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Multifunctional Compartments in the Nucleus: Insights from DNA and RNA Localization

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The long-standing view of the mammalian nucleus is rapidly changing. Through the work of several labs our knowledge of how genes, chromosomes and RNA metabolic components integrate into the structure of the nucleus has expanded greatly. Nuclear structure may be considered in terms of two different but related general aspects: spatial compartmentalization, meaning the localization or concentration of specific sequences or macromolecules at a discrete site, and matrix association; meaning the association of macromolecules with insoluble nuclear components which resist biochemical fractionation [Berezney and Coffey, 1974; Fey et al., 1986; reviewed in Nickerson et al., 1995; Stein et al., 1994]. These two may be intimately related in that, to the extent to which nonrandom nuclear compartmentalization exists, it is plausible that the insoluble matrix material provides a structural framework for much if not all of the compartmentalization.

From our perspective, to explore fundamental aspects of nuclear structure it was essential to investigate the distribution of specific genes and RNAs relative to the other functional and structural components of the nucleus. To open an avenue which made this possible, we focused on the development of powerful in situ hybridization technology for the detection of specific genes and RNAs [Lawrence et al., 1988, 1989; Xing et al., 1993, 1995], substantially expanding the capabilities of non-isotopic labeling techniques that had been developed previously [Langer-Safer et al., 1982; Landegent et al., 1987; Manning et al., 1975].

The localization of genes and RNAs has provided a number of new insights into nuclear structure, some of which challenge our earlier views. This prospectus will discuss some of our current thinking concerning two aspects of nuclear structure for which major new insights have been obtained by studies of the precise nuclear distribution of DNA and RNA. These results enhance our view of how RNA metabolism for specific genes is spatially integrated into nuclear structure, and provide substantial new evidence for the involvement of a specific type of nuclear RNA in the underlying structure of the nucleus and chromosome.

A particularly revealing discovery came from the investigation of RNA from the *XIST* gene. The *XIST* gene was initially identified as a potential candidate for regulation of X chromosome inactivation as it maps to the X inactivation center and is the only gene expressed exclusively from the inactive X chromosome (Xi) [Brown et al., 1991]. Unexpectedly, it was shown that the *XIST* gene had no open reading frame, and the *XIST* RNA was exclusively nuclear [Brown et al., 1992; Brockdorff et al., 1992]; suggesting that this RNA could represent a novel class of functional nuclear RNAs. Subsequently, there was a growing body of developmental and clinical evidence suggesting that the *XIST* RNA was, in fact, involved in the process of X inactivation [see for example Kay et al., 1993; 1994; McCarey and Dilworth, 1992; Richler, 1992; Salido et al., 1992; Migeon et al., 1994; Wolff et al., 1994]. We reasoned that if *XIST* RNA itself has a direct role in X inactivation, it might be expected that it would be spatially coincident with all or most of Xi, the site of its purported function. Examining, in 2 and 3-D space, the relationship of the *XIST* RNA to Xi, it was shown that the RNA and Xi not only occupied identical planes of focus, but the size and shape were almost identical [Clemson et al., 1996]. The strict relationship between the *XIST* RNA and Xi is unique as other protein coding RNAs do not 'paint' their

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parent chromosomes. These results on *XIST* RNA expand our concepts of the likely relationship between RNA, nuclear structure or compartmentalization, and gene expression.

Much evidence shows that the bulk of hnRNA is non-polyadenylated and turns over within the nucleus with no known function; whereas at least the vast majority of poly A RNA encodes pre-mRNA [Herman et al., 1978; Salditt-Georgieff et al., 1981; Harpold et al., 1981; Lewin 1990]. Interestingly, we have found evidence for two populations of *XIST* RNA in the nucleus, one that represents relatively short-lived nascent transcripts that contain introns, and a processed RNA that is apparently stable [Clemson et al., 1996]. Some studies have suggested a fundamental role of RNA in nuclear architecture [for example Nickerson et al., 1989]. Several results suggest that the *XIST* RNA may be a candidate for such a structural RNA. These include the findings that while the *XIST* RNA consistently "paints" the whole inactivated interphase chromosome, it is retained after digestion of chromosomal DNA and does not appear to hybridize substantially to Xi DNA [Clemson et al., 1996]. Apparently the *XIST* RNA is associated with insoluble nuclear components closely aligned with Xi, but is unlikely an integral part of the chromatin itself. These results not only provide insight into the mechanisms governing dosage compensation and X inactivation in mammalian females but also potentially identify a specific precedent for RNA involvement in nuclear/chromatin packaging.

From these results we proposed that the stable component of the *XIST* RNA is functional in the nucleus, perhaps acting as a bridge to insoluble structures of the nuclear matrix [Clemson et al., 1996]. In this manner it could possibly contribute to the definition of the chromosome territory in a variety of novel ways including: defining the shape and size of the chromosome; contributing to the condensation of the chromatin into heterochromatin, or by directly regulating the transcriptional activity of the Xi chromatin. As discussed further below, the inactive X chromosome shows a reproducible sequestration relative to other nuclear components implicated in RNA metabolism, which are concentrated and compartmentalized in discrete regions of the nucleus. This suggests the intriguing possibility that the *XIST* RNA could contribute to the transcriptional silencing of Xi by sequestering it to a certain nuclear territory devoid of RNA metabolic components. Some current observations concerning the compartmentalization of the nucleus, and *XIST* RNA's relationship to this, are considered below.

While in the traditional established view of the nucleus, the nucleolus is the singular well-recognized nuclear compartment, there have been hints for over two decades that the nucleoplasm contains other nuclear compartments. Early electron microscopic studies described various types of non-membrane bounded nuclear structures, such as nuclear bodies and interchromatin granule clusters [Bernhard, 1969; Fakan and Puvion, 1980]. In other early work, different antigens detected with autoimmune sera were observed to produce various types of spotted or "speckled" patterns within the nucleus [Lerner et al., 1981; Spector et al., 1983; Nyman et al., 1986]. In several cases the structures visualized by e.m. have been found to correlate to structures identified by immunofluorescence to specific antigens. For example, antibodies to snRNPs or the spliceosome assembly factor SC-35 create a heterogeneous pattern similar to the autoimmune serum "speckles" with about 30–40 sites of very high concentration, most or all of which correspond to ultrastructural regions termed interchromatin granule clusters (IGCs) [Spector et al., 1991; Fu and Maniatis, 1990, reviewed in Fakan, 1994]. Similarly, an antibody to the coilin protein identifies a spot-like pattern shown to correspond to 1–6 coiled bodies seen by e.m., which are most prominent in transformed cells [Raska et al., 1991]. Antibodies, including those to a protein mutated in promyelocytic leukemia, detect ND 10 (nuclear dot 10), also called PML domains, corresponding to a subset of approximately 10 ultrastructural nuclear bodies that have now been linked to the primary genetic defect in Promyelocytic leukemia. While the function of these domains is unresolved, they all provide evidence that the nucleoplasm is compartmentalized into distinct and different regions in which macromolecules involved in specific biochemical processes concentrate. Although we are just beginning to elucidate the structural and functional nature of these compartments, their existence is now well established and their potentially critical significance for the global function of the cell is increasingly appreciated.

In considering the overall structure of the nucleus two fundamentally distinct possibilities may exist which bear both on the potential func-

tions of the aforementioned nuclear compartments as well as the relationship of the genome to nuclear structure as a whole. First, the genome may be spatially arranged in a manner which is independent of compartments enriched in other nuclear constituents, such as splicing components. These domains or speckles could be accumulations of excess, inert factors for example. If this were the case, the domain or compartment need not show any particular relationship to specific genetic loci, which might be randomly localized with respect to them. Alternatively, the localization of specific genomic sequences may be structurally and functionally integrated with distinct "compartments" enriched in RNA and RNA metabolic components. The latter hypothesis is one we have forwarded based on the thinking that some nuclear compartments enriched in RNA metabolic factors are associated with sites of enhanced transcription and processing. If so, there would necessarily be a non-random spatial relationship of the factors that identify these compartments with specific genetic loci. An intriguing extension of this is that some genes might cluster at sites of high metabolic activity, possibly facilitating the expression of their RNAs.

To distinguish between these different types of global nuclear architecture, we investigated whether specific genomic loci show random or non-random relationships relative to two different nuclear compartments. Results demonstrated that for both domains enriched in SC-35 and poly A RNA (discussed below) [Xing et al., 1993; Xing et al., 1995] and for coiled bodies [Smith et al., 1995] there is a highly non-random spatial association of specific genetic loci with these nuclear compartments; some loci preferentially localize with these domains whereas others do not. Frey and Matera [1995] also found that the coiled body associated with specific genetic loci and work from that lab and our own is in agreement that the localization of certain snRNA and histone genes with the coiled body represents a specific association, but that the relationship is variable and likely dynamic, such that the frequency of association can vary even between closely related cell lines. The co-localization of specific genetic loci with nuclear bodies or domains is reminiscent of the relationship of nuclear spheres with histone loci previously described for lampbrush chromosomes [Gall et al., 1981].

We believe that the demonstration of a non-random relationship between specific genetic loci and these nuclear domains is a finding with fundamental and far-reaching implications for how the mammalian nucleus is structured. At the very least, it provides insight into the functions likely associated with some of these nuclear substructures, but it also raises the possibility that nuclear structure involves a higher-level organization of the genome relative to compartments in which factors or functions related to RNA metabolism are most concentrated. A full treatment of this subject is beyond the purview of this article, however we will briefly discuss evidence for and against three models for the potential relationship of (pre)-mRNA metabolism to the domains enriched in snRNPs, SC-35, and poly A RNA.

Is There (pre)-mRNA Metabolism Spatially Associated With Splicing Factor/poly A RNA Rich Domains?

Whether the 20–50 prominent nuclear domains greatly enriched in spliceosome assembly factor SC-35, snRNPs, and poly A RNA represent only accumulations of inert splicing factors or are, alternatively, directly associated with pre-mRNA metabolism has been a question of much recent interest and debate. For clarity we will refer to those regions here as SC-35 domains, to distinguish them from coiled bodies which also contain snRNPs but not SC-35 and poly A RNA: however, we have also referred to the SC-35 domains as transcript domains [Carter et al., 1991; 1993] and others have referred to them as snRNP speckles [Lerner et al., 1981; Huang and Spector, 1991] or even "splicing islands" [Nyman et al., 1986]. Before considering the function(s) of this splicing factor rich compartment, a brief comment about its structure is warranted. One view is that it represents a "reticulum" or continuous network from the nucleolus to the nuclear envelope, along which transport may occur, and that the marked "speckled" distribution only appears to be composed of discrete separate spots because the "connections" between them are out of the focal plane [Spector, 1990]. An alternative view based on digital imaging and 3-D reconstruction [Carter et al., 1993], is that these 20–50 prominent domains (approximately 0.5–3.0 microns in diameter) are separate entities with relatively discrete boundaries, which do not connect directly to each other or to the nuclear envelope.

There are clearly much weaker concentrations of poly A RNA and splicing factors distributed throughout the nucleoplasm; whether these serve to interconnect domains in any significant structural or functional sense remains to be shown. Interestingly, the prominent domains have a reproducible higher-level topography, in that they lie in a single focal plane just below the nuclear midline in many cultured cells [Carter et al., 1993]. It is widely agreed that these poly A RNA rich domains reside in regions of little to no DNA density, likely at the periphery of chromosomal territories [Fakan and Puvion, 1980; Spector, 1990; Carter et al., 1991, 1993; Cremer et al., 1993; Wansink et al., 1993; Clemson et al., 1996].

The models, shown in Figure 1, focus on domains of very high concentrations of splicing factors. While many or most of these correspond to interchromatin granule clusters [Fakan and Puvion, 1980; Visa et al., 1993; Davis et al., 1993; Spector et al., 1991], it cannot a priori be assumed that all of them do.

Model 1—The first model incorporates what has been the most long-standing and strongly held view of these domains, which proposes that they are accumulations of inert splicing factors unassociated with ongoing RNA metabolism, which exist for storage (and possibly assembly) of splicing factors. As discussed elsewhere [Fakan, 1994; Spector, 1993; de Jong et al., 1990], the IGC have long been viewed to be unassociated with pre-mRNA; transcription and splicing would presumably occur randomly dispersed throughout the interdomain space. This view is based on the established findings which show that there is little if any labeling of interchromatin granules or domains with short pulses of uridine [reviewed in Fakan, 1994; Wansink et al., 1993]. While the IGC label much less than the perichromatin fibrils dispersed throughout the inter-domain space, some studies made mention of label at the surface of the IGC and sometimes inside [see for example, Fakan and Bernhard, 1971; Dundr and Raska, 1993; Hendzel and Bazett Jones, 1995]. More recently, Br-UTP incorporation has been studied relative to SC-35 or snRNPs at the light microscopic level. Although one study did not exclude labeling of the splicing factor rich domains [Jackson et al.,

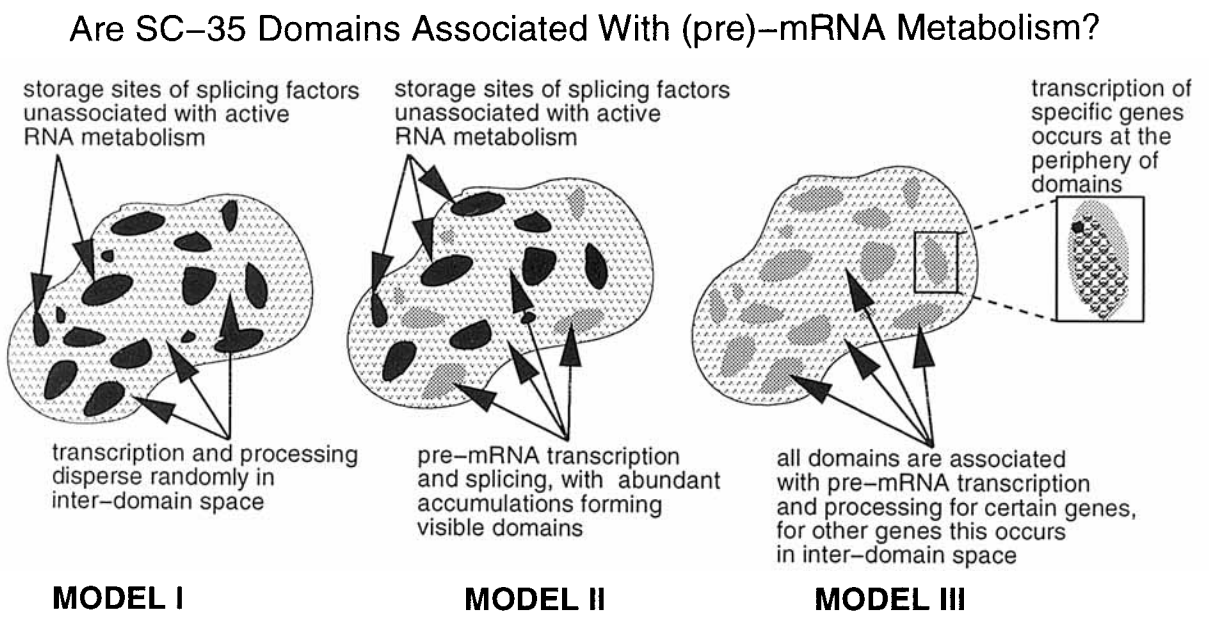

Fig. 1. In the models shown, SC-35 domains refer to approximately 20–40 sites highly enriched in splicing factors and poly A RNA, generally about 1–3 microns in diameter, as viewed by light microscopy. In *Model I,* these domains are not associated with pre-mRNA, but are accumulations of inert factors associated with storage (or assembly) of splicing factors, and corresponding generally to interchromatin granule clusters (IGC). In this model localization of specific pre-mRNAs may show no relationship to the domains. In *Model 2,* domains viewed by light microscopy are functionally distinct entities, with some representing storage sites (IGC) and other representing sites of transcription and splicing of highly active genes (large clumps of perichromatin fibrils). In *Model 3* domains are generally associated with pre-mRNA metabolism for specific active genes. As part of this model, we further propose (inset) that the SC-35 domains are subcompartmentalized, with a region of high transcription at the periphery and other functions (such as spliceosome re-assembly, distribution, or RNA transport) at the center, which likely corresponds to IGC [Xing et al., 1995].

1993] another was interpreted to suggest that the SC-35 domains were unassociated with (pre)mRNA [Wansink et al., 1993]. The demonstration that domains enriched in splicing components became more round and prominent upon specific inhibition of splicing was taken as further evidence that these regions were normally unassociated with RNA splicing [O'Keefe et al., 1994]. Further points which favor this model include the report that the essential splicing factor U2AF [Zamore and Green, 1991] apparently does not concentrate in these regions.

The first major challenge to this model came with results of in situ hybridization studies which indicated a markedly increased concentration of poly A RNA at 20–40 sites throughout the nucleoplasm, in addition to more dispersed signal throughout the nucleus [Carter et al., 1991; 1993]. The prominent "transcript domains" enriched in poly A RNA coincide with the highest concentration of snRNP antigens [Carter et al., 1991] and SC-35 [Carter et al., 1993]. Results from other labs have since confirmed, at the e.m. level, that high concentrations of poly A RNA are indeed found in what are deemed the IGC as visualized by e.m. and SC-35 domains by light microscopy [Visa et al., 1993; Huang and Spector, 1994].

To address whether the poly A RNA detected in these studies represents pre-mRNA, which is assumed to have a short nuclear half-life after transcriptional arrest, attempts have been made to use drugs which inhibit transcription. From such experiments Puvion's lab concluded that the bulk of poly A RNA in the IGC was short-lived (sensitive to actinomycin D) and likely represented (pre)-mRNA [Visa et al., 1993]. In contrast, Huang and Spector reported (1994) that the poly A RNA in domains was long-lived after inhibition and, therefore, was not pre-mRNA but likely a form of putative structural poly A RNA associated with splicing factor storage. Our laboratory observed contradictory results from transcriptional inhibition experiments; results varied with the drug, cell-type and experiment [Lawrence et al., 1993; Moen et al., 1995a]. In our view, the informativeness of this type of approach is further compromised by major obstacles to their interpretation, due to several potential secondary effects of both drugs and the global nuclear impact of transcriptional inhibition [for example, Brasch, 1990; discussed in Moen et al., 1995a; Xing et al., 1995; Clemson et al., 1996].

The biochemical evidence to date supports that most poly A RNA is in pre-mRNA [Herman et al., 1978; Salditt-Georgieff et al., 1981; Harpold et al., 1981; Lewin 1990], however, as suggested previously [Lawrence et al., 1993] the discovery of *XIST* RNA suggests that some of the nuclear poly A RNA may be long-lived and structural in nature [discussed in Clemson et al., 1996]. However, as discussed below studies of individual pre-mRNAs provide strong evidence that some of the poly A RNA in SC-35 domains is (pre)-mRNA.

Another major challenge to the tenet that the poly A RNA rich domains are storage sites unassociated with pre-mRNA metabolism comes from the localization of specific genes and RNAs, an approach which circumvents the limitations in interpretation of more global approaches. Individual pre-mRNAs show different nuclear formations ranging from small foci or elongated tracks [Lawrence et al., 1989], likely reflecting factors such as the size and abundance of the primary transcript, extent and efficiency of processing, level of transcription, and position of the gene within the nucleus [reviewed in Xing and Lawrence, 1993]. The most clear-cut demonstration that at least some SC-35 domains contain high levels of cellular (pre)-mRNA is provided by the recent demonstration that collagen type 1α1 RNA, which represents approximately 4% of total mRNA, is essentially always accumulated within a large prominent SC-35 domain [Xing et al., 1995]. We find that the transcription of this gene occurs at the periphery of the domain, whereas the RNA localizes throughout the central domain, a finding that may be key to reconciling these results with earlier e.m. observations (see below). We have observed other pre-mRNAs which localize within the central region of domains [for example, Moen et al., 1995b; Coleman et al., in preparation] and Wang et al. [1991] showed that microinjected intron containing globin RNA concentrated in these domains. In addition, several other endogenous pre-mRNAs associate with domains at their periphery with a probability well-above random expectation. Huang and Spector [1991] reported that shortly after induction, accumulations of c-fos RNA associated with regions enriched in splicing factors. Xing et al. directly identified the fibronectin RNA "track" as the site of transcription and splicing, and showed that it localized at the periphery of poly A RNA/SC-35 domains with a variable but much higher than random

frequency [Xing et al., 1993]. The beta-actin RNA transcription site also preferentially associates with the domain periphery whereas several inactive genes show no such association [Xing, 1993, 1995]. These results are in contrast to one study which argued that this relationship was random based on limited analysis of the beta-actin gene transcription site, which was interpreted to show a random relationship to domains [Zhang et al., 1994]. We find that different genes show markedly different distributions relative to these regions, which is reproducible for the gene and cell-type in question [see for example, Xing et al., 1993, 1995; Clemson et al., 1996]. Importantly, whether or not an endogenous active gene shows a preferential association is a result of its specific sequence, not simply a function of whether it contains an intron or is highly abundant [see Moen et al., 1995a and b; Clemson et al., 1996]. In toto, localization of specific genes and pre-mRNAs strongly supports that there is pre-mRNA transcription and splicing for some specific genes associated with prominent SC-35 domains, whereas other genes and RNAs are spliced and processed within the interdomain space, presumably by the lower levels of splicing factors present there.

Based on the evidence described above, we believe that model 1 is no longer tenable. Clearly, at least some of the domains are associated with active pre-mRNA metabolism. This idea is recently gaining wider acceptance over the earlier conceptions of these regions as inert factors. The question then becomes: is it some of these regions or essentially all of these regions that are associated with pre-mRNA? Hence, is model 2 or model 3 correct?

Model 2—Model 2 suggests that the prominent SC-35 domains are heterogeneous, with only some of them representing accumulations of inert factors unassociated with pre-mRNA, which would correspond to IGCs seen by electron microscopy. Accordingly, other domains would reflect high-levels of pre-mRNA splicing, presumably corresponding to large perichromatin fibrils where nascent transcripts are accumulated on the gene. Depending upon the level of splicing required and the efficiency with which it is done, the transcript may nucleate the formation of a large accumulation of splicing factors and of poly(A) RNA. In the case of collagen RNA it is easiest to argue that the RNA accumulation could be responsible for the accumulation of splicing factors, since this is a highly active gene and the splicing factors are largely coincident with the RNA. In other cases, such as fibronectin and actin, it is harder to argue that the RNA created this accumulation of splicing factors since the RNA accumulation is found at the periphery of and is smaller than the domain. In any case, in the simplest view of model 2, some light microscopic domains would correspond to pre-mRNA (large perichromatin fibrils, not IGCs); however, it is not clear that the dimensions of perichromatin fibrils [Fakan and Puvion, 1980] are consistent with this view.

So what evidence is there that storage or assembly sites, unassociated with pre-MRNA, do in fact exist? What is the evidence that prominent SC-35 domains are comprised of two or more distinct populations? Two major lines of evidence outside of the aforementioned uridine labeling study are taken to support the presence of storage sites. The first is the demonstration that in adenovirus infected cells, splicing factors moved from areas of high concentration to the areas of adenoviral infection where high levels of transcription and processing are presumably occurring [Spector, 1993]. The favored interpretation of these results was that the splicing factors were moving from a storage compartment to a site of transcription and splicing. If so, the speckles would have to represent two separate and distinct components: those associated with transcription and those associated with storage. However, perhaps the most straightforward interpretation of these results is that splicing factors move from one site of high pre-mRNA metabolism to another site of viral RNA metabolism, as viral transcription and splicing dominates the metabolic activity of the cell. Therefore, rather than interpret this as movement from storage to pre-mRNA, these results are easily interpreted in terms of movement from endogenous pre-mRNA to viral pre-mRNA.

The other evidence for the existence of storage sites was based on results from the inhibition studies. In cells inhibited for transcription or splicing, the number of splicing factor/poly(A) rich domains decreased and those few remaining became more prominent in inhibited cells; this was interpreted to show that the remaining domains correspond to what were storage sites in untreated cells. However, Xing et al. [1995] provided a direct test of this idea, e.g., that domains remaining in inhibited cells correspond to storage sites in uninhibited cells. We showed that the collagen gene is associated with the

large accumulation of splicing factors in both the untreated and in the inhibited cells [Xing et al., 1995], demonstrating directly that the domain with which collagen transcription and splicing are associated is the same domain which remains in inhibited cells. Therefore, this result brings into question the interpretation that the domains which remain are solely storage sites. We have proposed that multiple functions may be associated with domains, with specific pre-mRNA metabolism predominantly at the periphery and spliceosome assembly and or RNA transport in the center (Xing et al., 1993; 1995). Alternatively, splicing factors may aggregate to form large rounded structures upon inhibition of transcription.

These considerations do not rule out the possibility that the domains are heterogeneous, as we have observed some indirect evidence for two populations of SC-35 domains, but only in drug inhibited cells where results are extremely difficult to interpret [Moen et al., 1995a]. Nor do they rule out that there may be nuclear domains devoted strictly to the storage of splicing components and that those splicing factors are recruited by some active recognition mechanism to distant sites of transcription and splicing (Model 2). However at this time the evidence for the latter is readily subject to alternative interpretation.

Model 3—Despite the apparent contradiction with long-held views and the uridine labeling studies, we assert that model 3, in which essentially all of the SC-35/poly A RNA rich domains are associated with pre-mRNA metabolism, is highly viable. The observation that essentially all of the SC-35 rich domains also contain prominent accumulations of poly(A) RNA [Carter et al., 1993], is most consistent with this model. Additional support comes from other findings demonstrating that these regions behave uniformly with respect to their content of other RNA metabolic components, such as matrix proteins which bind pre-mRNA (Blencowe et al., 1994) and the hyperphosphorylated form of RNA polymerase II [Bregman et al., 1995]. Of the pre-mRNAs we have investigated thus far, more than half have been associated with the domains, suggesting that this association is not a coincidental location of one or two active genes, but a phenomenon which may involve several different domains and genes. However, more work will be required on the localization of specific genes and RNAs before we can unambiguously distinguish between model 2 or model 3.

But how can model 3 be reconciled with the aforementioned uridine labeling studies, which indicate that IGC or SC-35 domains show very low levels of incorporation? We consider a few factors which help to reconcile these apparently contradictory ideas. First, most of the pre-mRNAs which do associate with domains concentrate at the domain periphery rather than within it [for example, Xing et al., 1993; 1995]. Even for an RNA which accumulates within the domain, transcription apparently occurs at the outer domain boundary. As proposed in Figure 1 (inset) showing the subcompartmentalization of the light microscopic domain, this is consistent with earlier observations of uridine label or perichromatin fibrils at the boundary of IGC, as well as with the light-microscopic observation that poly A RNA rich "transcript domains" are slightly larger than the SC-35 core [discussed above and Carter et al., 1993]. We propose that there are high levels of transcription of specific genes at the periphery of a larger domain containing high concentrations of multiple factors involved in RNA metabolism. But, importantly, poly A RNA localizes throughout the domain, not just at the periphery [Carter et al., 1993]. To reconcile this with the uridine labelling studies a major consideration is that the uridine labeling studies detect a rather crude fraction of undefined nuclear RNA [discussed in Carter et al., 1993; Visa et al., 1993; Moen et al., 1995a; Xing et al., 1995]. Substantial evidence indicates that most hnRNA molecules are not poly-adenylated and never exit the nucleus [Herman et al., 1978; Salditt-Georgieff et al., 1981; Harpold et al., 1981]. Hence, much of the uridine label may be incorporated into nuclear RNA molecules with no known function, in addition to the large fraction which would be in excised introns rapidly removed from pre-mRNA. Therefore the distribution of uridine label does not necessarily accurately reflect the distribution of polyA RNA.

Finally, as previously demonstrated, there is clearly poly A RNA throughout the interdomain space [Carter et al., 1991, 1993], which collectively may represent more poly A RNA than within domains. An important specific example is provided by XIST RNA. Even though most of the XIST RNA apparently represents structural RNA that is stable after transcriptional inhibition, it consistently avoids poly A RNA rich SC-35 domains [Clemson et al., 1996]. This re-

sult contrasts with the suggestion that poly A RNAs which remain after transcriptional inhibition may preferentially localize to these domains [Huang et al., 1994]. Despite clear evidence that RNAs containing splice junction sequences have an affinity for these regions [Wang et al., 1991], XIST RNA, which undergoes splicing (Brown et al., 1992), remains restricted to a nuclear territory devoid of these large discrete domains enriched in splicing factors. This result, combined with the clear demonstration that the XIST is morphologically associated with the inactive X chromosome, becomes particularly intriguing in light of the recent demonstration that XIST is absolutely required for X inactivation [Penny et al., 1996]. Since our results show that XIST RNA is not associated with SC-35 domains, and that it is associated with Xi [Clemson et al., 1996], it is possible that most or all Xi genes are sequestered in a region of the nucleus away from these domains.

The finding that the active XIST gene is not associated with SC-35 illustrates the additional important point that transcription and processing for some intron-containing genes does not require nor correlate with large spatial accumulations of SC-35 as has also been observed for specific protein coding RNAs (discussed above). This is in keeping with uridine labeling studies which show transcription occurs throughout the nucleoplasm [for example, Fakan and Puvion, 1980; Fakan and Bernhard, 1971]. However, XIST RNA serves to illustrate that transcription may not only be for messenger RNA, while at the same time providing specific precedent for the compelling possibility of an RNA functioning as a component of nuclear architecture.

Irrespective of the varying interpretations of recent results it is clear that the study of nuclear compartmentalization and structure is an exciting and complex field that will yield many new insights into the process of cellular organization and function. Future experiments will help distinguish between the models we have presented here, specifically between model 2 and model 3; and will elucidate the mechanism of potential structural RNAs such as XIST RNA.

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