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Specialized Transcription Factories within Mammalian Nuclei

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ABSTRACT: Recent evidence suggests that active RNA polymerases are concentrated in discrete 'factories' where they work together on many different templates. The evidence that such factories specialize in the transcription of particular groups of genes is reviewed.

KEY WORDS: coiled body, nucleolus, PML body, RNA polymerase, transcription.

I. INTRODUCTION

Until recently, it was assumed that an RNA polymerase bound to a promoter and then tracked like a locomotive along the template. This assumption was not based on any direct experimental evidence, but from the perception that the polymerase was small relative to the size of the template, and that the smallest component should move. However, we now know that some RNA polymerases are enormous machines that dwarf the template. For example, the eukaryotic polymerase II is composed of a multisubunit core associated with many tens — perhaps hundreds — of other proteins involved in transcription, as well as related functions like splicing, polyadenylation, chromatin-remodeling, and DNA repair (Maldonado et al., 1996; McCracken et al., 1997; Scully et al., 1997; Cho et al., 1998; Myers and Young, 1998). Moreover, a number of such machines are themselves organized into still larger 'factories', where they make many transcripts (Cook, 1999). There are obvious advantages in concentrating molecules responsible for such a vital process in a few places within the nucleus, so that the molecules can act together. Here, we argue that nature carries this concentration even further, so that particular factories become dedicated to the transcription of particular groups of genes. We begin with theoretical arguments and go on to discuss several examples of such dedication.

II. CONTROLLING THE RATE OF INITIATION

In traditional models for transcription, a polymerase would diffuse through the nucleoplasm, collide with the appropriate complex at a promoter, and initiate. However, when the polymerase is fixed, it is now the template that must diffuse (Figure 1A). Is it feasible that a promoter in a chromatin loop could do so? An experiment involving the use of the green fluorescent protein (GFP) fused with the *lac* repressor and multiple copies of the *lac* operator DNA inserted as a tandem array in a yeast chromosome shows that it is (Marshall et al., 1997). The GFP-repressor binds tightly to the operator, so the array — that is presumably embedded in euchromatin — appears as a small fluorescent spot in a living (interphase) cell. This spot can be followed as it diffuses throughout a region with a radius of ~300 nm, but further diffusion seems to be constrained by neigh-

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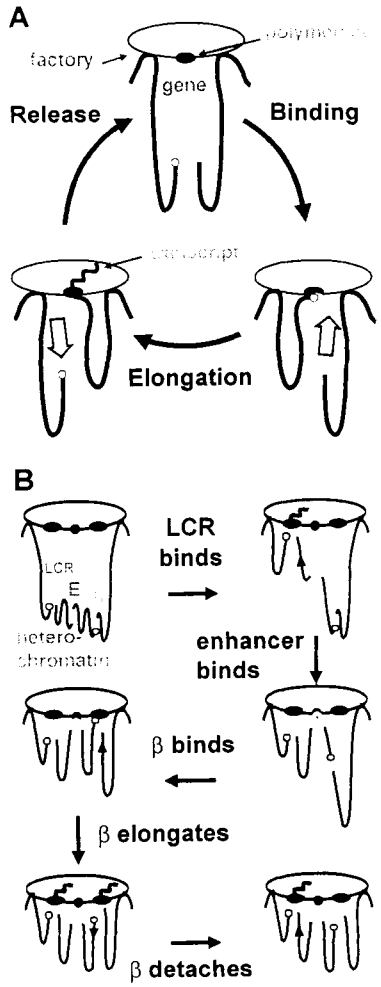

FIGURE 1. Models for transcription (A) and gene activation (B) involving polymerases fixed in factories. (A) chromatin fiber is tied in loops (only one is shown) to a factory. The promoter (small circle) binds to one of the polymerases in the factory, and the transcript is generated as the template slides (open arrows) through the polymerase; at termination, the template detaches so the cycle can repeat. (Reprinted with permission from Cook 1999, copyright 1999 AAAS). (B) Most of a long chromatin loop containing the β-globin gene, its enhancer (E) and locus controlling region (LCR) is condensed as heterochromatin. The loop is attached to a transcription factory. The gene is initially remote from polymerases (small ovals) in the factory and so has a low probability of attaching to them; therefore, it is unlikely to be transcribed. During erythroblast development, the concentration of critical activators rises above certain levels; they bind to the LCR, increasing its affinity for other transcription factors in the factory. This increased affinity is reflected by a higher probability of binding; when the LCR binds, the enhancer is inevitably brought closer to the factory surface. Once the transcription unit within the LCR has attached, it is transcribed; the associated movement of the LCR through the polymerase (arrow) 'opens' up the hitherto heterochromatic loop. The enhancer — now closer to the factory surface — has a higher probability of binding; when it binds, the associated gene is also brought closer, increasing its chances of binding. Once the β-globin promoter has attached, the gene slides (arrows) through the polymerase as the transcript (wavy line) is elongated. At termination the gene detaches; however, as the promoter remains close to the factory, it still has a high probability of reattaching. (From Iborra et al. [1996b] with permission of Academic Press, Inc.)

boring chromosomes. Therefore, a typical (euchromatic) chromosomal loop of 50 kbp in a mammalian cell has a diffusion constant of ~10^{-12} cm^2/s, and can move 200 nm in ~30 seconds. We might also expect that a promoter in a heterochromatic loop would be effectively immobilized, and so unable to get to a factory.

Once a promoter has bound to a factory, an initiation complex containing appropriate transcription factors would assemble, and polymerization would begin as the enzyme reels in the template and extrudes the transcript into the factory (Figure 1A). Once termination has occurred, the template would dissociate. However, now the chances that the promoter can attach again are high; on termination, it necessarily lies near a factory containing the appropriate complex of transcription factors, and its chromatin has been 'opened' by the previous cycle. It is obvious from this model that proximity to a factory is a major factor affecting the rate of initiation, and an example of how an inactive gene might become active is illustrated in Figure 1B. Gene activation then depends on increasing the chances that a gene can attach, with the major determinants being:

1. Gene-factory distance. The closer a promoter is to a polymerization site on the surface of a factory, the more likely it is to attach. Genes out in long loops will attach rarely.
2. Affinity of the promoter for the polymerizing site (as in the conventional model). However, here, once a battery of factors required for optimal transcription has been assembled in a factory, they are probably retained there. Therefore, we might expect that factories

... soon associate with particular groups ... transcription factors to become dedicated ... the transcription of particular groups of genes that utilize those factors.

The mobility of the promoter, which is related ... the degree of heterochromatinization. Long, heterochromatic loops condense on to the nuclear lamina or nucleolus where they become effectively immobile and unlikely to encounter a factory. However, transcription of a gene at the base of such a heterochromatin loop will drag that loop through nuclear space, 'opening' it up to improve its chances of encountering a factory (Figure 1B).

III. NUCLEOLI — PROTOTYPIC FACTORIES

The nucleolus is the prototypic multifunctional factory dedicated to ribosome production (Shaw and Jordan, 1995). A (triploid) HeLa cell contains ~540 45S rRNA genes arranged in tandem repeats on >10 different chromosomes (Table 1). Some of these genes are carried on chromosomes that do not associate with nucleoli; they are transcriptionally inactive. However, ~120 genes on the other chromosomes do form nucleoli. In the electron microscope, nucleoli contain three characteristic zones: a 'fibrillar center' and associated 'dense fibrillar component', which are embedded in a region where ribosomes mature — the 'granular component'. Each of the ~30 fibrillar centers contains a store of polymerase I, and a fraction of the molecules at the surface are associated with ~4 active transcription units (Hozák et al., 1994; Jackson et al., 1998). Each individual unit is associated with ~125 engaged polymerases, and their transcripts can be visualized after extension in Br-UTP as crescent-shaped structures that are equivalent to the dense fibrillar component (Figure 2; Pombo et al., 1999). As a result, a nucleolar factory with a fibrillar center at its core contains ~500 active pols and ~4 transcription units. Presumably, such templates are stripped off fibrillar centers to give the characteristic 'Christmas trees' with their ~125 'branches' that are seen in 'Miller' spreads (Miller and Bakken, 1972). Once a transcript has been made, it is processed and incorporated into the mature ribosomal subunits in the surrounding granular component.

These factories grow and shrink in response to the demand for ribosomes; as transcription increases, factories become smaller and more numerous. For example, the ~234 factories (i.e., fibrillar centers) in a fibroblast fall to ~156 on serum starvation (Jordan and McGovern, 1981), while the ~9 in a peripheral blood lymphocyte rise to ~80 as it is stimulated to divide (Haaf et al., 1991). This means that increasing transcription

TABLE 1
The Numbers of Polymerases and Transcription Factories in a HeLa Cell

	TU[1] (active)	pols (active)	Factories (location)	Active pols/ factory (TUs/factory)	Active pols/TU
pol I	540	?	30	500	125
	(120)	(15,000)	(nucleolus)	(4)	
pol II	?	320,000	8,000	8	1
	(65,000)	(65,000)	(nucleoplasm)	(8)	
pol III	?	?	2,000	5	1
	(10,000)	(10,000)	(nucleoplasm)	(5)	

Note: TU: transcription unit. [1]: Values given are for gene number, but some polymerases transcribe non-genic regions. For example, up to three-quarters of engaged pol II is probably transcribing non-genic regions (Jackson et al., 1996).

Data from Hozák et al. (1994), Jackson et al. (1998), Pombo et al. (1999), and Kimura et al. (1999).

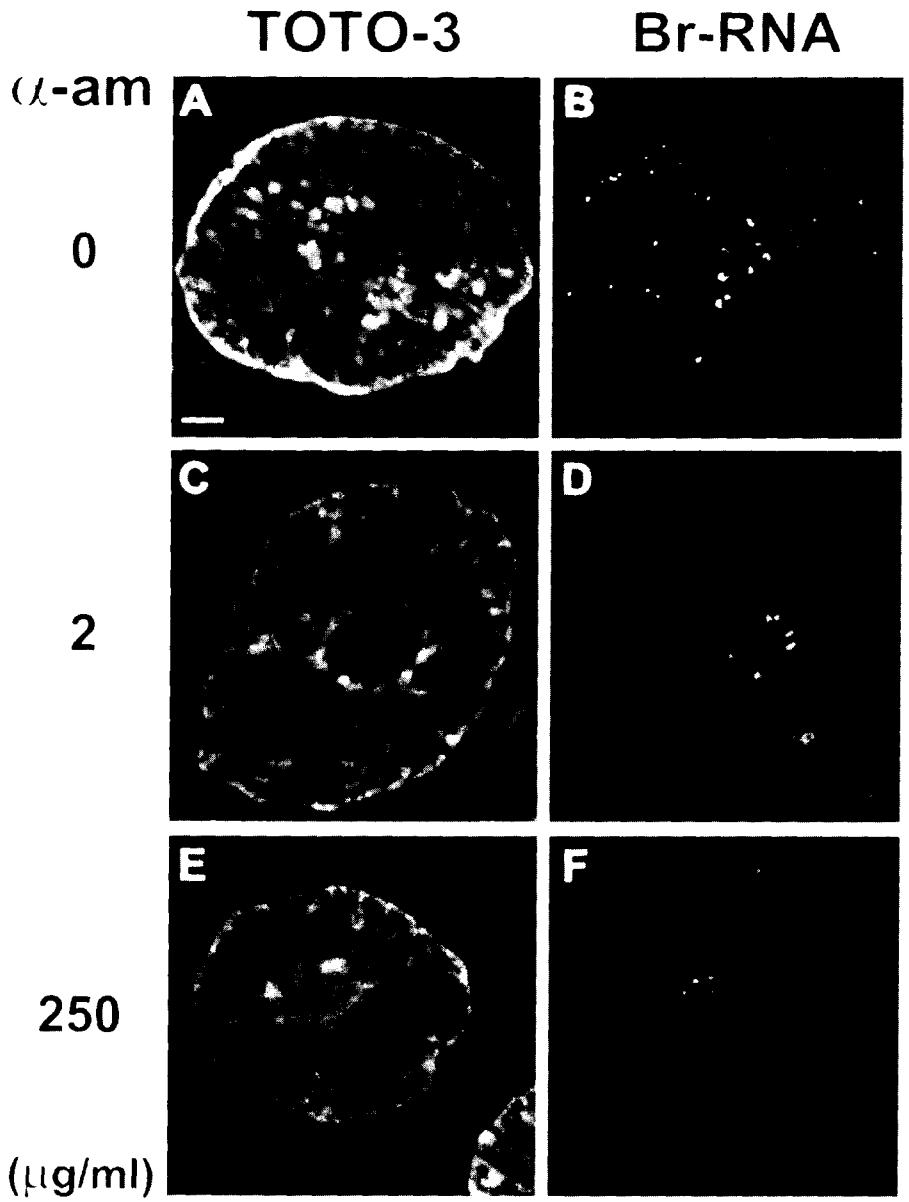

FIGURE 2. Transcription factories in a HeLa cell. Cells were permeabilized, nascent transcripts extended in Br-UTP ± α-amanitin as indicated, and cryosections (~90 nm) prepared; then, Br-RNA was indirectly immunolabeled with Cy3 (nucleic acids were counterstained with TOTO3), and red and far-red images of a cryosection collected using a 'confocal' microscope; pairs of images are shown without background subtraction (thresholding). Bar: 2.5 μm. (Reproduced from Pombo et al. [1999] with permission of Oxford University Press). (A,B) Foci containing Br-RNA are seen in nucleoli, the nucleoplasm, and mitochondria. (C,D) Sufficient α-amanitin to inhibit pol II reduces the density of nucleoplasmic foci, without affecting nucleolar or mitochondrial foci. (E,F) 250 μm/ml α-amanitin inhibits pol II and III and abolishes nucleoplasmic labeling (but not nucleolar or mitochondrial foci).

increases the total area on the surface of fibrillar centers, and so the number of polymerases accessible to promoters.

These nucleolar factories provide clear precedents for the concentration of particular polymerases and transcription/processing factors within a specific region within the nucleus. Moreover, the 45S rRNA genes must associate with the specific region in order to be transcribed, as genes incorporated into nucleoli are active, while those that are not are inactive.

IV. EXTRA-NUCLEOLAR FACTORIES CONTAINING POLYMERASES II OR III

It is attractive to suppose that nascent transcripts generated by pols II and III are also made and processed in analogous factories in the nucleoplasm. Convincing evidence for such a concentration only became available with the introduction of high-resolution techniques for localizing nascent RNA. In one approach, cells are lysed, and engaged polymerases allowed to extend nascent transcripts in Br-UTP or biotin-CTP; then, incorporated analogues are immunolabeled with antibodies conjugated with fluorochromes or gold particles (e.g., Jackson et al., 1993; Wansink et al., 1993; Hozák et al., 1994; Iborra et al., 1996a; Pombo et al., 1999). The analogues are found to be concentrated in a limited number of discrete sites — the factories — with diameters of 40 to 80 nm (Figure 2). A second approach using Br-U and living cells gives the same results (Jackson et al., 1998).

Can most factories be detected using such procedures? Two reasons suggest they can (Jackson et al., 1998; Pombo et al., 1999). First, we would expect that if only some factories were being detected, different methods with different thresholds of detection would yield different counts, but many different methods gave roughly the same estimates. The methods included the use of intact and permeabilized cells, different analogues incorporated with different efficiencies (i.e., Br-U in vivo, Br-UTP or biotin-CTP in vitro), nuclease digestion to remove chromatin or extraction with 2 *M* NaCl to improve immunodetection, pre- and postembedment immunolabeling on or within sections of varying thickness, different antibodies tagged with fluorochromes or gold particles, light and electron microscopy, and different stereological procedures (e.g., Iborra et al., 1996; Jackson et al., 1998; Pombo et al., 1999). Second, roughly the same number are seen after incorporating the analogues for different periods; if only a few factories were being seen, increased incorporation should raise more above the threshold (Iborra et al., 1996a; Pombo et al., 1999).

Quantitative analysis is now giving us a rough indication of the total numbers and types of these factories in HeLa cells (Table 1). Thus, the catalytic subunit of pol II can be detected by immunoblotting, and ~320,000 molecules were shown to be present in a typical cell by comparison with known weights of purified protein (Kimura et al., 1999). Two types of evidence show that most of these molecules of pol II are inactive. First, most can be extracted from the cell by treatment with sarkosyl, without loss of much activity; then, ~65,000 molecules of the hyperphosphorylated form — which is believed to be the active form — remain behind (Kimura et al., 1999). Second, each cell contains ~65,000 and ~10,000 nascent transcripts made by pols II and III, respectively (Jackson et al., 1998; Pombo et al., 1999). High-resolution light and electron microscopy shows that these transcripts are contained in only ~10,000 sites (Pombo et al., 1999).

However, are the active forms of pols II and III each concentrated in their own dedicated factories? The results of two different approaches suggest that they are (Pombo et al., 1999). In the first, site densities were compared after inhibiting pol II with α-amanitin. If the two pols were intermingled within a site and if most sites were detected, we would expect inhibition to reduce the intensity of labeling within a site, but to have little effect on site density. However, site density fell to one-fifth, consistent with four-fifths the sites being dedicated to pol II transcription (Figure 2). More convincing evidence was obtained by exploiting steric hindrance between antibody probes; cryosections were preincubated with one antibody to see if access of a second was blocked. It was found that an anti-pol II antibody blocked access to Br-RNA made by pol II, but not to pol

III protein or its transcripts; conversely, an anti-pol III blocked access to pol III transcripts, but not to pol II protein or its transcripts.

How many polymerases are engaged on a typical transcription unit? Polymerase III units are only ~100 bp long, and so are unlikely to associate with >1 engaged complex. Most polymerase II units also seem to associate with only one (Laird and Chooi, 1976; McKnight and Miller, 1979; Fakan et al., 1986; Jackson et al., 1998), although activated heat-shock and actin genes can be transcribed by more (O'Brien and Lis, 1991; Femino et al., 1998). Even the adenoviral unit — which is one of the most active known — is associated with only one engaged polymerase every 7.5 kb, which is the length of a typical transcription unit in the host (Wolgemuth et al., 1981; Beyer et al., 1981).

OPT AND OTHER DOMAINS

It was suggested earlier that some transcription factors might become concentrated within particular factories, so that genes activated by those factors could only be transcribed if they associated with those particular factories. There is growing evidence that this is the case, and the OPT (**O**ct1/**P**TF/**t**ranscription) domain provides the best example (Pombo et al., 1998). Immunofluorescence shows that Oct1 and PTF are found in 1–3 domains (diameter ~1.3 μm) that appear during G1 phase and disappear in S phase in HeLa cells. Each OPT domain typically contains 2 to 3 factories where Br-UTP is incorporated into nascent transcripts, as well as RNA polymerase II, TBP, and Sp1. Particular chromosomes (e.g., chromosome 6) associate with the domains more than others, including the largest (i.e., chromosome 1). Therefore, these domains seem to act like nucleoli to bring particular genes on specific chromosomes together to a region where the appropriate transcription and processing factors are concentrated, thereby facilitating the expression of those genes.

Which other nuclear domains are transcriptionally active? A structure lying next to the nucleolus — the perinucleolar compartment — is transcriptionally active (Huang et al., 1998). It is rich in snRNAs and hnRNP proteins, but it is not yet known if any particular genes associate with it when they are transcribed. Another domain — the coiled body (diameter ~1 μm) — was discovered by Ramón y Cajal at the turn of the century; it appears as a network of coiled fibers, hence the name (Matera, 1999). Coiled bodies are found in both animals and plants and contain many different proteins, including p80 coilin (the diagnostic marker), various transcription factors, as well as small ribonucleoproteins. However, they are not themselves transcriptionally active, although nascent snRNAs are found immediately next to them in mammalian cells, and active histone genes associate with the analogous structures called a sphere organelle in amphibian oocytes. Therefore, coiled bodies could be stores much like fibrillar centers of nucleoli with which certain genes must associate before they can be transcribed or processed. Interchromatin granule clusters appear in the electron microscope as accumulations of dense granules (each ~20 nm), and correspond to the 'speckles' seen by light microscopy (Misteli and Spector, 1998). Although they are rich in splicing factors and often lie close to sites of transcription (Smith et al., 1999), they do not themselves contain nascent transcripts or engaged RNA polymerase II (Pombo and Cook, 1996). However, newly made transcripts seem to associate with their surface as soon as they are made (Jolly et al., 1999). PML bodies are defined by their content of **pro**myelocytic **l**eukemia protein (Seeler and Dejean, 1999; Matera, 1999). They are roughly similar in size to coiled bodies but are usually more abundant, so a mammalian cell in tissue culture may have 1 to 3 coiled bodies and 10 to 20 PML bodies, and the PML bodies split up when cells experience the stresses associated with heat shock and inflammation. One report suggests that they are transcriptionally active (LaMorte et al., 1998), and another that their surface can provide a site of efficient viral transcription and replication in infected cells (Maul, 1998).

None of the extra-nucleolar domains discussed above yet have well-defined roles. They all seem to be dynamic structures. Most importantly, these domains are all defined by a local concentration of a particular marker. However, these markers are also found elsewhere in the nucleus, usually in

many thousands of smaller sites. Therefore, the major domains discussed above may simply be uninteresting depots where components are stored. Thus, a component like p80 coilin may be stored in a coiled body, but function elsewhere. Note in this context that microinjecting antibodies directed against p80 coilin disrupts coiled bodies, without obvious detriment to the cell (Almeida et al., 1998).

VI. DYNAMIC FACTORIES, SKELETONS, AND ATTACHMENTS

As we have seen, pol I factories are dynamic, splitting to increase their numbers in response to increasing demand. Therefore, pol II and pol III factories probably behave similarly. As a nucleoskeleton links one factory to another, this means that new connecting elements may also be polymerized concurrently to integrate the new factories into the existing structure (Cook, 1995).

Most models for looping the chromatin fiber involve permanent (i.e., static) attachments of specific motifs like **m**atrix/scaffold **a**ttachment **r**egions (i.e., MARs and SARs) to underlying structures (Jackson et al., 1992). However, in Figure 1, the DNA sequences mediating attachment change continually. Moreover, two kinds of evidence suggest that potentially active (pol II) transcription units spend most of their time unattached (and so untranscribed). First, if initiation occurs frequently, many units would be associated with many pols, but the associations seen seldom involve more than one (Jackson et al., 1998; Table 1); therefore, initiation (and so attachment) occur rarely. Second, 20 years of analysis of nucleoids and matrices isolated in hypertonic buffers, and of nuclei in 'physiological' buffers, has failed to uncover any sequence that is always attached in all cells in the population (Jackson et al., 1992). (SARs provide the exception, but they probably associate artifactually with 'scaffolds' during preparation [Jackson et al., 1990; 1992]). Thus, a motif that was permanently bound in all cells in the population would be enriched 10× when all but 10% chromatin was detached. However, no DNA sequences are ever enriched to this extent. Rather, enrichments are generally ≤ 5× (Jackson et al., 1996), implying that even the most frequently attached sequences are attached (and so transcribed) less than half the time. Note also that only ~120 of the ~540 pol I units in a HeLa cell are active at any moment (see above).

The chance nature of attachments, and the continual splitting/fusion of factories, means that the organization changes from moment to moment and from cell to cell. Thus, each cell in a clonal population of erythroblasts contains the same linear array of transcription units strung along the chromosome, and roughly the same number of factories with their halos of loops. However, β-globin gene may be out in a loop at one moment and attached the next (Figure 1B), perhaps even to the 'wrong' factory. Therefore, the precise attachments around the gene in another cell will rarely be the same. However, an exquisite functional order underlies the apparent chaos; in the population, a factory with the appropriate polymerizing machinery is always within reach.

CONCLUSIONS

All life forms concentrate molecules so that those molecules can react together. At the cellular level, it is commonplace that particular functions are concentrated in particular locales. Then, we might expect the machinery involved in transcription to be concentrated in discrete sites, and that different discrete sites would become even more specialized. Indeed, the nucleolus contains a concentration of RNA polymerase I in a particular site dedicated to the transcription of 45S rRNA genes, and polymerases II and III are also concentrated in their own dedicated factories. It also seems that these factories further specialize as some contain high concentrations of particular transcription factors. It follows that certain genes may only be transcribed by association with the appropriate transcription factory.

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