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SURVEY AND SUMMARY

Transcription factories in the context of the nuclear and genome organization

S. V. Razin[1,*], A. A. Gavrilov[1], A. Pichugin[2], M. Lipinski[2], O. V. Iarovaia[1] and Yegor S. Vassetzky[2,*]

[1]Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia and [2]UMR8126, Univ. Paris-Sud 11, CNRS, Institut de cancérologie Gustave Roussy, 94805 Villejuif, France

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ABSTRACT

In the eukaryotic nucleus, genes are transcribed in transcription factories. In the present review, we re-evaluate the models of transcription factories in the light of recent and older data. Based on this analysis, we propose that transcription factories result from the aggregation of RNA polymerase II-containing pre-initiation complexes assembled next to each other in the nuclear space. Such an aggregation can be triggered by the phosphorylation of the C-terminal domain of RNA polymerase II molecules and their interaction with various transcription factors. Individual transcription factories would thus incorporate tissue-specific, co-regulated as well as housekeeping genes based only on their initial proximity to each other in the nuclear space. Targeting genes to be transcribed to protein-dense factories that contain all factors necessary for transcription initiation and elongation through chromatin templates clearly favors a more economical utilization and better recycling of the transcription machinery.

In the era of classical molecular biology, the nucleus was perceived by many as a kind of miniature test tube, where soluble enzymes and structural proteins, histones included, would freely interact with an immobile DNA. This perception has progressively evolved towards a different view of the nucleus as a highly organized solid-state system, rigid and flexible at the same time, with enzymes taking part in complex factories that process and displace DNA (1). Transcription in the nucleus is highly compartmentalized. There are three distinct RNA polymerases in the eukaryotic cell nucleus. Transcription factories which harbor RNA polymerases I, II and III are strictly specialized. Ribosomal genes are processed in the nucleoli where RNA polymerases I and their co-factors are organized in small (200–500 nm) fibrillar centers. During transcription, rDNA slides over the surface of these centers, while newly synthesized transcripts are released into adjacent dense fibrillar compartments (2).

Transcribing (elongating) RNA polymerase II and III (Pol II and Pol III) molecules have also been demonstrated to be organized in clusters (3–5) which transcribe several genes and are usually referred to as transcription factories (4). Pol III transcription is organized in the nucleoplasm in approximately 2000 transcription factories. These factories do not contain any of the hyperphosphorylated form of the largest subunit of pol II (5).

From recent studies, it appears that the inclusion of individual genes into transcription factories is everything but random, and bears consequences on the spatial organization of the genome (6,7). Still, the structure and even the protein composition of transcription factories remain largely uncharacterized and their mechanism of assembly and disassembly poorly understood. In the present review, we critically analyze available data with special attention to apparent contradictions and problems that need further examination.

A SHORT HISTORY OF TRANSCRIPTION FACTORIES

Elongating Pol II have long been shown to form nuclear clusters, also referred to as 'foci' (3,4) or transcription factories which contain newly synthesized RNA (8,9) and thus, correspond to sites of active transcription where several Pol II molecules operate simultaneously. Elongating Pol II and transcription factories have been reported to be associated as a whole with the nuclear matrix, or nuclear skeleton (10–12), suggesting that transcribed DNA would move along immobilized transcription complexes (13). In

*To whom correspondence should be addressed. Yegor S. Vassetzky. Tel: +33 0 1 42 11 62 83; Fax: +33 01 42 11 54 94; Email: vassetzky@igr.fr
*Correspondence may also be addressed to Tel: +7 499 135 97 87; Fax: +7 499 135 41 05; Email: sergey.v.razin@usa.net

experiments where electroelution was applied to agarose-embedded nuclei, transcription complexes and associated transcribed genes were found to remain inside the nucleus even after most of the chromatin had been removed from the permeabilized nuclei (14,15). Interestingly, immobilizing RNA polymerase on agarose matrices did not affect its transcription efficiency (16). In other reports, it has also been shown that at least a portion of Pol II molecules are directly attached to the high salt-insoluble nuclear skeleton (14,15). However, over 20 years later, the true nature of this nuclear skeleton, or matrix, still remains to be deciphered. Moreover, even though partners of Pol II have been intensively looked for using GST-pull down and co-immunoprecipitation experiments (17), nuclear matrix structural proteins remain to be identified as obvious candidates for association with Pol II. Still, the immobility of Pol II molecules in the nucleus appears to be real and their association with DNA drastically decreases their diffusion rate throughout the nucleus (3,4,18).

Immuno-electron microscopy observations have revealed a size of ~70–80 nm (4) for transcription factories, each containing from 4 to 30 elongating RNA polymerases and nascent transcripts (4,19). Other authors have generated estimations of eight Pol II molecules per transcription factory in average (20). More recently, whether transcription factories really exist in the absence of transcription has been questioned. Heat shock was used to interrupt most Pol II transcription at the stage of initiation without resulting in any visible disorganization of Pol II-containing foci (21). In this study, immunofluorescence experiments were carried out using antibodies that recognize both phosphorylated and non-phosphorylated forms of the Pol II C-terminal domain (CTD). The number of transcription factories remained similar in heat-shocked, as compared with control, cells. Using 5,6-dichlorobenzimidazol 1-β-D-ribofuranoside (DRB) to inhibit transcription elongation did not affect the integrity of transcription factories either. From these observations, the authors concluded that transcription factories indeed exist in the absence of ongoing transcription. However, only ~30% of *Hbb,* and 50% of *Eraf,* alleles initially associated with transcription factories remained so after heat shock(21). This would indicate that some elongating Pol II complexes are arrested but not disassembled under heat shock conditions. It is well known that under such conditions, the initiation of transcription is rapidly blocked (22). The fate of Pol II elongation complexes is less clear. Some complexes may well terminate the ongoing transcription process, resulting in the release of a portion of *Hbb* and *Eraf* alleles from transcription factories. As in the case of DRB treatment, other alleles would remain associated to such arrested elongation complexes and would thus be retained in transcription factories. Most active transcription factories each contain approximately eight transcribing Pol II molecules in a cell cultivated *in vitro* (20), but probably much more in tissues (23). Even with 70% of Pol II molecules released under heat shock conditions, the remaining 30% would still be detectable in transcription factories that presumably should appear smaller. Although not discussed in Mitchell and Fraser's (21) publication, this is exactly what can be seen.

That the association of a gene with a transcription factory be mediated by some factor, other than a Pol II complex cannot be excluded either. Peter Cook (29) has proposed that both RNA polymerases and transcription factors might tether chromatin to the transcription factories. The localization in the nucleus of murine beta-globin genes and of the housekeeping gene *Rad23a* has been analyzed by Palstra *et al.* (24) using the 3C/4C technique and cryo-FISH (fluorescent *in situ* hybridization) in the absence and the presence of transcription inhibitors. Once established, the long-range interactions of the active β-globin locus with other active genes did not depend on an ongoing transcription or on Pol II binding to regulatory elements. Indeed, these long-range interactions persisted after transcription arrest as well as the association of key erythroid factors with globin genes (24).

To address the question whether transcription factories persist after transcription arrest, other observations must also be taken into account. In control cells, ~25% of the total Pol II population diffuse slowly, the vast majority being highly mobile throughout the nucleus (25,26). Under heat shock conditions, only a rapidly diffusing form of Pol II molecules is detected (26). This very mobile population is clearly distinct from Pol II molecules present in transcription factories where the high protein concentration is expected to prevent any rapid diffusion. Under normal conditions, the number of Pol II molecules that are slowly diffusing is roughly similar to that of phosphorylated active Pol II present in transcription factories (25,26). If these two populations are the same, the question thus ensues as to how to explain both the disappearance of the slowly diffusing Pol II fraction (25) and the persistence of transcription factories (21) under heat shock conditions.

REPOSITIONING OF GENES NEXT TO TRANSCRIPTION FACTORIES AS A MECHANISM OF TRANSCRIPTIONAL ACTIVATION

In FISH experiments, genes under transcription and transcribing Pol II complexes are observed within transcription factories (7,21,23,27,28), whereas potentially active but non-transcribed alleles are localized away from transcription factories. It has thus been proposed that genes should be moved to pre-existing transcription factories to be transcribed (29–32). Indeed, it was shown that activation of *c-Fos* and *c-Myc* transcription in B lymphocytes stimulated to proliferate correlated with their repositioning to transcription factories where immunoglobulin heavy chain (*IgH*) genes were already being transcribed. Such a displacement of activated genes to pre-existing transcription factories has also been reported by others (28,33). It has been suggested that activation-related gene displacements could be mediated by actin–myosin motors (34). However, further studies are necessary to delineate the exact mechanisms underlying such displacements.

The timing of assembly of Pol II pre-initiation complexes on promoters of activated genes also remains unclear. This assembly may take place prior to, or following, the displacement of genes towards transcription factories.

The latter possibility seems to better fit the current transcription factory model with gene displacement occurring first and facilitating access to Pol II (29–31). On the other hand, this model fails to explain the role of the large pool of Pol II molecules rapidly diffusing throughout the nucleus (25,26). Hence, we favor the alternative hypothesis that a Pol II molecule must interact first with a gene promoter to form a pre-initiation complex followed by displacement of the complex towards a transcription factory by simple diffusion or by a more sophisticated mechanism. Transcription will be facilitated by the presence in transcription factories of chromatin remodeling factors and additional transcription-enhancing components. Also worth considering is the idea that transcription factories can be advantageously localized near splicing factors-containing compartments (35,36) or close to RNA traffic pathways (37).

TRANSCRIPTION FACTORIES AND THE SPATIAL ORGANIZATION OF THE GENOME

The number of simultaneously transcribed genes is significantly higher than that of transcription factories (38) implying that different genes are transcribed within any one factory. It has been shown that interaction of genes with transcription factories is neither random, nor entirely specific (7). On what basis are genes directed towards the same or different factories? Their chromosome localization and the organization of the interphase nucleus in chromosome territories certainly play a role (39–42). On the other hand, directing functionally related genes to specific transcription factories would facilitate their coordinated expression. That some tissue-specific transcription factors can be shared by different transcribed genes within the same factories would be another advantage. Such preferential assembly in the same factories would in turn determine the spatial organization of the genome in the interphase nucleus (43–45).

Co-expression of neighbor genes may play a key role in the organization of transcription factories. The basic form of co-expression of a few neighbor genes relies on their belonging to a single operon. In *Caenorhabditis elegans,* 15% of the genes constitute operons that are transcribed as polycistronic messages (46). The primary transcripts are then processed into monocistronic RNAs, all genes within one operon sharing the same regulatory elements. Such an organization in operons could have paved the evolutionary way for transcription factories. Although, rarely organized into classical operons in higher eukaryotes, genes positioned in *cis* on a chromosomal fragment are often co-expressed (47–49). Organizing housekeeping genes in clusters (50) is clearly advantageous for the cell, with high and constant levels of gene expression resulting in the persistence of specific transcriptionally active compartments within the nucleus. Indeed, the promoters of actively transcribed housekeeping genes clustered on mouse chromosome 11 are observed in close proximity to each other, probably within a single transcription factory (33). The type of genome organization seen in ancient multicellular organisms may have left its print in the clusterization of co-expressed transcription units. In yeast, target genes regulated by a given transcription factor tend to cluster on a specific chromosome (51). This specific gene organization allows for efficient control of transcription within the nuclear space. During evolution, the appearance of clearly separated lineages for somatic and sex cells was probably the first manifestation of a process leading to cell differentiation. Interestingly, whereas tissue-specific genes are generally found scattered throughout the genome, sex-related genes exhibit a distinct pattern of chromosomal localization as exemplified in the case of the highly clustered mouse and *Drosophila* testis genes (49,52,53). Clusters of oocyte-specific genes have also been reported (54).

Co-expressing genes clustered on chromosomal fragments would have provided a selective advantage which may explain, why large portions of syntenic genetic regions often containing co-regulated genes have been maintained throughout evolution. The situation is more complex, however, for genes exhibiting a tissue-specific expression pattern. With the exception of paralogous genes (globin, immunoglobulin, olfactory receptor genes, etc.) which are the products of gene duplication events, tissue-specific genes do not appear to be clustered in the genome (55). This could explain why tissue-specific genes cannot form separate and functionally specific transcription factories. Indeed, during erythroid differentiation, erythroid-specific α-globin genes are displaced to pre-existing transcription factories for transcription (33).

After mitosis, the onset of transcription mediated by Pol I and its associated transcription factors, including upstream binding factor (UBF), triggers transcript clusterization and the formation of nucleoli. This is facilitated by the organization of ribosomal genes in clusters (56). The mechanisms of maintenance and reorganization of Pol II transcription factories during cell division have yet to be studied. A stable transcriptional landscape can result from continuous interactions between different genes on a mitotic chromosome, a hypothesis that can be tested. The reassembly of co-expressed genes into transcription factories could depend on the presence of specific genomic marks determining participants and assembly centres that will reconstitute and/or displace the transcription factories. It is proposed that the nuclear skeleton/matrix includes assembly centers recognized by CpG islands thus, initiating the assembly of the whole transcription machinery and formation of factories. As an example, non-methylated CpG islands within the alpha-globin gene domain have been found to be preferentially associated with the nuclear matrix in erythroid cells (57).

We will now briefly review the various methods that are currently being used to study how the eukaryotic genome is spatially organized. Chromatin conformation capture (3C) is the current method of choice (58–60) to determine whether any two DNA sequences are located in spatial proximity to each other in the eukaryotic nucleus. This method has limitations, however. First, it is not quantitative, i.e. it does not provide information on the proportion of cells in which the DNA sequences under study are

indeed located in close proximity. It only determines an average interaction pattern within a given cell population. Second, the 3C technique allows for investigating a possible interaction between only two DNA sequences at a time. Many 3C-derivative methods have been developed. The 4C method aims at disclosing a complete pattern of DNA–DNA interactions for a DNA sequence of interest (61,62). The 5C method simultaneously probes, by pairs, interactions of hundreds of different sites under study (63). The HiC method is a genome-wide version of 3C that ensures identification of all possible DNA–DNA interactions (the 'interactome') for a given cell population (64). Finally, the ChiP-loop, 6C and its genome-wide variant ChIA-PET methods all include an extra step of antibody precipitation targeting proteins potentially mediating interactions (65–68). All are based on a proximity ligation procedure that retains most of the restrictions inherent to the original 3C protocol. Insofar as genomic elements are examined pairwise, the simultaneous interactions between more than two elements can only be predicted, not demonstrated. Let's consider three DNA fragments (A, B and C) with preferential associations observed between A and B, A and C and B and C. One interpretation would have the three fragments participating in the assembly of a single chromatin hub (A–B–C) (58–60); another, equally plausible, would have each fragment pair corresponding to distinct complexes formed in a proportion of the cells only. The problem with such approaches is that they describe an 'average cell' which is unlikely to exist in nature (69).

The localization of several genes within any one transcription factory can be studied in individual cells using immuno-FISH (6,7,23,27). This technique allows for evaluations of the proportion of cells where genes of interest are colocalized but due to the complexity of the method and the fact that it is very time-consuming, the number of analyzed nuclei rarely exceeds 200 (27), making it difficult to obtain statistically reliable data, especially at low co-localization frequencies. Another problem lies in the low resolution of optical microscopy which can result in false gene co-localizations (two genes located in two neighbor transcription factories wrongly seen as unique). Immuno-electron microscopy studies of HeLa cells have revealed a total number of approximately 2000–2400 transcription factories active at a given time (4,19), an estimate that is 5-fold greater than the number of transcription factories observed using optical microscopy.

Data generated using both 3C and FISH techniques lead to the conclusion that the organization of genes into transcription factories is not random, but not rigidly determined either. How genes are transcribed in transcription factories has been best studied in the case of erythroid-specific genes. Both 3C and FISH data have revealed a very complex pattern of association of transcribed genes that cannot be explained, but through the existence of numerous variants of transcription factories being implicated in the transcription processes (7). While certain association preferences do exist between specific genes, the same genes can have a number of additional partners (7,23). When analyzed globally, association patterns occurring in a given transcription factory can involve neighbor genes as well as genes located at considerable distances on the same or even different chromosomes (7). Such a clusterization has also been reported for functionally related genes located on different chromosomes (70) but with a preference for genes located in *cis* (24,33,70).

MODELS OF TRANSCRIPTION FACTORIES

According to the current model, transcription factories are considered as relatively stable compartments which can persist in the absence of transcription (21), although this last assumption is not unanimously accepted (29). Transcription factories may contain only pre-initiation Pol II complexes ($S5p^{+}S2p^{-}$ transcription factories) or both pre-initiation and elongating Pol II complexes ($S5p^{+}S2p^{+}$ transcription factories) (71). Experimentally, there is no way to check whether these transcription factories also contain non-engaged ($S5p^{-}S2p^{-}$) Pol II molecules. In any case, Pol II molecules become available again after completion of their round of transcription. The important assumption in this classical transcription factory model is that the promoter is tethered to pre-existing transcription factories, thereby providing access to Pol II molecules (29–32). The local concentration of Pol II within transcription factories has been estimated to be ~1000-fold higher than in the nucleoplasm (72). It was thus suggested that promoters located in the vicinity of transcription factories would have a much better chance to attract Pol II and form pre-initiation complexes (29). Conversely, promoters located away from transcription factories would have to relocate from their initial positions to transcription factories where the pre-initiation complexes would then be formed. Thus, the relocation of a gene to a transcription factory would be a prerequisite for transcriptional activation. The composition of transcription factories beyond Pol II and its co-factors remains unknown, but it has been reported that transcription is carried out in protein-rich nuclear compartments (73). An important prediction in the frame of the current transcription factory model is that transcribed genes should be reeled through immobile transcription factories (1). This prediction has recently been verified in elegant experiments performed by Cook and collaborators (74). This model would seem inconsistent, however, with recent genome-wide studies that have revealed that the promoters of many non-transcribed genes are nonetheless associated with Pol II molecules (75). This apparent contradiction has been solved by the demonstration that the number of transcription factories containing pre-initiation Pol II complexes only ($S5p^{+}S2p^{-}$ transcription factories) exceed the number of transcription factories that actually perform transcription ($S5p^{+}S2p^{+}$ transcription factories) (71). On the other hand, the assumption that transcription factories persist in the absence of transcription (21) would directly contradict the observation that under such conditions, the pool of Pol II molecules with a slow diffusion rate, presumably incorporated in transcription factories, decreases to an almost zero level (26). In addition, the classical transcription factory model

cannot explain why a large pool of rapidly exchangeable Pol II molecules—i.e. Pol II which are not immobilized in transcription factories and which represent ~75% of the total pool of nuclear RNA Pol II (25,26)—should be present at all in the nucleus. Worse, according to the model, genes would migrate to pre-existing transcription factories in order to get access to an available Pol II, without ever making contact with any of the vastly abundant free Pol II molecules roaming in the nucleoplasm. In conclusion, this classical model of transcription factories calls for modifications that will take all experimental data available into account.

The model of specialized RNA pol II transcription factories can be seen as a variant of the classical model. It is based on the assumption that tissue-specific transcription factors could form centers nucleating the assembly of specialized transcription factories dedicated to the transcription of certain subsets of tissue-specific genes. The model was initially designed from experimental observations with autonomously replicating plasmids harboring different genes expressed under the control of different promoters. Once transfected into eukaryotic cells, such plasmids were targeted to distinct subsets of transcription factories depending on the type of promoter used and on the presence or absence of introns in the transcription unit (43). A more recent study of the so-called transcription-dependent interactome suggests that in erythroid cells, some level of functional specialization exists with transcription factories mediating transcription of erythroid-specific genes (7). However, if real, such a specialization would be anything but strict. In this regard it, is of note that a preferential association of more than two erythroid-specific genes in any given transcription factory has not been observed (7). Furthermore, in that particular study, a co-localization of the *Hbb* gene with another erythroid-specific gene in a transcription factory was as likely—or unlikely—as its co-localization with a housekeeping gene. Indeed, out of approximately 5000 genes expressed in erythroid cells (76), the number of different genes that can be found in a given transcription factory already containing β-globin genes, was estimated to be as high as approximately 700; for β-globin genes, the number of 'transcription factory-mates' was even greater, reaching almost 1300 (7) and few of them were erythroid-specific. Taken together, these results do not support the assumption of a preferential association of transcribed lineage-specific genes constituting an important driving force in the organization of transcription factories. What is clear from a careful analysis of interaction partners for globin genes is that the transcriptional interactome is very plastic, to say the least. Whether this plasticity corresponds to events occurring in single cells, or rather provides an average representation of whole cell populations is difficult to decide. The variability in the pattern of association between various genes strongly suggests that these associations result from functional processes such as transcription, DNA repair or replication, rather than being a pre-requisite for these processes to take place.

There could exist a certain preference for association in a given transcription factory of various genes regulated by the same set of transcription factors (6,7), but the spatial proximity of the genes in the nuclear space appears to be of greater importance. Indeed, in a given transcription factory, the levels of association of genes lying on the same chromosome and, even more, on the same arm of a given chromosome, is more relevant than the levels of interchromosomal gene associations, whatever their lineage specificity (6,7,23). Thus, the probability for genes to be associated in a given transcription factory is primarily determined by their positioning within the nucleus (39–42), or according to some properties of the chromatin fibers (77). In this respect, it is of interest that the α- and β-globin genes have different preferential transcription factory partners (7). This would be difficult to explain within the framework of the specialized transcription factory model with nucleation centres containing tissue-specific transcription factors. Furthermore, a study of transcriptionally activated estrogen-responsive genes did not reveal any evidence of co-recruitment to the same transcription factory (78). Therefore, not all specific transcription factors may share such a nucleating effect on the formation of transcription factories.

It rather appears that genes located close, or relatively close, to each other in the nuclear space have a greater probability of being incorporated into the same transcription factory. This readily accounts for the above mentioned observations. Indeed, the α- and β-globin genes have different neighbor genes since they sit on different chromosomes; therefore, they have different nuclear environments and they do interact with different partners in the nuclear space. Homologous chromosomes have been reported to occupy territories away from each other in the nuclear space (39) although the situation may be different in specific cases such as meiosis (29). This easily explains why homologous alleles are rarely (if ever) attracted to the same transcription factory. If constraints imposed by the organization of the genome in the nuclear space are removed, then co-regulated genes would more readily assemble within the same transcription factory. This is indeed what happens in the case of transfected plasmids which diffuse throughout the nucleus in the search for appropriate transcription factories (43).

We propose a modified transcription factory model, whereby transcription factories would result from the aggregation of Pol II-containing pre-initiation complexes localized next to each other in the nuclear space. This aggregation could be triggered by the phosphorylation of the Pol II CTD domain at Serine 5. A transcription factory would then incorporate genes without any bias as to their housekeeping or tissue-specific status, only provided that they are nearby, in proximity to each other and ready to be transcribed. Clusters of transcribing Pol II would form in nuclear localizations most favorable for co-transcriptional splicing and subsequent export of neo-synthesized mRNAs to the cytoplasm. Clusterization of active transcriptional complexes may be advantageous for a more rational use of auxiliary factors necessary for transcription of chromatin templates such as chromatin remodeling complexes, histone chaperones and histone-modifying enzymes. According to this model, gene promoters would first recruit soluble Pol II molecules from the nucleoplasm (75,79) before the genes are moved to

pre-existing transcription factories or serve as nucleation centers for new transcription factories.

The partitioning of genes between distinct transcription factories, determined substantially by their respective positions in the nucleus, would explain why mixed transcription factories are the most abundant, if not the only, type of Pol II transcription factories. The lineage-specific genes would share transcription factories by pure chance, because in differentiated cells the number of transcribed lineage-specific genes is quite important in comparison with the limited number of transcription factories. Indeed, in erythroid cells, it has been reported that there are only approximately 100–300 transcription factories (Pol II foci) per nucleus (23), contrasting with HeLa cells where this number can reach 8000 (20).

Differing from the specialized transcription factory model, this principle of transcription factory organization readily accounts for the numerous interaction partners identified for the *Hba* and *Hbb* genes in transcription factories (7). The model also makes sense of the large amount of highly diffusible Pol II molecules detected in the cell nucleus.

The currently preferred model of transcription factories suggests that engaged Pol II transcription complexes are fixed on the nuclear skeleton. Consequently, the transcribed genes should be reeled 'through' the transcription factories. (13,14,74,80). Cook and Gove (16) had reported on an immobilized Pol II enzyme being capable of mediating transcription. However, the RNA polymerase was from bacteriophage T7 and the template was a short DNA, not chromatin. In more recent views, a Pol II transcribing a chromatin template partially follows the path of the DNA around nucleosomal histones (81). Thus, a Pol II molecule that is immobilized because it is attached to a nuclear skeleton should not only reel the chromatin fiber, but would also rotate this fiber in a very complex manner. Physically, the transcription of chromatin templates by immobilized Pol II appears hardly possible without eviction of nucleosomes, and there are good reasons to believe that nucleosomes are not evicted, at least by a single transcription complex (81,82). Other considerations also question the feasibility of chromatin transcription by fully immobilized Pol II molecules (83). We rather favor a model, whereby the polymerase is not attached to a nuclear matrix but active in a protein dense subcompartment of the nucleus. In such a context, a transcribing Pol II molecule would retain enough mobility to 'manoeuver' over a chromatin template without being mobile enough to escape the transcription factories where the environment gets even more crowded as the synthesized mRNAs become longer and more abundant. In such a scenario, the tracking force exerted by the Pol II molecule would be used at least partially for displacing the transcribed chromatin fragment. From this displacement, a reorganization of the corresponding chromosomal territory would follow, the repositioning of the promoter sequence to its starting place in the nuclear space occurring only after termination of transcription and release of the local tension. With their limited resolution, current experimental procedures with live cells are not sensitive enough to detect such subtle movements and Pol II molecules appear immobile. Electron microscopy can only be performed on fixed cells where elongating Pol II molecules appear clustered (4) but whether Pol II are truly immobile cannot be determined. An association of Pol II and transcription factories has been previously reported with the nuclear matrix (84) but using fixation procedures that use high salt concentration, copper ions etc. which can cause artefacts (85–88). Although nuclear matrices thus prepared do retain many functional compartments including speckles and transcription factories as visualized in the nucleus of living cells (89,90), it is still questionable whether these compartments contain molecules really immobilized on the nuclear matrix. Further studies are necessary to solve the contradictions that have been summarized here.

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