Protein Dynamics: Implications for Nuclear Architecture and Gene Expression

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Studies of nuclear architecture reveal that the dynamic properties of proteins in the nucleus are critical for their function. The high mobility of proteins ensures their availability throughout the nucleus; their dynamic interplay generates an ever-changing, but overall stable, architectural framework, within which nuclear processes take place. As a consequence, overall nuclear morphology is determined by the functional interactions of nuclear components. The observed dynamic properties of nuclear proteins are consistent with a central role for stochastic mechanisms in gene expression and nuclear architecture.

Gene expression is a multistep process involving chromatin remodeling, transcription, RNA processing, RNA export, and translation in the cytoplasm. Each of these steps is carried out by highly specialized, elaborate machinery, typically consisting of tens or hundreds of components. How these processing complexes form at the right time and in the right place and how gene expression is integrated into the architectural framework of the cell nucleus are fundamental, unanswered questions in biology.

One can envisage conceptually different ways of how genes are expressed in the nucleus. The mere presence of substrates—DNA and RNA—and the processing factors that act on them may be sufficient for "things to just happen." Alternatively, in analogy to the compartmentalized organization of the cytoplasm, the existence of numerous intranuclear compartments suggests that particular processes occur in specific locations within the nucleus. To distinguish between these extremes, it is crucial that the basic biophysical properties of the nucleus and its components are determined. How crowded is the nucleus? How do proteins and RNAs move in the nucleus? How do proteins find their targets? How are nuclear compartments formed and maintained? How does nuclear structure affect gene expression? Recent experiments have answered some of these long-standing questions. What we find is an intriguing, somewhat provocative, and elegant new view of the cell nucleus.

Looking into the Nucleus with New Tools

Much of what we know about the nucleus comes from microscopy studies, because the organelle does not lend itself easily to biochemical analysis. DNA in the form of chromatin is organized in distinct chromosome territories (*1*), and many proteins exist in a soluble pool in the nucleoplasm, but also in a more insoluble fraction associated with distinct intranuclear compartments (*2–4*) (Fig. 1). Morphologically well characterized nuclear compartments include the nucleolus (*5*, *6*), the splicing factors compartments (*3*, *4*), and the large family of small nuclear foci, including the Cajal body (CB) (*7*) and the promyelocytic leukemia (PML) body (*8*) (Fig. 1). With the exception of the nucleolus, which represents the site of ribosomal RNA (rRNA) transcription, the functions of these compartments have remained largely elusive. The development of in vivo microscopy techniques using genetically encoded fluorescent tags has opened the door to probe nuclear architecture and function in living cells (*9*, *10*). These powerful methods have recently been combined with photobleaching techniques such as FRAP, allowing one for the first time not only to visualize protein dynamics, but in combination with kinetic modeling, to quantitatively determine biophysical properties of nuclear proteins in intact cells (Fig. 2). These experiments have given important new insights into nuclear architecture and function.

Proteins Roam the Cell Nucleus

Considering the high DNA content and the large amounts of RNAs and proteins, one might intuitively think of the nucleus as a viscous, gel-like environment. If this were true, the movement of proteins within the organelle might be severely restricted and specific transport mechanisms might be required to deliver proteins to their destinations. Photobleaching experiments have now shown that many proteins are highly mobile within the nucleus. The difference between the diffusional mobility of nonphysiological solutes in the nucleus compared with an aqueous solution is only about fourfold (*11*, *12*), and fluorescently tagged, biologically active proteins move rapidly throughout the nucleus (*13–18*). The fact that proteins involved in diverse nuclear functions such as chromatin remodeling, transcriptional activation, pre-mRNA splicing, rRNA processing, and DNA repair move rapidly in vivo suggests that high mobility is a general feature of proteins in the mammalian cell nucleus.

Nuclear mobility of proteins is energy-independent and therefore likely occurs by a diffusion-based, passive, nondirected mechanism (*14*, *17*, *18*) (Fig. 3A). This observation does not rule out that some proteins, or fractions of a protein population, are transported by active, directed mechanisms. However, active transport mechanisms might not be necessary, because diffusion is a very effec-

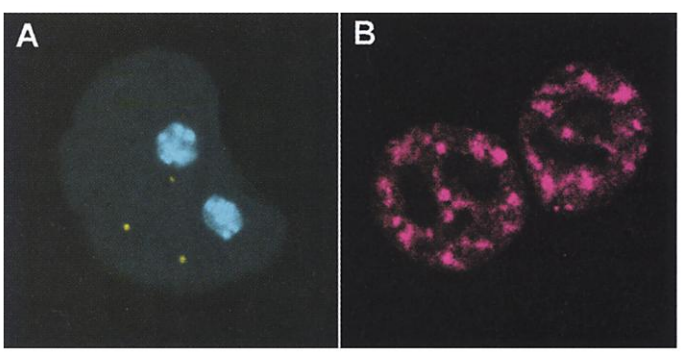

Fig. 1. Nuclear compartments. The nucleus contains morphologically defined compartments. **(A)** The nucleolus (blue) represents the site of ribosomal gene transcription and rRNA processing. It is formed by the coalescence of multiple chromosomes containing ribosomal genes in the nuclear space. A different type of compartment is formed by members of a family of small nuclear foci, represented by the Cajal bodies (yellow). The function of these nuclear foci is unclear. **(B)** Pre-mRNA splicing factors are concentrated in splicing factor compartments, or speckles (purple), which serve as assembly and/or recycling sites for spliceosomal components. Images of living cells expressing green fluorescent protein (GFP)–fusion proteins are shown.

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tive mode of transport. A monomeric protein can traverse the nucleus in a few seconds (*11*, *14*); even large molecular complexes the size of spliceosomes or ribosomes can readily diffuse from the center of the nucleus to its periphery in a few minutes. Their high mobility allows molecules to find their targets in the nucleus by diffusing through the nucleoplasmic space until they encounter an appropriate binding site. This behavior effectively constitutes a scanning mechanism, which does not require any specific targeting signals or signal recognition machinery. In addition, diffusion provides an energetically economical way of movement.

High mobility by diffusion is not a feature unique to proteins. Polyadenylated RNAs in mammalian cells, presumably in the context of ribonucleoprotein (RNP) particles, move with characteristics typical of energy-independent, diffusion-mediated mobility (*19*), and RNP particles in *Chironomus tentans* move through nuclear space in a random pattern indicative of a diffusion-based mechanism (*20*). Contrary to the view that the nuclear environment is largely occupied by chromatin and leaves little space for nuclear components to move freely, dextran mobility measurements indicate that the volume not accessible to diffusing proteins and RNAs in the nucleus is less than 15% (*12*). The implication is that diffusional mobility is sufficient to ensure the distribution of proteins throughout the entire cell nucleus.

FRAP experiments demonstrate that most proteins move more slowly than would be expected on the basis of their molecular weight alone (*14*, *17*, *18*, *21*) (Fig. 3A). What slows proteins down? Because biologically inactive molecules move 10 to 200 times as fast as a similar-sized, physiologically active protein (*13*, *14*, *21*), it can be excluded that the slowed movement is simply caused by their collision with physical obstacles in the nucleus such as chromatin or a nuclear scaffold. The apparent, not absolute, mobility measured in FRAP is a combination of the diffusional mobility and the specific biological properties of a protein. Incorporation of a protein into a larger complex reduces the protein's apparent mobility, although formation of complexes has only a small effect unless a protein is incorporated into a very large complex such as a small nuclear RNP (snRNP) or a ribosome (Fig. 3A). A second, more important, reason for slowed protein mobility is the interaction of proteins with nuclear components, which are relatively more immobile (Fig. 3A). This behavior is particularly relevant for chromatin-binding proteins. Chromatin is probably the major immobile component in the nucleus, but proteins may also be slowed down by their transient interactions with a putative karyoskeleton (*18*, *21*). Regardless of the nature of the immobile component, repeated transient interactions of a protein with relatively more immobile nuclear components results in the slowed, saltatory movement of proteins through the nucleus (Fig. 3A). The effective mobility of a protein is thus greatly determined by its interactions with other nuclear components.

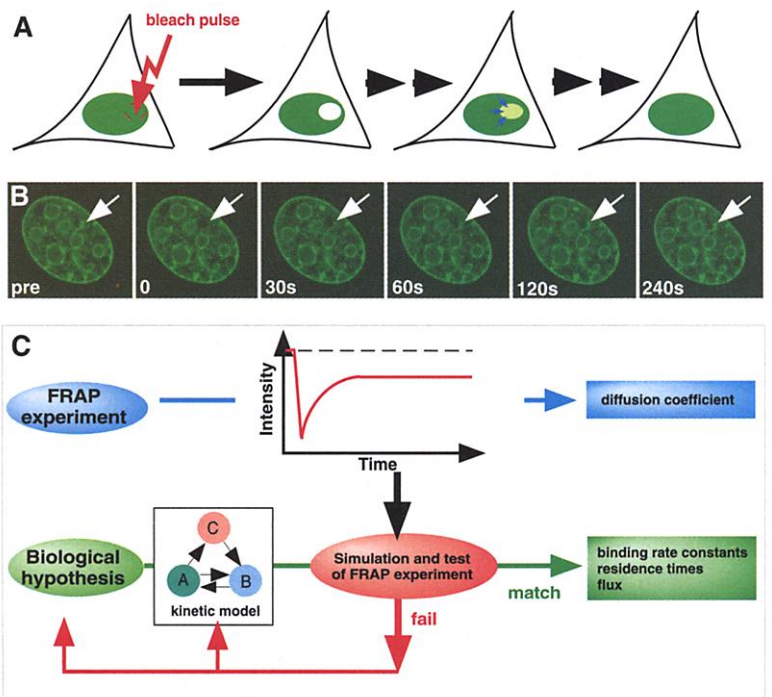

Fig. 2. FRAP as a tool to study protein dynamics. **(A)** In a FRAP experiment, a small area of a cell expressing a fluorescently tagged protein is rapidly and irreversibly bleached using a targeted laser pulse. Bleaching generates a region devoid of fluorescence signal. The recovery of the fluorescence signal is measured as a function of time using time-lapse microscopy. Recovery of fluorescence is due to the influx of unbleached molecules into the bleached area. The kinetics of recovery contain information about the apparent mobility of the labeled proteins. **(B)** FRAP on the linker histone H1. The area indicated by an arrow was bleached, and recovery was monitored for 240 s. The observed recovery demonstrates the exchange of GFP-H1 between chromatin and the nucleoplasm in a living cell. **(C)** FRAP data can be used in combination with kinetic modeling to obtain information on various biophysical properties of proteins in living cells. A biological hypothesis can be translated into a system of differential equations that constitute a kinetic model describing the hypothesis. The FRAP experiment is simulated using the kinetic model. If the model fails, either the biological model or the kinetic model can be adjusted and the simulation repeated. If the model can account for the FRAP data, best fits for the parameters (association and/or dissociation rates, binding constants, flux, and fractions of populations in a given kinetic compartment) that define the kinetic system can be obtained.

Nuclear Compartments Are in Perpetual Flux

A fundamental feature of the cell nucleus is the presence of distinct compartments. Nuclear compartments are similar to cytoplasmic compartments in that they are enriched in distinct sets of "resident" proteins, they can be identified morphologically both in fixed and in living cells, and some nuclear compartments can be isolated biochemically (*22*, *23*). But nuclear compartments differ fundamentally from most cytoplasmic compartments in that they are not delineated by membranes.

The key to understanding compartmental integrity in the absence of membrane boundaries may be the dynamic nature shared by all nuclear compartments. Most compartments, including the nucleolus, the splicing factor compartments, and CBs, disassemble during M phase and reassemble rapidly in daughter cells, indicating high structural plasticity (*24*, *25*). In addition, although the overall position of splicing factor compartments within the nucleus is maintained, each compartment undergoes continuous changes in shape, suggesting high internal dynamics (*21*, *26*). Although nuclear compartments are stable structures, FRAP experiments have now revealed that the components of nuclear compartments are in continuous flux between the compartment and the nucleoplasm (*14*–*16*, *21*) (Fig. 3B). For most proteins, the exchange is rapid, and the residence time of most proteins in compartments is on the order of a minute or less (*14*, *16*, *21*). Estimating the size of the outward flux by using kinetic modeling reveals that on average about 12,000 molecules of SF2/ASF and about

10,000 molecules of the rRNA processing factor fibrillarin leave their respective compartments each second (*14*). Compartments are thus extremely dynamic, yet overall stable, structures, and their morphology represents the equilibrium of release and binding of proteins.

Self-Organization of Nuclear Compartments

The size of the flux through a compartment is determined by a protein's on and off rates. These exchange rates, in turn, are strongly influenced by the functional activity of proteins both inside and outside of a compartment. Thus the functional status of exchanging proteins critically determines composition and morphological appearance of a compartment (Fig. 3B). These properties together with the rapid flow of proteins through compartments are consistent with the hypothesis that compartments are formed and maintained by principles of self-organization.

Several observations suggest self-organization as a mechanism for compartment formation and maintenance. The structural appearance of the nucleolus is closely related to the transcriptional activity of ribosomal genes (*5*, *6*, *24*). Nucleolar integrity is lost upon exposure to inhibitors of the nucleolar RNA polymerase I. Conversely, introduction of extrachromosomal ribosomal DNA into yeast or *Drosophila* results in the spontaneous formation of mini-nucleoli. Evidence for a role of self-organization in formation of nuclear foci comes from the observation that major components of several small nuclear bodies have the capacity to self-interact (*27*–*29*). For the splicing factor compartments, inhibition of RNA polymerase II transcription or pre-mRNA splicing results in the accumulation of splicing factors in a few, grossly enlarged splicing factor compartments. Upon reversal of the block, small, morphologically normal splicing factor compartments re-form spontaneously (*30*).

Involvement of self-organization in the formation of nuclear structures provides an elegant mechanism not only to concentrate factors where they are needed, but also to segregate factors away from sites where they are not wanted (*4*). The nucleolus serves as an example for a compartment whose formation is the consequence of a particular function (rRNA transcription). In contrast, splicing factor compartments appear to form independently of a direct function, but their morphology is determined by the functional status of their components (Fig. 3B). Splicing factor compartments serve to facilitate assembly/recycling of splicing components and are used to regulate splicing factor concentration in the nucleoplasm by sequestering factors away from their sites of action. A continuous flux of proteins dissociates from them, and the released splicing factors roam the nucleoplasm until they encounter a pre-mRNA. As expected for a self-organizing system, reduction of pre-mRNAs by inhibition of RNA polymerase II results in the accumulation of splicing factors in the compartments (*26*, *30*) (Fig. 3B). A similar function in controlling nucleoplasmic concentration has been proposed for several types of chromatin remodeling factor foci (*31*).

Dynamics of Chromatin-Binding Proteins

Structural chromatin-binding proteins such as histones or high mobility group (HMG) proteins are generally considered to be stably bound to DNA. Could it be that the apparently stable occupancy of sites in chromatin is as fluid as that in nuclear compartments (Fig. 3C)? In vitro experiments and microinjection experiments on the linker histone H1 showed that H1 molecules can be transferred within hours from one chromatin-binding site to another, indicating the potential dynamic nature of their association with chromatin (*32*, *33*). Photobleaching experiments have now confirmed that H1 molecules continuously exchange from chromatin, but they also suggest that the exchange is much more rapid than previously anticipated (*34*, *35*). The behavior of H1 can be summarized in a "stop-and-go" model, in which an H1 molecule binds chromatin for about 1 to 2 minutes, falls off, and then diffuses freely through the nucleoplasm for a short period of time until it encounters a free binding site (Fig. 3C). The same type of stop-and-go binding applies to members of the HMG proteins, although these proteins have a residence time on the order of seconds rather than minutes and have a significantly larger unbound fraction (*14*). Exceptions to the highly dynamic chromatin-binding proteins are the core histones, which generally reside on chromatin for several hours (*36*).

Transfer of histone H1 between binding sites occurs through a freely mobile intermediate (*35*). This type of "jumping" mechanism is consistent with proteins encountering their binding sites by roaming the nuclear space. Three-dimensional "scanning" by jumping appears to be a general feature of DNA binding proteins, because Eco RV finds its specific targets sites in a similar manner even in vitro (*37*). As in compartments, the residence time of proteins on chromatin is determined by the functional status of the protein and/or the properties of chromatin. For example, the residence time of H1 and HMG proteins on chromatin is significantly reduced when core histones are hyperacetylated and chromatin is remodeled (*34*, *38*). Similar to the situation in compartments, the rapid exchange of proteins generates a dynamic, but stable, configuration of proteins on chromatin.

The transient interaction of structural chromatin-binding proteins such as histone H1 and the HMG proteins may contribute to the dynamics of chromatin itself. It is difficult to imagine how a chromatin fiber can undergo conformational changes with structural proteins, which prevent access of other factors to the fiber, statically bound to it. The dynamic exchange of chromatin-binding proteins makes the local and global reorganization of chromatin possible. Whenever a protein dissociates, the opportunity arises for a different factor, be it a different struc-

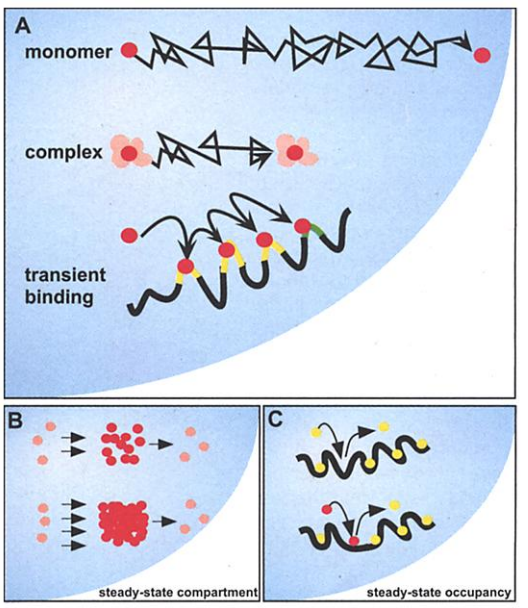

Fig. 3. Apparent mobility of nuclear proteins and steady-state compartments. (**A**) Proteins diffuse through the nucleus. The mobility of a protein is determined by its biological properties. Mobility of a monomeric protein is higher than for a complexed protein or for a protein that transiently binds to immobile components in the nucleus such as chromatin. Transient binding results in a saltatory, stop-and-go mode of mobility. As proteins diffuse through the nuclear space, they are slowed down by their transient interaction with low-affinity binding sites (yellow) before they find a specific, high-affinity site (green). (**B**) Proteins are continuously exchanged between the nucleoplasm and a compartment, generating a steady-state compartment. The morphology of the compartment is determined by the ratio of influx and efflux of proteins. Increased influx, for example, results in accumulation of proteins in the compartment. (**C**) Steady-state occupancy of a chromatin-binding protein is generated by the continuous exchange of proteins from the binding site. Replacement of a chromatin-binding protein (yellow) with a different factor (red) may induce alterations in chromatin structure.

tural protein or a remodeling activity, to gain access. The presence of linker histone H1 on chromatin prevents acetylation of core histones and inhibits chromatin remodeling by SWI/SNF (*39*, *40*). Each time an H1 molecule dissociates, SWI/SNF has an opportunity to access chromatin. Controlling the occupancy of chromatin-binding proteins by posttranslational modification may act as a regulatory mechanism for gene expression. In *Tetrahymena*, histone H1 phosphorylation mimics loss of H1 from chromatin and results in increased gene expression (*41*). Hyperacetylation of HMG-14 and HMG-17 results in their reduced affinity for nucleosomes (*42*, *43*). Conversely, statically bound proteins might contribute to transcriptional silencing. Although FRAP experiments demonstrate that a larger fraction of H1 is stably bound to heterochromatin than to euchromatin (*34*), it remains to be determined whether more specific heterochromatin proteins such as HP-1 or MENT are immobile in heterochromatin (*44*, *45*). These considerations suggest that the dynamic exchange of proteins on chromatin is essential for transcriptional activators to gain access to chromatin and that controlling the exchange rate of a protein on chromatin might contribute to regulation of gene expression.

Transcriptional activators are even more dynamic than structural chromatin-binding proteins. For example, steroid receptors such as glucocorticoid receptor (GR) and estrogen receptor (ER) regulate transcription by interaction with cofactors, including chromatin remodeling activities and the basal transcription machinery. Biochemical evidence has suggested that these receptors bind their response elements stably as long as ligand is present (*46*). However, recent photobleaching experiments on an array consisting of multiple GR response elements demonstrate that, although GR molecules are present at these binding sites for as long as the target genes are activated, the GR molecules are continuously and rapidly exchanged (*47*). Similar observations, although not in the context of a defined promoter, have been made for ER (*48*). The view of steroid receptors stably bound to their DNA target must be revised in favor of a "hit-and-run" model in which receptors reside for only short periods of time on the response elements. Modifications of the receptor or its interacting proteins significantly alter its dynamic binding properties and thus may affect its regulatory role in transcriptional activation (*48*).

Stochastics in Gene Expression and Nuclear Architecture

Movement of proteins within the nucleus occurs by passive, randomly directed diffusion. That a molecule encounters a particular compartment or a binding site therefore becomes a chance event. I suggest that recruitment of proteins to their binding sites, be it in a compartment or on chromatin, is determined by stochastic interactions. The dynamic properties of proteins in the nucleus are consistent with stochastic mechanisms in gene expression (*49*–*52*).

Activation of a gene requires remodeling of chromatin and subsequent assembly of the transcription machinery (*53*). Both of these processes are driven by the sequential interaction of proteins or preassembled modules. The probability of forming an assembly intermediate is influenced by the residence time of the assembling complex and the availability of each component at the assembly site, which, in turn, is determined by its mobility within the nucleus (Fig. 4). If a factor is not available within the residence time of a previous intermediate, the assembly process may be terminated (Fig. 4). During chromatin remodeling, a transcriptional activator, such as GR, which facilitates recruitment of remodeling complexes, associates only transiently with chromatin. If no chromatin modifying activity is recruited within the residence time of the activator, the activator dissociates (Fig. 4). The same is true for the steps during assembly of the core transcriptional apparatus. Evidence that the assembly of the core transcription machinery on remodeled chromatin involves stochastic events comes from the observation that stochastic activation of reporter genes occurs on nonchromatinized plasmids in vivo (*51*). In vitro, only a small fraction of templates are successful in forming preinitiation complexes, indicating that transcription machinery assembly is a relatively inefficient process (*54*). Furthermore, in vitro observations in *Drosophila* extracts show that assembly of the core machinery takes several minutes, whereas the transition from initiation to elongation occurs within seconds (*55*). The relatively low efficiency of transcription apparatus assembly is counterbalanced by the cooperative nature of binding of many transcription factors and by the formation of immobilized transcription factories, which effectively act to increase the local concentration of components (*53*, *56*).

Stochastic binding of proteins to nuclear

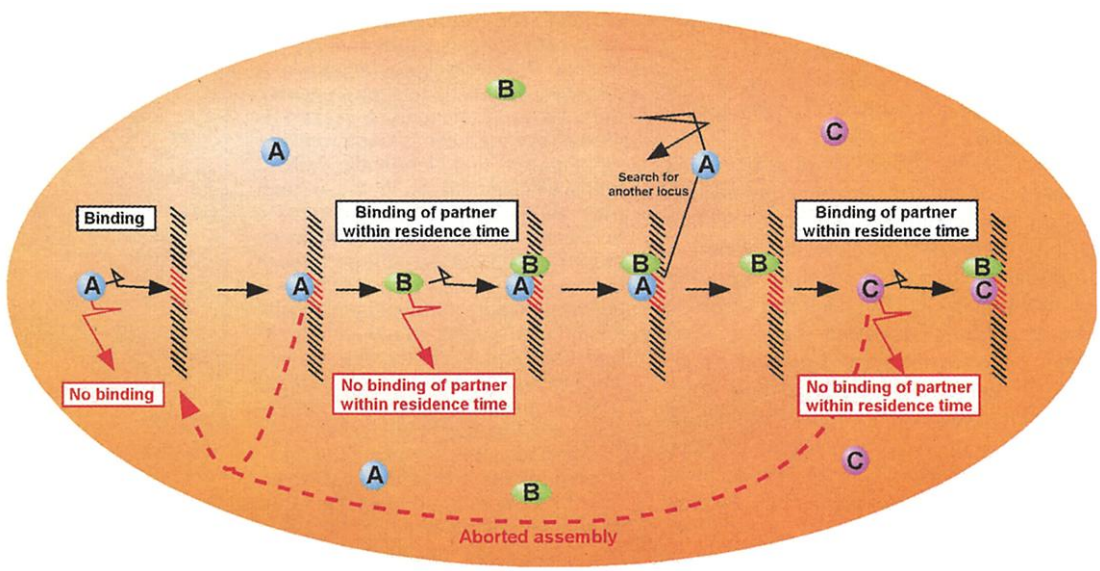

Fig. 4. Repeated transient interactions during gene activation. Chromatin remodeling and assembly of the transcription machinery require targeting of numerous components to specific DNA sequences. The rate of transcriptional activation is determined by the availability of any transcription factor at a locus. The random, diffusional mobility of proteins makes remodeling and assembly of the transcription apparatus stochastic. A transcriptional factor A binds transiently to its target sequence. If no binding partner B is recruited within the residence time of A, B will dissociate prematurely. Analogously, for the next step, C needs to bind within the residence time of B. The regulator A can either be a transcriptional activator, a cis-regulatory factor, or a component of the transcription machinery, and the binding partners B and C may be a chromatin remodeling activity, an interacting cis-regulatory factor, or a second component of the basal transcription machinery, respectively. Cooperative protein-protein interactions and the formation of transcription factories may facilitate the assembly and stabilize assembly intermediates.

compartments may also critically determine nuclear architecture. Less than five nucleoli or CBs and rarely more than 40 splicing factor compartments are found in a mammalian nucleus. If association of proteins with their compartments is stochastic, a prediction is that the number of molecules present in a compartment will undergo fluctuations. This is difficult to visualize and measure, but evidence for fluctuations exists. In time-lapse observations of splicing factor compartments, random changes in shape and fluorescence intensity at the periphery of each compartment were clearly observed (*26*). These changes were interpreted to represent the continuous dissociation and association of splicing factors. Similar fluctuations in fluorescence intensity of components of CBs have been reported (*57*). In addition, the number of CBs and PML bodies is variable within a clonal cell population, and at least two distinct size classes of CBs have been observed (*57*, *58*). These variations might reflect the stochastic interaction of proteins with their compartments.

An advantage of such dynamic and probabilistic behavior of proteins in the nucleus is the potential to respond promptly to external cues transmitted by signaling cascades. A modest increase in the abundance of a modified protein results in a relatively high probability of encountering its proper target. If proteins were statically bound to their targets, mechanisms would have to exist to release proteins from their binding sites before a modified protein could bind. The inherent short residence time of many proteins ensures the repeated availability of binding sites, as well as the factors. Analysis of cis-regulatory elements in a wide variety of organisms indicates that the number of functional interaction partners in upstream regulatory regions of genes is in the range of four to eight (*59*). The combinatorial interaction of a group of activators or repressors in a probability driven manner generates a spectrum of responses within a cell population. The frequency of the predominant interaction, and thus the physiological outcome, can be shifted by alteration of the interaction properties of proteins in response to signaling events. A network of transient interactions of several partners results in a high degree of plasticity, which facilitates rapid activation of particular genes or switching between gene expression programs.

The New Nucleus

The cell nucleus during interphase has for a long time been thought of as a homogenous, static organelle. Now, a new view of the nucleus is emerging. It has become clear that it is not only a structurally and functionally heterogeneous organelle, but that many nuclear components are highly dynamic. The dynamic nature of nuclear components provides a framework for the understanding of nuclear architecture and gene expression in vivo. High mobility provides the basis for a simple, energetically economical system to ensure the availability of proteins throughout the organelle. The combination of high mobility and high exchange rate ensures targeting of proteins to their site of action by simple diffusion during which proteins effectively scan the nucleus for appropriate binding sites without the requirement for directed targeting, specific signals or signal recognition machinery. These features favor stochastic, combinatorial use of components and generate a robust system that can respond quickly to external cues. From these considerations it appears likely that the overall structural stability of the nucleus is generated by the stochastic interaction of its components and that nuclear architecture is governed by principles of self-organization. The resulting structural and functional plasticity may be crucial for accurate execution of gene expression programs.

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