

NUCLEAR COMPARTMENTALIZATION AND GENE ACTIVITY

Claire Francastel*, Dirk Schübeler*, David I. K. Martin‡ and Mark Groudine*§

The regulated expression of genes during development and differentiation is influenced by the availability of regulatory proteins and accessibility of the DNA to the transcriptional apparatus. There is growing evidence that the transcriptional activity of genes is influenced by nuclear organization, which itself changes during differentiation. How do these changes in nuclear organization help to establish specific patterns of gene expression?

HETEROCHROMATIN

A condensed form of chromatin; the degree of compaction is similar to that of mitotic chromosomes. It is usually found around the centromere.

INTERPHASE

The period between two mitotic divisions.

Cellular differentiation is the process by which a cell acquires a new phenotype to accomplish specific functions, and it is accompanied by activation of a specific subset of genes and silencing of the remainder. As genes are silenced, the extent of chromatin condensation increases, and extended regions of DNA are packaged in a transcriptionally inactive form often referred to as HETEROCHROMATIN¹. The amount and distribution of condensed chromatin is similar in terminally differentiated cells of the same lineage, but it varies in the nuclei of different cell types, indicating that nuclear organization may be cell-type specific² (FIG. 1).

These observations have led to the idea that the topological organization of the INTERPHASE nucleus is related to the differentiated state of the cell, and that this spatial organization is involved in the establishment of the tissue-specific pattern of gene expression during cellular differentiation. Clearly, many overlapping pathways are involved in regulating gene expression^{3–6}. Much research is focused on determining the proteins (*trans*-acting factors) and DNA sequences (*cis*-acting elements) involved in the dynamic localization of genes within the nucleus, and we are just beginning to understand the link between the structure and

*Fred Hutchinson Cancer Research Center, 1,100 Fairview Avenue North, Seattle, Washington 98109, USA.

‡The Victor Chang Cardiac Research Institute, 384 Victoria Street Darlinghurst, Sydney, New South Wales 2010, Australia.

§Department of Radiation Oncology, University of Washington School of Medicine, Seattle, Washington 98195, USA. Correspondence to M.G. e-mail: markg@fhcrc.org

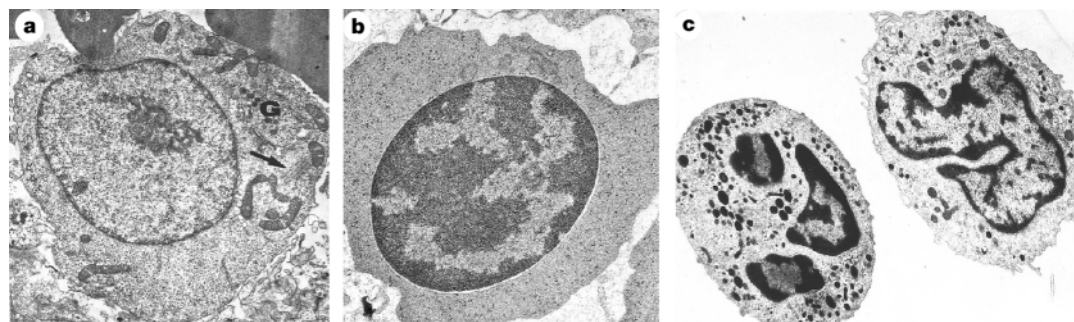


Figure 1 | **Patterns of chromatin condensation in haematopoietic cells.** The electron micrographs show haematopoietic cells from normal human bone marrow⁸², and illustrate the distinct patterns of chromatin condensation in distinct terminally differentiated cells. **a** | Proerythroblast (immature red blood cell). The nucleus contains almost no heterochromatin. **b** | Late erythroblast. Heterochromatin in the nucleus is predominant and appears as darkly staining regions. **c** | The nucleus of a monocyte (right) appears less condensed than that of a granulocyte (left). (Figure adapted with permission from REF. 82 © Harcourt Brace, Madrid, Spain.)

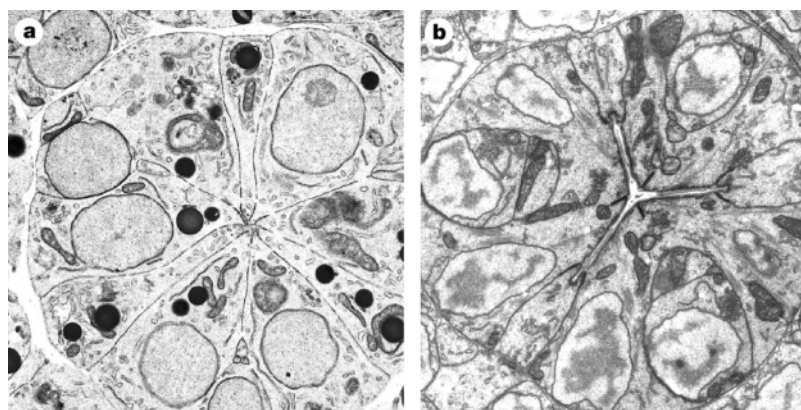


Figure 2 | Chromatin condensation accompanies cellular differentiation. Electron micrographs of *Caenorhabditis elegans* pharyngeal cells **a** | before and **b** | after commitment to the pharyngeal lineage. **a** | Cells are committed to give rise to pharyngeal cells, but have not yet undergone terminal differentiation. The nucleus seems to contain almost exclusively euchromatin, with a thin rim of heterochromatin at the nuclear periphery. **b** | In terminally differentiated cells, the nucleus appears more compact and condensed chromatin forms clumps inside the nucleus. (Images courtesy of J. Priess, Fred Hutchinson Cancer Research Center, Seattle, USA.)

function of chromatin and large-scale changes in the nucleus itself.

We propose that tissue-specific ENHANCERS prevent active genes from being included in regions of transcriptionally inactive condensed chromatin that form during cell differentiation. This allows a subset of genes to be active in the appropriate lineage, whereas the remainder are silenced, and ensures, for example, that globin genes are expressed only in red blood cells, and immunoglobulin genes only in B cells.

Gene potentiation during differentiation

Modulation of cell- and stage-specific gene expression during differentiation is thought to involve inactivation of those genes that do not need to be expressed in a given lineage. Conversely, those sets of genes that need to be activated are placed or maintained in a decondensed — or ‘potentiated’ — state in precursor cells of that lineage. Potentiation is characterized by an ‘open’ chromatin structure, meaning that a locus is accessible to the tissue-specific factors required for its appropriate expression⁷.

Haematopoiesis has been used as a model system to

study the putative coupling of chromatin condensation and gene repression during the differentiation of somatic cells. The haematopoietic programme is initiated from a stem cell with self-renewal and differentiation capacities, which can give rise to several distinct lineages. In multipotent haematopoietic stem cells, many genes relevant to different lineage fates are transcribed before the decision to commit to any one lineage^{8,9}. In other words, before the commitment decision, a general ‘permissive’ state exists in which genes specific to several lineages may be in an open chromatin configuration. Moreover, the subsequent activation of genes for any one lineage is probably coincident with closing of those gene domains associated with other potential cell fates. Indeed, this relationship may be a general feature of gene regulation during cellular differentiation. For example, microscopic analysis of the developing pharynx in the nematode worm *Caenorhabditis elegans* reveals a uniform structure of EUCHROMATIN in the nuclei of those cells committed to the pharyngeal cell fate (FIG. 2a). However, differentiation of these cells is associated with activation of pharyngeal-specific genes and extensive formation of heterochromatin, indicating that other domains may be inactivated (FIG. 2b).

So gross changes in chromatin structure accompany cellular differentiation. Chromatin condensation and gene silencing correlate with a change in the nucleosomal structure characterized by the deacetylation of core histones^{10–13} and accumulation of linker histones^{14,15} (BOX 1). For example, differentiation of embryonic stem cells is accompanied by a progressive deacetylation of histone H4 in the heterochromatin around centromeres¹⁶. Conversely, acetylation of histone tails marks ‘open’ (nuclease-sensitive) domains and is also involved in promoter activation (FIG. 3).

Although gene potentiation is necessary for transcription, it is not in itself sufficient for full activation of a promoter. For example, the α -globin locus resides in a region that is constitutively open, but this gene is not transcribed in non-erythroid cells¹⁷. In haematopoietic cell lines, the nuclease sensitivity of potentially active genes — for example the β -globin locus and *c-myc* — correlates with their level of histone H4 acetylation, regardless of their transcriptional activity^{18,19}. Therefore, chromatin opening and histone acetylation could represent the first step of a two-step mechanism to achieve gene expression — gene potentiation before transcriptional activation of the promoters.

Whether activation of globin genes in normal differentiating red blood cells is a multistep process is not clear, but there is evidence that it might be. Studies in mouse erythroleukaemia (MEL) cells, comparing the mouse β -globin locus before and after induction of differentiation, reveal that it can exist in two distinct structural states. The first is a potentiated chromatin structure in cells that are committed to the erythroid lineage but not terminally differentiated. The second is an activated state seen in terminally differentiating cells²⁰. In the potentiated state, the β -globin locus is more sensitive to nuclease digestion than in non-erythroid cells²¹, where the nucleosomes are arranged in a regular

ENHANCERS

Increase transcription of a linked promoter if placed in either orientation, upstream or downstream.

EUCHROMATIN

Chromatin that appears less compact than mitotic chromosomes. Active genes are contained within euchromatin.

Box 1 | Nucleosome structure and function

The nucleosome is the fundamental subunit of all chromatin, giving it the characteristic ‘beads-on-a-string’ appearance. Each nucleosome is composed of about 200 base pairs of DNA coiled roughly twice around an octamer of ‘core’ histone proteins — two molecules each of histones H2A, H2B, H3 and H4. The ‘linker’ histone H1 can be associated with each nucleosome; it seems to mediate the packing of adjacent nucleosomes during chromatin condensation.

The structure and function of chromatin can be modulated by acetylation (and deacetylation) of histones. Acetylation is a covalent modification of lysine residues — addition of an acetyl group — in the histone tails. Acetylated histones are found in regions of decondensed chromatin, whereas deacetylated histones are found in regions of condensed chromatin. Acetylation is mediated by histone acetyltransferases (HATs), and deacetylation by histone deacetylases (HDACs). Because changes in histone acetylation are associated with changes in chromatin structures, these enzymes are important cofactors in the regulation of gene expression.

(phased) pattern along the β -globin gene. In MEL cells, however, phasing is disrupted both before and after induction of differentiation, and this correlates with an increased generalized sensitivity to DNaseI (REF. 22). Moreover, in uninduced cells, the non-transcribed β -globin gene contains a hypersensitive site in the intervening sequence II, indicating the binding of a non-histone protein. In induced cells, however, transcription of the β -globin gene is associated with the appearance of a hypersensitive site at the promoter and an increase in general sensitivity to nucleases^{21,23}.

How are tissue-specific genes maintained in an open chromatin configuration before promoter activation? Although the molecular mechanisms behind this dynamic alteration of chromatin are not clear, a facet of chromatin opening and gene activation that has become apparent only in recent years deserves consideration — namely that active genes are localized in nuclear compartments that are permissive for transcription.

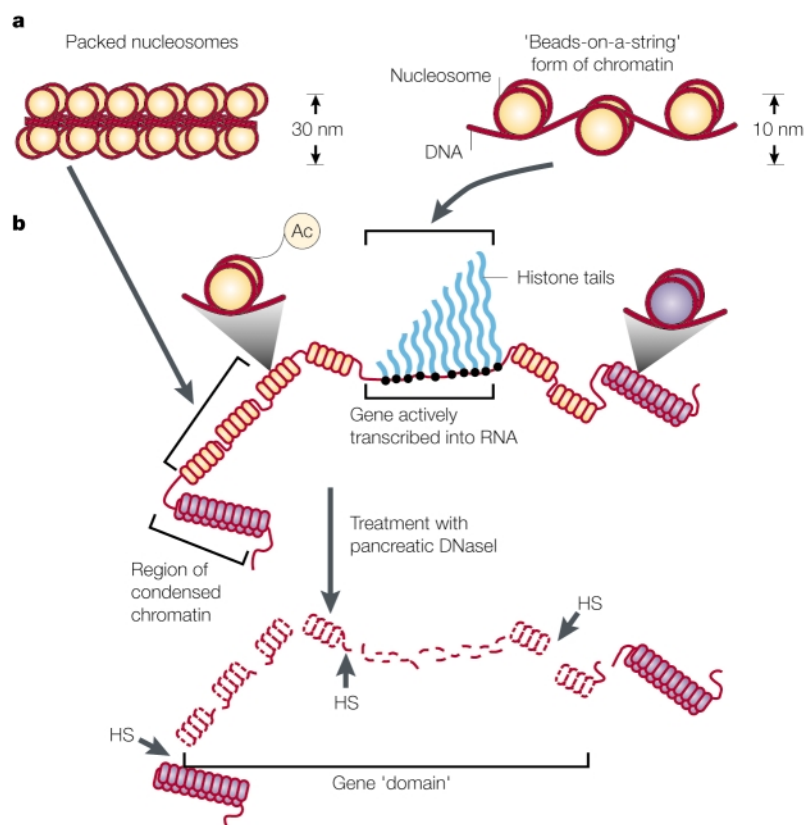


Figure 3 | Chromatin structure and gene expression. **a** | Organization of chromatin into the tightly condensed 30-nm fibre and 'beads-on-a-string' 10-nm fibre. **b** | Region of DNA containing an actively transcribed gene. The region encompassing the transcribed gene probably adopts the 10-nm configuration, whereas sequences up- and downstream are organized into a 30-nm fibre. Transcription involves changes in the chromatin structure that can be detected by digestion with the endonuclease DNaseI. The transcribed regions also contain hypersensitive (HS) sites, which may be associated with a small stretch of DNA devoid of nucleosomes or simply reflect a different nucleosomal structure (positioning or histone modification). They usually correlate with non-histone proteins bound to the DNA. Regions of general DNaseI sensitivity correlate with acetylation of the histone tails, defining a structural and functional 'domain' for gene activity that is characteristic of 'open' chromatin. This domain is embedded in regions of higher chromatin compaction that are more resistant to DNaseI digestion. This compacted structure correlates with the presence of deacetylated histones (blue wavy lines), and is characteristic of 'closed' chromatin. (Figure adapted from REF. 83.)

Changes in nuclear structure and differentiation. Although the nucleosomal organization of chromosomes has been the focus of much study, the higher-order structure of chromosomes in the interphase nucleus is only poorly understood. Recent advances in fluorescence *in situ* hybridization (FISH) have allowed individual genes in the interphase nucleus to be visualized and topographically analysed (FIG. 4), contributing to the increasing awareness that spatial context within the nucleus is important in modulating gene expression^{24–26}.

Two main levels of nuclear organization can be distinguished. First, chromosomes and genes are confined to discrete nuclear zones within the nuclear volume, referred to as 'chromosome territories'^{27–29}. These territories occupy non-random positions in the interphase nucleus that are specific to certain cell types^{30–32}. Second, many nuclear functions — such as replication, RNA processing and transcription — take place in well-defined compartments in the nucleus³³.

How might this higher-order organization regulate gene expression? One view is that active and inactive regions of the genome, as well as protein factors involved in activation or repression of gene expression, are compartmentalized within the nucleus. Nuclease-sensitive regions of chromatin and sites of active transcription are located in clusters scattered throughout the nucleus^{33–35}. Whereas chromosomes with few genes are often associated with the nuclear periphery, gene-rich chromosomes reside in a more internal nuclear position³⁶. Genes on the same chromosome are further compartmentalized into distinct active and inactive domains within a chromosome territory: non-coding sequences are found in the interior or randomly distributed in the chromosome territory^{37,38}, whereas potentially active genes, regardless of their transcriptional activity, are preferentially located at the periphery of chromosome territories. The surface area of the chromosomes interacts with the nuclear space between the chromosome territories, the so-called interchromosomal domain, where gene transcription and messenger RNA splicing are thought to occur^{38,39}. Moreover, transcriptionally active gene clusters can be found on large chromatin loops extending outwards from the surface of the chromosome territory, indicating that genomic sequences may be recruited to environments that are permissive for transcription⁴⁰.

If active transcription is concentrated in a fraction of the nucleus, it is conceivable that RNA polymerase II and specific transcription factors would also accumulate in these nuclear compartments for active transcription. Indeed, a significant fraction of active transcription has been shown to colocalize with RNA polymerase II or with basal transcription factors⁴¹. Furthermore, nuclear regions containing transcription factors, as well as TATA-box binding protein (TBP), RNA polymerase II and nascent RNA, have been described⁴².

However, a large fraction of transcription-factor molecules are not associated with sites of active transcription, nor with RNA polymerase II; the function of these sites is not yet clear³⁸. One possibility is that they are storage sites from which proteins can be recruited. In

METHYL-DNA-BINDING DOMAIN PROTEINS

Proteins that bind specifically to methylated DNA through a methyl-DNA-binding domain. Some of these proteins are involved in transcriptional repression of methylated DNA.

SATELLITES

Relatively short DNA sequences that are highly repeated in long tandem arrays.

CONSTITUTIVE HETEROCHROMATIN

The fraction of heterochromatin that stays compact through the cell cycle. It is mainly composed of repetitive sequences (satellite DNA; see above), and is concentrated in characteristic regions such as centromeres.

CHROMOCENTRES

Aggregates of constitutive heterochromatin from different chromosomes.

this case, they may be specific for particular transcription factors, as most of the factors investigated occupy non-overlapping sites. Interestingly, a subset of transcription-factor-rich zones colocalize in the nuclei of neurons in the hippocampus, and this may reflect their coordinated action on genes involved in neuronal excitability⁴³.

Factors involved in gene silencing have also been shown to colocalize with each other and with constitutive heterochromatin. These factors include proteins associated with heterochromatin (**heterochromatin protein 1 (HP1)**, **SUV39H1** and **Ikaros**)^{44–46}, METHYL-DNA-BINDING DOMAIN PROTEINS (**MBDs**) and histone deacetylases (**HDACs**). For example, MBD proteins associate with major SATELLITES in embryonic stem cells^{47,48}, and HDACs (BOX 1) colocalize with centromeric DNA⁴⁹.

Current evidence supports the idea that the eukaryotic nucleus is functionally divided into heterochromatin compartments that repress transcription, and compartments in which transcription is permitted. How are these discrete compartments and the specific spatial relationship between them established to produce a precise pattern of gene expression?

Some evidence indicates that centromeres and CONSTITUTIVE HETEROCHROMATIN provide a structural framework for this nuclear architecture, and that changes in this architecture are involved in cellular diversification. During interphase, the constitutive heterochromatin,

which is found mainly around the centromeres, stays condensed. The position of the centromeres in interphase nuclei seems to be cell-type specific, and it can change during the cell cycle, during differentiation, or with cellular transformation^{30,50–52}. In mammalian cells, centromeres tend to cluster in so-called CHROMOCENTRES. The specific combinations of centromeres found in chromocentres are different in fibroblasts and haematopoietic cells⁵². Moreover, they also seem to be distinct in mature cells of different haematopoietic lineages. Just as the organization and location of centromeres change with various cellular states, so a dynamic positioning of chromosomes, specific chromosomal domains or *trans*-acting factors has also been observed during cell-cycle progression, differentiation and malignant transformation^{51–58}. For example, during nerve growth factor (NGF)-induced differentiation of rat pheochromocytoma cells, changes in gene expression correlate with redistribution of DNaseI hypersensitive domains³⁴, and, in hormone-induced differentiation of pre-adipocytes, the *trans*-acting factor **C/EBP** is relocated into discrete nuclear foci⁵⁹.

Nuclear compartmentalization

Dynamic nuclear architecture could reflect changes in the physical position of tissue-specific genes during cell differentiation, which affect the accessibility of genes to regulatory factors and the transcriptional machinery. This, in turn, may facilitate nuclear functions such as gene transcription or silencing.

Experimental evidence supports the idea that positioning of a gene near heterochromatin compartments promotes gene silencing. For example, in *Drosophila melanogaster* the insertion of a block of heterochromatin into one allele of the euchromatic **brown** gene results in the association of both alleles with centromeric heterochromatin, leading to transcriptional inactivation of the wild-type allele^{60–62}. During differentiation of mouse lymphocytes, gene silencing is heritable and associated with repositioning of genes close to centromeres^{45,63}. In addition, the human β -globin gene domain, in its native context, can be found in distinct locations in the interphase nucleus relative to the heterochromatin compartment¹⁹. Its positioning in the erythroid nucleus correlates with the chromatin and general acetylation configurations of the locus, but not with transcription of the β -globin genes¹⁹. Together with the fact that suppression of transgene silencing and maintenance of its open chromatin configuration require distance away from centromeric heterochromatin, these results indicate that stably inherited chromatin opening of a locus might be mediated by its sequestration in a permissive compartment. These findings also argue against the idea that nuclear relocation is the consequence of transcription.

Other silencing systems — such as the Sir-dependent, telomeric and mating-type-locus silencing in yeast — are associated with compartmentalization of the silenced gene. Telomeric silencing occurs at the nuclear envelope in compartments where telomeres are clustered and the concentration of Sir proteins is high^{64–68}.

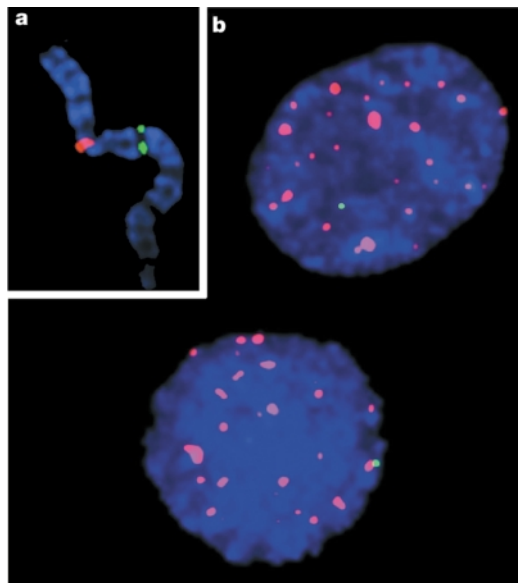


Figure 4 | **Fluorescence *in situ* hybridization to study gene localization.** **a** | Chromosome from a metaphase spread. Centromeric sequences are detected with a Texas-red-conjugated probe (red), and a transgene — integrated in the genome of a human erythroleukaemia cell line — is localized with a fluorescein isothiocyanate-conjugated probe (green). The transgene is integrated on the long arm of the chromosome distant from its centromere. **b** | Fluorescence *in situ* hybridization experiments in interphase cells reveal that the transgene is in close proximity to centromeric heterochromatin in silent cells (bottom cell), but it is found away from this compartment in expressing cells (upper cell). Silencing of the transgene is therefore accompanied by its relocalization, in the interphase nucleus, close to the heterochromatin compartment⁷⁰.

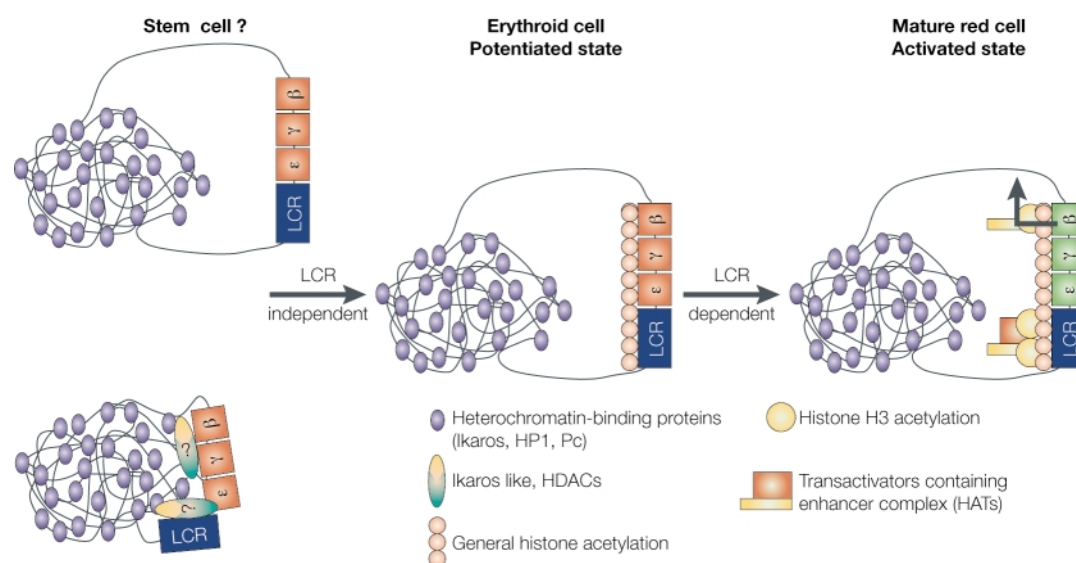


Figure 5 | **Activation at the β -globin locus: a multistep process?** Chromatin opening and transcriptional activation at the β -globin locus can be dissociated^{80,81}, indicating that they are achieved through distinct mechanisms. First, an open chromatin configuration, marked by a locus-wide acetylation, is mediated by positioning of the locus away from centromeric heterochromatin. The resulting open chromatin structure favours the binding of tissue-specific *trans*-acting factors to enhancers and the promoter, concomitant with a local hyperacetylation of histone H3 and promoter activation. Although sequences outside the locus control region (LCR) are sufficient to promote the first step, gene activation requires an intact LCR. The demonstration that the β -globin locus can adopt distinct structural correlates, together with the demonstration that erythroid differentiation in a model system is a multistep process, supports the idea that activation of globin genes in normal differentiating red blood cells is also a multistep process. The configuration of the chromatin structure in a multipotent stem cell, as well as the structure and localization of the β -globin locus in precursor cells, remains to be determined. (HDACs, histone deacetylases; HP1, heterochromatin protein 1; Pc, Polycomb, HATs, histone acetyltransferases.)

Furthermore, positioning of a locus to the nuclear periphery can provoke Sir-mediated silencing⁶⁹.

In centromeric silencing, by contrast, there is no direct evidence that association with centromeric heterochromatin is the cause of silencing, nor is there evidence that localization away from heterochromatin is required for transcriptional activity. For example, an active transgene may be located close to centromeric heterochromatin. However, transcription and the open chromatin structure of a transgene are unstable in this compartment⁷⁰. Conversely, a silent transgene can be located away from centromeric heterochromatin⁷⁰, and transient gene silencing during differentiation of B-cell lines is not associated with relocation close to centromeres⁶³.

So although there is evidence that positioning to specific compartments may affect transcriptional activity or gene silencing, such compartmentalization does not seem to be an absolute requirement. In general, however, stably inherited gene activity and open chromatin configurations seem to be associated with positioning away from centromeric heterochromatin, whereas stably inherited gene silencing requires positioning close to centromeric heterochromatin. If there are indeed specific mechanisms to place and keep genes in a silent compartment, the potentiated state would be determined by the positioning of a gene in an active compartment of the nucleus. This, in turn, would allow tissue-specific transcription factors to induce the precise programmes of stable gene expression typical of differentiated cells.

Role of *cis*-acting elements

Finally we can ask how modifications of the chromatin structure are achieved over broad regions⁷¹, and what sequences are involved in mediating an open or closed structure. Sequences that mediate silencing have been described in yeast and *Drosophila*, and the observations indicate that the formation of an inactive structure may involve assembly of proteins that initially bind to SILENCER ELEMENTS. Such a repressive structure may then propagate along the chromatin.

Mating-type silencing in yeast involves the nucleation of a repressive chromatin structure at silencer elements, with subsequent spreading of this structure. Interestingly, silencing at the mating-type loci is associated with both a silenced chromatin structure and localization of silenced regions near the nuclear periphery. This indicates that nuclear compartments and the spreading of specific chromatin structures are not incompatible phenomena but that both define the silenced state⁶⁶. Telomeric silencing in yeast involves similar spreading of silenced chromatin, mediated by Sir proteins, and is associated with histone hypoacetylation^{25,72}. Similar nucleation and spreading effects are thought to account for silencing of genes located in or near heterochromatin in higher eukaryotes and for **Polycomb-mediated gene silencing in *Drosophila*** — a system required for maintaining the inactive state of genes containing a Polycomb response element (PRE). So far, the PRE is the only clear example of a *cis*-element responsible for silencing in higher eukaryotes^{73,74}.

Numerous studies have shown that enhancers can

SILENCER ELEMENTS
Cis-acting elements that are involved in silencing, most probably by directly recruiting repressive proteins.

LOCUS CONTROL REGION
Defined by its ability, in transgenic assays, to confer high-level, tissue-specific expression on a linked promoter, at all integration sites.

FACULTATIVE HETEROCHROMATIN
Fraction of chromatin that is condensed and inactive in a given cell lineage, which may be decondensed and active in another.

ANEUPLOIDY
The ploidy of a cell refers to the number of sets of chromosomes that it contains. Aneuploid karyotypes are those whose chromosome complements are not a simple multiple of the haploid set.

counteract such silencing events^{75–77}. The demonstration that an enhancer is sufficient for both localization of a transgene away from centromeric heterochromatin and suppression of transgene silencing indicates that enhancers may maintain gene expression by preventing localization close to the repressive heterochromatin compartment⁷⁰ (FIG. 5).

The situation in a multigene endogenous locus seems to be more complex when compared with the simplified structure of a transgene. The human β -globin LOCUS CONTROL REGION (LCR), although essential for transcriptional activation, is not required to relocate the native locus away from heterochromatin¹⁹. The apparent paradox between results obtained with the native locus and those with transgenes can be explained by the existence of numerous factor-binding sites throughout the native locus, which probably alter subnuclear location and chromatin structure. So *cis*-acting elements other than the LCR, but with similar function, may maintain the β -globin locus in an open chromatin/acetylated configuration, localized away from centromeres.

There are various means by which genetic regulatory elements, such as enhancers and LCRs, could cause a gene to localize away from heterochromatin. Initial transcription-factor binding to the enhancer might promote recruitment of a locus to a nuclear compartment enriched in chromatin remodelling complexes and histone acetyltransferases (HATs; for example, HAT1), as well as other elements of the transcriptional machinery.

Just as there are transcription-factor-rich sites, there are regions of the nucleus that are devoid of, or contain low concentrations of, such factors. These areas might be the default location of a gene or transgene if transcription factors do not bind to its enhancer elements. Alternatively, the lack of a suitable transcription factor could result in failure of a gene to be targeted to a transcription-rich region of the nucleus. The instability of gene expression associated with localization near heterochromatin may result from this low concentration of

positive transcriptional regulators in the heterochromatin compartment, and from a high concentration of negative regulators. Perhaps the simplest mechanism for activation by regulatory elements is one in which transcriptional activators bound to regulatory elements disrupt local interactions between a locus and heterochromatin, permitting the gene to move into the active compartment.

Future directions

The chromosomal and nuclear position of a gene can influence its activity, and the position of a gene within the nucleus can be dictated by the *cis*-acting sequences linked to it. Tissue-specific enhancers may prevent a linked gene from being included in FACULTATIVE HETEROCHROMATIN, which forms during cell differentiation, therefore allowing it to be active in the appropriate lineage. If the precise topological conformation of genes within the nucleus is disrupted, the result may be phenotypic changes or disease. For example, cancer involves disruption of stable patterns of gene expression, and can be accompanied by a global disorganization of chromosomal positioning in the nucleus⁷⁸. Chromosomal abnormalities and ANEUPLOIDY, which often affect nuclear architecture, can influence gene expression not only of the affected chromosome, but also of nearby chromosomal regions⁷⁹. So investigations into links between structural and functional aspects of interphase chromosomes promise to reveal fundamental aspects of biology, as well as insights into the development of disease.

Links

DATABASE LINKS [Histone H4](#) | [\$\alpha\$ -globin](#) | [\$\beta\$ -globin](#) | [*c-myc*](#) | [TBP](#) | [RNA polymerase II](#) | [HP1](#) | [SUVAR39H1](#) | [Ikaros](#) | [MBDs](#) | [HDACs](#) | [C/EBP](#) | [brown](#) | [HAT1](#)
FURTHER INFORMATION [Polycomb-mediated gene silencing](#)
ENCYCLOPEDIA OF LIFE SCIENCES [Nucleosomes: detailed structure and mutations](#)

- John, B. *The Biology of Heterochromatin* (ed. Verma, R. S.) (Cambridge Univ. Press, Cambridge, 1988).
- Leitch, A. R. Higher levels of organization in the interphase nucleus of cycling and differentiated cells. *Microbiol. Mol. Biol. Rev.* **64**, 138–152 (2000).
- Felsenfeld, G. Chromatin as an essential part of the transcriptional mechanism. *Nature* **355**, 219–224 (1992).
- Gregory, P. D. & Horz, W. Chromatin and transcription — how transcription factors battle with a repressive chromatin environment. *Eur. J. Biochem.* **251**, 9–18 (1998).
- Kadonaga, J. T. Eukaryotic transcription: An interlaced network of transcription factors and chromatin-modifying machines. *Cell* **92**, 307–313 (1998).
- Wolffe, A. P. Transcription: In tune with the histones. *Cell* **77**, 13–16 (1994).
- Kramer, J. A., McCarrey, J. R., Djakiew, D. & Krawetz, S. A. Differentiation: The selective potentiation of chromatin domains. *Development* **125**, 4749–4755 (1998).
- Jimenez, G., Griffiths, S. D., Ford, A. M., Greaves, M. F. & Enver, T. Activation of the β -globin locus control region precedes commitment to the erythroid lineage. *Proc. Natl Acad. Sci. USA* **89**, 10618–10622 (1992).
- Hu, M. *et al.* Multilineage gene expression precedes commitment in the hemopoietic system. *Genes Dev.* **11**, 774–785 (1997).
- Lee, D. Y., Hayes, J. J., Pruss, D. & Wolffe, A. P. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. *Cell* **72**, 73–84 (1993).
- Wolffe, A. P. & Pruss, D. Targeting chromatin disruption: Transcription regulators that acetylate histones. *Cell* **84**, 817–819 (1996).
- Turner, B. M. Histone acetylation as an epigenetic determinant of long-term transcriptional competence. *Cell. Mol. Life Sci.* **54**, 21–31 (1998).
- Wolffe, A. P. Packaging principle: How DNA methylation and histone acetylation control the transcriptional activity of chromatin. *J. Exp. Zool.* **282**, 239–244 (1998).
- Crane-Robinson, C. How do linker histones mediate differential gene expression? *Bioessays* **21**, 367–371 (1999).
- Wolffe, A. P., Khochbin, S. & Dimitrov, S. What do linker histones do in chromatin? *Bioessays* **19**, 249–255 (1997).
- Keohane, A. M., O'Neill, L. P., Belyaev, N. D., Lavender, J. S. & Turner, B. M. X-inactivation and histone H4 acetylation in embryonic stem cells. *Dev. Biol.* **180**, 618–630 (1996).
- Vyas, P. *et al.* *Cis*-acting sequences regulating expression of the human α -globin cluster lie within constitutively open chromatin. *Cell* **69**, 781–793 (1992).
- O'Neill, L. P. & Turner, B. M. Histone H4 acetylation distinguishes coding regions of the human genome from heterochromatin in a differentiation-dependent but transcription-independent manner. *EMBO J.* **14**, 3946–3957 (1995).
- Schübeler, D. *et al.* Nuclear localization and histone acetylation: A pathway for chromatin opening and transcriptional activation of the human β -globin locus. *Genes Dev.* **14**, 940–950 (2000).
- Indicates that positioning away from the heterochromatin compartment may mediate open chromatin configuration and histone acetylation of the human globin locus.
- Reddy, P. M. & Shen, C. K. Erythroid differentiation of mouse erythroleukemia cells results in reorganization of protein–DNA complexes in the mouse β -major globin promoter but not its distal enhancer. *Mol. Cell. Biol.* **13**, 1093–1103 (1993).
- Cohen, R. B. & Sheffery, M. Nucleosome disruption precedes transcription and is largely limited to the transcribed domain of globin genes in murine erythroleukemia cells. *J. Mol. Biol.* **182**, 109–129 (1985).
- Benezra, R., Cantor, C. R. & Axel, R. Nucleosomes are phased along the mouse β -major globin gene in erythroid and nonerythroid cells. *Cell* **44**, 697–704 (1986).
- Sheffery, M., Rifkind, R. A. & Marks, P. A. Murine erythroleukemia cell differentiation: DNase I hypersensitivity and DNA methylation near the globin genes. *Proc. Natl Acad. Sci. USA* **79**, 1180–1184 (1982).
- Lamond, A. I. & Earnshaw, W. C. Structure and function in the nucleus. *Science* **280**, 547–553 (1998).
- Cockell, M. & Gasser, S. M. Nuclear compartments and gene regulation. *Curr. Opin. Genet. Dev.* **9**, 199–205 (1999).

26. Marshall, W. F., Fung, J. C. & Sedat, J. W. Deconstructing the nucleus: Global architecture from local interactions. *Curr. Opin. Genet. Dev.* **7**, 259–263 (1997).
27. Munkel, C. *et al.* Compartmentalization of interphase chromosomes observed in simulation and experiment. *J. Mol. Biol.* **285**, 1053–1065 (1999).
28. Manuellidis, L. A view of interphase chromosomes. *Science* **250**, 1533–1540 (1990).
29. Zink, D. *et al.* Structure and dynamics of human interphase chromosome territories *in vivo*. *Hum. Genet.* **102**, 241–251 (1998).
30. Manuellidis, L. Different central nervous system cell types display distinct and nonrandom arrangements of satellite DNA sequences. *Proc. Natl Acad. Sci. USA* **81**, 3123–3127 (1984).
- One of the first papers showing that the spatial organization of centromeres is non-random and cell-type specific, and indicating that this could represent specific functional capacities.**
31. Manuellidis, L. & Borden, J. Reproducible compartmentalization of individual chromosome domains in human CNS cells revealed by *in situ* hybridization and three-dimensional reconstruction. *Chromosoma* **96**, 397–410 (1988).
32. Haaf, T. & Schmid, M. Chromosome topology in mammalian interphase nuclei. *Exp. Cell Res.* **192**, 325–332 (1991).
33. Sadoni, N. *et al.* Nuclear organization of mammalian genomes. Polar chromosome territories build up functionally distinct higher order compartments. *J. Cell Biol.* **146**, 1211–1226 (1999).
34. Park, P. C. & De Boni, U. Transposition of DNase hypersensitive chromatin to the nuclear periphery coincides temporally with nerve growth factor-induced up-regulation of gene expression in PC12 cells. *Proc. Natl Acad. Sci. USA* **93**, 11646–11651 (1996).
35. de Graaf, A. *et al.* Three-dimensional distribution of DNase I-sensitive chromatin regions in interphase nuclei of embryonal carcinoma cells. *Eur. J. Cell Biol.* **52**, 135–141 (1990).
36. Croft, J. A. *et al.* Differences in the localization and morphology of chromosomes in the human nucleus. *J. Cell Biol.* **145**, 1119–1131 (1999).
- Analyses the influence of the cell cycle and the inhibition of transcription on the morphology and distribution of specific human chromosome territories.**
37. Kurz, A. *et al.* Active and inactive genes localize preferentially in the periphery of chromosome territories. *J. Cell Biol.* **135**, 1195–1205 (1996).
38. Verschure, P. J., van Der Kraan, I., Manders, E. M. & van Driel, R. Spatial relationship between transcription sites and chromosome territories. *J. Cell Biol.* **147**, 13–24 (1999).
- Shows that transcription is compartmentalized, and that sites of active transcription are distinct from sites of inactive chromatin.**
39. Zirbel, R. M., Mathieu, U. R., Kurz, A., Cremer, T. & Lichter, P. Evidence for a nuclear compartment of transcription and splicing located at chromosome domain boundaries. *Chromosome Res.* **1**, 93–106 (1993).
40. Volpi, E. V. *et al.* Large-scale chromatin organization of the major histocompatibility complex and other regions of human chromosome 6 and its response to interferon in interphase nuclei. *J. Cell Sci.* **113**, 1565–1576 (2000).
- This paper finds a correlation between the formation of large external chromatin loops, outside a chromosome territory, and the transcriptional activity of a gene cluster.**
41. Grande, M. A., van der Kraan, I., de Jong, L. & van Driel, R. Nuclear distribution of transcription factors in relation to sites of transcription and RNA polymerase II. *J. Cell Sci.* **110**, 1781–1791 (1997).
42. Pombo, A. *et al.* Regional and temporal specialization in the nucleus: A transcriptionally active nuclear domain rich in PTF, Oct1 and PIKA antigens associates with specific chromosomes early in the cell cycle. *EMBO J.* **17**, 1768–1778 (1998).
43. van Steensel, B. *et al.* Partial colocalization of glucocorticoid and mineralocorticoid receptors in discrete compartments in nuclei of rat hippocampus neurons. *J. Cell Sci.* **109**, 787–792 (1996).
44. Aagaard, L. *et al.* Functional mammalian homologues of the *Drosophila* PEV-modifier *Su(var)3-9* encode centromere-associated proteins which complex with the heterochromatin component M31. *EMBO J.* **18**, 1923–1938 (1999).
45. Brown, K. E. *et al.* Association of transcriptionally silent genes with Ikaros complexes at centromeric heterochromatin. *Cell* **91**, 845–854 (1997).
- Ikaros, a protein associated with heterochromatin foci in the nucleus of mouse B lymphocytes, is also associated with transcriptionally inactive genes, indicating a different mechanism for gene silencing.**
46. Wreggett, K. A. *et al.* A mammalian homologue of *Drosophila* heterochromatin protein 1 (HP1) is a component of constitutive heterochromatin. *Cytogenet. Cell Genet.* **66**, 99–103 (1994).
47. Hendrich, B. & Bird, A. Identification and characterization of a family of mammalian methyl-CpG binding proteins. *Mol. Cell Biol.* **18**, 6538–6547 (1998).
48. Lewis, J. D. *et al.* Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. *Cell* **69**, 905–914 (1992).
49. Kim, J. *et al.* Ikaros DNA-binding proteins direct formation of chromatin remodeling complexes in lymphocytes. *Immunity* **10**, 345–355 (1999).
- Ikaros can recruit histone deacetylases and chromatin remodelling complexes to regions of heterochromatin in the nucleus of mouse T lymphocytes.**
50. Shelby, R. D., Hahn, K. M. & Sullivan, K. F. Dynamic elastic behavior of α -satellite DNA domains visualized *in situ* in living human cells. *J. Cell Biol.* **135**, 545–557 (1996).
51. Vourc'h, C., Taruscio, D., Boyle, A. L. & Ward, D. C. Cell cycle-dependent distribution of telomeres, centromeres, and chromosome-specific subtelomeric domains in the interphase nucleus of mouse lymphocytes. *Exp. Cell Res.* **205**, 142–151 (1993).
52. Alcobia, I., Dilao, R. & Parreira, L. Spatial associations of centromeres in the nuclei of hematopoietic cells: Evidence for cell-type-specific organizational patterns. *Blood* **95**, 1608–1615 (2000).
53. Manuellidis, L. Indications of centromere movement during interphase and differentiation. *Ann. NY Acad. Sci.* **450**, 205–221 (1985).
54. Minc, E., Allory, Y., Worman, H. J., Courvalin, J. C. & Buendia, B. Localization and phosphorylation of HP1 proteins during the cell cycle in mammalian cells. *Chromosoma* **108**, 220–234 (1999).
55. Kozubek, S. *et al.* Distribution of ABL and BCR genes in cell nuclei of normal and irradiated lymphocytes. *Blood* **89**, 4537–4545 (1997).
56. Neves, H., Ramos, C., da Silva, M. G., Parreira, A. & Parreira, L. The nuclear topography of ABL, BCR, PML, and RAR α genes: Evidence for gene proximity in specific phases of the cell cycle and stages of hematopoietic differentiation. *Blood* **93**, 1197–1207 (1999).
57. Bartova, E. *et al.* Nuclear topography of the *c-myc* gene in human leukemic cells. *Gene* **244**, 1–11 (2000).
58. Bridger, J. M., Boyle, S., Kill, I. R. & Bickmore, W. A. Remodelling of nuclear architecture in quiescent and senescent human fibroblasts. *Curr. Biol.* **10**, 149–152 (2000).
59. Tang, Q. Q. & Lane, M. D. Activation and centromeric localization of CCAAT/enhancer-binding proteins during the mitotic clonal expansion of adipocyte differentiation. *Genes Dev.* **13**, 2231–2241 (1999).
60. Dernburg, A. F. *et al.* Perturbation of nuclear architecture by long-distance chromosome interactions. *Cell* **85**, 745–759 (1996).
61. Csink, A. K. & Henikoff, S. Large-scale chromosomal movements during interphase progression in *Drosophila*. *J. Cell Biol.* **143**, 13–22 (1998).
62. Csink, A. K. & Henikoff, S. Genetic modification of heterochromatic association and nuclear organization in *Drosophila*. *Nature* **381**, 529–531 (1996).
63. Brown, K. E., Baxter, J., Graf, D., Mermenschlager, M. & Fisher, A. G. Dynamic repositioning of genes in the nucleus of lymphocytes preparing for cell division. *Mol. Cell* **3**, 207–217 (1999).
64. Aparicio, O. M., Billington, B. L. & Gottschling, D. E. Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. *Cell* **66**, 1279–1287 (1991).
65. Chien, C. T., Buck, S., Sternglanz, R. & Shore, D. Targeting of SIR1 protein establishes transcriptional silencing at HM loci and telomeres in yeast. *Cell* **75**, 531–541 (1993).
66. Gotta, M. & Gasser, S. M. Nuclear organization and transcriptional silencing in yeast. *Experientia* **52**, 1136–1147 (1996).
67. Laroche, T. *et al.* Mutation of yeast *Ku* genes disrupts the subnuclear organization of telomeres. *Curr. Biol.* **8**, 653–656 (1998).
68. Maillet, L. *et al.* Evidence for silencing compartments within the yeast nucleus: A role for telomere proximity and Sir protein concentration in silencer-mediated repression. *Genes Dev.* **10**, 1796–1811 (1996).
69. Andrulis, E. D., Neiman, A. M., Zappulla, D. C. & Sternglanz, R. Perinuclear localization of chromatin facilitates transcriptional silencing. *Nature* **394**, 592–595 (1998).
70. Francastel, C., Walters, M. C., Groudine, M. & Martin, D. I. A functional enhancer suppresses silencing of a transgene and prevents its localization close to centromeric heterochromatin. *Cell* **99**, 259–269 (1999).
- Stable transgene expression requires positioning away from the heterochromatin compartment and the binding of transcription factors to the enhancer.**
71. Bulger, M. & Groudine, M. Looping versus linking: Toward a model for long-distance gene activation. *Genes Dev.* **13**, 2465–2477 (1999).
72. Lustig, A. J. Mechanisms of silencing in *Saccharomyces cerevisiae*. *Curr. Opin. Genet. Dev.* **8**, 233–239 (1998).
73. Moehrl, A. & Paro, R. Spreading the silence: epigenetic transcriptional regulation during *Drosophila* development. *Dev. Genet.* **15**, 478–484 (1994).
74. Loo, S. & Rine, J. Silencing and heritable domains of gene expression. *Annu. Rev. Cell Dev. Biol.* **11**, 519–548 (1995).
75. Aparicio, O. M. & Gottschling, D. E. Overcoming telomeric silencing: a trans-activator competes to establish gene expression in a cell cycle-dependent way. *Genes Dev.* **8**, 1133–1146 (1994).
76. Festenstein, R. *et al.* Locus control region function and heterochromatin-induced position effect variegation. *Science* **271**, 1123–1125 (1996).
77. Walters, M. C. *et al.* Transcriptional enhancers act in *cls* to suppress position-effect variegation. *Genes Dev.* **10**, 185–195 (1996).
78. Linares-Cruz, G. *et al.* p21WAF-1 reorganizes the nucleus in tumor suppression. *Proc. Natl Acad. Sci. USA* **95**, 1131–1135 (1998).
- Global disorganization of nuclear architecture can be associated with cancer, but is reversible during tumour suppression.**
79. Qumsiyeh, M. B. Impact of rearrangements on function and position of chromosomes in the interphase nucleus and on human genetic disorders. *Chromosome Res.* **3**, 455–465 (1995).
80. Epner, E. *et al.* The β -globin LCR is not necessary for an open chromatin structure or developmentally regulated transcription of the native mouse β -globin locus. *Mol. Cell* **2**, 447–455 (1998).
81. Reik, A. *et al.* The locus control region is necessary for gene expression in the human β -globin locus but not the maintenance of an open chromatin structure in erythroid cells. *Mol. Cell Biol.* **18**, 5992–6000 (1998).
82. Rozman, C., Woessner, S., Fellu, E., Lafuente, R. & Berga, L. *Cell Ultrastructure for Hematologists* (ed. Ediciones Doyma, S. A.) (Barcelona, Spain, 1993).
83. Alberts, B. *et al.* *Molecular Biology of the Cell* 3rd edn (Garland, New York, 1994).

Acknowledgements

The authors thank Matthew Lorincz and Bas van Steensel for their useful comments on this manuscript. This work was supported by a special fellowship to C.F. from the Leukemia and Lymphoma Society, a fellowship from the Deutsche Forschungsgemeinschaft to D.S., and NIH grants to M.G.