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REVIEW

Transcription Factories and Spatial Organization of Eukaryotic Genomes

S. V. Razin[1,2]*, A. A. Gavrilov[1], and O. V. Yarovaya[1]

[1]*Institute of Gene Biology, Russian Academy of Sciences, ul. Vavilova 34/5, 119334 Moscow, Russia; fax: (499) 135-4105; E-mail: sergey.v.razin@usa.net*
[2]*Biological Faculty, Lomonosov Moscow State University, 119991 Moscow, Russia*

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Abstract—The phenomenon of association of transcribed genes into so-called transcription factories and also the role of these associations in spatial organization of the eukaryotic genome are actively discussed in the modern literature. Some authors think that the association of transcribed genes into transcription factories constitutes a major factor supporting the function-dependent three-dimensional organization of the interphase genome. In spite of the obvious interest in the problem of spatial organization of transcription in the eukaryotic cell nucleus, the number of experimental studies of transcriptional factories remains rather limited and the results of these studies are often contradictory. In the current review we have tried to critically re-evaluate the published experimental results that constitute the basis for current models and also the models themselves. We have especially analyzed the existing contradictions and attempted to explain them whenever possible. We also discuss new models that can explain the biological significance of clustering of transcribed genes and show possible mechanisms of the origin of transcription factories in the course of evolution.

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TRANSCRIPTION OF EUKARYOTIC GENES: WHAT MOVES AND WHAT REMAINS MOTIONLESS?

From first appearances the response to the question appears obvious. One can recollect famous electron microscopic photos of transcribing ribosomal genes [1] and come to the following conclusion: the elongating complex of RNA polymerase moves along the DNA molecule, which remains motionless. The same is written in the majority of molecular biology textbooks. But it is reasonable to mention that the electron microscopic photos of transcribing ribosomal genes were obtained under conditions providing for destruction of various intranuclear structures (low ionic strength, ionic detergent). In the cell nucleus ribosomal genes are transcribed in the nucleoli where RNA polymerase I and transcriptional factors required for its activity are concentrated in rather small (200-500 nm) compartments called "fibrillar centers". Some data suggest that during transcription rDNA is moved along the surface of the fibrillar centers. And the newly synthesized transcripts are released into the compartments adjacent to fibrillar centers ("dense fibrillar component") [2].

The majority of eukaryotic genes are transcribed by RNA polymerase II. For a rather long time data have been published indicating that the elongating complexes of RNA polymerase II are fixed on skeletal structures of the nucleus [3, 4]. Based on these data, the DNA molecule was supposed to move along the immobilized transcriptional complex [5]. This hypothesis provoked many discussions. It should be noted that the preferential localization of the elongating complexes of RNA polymerase II within skeletal structures of the nucleus (nuclear matrix) is not a clear proof of immobilization of these complex on a protein framework. Strictly speaking, elongating transcriptional complexes cannot be solubilized when nuclei are extracted with nonionic detergents and concentrated salt solutions. This can be a consequence of aggregation of transcriptional complexes themselves and/or of newly synthesized RNA molecules associated

* To whom correspondence should be addressed.

with them in concentrated salt solutions [6]. This viewpoint was disproved by experiments, which analyzed the possibility of electroelution of transcribing DNA sequences and transcriptional complexes bound with them from nuclei placed into agarose blocks. It occurred that at the physiological ionic strength chromatin fragments bound with transcriptional complexes could not be removed from nuclei under the influence of electric field (in contrast to chromatin fragments not bound with transcriptional complexes) [7, 8]. Thus, the progressively accumulated indirect data indicated that the transcription could be catalyzed by RNA polymerase II molecules immobilized on skeletal structures of the nucleus. In this connection is reasonable to ask whether the immobilized RNA polymerase II molecule can perform transcription. The answer to this question was given by a model experiment in which the RNA polymerase molecule was fixed on a Sepharose carrier. This molecule was shown to effectively perform transcription [9, 10].

TRANSCRIPTION FACTORIES

Analysis of distribution of newly synthesized RNA in nuclei of eukaryotic cells revealed its concentration in a limited number of places (transcription foci) [11, 12]. In the same foci elongating complexes of RNA polymerase II are concentrated, which can be visualized by immune staining with antibodies to the phosphorylated form of RNA polymerase II [10, 13, 14]. By analogy with the earlier identified replication factories [15], clusters of the elongating RNA polymerases were called transcription factories [10]. Clustered places elongating complexes of RNA polymerase II (transcription factories) were also found by immunoelectron microscopy of nuclei upon a short-term inclusion of a biotinylated precursor into the synthesizing RNA. The diameter of transcription factories was 70-80 nm [16]. Each transcriptional factory contained from 4 to 20 elongating polymerases (as judged from the number of detected newly synthesized transcripts) [16]. Based on calculations (comparison of the total number of synthesized transcripts and the total number of transcription factories in the nuclei of HeLa cells) the same authors found that the average number of RNA polymerase II molecules in one transcription factory was 30 [17]. The total number of transcription factories in nuclei of HeLa cells was 2000-2400 [16, 17]. This evaluation is significantly different from previous results obtained by the same and other authors (300-500), which were based on calculations of transcription foci with a fluorescence microscope [11, 12].

The clustering of elongating complexes of RNA polymerase II clearly demonstrated in the above-cited works evokes many questions, in particular, whether transcription factories exist as independent nuclear compartments in the absence of transcription. The alternative possibility is the clustering of elongating transcription complexes for some other reason. In this case, transcription factories will exist as long as the transcription continues. This hypothesis was experimentally tested by Mitchell and Fraser [18]. They found that under heat shock conditions when inclusion of labeled precursors into RNA was almost completely suppressed the phosphorylated RNA polymerase II was retained in isolated foci (transcription factories). Moreover, the number of transcription factories was virtually the same in the control cells and in the cells cultured under heat shock conditions. Similar observations were also recorded upon the inhibition of transcription with 5,6-dichlorobenzimidazole (DRB) [18]. These findings directly confirmed the model of the existence of transcription factories (agglomerates of phosphorylated RNA polymerase II) independently of the transcription process.

Nevertheless, this model is not beyond debate. In the framework of this model it is difficult to explain why only the rapidly diffusing population of RNA polymerase II can be detected under conditions of heat shock [19]. This population does not contain phosphorylated forms of the enzyme [19] and cannot be represented in transcription factories even because the concentration of any protein in some foci revealed by immunostaining is always determined by the rate of protein exchange between the foci and the remaining part of the nucleoplasm, which is lower than the exchange rate of this protein within the nucleoplasm. In cells cultured under usual conditions, in addition to the rapidly exchanging population of RNA polymerase II (~75% of the total number of RNA polymerase II molecules), a slowly exchanging population was also present (~25% of the total number of RNA polymerase II molecules) [19, 20]. By quantitative immunoblotting the total number of molecules in the slowly exchanging population of RNA polymerase II was calculated to be approximately the same as the number of phosphorylated molecules of this enzyme [19, 20]. Note that just the phosphorylated form of RNA polymerase II is preferentially present in transcription factories. But the complete disappearance of the slowly exchanging fraction of RNA polymerase II under conditions of heat shock [20] is difficult to connect with the observation that the number of transcription factories detected in the nuclei is the same under normal and under heat shock conditions [18]. Further experiments are required to explain this contradiction.

GENE DISPLACEMENT TO TRANSCRIPTION FACTORIES AS A STAGE OF TRANSCRIPTION ACTIVATION

Because transcribing molecules of RNA polymerase II are concentrated in transcription factories, the transcribed genes should also be there. This was confirmed using the *in situ* fluorescent hybridization technique

(FISH) [21-24]. To discriminate the transcribing and silent alleles, DNA-FISH with samples to the genes under study and RNA-FISH with samples to short-lived intron sequences were performed concurrently. These experiments demonstrated not only that active genes are colocalized with transcription factories, but also that the transcription occurs not constantly but within relatively short periods followed by periods of silence. Potentially active but not transcribed at the moment genes are localized far from transcription factories. Some cells can be significantly different in the spectra of genes transcribed at a particular time. Analysis of fixed cells by the FISH technique revealed cells with expression of both alleles of the gene under study, cells with expression of one allele, and cells without the gene expression [21, 22, 25-27]. It was also demonstrated that inhibition of transcription under conditions of heat shock was associated with a partial dissociation from transcription factories of earlier transcribed genes [18]. However, this result is not quantitatively convincing. In fact, according to data of the above-cited work, in cells of mouse embryonic liver ~60% of *Hbb* (the actively expressed gene of globin) alleles were associated with transcription factories. Under heat shock conditions this fraction decreased to 20% but remained significant. In the case of another erythroid-specific gene (*Eraf*) ~43% of alleles were associated with transcription factories before the heat shock, and ~22% of alleles remained associated with them under heat shock conditions.

In other experiments from Fraser's laboratory, it was studied whether the start of transcription correlates with the displacement of genes to pre-existent transcription factories. As a model system, induction of expression of the genes *c-Fos* and *c-Myc* upon stimulation of resting B-cells by interleukin-4 was used. Upon the stimulation, the number of expressing alleles of *c-Fos* and *c-Myc* increased 2-3-fold (from 20 to 53% for the *c-Fos* gene and from 26 to 75% for the *c-Myc* gene) [22]. Both genes were preferentially displaced into the transcription factories, which transcribed genes of immunoglobulin heavy chains. The choice of just these transcription factories seemed to be determined by some specific features of the spatial organization of DNA in the nucleus and was not directly associated with the level of *c-Myc* transcription. In fact, before the stimulation of the resting B-cells by interleukin-4 nearly 25% of the transcribed alleles of *c-Myc* were colocalized with the transcribed alleles of *Igh*. The level of *c-Myc* expression is extremely low (on average, <1 transcribed allele per cell). Upon the stimulation the number of transcribed alleles of *c-Myc* increased significantly. However, the percent of transcribed *c-Myc* alleles colocalized with the transcribed *Igh* alleles remained virtually constant (~25%). This means that independently of the total number of transcribed alleles of *c-Myc* each fourth transcribed allele was attracted to the transcription factory performing the transcription of *Igh* [22]. Certainly, it was possible, in particular, because a significantly greater number of *Igh* alleles were transcribed compared to the number of transcribed *c-Myc* alleles.

Thus, the existence of "vacant" transcription factories containing active *Igh* alleles and ready to "accept" *c-Myc* alleles for transcription was not a limiting factor. The displacement of transcribed genes to preexisting transcription factories was also shown in other works [23, 28]. The mechanism of gene displacement to transcription factories is not clear. In work [29] the gene displacement associated with the activation of transcription was shown to be mediated through actin-myosin motors.

It remains absolutely unclear when the pre-initiatory complex of RNA polymerase II is assembled on promoters involved in gene transcription. This might occur both before and after attracting these genes to transcription factories. The second possibility seems to be preferential in the context of the transcription factory model. In fact, it would be reasonable to suppose that genes are displaced to the pre-existent transcription factories just to provide their availability for RNA polymerase. But within this scenario it is difficult to explain the presence in the nucleus of a rather impressive population of rapidly diffusing RNA polymerase II molecules, which includes ~70% of the molecules of this enzyme [19, 20]. One may suggest that this enzyme first forms pre-initiation complex on a promoter, and afterwards the gene is displaced to a transcription factory. The elongation inside transcription factories may be favorable because these compartments may contain complexes of chromatin remodeling and other factors required for effective transcription of DNA organized into nucleosomes. Moreover, transcription factories can be located near compartments containing splicing factors [30, 31] and near transport pathways used for displacement of newly synthesized RNA [32].

TRANSCRIPTION FACTORIES AND SPATIAL ORGANIZATION OF THE GENOME

Since the number of transcribed genes is significantly higher than the number of transcription factories [33], it is obvious that one transcription factory has to contain various genes. The question arises whether the distribution of transcribed genes among the individual transcription factories is random. If the association of transcribed genes into individual transcription factories is not random, it is reasonable to ask what determines the combination of particular genes into transcription factories. Some regulations in the gene distribution among transcription factories can be determined by specific features of the chromosome positions in the interphase nucleus and of the spatial organization of individual chromosome territories [34-37]. On the other hand, the combining of functionally linked genes into the same transcription factory can promote additional possibilities for regulation of coordinated expression of these genes and thus be biolog-

ically significant. Therefore, it seems that just the necessity for concentrating particular genes in the individual transcription factories stipulates specific features of the spatial organization of the genome in the interphase nucleus [38, 39]. All these questions are actively discussed in the literature [40]. To look into the problem, it is necessary to mention approaches that are used for investigating the three-dimensional organization of the genome.

Among these approaches fixation of chromosome conformation (3C) is especially important [41-44] (Fig. 1a). This approach allows us to elucidate whether two distant sequences on the DNA molecule are immediately adjacent in the cell nucleus. A series of similar experiments also promotes analysis of the probability of the spatial colocalization of the DNA sequence under study with some different sequences. The 3C-based full-genome approaches (4C, 5C) [45, 46] allow researchers to identify the whole spectrum of sequences that can contact (be within the common functional complex) with the DNA sequence under study and to establish relative frequencies of associations between the DNA sequence under study and every partner.

For correct interpretation of results obtained with the 3C approach, it is necessary to understand that it has some shortcomings. First, as any biochemical method, the 3C approach can give only an averaged picture of events occurring in different cells of the population. Second, this approach does not allow researchers to assess absolute values. Interactions between various regions of the genome can be judged based on the shape of the curve that presents relative frequencies of ligation of different fragments of the genome. Two- or threefold difference is often considered sufficient for conclusion about the interaction between some fragments of the genome and the lack of interaction between its other fragments [47, 48]. Thus, in one of the classic works characterizing the activator complex of the mouse β-globin gene domain the experimentally found frequency of associations between the embryonic globin gene *y* with the region of control of the β-globin gene locus in erythroid cells of 10.5-day-old embryos was only twice as high as in brain cells where globin genes are not expressed [48]. Based on this finding, it was concluded that the erythroid cells of 10.5-day-old mouse embryos contain a specific activator complex that includes both embryonic globin genes and regulatory elements of the domain [48]. Unfortunately, it is impossible to elucidate whether the embryonic globin genes interact with the locus-controlled region in all cells or only in a small part of the cell population. To answer this question, it is necessary to normalize data on the experimentally found frequency of associations of two genome fragments that are exactly known to be localized within the same complex in all cells. Although there are experimental approaches for creating such a control, nothing of the kind has been performed up to now. Finally, the third fundamental shortcoming of the 3C procedure is its inability to demonstrate the existence of multicomponent complexes including more than two DNA fragments. A conclusion about the existence of such complexes is based on indirect data. Thus, if in two experiments the fragment *a* is shown to interact with both fragments *b* and *c* and in a third experiment fragment *b* is shown to interact with fragment *c*, it is concluded that all three fragments are localized within the same complex *a*–*b*–*c* [42, 47-49]. But really these results can also mean that in different cells of the population there are interactions in pairs: *a*–*b*, *a*–*c*, and *b*–*c* (Fig. 1b).

The question about colocalization of two or more genes within the same transcription factory can be analyzed using the FISH approach to visualize genes under study (or short-living intron transcripts) and immunofluorescent staining to detect the phosphorylated form of RNA polymerase II [21, 22, 24, 50]. This approach allows us to directly calculate the relative number (percent in population) of cells with colocalized genes under study. But the method is rather laborious. Therefore, in the majority of works 200 or fewer signals are calculated [22]. Correspondingly, at low percent of cells with colocalized genes the statistical significance of results is not high. Another problem is associated with the resolution of light microscopy. False-positive results can be obtained (two genes located within different but closely located transcription factories can be recorded as genes located within the same transcription factory). In this relation it is reasonable to remember that the number of transcription factories detected in HeLa cells with an optical microscope was about fivefold lower than the number of transcription factories detected in the same cells by electron microscopy [16, 17].

The results obtained using both the 3C technique and the FISH method suggest that organization of active genes into transcription factories is not random. However, this organization is not strictly determined. Organization into transcription factories is best studied for erythroid-specific genes. By biochemical (4C) and cytological (FISH) approaches a preferential localization of erythroid-specific genes was revealed within the same transcription factory in erythroid mouse cells expressing α- and β-globin genes. The colocalization frequencies of different erythroid-specific genes within the same transcription factory significantly varied (from 2.1 to 14.4%) [24, 50]. The frequency of colocalization within the same transcription factory of α- and β-globin genes was 7%. To correctly evaluate these results, it should be taken into account that the frequency of random colocalization of two genes within the same transcription factory is 1-2% according to calculations of the same authors [51].

Note that preferential partners for transcription factories were different for genes of α- and β-globins. Frequencies of colocalization of three erythroid-specific genes in the same transcription factory were never higher than that calculated based on frequencies of colocaliza-

tion of different gene pairs, but for some genes it could be significantly lower. Thus, the presence of particular gene pairs within the transcription factory had a negative influence on the probability of inclusion into this factory of other erythroid-specific genes [24].

The most unexpected result of the cited work was the demonstration that preferential partners of globin genes from the transcription factories were localized on different chromosomes. It was reported earlier that the genes preferentially interacted with other genes and regulatory sequences localized on the same chromosome, i.e. inside the same chromosome territory [52]. The authors interpreted their results in the context of the model of specialized transcription factories [38]. Organizing stimuli of

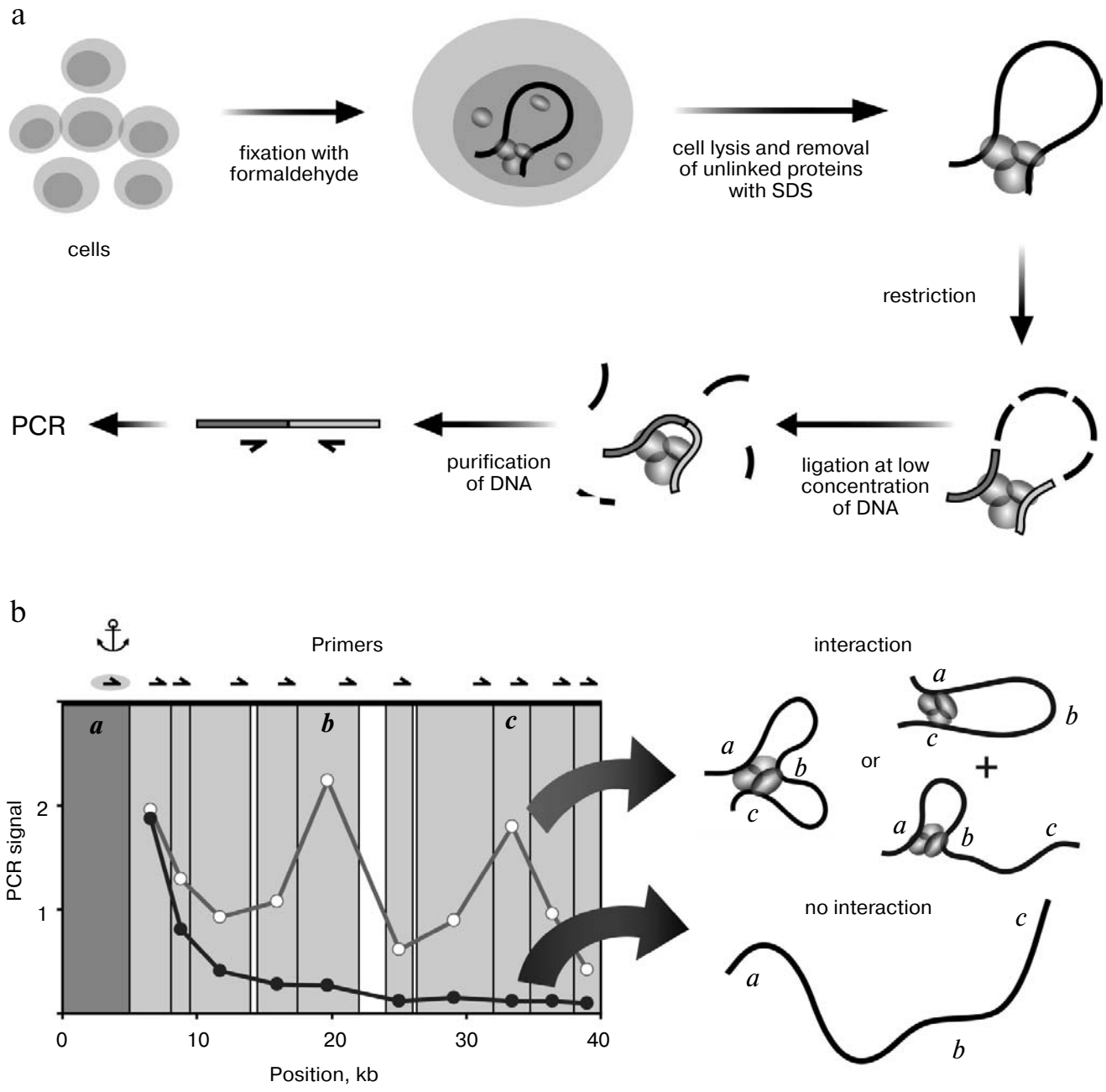

Fig. 1. General principle of 3C experiments (a) and interpretation of results (b). b) Hypothetical case when fragment *a* interacts with fragments *b* and *c* in some cells (gray curve) and does not interact with these fragments in other cells (black curve). The curves show the dependence of probability of fragment *a* (the so-called "anchor fragment") cross-ligation with some fragments localized to the right of fragment *a*. The vertical black lines indicate positions of sites of DNA cleavage by restrictases. The interpretation of the 3C analysis results is shown to the right. The approach cannot differentiate situations when fragments *a*, *b*, and *c* form one complex and when these fragments interact in pairs *a*–*b* and *a*–*c*.

such transcription factories seem to be tissue-specific transcription factors (Klf1 in erythroid cells) [24]. This hypothesis is inconsistent with the presence in the transcription factories transcribing globin genes of actively transcribed non-erythroid genes, in addition to erythroid-specific genes [21].

The studies on the spatial organization of erythroid-specific genes in globin-producing cells resulted in the most important conclusion about the extreme plasticity of this organization. Although there is a slight preference for association between particular genes, these genes can form complexes with other partners, and the number of interaction partners found by different approaches is very great [21, 24]. At present it is difficult to establish whether the genome spatial organization is dynamic within the same cell or different variants of this organization are realized in different cells of the population. In any case, the absence of a strict determination in the spectrum of spatial association of different genes indicates that such associations arise passively as a consequence of functional processes and are not a prerequisite for these processes.

TRANSCRIPTION FACTORIES AND ELONGATED ZONES OF SYNTENIC GENE LOCALIZATION IN GENOMES OF DIFFERENT ORGANISMS

In addition to the above-discussed model of specialized transcription factories, the necessity of clustering of several transcription units can be also explained otherwise. The most interesting is the hypothesis that the crucial role in organization of transcription factories is played by coexpression of neighboring genes. The most primitive form of coexpression of neighboring genes is their association into an operon. Fifteen percent of genes of *C. elegans* are operons transcribing as a polycistronic template [53]. The primary transcript is processed with production of monocistronic RNAs, and genes within the same operon are controlled by common regulatory systems. It is reasonably to suppose that such operons are evolutionary precursors of transcription factories. Transcription units of higher eukaryotes are not united into classic operons. But analysis of transcription databases reveals a frequent coexpression of neighboring genes in higher organisms [54-56]. The biological sense of clustering of housekeeping genes is clear [57]. The high and constant level of their expression and clustering in the genome are responsible for supporting in the nucleus of special compartments where transcription goes actively and constantly. However, clustering of actively transcribed sequences is rather a reasonable prerequisite for their uniting into the same transcription factory. In fact, promoters of the housekeeping genes clustered on the mouse 11th chromosome are closely located. This resulted in the conclusion that they are associated within the same transcription factory [28].

It seems that genomes of ancient multicellular organisms were organized as polycistronic operons; this type of the genome organization left its imprint in contemporary organisms as clustering of coexpressing transcription units. It seems that the most ancient specialization of cells in primitive multicellular organisms was their separation into sex and somatic cells. Therefore, in addition to clustering of coexpressed housekeeping genes associated into the same transcription factory, it is reasonable to expect clustering of genes specific for sex cells. In fact, the clustering level of genes expressed in testes of mice [58] and fruit fly [56, 59] is rather high. Clusters of oocyte-specific genes are also described [60]. Study on their spatial structure using the 3C approach is promising for determination of the topography of transcription factories in these regions of the genome.

While the clustering of housekeeping genes seems to be a sufficient and reasonable prerequisite for their association within the same transcription factory, the situation with tissue-specific genes appears much more complicated. Not considering paralogous genes, which appeared as a result of tandem duplications (globins, immunoglobulins, olfactory receptors), one can conclude that tissue-specific genes do not tend to cluster within the genome (except the clustering of genes coexpressed in cartilaginous tissue [61]). It seems that dispersed localization in the genome prevents formation by tissue-specific genes of structurally and functionally separate transcription factories. In fact, "strictly" erythroid-specific genes of α-globins are reported to use pre-existent transcription factories and be attracted to them during erythroid differentiation [28].

If coexpression of clustered genes is really favorable for the organism, it explains the support of elongated regions of genomic synteny during evolution, i.e. existence in different species of rather elongated regions of the genome with the same sequences in the gene localization. Such syntenic regions often include coexpressed genes.

Mechanisms of both support and reorganization of transcription factories during cell division are still beyond researchers' attention. Support of an unchanged transcription landscape upon cell division can be provided by persistence of the existent interactions of the genome elements in the mitotic chromosome. This hypothesis can be easily tested. The *de novo* association of coexpressed genes after cell division within the same transcription factory suggests, on one hand, the marking in the genome that determines the partners and, on the other hand, the presence of a place for assemblage and mechanisms for moving transcription units and their regulatory elements within the nucleus. The nuclear matrix seems to be just such a place permitting the search for transcription factory partners in a plane instead of a three-dimensional space. CpG-islets capable of interacting with the nuclear matrix are rather attractive candidates as "reference" points for initiating the assemblage of a transcription factory.

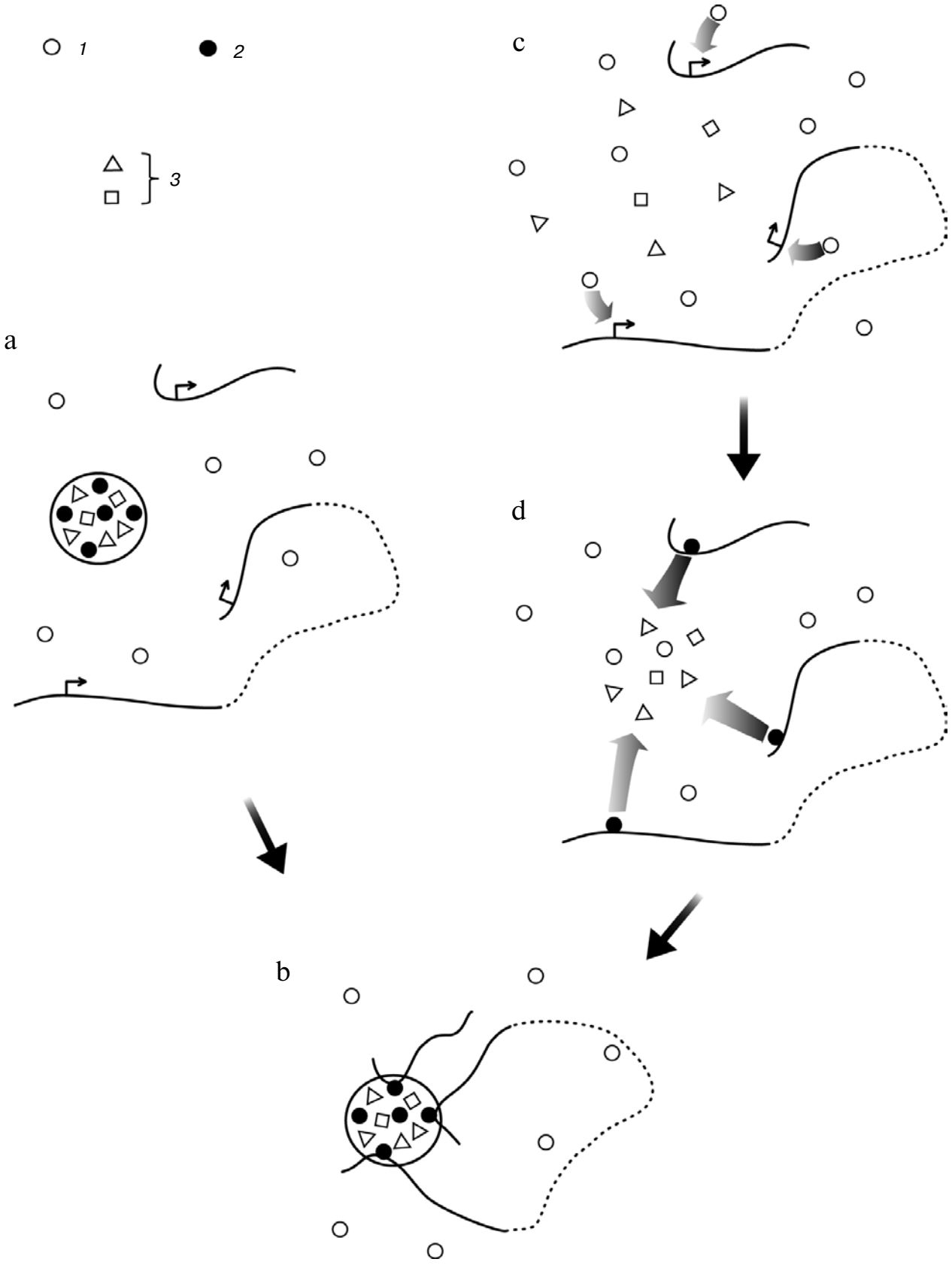

Fig. 2. Two models of transcription factories: *1*, *2*) rapidly exchanged and phosphorylated RNA polymerases, respectively; *3*) other proteins.

In conclusion, we shall try to answer the question: what are transcription factories? According to the traditional viewpoint, transcription factories are compartments where transcription complexes are concentrated (Fig. 2a). To be transcribed, genes are first moved to transcription factories, and after that they can be bind with RNA polymerase (Fig. 2b). Individual genes are supposed to be transcribed within short time intervals whose duration is determined by the duration of the gene association with the transcription factory [51]. The components of transcription factories (besides RNA polymerase II) are still unknown. It is only reported that transcription occurs in protein-enriched compartments of the nucleus [62].

All experimental results can be explained also in the context of a fundamentally different model of transcription factories. This model suggests that transcription factories arise as a result of association of genes on whose promoters the pre-initiatory complex of RNA polymerase II is already assembled (Fig. 2d). Uniting these genes within some nuclear compartments can be necessary first of all for the more rational use of different accessory proteins (complexes of chromatin remodeling, FACT, etc.) that are required for effective transcription of DNA organized as chromatin. The final result—clustering of RNA polymerase II molecules involved in elongation—will be the same as in the traditional model (Fig. 2b). However, this model explains the existence of a considerable population of rapidly exchangeable (not phosphorylated) RNA polymerase II (Fig. 2c). Just such polymerase sits on the promoter, and then the C-terminal domain is phosphorylated. Recently published data suggest that transcription factories can unite genes located rather distantly, including genes located in different chromosomes. It was also shown that transcription factories could arise as a result of clustering of neighboring genes.

There is a viewpoint that transcription is catalyzed by RNA polymerase tightly anchored on skeletal structures of the nucleus. But the modern findings do not undoubtedly confirm that transcription is catalyzed by just such RNA polymerase. In any case, RNA polymerase, which performs the elongation, will be exchanging slowly because it is linked with a DNA molecule (more precisely, with the chromatin fibril). The resolution of fluorescent microscopy is insufficient for the statement that RNA polymerase II is not displaced inside transcription compartments, which can be detected in the nuclei by immune staining with antibodies against phosphorylated RNA polymerase II. These compartments are rather large. Polymerase can be supposed to move along the chromatin fibril while the fibril slowly changes its location (moves in the direction opposite to the direction of the polymerase movement) to keep the transcription complex within the limits of the compartment containing all protein complexes required for transcription. Electron microscopy photos show the position of molecules at the moment of cell fixation. These photos undoubtedly confirm the clustering of the elongating molecules of RNA polymerase II [16]. But they do not allow us to determine whether the RNA polymerase II molecule is immobilized.

As to association of RNA polymerase II and transcription factories with the nuclear matrix [63], it must be taken into account that the procedure of preparing nuclear matrix includes elements of fixation (aggregation of molecules at high ionic strength, stabilization with copper ions, stabilization with agents locking disulfide bonds) [64-67]. Discussion of the vast literature concerning this problem is beyond the limits of this review. Although there is no doubt that in nuclear matrix preparations many functional compartments are retained that can be visualized in the nucleus of living cells (including "speckles" and transcription factories) [68, 69], it cannot be stated now that these compartments consist of molecules immobilized on the nuclear matrix. Future studies are needed to solve the questions and remove contradictions associated with the concept of "transcription factory".

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