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PROSPECTS

Nuclear Neighbours: The Spatial and Functional Organization of Genes and Nuclear Domains

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Abstract It is becoming clear that the cell nucleus is not only organized in domains but that these domains are also organized relative to each other and to the genome. Specific nuclear domains, enriched in different proteins and RNAs, are often found next to each other and next to specific gene loci. Several lines of investigation suggest that nuclear domains are involved in facilitating or regulating gene expression. The emerging view is that the spatial relationship between different domains and genes on different chromosomes, as found in the nucleolus, is a common organizational principle in the nucleus, to allow an efficient and controlled synthesis and processing of a range of gene transcripts. J. Cell. Biochem. 70:159–171. © 1998 Wiley-Liss, Inc.

Key words: nucleus; nuclear domain; genome; nucleolus; coiled body

The cell nucleus is one of the most clearly discernable features of the eukaryotic cell when observed through a light microscope. Various staining, immunolabelling, and in situ hybridization techniques in combination with sophisticated light and electron microscopy have shown that the nucleus itself contains separate subdomains, each with its own appearance and composition [de Jong et al., 1996]. The function of these various subdomains, however, is still unclear. Only nucleoli are known to have a defined function in the production and maturation of ribosomal RNA (rRNA) and the assembly of ribosomes. Recent studies have indicated that the nucleus is not only organized in domains but that these domains have a defined spatial relationship with each other and with the surrounding genomic DNA. Characteristic for this level of organization is that specific nuclear domains and genomic loci are positioned next to each other with little or no overlap. These discoveries have shed new light on the function of various nuclear domains and have revealed new aspects of nuclear organization.

Of the various different nuclear domains that have been identified and studied over the years, the most prominent and well characterized is the nucleolus. Interestingly, the nucleolus itself is also divided into different compartments: the fibrillar centres, the dense fibrillar components, and the granular component, each with its own morphology and protein composition [Scheer and Benavente, 1990]. Ribosomal RNA genes on different chromosomes are grouped together in the nucleolus to facilitate the expression and maturation of the rRNA. This well-defined higher order organization has been the first example of how different domains and chromosomes can be spatially organized to allow efficient gene expression and RNA processing. This prospect paper will give an overview of similar spatial and functional arrangements of other nuclear domains and genes. It will make clear that the spatial organization of protein factors and genomic loci in separate yet closely associated nuclear domains is probably a common organizational principle in the nucleus.

THE NUCLEOLUS AND COMPANY

Several nuclear domains have been reported to occur closely associated with the nucleolus: the perinucleolar compartment (PNC), the hnRNP L domain, the OPT domain, and coiled bodies. Coiled bodies have a complex and dynamic spatial relationship with the nucleolus and several other nuclear domains which will be discussed later in this paper. The PNC is an irregularly shaped domain that contains high

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concentrations of the polypyrimidine tract binding protein (PTB), also known as hnRNP I, together with specific RNA polymerase III transcripts (i.e., Y RNAs and the RNA components of RNase P and RNase MRP [Ghetti et al., 1992; Matera et al., 1995]). Images obtained from electron microscopy have shown that PNCs are in direct contact with the surface of the nucleolus and occasionally extend into the nucleolus [Huang et al., 1997]. Another hnRNP protein, hnRNP L, has a significant homology with hnRNP I and is also found concentrated in large domains at the periphery of nucleoli [Piñol-Roma et al., 1989]. However, this domain does not colocalize with the PNC [Ghetti et al., 1992]. A third large, irregularly shaped domain has been reported to be enriched in the transcription factors Oct1 and PTF and contains RNA polymerase II and III transcription sites [Grande et al., 1997; Pombo et al., 1998]; hence, it is called the Oct1/PTF/transcription (OPT) domain. The PNC, hnRNP L, and OPT domains form separate nuclear compartments around the nucleoli and do not coincide (Fig. 1A). They are usually present only in a fraction of cells in an unsynchronized tissue culture; Huang et al. [1997] showed that PNCs predominantly occur in cancer cells and are rarely found in normal primary cells, while Pombo et al. [1998] found that the OPT domain is present only in late G1 and early S phase of the cell cycle. The different domains can occur separately in different nuclei but also together in one nucleus.

The function of these nuclear domains is still unclear. However, their specific association with the nucleolus indicates that there is a functional relationship between these domains and the nucleolus. Interestingly, the OPT domain has been shown to be preferentially associated with chromosomes 2, 6, and 7 and to contain specific transcription factors and sites of RNA synthesis [Pombo et al., 1998]. It has been proposed that the organizational principles that govern the coordinated expression of rRNA genes in the nucleolus also underlie the expression of a set of specific genes in the OPT domain [Pombo et al., 1998]. It will become clear that the nucleolus may be considered a paradigm for the functional compartmentalization of RNA synthesis and processing in the eukaryotic cell nucleus.

THE COILED BODY AND THE NUCLEOLUS

One of the more illustrious nuclear domains that has been found associated with the nucleolus is the coiled body. It owes its name to its distinct appearance in the electron microscope but was already observed by light microscopy in 1903 by Ramón y Cajal. The coiled body is a small spherical structure of 0.1–1.0 μm and is present in virtually every cell type in plants and animals [Gall et al., 1995]. There are usually one to five coiled bodies per nucleus, which can be associated with the nucleolus or located in the nucleoplasm. Coiled bodies are enriched in many different proteins, including the protein p80-coilin. P80-coilin is primarily concentrated in coiled bodies, thus forming a hallmark for this nuclear compartment [Andrade et al., 1991; for reviews see Lamond and Carmo-Fonseca, 1993; Bohmann et al., 1995]. Electron microscopical images have demonstrated that coiled bodies can be intimately connected with the nucleolar periphery, often appearing to bud off from or fusing with the edge of the nucleolus [Raška et al., 1990]. It has been shown that the nucleolar association of coiled bodies is dynamic and can be enhanced by various procedures. Hepatocytes from roosters injected with the hormone estradiol showed an increased number of coiled bodies after 48 h, many of which were in contact with the periphery of the nucleolus. Four weeks after injection, the increased number and nucleolar association had subsided again [Ochs et al., 1995]. Similarly, the hepatocytes of hibernating dormice display an increased number of coiled bodies that were mostly found inside the nucleolus. The nucleolar coiled bodies rapidly disappeared when the animals were aroused from hibernation [Malatesta et al., 1994]. A comparable accumulation of coiled bodies in the nucleolus has been achieved by treating HeLa cells with the phosphatase inhibitor okadaic acid [Lyon et al., 1997], and in certain human breast carcinoma cells these nucleolar coiled bodies form spontaneously [Ochs et al., 1994]. Coiled bodies are also enriched in proteins that are typically found in the nucleolus (i.e., fibrillarin [Raška et al., 1991], Nopp140 [Meier and Blobel, 1992], NAP57 [Meier and Blobel, 1994], and ribosomal protein S6 [Raška et al., 1990]). These observations demonstrate that there is a spatial and functional relationship between the coiled body and the nucleolus. However, coiled bodies lack

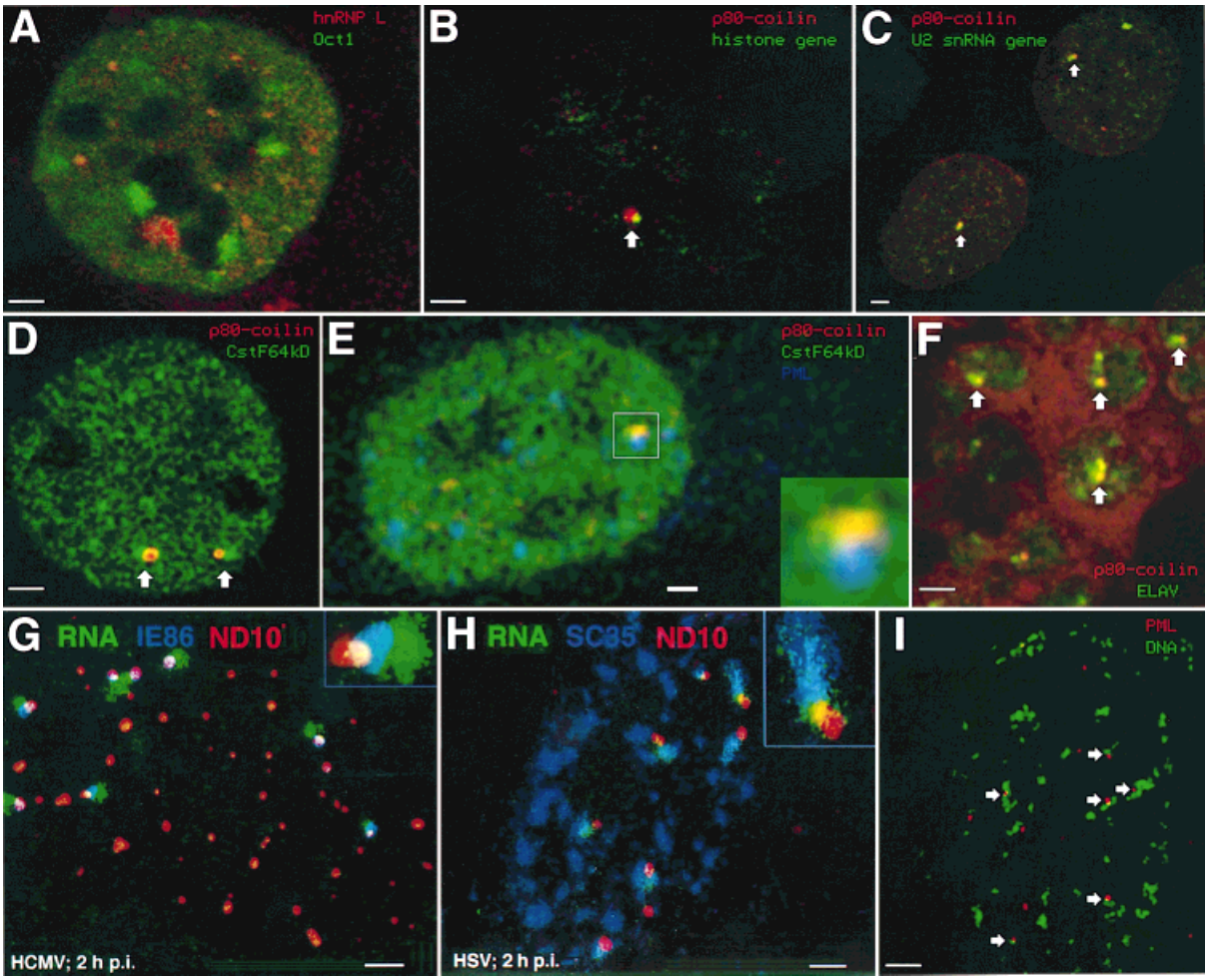

Fig. 1. Double- and triple-labelled cells reveal closely associated domains and genomic loci in their nucleus. Bars represent 2 µm. **A:** HnRNP L and the transcription factor Oct1 can be found concentrated in different domains at the periphery of nucleoli (visible here as dark areas in the nucleus). **B:** The histone genes and (**C**) the U2 snRNA genes are often located adjacent to small domains, known as coiled bodies (arrows), visualized by labelling for the protein p80-coilin. **D:** Coiled bodies are also observed adjacent to domains, called cleavage bodies (arrows), that are enriched in RNA 3' cleavage factors such as CstF 64 kD. **E:** Domains enriched in the protein PML, called PML bodies or ND10, can be found adjacent to coiled bodies and cleavage bodies, thus forming a trio of different nuclear domains. (Reproduced from EMBO Journal, vol. 15, pp. 2883–2892 by permission of Oxford University Press. **F:** Coiled bodies in the neurons of *Drosophila melanogaster* are associated with a domain enriched in the protein ELAV (arrows), known as the ELAV dot and the ELAV web. (Image provided by Dr. Y. Yannoni.) **G:** In cytomegalovirus-infected cells, PML bodies (ND10) are adjacent to sites of viral transcription (RNA) and domains enriched in the viral protein IE86. (Reproduced from The Journal of Cell Biology, 1997, vol. 138, pp. 5–16 by copyright permission of The Rockefeller University Press.) **H:** The PML bodies (ND10) are also associated with domains enriched in splicing factors (SC35) which overlap with the viral transcripts (RNA), shown here for a herpes simplex virus–infected cell. (Reproduced from The Journal of Cell Biology, 1997, vol. 138, pp. 5–16 by copyright permission of The Rockefeller University Press.) **I:** In uninfected cells, PML bodies are found associated with DNA that replicates in middle–late S phase (arrows), visualized here by BrdU labelling. All images are confocal sections. (Reproduced from Grande et al., 1996, by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

several other nucleolar proteins (i.e., RNA polymerase I, nucleolin, B23 [Raška et al., 1991] and No55 [Ochs et al., 1996]). Moreover, coiled bodies are often located in the nucleoplasm, and they are enriched in proteins that are typically found in the nucleoplasm (e.g., snRNP splicing factors [Carmo-Fonseca et al., 1991, 1992] and RNA polymerase II transcription factors [Grande et al., 1997; Jordan et al., 1997]). It is therefore likely that coiled bodies also have a nucleoplasmic function. It has been hypothesized that coiled bodies may migrate through the nucleoplasm and periodically contact the nucleolus as part of their elaborate nuclear

function. Alternatively, coiled bodies may form and disassemble in different locations wherever their presence may be required. It is likely, however, that the kinetic properties of their different functions may result in a steady-state distribution between the nucleoplasmic and the nucleolar-associated state. This distribution may be different between cell lines and could be shifted by changing the metabolic state of the cells as described above.

The relationship between coiled bodies and nucleoli is one of several spatial associations coiled bodies have with different nuclear domains. It is nonetheless a good example of an intimate and dynamic relationship between distinct nuclear domains.

THE COILED BODY IN THE NUCLEOPLASM

Recently, a number of reports have given important new insight into the possible nucleoplasmic function of the coiled body. These studies indicate that nucleoplasmic coiled bodies can be associated with several nuclear domains and a selection of genomic loci. Frey and Matera [1995] have shown that the histone gene clusters, located on chromosome 1 and 6, are frequently found adjacent to coiled bodies [Frey and Matera, 1995] (Fig. 1B). Importantly, they also demonstrated that coiled bodies themselves are enriched in U7 snRNA, which is essential for 3' processing of the histone transcripts [Marzluff, 1992]. Also, the homologues of coiled bodies in amphibian oocytes, called sphere organelles or C snurposomes, contain U7 snRNA and are associated with the histone loci on lampbrush chromosomes [Wu and Gall, 1993; Callan et al., 1991]. The histone genes probably make use of the high concentrations of U7 snRNP, and possibly other RNA processing factors, present in the coiled body. These findings show a striking spatial and functional relationship between nuclear domains and specific genomic loci, in which the loci are adjacent to the domains and not overlapping with them.

The genes for U1 and U2 snRNA, which are arranged as multicopy gene clusters on chromosomes 1 and 17, respectively, are also often found adjacent to coiled bodies (Fig. 1C) [Frey and Matera, 1995; Smith et al., 1995]. Additionally, we found that the snRNA gene-specific transcription factor PTF and the TATA binding protein (TBP), which are necessary for snRNA gene expression [Henry et al., 1995; Lobo and Hernandez, 1994], are both concentrated in and around coiled bodies and overlap with the snRNA genes adjacent to the coiled bodies [Schul et al., 1998a]. This indicates that the snRNA genes adjacent to coiled bodies are probably active, similar to the histone genes. We have recently found also that genes that produce small nucleolar RNA (snoRNA) are preferentially associated with coiled bodies [Schul et al., manuscript in preparation]. Similarly, Matera and coworkers found the U3 snoRNA genes frequently adjacent to coiled bodies [Gao et al., 1997]. Interestingly, coiled bodies are enriched in fibrillarin which can bind to snoRNA to form snoRNP complexes [Jansen et al., 1991]. SnoRNP complexes are found concentrated in the nucleolus [Matera et al., 1994] and have recently been shown to be involved in rRNA cleavage and methylation [Kiss-László et al., 1996; Maxwell and Fournier, 1995]. It should be noted that the relationship between coiled bodies and snoRNA-producing genes provides, for the first time, a link between the nucleoplasmic role of the coiled body and its nucleolar association.

Although various experiments have confirmed that coiled bodies do not contain DNA or nascent RNA [Raška et al., 1991, 1995; Moreno Diaz de la Espina et al., 1982; Jordan et al., 1997], immunofluorescent labelling of transcription sites by incorporation of 5-bromo-UTP (BrUTP) into newly synthesized RNA has revealed that the immediate surroundings of coiled bodies do contain several active genes [Schul et al., 1996; Jordan et al., 1997]. These active genes probably include the U1 and U2 snRNA, the histone, and the snoRNA-producing genes. Importantly, Smith et al. [1995] reported that several U1 and U2 snRNA genes can be found grouped around a single coiled body, similar to the clustering of rRNA genes from different chromosomes in the nucleolus. Gao et al. [1997] describe a similar association of U2 snRNA genes and U3 snoRNA genes with one coiled body. These findings provide a strong indication that a nucleolus-like organization of related genes and protein factors in closely associated domains is a common principle of efficient gene expression in the nucleus.

Indications that the coiled body is indeed involved in the production and maturation of transcripts from nearby genes has come from the immunolabelling of factors involved in the 3' processing of polyadenylated RNAs. The 100 kD subunit of the cleavage and polyadenylation

specificity factor (CPSF) and the 64 kD subunit of the cleavage stimulation factor (CstF) have been found concentrated together in small spherical domains that resembled coiled bodies [Schul et al., 1996]. These domains, called cleavage bodies, were often adjacent to coiled bodies but could also be found partially or completely overlapping with coiled bodies (Fig. 1D). Interestingly, the cleavage bodies adjacent to coiled bodies contained newly synthesized RNA, visualized by BrUTP labelling, while inhibition of RNA polymerase II transcription resulted in the complete colocalization of coiled bodies and cleavage bodies. We have proposed the model that the cleavage factors CstF 64 kD and CPSF 100 kD are concentrated in coiled bodies and can be distributed to one or more active genes adjacent to the coiled body, but relocate to the coiled body when the genes are inactive [Schul et al., 1996]. We now have indications that the cleavage bodies are affiliated with a cell cycle–regulated gene that can associate with coiled bodies [Schul et al., manuscript in preparation]. These results support the idea of a dynamic spatial and functional organization of genes and ultrastructural domains in the nucleus.

More and more reports are coming in on nuclear domains that can be found adjacent to coiled bodies, some of them linked to serious pathological phenotypes and developmental disorders. Liu and Dreyfuss [1996] have found that the protein SMN is concentrated in a few small round nuclear domains, called gemini of coiled bodies or gems, which were often found next to coiled bodies or partially overlapping with them. Patients with spinal muscular atrophy, a severe neuromuscular disorder, have a disruption in one of the genes for SMN and have a significant reduction in the number of gems in their cell nuclei [Coovert et al., 1997; Lefebvre et al., 1997]. Interestingly, the SMN protein can interact with fibrillarin, one of the proteins enriched in coiled bodies.

The protein PML has also been found concentrated in many small round nuclear domains, called PML bodies, ND10, or PODs. PML bodies are associated with various nuclear components and processes similar to coiled bodies which will be discussed later in this paper. We will mention here only that PML bodies are often found adjacent to coiled bodies and cleavage bodies [Grande et al., 1996; Ishov and Maul, 1996; Schul et al., 1996], sometimes forming trios of these nuclear domains (Fig. 1E). Importantly, in patients with acute promyelocytic leukemia, the PML protein is fused to the retinoic acid receptor α due to a t(15;17) chromosomal translocation which has fused the genes of both proteins [Warrel et al., 1993]. These patients have no PML bodies, but the administration of retinoic acid results in the reappearance of the PML bodies and remission of the patients [Dyck et al., 1994; Koken et al., 1994; Weis et al., 1994].

Berciano et al. [1996] used light and electron microscopy to look at the nuclei of Schwann cells from a patient with acute Guillain-Barré syndrome, which is characterized by severe segmental demyelination and damage to axons. The patient's cells showed an increased number of nucleoplasmic coiled bodies compared to control cells. Interestingly, these coiled bodies were regularly found associated with other nuclear bodies which had a fibrillar or granular appearance. Another remarkable nuclear domain that has been reported to associate with coiled bodies occurs in the neurons of *Drosophila melanogaster* and is enriched in the protein ELAV. The ELAV protein has an RNA-binding domain and is thought to be involved in alternative splicing of specific transcripts [Koushika et al., 1996]. ELAV is essential for the formation and the maintenance of the nervous system of the fly, and hypomorphic mutations of the *elav* gene produce aberrant eye structures, defective electroretinograms, and flight defects [Campos et al., 1985]. Immunofluorescent labelling showed that ELAV is concentrated in domains that contain a brightly labelled dot, often positioned adjacent to the nucleolus, and a more diffusely labelled area close to the ELAV dot, referred to as the ELAV web. Coiled bodies are often found in the ELAV web and sometimes partially or completely overlap with the ELAV dot (Fig. 1F) [Yannoni and White, 1997].

Over the years, several investigators have remarked about the occurrence of small, round nuclear domains located next to each other. Brasch and Ochs [1992] observed paired nuclear bodies in the nucleoplasm of hormone-treated cells studied by electron microscopy, and Ascoli and Maul [1991] found doublets of different nuclear dots when stained with particular autoimmune sera. Ishov and Maul [1996] reported that Hep-21 cells contained foci enriched in the transcription factor NF-1, mostly located adjacent to coiled bodies. It should be emphasized

that although coiled bodies can be closely associated with several different nuclear domains and can even be found inside nucleoli, the coiled body remains a separate nuclear compartment with its own distinct morphology and protein composition. The cleavage bodies, gems, PML bodies, and ELAV dots are just a few striking examples of nuclear domains that have a complex and dynamic spatial relationship with coiled bodies and with the RNA synthesis and processing machinery. In the final paragraph we will propose a model in which we have incorporated the various associations of coiled bodies with specific genes and nuclear domains.

PML BODIES AND THEIR VIRAL NEIGHBOURS

The aforementioned PML bodies are similar in size and shape to coiled bodies but occur in higher numbers, usually 10–30 per nucleus, in virtually every cell type. They are enriched in several proteins (i.e., PML, sp100, PIC1, and Int-6 [Stuurman et al., 1992; Szostecki et al., 1987; Boddy et al., 1996; Desbois et al., 1996]), which can be continuously incorporated into and released from these domains [Stuurman et al., 1997]. However, neither the function of the PML bodies nor the exact function of the proteins is known. Similar to coiled bodies, PML bodies are found associated with other nuclear domains, including coiled bodies, cleavage bodies, and irregularly shaped domains enriched in the protein 1SG20 [Gongora et al., 1997].

Important new insight into the nuclear function of PML bodies has come from virus-infection studies. The spatial association of nuclear domains during virus infection turns out to be one of the most striking examples of higher order nuclear organization. Infection of cells with DNA viruses such as adenovirus 5 (Ad5), herpes simplex type-1 (HSV-1), cytomegalovirus (HCMV), and simian virus 40 (SV40) causes the disruption of PML bodies. To this end, each different virus has its own type of immediate-early protein that first concentrates inside PML bodies and then causes their disruption [Maul et al., 1993; Everett and Maul, 1994; Carvalho et al., 1995; Korioth et al., 1996]. However, this disruption is a late event that happens many hours after infection. In the early stages of viral infection, PML bodies are located adjacent to the viral DNA and domains enriched in viral proteins. Ishov and Maul [1996] showed that input Ad5 viral DNA is found concentrated in domains next to PML bodies 4 h after infection. Infection with an Ad5 virus, containing a mutation in the E4ORF3 gene which disables the virus's ability to disrupt PML bodies, revealed that all viral replication sites are eventually accompanied by a PML body. Also SV40, HSV-1, and HCMV DNA were found concentrated adjacent to PML bodies in early stages of infection. Electron microscopy data have confirmed that PML bodies are in direct contact with the domains containing the viral DNA [Ishov and Maul, 1996]. Interestingly, these studies also showed that coiled bodies were regularly located adjacent to PML bodies, although these coiled bodies were never in contact with the viral domains.

Recent studies have revealed that there are several different nuclear domains closely associated with sites of viral transcription and replication. Ishov et al. [1997] showed that the cytomegaloviral protein IE86 is concentrated in domains next to PML bodies and next to sites containing viral DNA (Fig. 1G). Carvalho et al. [1995] showed the accumulation of large T antigen next to PML bodies in SV40-infected cells. Interestingly, cells transformed with just the gene for the SV40 large T antigen show an accumulation of this protein adjacent to PML bodies without the presence of viral DNA or other viral proteins [Jiang et al., 1996]. Similarly, Lukonis and Keller [1997] found that the HSV-1 protein UL29 independently localized adjacent to PML bodies. The viral proteins can apparently form domains inside the nucleus which specifically associate with other nuclear domains (i.e., PML bodies).

Ishov et al. [1997] demonstrated that, in infected cells, the immediate early transcripts of the human cytomegalovirus appear only adjacent to PML bodies. The areas containing the viral transcripts did not overlap with the aforementioned IE86-enriched domains which are also found adjacent to PML bodies. These three domains therefore form closely associated clusters in the nucleus (Fig. 1G). Ishov et al. [1997] additionally showed that the viral transcripts extend into large, irregularly shaped domains enriched in splicing factors, referred to as nuclear speckles (described more elaborately in the next section and in Spector [1993]). Consequently, several speckles were found next to PML bodies and next to the domains enriched in viral DNA. Additionally, the viral transcripts were found to extend into the speckles (Fig. 1H). Detailed observations indicated that the

IE86 domain interposed between the PML body and the nuclear speckle or was located immediately beside the apparent attachment between the PML body and the nuclear speckle. Ishov et al. [1997] proposed that viral genomes are positioned at preexisting sites with high transcriptional potential where transcripts pass through several specialized domains for efficient RNA processing. An adapted form of this model will be presented in the last section of this paper.

These studies on PML bodies in virus-infected cells clearly indicate a functional relationship between PML bodies and viral infection. The finding that interferon upregulates the expression of all constituents of the PML body [Guldner et al., 1992; Chelbi-Alix et al., 1995; Lavau et al., 1995] and the observation that viruses disrupt PML bodies before viral replication begins point to a role for PML bodies in the cell's defence mechanism against viruses.

The observations on PML bodies and sites enriched in viral DNA and proteins form another clear example of the spatial association of different nuclear domains with a complex functional relationship. Interestingly, there are indications that PML bodies are not only associated with viral DNA. Grande et al. [1996] have shown that PML bodies are often found juxtaposed to human genomic DNA that replicates in middle–late S phase (Fig. 1I). It is believed that different genomic elements replicate during a defined stage in S phase, with the most active genes first and the centromeres last. However, it is unknown what DNA replicates at middle–late S phase [Goldman et al., 1984; Hatton et al., 1988]. Although it is unclear what the role PML bodies play in the nucleus, the spatial association with other nuclear domains and specific genomic or viral loci is probably a fundamental aspect of their nuclear function.

NUCLEAR SPECKLES AND ASSOCIATED GENES

The association of genes and transcripts with the nuclear speckles is a phenomenon that not only occurs in combination with viral infection. There are about 10–50 speckles in each cell nucleus, and they are enriched in splicing factors [Fu and Maniatis, 1990; Spector et al., 1991], hyperphosphorylated RNA polymerase II [Bregman et al., 1995; Mortillaro et al., 1996], and poly(A) RNA [Carter et al., 1991]. Although this suggests a role for the speckles in the formation and maturation of mRNA, speckles contain little newly synthesized RNA [Fakan and Bernhard, 1971, 1973; Wansink et al., 1993] and are devoid of DNA [discussed in Thiry, 1995]. The function of speckles has remained unclear. Recent findings, however, have shown that highly active genes are often found adjacent to nuclear speckles, indicating that the periphery of the speckles may be a site of proficient transcription and RNA processing.

Evidence towards these ideas has come from localization studies of specific genes and gene transcripts. This demonstrated that active fibronectin, β-actin, and collagen Iα1 genes are frequently located at the periphery of nuclear speckles, while inactive genes are rarely found there [Xing et al., 1993, 1995]. As expected, transcripts of the fibronectin, β-actin, collagen Iα1, and *c-fos* genes were found closely associated with the speckles [Huang and Spector, 1991; Xing et al., 1993, 1995]. Visualization of sites of transcription by immunofluorescent detection of BrUTP-labelled nascent RNA has confirmed that RNA synthesis takes place only at the periphery of speckles and not in the interior [Wansink et al., 1993; Pombo and Cook, 1996]. It should be noted, however, that these studies clearly indicate that many sites of RNA synthesis are in between the speckles, not associated with their periphery. Also, in situ hybridization studies have shown that not all active genes are found associated with the nuclear speckles [Zhang et al., 1994; Dirks et al., 1997].

We have found that factors involved in RNA polyadenylation (e.g., poly(A) polymerase and poly(A) binding protein II) occur together in elevated amounts at the edge of the speckles [Schul et al., 1998b]. Interestingly, Carter et al. [1993] noticed that the nuclear domains enriched in poly(A) RNA were found not only to colocalize with speckles but also to extend beyond the edge of the speckles to the immediate surroundings, probably overlapping with the genes at the speckle's periphery [Carter et al., 1993]. The same distribution has been found for the poly(A) binding protein II [our unpublished results]. Taken together, these findings indicate that the periphery of the speckles is a specific compartment where active genes may be efficiently supplied with the RNA processing factors they need. It is unclear, however, whether the association between genes and speckles can exist without RNA synthesis. Xing et al. [1995] showed that the collagen gene remains closely associated with nuclear speckles after transcription has been inhibited and

no collagen transcripts can be detected, but Dirks et al. [1997] found that HCMV immediate-early genes were accompanied by speckles only when they were active. A model towards the spatial organization of genes and gene transcripts in relation to nuclear speckles is presented in the next section.

In conclusion, the coordination of genes and RNA processing factors in and around nuclear speckles is yet another example of the spatial and functional organization of distinct nuclear domains and genomic loci to facilitate the production and processing of RNA transcripts.

An indication that speckles are associated not only with active genes but also with specific nuclear domains comes from electron microscopical studies. In the electron microscope, nuclear speckles mostly appear as clumps of 20–25 nm large granules located in the interchromatin space, referred to as interchromatin granule clusters (IGCs) [Thiry, 1995]. Visa et al. [1993] identified in HeLa cells a nuclear domain adjacent to IGCs with a distinct fibrillar appearance, called the interchromatin granule-associated zone. This domain was found to be enriched in U1 small nuclear RNA, p80-coilin, PML, and sp100 [Visa et al., 1993; Puvion-Dutilleul et al., 1995a,b], which are also found concentrated in IGCs, coiled bodies, and PML bodies. Although the function of the interchromatin granule-associated zone is still completely unknown, there is an obvious relationship in composition and spatial association with other nuclear domains.

NUCLEAR DOMAINS AND GENE EXPRESSION

It is widely accepted that the cell nucleus contains different domains that are in a direct or indirect way involved in the synthesis and/or processing of RNA. The view that these domains have specific spatial relationships with each other and with defined genomic loci has only recently emerged. Only the nucleolus was known to be a nuclear compartment where different domains (i.e. the fibrillar centres, dense fibrillar component, and the granular component) and specific genes from different chromosomes are spatially and functionally associated with each other in order to produce RNA in an efficient and coordinated manner (Fig. 2A). It now turns out that this higher order organization of nuclear domains and genomic loci is probably a more general nuclear principle of facilitating and/or regulating gene expression in the nucleus.

One striking example, the coiled body, has not only a dynamic spatial relationship with the nucleolus but can also be associated with several genes in the nucleoplasm. There it probably functions as a coordination centre from where specific transcription and processing factors are supplied to neighbouring genes to facilitate and possibly regulate their expression (Fig. 2B). Other nuclear domains (e.g., cleavage bodies, gems, ELAV domains, and PML bodies) can be juxtaposed to coiled bodies. These domains may cooperate with the coiled body or perform a separate function on coiled body–associated genes. These functions could include highly efficient initiation of transcription or splicing, alternative splicing, unusual 3' processing, or RNA modification. At the coiled body, genes from different chromosomes can be grouped together to allow efficient and controlled transcription and RNA processing, comparable to the organizational structure of the nucleolus.

Little is known about the function of PML bodies, but there are indications that they may play a role in the defence against viral infection, as discussed earlier. Strikingly, the viral expression system makes use of the spatial organization of viral and endogenous nuclear domains to facilitate the production of viral RNA and DNA. PML bodies might associate with these domains to regulate and/or suppress the high levels of RNA production there. Similarly, in uninfected cells, PML bodies may be associated with genes that need to be carefully controlled or suppressed, including possible oncogenes (Fig. 2C). Interestingly, PML can suppress cell growth and suppress oncogenic transformation by cooperative oncogenes, and it suppresses transformation of NIH 3T3 cells by the activated neu oncogene [Mu et al., 1994, 1996; Liu et al., 1995]. Some of the PML body–controlled genes may also be associated with coiled bodies, explaining the spatial association of PML bodies and coiled bodies. However, little is known about the putative PML body–associated sequences, only that they are probably replicated at middle–late S phase. Nonetheless, we again see nuclear domains and specific (viral) genomic loci grouped together to organize and regulate gene expression.

Finally, nuclear speckles are also found associated with specific genes and nuclear domains. Speckles are enriched in hyperphosphorylated

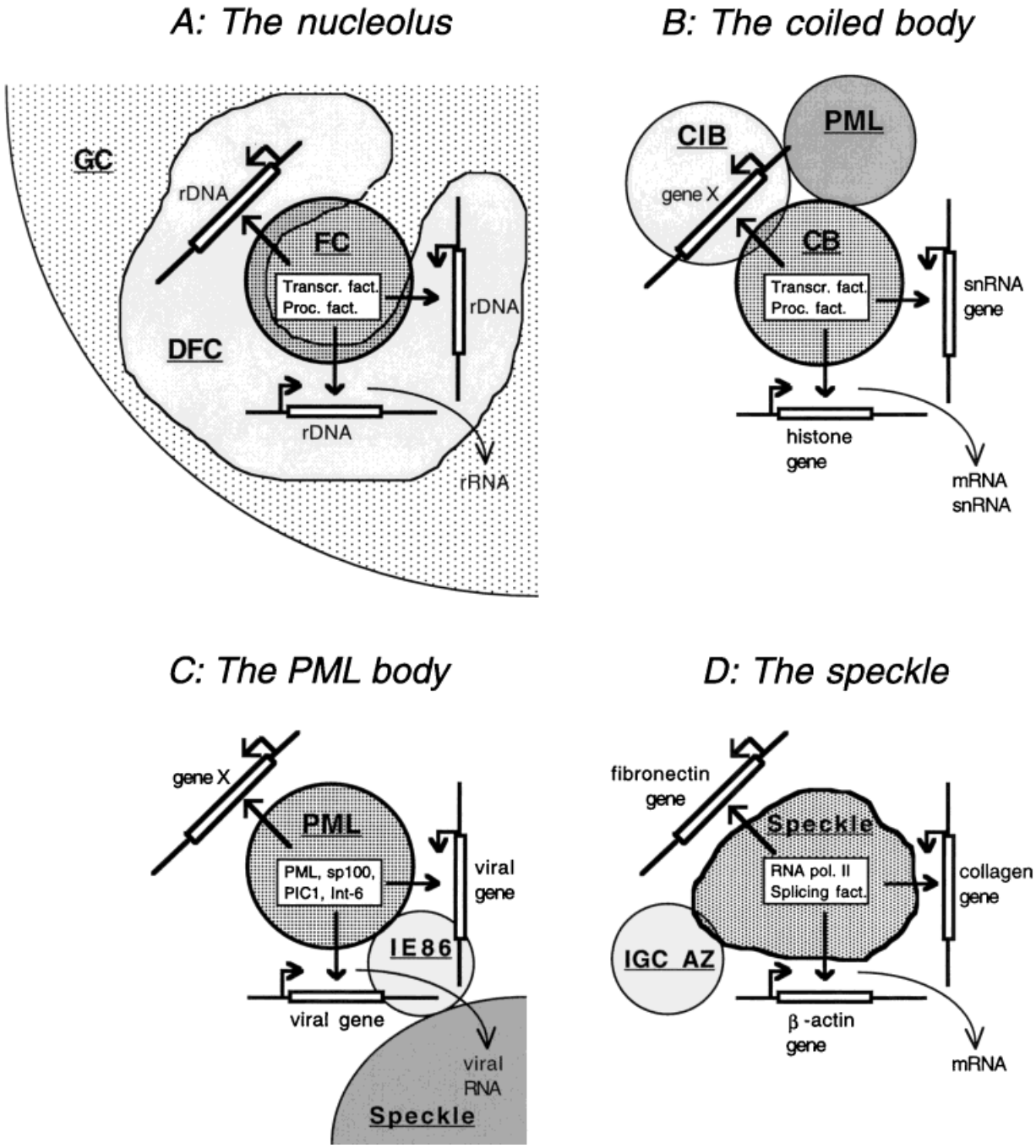

Fig. 2. Drawings representing a proposed common principle in the spatial organization of specific genes and domains in the nucleus. **A:** The nucleolus contains rRNA genes (rDNA) from different chromosomes that are located in closely associated nucleolar domains (i.e., the fibrillar centers (FC) and the dense fibrillar components (DFC)) where transcription and processing factors are concentrated to allow efficient and regulated rRNA production. The rRNA subsequently passes to another nucleolar compartment, the granular component (GC). **B:** Coiled bodies (CB) can have a similar arrangement of genes from different chromosomes around them. Among these are the U1 and U2 snRNA genes, the histone genes, and probably other genes (gene X). There are transcription and processing factors present in coiled bodies that can facilitate and regulate the expression of the associated genes. Other nuclear domains such as cleavage bodies (ClB) and PML bodies (PML) are found associated with coiled bodies and may be involved in the synthesis or processing of RNA transcripts. **C:** Comparably, in virus-infected cells, PML bodies (PML) are adjacent to the viral DNA and to domains enriched in viral proteins (IE86). Viral transcripts are passed to another associated nuclear domain (speckle), which is enriched in splicing factors. The various proteins in PML bodies may be involved in repressing the viral genes and may play a similar role on specific endogenous genes (gene X). **D:** Nuclear speckles can be associated with highly active genes from different chromosomes (e.g., the fibronectin gene, the collagen Iα1 gene, and the β-actin gene). RNA polymerase II and splicing factors are probably distributed from speckles to adjacent genes to facilitate and/or regulate the expression of these genes. Other domains, such as the interchromatin granule cluster–associated zone (IGC AZ), may play an additional role in this process. The common organizational principle among these nuclear conglomerates is the association of genes, located on different chromosomes, with one or more nuclear domains from where specific factors may be distributed to the genes to allow efficient and regulated gene expression.

RNA polymerase II, splicing factors, and several polyadenylation factors. They probably function as efficient suppliers of these factors to highly active genes that are associated with the periphery of the speckles (Fig. 2D). The rapidly produced transcripts are probably only temporarily associated with the speckles since speckles contain little newly synthesized RNA. Speckles may also play a role in alternative splicing of transcripts, as proposed by Melcak and Raška [1996]. The role of nuclear domains associated with speckles (e.g., the interchromatin granule-associated zone) is still unclear. However, the coordinated and efficient production of RNA from different genes around an organizational centre is again comparable to the association of genes around coiled bodies and in the nucleolus.

Many questions about nuclear domains are still unanswered. How are the components of a nuclear domain held together without an enveloping membrane? It could be protein-protein interactions between the various constituents of a domain, it could be binding of the proteins to local concentrations of specific RNAs, or there could be components inside the domains that form the structural framework of these nuclear compartments. The next question is how these large domains are associated with each other or with specific genomic loci and yet are discernable as separate compartments in the light microscope. Again, interactions between proteins and RNAs may be involved, although it seems clear that these interactions are not strong enough to disrupt the cohesion of the individual domains. It is also possible that different domains are associated with the same genes without direct interactions between the domains themselves. Whatever the mechanisms, it appears there is an important role for these domains as distinct nuclear entities on a macromolecular level.

How dynamic and mobile are these domains, and how are they assembled or disassembled in the nucleus? Still very little is known about how, where, and when these domains are formed. The clearest evidence with regards to the dynamics of nuclear domains has come from experiments by Spector and coworkers, who used fusions between the green fluorescent protein and proteins enriched in nuclear domains, allowing the visualization of domains in living cells. This has revealed for both the perinucleolar compartment and the nuclear speckles that, although these domains are dynamic, they persist for hours as distinct nuclear compartments while they change shape or move through the nucleus [Huang et al., 1997; Misteli et al., 1997]. It is completely unclear whether the domains exist as independent and stable nuclear compartments which supply factors to genes that associate with them or whether they are formed near genes only when they are required [discussed by Singer and Green, 1997]. It is evident that we are only just beginning to understand the spatial and functional organization of the nucleus and the role this organization plays in gene expression. New and more refined techniques should allow a detailed study of nuclear components and nuclear domains in time and space.

In summation, proteins and RNAs are not only concentrated in domains in the nucleus, but these domains are also organized relative to each other. The spatial association between nuclear domains can be temporary and dynamic and is dependent on the activational state of the cell. In addition, the nuclear domains can be associated with specific genes, probably to facilitate or regulate the production and maturation of RNA. The spatial and functional organization of different domains and specific genomic loci in higher-order structures as found in the nucleolus is probably a common organizational principle for controlled and efficient gene expression in the cell nucleus.

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