INTRODUCTION

Functional compartmentalization in the eukaryotic cell is readily accepted from observation of membrane-bounded organelles that can be fractionated and their properties determined in isolation. The existence of discrete compartments within a given organelle is less immediately apparent, but nonetheless real. Within the eukaryotic nucleus several independent approaches point to the compartmentalization of particular activities such as transcription, RNA processing and replication assembles morphologically distinct nuclear organelles with defined functional properties. These observations indicate a very high level of structural organization for the various metabolic activities occurring within the nucleus. We discuss the possible existence of novel regulatory functions inherent to nuclear architecture itself.

Key words: Nucleus, Compartmentalization, Chromosome, Replication, Transcription, Splicing, Nuclear export

DNA SYNTHESIS

The most dramatic example of the compartmentalization of nuclear function is seen with replication. Analysis of DNA synthetic sites with bromodeoxyuridine or biotinylated dUTP reveals only 150 foci of incorporation within each nucleus during S phase (Nakamura et al., 1986). The foci are clearly defined with a clear and relatively uniform separation from each other. When replication initiates, these foci are small and appear as ‘dots’, as time progresses they become more diffuse (Manders et al., 1992; O’Keefe et al., 1992). These foci contain accumulations of the proteins necessary for replication: DNA polymerase α, PCNA, and RP-A as well as regulatory molecules such as cyclin A, cdk2, and RPA70 (Adachi and Laemmli, 1992; Hozak et al., 1993; Cardoso et al., 1993; Sobczak-Thepot et al., 1993). Immunolabelling synthetic sites with gold particles suggests that nascent DNA is extruded from the replication foci (Hozak et al., 1993). This implies that DNA moves through a fixed architecture containing the molecular machines directing replication. The advantages of the compartmentalization of DNA replication include a concentration of the necessary regulatory, structural and enzymatic components required to duplicate both DNA and chromosomal structure. The staged assembly of a functional replication elongation complex occurs within a defined macromolecular complex; this allows many check points and controls to be built into the initiation of replication (Almouzni and Wolfe, 1993).

The essential role of nuclear architecture in determining the functional properties of DNA is perhaps most apparent in connection with chromosomal replication in Xenopus laevis eggs. Injection of prokaryotic DNA into an egg or incubation of the DNA in an egg extract leads to the assembly of a pseudonucleus competent to replicate DNA (Forbes et al., 1983; Blow and Laskey, 1986). Importantly, replication is regulated spatially in that it occurs at discrete sites containing clusters of replication forks (Cox and Laskey, 1991). There is a remarkable similarity between the number and distribution of replication ‘foci’ in the pseudonuclei and those observed in replicating eukaryotic nuclei in tissue culture cells (Nakamura et al., 1986; Mills et al., 1989). The assembly of functional replication origins is not necessarily dependent on defined DNA sequences in the chromosomes, but on features of nuclear architecture that can be assembled even on prokaryotic DNA. The implication is that general features of nuclear architecture...
can impose a particular function, in this case that of replication. It should be noted that the early Xenopus embryo is a special case in which normal somatic controls might have been relaxed. Chromatin loop attachments to the chromosomal axis and the number of chromosomal origins of replication are much more frequent in the chromosomes of early embryonic nuclei in Xenopus compared to somatic cell nuclei (Micheli et al., 1993; Laskey et al., 1983). Only when the cell cycle lengthens at the mid blastula transition are normal controls established (see Hyrien et al., 1995).

In normal somatic nuclei the replication foci do not all engage in replication simultaneously, some are utilized early in S-phase and others late in S-phase (Nakamura et al., 1986; Ariel et al., 1993). This reflects differential replication timing, which is an important regulatory step in maintaining local chromatin organization and gene activity (Wolffe, 1991). The molecular mechanisms controlling the differential utilization of origins are presently unknown (Gilbert, 1986; Gintz and Korn, 1986; Wolffe, 1993). However, comparable phenomena occur in yeast (Newlon et al., 1993), where origin utilization is found to be dependent on chromosomal position. This is an important area for future study.

These observations on DNA replication, the dependence on nuclear architecture and the movement of the DNA strand through a fixed site have led to speculation that comparable phenomena also govern transcription (Cook, 1994; Hughes et al., 1995). The concentration of RNA polymerase within defined nuclear compartments together with other components of the transcriptional machinery lends some support to these ideas (see later). RNA polymerase may be much less mobile and chromatin more mobile than generally considered. An important additional point is that the assembly of a particular nucleoprotein architecture that favors one biological process, e.g. replication, might exert an exclusionary or repressive influence on another, e.g. transcription (see Wansink et al., 1994). In fact in the S. cerevisiae chromosome, components of the origin recognition complex required for replication exert a silencing effect on transcription (Fox et al., 1993).

THE NUCLEOLUS AS A PARADIGM FOR NUCLEAR COMPARTMENTALIZATION

Ribosomal gene transcription, rRNA processing and preribosomal particle assembly occur in the nucleolus (Scheer and Benavente, 1990). All of these events involve the assembly of macromolecular machines that localize within this specialized nuclear compartment. The molecular mechanisms that direct particular proteins and enzyme complexes to this compartment and retain them there are largely unknown (see Hatanaka, 1990). One simple hypothesis is that the majority of the nucleolar architecture is generated from the activities of the transcriptional machinery itself which assembles reiterated regulatory nucleoprotein complexes on rDNA. Ribosomal RNA genes are tandemly arrayed with approximately 250 copies of a 44 kb repeat in man (Scheer and Benavente, 1990). Thus, more than 10^6 bp of rDNA and associated proteins could provide the framework for the nucleolus. Once transcription itself is in progress, additional features of nucleolar architecture would potentially follow from the accumulation and activities of the molecular machines that process pre-rRNA and that assemble ribosomes.

Morphologically the nucleolus has three major organizational areas: (1) the nucleolar fibrillar centers, which are surrounded by (2) a dense fibrillar region, and (3) the granular region. Numerous localization studies using specific antibodies and hybridization probes indicate that the nucleolar fibrillar centers are the sites where the ribosomal RNA genes, RNA polymerase I, the class I gene transcription factor UBF and topoisomerase I are localized (Scheer and Rose, 1984; Raska et al., 1989; Rendon et al., 1992; Thiry, 1992a,b). Accumulation of these particular macromolecules leads to the inference that the nucleolar fibrillar centers are the assembly sites for the regulatory nucleoprotein complexes that direct transcription (Fig. 1A). The dense fibrillar component that surrounds the nucleolar fibrillar center consists of nascent ribosomal RNA and associated proteins. It is in the dense fibrillar component that RNA precursors such as [3H]uridine or biotinylated ribonucleotides are initially found on pulse labelling (Thiry and Goessens, 1991). Specific hybridization probes localize unprocessed nascent transcripts and associated processing machinery to the dense fibrillar component (Ochs et al., 1985; Kass et al., 1990; Puvion-Dutilleul et al., 1991). Mature 28 S and 18 S rRNA, partially processed transcripts and intermediates in ribosome assembly are found in the granular region. These assembly intermediates are visualized as particles 15-20 nm in diameter. Movement of preribosomal subunits from the nucleolar granular region to the cytoplasm might be facilitated by proteins that move within specific pathways or tracks from the nucleolus to the nuclear envelope (Meier and Blobel, 1992).

This hierarchical organization of the nucleolus with particular morphologically distinct compartments reflecting accumulations of specialized molecular machines and their substrates provides an extremely useful model with which to consider the functional compartmentalization of mRNA synthesis, processing and export.

MAKING mRNA

The synthesis of mRNA within the nucleus and the subsequent delivery of the mature transcript to the translational machinery within the cytoplasm also involves the concerted and coordinated activities of multiple molecular machines. Transcription requires the assembly of a regulatory nucleoprotein complex, containing the promoter region, associated coactivators and the RNA polymerase holoenzyme. The polymerase must initiate RNA synthesis and traverse the gene. The pre-mRNA must be processed through the addition of a m7G(5'cpp) cap, removal of introns (splicing) and polyadenylation. These various biochemical events are interdependent since transcription by RNA polymerase II is a prerequisite for both efficient splicing and polyadenylation (Sisodia et al., 1987), and the 5' cap and associated proteins also facilitate splicing and mRNA export (Izaurralde et al., 1994; Lewis et al., 1995).

The transcriptional machinery that synthesizes pre-mRNA localizes with the perichromatin fibrils found at the boundaries of condensed chromatin domains. Perichromatin fibrils are nuclear ribonucleoprotein complexes with a diameter varying from 3 nm to 20 nm. They are enriched in nascent pre-mRNA
radiolabelled with [3H]uridine or bromouridine, and fibril density correlates with transcriptional activity (Bachellerie et al., 1975; Fakan, 1994; van Driel et al., 1995). Components of the splicing machinery are found with the perichromatin fibrils (Fakan et al., 1984) consistent with the assembly of the splicing machinery initiating at the site of transcription. This is visualized through immunofluorescent probe detection of the splicing machinery as diffuse nucleoplasmic staining (Spector, 1993). Considerable morphological and molecular biological evidence indicates that splicing occurs concomitant with transcription (Beyer and Osheim, 1988; Le Maire and Thummel, 1990; Waurin and Schibler, 1994).

The localization of specific transcripts such as fibronectin pre-mRNA using hybridization probes reveals elongated ‘tracks’ or more compact ‘dots’ at one or two discrete sites corresponding to the chromosomal copies of the gene (Xing et al., 1993, 1995; Huang and Spector, 1991). Simultaneous RNA and DNA hybridization demonstrates that transcribing genes directly localize with the RNA tracks or dots, with the gene at one end of the track (Xing et al., 1993, 1995; Xing and Lawrence, 1993). Moreover probes for introns only detect the track near the gene, suggesting that splicing occurs along the track. These results suggest a model of cotranscriptional assembly of the splicing machinery onto pre-mRNA at the perichromatin fibrils, with splicing continuing as the pre-mRNA is released from the gene.

RNA tracks can have a very close association with discrete structures known as interchromatin granules or ‘speckles’ (Xing et al., 1993). These speckles are sites at which the splicing machinery accumulates together with intron-containing pre-mRNA and polyadenylated mRNA (Spector, 1990; Fu and Maniatis, 1990; Carter et al., 1991, 1993; Visa et al., 1993a; Wang et al., 1991). Thus speckles potentially represent sites of pre-mRNA processing and of mature mRNA accumulation in the nucleus.

Lawrence and colleagues have suggested that actively transcribing genes have a non-random association with the speckles (Xing and Lawrence, 1993; Lawrence et al., 1993; Xing et al., 1995), the implication of this association being that in certain instances speckle structures represent the sites of transcription itself. An immediate limitation to this latter hypothesis is that there are only 20-50 speckles scattered in a
punctate distribution throughout the nucleus. Clearly not every active gene can be associated with these structures. However, out of ten transcribing genes investigated in the Lawrence laboratory, seven are associated with speckles (Xing et al., 1995). It has also been hypothesized that these transcription domains might contain several actively transcribing genes at any one time (Xing et al., 1993; Xing and Lawrence, 1993). In contrast to this view, the bulk of nascent pre-mRNA labelled with bromouridine accumulates in a distinct pattern which does not correspond to that of speckles containing snRNPs (Jackson et al., 1993; Wansink et al., 1993). Adenoviral and actin transcripts can be visualized as discrete dots in the nucleus, with no apparent association with snRNP speckles (Zhang et al., 1994). However, a more detailed study indicates a clear association between actin transcripts and speckles (Xing et al., 1995). An additional complication is that not all RNA detected in these assays is pre-mRNA, but an ill defined proportion might correspond to a large pool of stable nuclear polyadenylated RNA involved in structural functions within the nucleus (Huang et al., 1994; Mattaj, 1994).

Much of the evidence presented in support of the association between sites of transcription and speckles is based on the transcription of very active genes (e.g. collagen, which comprises 4% of total mRNA in fibroblasts; and the induced expression of the fos gene after serum starvation). It could be envisaged that the speckles, rich in splicing components, act as processing factories closely associated only with the most actively transcribing genes.

Once synthesized and assembled with the splicing machinery, the pre-mRNA has to reach the nuclear envelope and enter the cytoplasm. The pre-mRNA is packaged not only with the splicing apparatus but also with heterogenous nuclear ribonucleoproteins (hnRNPs). These proteins provide the ‘workbench’ on which mRNA is processed. Like the packaging of DNA with histones, the resulting architectures are important for the maturation of mRNA (Dreyfuss et al., 1993). The preparation of nuclear matrix, a process that removes the vast majority of chromatin from the nucleus, retains pre-mRNA, hnRNPs and some elements of the splicing machinery (Huang and Spector, 1991; Mattern et al., 1996). This is indicative of both the abundance of and major structural role for hnRNPs in the nucleus. In general, hnRNPs are diffusely distributed throughout the nucleoplasm, however, a subset overlap snRNP speckles, and some even shuttle with mRNA into the cytoplasm before returning to the nucleus (Visa et al., 1996; Pinol-Roma and Dreyfuss, 1992). The movement of a specific pre-mRNA from gene to cytoplasm has been reconstructed based on the export pathway of the Balbiani ring (BR) pre-mRNP particles in the dipteran Chironomus tentans (Mehlin and Daneholt, 1993). BR genes are easily visualized as two giant puffs in the polytene chromosomes of the salivary glands. The large nascent transcripts from these puffs are assembled with hnRNPs and the splicing machinery during transcription to form a thin fiber, which, with elongation, becomes thicker and bends into a ring-like structure. The mature pre-mRNP granule, now thought to contain spliced RNA is released into the nucleoplasm making its way to the nuclear envelope, where it positions itself against a nuclear pore and becomes elongated into a rod-shaped structure which goes through the pore in a 5’-head-first manner. As the pre-mRNP granule emerges on the cytoplasmic side it immediately becomes associated with ribosomes (Mehlin and Daneholt, 1993). The important point for this discussion is that the entire process occurs within precise nucleoprotein architectures.

How does the pre-mRNA reach the nuclear membrane from the sites where transcription takes place? Estimates of the rate of movement of pre-mRNA have been made using a highly expressed hybrid gene in Drosophila salivary glands, which gives a strong signal at the site of transcription and a more diffuse channel-like network pattern throughout the nucleoplasm (Zachar et al., 1993). These results have been interpreted to suggest that simple diffusion alone could account for the dispersal of mRNA. However, there is also evidence to suggest that pre-mRNA movement through the nucleoplasm occurs in a directed fashion. For example, the need for particular structural features in the pre-mRNAs for their movement into the cytoplasm (Elliot et al., 1994) is inconsistent with a simple diffusion model. Furthermore, the tight association of transcripts, hnRNPs, and functional processing components (e.g. the splicing machinery) with the nuclear matrix argues against the pre-mRNA being freely diffusible in the nucleoplasm. Huang and Spector (1991) were able to visualize ‘tracks’ corresponding to fos gene transcripts frequently extending to the nuclear envelope and exiting over a limited area. This would appear to give credence to one aspect of the gene gating model proposed by Blobel (1985). This postulates that due to overall three-dimensional architectural constraints in the nucleus, genes will associate with a specific region of the nuclear envelope, hence their transcripts are ‘gated’ to exit at a defined set of nuclear pores. Though the organization of genes relative to the nuclear envelope remains largely unproven, the findings of Huang and Spector (1991) are consistent with the ‘gating’ hypothesis. In contrast, Lawrence and colleagues saw no significant evidence for their transcript ‘tracks’ making contact with the nuclear envelope (Xing and Lawrence, 1993; Xing et al., 1995). Collagen mRNA was visualized as ‘studding’ or ‘encircling’ the nuclear envelope (Xing et al., 1995), indicative of an exit at many nuclear pores. It has been suggested that the lack of tracks visibly extending to the nuclear envelope may be due to the fact that somewhere along the transport pathway, pre-mRNA rapidly disperses in many directions (Xing and Lawrence, 1993; Xing et al., 1995). Once again evidence of specificity in a nuclear process is indicative of a high degree of structural organization. Not all mRNAs might require such specificity in their export pathway.

The process of mRNA synthesis has many parallels with that of rRNA: sites of synthesis can be visualized, the pre-mRNA is packaged with processing machinery cotranscriptionally, and then for certain mRNAs processing within a defined structure takes place before release for export from the nucleus. The various structures visualized reflect the molecular machines active at those sites (Fig. 1B). Moreover the structures are dynamic with a constant vectorial flow from the sites of synthesis to the next step on the way to the cytoplasm. Components can also recycle between the different functional compartments (e.g. Pinol-Roma and Dreyfuss, 1992). Everything happens as a nucleoprotein complex that is visually identified as a functional and morphologically discrete compartment. Does it matter that transcription and splicing/processing occur in particular domains? The advantages of compartmentalization are similar to those discussed earlier for replication. There is a concentration of the necessary regulatory, structural and
enzymatic components required to transcribe or splice mRNA. The organization of the components within an architectural framework provides many more opportunities for regulation compared to a freely diffusible state. Clearly transcription and splicing can occur in dilute solutions (1 µg/ml) within an in vitro reaction tube, however, the efficiency with which these events occur is much less than that achieved in vivo. Organization and channeling of macromolecules from one site of enzymatic activity to another within a specific architecture is clearly advantageous within a nucleus containing nucleoprotein at >50 mg/ml.

**CHROMOSOMAL TERRITORIES FOR ACTIVE OR REPRESSED CHROMATIN**

The functional organization of the chromosome into discrete domains has been increasingly recognized through experiments in *Drosophila* that make use of the phenomenon of position effect variegation (Schaffer et al., 1993). These experiments employ a powerful combination of techniques including genetic analysis and cytological observation of the large polytene chromosomes. The introduction of a normally active gene into a chromosome at a position adjacent to a transcriptionally inactive, condensed heterochromatin domain will lead to a significant repression of the transcription process. This is believed to occur through a spreading of heterochromatin structure into the normally active gene. Thus, the expression of a gene depends on its chromosomal position, hence the term ‘position effect’. This phenomenon provides a useful screen for genes and their products that will suppress or enhance the repression of transcription due to position effects on a suitable reporter gene, often one influencing *Drosophila* eye coloration. Many of the genes influencing position effect have been characterized and have been found to encode structural components of chromatin itself or to be capable of modifying the organization of chromatin through enzymatic mechanisms.

Position effect variegation is now recognized as a universal phenomenon in eukaryotic chromosomes. Genes integrated into yeast chromosomes near the silent mating loci or close to the telomeres are repressed in a way that reflects their proximity to these sites in the chromosome. This silencing effect can spread over at least 5-10 kb of contiguous DNA, but not as much as 20 to 30 kb in yeast. As in *Drosophila*, the genes influencing position effect in yeast encode structural components of chromatin or enzymes associated with the modification of chromatin (Laurensen and Rine, 1992). An important advance is the recognition that certain structural components of repressive chromatin might influence the position of that portion of the chromosome within the nucleus (Palladino et al., 1993). Thus the compartmentalization of chromosomes might have a direct role in regulating gene activity.

Early cytological experiments demonstrated the positioning of telomeres at the nuclear envelope in salivary gland cells of salamanders (Rabl, 1885). The telomeres of *Drosophila* polytene chromosomes and those of *Schizosaccharomyces pombe* chromosomes in G2 phase of the cell cycle also show comparable localization of the telomeres at the nuclear periphery (Hochstrasser et al., 1986; Funabiki et al., 1993). Advances in confocal immunofluorescence microscopy and molecular genetics have allowed the demonstration that two proteins, silent information regulators (SIR) 3 and 4 are required for the perinuclear localization of *Saccharomyces cerevisiae* telomeres (Palladino et al., 1993). These proteins are also required for the heritable inactivation of genes within specific chromosomal domains located at the silent mating type loci and telomeres of *S. cerevisiae*. Thus a connection is made between the location of a particular chromosomal territory in the nucleus and transcriptional repression per se.

Experiments designed to examine the localization of active genes in the nucleus clearly demonstrate that these are predominantly found within the nuclear interior (Spector, 1993). Early suggestions that active genes were preferentially located at the nuclear periphery are probably based on experimental artefact (Hutchison and Weintraub, 1985). With respect to specific active genes, Lawrence and colleagues have suggested that some genes occupy non random positions (Lawrence et al., 1993). For example: three active genes with very different localizations are the whole EBV genome and the *neu* oncogene (transcriptionally active) which are positioned within the inner 50% of the nuclear volume, whereas the dystrophin gene is at the extreme nuclear periphery. However, three inactive genes (encoding albumin, cardiac myosin heavy chain, and neurotensin) all localize in constitutive heterochromatin at the nuclear periphery or near the nucleolus (Xing et al., 1995). UV microirradiation and in situ hybridisation experiments extend the experiments examining telomeres or specific genes to suggest that individual chromosomes occupy broad, but discrete territories within the nucleus (Cremer et al., 1993; Heslop-Harrison and Bennett, 1990; van Driel et al., 1995).

The spatial relationship between chromosome territories and other subnuclear compartments has been investigated by Cremer and colleagues (Zirbel et al., 1993). It was shown that the splicing machinery subcompartments were associated with the periphery of chromosome territories and were excluded from their interior (Zirbel et al., 1993). Similarly, a specific gene transcript visualized as an RNA track was shown to be preferentially localized on the surface of the chromosome territory and a very limited survey of the localization of individual genes again placed them to the exterior of chromosomal territories (Zirbel et al., 1993; Cremer et al., 1993). On the basis of the above evidence, Cremer and colleagues have postulated that the interchromosome space excluded by the chromosomal territories defines an interconnected functional compartment for transcription, splicing, maturation and transport (Fig. 2). This compartment is intimately associated with actively transcribing genes localized on the surface of the territory, presumably on extended loops (Zirbel et al., 1993; Cremer et al., 1993). This is an intuitively appealing model which potentially encompasses the observations of Lawrence and colleagues regarding the non-random distribution of genes. However, the nature of the functional interface between an active gene in a chromosome territory and the interchromatin compartment remains unclear, primarily due to our poor understanding of the higher order organization and compaction of DNA into chromosomes.

**NUCLEAR BODIES AND DOMAINS**

The compartmentalization of transcription, RNA processing
and replication components within the nucleus lends credence to the existence of specialized functional roles for any morphologically distinct structure in which a protein of interest accumulates. Nuclear bodies, originally described at an ultrastructural level, represent such structures in search of a function.

Coiled bodies
Morphologically defined as a tangle of coiled threads (Monneron and Bernhard, 1969), coiled bodies are often associated with the periphery of nucleoli. A number of nucleolar proteins and RNAs important for rRNA modification and processing are found in the coiled body including fibrillarin and the U3 small nucleolar RNA (Jimenez-Garcia et al., 1994). snRNPs including U7 snRNA also accumulate in this structure, as do specialized proteins such as p80-coilin (Frey and Matera, 1995; Bohmann et al., 1995). Accumulation of these proteins suggests a role in RNA processing, however, rRNA and mRNA have not been detected in these structures (Huang et al., 1994; Jimenez-Garcia et al., 1994). Coiled bodies are dynamic, they disassemble at mitosis and reassemble in G1 during the cell cycle, they also increase in abundance in response to growth stimuli (Carmo-Fonseca et al., 1993; Lamond and Carmo-Fonseca, 1993).

The nuclei of amphibian oocytes contain structures known as C-snurposomes with many similarities to mammalian coiled bodies (Bauer et al., 1994; Gall et al., 1995). These nuclear organelles are found attached to the histone gene loci of lampbrush chromosomes, and since U7 snRNA is involved in the 3’-end modification of histone pre-mRNAs, it has been suggested that this is one function of the C-snurposome and of coiled bodies (Gall et al., 1995; Frey and Matera, 1995). Alternatively the C-snurposome or coiled bodies might represent assembly sites for the molecular machines that process various RNAs (Bohmann et al., 1995).

PML (promyelocytic leukemia) nuclear bodies
Acute PML is a haemopoietic malignancy that is most often associated with a t(15; 17) chromosome translocation which results in an inframe fusion of the PML gene to that of the retinoic acid receptor α (RARα) (Warrell et al., 1993). The

![Diagram of chromosomal territories in the nucleus.](image)
PML protein itself contains a zinc binding RING finger, two cysteine rich domains and a C-terminal coiled-coil domain (Lovering et al., 1993; Reddy et al., 1992; Perez et al., 1993). In cells from normal individuals, PML predominantly accumulates in a novel nuclear body consisting of a dense fibrillar ring surrounding a central core (Koken et al., 1994; Weis et al., 1994; Dyck et al., 1994). PML is also found with U1 snRNA and p80-coilin in a distinct compartment or zone surrounding interchromatin granules or speckles (Visa et al., 1993b; Puvion-Dutilleul et al., 1995). PML nuclear bodies are dynamic with respect to the cell cycle and there appears to be a correlation between their prominence and proliferative states (Koken et al., 1995; Terris et al., 1995). Viral infections can disrupt PML bodies (for example Puvion-Dutilleul et al., 1995; Kelly et al., 1995). For adenovirus, this disruption may be a crucial step for replication of the viral genome (Doucas et al., 1996). Treatment of cells with interferon induces PML expression and represses viral replication, thus offering an additional contribution to their antiviral activities (Guldner et al., 1992; Stadler et al., 1995).

When PML is fused to RARα in acute promyelocytic leukemia patients the normal distribution of PML in defined nuclear bodies is disrupted and a ‘micropunctate’ pattern is observed (Weis et al., 1994; Koken et al., 1994; Dyck et al., 1994). Any wild-type PML protein is also sequestered into this micropunctate pattern. Treatment of cells with retinoic acid facilitates the restoration of the normal nuclear body distribution of PML through a mechanism that is not understood (Dyck et al., 1994; Weis et al., 1994; Koken et al., 1994). This correlates with the fact that patients with acute promyelocytic leukemia go into remission following treatment with retinoic acid. It has been suggested that wild-type PML functions to suppress growth, slowing down the growth rate of transformed cell lines and suppressing their tumorigenicity (Koken et al., 1995). The molecular mechanisms by which this would be achieved remain unknown.

**WT1 nuclear domains**

Wilms’ tumor is a childhood kidney malignancy frequently associated with congenital urogenital abnormalities, thus indicating underlying developmental deficiencies. It is a complex genetic disease, with at least three genetic loci contributing to it. So far only one gene has been isolated, the WT1 tumor suppressor gene (Hastie, 1994). Mice homozygous for the WT1 knock-out die before day 15 of gestation with a clear failure to develop kidneys, gonads and a normal mesothelium (Kreidberg et al., 1993).

The product of the WT1 gene was originally thought to be a transcription factor since it contains an N-terminal proline/glutamine-rich domain, frequently associated with transcriptional activators, and four C-terminal zinc-fingers, very closely related to those found in transcription factors such as Sp1, EGR1 and EGR2. WT1 has been shown to bind to a GC-rich motif in vitro and to repress transcription in transient transfection assays in promoters that contain this motif. As yet, no physiological gene target for WT1 activity has been identified.

The subnuclear localization of WT1 in a mouse mesonephric cell line, as well as in fetal kidney and testis, showed a distinct punctate pattern as well as a diffuse nucleoplasmic staining (Larsson et al., 1995; Englert et al., 1995). Double staining clearly showed that WT1 did not occupy the nuclear transcription factor domains that were stained by Sp1. Instead, by using an anti-Sm antibody that detects snRNPs, WT1 was shown to colocalize with the snRNP ‘speckles’ (Larsson et al., 1995). WT1 was also co-immunoprecipitated with an anti-p80-coilin antibody, suggesting that WT1 is also present in coiled bodies. One recent study in transfected osteosarcoma cells showed that WT1 colocalized with only a subset of the snRNP speckles which did not stain with a monoclonal antibody against the essential non-snRNP splicing protein SC-35 (Englert et al., 1995). This raises the possibility that the WT1-rich speckles constitute a novel nuclear subcompartment that also contains snRNPs. The distribution of WT1 in the nucleus has been shown to be a dynamic one which paralleled, to a large extent, that of snRNPs. For example, microinjection in the nucleus of anti-snRNA oligonucleotides (Larsson et al., 1995) or heat shock (Charliu et al., 1995) led to an identical rearrangement for both WT1 and snRNPs in both cases. In contrast, treatment with actinomycin D, a transcriptional inhibitor, led to a re-distribution of WT1 that overlapped that observed for p80-coilin, a coiled body hallmark. In both cases, the proteins were seen to be re-distributed around the nucleolus remnants, as opposed to the majority of snRNPs which concentrated in large foci (Larsson et al., 1995).

Alternative splicing gives rise to four WT1 isoforms dependent on the inclusion or omission of two motifs: 17 amino acids encoded by exon 5 included N-terminally to the zinc-fingers, and/or the KTS motif (for lysine-threonine-serine) included between zinc-fingers 3 and 4. The presence or absence of the KTS motif significantly affects the DNA binding properties of WT1, with the +KTS WT1 isoforms having a significantly lower affinity for binding to DNA. In addition, the +KTS isoforms were seen to distribute mostly in a speckled pattern, whereas the −KTS isoforms were distributed more diffusely (Charliu et al., 1995). DNA binding-defective −KTS isoforms accumulate in the speckles, thus suggesting a correlation between DNA-binding affinity and subnuclear localization (Larsson et al., 1995; Charliu et al., 1995). Moreover, DNA-binding −KTS isoforms are sequestered into a speckled distribution by a WT1 mutant lacking the zinc-finger domain (Englert et al., 1995). These findings are significant, since naturally occurring dominant negative mutations that give rise to developmental abnormalities, have been mapped within the zinc-finger domain (Hastie, 1994). These are likely to affect not only DNA binding by the mutant WT1, but also the subnuclear distribution of any wild-type −KTS protein.

This work therefore suggests a clear subcompartmentalization of WT1 isoforms relating to WT1 function. The dynamic association of WT1 with the splicing machinery, suggests a previously unappreciated role for WT1 in post-transcriptional gene regulation, as well as in transcription per se (Charliu et al., 1995).

**PERSPECTIVE – A NUCLEAR FRAMEWORK**

The experimental data discussed here illustrate the diversity of nuclear events in their structural context. There are two major landmarks in the nucleus: (1) the nuclear envelope, associated lamina and nuclear pores (Gerace and Burke, 1988; Dingwall,
might reflect roles for these proteins that are yet to be defined. For example, a potential role for WT1 in post-transcriptional gene regulation emerges from the colocalization of this protein with speckles. The clear significance of these gene products for human disease should further stimulate research on novel functions for nuclear organelles.

In conclusion, the nucleus is revealed to have a structure far removed from an amorphous bag of chromosomes. The nuclear components and structures are assembled and are utilized with a precise temporal and spatial order. Effective nuclear metabolism appears to require a high degree of organization. It is likely that much insight into both transcription and replication will follow from definition of what this organization is, and how it is assembled and regulated.

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