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The Tpr Protein: Linking Structure and Function in the Nuclear Interior?

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As the organelle housing the genome, the nucleus plays a fundamental role in the operation of the cell. A large number of essential and complex functions occur there, including DNA packaging and replication, RNA transcription, RNA processing, and mRNA transport. The number and complexity of these tasks has long led to suggestions of internal nuclear structures that organize and facilitate these functions (e.g., see Comings 1968). During the past several years, considerable new evidence has accumulated for nuclear functions occurring in discrete spatial domains (for a review, see Strouboulis and Wolffe 1996). These observations raise, once again, long-standing questions about whether extrachromosomal structures exist within the nucleus to spatially organize nuclear functions. Is the nucleus like a bag of chromosomal spaghetti, where nuclear functions occur only via freely diffusing factors that self-associate to form the observed nuclear domains? Or is the nucleus a very highly structured organelle where, for instance, a nucleoskeleton ties function to discrete spatial positions?

Morphological and biochemical evidence has long suggested a nuclear skeleton or "nuclear matrix" that might organize nuclear functions. In this skeleton, networks of filamentous proteins provide structural continuity between the nuclear interior and the nuclear periphery. It is hypothesized that this skeleton provides binding sites for any number of functional complexes, and, indeed, many components of nuclear spatial domains have been found associated with nuclear matrix preparations (e.g., see Berezney et al. 1995). What has been missing is the identification of the molecular constituents of the nuclear matrix structures and the demonstration that the structures formed by these gene products play a role in nuclear function.

It was against this background that my laboratory began studies of a large (262-kD) predicted filamentous protein in the nuclear interior, a protein now known as "Tpr." Much to the surprise of my colleagues and me, both the characteristics of Tpr and recent work in the nuclear structure field combine to suggest an alternative formulation of a nuclear skeleton. Here, nuclear pore complex–associated filamentous proteins provide structural connectivity between the nuclear interior and nuclear periphery in the channels between chromosomes. This model is an attractive means of linking the major function of the nucleus—that is, gene expression—to known but poorly characterized structures in the nuclear interior. Furthermore, there is a direct path to testing this model by use of in vivo analysis tools such as genetic and in vivo imaging, which are readily available in a metazoan model organism such as *Drosophila melanogaster.*

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The Controversial Nuclear Matrix

Much of the controversy about internal nuclear structures that might organize function results from 2 decades of technically difficult biochemical approaches to electron micrograph (EM)–observable structures named "nuclear matrix" or "nuclear skeleton." As first characterized by Berezney and Coffey in the mid-1970s (Berezney and Coffey 1974, 1977), nuclear matrices result from selective extraction procedures employing salts, detergents, and nucleases to produce an insoluble nuclear remnant. Many features of these remnants appear enticing. For example, whole-mount EMs can show fibers in the nuclear interior that are anastomosed with the nuclear lamina (reviewed by Nickerson and Penman 1991). The internal nuclear fibers can have the width and repeat spacing expected for intermediate-filament-like proteins (e.g., see Jackson and Cook 1988). Two-dimensional protein gels of these remnants show both distinctive patterns of proteins in different cell types (e.g., see Fey and Penman 1988) and shifting patterns of proteins during the development of cancers (e.g., see Partin et al. 1993), much as one would expect if these proteins play a role in changing patterns of gene expression.

However, historically the great difficulty in these biochemical approaches has been the wide divergence in the structures produced and in the proteins isolated by different groups (reviewed by Cook 1988). The scientific

discussion has long been dominated by demonstrations of artifact in different preparation methods (e.g., see Kaufmann et al. 1986; Belgrader et al. 1991), so much so that the entire notion of any internal nuclear skeleton has often been called into question (e.g., see Cook 1988). This field is thus in the difficult position of having provided a strong indication of what an internal nuclear skeleton is likely to be like, while, at the same time, being unable to show unequivocally that such a structure exists and performs essential functions in the living cell.

Most Nuclear Matrix Proteins Appear to Be Components of RNA Metabolism, Not the Expected Skeletal Proteins

One way to unequivocally demonstrate the existence of a nuclear skeleton would be to identify specific proteins that form the expected matrix structures and that also perform demonstrable functions (e.g., via mutant genetic phenotypes). Cell biologists began on this course during the early 1990s, using traditional "reverse genetics" approaches. Surprisingly, most of the proteins identified have roles in RNA metabolism and no obvious skeletonlike characteristics. For example, van Driel's group (Mattern et al. 1996) recently completed a systematic study of the 21 most abundant proteins of the nuclear matrix of HeLa S3 cells. These proteins represent approximately three quarters of the mass of the matrix internal to the nuclear lamina. Of the 21 proteins, 16 are known hnRNP proteins, 1 is an abundant nucleolar protein (B23 or numatrin or nucleophosmin), and the remaining 4 are unidentified. Generally similar results have been obtained by other groups, with several SR-related proteins (e.g., see Blencowe et al. 1995) and a hyperphosphorylated form of the RNA polymerase II large subunit (Mortillaro et al. 1996; Vincent et al. 1996) also being identified as matrix proteins.

Although hnRNP proteins have long been known to be associated with the nuclear matrix, where are the filamentous proteins that should be at the core? Early candidates were the mammalian NuMA protein (reviewed by Cleveland 1995) and the Nuf1p protein of budding yeast (Mirzayan et al. 1992). Both of these proteins are localized to the nuclear interior and have the large coiled-coil secondary-structural motifs expected for filamentous proteins. However, the major function of both proteins appears to be in mitosis, with no obvious interphase function.

In this void of candidate nuclear skeletal proteins, the Tpr protein (Byrd et al. 1994; Cordes et al. 1997; Zimowska et al. 1997) has assumed the role of a protein to watch. *Drosophila* Tpr isolates exclusively with the nuclear matrix fraction of a traditional nuclear matrix preparation (Zimowska et al. 1997). The nuclear interior-staining pattern of *Drosophila* Tpr is decidedly nonuniform, often having a linear or fibrous appearance when observed with immunofluorescence (Zimowska et al. 1997). The Tpr cDNA sequence predicts two distinct structural domains: a large N-terminal domain (180 kD) strongly predicted to form a coiled-coil and an acidic C-terminus (82 kD) predicted to form a random coil (Byrd et al. 1994; Zimowska et al. 1997). The Tpr coiled-coil domain therefore may assemble into a skeletal structure, leaving the C-terminus free to interact with other macromolecules.

The localization of Tpr within the nucleus also offers an unexpected twist: *Drosophila* Tpr is excluded from the chromosomal and nucleolar spaces but generally is found in all other regions (Zimowska et al. 1997). Because these extrachromosomal regions are the major sites of RNA metabolism and transport within the nucleus (e.g., see Zachar et al. 1993), it is worth considering a novel model of nuclear structure, in which Tpr, RNAs, and RNA-binding proteins fill the spaces between chromosomes.

Chromosomal Territories and Interchromosomal Channels as Determinants of Nuclear Functional Domains

During the past decade, chromosome-painting methods have revealed a simple but profound fact: within the interphase nucleus, individual chromosomes occupy distinct, nonoverlapping regions (see Kurz et al. 1996); that is, unlike the simplistic view from a traditional EM image, the euchromatic regions of different chromosomes do not intermingle within the nuclear interior but, rather, remain separate from each other in distinct volumes occupied by each chromosome. One immediate consequence is that the chromosomes themselves segregate the nuclear interior into a continuous region that is simply the spaces between the chromosomes. Such interchromosomal channels provide a continuous path for molecular exchange from the deep nuclear interior to pore complexes in the nuclear periphery, or vice versa (fig. 1*a;* also see, e.g., Bridger et al. 1998). For some time now, there have been demonstrations that these channels, alternately labeled the "interchromosomal channel domain" (Zirbel et al. 1993) or the "extrachromosomal channel network" (Zachar et al. 1993), concentrate activities required for mRNA metabolism (e.g., pre-mRNA splicing factors) and provide a nonrandom pathway for mRNA to be transported out of the nucleus (fig. 1*a;* for reviews, see Kramer et al. 1994; Razin and Gromova 1995; Strouboulis and Wolffe 1996). Given the initial evidence that most genes (active and inactive) appear to be localized at the edges of the chromosomal territories (Kurz et al. 1996), these interchromosomal channels provide a simple means of concentrating and coordinating gene transcription, mRNA processing, and

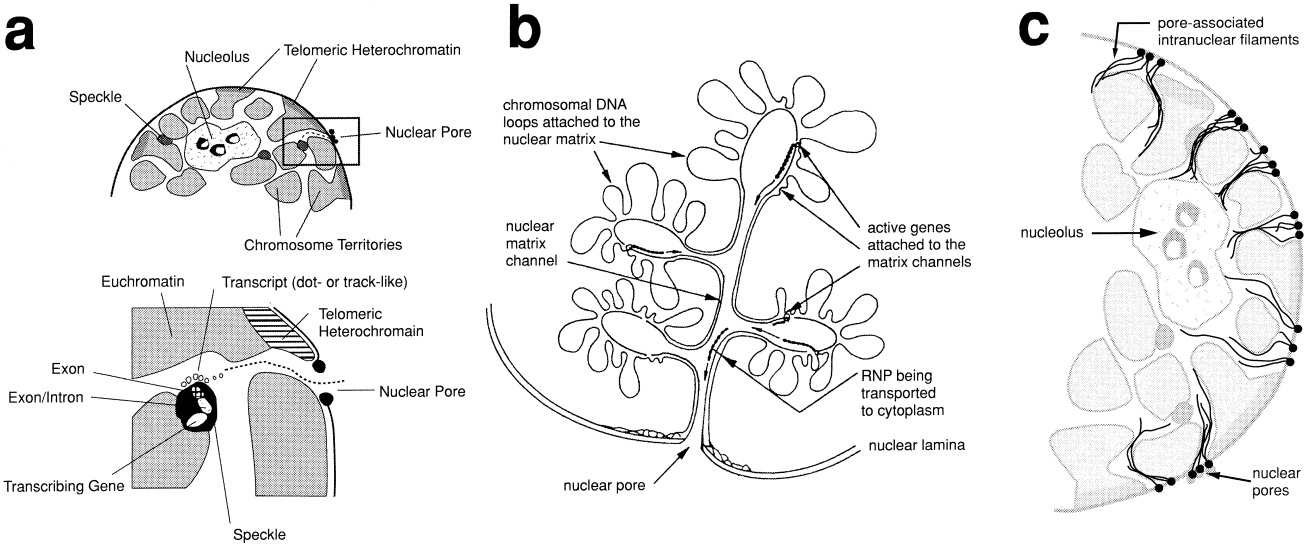

Figure 1 Three models for functional compartmentalization of the nucleus in which chromosomes are major determinants of the compartments. Models can be formulated by use of either the chromosomal channels alone (*a* [from Strouboulis and Wolffe 1996]), chromosomal channels lined with a nuclear matrix (*b* [from Razin and Gromova 1995]), or chromosomal channels filled with nuclear pore complex–associated intranuclear filaments (*c* [as suggested in this review]). In *a,* approximately one-half of a nucleus is shown in the upper section, and the bottom section shows an enlarged view of the rectangle in the upper section; in *c,* nuclear pore complexes and nuclear pore–associated intranuclear filaments are shown superimposed on the half nucleus shown in the upper section of *a,*which has been rotated 90° clockwise in the image plane.

mRNA transport in the same region of the nuclear interior.

Such interchromosomal channel networks need not exclude the existence of a nuclear skeleton or matrix. Indeed, Razin and Gromova (1995) have proposed an alternative formulation of the nuclear matrix that places nuclear matrix proteins on the surfaces of chromosomes (as opposed to the centers of chromosomes), lining the interchromosomal channels (fig. 1*b*). This model makes it possible to reconcile all the available matrix data, particularly that which indicates that chromosomal DNA is organized as a series of loops tethered to a matrixlike structure. Thus, perhaps a better way to find a nuclear matrix is to seek the expected filamentous proteins in the spaces between the chromosomes.

Nuclear Pore Complex–Associated Intranuclear Filaments

Classic EM studies of amphibian oocyte nuclei have long shown filaments extending from the inner face of nuclear pore complexes to a considerable distance into the nuclear interior (as far as 1 μm; e.g., see Franke and Scheer 1970). These filaments, which may stretch from pore complexes to the nucleolus, contain both the Tpr protein and another nuclear pore complex protein, Nup153 (Cordes et al. 1993, 1997). Because Nup153 is implicated in nuclear export (Bastos et al. 1996), it seems likely that the long intranuclear filaments form a channel or act as tracks for movements of molecules between the nuclear interior and the pore complexes. Indeed, Ris (1997) has observed these same *Xenopus* oocyte nuclei with high-resolution field-emission scanning EM and has found that the nucleoplasmic faces of pore complexes are interconnected by 50-nm channels formed from eight 6-nm filaments. Channels from several pore complexes merge to form a common channel that then runs deeper into the nuclear interior. Such channels deep in the nuclear interior could provide the filamentous proteins expected at the core of a nuclear matrix structure.

An Alternate Model for a Nuclear Matrix: Pore Complex–Associated Intranuclear Filaments in the Interchromosomal Channel Network

A nuclear matrix model based on pore complex–associated intranuclear filaments in the interchromosomal channels is sketched in figure 1*c*. This sketch is intentionally vague, to emphasize the general notion rather than exhaustive details. This model is attractive because it uses filamentous structures known to exist and integrates nuclear structure with gene expression, a nuclear function of paramount importance. Furthermore, both the RNA metabolism function of most nuclear matrix proteins characterized to date and the RNase sensitivity of traditional matrix preparations are anticipated in this model. Finally, note that, because the

nuclear matrix is generally observed only after DNase treatment, a matrix that exists only in the spaces between chromosomes, such as is shown in figure 1, would not be obvious in traditional preparations.

Potentials and Problems of Tpr as Part of an Interchromosomal Channel Nuclear Skeleton

Are the functions of Tpr consistent with a role in an interchromosomal channel–based nuclear matrix? Can even the more modest hypothesis of a Tpr role in nuclear transport be documented? Currently, Tpr functional studies are only beginning, and thus all the intriguing possibilities remain open.

The initial functional evidence is that Tpr plays some role in intranuclear transport. As this review was being written, four groups were reporting data implicating Tpr either in the export of mRNA or in the import of proteins. Overexpression of either full-length mammalian Tpr or certain Tpr-deletion constructs in tissue-culture cells leads to accumulation of poly(A)+ RNA within the nucleus (B. Burke, personal communication). This may mean that some soluble factor required for export binds to the overexpressed Tpr and is not available for its usual function. Similarly, my laboratory has observed that overexpression of *Drosophila* Tpr full-length or deletion constructs in yeast, mammalian tissue-culture cells, and fly salivary glands leads to an accumulation of poly(A)+ RNA (G. Zimowska, V. Lamian, and M. R. Paddy, unpublished data). Surprisingly, however, my laboratory has yet to find conditions in which overexpression of *Drosophila* Tpr or of *Drosophila* Tpr–deletion constructs produces organismic death, aside from the trivial case of overexpressed protein blocking transport through pore complexes (G. Zimowska, V. Lamian, and M. R. Paddy, unpublished data). Similarly, Cordes has found that none of a large number of Tpr-deletion constructs leads to cell death when overexpressed in tissue-culture cells (V. Cordes, personal communication). Taken together, these results may indicate that Tpr plays only an accessory—and not an obligatory—role in nuclear export (i.e., it may facilitate but not be required for export). Alternately, it is possible that Tpr plays some other role upstream of mRNA export, a role that either remains unrecognized or is masked by the endogenous Tpr protein present in all these experiments. Clearly, there is a need to repeat these experiments in a Tpr genetic-null background.

Surprisingly, Forbes's group finds that Tpr is a major physiological binding site for importin β, which, in combination with importin α, is required for import of classic nuclear localization signal–containing proteins (Shah et al. 1998). This result was unanticipated, because it is unclear why the nucleus would require that binding sites for importin β be at any place other than the nucleoplasmic face of the pore complex. Shah et al. (1998) have hypothesized that Tpr participates either in binding of importin α/β after release of the nuclear localization signal–containing protein or else in the recycling of importin β back to the cytoplasm. Thus, Tpr may participate in nuclear trafficking in both directions.

If Tpr acts in mRNA export, it touches on a classic debate: Does mRNA export from the nucleus occur along a defined path or track, or does it occur by simple diffusion (contrast the review of Xing and Lawrence [1993] with that of Kramer et al. [1994])? Most theoretical and experimental studies suggest that mRNA transport occurs by simple diffusion (e.g., see Zachar et al. 1993; Politz et al. 1998). However, Ris's recent images of pore complex–associated intranuclear channels evoke earlier work suggesting a defined path or track for RNA export (Xing and Lawrence 1993).

If Tpr were part of a filamentous track or path for mRNA export, one would expect Tpr-containing intranuclear filaments to run continuously from pore complexes to deep into the nuclear interior. Currently, there is no evidence for this. Indeed, *Drosophila* appears unique in the extent to which Tpr is localized throughout the nuclear interior, not merely adjacent to pore complexes. Furthermore, the preliminary data from my laboratory suggest that, at certain developmental stages, Tpr is found in a form other than the intranuclear filaments. In some stages of larval salivary-gland development, Tpr is localized to small spherical structures that often border the nucleolus (Zimowska et al. 1997; G. Zimowska and M. R. Paddy, unpublished data). This may represent a storage form of newly synthesized Tpr, Tpr complexed with pre-mRNA, or Tpr cycling on and off pore-associated intranuclear filaments. If these preliminary observations are confirmed, a simple, static intranuclear-filament structure for Tpr will not suffice. A dynamic nuclear matrix has often been proposed (e.g., see Mattern et al. 1996), and the true structure of a dynamic matrix might never be made clear in traditional static (fixed-sample) structural methods. In vivo imaging of either Tpr green-fluorescence protein chimeras or Tpr tagged with photoactivatable fluorescence probes should provide a direct path toward examination of any dynamic Tpr structures or any mRNA transport along an intranuclear filament.

Conclusion

Although descriptive evidence continues to mount that many nuclear functions are organized in discrete spatial domains, the existence of a nuclear skeleton or matrix to direct this organization remains unproved. What I have suggested here is that the broad outlines of a nuclear matrix already may be evident if we relax our expectations of what a nuclear matrix must be (i.e., a rigid nuclear skeleton) and, instead, focus on two structures already known to exist—interchromosomal channels

and pore complex–associated intranuclear filaments. Such a matrix model unifies transcription at the surfaces of chromosomes, mRNA metabolism, and nuclear transport, through common structural elements. Of course, at the heart of any nuclear matrix model must be the long-awaited filamentous proteins, to provide the expected skeletal functions. The fact that the Tpr protein, with its predicted large (~180-kD) coiled-coil filamentous domain, has been localized to the pore-associated intranuclear filaments provides hope that, at last, one of these filamentous proteins has been isolated. Tpr now must run the gauntlet of molecular functional and structural analysis, to see whether this hope can be realized.

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