, M., Simons, J.P., and Clark, A.J. (1992) *Biochem. J.* 286, 31-39.

Wilmut. I., and Whitelaw, C.B.A. (1994) *Reprod. Fertil. Dev.* 6, 625-630.

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Organizers: S. Moncada and S. Lamas.

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