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Review

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Beyond the Sequence: Cellular Organization of Genome Function

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Genomes are more than linear sequences. In vivo they exist as elaborate physical structures, and their functional properties are strongly determined by their cellular organization. I discuss here the functional relevance of spatial and temporal genome organization at three hierarchical levels: the organization of nuclear processes, the higher-order organization of the chromatin fiber, and the spatial arrangement of genomes within the cell nucleus. Recent insights into the cell biology of genomes have overturned long-held dogmas and have led to new models for many essential cellular processes, including gene expression and genome stability.

Introduction

We usually think of genomes abstractly as one-dimensional entities that are purely defined by their linear DNA sequences. Reality, of course, is far more complex. The DNA helix is folded hierarchically into several layers of higher-order structures that eventually form a chromosome (Woodcock, 2006). In this way, DNA is compacted and can be accommodated in the limiting space of the cell nucleus. The spatial arrangement of the chromatin fiber and the genome as a whole dramatically affects the function of DNA, and knowing the sequence of a genome is insufficient to understand its physiological function.

In addition to the complex arrangement of the genetic information itself, the cellular factors that read, copy, and maintain the genome are organized in sophisticated patterns within the cell nucleus (Lamond and Spector, 2003; Misteli, 2005). Many transcription factors, chromatin proteins, and RNA-processing factors are compartmentalized and accumulate in distinct nuclear domains; specific nuclear processes such as transcription and replication occur at spatially defined locations in the nucleus. The organizational properties of genomes and the machineries that act on them create an elaborate architectural environment in which genomes must function. How they do so is one of the great challenges in modern cell biology.

Uncovering the cell biology of genomes is fundamental. Although comparative genome analysis and large-scale mapping of genome features have yielded insights into the physiological role of genetic information, these efforts shed little light onto the Holy Grail of genome biology, namely the question of how genomes actually work in vivo. The elucidation of the cellular organization of genomes and its impact on genome regulation is a logical next step after the completion of sequencing projects. Understanding genome function within its architectural framework is also highly relevant for biotechnological applications that range from stem cell differentiation to somatic cloning and gene therapy as all of these processes involve massive reorganization of nuclear architecture. Knowledge of the functional interplay between genome organization and activity will significantly contribute to making these applications more efficient and controllable.

Cellular organization of genome function occurs at three hierarchical levels: the spatial and temporal organization of nuclear processes themselves, including transcription, RNA processing, DNA replication, and DNA repair; the organization of chromatin into higher-order domains; and the spatial arrangement of chromosomes and genes within the nuclear space. Each one of these levels has regulatory potential, and all are interdependent. Several simple questions serve as guideposts to unravel the complex structure-function interplay of the genome in the cell: How are genome processes and genomes organized in 3D space? What are the fundamental principles of organization? What are the molecular mechanisms that give rise to the organization patterns? What are the physiological consequences of spatial genome organization? Emerging answers to these questions are now leading to unprecedented insights into genome biology and to new, unexpected models of genome function.

Cellular Organization of Nuclear Processes

A hallmark of many nuclear processes is their spatial compartmentalization. Most nuclear events do not occur ubiquitously throughout the nucleus but are limited to specific, spatially defined sites that often occur in dedicated nuclear bodies (Lamond and Spector, 2003; Misteli, 2005). Remarkably, common mechanisms appear to organize some of the vastly different, fundamental nuclear processes.

The Organization of Transcription

The most fundamental of all genome functions is transcription. Surprisingly, there is still much uncertainty as to how transcription is organized within the nucleus (Cook, 1999; Chakalova et al., 2005). Visualization of

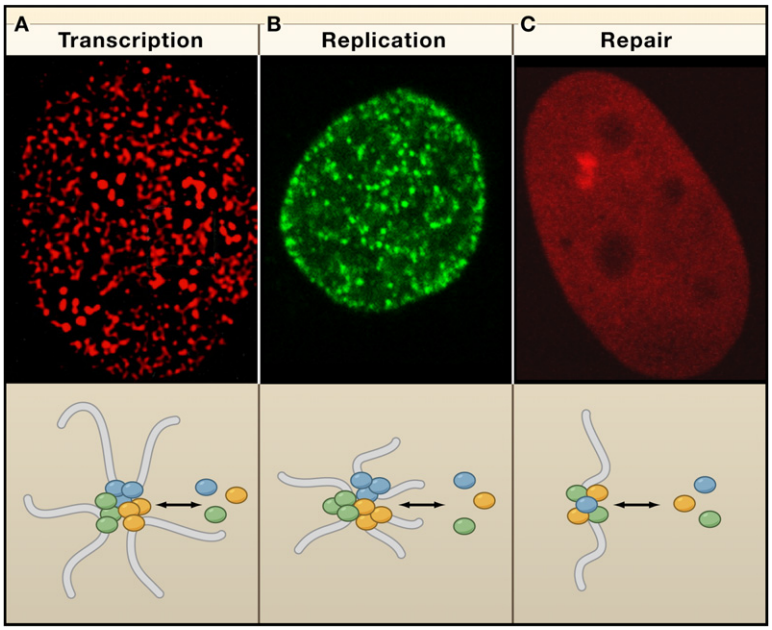

Figure 1. Compartmentalization of Nuclear Processes

Transcription, replication, and DNA repair are compartmentalized. (A) Transcription sites visualized by incorporation of bromo-UTP, (B) replication sites visualized by incorporation of bromo-dUTP, and (C) repair sites visualized by accumulation of repair factor 53BP1 at a double-strand break (DSB) are shown. In all cases, components are dynamically recruited from the nucleoplasm as single subunits or small preassembled subcomplexes. (A) is reprinted with permission from Elbi et al., 2002, (B) is courtesy of Rong Wu and David Gilbert at Florida State University, and (C) is courtesy of Evi Soutoglou from the National Cancer Institute, NIH.

transcription sites reveals the presence of several thousand distinct sites that appear to be randomly dispersed throughout the nuclear volume (Wansink et al., 1993; Figure 1A). Influenced largely by in vitro analysis of the transcription machinery, it was long assumed that this distribution represents RNA polymerases (RNA pol) elongating along genes. But an alternative and increasingly plausible view is that these sites correspond to subnuclear transcription centers (Cook, 1999; Chakalova et al., 2005; Figure 1A). As originally proposed by Cook, these "transcription factories" are transcription hot spots that harbor enough transcription factors and polymerases to serve multiple genes (Cook, 1999). The organization of transcription in centralized structures that contain multiple transcription machineries is consistent with the presence of an estimated 65,000 active RNA pol II molecules but fewer than 10,000 transcription sites in a HeLa cell. Considering that most active RNA pol II genes only contain one active polymerase at any time, transcription factories would contain between 6 and 8 actively elongating polymerases and would probably transcribe multiple genes at a time (Cook, 1999). The organization of RNA pol II transcription into distinct sites is not unprecedented, as it is analogous to the well-established clustering and compartmentalization of ribosomal RNA genes, which are transcribed by RNA pol I within the nucleolus in large, specialized transcription centers (Raska et al., 2006).

The compartmentalization of transcription has the obvious advantage of concentrating the required factors to ensure efficient interactions amongst components of the transcription machinery (Cook, 1999; Chakalova et al., 2005). An attractive possibility is that different transcription factories contain distinct sets of transcription components and thus create distinct transcriptional environments. Regulation of multiple genes may then be coordinated by their association with shared transcription factories of particular composition. At present, however, this idea is largely hypothetical as little evidence exists for differential composition amongst transcription sites.

The Dynamic Nature of Transcription Complexes

Despite the organization of transcription into structural compartments, biochemical-analysis and in vivo-imaging approaches have recently revealed that the transcription machinery is surprisingly dynamic and significantly determined by stochastic events (Misteli, 2005). These properties are based on the highly transient interaction of proteins with chromatin. In vivo analysis of many transcription factors and chromatin proteins suggests that most of them undergo rapid cycles of binding and unbinding on chromatin, with dwell times on the order of only a few seconds (Phair et al., 2004). Upon unbinding, proteins are able to freely diffuse through the nuclear space, which allows them to scan the genome for specific binding sites by using a hit-and-run mechanism (Misteli, 2001b; Hager et al., 2002).

Direct evidence for the highly dynamic nature of transcription complexes comes from observing transcription factors on their specific target genes in living cells. The glucocorticoid- and estrogen-receptor transcriptional coactivators bind to their specific response elements in the promoter regions of target genes with residence times on the order of only a few seconds (McNally et al., 2000; Stenoien et al., 2001), and several of their interaction partners bind equally transiently to the promoter (Becker et al., 2002). In addition, binding of NFκB on its cognate sites is highly transient (Bosisio et al., 2006). These findings on artificial promoter arrays are strongly corroborated by analysis of the assembly dynamics of RNA pol I subunits on endogenous ribosomal RNA genes (Dundr et al., 2002).

All RNA pol I subunits undergo rapid exchange at the promoter and stably associate with chromatin only when they are incorporated into an elongation complex. Assembly of the polymerase appears to occur in a stepwise process by largely stochastic collisions of subunits with the polymerase machinery at the promoter (Dundr et al., 2002). Further evidence for dynamic subunit assembly of transcription complexes comes from observation in *Drosophila*, where heat shock factor (HSF) becomes rapidly recruited and immobilized on its target genes upon heat shock, and HSF dynamics appear to differ from the polymerase proper (Yao et al., 2006). These observations challenge the traditional view of the holoenzyme being recruited to a gene in a single step, although they do not rule out that assembly occurs, at least in part, from preassembled subcomplexes (Schneider and Nomura, 2004).

Organization of DNA-Replication and -Repair Sites

The organization of transcription into distinct sites and their highly dynamic nature might be surprising at first, particularly in light of the more static view from traditional in vitro experiments. But similar principles of organization and dynamics also apply to other essential nuclear processes including DNA replication and repair.

Replication occurs at nuclear sites referred to as ''replication factories'' (Cook, 2002; Figure 1B). These factories associate with multiple replication origins and contain the entire replication machinery as well as additional factors involved in chromatin assembly and cell-cycle regulation. In a manner similar to transcription factories, replication factories form by recruitment of replication factors from an unbound, freely diffusing nucleoplasmic pool during S phase, and assembly occurs in a stochastic fashion from single subunits rather than from recruitment of preassembled replication machineries (Sporbert et al., 2002; Figure 1B). Once assembled, some components of the replication machinery, including the PCNA clamp, are stably incorporated for the duration of the replication cycle, which is typically on the order of a few minutes, whereas others rapidly exchange with the nucleoplasm (Sporbert et al., 2002; McNairn et al., 2005). The differences in residence times most likely reflect the specific temporal requirement of each factor in the replication process. The formation and maintenance of replication factories is entirely driven by the replication process alone, which strongly suggests that the replication factories are self-organizing structures (Kitamura et al., 2006).

The highly dynamic nature of replication factories is critical for their proper function as the plasticity of these sites is essential for progression of replication along chromosomes. Elegant photobleaching studies have demonstrated that a replication factory persists for a few minutes before it disassembles (Sporbert et al., 2002). A new factory is then assembled de novo from the unbound pool of factors. Remarkably, the new factory forms immediately adjacent to the previous one, thus ensuring ordered spreading of replication. The ability of the replication machinery to progress thus relies entirely on the dynamic nature of replication foci and their ability to rapidly disassemble and then reassemble at a new site (Sporbert et al., 2002).

DNA repair is similarly compartmentalized. It involves the rapid recruitment to sites of damage of key factors from a diffuse pool to form spatially defined repair foci in which DNA repair eventually occurs (Essers et al., 2006; Figure 1C). These repair centers may form at a single site of DNA damage, but observations in *Saccharomyces cerevisiae* suggest that a repair focus may also serve multiple damaged sites (Lisby et al., 2003). Whether this also applies to mammalian cells remains to be seen. Repair foci form in a highly dynamic fashion, and factors rapidly accumulate at damage sites upon induction of double-strand breaks (DSBs; Houtsmuller et al., 1999; Politi et al., 2005; Bekker-Jensen et al., 2006). The recruitment of a multitude of factors occurs from single subunits rather than from preassembled repair machinery as demonstrated by the differential kinetics of recruitment of various factors (Politi et al., 2005). As for replication and transcription factors, recruitment is not a directed process but occurs via the capturing of freely diffusing molecules from the nucleoplasm. The repair factors remain associated with the repair centers for various periods of time depending on their function and then diffuse away once they have completed their task (Houtsmuller et al., 1999; Politi et al., 2005).

The Stochastic, Self-Organizing Nature of Nuclear Processes

The emerging view from these studies is that assembly of large macromolecular complexes on chromatin occurs via recruitment of soluble subunits from a nucleoplasmic pool. This is accomplished by stochastic interactions of single subunits or small preformed subcomplexes (Misteli, 2001b; Figure 1). Stochastic assembly from subunits intuitively seems to be an inefficient way to ensure the establishment of functional machinery. However, given the ability of most nuclear proteins to rapidly roam the nucleus for specific binding sites, even relatively low-abundance proteins frequently encounter specific target sites (Misteli, 2001b). Considering that many genes only fire sporadically and that most replication and repair sites only require the presence of a few copies of a particular component, probabilistic interactions of factors with chromatin are sufficient to sustain their functionality. In addition, although the binding of each single subunit may be relatively inefficient, the presence of assembled intermediates most likely facilitates the incorporation of subsequent subunits into an assembling complex in a cooperative fashion (Dundr et al., 2002; Agresti et al., 2005).

An important and often neglected factor that facilitates stochastic interactions and makes them more efficient in vivo is molecular crowding. The estimated protein concentration in the nucleus is an exceedingly high 100–400 mg/ml. In addition, within the nonhomogenous topology of chromatin and nuclear bodies, molecules may be spatially trapped and corralled, which further favors their stochastic interactions. Molecular crowding greatly increases the effective concentration of a component by

several orders of magnitude, and rates of protein-protein and protein-DNA interactions are greatly elevated (Minton, 2000).

An additional property of molecularly crowded systems is the emergence of discrete phases that are formed by dynamic protein aggregates. Theoretical considerations indeed suggest that molecular crowding may be the driving force behind the formation of transcription and replication factories (Minton, 2000; Marenduzzo et al., 2006). Experimental evidence for a significant role of molecular crowding in the nucleus comes from the observation that expansion of the nuclear volume leads to the disassembly of several nuclear compartments, such as the nucleolus, as well as inhibition of nuclear processes including RNA pol I transcription (Hancock, 2004). Remarkably, introduction of inert macromolecules restores these structures morphologically and rescues RNA pol I transcription (Hancock, 2004). The absence of molecular crowding is likely one of the key factors for the dramatically reduced efficiency of in vitro transcription, splicing, and replication systems compared to that of the in vivo situation. The precise role of molecular crowding in gene expression remains to be elucidated.

Several organizational properties of transcription, replication, and repair strongly point to the possibility that the compartmentalization of these essential nuclear processes occurs via self-organization (Misteli, 2001a). All processes occur in highly dynamic steady-state structures, and the formation of the functional compartments is entirely dependent on their respective functions. Replication factories do not exist outside of S phase and form rapidly as cells initiate replication (Sporbert et al., 2002; Kitamura et al., 2006). In addition, their formation kinetics correlate with the rate of replication progression (Kitamura et al., 2006). Similarly, repair foci form rapidly upon induction of DNA damage, and their extent is related to the degree of global damage (Bekker-Jensen et al., 2006). Although the situation is less clear for transcription, we know that RNA pol I-mediated expression of ribosomal RNA gene clusters is sufficient to give rise to the nucleolus, which is one of the most prominent nuclear compartments (Karpen et al., 1988; Misteli, 2001a). These properties are hallmarks of self-organizing structures.

The similarities in spatial and temporal properties of the various nuclear processes indicate that the organizational principles involved in their biogenesis are universal. In fact, it seems likely that the same principles apply to virtually all nuclear structures as many nuclear bodies, including the nucleolus, Cajal bodies, PML bodies, and splicing-factor speckles, all share a high degree of dynamic protein exchange and stochastically recruit factors from the nucleoplasm, which is reminiscent of the dynamic behavior of transcription, replication, and repair sites (Misteli, 2005). It thus appears that compartmentalization of nuclear processes, likely via self-organization, into well-defined yet dynamically malleable sites is one of the fundamental principles of organizing genome function in vivo.

Higher-Order Chromatin Organization

Chromatin is organized into higher-order structures, although much of the details of the folding geometry are unclear (Cremer et al., 2006; Woodcock, 2006). It is known that the 10 nm nucleosomal fiber is folded helically into a fiber of around 30 nm and further into a 60–130 nm chromonema fiber. The characteristics of the fiber beyond this level have not been resolved. An indication for subsequent organization levels comes from the observation that early- and late-replicating chromosome domains of about 1 Mb in size are physically separate and are maintained over several cell cycles (Sadoni et al., 1999; Cremer et al., 2006). In addition, gene-rich and gene-poor stretches of chromosomes are physically separated from each other (Boutanaev et al., 2005; Shopland et al., 2006). Regardless of the precise geometry of higher-order chromatin, the folding of the fiber is critically important for genome function.

Chromatin as an Accessibility Barrier

A link between gene activity and chromatin structure originates from the observation that active genes are often found in largely decondensed euchromatin and silenced genes in condensed heterochromatin. The most common view for how chromatin folding may act as a regulatory mechanism is via preventing the access of regulatory factors by excluding them from condensed chromatin domains (Dillon and Festenstein, 2002). Although this model is attractive it is probably an oversimplification. Several large-scale mapping studies have found an incomplete correlation between gene activity and higher-order chromatin condensation. Comparison of gene-expression profiles with chromatin structure after biochemical separation of open and condensed regions reveals a correlation with gene density, rather than activity, with decondensed chromatin representing gene-rich regions and condensed regions gene-poor stretches of the genome (Gilbert et al., 2004). Similarly, higher-order chromatin condensation and gene expression only weakly correlate when probed by genome-wide micrococcal nuclease and DNase mapping (Sabo et al., 2004; Weil et al., 2004).

The idea of higher-order structure as a regulator of accessibility is also challenged by recent observations of the diffusional mobilities of proteins in the nucleus. In both *S. cerevisiae* and mammalian cells, heterochromatin proteins can readily diffuse into and bind to their sites in highly condensed heterochromatin (Cheutin et al., 2003, 2004; Festenstein et al., 2003). Similarly, inert diffusion probes that correspond to macromolecular complexes of several hundred kilodaltons can gain ready access to condensed chromatin (Verschure et al., 2003; Gorisch et al., 2005). These observations strongly suggest that the higher-order folding of chromatin per se does not present an insurmountable accessibility barrier to nuclear proteins and that the true accessibility barrier in chromatin lies at the level of the 10 nm nucleosome fiber or below.

Genome Regulation via Local Chromatin Loops

Chromatin loops are a ubiquitous structural element of chromatin (van Driel et al., 2003; Fraser, 2006; Figure 2).

They are attractive organizational and regulatory features because they provide structural support to the chromatin fiber and at the same time bring distantly located sequence elements into spatial proximity, which allows for regulatory communication between these sites. Vice versa, loops can spatially segregate genome regions from each other and ensure their independent function. Loops have been implicated in virtually all levels of chromatin organization and function ranging from kilobase-sized loops involved in the interaction of upstream elements with promoters to giant loops of hundreds of kilobases that might contribute to gene placement away from the chromosome body and into distinct nuclear environments (van Driel et al., 2003; Cremer et al., 2006). The existence and physiological relevance of the various types of loops is at times difficult to ascertain as they often cannot be detected under native conditions and are generally refractory to visualization in situ. Regardless, the relevance of loops in several gene-regulatory events has recently been reinforced (Fraser, 2006).

Local chromatin loops are critical in both positive and negative gene regulation (Fraser, 2006; Figure 2A). The prototypical example is the β-globin gene, whose enhancer physically interacts with the main body of the gene ~50 kb downstream concomitantly with activation (Wijgerde et al., 1995). Loop formation is not merely a consequence of transcriptional activation given that it occurs prior to gene activation when erythroid progenitor cells become lineage committed (Palstra et al., 2003). The purpose of looping is to bring together far-upstream locus-control regions, promoter-proximal regulatory elements, and the gene body itself to form a "transcription hub" that presumably creates an environment of high transcriptional activity by concentrating relevant transcription factors. In an extension of this idea, the thymocyte-specific SATB1 protein is responsible for tethering regulatory sequences of a number of target genes via formation of a multitude of loops, whose formation is directly linked to the proper regulation of the target genes (Cai et al., 2003, 2006). Chromatin loops may also contribute to gene silencing, as looping of imprinting-specific regions occurs parent specifically in the insulin-like growth factor 2, H19 gene cluster (Murrell et al., 2004), and the maternally expressed *DLX5* locus (Horike et al., 2005).

Chromatin looping might in fact be more prevalent and important for proper gene expression than is commonly thought. Recent analysis of the in vivo topology of several genes in *S. cerevisiae* and in humans suggests that active loci fold back onto themselves, bringing their 3′ end in physical proximity to their 5′ beginning (O'Sullivan et al., 2004; Ansari and Hampsey, 2005; Martin et al., 2005; Figure 2B). This behavior is consistent with the now widely accepted view that 3′ end-processing and RNA-processing factors physically interact with the transcription machinery (Bentley, 2005). Furthermore, gene looping explains the observation that termination- and 3′ end-processing factors often affect transcription and have been found to interact with promoter regions. Gene looping would provide an effective way to coordinate transcription and RNA processing and would facilitate reinitiation. The possibility that local looping is a general characteristic of active genes is of particular interest in light of the transcription-factory model in which the transcription machinery is concentrated in distinct sites from which genes loop out (Fraser, 2006). How prevalent looping of active genes is remains to be seen.

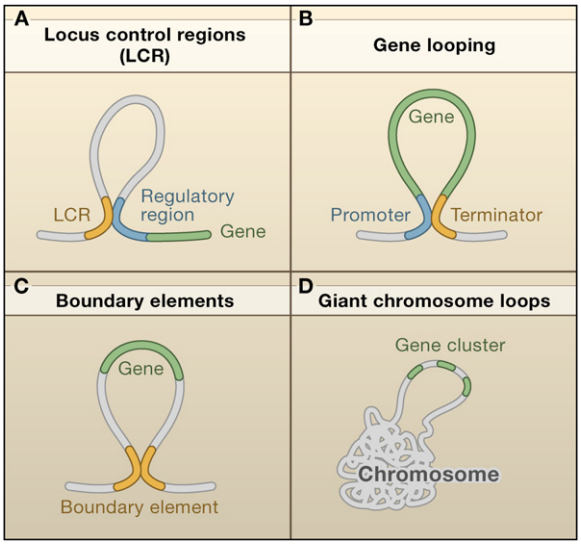

Figure 2. Local Organization of Chromatin
Local chromatin loops are essential for (A) transcriptional activation and repression, (B) coordination of initiation and termination/3′ end processing, and (C) boundary function. (D) Giant loops displace gene clusters from the chromosome body.

Local chromatin looping is likely also involved in maintaining the individuality and specific gene-expression properties of neighboring genes and genome regions (Labrador and Corces, 2002). Insulators and boundary elements are operationally defined gene-flanking sequences, and they protect a locus from the influence of its neighbors. One model to do so envisions the physical interaction of the insulator sequences generating a loop that contains the gene (Figure 2C). Physical interaction between flanking insulator regions has been demonstrated for the *Drosophila* scs and scs′ insulators (Blanton et al., 2003). Furthermore, the looping out of sequences located between two gypsy insulators can be visualized by light microscopy, and the introduction of an extra gypsy insulator into the loop leads to the formation of two smaller loops, which strongly suggests that the gypsy insulators form the basis of the loops (Byrd and Corces, 2003). Loops may also define the boundary between heterochromatic and euchromatic regions of the genome. In *Schizosaccharomyces pombe*, such boundaries are frequently characterized by binding of the RNA pol III transcription initiation factor TFIIIC, which localizes in several foci at the nuclear periphery. It has been suggested that boundary regions are clustered in TFIIIC foci, thereby organizing the

intervening, active sequences into loops that protrude into the nuclear interior (Noma et al., 2006).

The Emergence of Large-Scale Chromatin Loops

In addition to local loops, larger chromatin loops are emerging as possible candidates to contribute to genome regulation (Chubb and Bickmore, 2003; Cremer et al., 2006; Figure 2D). Giant loops of several megabases that emanate from the chromosome body have been suggested to represent a fundamental organization unit of chromatin (Chubb and Bickmore, 2003; Cremer et al., 2006). These loops are thought to segregate genome regions from each other and place them in distinct nuclear environments, presumably to optimize their activity. The most prominent examples of giant loops are highly expressed gene clusters such as the human major histocompatibility complex II and the mouse epidermal differentiation complex (Volpi et al., 2000; Williams et al., 2002). Both of these regions become expelled from their chromosome territory upon activation. Similarly, extrachromosomal loops are induced upon activation of the mouse Hox cluster (Chambeyron and Bickmore, 2004). The remarkable synchrony of this movement with the activation kinetics of the Hox cluster strongly suggests a functional link, although the precise role of this dramatic change is unknown (Chambeyron and Bickmore, 2004). Even though it is clear that neither all highly transcribed regions nor all transcribed gene clusters form giant loops, a recent high-resolution in situ hybridization method revealed a higher degree of intermingling between neighboring chromosomes than previously was assumed, which suggests that large chromatin loops might be more prevalent than commonly anticipated (Branco and Pombo, 2006). The recent development of methods to probe the physical association of genome regions in a unbiased and genome-wide scale should lead to rapid progress in our still-rudimentary understanding of the functional significance of chromatin loops (Simonis et al., 2006; Wurtele and Chartrand, 2006; Zhao et al., 2006).

A defining feature of all chromatin loops is their requirement for a tether at their base. Tethering occurs by several mechanisms. The gypsy-insulator and TFIIIC bodies are generally found associated with the nuclear periphery, which allows for the possibility of tethering to the nuclear edge (Byrd and Corces, 2003; Noma et al., 2006). The nuclear pore may serve as a tether given that synthetic boundary constructs interact with the nuclear-pore complex in *S. cerevisiae* (Ishii et al., 2002). On the other hand, an array of the chicken HS4 insulator and its flanking sequences associates with the nucleolus, and this localization is mediated by CTCF, one of the major insulator-binding proteins (Yusufzai et al., 2004). An intriguing possibility is that transcription and replication factories themselves may serve as bases of loops (Cook, 2002; Chakalova et al., 2005). Transcription and replication sites may in fact be the major tethering sources for chromatin loops as they are highly abundant and found throughout the nucleus. Furthermore, theoretical analysis of the entropy involved in the formation of loops by tethering to DNA and RNA pol clusters suggests that these are energetically favorable arrangements (Marenduzzo et al., 2006). Evidence for polymerase-mediated loops exists in both prokaryotes and eukaryotes ranging from yeast to *Drosophila* to humans (Cook, 2002). This indicates that transcription site- and replication site-mediated loop formation may be a universal and intrinsic principle of chromatin organization in the cell nucleus.

Spatial Organization of Genomes

The most global level of cellular genome organization is the arrangement of genome regions within the 3D space of the cell nucleus (Cremer et al., 2006; Meaburn and Misteli, 2007). The nonrandom nature of spatial genome organization is indicated by the age-old observation of segregation of transcriptionally active and inactive regions into physically separate domains of euchromatin and heterochromatin, respectively. Recent more-detailed mapping studies of smaller genome regions have significantly extended this concept and have made it clear that chromosomes, genome regions, and single genes are nonrandomly arranged within the nucleus (Cremer et al., 2006). Changes in positioning patterns occur during differentiation and development, which strongly suggests a link between positioning and genome function (Parada et al., 2004; Cremer et al., 2006).

Internal versus Peripheral Genome Positioning

A simple way to assess the position of a genome region within the nucleus is by determining its distance from the nuclear periphery. A general correlation between transcriptional silencing and localization toward the nuclear edge has long been suggested based on the observation that early-replicating and presumably transcriptionally active R bands are generally found toward the center of the nucleus, whereas late-replicating, inactive G bands are often located toward the periphery (Ferreira et al., 1997; Sadoni et al., 1999; Gilbert et al., 2004). In addition, human lymphocytes show a strong correlation between the radial position of human chromosomes and their gene density, with gene-poor chromosomes positioned toward the nuclear periphery and gene-rich chromosomes located in the nuclear interior (Boyle et al., 2001). Although similar correlations have been made in other cell types, chromosome positioning has been correlated with properties other than gene density, such as chromosome size (Bolzer et al., 2005). However, gene density or chromosome size alone clearly cannot explain the position of a chromosome given that the position differs between cell types and tissues where these properties are unchanged (Cremer et al., 2003; Parada et al., 2004).

Similar to that of chromosomes, the position of single genes relative to the nuclear periphery is nonrandom and has been linked to their functional status. For example, the IgH locus is preferentially associated with the nuclear periphery in B cell progenitors where it is silent, but it moves toward the interior when it becomes potentiated in B cell precursors (Kosak et al., 2002; Ragoczy et al., 2006). Similarly, the CD4 locus repositions from the periphery to

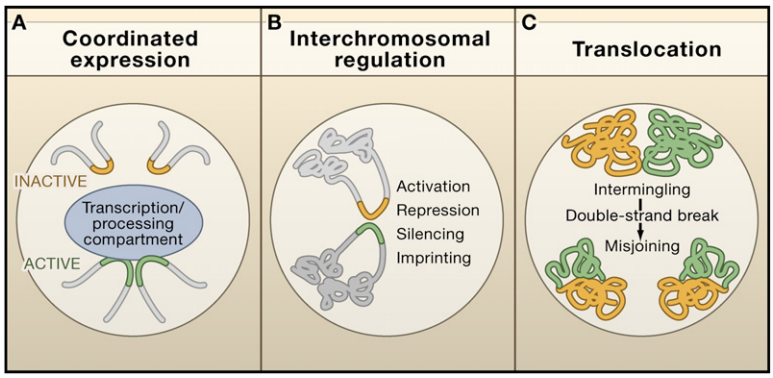

Figure 3. Functional Consequences of Global Chromatin Organization

(A and B) Spatial clustering of genes on distinct chromosomes facilitates their expression by (A) association with shared transcription and processing sites or (B) physical interactions with regulatory elements on separate chromosomes. (C) The physical proximity of chromosomes contributes to the probability of chromosomal translocations.

the nuclear interior during T cell differentiation, and Hox1b and Hox9 become internalized roughly concomitantly with their transcriptional activation (Chambeyron and Bickmore, 2004; Kim et al., 2004). On the other hand, the radial position of a gene is generally not directly related to its activity as indicated by the fact that in most cells the two alleles are positioned differently yet their functional properties appear to be similar (Roix et al., 2003). In addition, in many cases, no repositioning occurs upon a change in gene activity.

An extreme case of positioning is the physical association of gene loci with the nuclear periphery. In *S. cerevisiae*, association with the periphery is sufficient, although not necessary, for transcriptional silencing and increases DNA-repair efficiency (Gartenberg et al., 2004; Therizols et al., 2006). In mammalian cells, transcriptional activity of the cystic fibrosis disease gene correlates strongly with its association with the nuclear envelope (Zink et al., 2004). The nuclear periphery, however, does not function exclusively as a repressive environment given that a large number of *S. cerevisiae* genes are repositioned to the periphery where they interact with nuclear-pore components when they become activated (Brickner and Walter, 2004; Casolari et al., 2004; Cabal et al., 2006). This association with the periphery does not appear to be absolutely essential for their expression, but it might primarily play a role in optimizing gene activity (Taddei et al., 2006).

The potential role of the nuclear periphery in genome regulation has become of particular importance due to the emergence of several human diseases that are caused by mutations in the *LMNA* gene, which encodes lamin A and lamin C, the two major architectural proteins of the peripheral lamina (Gruenbaum et al., 2005). Although the nuclear lamina has traditionally been considered to have purely structural properties, recent observations allow for the possibility that it more directly contributes to gene regulation by tethering specific genome regions. In *Drosophila*, defined genome regions containing clusters of closely spaced genes have been identified that preferentially associate with the periphery and whose expression is affected by this interaction (Pickersgill et al., 2006). Peripheral localization of genome regions might occur directly via interactions between lamin A and core histones or more indirectly via chromatin-adaptor proteins (Gruenbaum et al., 2005). Interestingly, a hallmark of at least one of the lamin A-mediated genetic diseases is the dramatic change in histone-modification patterns and the almost complete loss of heterochromatin (Scaffidi and Misteli, 2005; Shumaker et al., 2006). How the nuclear lamina affects chromatin structure and epigenetic status is one of the most intriguing questions in the field.

Relative Positioning: The Power of Proximity

In contrast to the somewhat uncertain role of radial positioning, the position of multiple genome elements relative to each other is rapidly emerging as an important determinant of function (Figure 3).

For a long time, the lone example of spatial gene clustering had been the ribosomal genes, which coalesce in the nucleolus to bring the ribosomal gene arrays located on several separate chromosomes into physical proximity. More recently, similar coalescence has been described for tRNA genes in *S. cerevisiae* (Thompson et al., 2003). Initial evidence for spatial clustering of RNA pol II-transcribed genes in mammalian cells has recently come from the observation of colocalization of coordinately activated genes in erythroid cells (Osborne et al., 2004). Upon transcriptional activation, multiple genes that were located over 30 Mb apart on the same chromosome relocalized and became associated with shared transcription sites. Similarly, the human α- and β-globin genes located on chromosomes 16 and 11, respectively, are in close spatial proximity when highly expressed, thus extending the concept of gene clustering to multiple chromosomes (Brown et al., 2006; Figure 3A).

Although the functional significance of association of multiple coregulated gene loci is still unclear, direct physical interactions between chromosomes are now known to have regulatory functions (Figure 3B). This new paradigm was recently established by analysis of the *Ifng* and *TH2* loci in naive T cells (Spilianakis et al., 2005). The TH2 locus control region on mouse chromosome 11 physically interacts with the Ifng locus on chromosome 10 in naive T-helper cells. Upon stimulation of naive T cells to differentiate, the two genome regions separate, and *Ifng* transcription commences. Similarly, in sensory neurons a single odorant receptor from a large repertoire is selected

for expression by physical association of an odorant-receptor-enhancer element on chromosome 14, with the selected receptor localized on another chromosome (Lomvardas et al., 2006; Figure 3B). These observations establish the concept of *trans*-regulation via interchromosomal communication and suggest that, in addition to the physical interactions amongst genome elements on the same chromatin fiber, interactions in *trans* between regulatory elements on separate chromosomes must be considered in transcriptional regulation. A slight complication with these observations is the fact that associations are generally only observed for single alleles and not in all cells of a population. It is possible that this is a reflection of the dynamic nature of gene loci, which are able to move over several micrometers by constrained diffusion (Chubb and Bickmore, 2003). Alternatively, differences between alleles may be due to the stochastic nature of gene expression in which one allele is not transcribed continuously but transcription fluctuates between the two alleles (Levsky and Singer, 2003).

Interchromosomal interactions are also emerging as novel contributors to imprinting decisions. Although imprinting control regions (ICRs) have been characterized as *cis*-regulators of nearby genes, it has recently become clear that they may also act in *trans* (Ling et al., 2006). The ICR on chromosome 7 not only regulates the expression of its flanking Igf2 and H19 loci on the same chromosome but it also interacts with an intergenic region located between the *Wsb1* and *Nf1* genes on chromosome 11. This interaction is mediated by the maternal ICR on chromosome 7 via binding of the boundary element protein CTCF (Ling et al., 2006). At a more global level, the physical interaction of X-chromosome homologs may be important in determining which of the two copies becomes silenced in mammalian X inactivation. Mapping of the location of the two X chromosomes in embryonic stem (ES) cells shows that the two homologs briefly come in close spatial proximity during the period in differentiation when X-inactivation choice occurs (Bacher et al., 2006; Xu et al., 2006). These results clearly point to an emerging role for physical proximity of genome regions in gene regulation.

One of the most important genome functions that is directly affected by the physical organization of the genome is the formation of chromosomal translocations (Meaburn et al., 2006). These occur when unrepaired DSBs from separate chromosomes undergo illegitimate joining. Formation of translocations requires the interaction, and thus physical proximity, of partner chromosomes. Spatial mapping of genome regions that frequently undergo translocations indicates a significant correlation between their proximity and translocation frequency (Bickmore and Teague, 2002; Cornforth et al., 2002; Parada and Misteli, 2002; Figure 3C). The breakage sites of several common translocations, including PML/RAR and BCR/ABL, are more frequently found in close spatial proximity in normal B cells prior to undergoing translocations than would be expected based on random positioning (Lukasova et al., 1997; Neves et al., 1999). A gradual correlation between translocation frequency and spatial proximity is also observed in Burkitt's lymphoma, where the *myc* locus is on average closest to its most frequent translocation partner *IgH*, whereas it is increasingly distal from its two minor translocation partners, *Ig*λ and *Ig*κ (Roix et al., 2003). Furthermore, tissue-specific proximity of chromosomes correlates with tissue-specific translocation frequency (Parada et al., 2004). Additional support for the idea that physical proximity enhances the formation of chromosomal translocations comes from the observation that the degree of intermingling amongst adjacent chromosomes strongly correlates with translocation frequency (Branco and Pombo, 2006).

A similar role for proximity has been implicated in recombination. Repair of DSBs by nonhomologous end joining or homologous recombination occurs significantly more efficiently between sites located on the same chromosome, which by definition are in close spatial proximity, than between loci on separate chromosomes (Richardson and Jasin, 2000; D'Anjou et al., 2004). In *S. cerevisiae* the MATa locus is on average in closer spatial proximity to its preferred recombination partner HML compared to its roughly equally distant, but less favored, partner HMR located on the same chromosome (Bressan et al., 2004). Interestingly, in *S. cerevisiae* there is no difference in the efficiency of intra- and interchromosomal rejoining of DSBs (Haber and Leung, 1996). This fact is most likely due to the fundamentally different nature of chromosome organization whereby mammalian chromosomes are confined to defined subvolumes of the nucleus, which are referred to as chromosome territories, but *S. cerevisiae* chromosomes appear to lack such territoriality (Haber and Leung, 1996).

Models of Cellular Organization of Genome Function

We have accumulated a considerable amount of information about the multiple levels of genome organization and nuclear architecture. But can we derive a comprehensive model of how genomes are organized and function in vivo? Such a model should account for the complex morphological features of the nucleus and should be consistent with the structural and dynamic properties of genomes. Two types of models should be considered: deterministic models and self-organizing models.

Deterministic Models

In a deterministic model, structure dictates function. Architectural features, such as compartments, are purposefully built from dedicated structural elements to provide an environment for a particular process (Figure 4A). Such a compartment is defined by stable structural elements, and its presence is independent of the ongoing function (Figure 4A).

Deterministic models of nuclear function are consistent with the observation of several relatively stable structures within the cell nucleus, such as the lamin network, the presence of short actin filaments, or the nuclear bodies, all of which might serve as structural scaffolds (Gruenbaum et al., 2005; McDonald et al., 2006). However, no dedicated

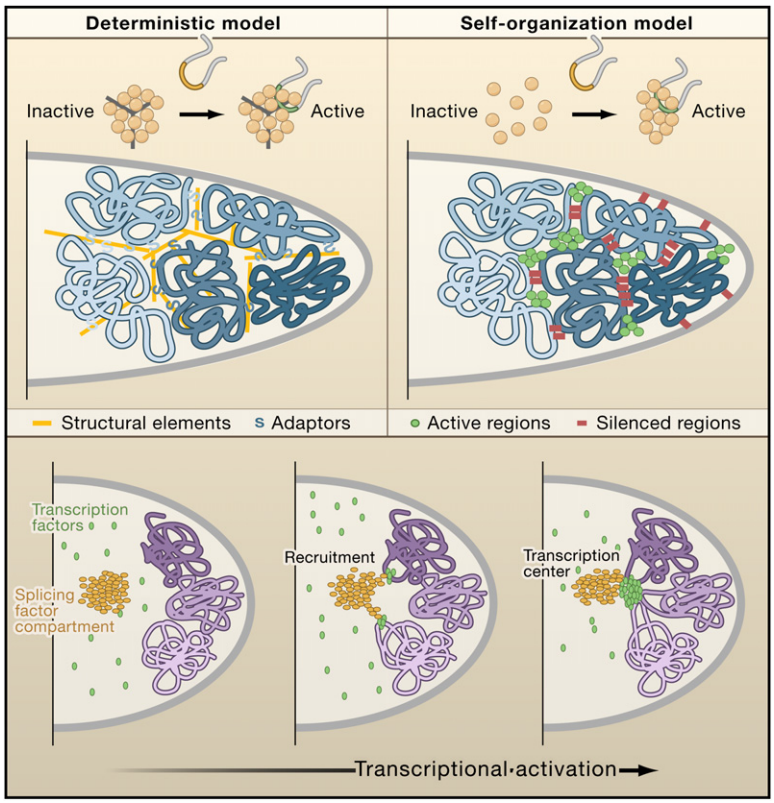

Figure 4. Models of Nuclear Organization

(Top left) In a deterministic model a functional site (transcription, for example) is preformed and contains structural elements. In this model, chromosome position is established and maintained by specific interactions of chromosomes with a scaffold.

(Top right) In a self-organization model the site forms around a poised gene as a consequence of its activation. In this model, chromosome position is determined by the interaction of functionally equivalent regions on distinct chromosomes.

(Bottom) Nuclear architecture is generated by self-organization. Transcription factors are predominantly unbound and diffuse freely though the nucleus in search of specific binding sites. Upon initial transcriptional activation of a particular gene, chromatin is remodeled, and transcription factors are recruited to the gene where they initiate formation of a transcription hub. As pre-mRNA is synthesized, splicing factors are recruited from their storage compartments. At high levels of transcription, multiple genes may coalesce to form a transcription center, which is closely associated with the splicing-factor compartment. The formation of the transcription center does not require the presence of a nuclear scaffold; chromatin is sufficient to serve as an attachment site. The configuration of splicing-factor compartment, transcription factory, and gene locus is generated in a self-organizing manner without the requirement for dedicated structural elements.

structural elements have been identified for any of the nuclear compartments, and the functional role of nuclear scaffolds is unclear. Elimination of some of the prime structural components of the nucleus, such as the lamins, has relatively little effect on the spatial organization of transcription and pre-mRNA splicing sites, although interference with the essential B-type lamins affects transcription and splicing (Spann et al., 1997; Sullivan et al., 1999; Vecerova et al., 2004). Along the same lines, although interference with nuclear actin-filament formation reduces transcription levels, the effect is moderate, and no global reorganization of transcription sites occurs (McDonald et al., 2006).

The same concerns apply to deterministic mechanisms of spatial genome organization (Figure 4, top). It is not trivial to think of mechanisms by which chromosomes are positioned in a specified, nonrandom manner. Such mechanisms would require recognition of each chromosome individually and their arrangement in particular patterns (Figure 4, top). No such recognition mechanisms are known. In fact, the observation that the chromosome-positioning patterns are not well conserved between cells in a population but are largely probabilistic suggests that no such mechanisms exist (Parada and Misteli, 2002; Cremer et al., 2006). Furthermore, the fact that chromosome-positioning patterns differ amongst cell types and tissue types would imply the existence of cell-type-specific organizing mechanisms; this seems unlikely.

A prediction of deterministic models is that structural elements should form prior to commencement of activities within those structures. The reassembly of the nucleolus after mitosis is a good example to test this prediction. Ribosomal gene expression ceases during mitosis and resumes at the telophase/G1 boundary. Rather than first forming a nucleolus into which ribosomal genes are recruited, the reforming nucleolus is nucleated around the reactivated ribosomal genes and then increases gradually in size concomitant with resumption of rRNA transcription, which strongly suggests that the structure of the nucleolus is interdependent on its function (Hernandez-Verdun et al., 2002). In sum, although the complex architecture of the genome and of nuclear processes seems to make a compelling case for deterministic organization, much of the current experimental evidence does not support such a model.

Self-Organization Models

Many nuclear properties, particularly recently discovered ones, are compatible with self-organization, and it has been suggested that the nucleus as a whole is a self-organizing system (Misteli, 2001a; Cook, 2002). Such systems are based on the dynamic interaction of their components and the mutual interplay between structure and

function. The morphological appearance and spatial organization of a self-organizing system is a reflection of the sum of all ongoing functions. At the same time the resulting structural features support and enhance ongoing activities in a self-reinforcing manner (Figure 4, top).

Evidence for self-organization of nuclear architecture and function exists at all levels of organization. Interference with virtually any nuclear process, including transcription, pre-mRNA splicing, and replication, leads to rapid changes in global architecture (Lamond and Spector, 2003). Furthermore, when new functional sites are generated within the nuclear space, structural elements often form de novo. A classic example is the ectopic expression of ribosomal genes on plasmids, which leads to the biogenesis of micronucleoli (Karpen et al., 1988). Similarly, replication factories form rapidly from dynamic components at replication origins, repair foci form upon induction of DSBs, and activation of genes may initiate the formation of transcription hubs (Houtsmuller et al., 1999; Sporbert et al., 2002; Chakalova et al., 2005). Therefore, several of the most prominent nuclear structures can form de novo, which is a hallmark of self-organizing systems.

The spatial positioning of genes and chromosomes can similarly be explained by self-organizing properties. The central idea is that the sum of all functional properties of a chromosome (i.e., the frequency and linear distribution of its active and inactive regions) determines its positioning. It can be envisioned that functionally equivalent regions from multiple chromosomes cluster within the nucleus. It is well-known that heterochromatic regions on distinct chromosomes frequently cluster in 3D space. Similarly, active chromosome regions may be constrained by shared transcription factories (Cook, 2002; Chakalova et al., 2005). Quantitative analysis demonstrates that the organization of chromatin fibers into loops constrained by transcription and replication factories represents a favorable arrangement and creates an entropy minimum, thus stabilizing the system overall (Marenduzzo et al., 2006). The sum of these interactions creates preferential associations amongst genome regions and chromosomes and constrains their motion. In this way, each genome region and each chromosome determines in a self-organizing fashion whom its neighbor is, and preferential, yet probabilistic, patterns of positioning emerge.

It is important to realize that self-organization models of nuclear architecture are not contradictory to the presence of relatively stable structures such as a lamina or a putative actin-based nucleoskeleton (Gruenbaum et al., 2005; McDonald et al., 2006). Stable structures may still serve as platforms onto which functional sites are assembled. Although such scaffolds may enhance the efficiency of nuclear processes, they might not be required; rather, the structural integrity of the nucleus might largely be generated by chromatin itself. Although it is generally assumed that transcription and replication factories are tethered to a nucleoskeleton of unknown identity, it is equally possible that chromatin itself serves as the attachment site. It is, for example, plausible that a transcription factory forms de novo on chromatin upon initiation of transcription and then attracts other transcribed genes to form a multigene transcription hub (Cook, 2002; Chakalova et al., 2005; Figure 4, bottom). Consistent with such a chromatin-driven self-organization scenario, it is well accepted that replication and DNA-repair machineries use chromatin as their nucleation site rather than as a dedicated nuclear scaffold.

The obvious weakness of self-organization models is the difficulty of testing them experimentally. Although in deterministically organized systems, structure and function can be separated and molecularly characterized, the intimate structure-function interplay in self-organizing systems prevents uncoupling by experimental means. Although much of the experimental data are consistent with self-organization, other approaches must be used to probe the self-organizing nature of genome organization and nuclear architecture. A promising strategy is the use of computational models. Sufficient data are being accumulated to constrain computational models and to make testable quantitative predictions (Gorski and Misteli, 2005). The first simple applications of these strategies are now being developed, and initial results indicate that the morphological appearance of nuclear-splicing-factor compartments can indeed be modeled by assuming principles of self-organization (Soula et al., 2005; Carrero et al., 2006).

Conclusions

The deceivingly simple question of how genomes function has become the Holy Grail of modern biology. Although sequencing efforts, molecular analysis, and in vitro biochemistry have identified the key players in virtually all genome processes, we have come to appreciate the importance of cellular organization in genome function. The degree of structural complexity in the mammalian cell nucleus is stunning. At first glance, the nonrandom organization of genomes and their interacting factors appears to complicate the task of coordinating genome functions as processes are compartmentalized and the appropriate components must be present in just the right place and at the right time to ensure efficient gene function. On the other hand, these apparent complications are counterbalanced by their potential as regulatory mechanisms. It is now clear that process compartmentalization, chromatin accessibility, and spatial sequestration of genes and their regulatory factors serve to modulate the output and functional status of genomes. New system-wide models of how genomes function in vivo based on stochastic and self-organizing behavior are emerging, and they must now be tested by comparing complete maps of transcriptional activity, epigenetic modifications, chromatin structure, and spatial positioning with cellular genome organization. The complex nature of these models requires a novel theoretical framework of biological processes and new experimental approaches, including visualization technology, analysis of dynamic events, and system-wide computational modeling, to test them. The

exploration of the principles of cellular genome organization and function will be one of the great challenges of this new kind of cell biology.

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Nuclear microenvironments in biological control and cancer

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Abstract | Nucleic acids and regulatory proteins are compartmentalized in microenvironments within the nucleus. This subnuclear organization may support convergence and the integration of physiological signals for the combinatorial control of gene expression, DNA replication and repair. Nuclear organization is modified in many cancers. There are cancer-related changes in the composition, organization and assembly of regulatory complexes at intranuclear sites. Mechanistic insights into the temporal and spatial organization of machinery for gene expression within the nucleus, which is compromised in tumours, provide a novel platform for diagnosis and therapy.

Nuclear microenvironments
Dynamic, microscopically visible, regulatory sites (domains) within the nucleus that are organized and assembled by scaffolding proteins.

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Solid tumours, leukaemias, and lymphomas show striking alterations in nuclear morphology as well as in the architectural organization of genes, transcripts and regulatory complexes within the nucleus[1–9]. From a mechanistic perspective, these cancer-related changes disrupt several levels of nuclear organization that include linear gene sequences, chromatin organization and subnuclear domains[1,4–6]. From a molecular organization perspective, point mutations and chromosomal translocations result in the rearrangement of promoter elements and coding sequences that can result in context-dependent gene activation or suppression[10–14]. Modifications in chromatin remodelling complexes, the persistent association of regulatory proteins with gene loci and DNA methylation epigenetically modulate genome accessibility to regulatory factors for the physiological control of cell fate and lineage commitment[1,4,5,9,15]. Similarly, there are cancer-related alterations in the organization and placement of macromolecular complexes at intranuclear sites where regulatory signals are assembled and integrated. From a treatment perspective, alterations in the composition, assembly and architectural organization of regulatory machinery within the cancer cell nucleus, including those related to hypothermia, change radio- and chemosensitivity[16]. Thus, cancer cells show modified nuclear architecture, suggesting a functional relationship between nuclear organization and gene expression that can facilitate tumour diagnosis and perhaps relate to therapeutic responsiveness. However, it is crucial to determine the extent to which cancer-associated changes in nuclear organization are cause or effect. Such insights will lead to an understanding of mechanisms that relate nuclear structure and function in normal and cancer cells. Whether tumour-related modifications in nuclear organization are a cause or consequence of transformation or tumour progression, they can provide diagnostic markers and targets for therapy. However, each parameter of nuclear organization is governed by different regulatory mechanisms. Therefore, it is unlikely that a single component of control can prevent cancer-related changes in nuclear structure or restore the subnuclear organization of regulatory machinery observed in normal diploid cells.

The complexity of nucleic acid and regulatory protein organization in nuclei provides necessary options for the fidelity of gene expression, replication and repair. It is well established that both nucleic acids and regulatory machinery are focally compartmentalized for combinatorial control of nuclear functions into specialized regions designated nuclear microenvironments. Accruing evidence suggests that the combinatorial assembly and organization of nuclear microenvironments is mediated by scaffolding proteins at several sites in target gene promoters as well as in subnuclear domains (reviewed in REFS 15,17–24). As outlined in BOX 1, such focal compartmentalization of regulatory machinery in nuclear microenvironments might regulate the dynamic temporal and spatial integration of physiologically responsive regulatory networks and provide threshold concentrations of factors that govern the extent to which genes are activated, suppressed or coordinately controlled.

At a glance

- The biological control of gene expression requires the temporal and spatial integration of dynamic processes. These include nuclear import, intranuclear targeting and chromatin remodelling that facilitate the organization and assembly of gene-regulatory machinery in microenvironments within the cell nucleus.
- Combinatorial assembly and organization of nuclear microenvironments is mediated by scaffolding proteins at several sites in target gene promoters as well as in subnuclear domains. Such focal compartmentalization of regulatory machinery in nuclear microenvironments might regulate the dynamic formation and activity of physiologically responsive regulatory networks and provide threshold concentrations of factors that govern the extent to which genes are activated, suppressed or coordinately controlled.
- Targeting of scaffolding proteins to specific sites within the nucleus supports their involvement in biological control and reflects the potential influence of cancer-related alterations on gene expression.
- Solid tumours, leukaemias and lymphomas show striking alterations in nuclear morphology as well as in the architectural organization of genes, transcripts and regulatory complexes within the nucleus. Examples of altered nuclear microenvironments include promyelocytic leukaemia (PML) bodies and acute myeloid leukaemia (AML) foci in leukaemias, the nucleolus in some solid tumours and extensive chromosomal rearrangements.
- Imaging principal nuclear compartments that are frequently rearranged in cancer combined with genomic and proteomic analyses can improve the biological and clinical relevance of regulatory signatures produced as a result of high throughput gene profiling of tumours.
- Mechanistic insights into the temporal and spatial organization of the nuclear machinery involved in gene expression, which is compromised in tumours, provide a novel platform for diagnosis and therapy.

General and tumour-type specific modifications in nuclear organization are longstanding indicators of cancer. Many, but not all cancer cells exhibit alterations in the number and composition of nucleoli, modified nuclear lamina and chromosomal rearrangements. For example, prostate cancer cells are typically diagnosed by the presence of several large nucleoli[25]. Chromosomal translocations and rearrangements are hallmarks of leukaemias and lymphomas[11,26]. However, it is only recently that we are gaining mechanistic insights into such longstanding, but descriptive, cancer-related changes in nuclear organization. Quantitative approaches are required for parameters of nuclear organization to serve as a basis for the diagnosis and treatment of cancer. This Review focuses on the modified compartmentalization of the nucleus in cancer and its possible implications for improving specific detection and selective treatment.

Perturbed subnuclear organization in cancer

Nuclear functions that include DNA replication and repair, as well as RNA synthesis and processing, depend on the architectural organization and assembly of regulatory proteins in subnuclear domains[17,27]. Apart from contributions to physiological control, proteins that reside in these subnuclear domains have been used to diagnose cancer. The cancer-related modifications to the compartmentalization of multifunctional protein complexes are shown by changes in the content and/or organization of promyelocytic leukaemia (PML) bodies (linked to cellular stress and apoptosis), Runx and acute lymphoblastic leukaemia 1 (ALL1) regulatory domains (involved in transcription), the nucleolus (site for ribosomal RNA synthesis), PCNA sites (implicated in DNA replication and repair), as well as BRCA1 nuclear foci (associated with DNA repair) in tumour cells[18,28–33] (FIG. 1 and see below). Similarly, tumour-related changes in sites for steroid hormone responsiveness might result in alterations that make hormone-responsive gene promoters more (or less) accessible to the steroid receptor complex — both the receptors and co-regulatory proteins. This is shown by oestrogen receptor localization, access to target genes and/or interaction with co-activators and co-repressors in breast cancer cells[34,35]. Despite the striking reconfiguration of regulatory compartments within the nuclei of cancer cells, not all components of nuclear organization appear to be disrupted in cancer. For example, RNA processing speckles, where the regulatory machinery for splicing resides, are generally unaltered in transformed and tumour cells, indicating that some architectural components of gene expression are maintained[24].

Although our knowledge of modified nuclear architecture and its possible link to cancer is increasing, several issues remain to be resolved. Are changes in nuclear organization a cause or effect of tumour progression? Do the tumour-related changes in nuclear organization that have been observed in monolayer culture reflect the organization of regulatory machinery in three-dimensional cultures and *in vivo*? Can these modifications be a general marker for identifying different cancers? How early during tumour progression can the altered parameters of nuclear architecture be determined? Can parameters of nuclear architecture be specific targets for therapy? Some of these obstacles to novel strategies for tumour diagnosis, prognosis and therapy can be overcome by recently developed capabilities for quantification, archiving and functionally relevant comparisons of image-derived data[36]. The combined application of imaging with genomic and proteomic analyses can improve the biological and clinical interpretation of regulatory signatures acquired from the high throughput gene profiling of tumours. Such gene profiles have been informative, but are sometimes ambiguous. Here we will focus on modifications in three principal nuclear compartments that are frequently rearranged in cancer.

Nucleolus. The nucleolus, where ribosomal gene expression takes place, is the most prominent example of subnuclear compartmentalization for cellular functions. The nucleolus is generated around the specific acrocentric chromosomes that contain the rDNA repeats. Changes in the composition, number, size, intranuclear localization and activity of nucleoli are observed in cancer cells, and can be used to distinguish several types of tumour cells from normal cells[37,38]. Historically, alterations in the number and size of nucleoli have been recognized as an early indication of cancer, perhaps reflecting changes in nuclear organization to support modified cellular requirements for protein synthesis.

Scaffolding proteins
Nuclear scaffolding proteins are regulatory factors that bind to DNA in a sequence-specific manner, associate with the nuclear matrix and interact with co-activators and co-suppressors to regulate transcription, replication and repair.

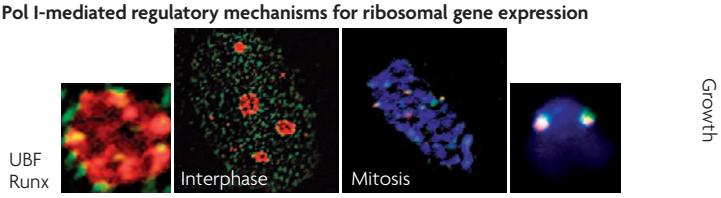
Runx
A family of three mammalian Runx transcription factors control three distinct lineage commitments (RUNX1 in haematopoiesis; RUNX2 in osteogenesis; and RUNX3 in neurogenesis and gut development) and regulate cell growth, differentiation and proliferation.

Acrocentric chromosomes
An acrocentric chromosome is one in which the centromere is located very near to one of the ends of the chromosome, thus making the short arm of the chromosome negligible. Human chromosomes 13, 14, 15, 21 and 22 are acrocentric chromosomes and all have genes that encode rRNAs.

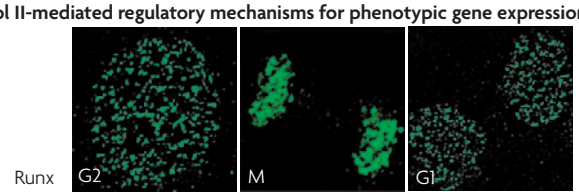
Box 1 | **Nuclear microenvironments within the cell nucleus**

The composition, organization and localization of nuclear microenvironments within the cell nucleus support transient and long-term requirements for gene expression. The residency of regulatory proteins and subnuclear placement of focally configured regulatory domains is not static. Rather, there are dynamic modifications in the representation of regulatory components to accommodate combinatorial responses to physiological cues. Five striking and broadly relevant examples of dynamically reconfigured microenvironments, where essential regulatory machinery for the control of growth, proliferation and differentiation is localized, are discussed. RUNX2 (shown in **a** in green), is an essential transcription factor for osteoblast differentiation, and it associates with ribosomal RNA genes (visualized by UBF immunostaining; shown in **a** in red) in the nucleolus in interphase nuclei. RUNX2 also associates with nucleolar organizing regions on metaphase chromosomes (shown in **a** in green) during mitosis to regulate growth properties of mesenchymal lineage cells[48]. In addition to regulating ribosomal RNA genes, Runx proteins (shown in **b** in green) also associate with phenotypic genes through successive cell divisions to maintain lineage commitment[118]. Two key transcription factors, histone nuclear factor-P (HiNFP shown in **c** in green) and nuclear protein ataxia telangiectasia locus (NPAT; shown in **c** in red), which are involved in cell-cycle progression, associate with histone gene clusters exclusively during the S-phase of the cell cycle, thus maximally inducing histone genes that are functionally coupled with genome duplication[17]. The ataxia telangiectasia mutated (ATM) kinase is central to the cellular response to DNA damage. Double stranded DNA breaks activate ATM (shown in **d** in red), which in turn phosphorylates several key downstream proteins. Activated ATM accumulates at specific subnuclear foci, whereas cells with intact DNA do not exhibit punctate ATM localization (irradiated compared with control, respectively)[17]. Several proteins, such as proliferating cell nuclear antigen (PCNA, shown in **e**), are involved in DNA replication from intranuclear domains during S-phase and alter their subnuclear localization in early, mid and late S-phase[30].

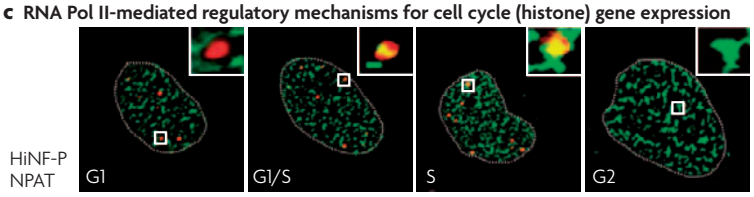
a RNA Pol I-mediated regulatory mechanisms for ribosomal gene expression



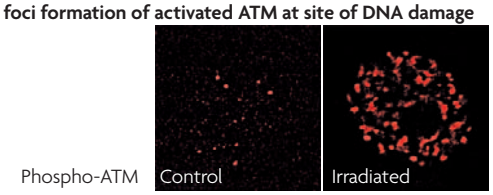
b RNA Pol II-mediated regulatory mechanisms for phenotypic gene expression



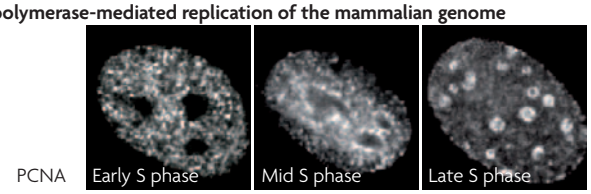
c RNA Pol II-mediated regulatory mechanisms for cell cycle (histone) gene expression



d Nuclear foci formation of activated ATM at site of DNA damage



e DNA polymerase-mediated replication of the mammalian genome



Changes in the size and number of nucleoli distinguish benign from highly metastatic prostate cancer cells. Prostate cancer cells are characterized by the presence of enlarged nucleoli that are particularly pronounced in high-grade prostate tumours[25]. Recent evidence that several tumour suppressors and oncoproteins, such as p53, MDM2, p19^{ARF} (encoded by *CDKN2A*), IRS, B23 (nucleophosmin) and MYC, are sequestered in the nucleoli of tumour cells suggests a cancer-related role for nucleoli that goes beyond protein synthesis. The presence of these regulatory proteins in nucleoli supports potential relationships between growth control, nuclear reorganization and cancer[37,39–45]. Several of these proteins are directed to the nucleolus by unique nucleolar targeting signals, indicating specific mechanisms that operate at the subnuclear level to organize and assemble functional protein complexes at specialized sites[42]. Relationships of nucleolar size, composition and function with cancer are apparent; however, it is unrealistic to associate a single set of modified parameters with all stages of tumour progression. Proximity, co-localization or functional interactions of these proteins remain to be conclusively demonstrated. The linkage between transformation, tumour progression and the cohort of regulatory proteins that reside in nucleoli must be mechanistically defined to understand crosstalk between the control of protein synthesis and aberrant proliferation in cancer[46].

Altered relationships between growth and phenotype in cancer cells necessitate investigating crosstalk between these fundamental parameters of biological regulation. Two prominent components of nuclear architecture support control of the ribosomal regulatory machinery — nucleoli and nucleolar organizing regions[47]. The demonstration that the cell-type specific Runx transcription factors functionally associate with rRNA genes in the nucleolus of interphase cells and with nucleolar organizing regions during mitosis provides a mechanistic link between growth, proliferation and differentiation[48]. RUNX1 and RUNX2 are master regulators of haematopoiesis and osteoblast differentiation, and also control cell proliferation[49–51]. In addition, RUNX1 is a frequent target of chromosomal translocations in acute myeloid leukaemia (AML), whereas the expression of RUNX2 is upregulated in breast and prostate cancer, particularly in highly aggressive tumours[26,52–57]. Therefore, cancer-related modifications in Runx proteins might influence the balance between cell cycle, growth control and phenotypic gene expression that is associated with the onset and progression of tumorigenesis.

A subnuclear structure, designated the perinucleolar compartment (PNC), is found in the periphery of the nucleolus both *in vitro* and *in vivo*[2]. Its location at the periphery of the nucleolus may reflect its involvement in the control of ribosomal gene expression, processing of gene transcripts or co-regulatory functions with mediators of gene expression that have recently been shown to be present in the nucleolus (such as MDM2, p53, MYC, RUNX1 and RUNX2). Although the PNC has been observed in several tumours, the presence of the

PNC positively correlates with the progression of breast cancer[58]. The function of the PNC has not been definitively established; however, a role in RNA metabolism is suggested by the localization of newly synthesized RNA pol III transcripts and RNA-binding proteins[59]. Despite an inconclusive link between the PNC and the regulation of gene expression, the presence of the PNC may be an informative diagnostic marker for breast cancer.

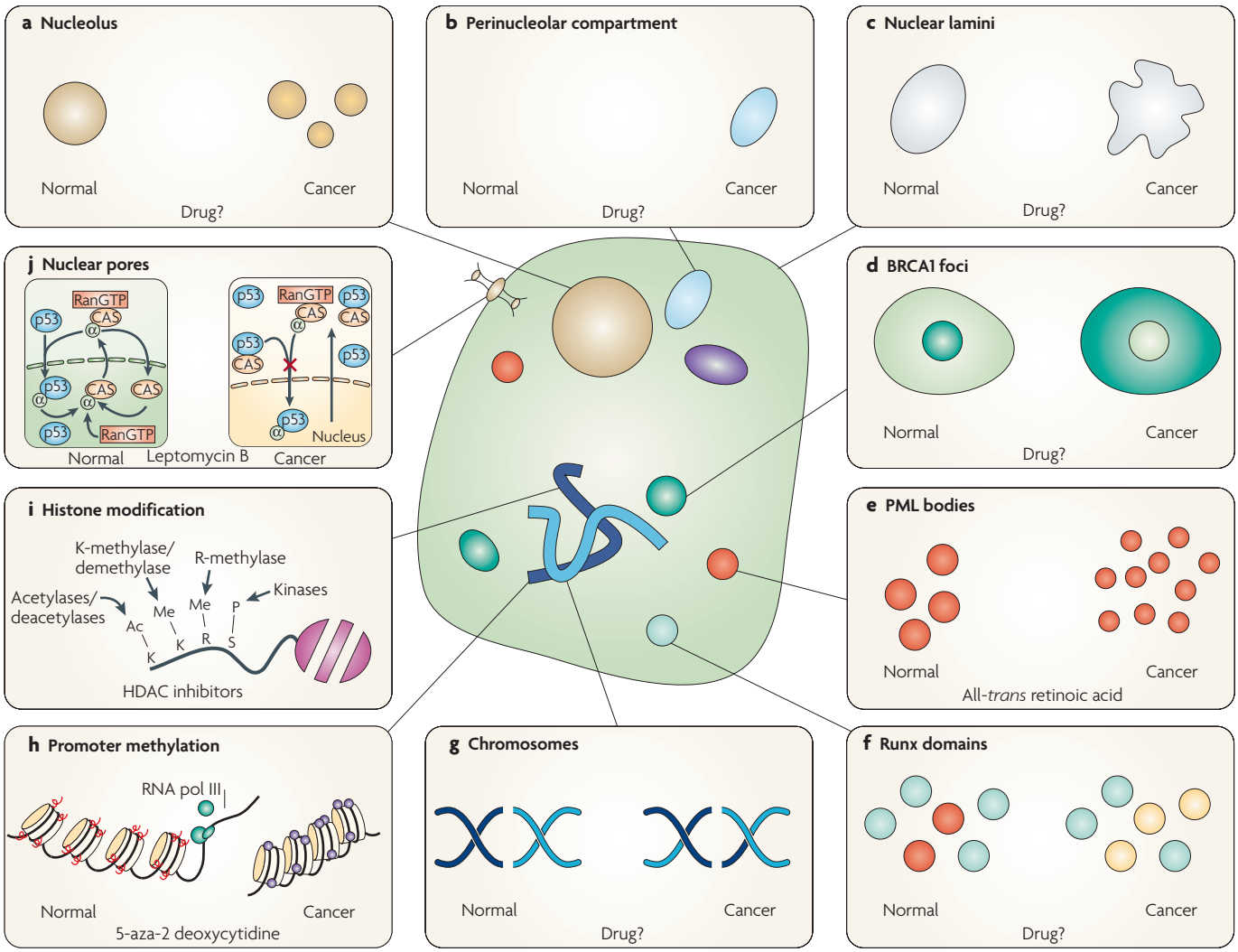


Figure 1 | **Perturbations in nuclear microenvironments in cancer.** Several nuclear microenvironments are schematically depicted in the green nucleus shown in the middle of the figure. Each surrounding box illustrates a distinct nuclear microenvironment that is modified in tumour cells. Drugs that selectively target perturbations in the organization of regulatory machinery within the nucleus and are currently in use for cancer treatment are indicated in each box. Question marks in several boxes indicate the current absence of an effective drug to target nuclear microenvironments for cancer treatment. **a** | Prostate cancer cells show multiple nucleoli (yellow circles) compared with normal prostate cells. **b** | The perinucleolar compartment (PNC, blue oval) can be present in breast and other cancer cells, but is absent in normal cells. **c** | A smooth nuclear envelope is often seen in normal cells, but a deformed nuclear lamina is evident in prostate tumour cells. **d** | Nuclear localized BRCA1 (shown as a dark green nucleus) is often retained in the cytoplasm of breast cancer cells (depicted as dark green cytoplasm). **e** | Promyelocytic leukaemia (PML bodies; red circles) are smaller in size and larger in number in acute promyelocytic leukaemia cells, a characteristic that is used as a diagnostic marker. The use of all-*trans* retinoic acid to treat patients with acute promyelocytic leukaemia has proven to be successful. **f** | Acute myeloid leukaemia (AML) patients with t(8;21) *RUNX1–ETO* translocation show aberrant intranuclear targeting of RUNX1. RUNX1 (depicted as green circles) is misdirected to ETO sites (shown as red circles in normal cells; yellow circles in cells from patients with AML). **g** | Chromosomes are often duplicated, translocated or deleted in cancer cells, shown here is a chromosomal translocation as an exchange of dark blue and light blue arms of two chromosomes. **h** and **i** | Methylation and histone modification of chromatin at tumour-suppressor as well as oncogenic gene loci can change gene expression levels. The most prominent group of drugs at different stages of clinical trials includes histone deacetylase and DNA methylase inhibitors that target aberrant chromatin architecture. **j** | There is a potential role for the nuclear export inhibitor drug leptomycin B, which blocks karyopherin-β family member CRM1 from exporting proteins out of the nucleus, as a therapeutic agent to treat cancer cells with mislocalized proteins.

Chromosomal territories. Mutations, deletions and chromosomal translocations have been functionally linked to cancer[10,11]. It has been generally recognized that chromosomes are non-randomly organized in the interphase nucleus as chromosomal territories. The location of genes within chromosomal territories has been associated with transcriptional status[12]. Recently, it has been shown that chromosomal territories are dynamic, rather than static, and intermingle with each other[60]. The differences between these observations may be due in part to technical variations between the procedures used to visualize chromosomal territories. For example, Cremer *et al.* define chromosomal territories principally using fluorescence *in situ* hybridization combined with high-resolution microscopy and quantitative analysis by an innovative series of algorithms they have developed[12]. Pombo and colleagues found evidence for the intermingling of chromosomal territories based directly on electron microscopy using immunogold labelling as well as fluorescence microscopy[61]. It remains to be conclusively demonstrated whether these territories are completely non-overlapping or are to some extent intermingled. This is important because of implications for the coordinated control of transcription that involves non-clustered genes that are not on the same chromosome, as well as the potential for gene rearrangements, as discussed below. In addition, chromosomal organization in the interphase nucleus may have implications for coordinated timing of replication at intranuclear sites for DNA duplication that seem to persist from one S-phase to the next[62].

Regardless of differences, either model for the organization of chromosomal territories is compatible with the facilitation of inter-chromosome rearrangements. For example, MYC, BCL2 and immunoglobulin loci, which are translocated in various B-cell lymphomas[11,63], have been shown to be preferentially positioned in close proximity relative to each other in normal B cells[13]. Thus, the spatial relationships of particular chromosomal territories might contribute to mechanisms that lead to translocations[14]. Consequently, chromosomal translocations might result in the disruption of defined inter- and intra-chromosomal interactions between and within specific genetic loci that regulate transcriptional activation or suppression[64]. For example, intra-chromosomal interactions between cytokine genes interleukin 4 (*IL4*), *IL5* and *IL13* result in coordinated transcriptional control[64,65]. Similarly, the inter-chromosomal interactions between the T-helper 2 (T_H2) locus control region on chromosome 11 and the interferon-γ (*IFNγ*) gene on chromosome 10 defines two alternative, yet functionally relevant, $CD4^+$ T-cell fates[66]. Because the products of each of these genes are involved in immune responsiveness, any chromosomal translocation involving these regions will have a significant impact on the human immune response.

Now there is a necessity to establish experimentally the mechanisms that determine distances and interactions between functionally related genes on independent chromosomes and facilitate chromosomal translocations[66]. This understanding will provide insights into parameters of gene regulation at the level of higher order chromatin organization. In addition, it will lead to a better comprehension of prevalent chromosomal translocations that include, but are not confined to, the *AML* translocations such as t(8;21; *ETO–AML1*); the ALL translocations such as t(4;11; *AF4–ALL1*); the chronic myeloid leukaemia (CML) translocations such as t(9;22; BCR-ABL), as well as the lymphoma translocations such as t(8;14; MYC–TCRα), within the context of transformation and tumour progression[63,67–69]. An in-depth knowledge of cellular and molecular mechanisms that influence the positioning of genes in the interphase nucleus will further explain chromosomal rearrangements that result in genomic instability, modified DNA replication and/or repair response, and aberrant chromosomal segregation. Aneuploidy and fragmented chromosomes might have significant implications for the territorial organization of the genome within the nuclei of transformed and tumour cells that may be linked to the progression of disease.

PML Bodies. Promyelocytic leukaemia (PML) is a tumour-suppressor protein that has been implicated in leukaemia and cancer pathogenesis[70]. PML organizes and assembles a multi-protein complex into a subnuclear compartment, designated the PML nuclear body[71]. Accruing evidence suggests that PML nuclear bodies are involved in the induction of apoptosis through the dynamic regulation of pro-apoptotic transcriptional events[72]. The functional and causal relationships between PML body formation and tumorigenesis are only partially understood. However, there is compelling evidence for the involvement of an altered PML protein in promyelocytic leukaemia[73,74]. The *PML* gene is rearranged in the 15;17 chromosomal translocation that is specific for acute promyelocytic leukaemia (APL), and as a consequence, *PML* fuses to the retinoic acid receptor-α (*RARα*) gene[73–75]. The chimeric promyelocytic leukaemia–retinoic-acid receptor (PML–RAR) fusion protein is associated with the dispersal and altered composition of PML bodies that leads to a modified cellular stress responses. Remission of the disease in patients with PML that are treated with all-*trans* retinoic acid is accompanied by the restoration of PML bodies to normal size, number and composition[76]. Therefore, the PML body provides an example of a subnuclear compartment that is disrupted in cancer and can be structurally and functionally reorganized after treatment. Restoration of physiological control is reflected by the recovery of competency for differentiation[76,77]. The extent to which other drugs (for example, arsenic trioxide) used to treat patients with PML restore PML domains remains to be determined[78]. In addition, characterizing the spectrum of regulatory proteins that reside in PML bodies and their tumour-related modifications that change their interactions within these domains might further refine cancer diagnosis and provide options for therapy that are based on combinatorial control.

T_H2 response
A T-helper-2 response involves the production of cytokines, such as IL4, which stimulate antibody production. T_H2 cytokines promote secretory immune responses of mucosal surfaces to extracellular pathogens and allergic reactions.

Intranuclear targeting
Directed movement of regulatory proteins within the nucleus to specific nuclear microenvironments.

Nuclear matrix targeting signal
(NMTS) The NMTS is a 30–40 amino acid sequence that directs regulatory proteins to nuclear matrix-associated sites and is unique in structure and sequence.

Nuclear matrix
An architectural network of ribonuclear proteins within the nucleus that is retained following the removal of soluble cytoplasmic and nuclear proteins as well as chromatin.

Altered subnuclear processes in cancer

The biological control of gene expression requires the temporal and spatial integration of dynamic processes. These include nuclear import, intranuclear targeting and chromatin remodelling that mediate the organization and assembly of regulatory machinery within the cell nucleus. Each of these processes can be compromised in cancer. Perturbations in the bidirectional nuclear–cytoplasmic exchange of transcription factors and signalling proteins are observed in cancer cells. Intranuclear targeting, which places regulatory proteins at strategic sites within the nucleus for the convergence of signalling pathways to support combinatorial control of gene expression, is modified in AML as a result of chromosomal translocations (that is, t(8;21)). Yet another example of architectural reorganization in nuclei of cancer cells is altered chromatin remodelling of target gene promoters. Here we discuss cancer-related perturbations in each of these processes and their potential as therapeutic targets.

Nuclear import. Nuclear proteins use many trafficking signals to organize and assemble regulatory complexes in distinct intranuclear domains (FIG. 2). This is a multistep process. A nuclear import signal ensures that newly synthesized proteins are translocated to the nucleus, a DNA binding motif dictates sequence specificity, and a nuclear matrix targeting signal (NMTS) mediates the interactions of regulatory proteins with components of nuclear architecture. Additional parameters that include post-translational modifications (such as sumoylation, acetylation and phosphorylation) and protein–protein interactions also contribute to the focal organization of regulatory complexes as well as their stability and/or activity[79–81]. One or more of these molecular localization signals can be compromised in cancer. In cancer cells, several nuclear proteins are sequestered in the cytoplasm, preventing the execution of regulatory signals within the nucleus. Examples of proteins retained in the cytoplasm of cancer cells include Smads, BRCA1 and FOXO3a in breast cancer, RUNX3 in gastric cancer, p53 in osteosarcoma and glioblastoma, BCR-ABL in CML and adenomatosis polyposis coli (APC) or β-catenin in colon cancer[82–89]. Each of these proteins exhibit clinically relevant mutations that compromise their nuclear localization. The prevalence of mutations in these regulatory factors that affect their nucleo-cytoplasmic localization in tumour cells is consistent with functional linkage of nuclear import to cancer.

Does the altered nuclear–cytoplasmic exchange of regulatory proteins provide a basis for cancer diagnosis and therapy? In fact, altered cellular localization has proven to be an effective therapeutic target in CML. The BCR-ABL chimeric protein in CML is sequestered in the cytoplasm, therefore preventing the execution of tyrosine kinase signalling within the nucleus. Blocking the nuclear export of this leukaemic protein by small-molecule inhibitors results in nuclear retention and apoptosis of CML cells[90]. Although altered cellular localization of regulatory proteins in some leukaemias and solid tumours is used for cancer diagnosis, the challenge in restoring normal localization is attaining specificity — blocking nuclear import or export of affected proteins in cancer cells can interfere with normal nucleo–cytoplasmic shuttling[83–85]. The development of small molecules that selectively target the nuclear entry or exit of regulatory proteins altered in cancer may be a viable option for treatment and prevention. However, it is unrealistic to expect that nucleo–cytoplasmic exchange can be an effective therapeutic target in all cancer types or that therapeutic effectiveness can be sustained on a long-term basis because the nucleo–cytoplasmic shuttling of proteins in normal cells may also be compromised.

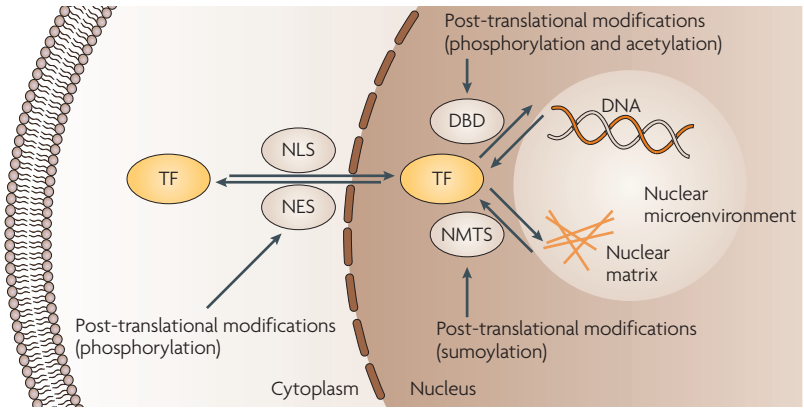


Figure 2 | **Several trafficking signals localize regulatory proteins to the right place at the right time.** Nuclear proteins use a hierarchy of trafficking signals to organize and assemble regulatory complexes in distinct intranuclear domains. A nuclear import signal (NLS) ensures that newly synthesized proteins (a transcription factor (TF) is shown here) are translocated to the nucleus; a DNA binding motif (DBD) dictates sequence specificity, and a nuclear matrix targeting signal (NMTS) mediates interactions of regulatory proteins with components of nuclear architecture. Unique nuclear microenvironments result from the activity of these trafficking signals. In addition, several post-translational modifications and protein–protein interactions are known to regulate the localization of proteins within the nucleus. NES, nuclear export signal.

Intranuclear targeting. Scaffolding proteins (such as Runx and ALL1 factors) are nuclear regulatory factors that assemble in focally organized nuclear microenvironments that are associated with the nuclear matrix[91,92]. Targeting scaffolding proteins to specific sites within the nucleus supports their involvement in biological control and reflects the potential influence of cancer-related alterations on gene expression. Multi-protein complexes that are present in these nuclear microenvironments contain basal transcription factors (such as the TATA-binding protein), chromatin-remodelling machinery (for example BRG1), signalling proteins that include Smads and YAP, growth factor receptors and steroid hormone receptors (for example, oestrogen receptor) and histone-modifying factors (for example, p300)[91,92].

We are only beginning to understand mechanistically how scaffolding proteins are assembled and organized as nuclear microenvironments. Other scaffolding proteins that reside in focal nuclear microenvironments include PCNA (involved in DNA replication), SATB1 (associated with base unpaired regions at the base of DNA loop domains), and BRCA1 (implicated in DNA repair)[93–98]. Although less is known about sequences that mediate

subnuclear targeting of these proteins, the focal organization of some scaffolding proteins within the nucleus is shown to be mediated by the subnuclear targeting signals. Subnuclear targeting signals function autonomously and are unique in sequence and structure, thus conferring specificity to the intranuclear localization of regulatory proteins[29,32,99–104]. Examples include Runx transcription factors, PIT1, YY1, SATB1, glucocorticoid receptor, vitamin D, androgen and oestrogen receptors, and DNA polymerase-α[29,99–105].

An example of altered subnuclear targeting in cancer cells is the gene that encodes the RUNX1 haematopoietic regulatory protein that is a frequent target of chromosomal translocations in patients with AML[52]. This fusion protein combines the N terminus of the RUNX1 haematopoietic transcription factor and MTG8-encoded protein (ETO) that is not expressed in haematopoietic cells. A significant proportion of patients with AML express a chimeric protein resulting from an 8;21 chromosomal translocation (AML–ETO)[26]. The fusion protein has a modified subnuclear targeting signal and organizes into nuclear microenvironments that are distinct from those in which wild-type RUNX1 resides[53,54]. Consequently, in addition to the gain and loss of other regulatory functions, the pathology of AML involves the intranuclear misrouting of RUNX1, which results from the loss of the C terminus and the acquisition of a variant subnuclear targeting signal. Another example of impaired subnuclear targeting of regulatory proteins because of a leukaemia-related translocation is provided by mixed lineage leukaemia (MLL) protein. MLL is a methyl transferase that regulates transcription globally and fuses with various proteins in both ALL and AML. Two of these proteins, each of which is a transcription elongation factor, are AF4 and AF9. Erfurth *et al.* have found that AF4 and AF9 interact at specific subnuclear sites that are distinct from known intranuclear domains. The MLL–AF4 fusion protein, although it maintains interaction with AF9, alters its intranuclear localization[106]. Therefore, MLL–AF4 fusion offers another mechanism, which is distinct from that of AML–ETO, but results in subnuclear misrouting of regulatory proteins in leukaemia cells. It remains to be determined whether other chimeric regulatory proteins in leukaemias also exhibit altered subnuclear localization, and whether such modified intranuclear targeting will offer a viable therapeutic target in those leukaemias.

An additional line of evidence for the significance of intranuclear targeting is provided by single amino acid substitutions in the NMTS of lineage-specific Runx regulatory proteins. These mutations modify subnuclear targeting of Runx proteins, and prevent osteolytic activity of metastatic breast cancer cells *in vivo* (in the case of RUNX2) or support the expression of a transformed phenotype in myeloid progenitor cells (in the case of RUNX1)[55–57]. These findings suggest that the therapeutic restoration of altered regulatory protein subnuclear targeting in leukaemia and solid tumours might improve the response of tumours to standard therapies. The effectiveness of this strategy requires a further understanding of the extent to which other regulatory proteins show modified subnuclear targeting in metastatic cancers.

Chromatin remodelling and epigenetic regulation of gene expression. Changes in chromosome structure that are associated with cancer go beyond translocations, mutations and amplifications. Cancer cells acquire alterations in chromatin remodelling machinery that affect the promoter accessibility of genes involved in the onset and progression of tumorigenesis[1,4,5,107]. Therefore, promoters of oncogenes such as *MYC* and Ras often become constitutively accessible to gene-regulatory machinery[108,109]. Consistent with this observation, several chromatin remodelling proteins (such as INI1) function as tumour suppressors[110]. Similarly, gene promoters of tumour-suppressor genes (for example, $p19^{ARF}$ in solid tumours and leukaemias, and RUNX3 in gastrointestinal carcinomas) are methylated, thus silencing these genes and contributing to tumour progression[111]. The methylation of tumour-suppressor gene promoters is increasingly used to diagnose stages of tumorigenesis[112]. Targeting the activity of DNA methyl transferases by small molecules (for example, 5 aza-cytidine) has proven to be effective in cancer treatment and prevention[112,113]. In addition, several drugs that target the activity of chromatin modifying histone deacetylases are in clinical trials at various stages (recently reviewed in REFS 114,115).

Another parameter of epigenetic control that is relevant to tumorigenesis is the retention of regulatory proteins, which include Sp family proteins[116] and Runx transcription factors, with target genes during mitosis[117]. This association of regulatory proteins with mitotic chromosomes provides a mechanism for the distribution of gene-regulatory machinery to progeny cells, epigenetically controlling cell fate and sustained lineage commitment as well as the maintenance of the transformed phenotype by supporting the resumption of transcription post-mitotically[48,118].

Taken together, epigenetic changes that alter chromatin structure have surpassed the proof of principle stage and now offer new dimensions for cancer diagnosis and therapy. With expanding use of epigenetically-mediated therapeutics for the treatment of cancer, broad-based effects must be considered as well as consequences from long-term treatments of tumours that will prevent recurrence but may not be curative.

Future perspectives

We are only beginning to understand the complexity of nuclear organization and nuclear structure–gene-expression relationships within the context of biological responsiveness. Although modified nuclear organization in cancer cells is well documented, cause or effect relationships remain to be conclusively determined. Nonetheless, accruing evidence suggests that each architecture-linked regulatory parameter is vulnerable to perturbations that can compromise the control of cell growth, proliferation and differentiation, and provides a potential target for therapy. Challenges to the use of this information for therapeutic exploitation include identifying methods of quantitative analysis that reproducibly capture subtle differences in subnuclear protein localization between normal and cancer cells, and the development of small-molecule inhibitors that specifically and selectively target

Intranuclear informatics
A mathematical alogrithm that uses 28 independent parameters to quantitatively assess subnuclear organization of regulatory proteins.

Architectural signature
A quantitative representation of subnuclear organization that is specific for each protein.

components of nuclear organization that are perturbed during tumorigenesis. These challenges can be overcome in part by the emerging capabilities of high-resolution imaging[119] and an integrated biological approach.

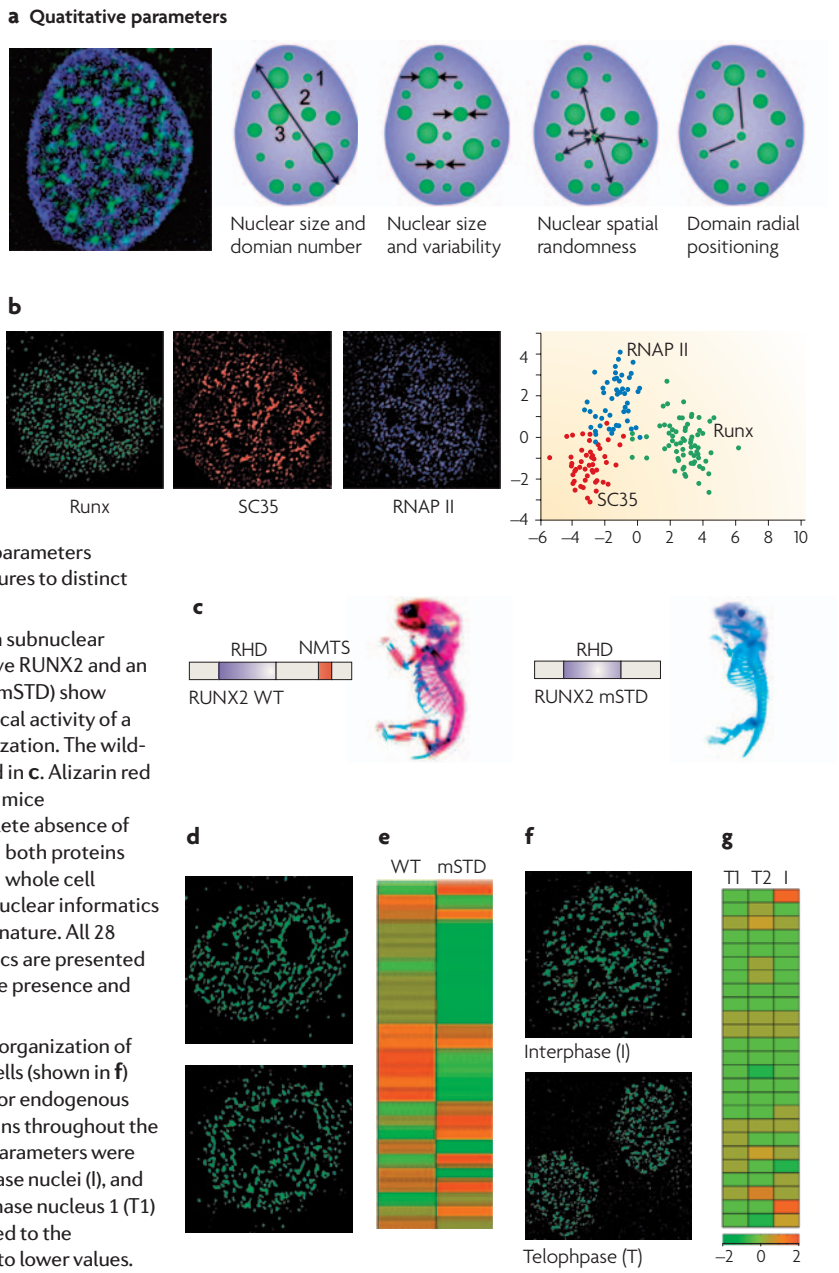
Recently, mathematical algorithms, designated intranuclear informatics, have been developed to identify and assign unique quantitative signatures that define regulatory protein localization within the nucleus[36] (BOX 2). Quantitative parameters that can be assessed include nuclear size and variability in domain number, size, spatial randomness and radial positioning.

Intranuclear informatics provides quantitative criteria to link protein subnuclear organization with biological function. The significance and implication of intranuclear informatics can be shown by three distinct biological examples (BOX 2). Regulatory proteins with different activities can be subjected to intranuclear informatics analysis, which assigns each protein a unique architectural signature. The overlap between the architectural signatures of different proteins is often correlated to their functional overlap. Alternatively, the subnuclear organization of a protein domain can be linked with subnuclear targeting, biological function and disease. For example, RUNX2 and its subnuclear targeting defective mutant (mSTD) show distinct architectural signatures, indicating that the biological activity of a protein can be defined and quantified as subnuclear organization. Finally, these data can be used to define functional conservation, for example,

Box 2 | **Intranuclear informatics**

Intranuclear informatics can be used to examine nuclear alterations in cancer cells compared with normal cells.

- The conceptual framework for the quantitation of subnuclear organization by intranuclear informatics. The four main groups of parameters examined (shown in **a**) are based on inherent biological variability.
- Regulatory proteins with different activities can be subjected to intranuclear informatics analysis, which assigns each protein a unique architectural signature. The overlap between the architectural signatures of different proteins is often correlated with their functional overlap. Shown in **b** are Runx transcription factor (green), SC35 splicing protein (red), and RNA Polymerase II (blue). These data obtained from intranuclear informatics can be presented in various forms such as a graph (shown in **b**) comparing two parameters: domain spatial randomness (on the X-axis) and domain radial positioning (on the Y-axis). One hundred nuclei (each represented as one circle on the graph) co-stained for RUNX2, SC35 and RNA pol II were analysed. As shown here, each of the three regulatory proteins exhibits distinct properties for the two parameters represented. We attribute these unique architectural signatures to distinct functional properties of these proteins.
- The subnuclear organization of Runx domains is linked with subnuclear targeting, biological function and disease. Biologically active RUNX2 and an inactive subnuclear targeting defective mutant of RUNX2 (mSTD) show distinct architectural signatures, indicating that the biological activity of a protein can be defined and quantified as subnuclear organization. The wild-type and mSTD RUNX2 proteins are schematically depicted in **c**. Alizarin red (bone) and alcian blue (cartilage) staining of skeletons from mice homozygous for wild-type and mSTD RUNX2 show a complete absence of mineralized bone in mSTD RUNX2 knock-in mice. Although both proteins exhibit similar patterns of subnuclear organization *in situ* in whole cell preparations (shown by the green fluorescence in **d**), intranuclear informatics shows that each protein exhibits a distinct architectural signature. All 28 quantitative parameters analysed by intranuclear informatics are presented in **e** as hierarchical clusters. The green colour represents the presence and red colour represents absence of a specific nuclear feature.
- Post-mitotic restoration of the spatially ordered subnuclear organization of Runx is functionally conserved. ROS 17/2.8 osteosarcoma cells (shown in **f**) were subjected to *in situ* immunofluorescence microscopy for endogenous RUNX2. RUNX2 is distributed at punctate subnuclear domains throughout the interphase and telophase nuclei. Subnuclear organization parameters were computed from deconvolved images for RUNX2 for interphase nuclei (I), and both progeny telophase nuclei, denoted arbitrarily as telophase nucleus 1 (T1) or telophase nucleus 2 (T2). A colour map (**g**) has been applied to the standardized data assigning red to higher values and green to lower values.



this technique can be used to show that the post-mitotic restoration of the spatially ordered Runx subnuclear organization is functionally conserved.

These architectural signatures have the potential to discriminate between the intranuclear localization of proteins in normal and cancer cells. Intranuclear informatics can be combined with proteomics (changes in protein–DNA and protein–protein interactions) and genomics (altered gene-expression profiles) to develop a novel platform for the identification and targeting of perturbed regulatory pathways in cancer cells.

Such an integrated biological approach that incorporates a temporal and spatial perspective to the control of gene expression within the three-dimensional context of nuclear organization can be both biologically and clinically informative. However, it would be naive to anticipate that such a strategy, as with conventional therapies, is not vulnerable to compensation on a long-term basis. The convergence and integration of signalling networks in nuclear microenvironments provides architecturally-based options for selectively targeting cancer-related changes in the control of transcription, replication and repair.

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Profiles and Legacies

Gene Expression in Nuclear Microenvironments for Biological Control and Cancer

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The central theme of my research has consistently been to investigate mechanisms that control proliferation and differentiation with emphasis on regulation that is compromised in cancer. Starting as a graduate student, I have been committed to exploring cell cycle and growth control and tissue-specific transcription. And, I have been fortunate, throughout my career, to have colleagues who nurtured an architectural perspective of gene expression providing a novel dimension to the problem from both fundamental biological and clinical perspectives.

It was a unique opportunity to contribute to the initial characterization of transcriptional regulation that mediates control of the cell cycle. The studies from our laboratory in the early 1970s provided insight into the molecular mechanisms regulating gene expression during the cell cycle at the G_1/S phase transition in normal and tumor cells. To mechanistically examine linkages between proliferation and differentiation we developed a foundation for addressing bone tissue-specific gene expression. Our research group has established aberrations that accompany the onset and progression of skeletal disease and changes in gene expression that are associated with breast and prostate tumor metastasis to bone. A major contribution from our laboratory has been to mechanistically define functional relationships between the subnuclear organization of nucleic acids and regulatory proteins. During the past several years our research group has focused on combinatorial organization and assembly of regulatory machinery for gene expression in nuclear microenvironments for epigenetic control of cell fate and lineage commitment in biological control and cancer.

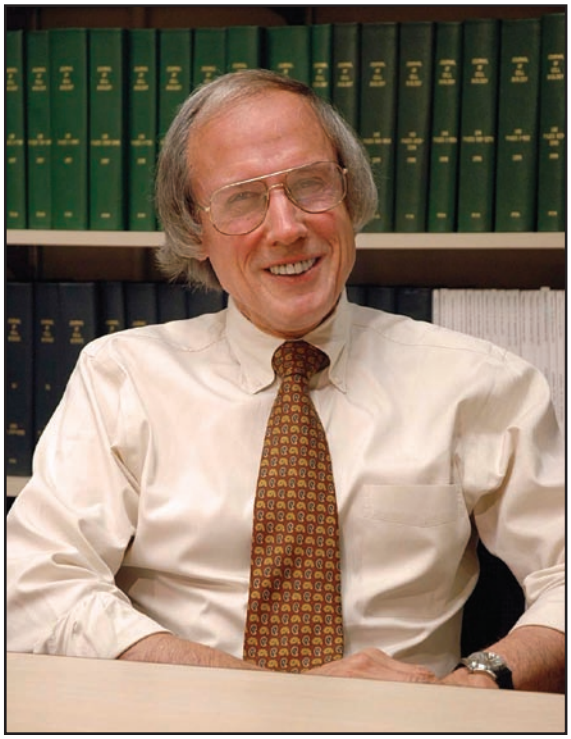
Progress in science does not occur in a vacuum. For me, the longstanding partnerships with Janet Stein, Jane Lian, Andre van Wijnen and Martin Montecino have been both effective and exceptionally meaningful. These are gifted scientists with skill sets and perspectives that have provided a broad-based platform to confront the challenges of growth control and tissue-specific gene expression that is compromised in cancer. Janet is an outstanding nucleic acid biochemist. Jane is a highly talented bone biologist, Andre is an innovative molecular biologist and Martin is an insightful chromatin biochemist. It is gratifying to look back at decades of collaboration and describe "our," rather than my initiatives and contributions.

I never lose sight of the mentors and collaborators who have been truly instrumental in development of strategies and experimental approaches. Sheldon Penman, since the late 1960s, has been the driving force behind pursuit of cell structure-gene expression relationships. Arthur Pardee, Renato Baserga and my thesis advisor Howard Rothstein guided our investigations into the regulatory mechanisms that are operative in cell cycle control; not simply as components of pathways but within the context of physiologically integrated networks and regulatory machinery that is dynamically organized and assembled. Art Pardee had the vision to dissect the components of combinatorial control and recognized the importance of multi-dimensional signaling mechanisms.

A collaboration with Carlo Croce over many years has provided a cancer genetic perspective. Sidney Weinhouse taught me the importance of relating biochemical and molecular mechanisms to cancer as a disease. His guidance solidified my commitment to tumor biology and pathology. A rewarding partnership and friendship with our Cancer Center Director, Dario Altieri, to build a Cancer Center with disease-based programs where tumor biology and pathology are pursued in a seamless manner, has been professionally and personally rewarding.

GARY S. STEIN, PH.D.

Gary S. Stein, PhD, is the Gerald L. Haidak and Zelda S. Haidak Professor of Cell Biology at the University of Massachusetts Medical School (UMMS), Chair of the Department of Cell Biology, Deputy Director for the UMASS Memorial Cancer Center, and a driving force behind establishing the UMMS Stem Cell Program. The central theme of Gary Stein's research has been to discover mechanisms controlling proliferation and differentiation. He pioneered characterization of transcriptional regulation that mediates cell cycle control. His studies have provided considerable insight into the molecular mechanisms governing gene expression during the cell cycle that are compromised in cancer. Gary Stein has had major and lasting impact in skeletal biology, where he established the foundation for addressing bone tissue specific gene expression, and provided valuable insight into aberrations that accompany the onset and progression of skeletal disease and tumor metastasis to bone. He has been instrumental in defining functional interrelationships between the localization of gene regulatory machinery in nuclear microenvironments and has made seminal contributions to mechanisms that direct transcription factors to intranuclear sites that support combinatorial organization, integration and assembly of machinery for activation or suppression of genes within the context of biological control and cancer.



CHARACTERIZATION OF TRANSCRIPTIONAL REGULATION THAT MEDIATES CELL CYCLE CONTROL

In the mid to late 1960s as a graduate student at the University of Vermont, working with Howard Rothstein, I initiated studying cell cycle control using early cleavage divisions of the zebrafish Brachydanio rerio and wound healing in lens epithelial cells as models. At that time, recombinant DNA technology had not been developed and the field of eukaryotic gene expression was in its infancy. Although interpretation of cell cycle regulatory mechanisms was dependent upon results from metabolic labeling and inhibitor studies, we made a series of observations that directed our focus to the G_1/S phase cell cycle transition.[1-4] With the demonstration by Hewson Swift and Ted Borun that histone protein synthesis is restricted to the S phase of the cell cycle, we developed control of histone gene expression as a paradigm for defining transcriptional regulatory mechanisms that are operative at the onset of S phase. During the past forty years we have made a series of contributions to understanding cell cycle and growth control that have impacted on fundamental regulatory mechanisms that support proliferation and directly relate to aberrant gene expression in cancer. By the combined application of in vivo and in vitro experimental approaches that include biochemical, molecular, cellular and in vivo genetic analysis, we have been utilizing the histone gene promoter to identify and characterize the requirements for transcriptional competency at the G_1/S phase transition point in response to factors that combinatorially regulate cell cycle progression and mechanisms that couple histone gene expression with DNA replication.[5-9]

We were one of the first laboratories to establish that transcriptional control is a key regulatory mechanism mediating the G_1/S phase cell cycle transition. We demonstrated that transcription factors modulate control of gene expression in a cell cycle dependent manner.[2] Subsequently, we cloned the first human cell cycle regulatory genes and used them to probe for proliferation-dependent signaling pathways operative at the onset of S phase.[10,11] These studies uncovered principal regulatory mechanisms that temporarily and functionally couple expression of multiple histone genes with DNA replication. We made the initial observation that remodeling of chromatin structure and nucleosome organization supports modifications in transcription for cell cycle progression.[12] This pursuit of nuclear organization as a component of transcriptional control provided us with an orientation that continues to guide our experimental strategies.

In collaboration with Lewis Kleinsmith our laboratory provided the initial observation that phosphorylation of transcription factors is a key component of cell cycle-related gene expression.[3] We identified a series of cell cycle regulatory elements and cognate transcription factor complexes that are responsible for upregulation of gene expression at the G_1/S phase transition. These studies have defined a novel cell cycle checkpoint at the initiation of S phase that is E2F independent as well as temporally, biochemically and functionally distinct from the R point late in G_1 when genes encoding enzymes for deoxynucleotide metabolism are upregulated (e.g. thymidine kinase, dihydrofolate reductase). We have characterized "R-point–S phase" signaling mechanisms.[13,14] Our research group has shown that regulatory factor complexes that mediate transcriptional control at the G_1/S phase transition are stringently cell cycle regulated in normal cells and constitutive in transformed and tumor cells where growth control has been abrogated.[15,16] The experimental approaches we employed included cell free systems, intact cell studies, gene expression profiling at the G_1/S phase transition and the first transgenic animal models for transcriptional control of cell cycle regulated genes.[6] An early lesson we learned is the necessity to pursue multidisciplinary approaches to address the challenges of combinatorial complexity that is operative in biological control.

GENE EXPRESSION CONTROLLING SKELETAL PROLIFERATION AND DIFFERENTIATION AND BONE CANCER

For many years our laboratory, to a large extent due to the insight and dedication of Jane Lian, has been actively engaged in defining molecular, cellular, biochemical, genetic and physiological mechanisms that regulate skeletal development and bone remodeling in vitro and in vivo. We have focused on skeletal pathology and tumors that originate in bone or preferentially interact with the bone microenvironment. Metastatic breast and prostate tumors are the two bone-seeking tumors that we have been examining.

Our laboratory was fortunate to be in a position to provide concepts and experimental approaches that have paved the way for resolving complexities of regulatory mechanisms that control osteoblast proliferation and differentiation including the identification of steroid hormone responsive promoter elements and bone tissue-specific transcription factors and coregulatory complexes.[15,17-20] A breakthrough for us to understand bone cell biology and pathology was the identification of distinct developmental stages (i.e. proliferation, extracellular matrix maturation, extracellular matrix mineralization and apoptosis) that reflect establishment and maintenance of the osteoblast phenotype. Our research group laid the foundation for addressing gene regulatory mechanisms that are operative during development of the osteoblast phenotype by characterizing the promoters of cell growth and bone specific genes as blueprints for responsiveness to physiological regulatory signals. We established osteoblast phenotype development as a widely utilized approach for studying signaling mechanisms operative during osteoblast differentiation, as well as for examining selective responsiveness to physiological and pharmacological mediators at specific stages of bone cell maturation. Our research team was the first to characterize perturbations in osteoblast growth and differentiation in vivo in rodent models of bone metabolic disease (osteoporosis and osteopetrosis) and cancer (osteosarcoma).[16] The sequential stages and developmental transitions of osteoblast maturation have proved to be a paradigm for many investigators to define regulatory parameters of bone biology and pathology.

To bridge the gap between regulatory mechanisms and clinical applications, our laboratory has developed a novel, transplantation-based approach for targeting gene therapy to bone using tissue-specific promoters. The combined insights obtained from characterizing bone-specific regulatory mechanisms in culture, in transgenic mice and in rodent knockout models, as well as in bone marrow-derived osteoblast precursor cells have permitted development of novel approaches for targeting gene therapy to bone using tissue-specific promoters.[21]

Building on our earlier observations that chromatin structure and nucleosome organization are dynamically modified to accommodate expression of genes during the cell cycle we demonstrated that chromatin remodeling of skeletal genes supports bone tissue-specific and steroid hormone-responsive transcription. With Martin Montecino, initially as a graduate student in our laboratory and now as a Professor at the University of Concepcion in Chile, we pioneered understanding of interrelationships between nuclear architecture and control of skeletal gene expression supporting bone cell differentiation. We have functionally linked remodeling of the chromatin structure and nucleosome organization of skeletal gene promoters with competency for interactions with physiologic mediators of transcription.[18,22]

NUCLEAR MICROENVIRONMENTS

Our pursuit of mechanisms that control proliferation and differentiation have been guided by the requirement to understand localization of regulatory complexes within the nucleus where the combinatorial components of gene expression are organized, assembled and integrated. Our appreciation for the role of nuclear organization in control of gene expression, particularly in relation to biological control and cancer has evolved during the past several years. There is recognition that extensive informational content is encoded in epigenetic signatures that go beyond DNA sequences. DNA methylation and histone modifications are providing signatures for epigenetic control of proliferation, differentiation, transformation and tumor progression as components of mechanisms that sustain aberrant gene expression.

We have been addressing regulatory parameters of transcription that are related to localization of nucleic acids and regulatory proteins within the cell nucleus. Relationships between nuclear structure and gene expression have been recognized for some time. Nucleoli are focal sites where the regulatory machinery for ribosomal gene expression resides. Functional compartmentalization of the cell nucleus is reflected by intranuclear sites that are dedicated to replication, repair, cell survival, and RNA processing. The changes in nuclear morphology that occur during hematopoietic cell differentiation are striking and reflect modifications in the organization of nucleic acids and regulatory proteins that support biological control. Transformation and tumor progression are frequently associated with altered nuclear organization. However, the challenge is to mechanistically understand the localization of regulatory complexes within the cell nucleus using criteria that are specific and quantitative. Our objective has been to develop regulatory parameters of nuclear organization into targets for tumor diagnosis and therapy.

Our group is actively engaged in exploring the intranuclear organization of regulatory domains with emphasis on modifications in cancer cells. Our strategy has been directed to the AML/Runx transcription factors that support tissue-specific gene expression (AML1 supports hematopoiesis, Runx2/AML3 supports osteogenesis and Runx3/AML2 supports gastrointestinal cell differentiation) and context-dependent tumor suppression. We have focused on two classes of nuclear microenvironments that mediate organization, assembly and integration of regulatory cues. First, we established that the Runx transcription factors bind to multiple sites of target gene promoters where they are strategically placed to scaffold coregulatory proteins for epigenetic control and serve as endpoints for key signaling pathways. Here, the regulatory signal is the Runx DNA binding domain. Second, we are pursuing a novel dimension to genetic and epigenetic control by intranuclear trafficking.[23,24] An initial component of a mechanism for localization of regulatory complexes within the nucleus came from our identification of a unique 31 amino acid intranuclear trafficking sequence which functions autonomously. Specificity of the Runx intranuclear trafficking signal is supported by a unique sequence and a unique crystal structure. To quantitatively define a signature for positioning of Runx regulatory proteins within the nucleus we developed a strategy that combines high resolution quantitative image processing with point mutations in Runx proteins that are determinants for temporal, spatial and functional parameters of control. Using emerging capabilities of high resolution imaging, we have quantitatively constructed

signatures for colocalization of Runx regulatory proteins within the nucleus that reflect the transformed phenotype.[24-26] We have demonstrated that the T(8;21) chromosomal translocation in AML leukemia disrupts the AML locus and results in aberrant intranuclear trafficking of AML transcription factors that compromise fidelity of tissue-specific gene expression. Our research group has further linked intranuclear trafficking of transcription factors with activity of tissue-specific genes using in vitro and in vivo genetic approaches. We have demonstrated that cancer cells exhibit altered subnuclear distribution of transcription factors supporting linkage of compromised intranuclear trafficking with tumorigenesis. We showed that replacement of the chromosome 21 encoded intranuclear trafficking signal in the AML transcription factors as a consequence of chromosomal translocation that occurs frequently in AML leukemia, redirects a major hematopoietic regulatory protein to sites within the nucleus for transcriptional suppression rather than activation. These findings provide evidence that subnuclear localization of regulatory proteins is linked with formation of osteolytic lesions by metastatic breast cancer cells and the leukemic phenotype.

Recent efforts in our laboratory have been directed to the extent that microenvironments with transcriptional regulatory machinery contribute to epigenetic control. We have been focusing on Runx/AML transcription factors and leukemia-related Runx/AML translocation-fusion proteins in parental and post mitotic progeny cells to investigate mechanistic parameters of cell fate and lineage commitment. We have observed that the wild type and translocation/fusion Runx/AML transcription factors are retained at chromosomal loci during mitosis providing a novel dimension to epigenetic retention of phenotypic gene expression for biological control. An analogous mechanism appears to be operative that sustains the transformed and tumor phenotypes of cancer cells and similar mechanisms epigenetically support cell fate, lineage commitment and coordinate control of proliferation, cell growth and tissue specific gene expression in a broad biological context.[27,28]

COLLABORATION IS KEY TO CURING CANCER

Collective insight into regulatory parameters that govern biological control of proliferation and differentiation and perturbations that are associated with cancer underscore the importance of combinatorial mechanisms. Evidence is accruing for temporal and spatial dimensions to control of gene expression, replication and repair with a requirement for architectural organization, assembly, integration and localization of regulatory machinery. Structure-function interrelationships are beginning to define regulatory networks that mediate physiological control and aberrations that are linked to the onset and progression of tumorigenesis.

It is now apparent that the informational content of macromolecules and macromolecular complexes goes beyond DNA sequences. There is emerging evidence that epigenetic codes and signatures for components of biological control include histone subtypes, post translational histone modifications, DNA methylation, the subnuclear localization of regulatory complexes and mitotic occupancy of regulatory factors for proliferation, cell growth and phenotypic target gene loci.

The challenge we face is to configure the data from high throughput screens in a manner that maximizes insight into mechanisms and therapeutic targets. The scope of the strategies is rapidly growing. The volume and complexities of information that is readily obtainable is extensive and expanding. Traditional boundaries between disciplines need not be an obstacle. Team approaches where partnerships between investigators with perspectives and skill sets that combine the power of genomic, proteomic, cellular, biochemical and molecular approaches provide the platform for novel insight into biological control and innovative options for cancer diagnosis and treatment. From a personal perspective, collaboration has been the most rewarding component of my career. I am confident that advances in understanding cancer biology and pathology through collaboration are more effective and meaningful than any cohort of individual contributions.

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