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Gene regulation and large-scale chromatin organization in the nucleus

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Abstract

Regulation of gene expression involves a number of different levels of organization in the cell nucleus. The main agents of transcriptional control are the *cis*-acting sequences in the immediate vicinity of a gene, which combine to form the functional unit or domain. Contacts between these sequences through the formation of chromatin loops forms the most basic level of organization. The activity of functional domains is also influenced by higher order chromatin structures that impede or permit access of factors to the genes. Epigenetic modifications can maintain and propagate these active or repressive chromatin structures across large genomic regions or even entire chromosomes. There is also evidence that transcription is organized into structures called 'factories' and that this can lead to inter-chromosomal contacts between genes that have the potential to influence their regulation.

Eukaryotic transcription takes place within the micro-environment of the cell nucleus. Nuclear organization has the potential to profoundly affect gene regulation by varying the distribution of genes, transcription factors and RNA polymerases. However, the nucleus does not contain membrane bound sub-compartments equivalent to the endoplasmic reticulum and the Golgi apparatus in the cytoplasm. This means that differences in the concentrations of nuclear components are mainly due to interactions between these components that have the effect of creating localized substructures or organelles. Experimental analysis has shown that there is a potential for rapid exchange of macromolecules between these structures. Understanding the role played by this type of organization in regulating gene expression presents a huge challenge and requires that particular care be taken to distinguish between genuine regulation and secondary epiphenomena. Here I review the different levels of transcriptional regulation in the nucleus and examine current thinking on how this regulation might be affected by nuclear structure.

Regulating transcription: from genes to chromatin domains

The multiple parameters that affect transcription in the nucleus can be arranged into a series of different levels. At the most basic level, there is regulation of the gene through the action of promoters and more distal regulatory sequences which are usually classified under the operational definition of enhancer. Assembly of the pre-initiation complex at the promoter is influenced by binding of sequence-specific transcription factors to promoter-proximal sequences and to distally located enhancer sequences. There is now a considerable body of evidence that the activating effect of enhancers is the result of direct contacts with promoters through looping out of the

intervening sequences (Figure 1A) (Dillon *et al*. 1997, Carter *et al*. 2002, Tolhuis *et al*. 2002). The general transcription factors (GTFs) that form the pre-initiation complex are recruited to promoters by interaction with these sequence-specific factors. There is some recent evidence that suggests that GTFs are also recruited to enhancers before transcription is activated. This suggests a model where binding of sequence-specific factors to distal elements (enhancers) recruits GTFs, which are then transferred to the promoter by direct contact and looping out of the intervening sequence (Szutorisz *et al*. 2005b). Formation of functional contacts between regulatory elements through local DNA looping introduces a specific level of higher order structure that is directly involved in regulating gene expression (Figure 1A). The size and nature of these loops would be expected to vary widely between organisms; for example, yeast genes are located close together and loops are likely to be a maximum of only a few kb whereas in mammals, the much larger genes and more distant regulatory sequences will form correspondingly larger loops that may extend over several hundred kb and will have quite different physical characteristics. *Drosophila melanogaster* lies somewhere between these two extremes. This highlights the need to be cautious when extrapolating insights about large-scale nuclear organization and gene regulation from model systems that have very different characteristics from mammalian nuclei.

The next level of functional organization is the gene expression domain. Analysis of a number of mammalian gene loci in transgenic mice has shown that inclusion of a sufficiently large amount of sequence flanking a gene or gene cluster gives copy-dependent expression that is largely independent of the position of integration of the transgene in the genome (Grosveld *et al*. 1987, Greaves *et al*. 1989, Sabbattini *et al*. 1999). Surprisingly, some of these functional domains seem to be able to drive efficient transcription even when they are integrated into condensed pericentromeric heterochromatin with variegation only observed when the gene was partially disabled by removing some activating sequences (Festenstein *et al*. 1996, Milot *et al*. 1996). The finding that cis-acting sequences are dominant over location fits well with recent observations showing that factors (including the heterochromatin binding protein, HP1) are highly mobile in the mammalian nucleus (McNally *et al*. 2000, Cheutin *et al*. 2003, Festenstein *et al*. 2003).

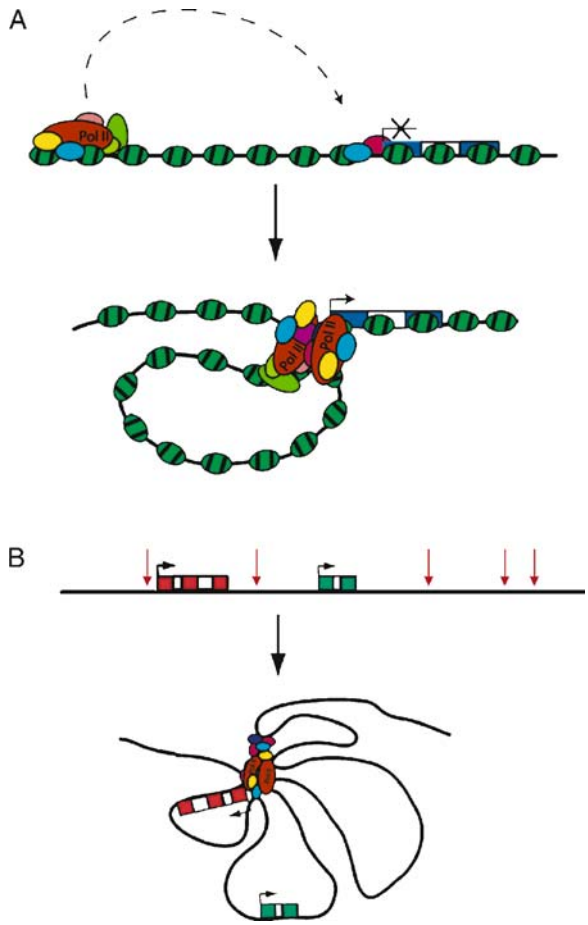

Figure 1. Organization of transcriptionally active chromatin. (**A**) Looping out of the DNA between enhancers and promoters brings these regions into contact with one another. The contacts, which are stabilized by specific interactions between transcription factors (coloured circles) bound to both elements, is thought to be critical for the activating effects of enhancers. (**B**) The Active Chromatin Hub (ACH) model (Tolhuis *et al*. 2002). The model proposes that clustered factor binding sites at DNase I hypersensitive sites (indicated by vertical arrows) form specific contacts through the formation of multiple loops. The result is a large structure or 'hub' which brings the activating elements in the locus together and effectively isolates the domain from contact with other sequences either *in cis* or *in trans*.

Further support for this conclusion has come from the finding that a λ5 transgene can be transcribed even when it is in direct contact with heterochromatin (Lundgren *et al*. 2000). These results place an important constraint on models that argue that nuclear location plays a significant part in regulating transcription and they provide evidence that the primary

determinants of expression patterns are the cis-acting sequences in the immediate vicinity of the genes.

The structure of functional gene expression domains continues to be a major area of research that impacts on a wide range of biological questions ranging from the minutiae of gene regulation mechanisms to the nature of the gene itself (Dillon 2003). There are two different kinds of model that have been put forward to explain domain organization. One model, which has gained wide currency, proposes that gene expression domains coincide with chromatin loops isolated by insulators that are anchored to nuclear attachment points (Burgess-Beusse *et al.* 2002, Labrador & Corces 2002). A major problem with this model is the fact that, to date, it has not been possible to demonstrate a role for insulators in isolating functional domains in metazoan organisms by deleting an insulator *in situ* and demonstrating an effect on gene expression. As an alternative to the 'strong' domain boundary model, we have proposed a 'weak' domain model that explains insulator distribution in terms of differential selection – enhancer blocking activity is known to be a secondary property of many enhancers and promoters and sequences that have this property will be selected against if they are located within the domain but not if they are located on the outside (Dillon & Sabbattini 2000). This means that insulators are more likely to be found at the edges of domains without necessarily being selected to act as domain boundaries. In some instances, these insulators might be recruited to block interactions between neighbouring genes but there will also be situations where the borders between domains will be 'fuzzy' and not defined by a discrete boundary (Fourel *et al.* 2004).

Functional dissection of mammalian gene expression domains has revealed that they contain multiple elements that combine to activate transcription. These elements frequently coincide with DNase I hypersensitive sites (HS) that are created by binding of specific combinations of tissue-specific and ubiquitous transcription factors to clustered binding sites (Philipsen *et al.* 1993, Boyes & Felsenfeld 1996). In the domains that have been characterized in detail, groups of 3–5 HS have often been found located outside, but immediately adjacent to, the region containing the genes. In the human *β-globin* and *CD2* and mouse *λ5-VpreB1* loci, these locus control regions (LCRs) play an essential role in activating expression (Grosveld *et al.* 1987, Greaves *et al.* 1989, Sabbattini *et al.* 1999), but there is evidence that they act in conjunction with DNase I HS that are distributed across the rest of the domain (Epner *et al.* 1998, Sabbattini *et al.* 1999). Thus, the functional unit can be considered to be a complex information processor that is able to 'read' the transcription factor profile of the cell and activate transcription in a manner that is dominant over negative chromatin effects and genomic position. This dominance is hierarchical in nature, with some factors gaining access to specific sequences and forming a platform for the binding of other transcription factors and general transcription complexes or for recruitment of enzyme complexes that remodel chromatin. Sequence-specific factors also have the capacity to recruit histone modifying enzymes that can mark entire domains with activating modifications such as histone H3 lysine 4 dimethylation (Anguita *et al.* 2001, Litt *et al.* 2001, Szutorisz *et al.* 2005a). Recent studies have also suggested that a number of DNase I HS in a domain can come together to form a complex structure containing multiple loops, which has been termed an Active Chromatin Hub (Figure 1B) (Tolhuis *et al.* 2002).

Chromosomes and chromosome territories

In eukaryotes, the unit of higher order gene organization is the chromosome. Each chromosome contains multiple genes arranged in a linear map, together with the centromere and the telomeres. In most eukaryotes (the budding yeast, *Saccharomyces cerevisiae*, is an exception), the centromeres are embedded in regions of chromatin that remain condensed throughout the cell cycle. Sequence analysis of these regions, which are termed pericentromeric heterochromatin, has shown that they are made up of satellite repeat sequences that vary widely in length between different organisms, and large numbers of transposable elements (Spradling 1994). Although they are gene-poor and depleted in histone modifications that are associated with transcription, a number of functional genes have been mapped to pericentromeric heterochromatin in *Drosophila* (Weiler & Wakimoto 1995, Elgin 1996). This indicates a need for caution in assuming that heterochromatin is inert and refractory to transcription. Telomeres also contain repeats and have been found to be organized into heterochromatin in some species (Gottschling *et al.*

1990). Blocks of heterochromatin are found away from the centromeres on some chromosomes – for example *Drosophila* chromosome 4 (Haynes *et al.* 2004).

The majority of genes are located in regions of euchromatin along the chromosomal arms and there has been considerable interest in the possibility that the regulation of these genes might be affected by their distribution relative to one another. Whole genome sequencing has allowed this issue to be addressed directly and evidence has been obtained that genes with similar patterns of expression show some degree of clustering (Caron *et al.* 2001, Spellman & Rubin 2002, Vogel *et al.* 2005). Comparison of human and mouse sequences has shown that the frequency of rearrangement resulting from chromosomal breaks within clusters of housekeeping genes is lower than would be expected from random events (Singer *et al.* 2005). There is also evidence of interspecies conservation of sequences in so-called gene deserts – large regions of DNA that lack genes or known regulatory sequences (Ovcharenko *et al.* 2005).

These observations have generated considerable enthusiasm for the idea that co-regulation of clustered genes by changes in chromatin structure across large genomic regions might be a general phenomenon in higher eukaryotes. However, translating the results of this type of statistical analysis into firm conclusions about mechanisms of gene regulation presents some problems. There are several possible explanations for co-expression of clustered genes. For example it has been suggested that a strongly expressed gene could cause ectopic expression of neighbouring genes, which would not be selected against if it had no harmful effects (Spellman & Rubin 2002). An example of this has been identified in the human growth hormone locus where the *Igβ* (*CD79*) gene is located between the growth hormone gene and its LCR. *Igβ* is expressed in B cells where it is involved in B cell receptor signalling, but high levels of expression have also been observed in the pituitary gland where it has no apparent function (Cajiao *et al.* 2004). Analysis of individual gene loci has also cast doubt on the existence of this type of large-scale organization. Genes with quite different patterns of expression are often closely juxtaposed. Examples include the mouse *λ5-VpreB1* and chicken *lysozyme* loci, both of which have widely expressed genes closely juxtaposed with genes that are highly tissue-specific (Chong *et al.* 2002, Minaee *et al.* 2005). In the *λ5-VpreB1* locus, the ubiquitously expressed *Topoisomerase-3β* gene and the pre-B cell specific *VpreB1* gene are divergently transcribed and have their start sites located only 1.5 kb apart (Minaee *et al.* 2005). The erythroid specific human *β-globin* locus is embedded in a region containing multiple olfactory receptor genes and neither gene locus appears to have acquired the expression pattern of the other (Bulger *et al.* 1999). These examples provide further support for a model where the primary determinant of patterns of gene expression is the binding of sequence-specific factors to cis-acting sequences in the neighbourhood of a gene, with different combinations of factors generating different patterns of expression. The roles that have been proposed for gene clustering also raise fundamental issues about how we define function with respect to gene regulation. At present, there is insufficient information about how natural selection acts on the mosaic of regulatory sequences that control expression of gene loci to draw broad conclusions about the functional effects of large-scale chromosomal organization on gene regulation. The next step for research in this area will be to extend the type of functional and epigenetic analysis that has been carried out on multi-gene loci to regions of several megabases containing large numbers of genes with different cell-type specificities.

In interphase nuclei, chromosomes are organized into discrete territories that are relatively immobile and occupy positions in 3-dimensional space that are conserved across species. Given that transcription factors have been shown to be highly mobile in the nucleus, it is possible that the DNA polymer is among the more static architectural features of the nucleus and plays a major role in maintaining nuclear structure through interaction with specific anchoring points, such as those that have been identified at the nuclear envelope (reviewed by Hetzer *et al.* 2005). This would require a modification of the commonly held perception that DNA is held in position by a fixed 'nuclear matrix'. In fact, the properties of DNA make it in many ways more suitable to act as a nuclear organizer than a protein-based matrix. This type of model does not however preclude a substantial amount of local movement of the DNA fibre in response to changes in folding induced by interaction with nuclear proteins and there is a considerable body of evidence that this happens (Marshall *et al.* 1997, Heun *et al.* 2001).

Higher order chromatin folding as a mechanism for regulating transcription

Co-ordinated folding and unfolding of higher order chromatin structures has the potential to regulate expression across large regions of DNA by impeding or permitting access of transcription factors and RNA polymerases to the DNA. The nature of this higher order folding is still relatively poorly understood. It has been shown that the condensed heterochromatin around the centromeres is organized into a regular nucleosome array with a reduced linker length and an increase of 10 bp in the size of the region protected from micrococcal nuclease digestion (Wallrath & Elgin 1995). The next level folding above that of the nucleosome was until recently considered to be the helical 30 nm fibre. However, electron microscopy of chromatin organization *in vivo* has provided evidence for an alternative 'two-start ribbon' conformation which leads to a zigzag higher order structure (Woodcock *et al*. 1984, Woodcock & Dimitrov 2001) and this has recently received further support from crystallographic analysis (Dorigo *et al*. 2004, Schalch *et al*. 2005). Further levels of folding are thought to lead to the formation of the visible constitutive heterochromatin that is present during interphase and the facultative heterochromatin that forms during terminal differentiation of many cell types (Belmont *et al*. 1999).

For disruption of higher order chromatin folding to act as a specific regulatory event, it is necessary for it to take place in a sequence-specific manner. This implies that the primary actors that initiate chromatin disruption must be sequence-specific transcription factors and there is evidence that this is the case. A DNase I hypersensitive site containing clustered factor binding sites was shown to be capable of disrupting the higher order structure of a λ5 transgene that was integrated into pericentromeric heterochromatin in mice and relocating the transgene to the outside of the heterochromatin complex (Lundgren *et al*. 2000). The frequency of relocation was found to be affected by the level of a transcription factor that is known to be involved in activating λ5 expression. It has also been shown that targeting acidic activators such as VP16 to an artificially created lac operator heterochromatin array results in visible large-scale chromatin decondensation of the heterochromatin through a mechanism that appears to be independent of transcription (Carpenter *et al*. 2004). However, *in vivo* analysis of chromatin compaction in *Saccharomyces cerevisiae* has suggested that decondensation of transcribing genes is a relatively transient event with the chromatin condensing immediately after transcription ceases (Bystricky *et al*. 2004). This may reflect variation in the chromatin characteristics of different organisms.

Epigenetic modifications and large scale chromatin organization

Epigenetic events such as modifications of the core histones and DNA methylation are critical for maintaining active or silent transcriptional states. Histone modifications can directly disrupt the structure of the chromatin fibre (Krajewski & Becker 1998) and are also recognized by transcription factors such as the bromodomain proteins, which bind to different acetylated residues of histone H3 (Yang 2004). Since histone modifications are non-specific, they have to be targeted to specific sequences by DNA binding factors. An additional mechanism that has been identified recently involves the production of short (20–25 bp) double stranded (ds) RNAs by transcription of a target sequence. These dsRNAs form a complex called the RITS complex which binds to the target DNA by virtue of the sequence homology with the dsRNA and recruits histone methyltransferases (HMTases) that are involved in generating silencing modifications (Grewal & Rice 2004). Non-coding micro-RNAs produced by transcription of micro-RNA genes may also be involved in targeting epigenetic modifications to specific regions.

Epigenetic modifications have the potential to activate or silence large regions of chromatin. Dosage compensation in *Drosophila* results in a two fold-increase in transcription of genes across the entire male X chromosome. This is due to targeting of histone H4 lysine 16 acetylation to the X chromosome by the MSL complex which contains proteins and RNA. Marking of the chromosome is accomplished by spreading of H4 acetylation, which is probably initiated from a limited number of MSL binding sites (Kelley 2004). In female mammalian cells, inactivation of one of the two X chromosomes provides another example of large-scale epigenetic silencing. The initiating event and distinctive feature of X inactivation is the coating of the inactive chromosome with a non-coding RNA, the Xist RNA,

which is produced in cis. Xist initates activation of the X chromosome by an unknown mechanism, which may involve histone deacetylation (reviewed by Heard 2004). Maintenance of silencing involves a transient recruitment of the Polycomb group proteins Eed and Enx1, which leads to methylation of histone H3 lysine 9 and 27 and long-term silencing (Silva *et al*. 2003).

Pericentromeric heterochromatin is also formed as a result of epigenetic marking that leads to condensation of megabase regions of chromatin (reviewed by Peters & Schubeler 2005). In mammals, the histone methyltransferase Suv 39h is recruited to the pericentromeric satellite repeats through a mechanism that is likely to involve production of dsRNA and the RNAi pathway. Suv 39h mediates histone H3 lysine 9 trimethylation and H3 lysine 27 monomethylation (Peters *et al*. 2003). The condensed structure of heterochromatin is stabilized by binding of the chromodomain protein HP1 which interacts with H3 methyl-lysine 9.

Spatial organization of transcription in the nucleus

The complexity of the transcriptional machinery means that it has significant potential for organizing the local environment around the transcribed gene. We have already seen that the formation of large loops when regulatory sequences come into contact can affect large-scale chromatin organization. The complexes that form between transcription factors and RNA polymerases that form at transcribed genes can also create structures that influence the overall structure of the nucleus.

The nucleolus is the best characterized example of this type of organization. The ribosomal RNA (rRNA) genes in humans are located in clusters of approximately 40 genes arranged as tandem arrays at five different chromosomal locations. These clustered genes are transcribed at very high levels by RNA polymerase I to produce the structural RNAs that are found in the ribosome. The transcribing rRNA genes come together to form the nucleolus in G_0 when rRNA transcription is activated following exit from mitosis. A detailed description of the structure of the nucleolus is beyond the scope of this review, but as well as being effectively a transcription factory for rRNA, it acts as a centre for rRNA processing and ribosome assembly (for reviews of this topic see Grummt 2003, Raska *et al*. 2004). In addition to these functions, there is evidence that the nucleolus acts a stress-sensor for the cell. Stress conditions that inhibit RNA Pol I lead to perturbation of nucleolar structure and release of ribosomal proteins into the nucleoplasm. These proteins associate with and block the activity of MDM2 ubiquitin ligase, which is normally responsible for ubiquitination of p53. As a result, p53 is stabilized, triggering cell cycle arrest and apoptosis (Mayer & Grummt 2005). The fact that the nucleolus has acquired these diverse roles emphasizes that structures of this type may have been selected to perform a number of different functions rather than having transcriptional regulation as their sole, or even their main function.

Transcription by RNA Pol II is not associated with the large-scale organization that is observed for Pol I transcription in the nucleolus, but there is a growing body of evidence that Pol II is organized into smaller structures termed transcription factories (reviewed by Martin & Pombo 2003). The evidence for Pol II transcription factories is based on observations that show that there are approximately eight times fewer sites of transcription in the nucleus than there are active RNA Pol II molecules. This suggests that there are around eight Pol II molecules per factory. Visualizing such small structures is difficult and there is still little information about the degree of organization of the Pol II molecules within factories. Presumably these factories must also be enriched for the general transcription factors that form the preinitiation and elongation complexes. A study that used a GFP-tagged large subunit of Pol II has provided evidence that a substantial fraction (~75%) of Pol II exchanges rapidly (association $t_{1/2}$: ~15 seconds) while the remainder is transiently immobile ($t_{1/2}$: ~20 minutes) (Kimura *et al*. 2002). This result (which needs to be confirmed by other methods) could be explained if the transcribing polymerase is constrained while the Pol II in the pre-initiation complex is in a state of dynamic exchange between factories.

Transcription factories have the potential to bring genes that are co-expressed into direct contact with one another, and two recent studies have suggested that tissue-specific genes that are activated in the same cell-type co-localize in the nucleus at a higher frequency than would be expected through random association (Osborne *et al*. 2004, Spilianakis *et al*. 2005). Osborne *et al*. used fluorescence *in situ*

hybridization to show that the erythroid-specific *α*- and *β-globin* and *eraf* loci were significantly closer together in erythroid cells compared with non-erythroid tissues. Chromosome conformation capture (3C) analysis was also used to measure associations. The technique, which uses formaldehyde crosslinking followed by ligation and PCR analysis of ligated products, showed evidence of an association between the *β-globin* and *eraf* loci in erythroid cells but not in brain. Spillianakis *et al*. used similar approaches to demonstrate an interaction between the T_H2 cytokine gene locus on mouse chromosome 11 and the interferon-γ (*Ifng*) gene on chromosome 10. The T_H2 locus contains the cytokine genes *Il4*, *ll5* and *Il13*, which are expressed following differentiation of naïve T cells into T_H2 cells, and the ubiquitously expressed *RAD50* gene which is involved in DNA repair. The cytokine genes in the TH2 locus are activated by an LCR, part of which is located in the 3′ region of the RAD50 gene. A strong interaction between the T_H2 and *Ifng* loci was detected by 3C analysis in naïve T cells and this interaction became much weaker when the cells were differentiated into T_H2 cells. The decrease was accompanied by an increase in interactions within the *Ifng* locus. The interaction between the T_H2 and *Ifng* locus was dependent on the presence of a DNase I hypersensitive site in the T_H2 LCR and deletion of this HS resulted in delayed activation of *Ifng* expression during differentiation. This provides the first direct evidence that this type of interaction has the potential to regulate gene expression. However, there are many questions that still have to be addressed before this can become a general paradigm. Nothing is known about the mechanisms that would allow such long-range interactions to affect expression, although it can be presumed that specificity is provided by interactions between factors bound to the interacting genes (Figure 2A). Another problem comes from the observations from genomic sequencing that cell-type specific genes are often located in gene dense regions that contain a number of genes with different cell-type specificities that frequently overlap with one another. An interaction between one of these genes and a gene on another chromosome will bring other closely linked genes along with it (Figure 2B). This suggests that gene regulation based on this type of long-range interaction is likely to be the product of rare and contingent evolutionary events that occurred under specific favourable circumstances and is unlikely to be a widespread phenomenon. It is notable in this regard that the mouse *Ifng* gene is located in a gene-poor region with the nearest adjacent genes located more that 1 Mb away, so effects on other genes would be minimized in this specific instance.

How does nuclear position affect transcription?

A good deal of effort has been expended on attempts to correlate position of genes within chromosome territories with their transcriptional status. The interchromosomal domain (ICD) model proposed that chromosomes are separated by spaces or lacunae that are enriched for RNA polymerases and transcription factors (Verschure *et al*. 1999). Actively transcribed genes would be located at the surface of the territory or would loop out into the ICD. Conversely, inactive genes would be located within the territory and this would make them inaccessible to factors and polymerases. The clearest example of a correlation between expression and position with respect to a chromosome territory comes from a study on the mouse *Hoxb* cluster (Chambeyron & Bickmore 2004). Expression of the genes during differentiation of ES cells into neural cells is accompanied by

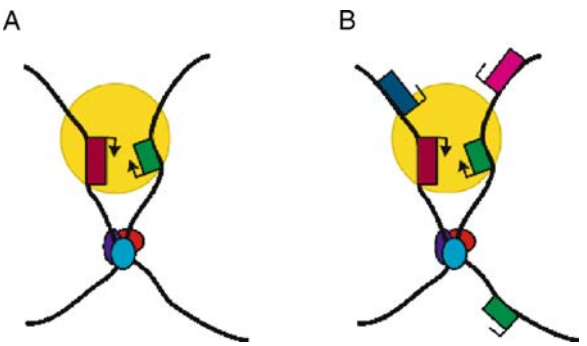

Figure 2. Interchromosomal contacts between genes on different chromosomes. Evidence from two recent studies (Osborne *et al*. 2004; Spilianakis *et al*. 2005) suggests that genes on different chromosomes can contact one other and that these contacts can affect regulation. Contacts are likely to involve specific interactions between transcription factors (coloured circles) bound to enhancer elements in each locus but they may also be a consequence of two loci being recruited to the same transcription factory (large yellow circles). The effects of such contacts could be similar to *in cis* effects of enhancers when the interacting loci are relatively isolated (**A**). They are likely to be more complex when the interacting genes are located in gene-dense regions with the result that other genes with different patterns of expression are pulled into the same region of the nucleus as bystanders (**B**).

extrusion of the locus from the territory with the genes that are expressed first being the first to relocate out of the territory. As with all correlative studies of this type, this result does not distinguish between cause and effect so it is unclear whether transcription is driving localization of the *Hoxb* genes or vice versa. We do know that this type of relocation is not universal since the human *WAGR* region, which contains the *ET1*, *RCN*, *PAX6* and *PAXNEB* genes, has been shown to be located deep within the chromosome 11 territory even when the genes are transcribed (Mahy *et al*. 2002). An alternative chromatin subdomain model proposes that the chromosome territory is subdivided into subdomains of condensed chromatin with active genes located on the surface of these condensed chromatin domains (reviewed by Williams 2003). Such a model has some similarities to models derived from chromatin structure studies that propose that local chromatin condensation plays a major role in regulating gene expression. Further problems with the ICD model have been raised by a recent study that shows that chromosomes intermingle extensively, raising doubts about the existence of the ICD (M. Branco and A. Pombo, personal communication).

Location of genes to the nuclear periphery and to pericentromeric heterochromatin has also been correlated with silencing. The suggestion that peripheral location plays a causal role in silencing was initially supported by a study that showed that tethering a partially disabled mating type silencer to the nuclear membrane enhanced silencing in *S. cerevisiae* (Andrulis *et al*. 1998). However, another study in *S. cerevisiae* has used immunoprecipitation and microarray analysis to show that components of the nuclear pore complex are associated with active genes (Casolari *et al*. 2004). The hypothesized interaction of transcribed genes with nuclear pores needs to be confirmed by other methods but the results suggest a complex relationship between nuclear location and expression status and raise the possibility that nuclear location may be a visible manifestation of a network of specific contacts between genes, transcription factors and different nuclear structures.

Conclusions

That nuclear organization can influence gene expression and regulation is no longer in any doubt. However, it is also becoming clear that it will be necessary to modify many of the theories that were based on the existence of discrete nuclear compartments that could sequester genes and factors. Instead, a picture is emerging of a highly dynamic nucleus in which factors exchange rapidly between different regions. In this type of environment, specific interactions between nuclear components are likely to be what drives the processes that give rise to visible higher order organization. Understanding the nature of these interactions and the functional roles that they play in regulating nuclear processes will be the major challenge for future studies in this area.

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