Transcription

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Transcription factories

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Abstract

There is increasing evidence that different transcription units are transcribed together in discrete nuclear structures known as transcription factories. Various new techniques enable us to detect and characterize these structures. We review the latest findings and discuss how they support a model for transcription and chromosome organization.

Introduction

The sequence of the human genome has now been obtained, but the way DNA with a contour length of approx. 2 m might be compressed into a nucleus approx. 200 000 times smaller is still a matter of debate. All agree on the first level of folding, in which DNA is wrapped around histone octamers to form nucleosomes, but the various properties of chromatin have complicated the analysis of the higher-order structure. First, chromatin is easily sheared when it is extracted from nuclei, and then it often aggregates into an intractable gel. Non-physiological buffers are often used to minimize this, but they can distort the structure. Secondly, many chromatin folds have dimensions below the resolution of the light microscope (i.e. ~200 nm), and use of the electron microscope with its higher resolution introduces additional problems of preserving structure *in vacuo*. As a result, many conflicting results have been obtained, and these have led to several different models. However, one enduring idea is that chromosomes are looped by attachment either to some subnuclear structure or to another part of the same chromosome (e.g. [1–3]), and there is now evidence that active transcription units strung along the chromosome come together to form a 'transcription factory' inevitably tying the intervening DNA into loops [4]. Here, we discuss how some new techniques are adding to our understanding of these transcription factories and genome organization.

Key words: chromosome conformation capture (3C), chromosome organization, genome organization, locus control region, transcription factory.

Abbreviations used: BrUTP, bromouridine triphosphate; 3C, chromosome conformation capture; 4C, 3C-on-chip; ESI, electron spectroscopic imaging; FISH, fluorescence *in situ* hybridization; GATA-1, GATA-binding protein 1; LCR, locus control region; NOR, nucleolar organizing region; UBF, upstream binding factor.

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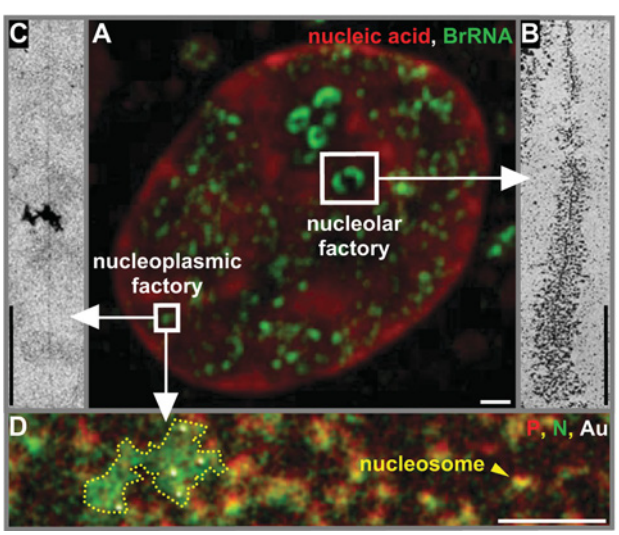
Transcription occurs in factories

The first evidence that the human genome might be looped came from experiments demonstrating the existence of supercoiling in the DNA of nucleoids made by removing histones with 2 M NaCl; such supercoils can only be maintained in a linear chromosome if two or more points are tethered to each other or to the substructure [5]. Furthermore, cutting linear (nucleosome-covered) chromosomes randomly should generate a few large fragments that are progressively cut into smaller ones. However, when nuclei are treated with a non-specific nuclease, few large fragments are released; instead, many cuts are required to release any DNA, and the kinetics fit a model in which at least two cuts are required to release one small fragment from a loop [6,7]. It has also been shown that an enhancer can activate its target promoter even when the two are carried on different plasmids, or separated by a biotin–streptavidin bridge [8,9]; we must then assume that in the normal context, a physical interaction between the enhancer and promoter on one chromosome will generate a loop.

The initial experiment that pointed to the transcription machinery acting as a critical molecular tie that maintained the loops involved growing cells in [^{3}H]uridine to pulse-labelled RNA and then removing most of the loop with a nuclease; essentially all (nascent) [^{3}H]RNA remained behind with the templates. This indicates that active genes lie very close to points of attachment [10]. Evidence suggesting that several different transcription units cluster together was provided by permeabilizing cells, and allowing engaged polymerases to extend their transcripts in BrUTP (bromouridine triphosphate);

Figure 1 | Different views of the nascent transcripts in HeLa cells

(**A**) Cells were permeabilized, nascent transcripts extended in BrUTP, cells cryosectioned (100 nm), the resulting BrRNA immunolabelled with FITC (green), nucleic acids counterstained with TOTO®-3 (red) and a fluorescence image collected on a confocal microscope. Newly made BrRNA is concentrated in factories in the cytoplasm (made by mitochondrial polymerases), nucleoplasm and nucleoli. Modified from [48] with permission; image courtesy of A. Pombo. (**B**, **C**) Conventional electron micrographs of spread transcription units. In (**B**), a crescent like one of the two in the nucleolar factory in (**A**) has been stripped off the underlying structure; approx. 125 transcripts can be seen engaged on the rDNA unit. Adapted from [49] with permission. © Society of the European Journal of Endocrinology (1972). In (**C**), one of the approx. 8 active transcription units in a nucleoplasmic factory like the one in (**A**) is shown; the template is associated with one polymerase and transcript. Adapted from [13] with permission. © 1998 The American Society for Cell Biology. http//www.molbiolcell.org. (**D**). Electron micrograph of a nucleoplasmic factory obtained using a specialized technique (ESI) that can detect endogenous phosphorus and nitrogen in unstained sections. HeLa cells were permeabilized, nascent transcripts extended in BrUTP, the resulting BrRNA immunolabelled with 5-nm gold particles; after sectioning (70 nm), maps of phosphorus (red), nitrogen (green), and the gold particles (white) marking BrRNA were collected. The image shows a merge of the three maps. Five gold particles mark BrRNA in a nitrogen-rich factory (perimeter indicated). Absolute numbers of nitrogen and phosphorus atoms within this perimeter can be calculated by using nearby nucleosomes as references (as they contain known numbers of atoms). Adapted from [43] with permission. Scale bars, 1 μm (**A**, **B**) and 100 nm (**D**).



(nascent) BrRNA was seen in a few discrete foci, the factories (Figure 1) [11,12]. Quantitative analysis then showed that a typical factory in the nucleoplasm of a HeLa cell contains approx. eight polymerases, each engaged on a different unit [13].

Clustering of active units ensures high local concentrations, enabling efficient interaction; for example, HeLa nuclei contain an approx. 1 μM pool of RNA polymerase II, but the local concentration in a factory is approx. 1 mM, so few transcripts would then be made outside factories. Two theoretical arguments suggest that components of the transcriptional machinery are likely to cluster, and so form factories [14]. First, many transcription factors dimerize, and if they also bind to two sites on DNA that are a few kb apart, they will inevitably loop the intervening DNA when they come together. As GFP (green fluorescent protein)-tagging shows that many transcription factors remain bound to DNA for only a second or so, such ties (and the resulting loops) would be transient. Secondly, two polymerases engaged several kb apart on one template are likely to come together spontaneously in the crowded nucleus through what physicists call the 'depletion-attraction'. Loops formed in this way would last for as long as the polymerases remain engaged, which can be for many hours in humans.

Results obtained with a new set of techniques now provide strong evidence for the existence of loops. For example, 3C (chromosome conformation capture) and its derivatives involve fixing cells with formaldehyde and then analysing which sequences tend to lie next to each other in three-dimensional nuclear space [15–21]. Contacts between *Hbb-b1* (encoding β-globin) and its LCR (locus control region) have extensively been studied [16,22]. The two are often found together in liver cells where both are transcribed, but not in brain cells where globin is silent (Figure 2A). (Many other LCRs, and functional elements such as enhancers, silencers and insulators, are also transcribed [23,24].) Then, it is easy to imagine that the LCR and *Hbb-b1* are bound to the same factory [17,25,26], and that the LCR acts by bringing its target genes closer to the relevant factory (Figure 2B).

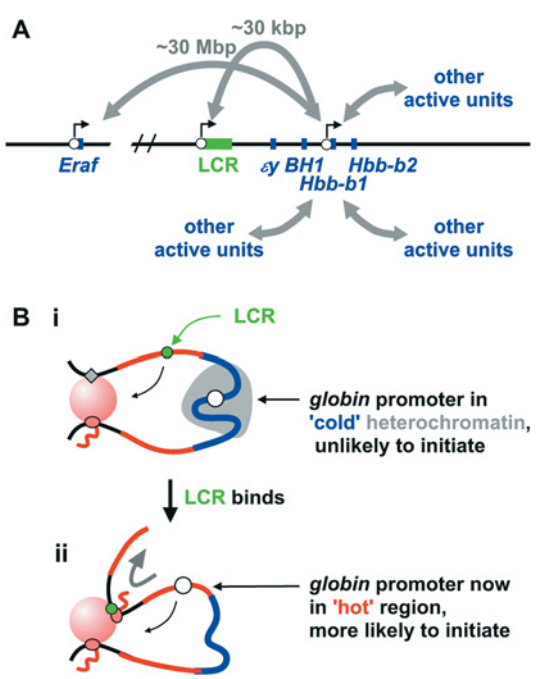
Hbb-b1 can also be found close to *Uros* (uroporphyrinogen III synthase) and *Eraf* (erythroid-associated factor), two genes located more than 20 Mbp away on the same chromosome (Figure 2A) [17,25]. Proximity depends on ongoing transcriptional initiation, suggesting that the genes are transcribed together in the same factory [27]. Many of the results described above have been confirmed using RNA TRAP (tagging and recovery of associated proteins) and RNA FISH (fluorescence *in situ* hybridization) [17,22]. 3C has also been used to show that EKLF (erythroid Krüppel-like factor), GATA-1 (GATA-binding protein 1) and FOG-1 (friend of GATA-1) [28,29] {but not another regulator, p45 NF-E2 (nuclear factor, erythroid-derived 2) [30]} contribute to the LCR–*Hbb-b1* interaction. Inhibiting transcription does not cause the LCR to disengage from the gene, suggesting that transcription may not be required to maintain the structure [27]. Note that it is unlikely that loops are generated by the fixation used for 3C, as another method, 'Dam identification', provides evidence for loops in living cells [31].

Specialized transcription factories

Can any gene be transcribed in any factory, or do factories specialize in transcribing different types of gene? Are genes that encode components of one biosynthetic pathway and which are expressed co-ordinately (e.g. *Hbb-b1*, *Uros* and *Eraf*) transcribed in the same factory? There is now good evidence that factories do specialize, but how many different types of factory there are remains to be established.

Figure 2 | A model for LCR function

(**A**) The interaction landscape of mouse *Hbb-b1* in fetal liver cells. The position of globin genes, its LCR and *Eraf* on chromosome 7 are indicated. Grey arrows: interactions detected by 3C and 4C between *Hbb-b1* on this and other chromosomes in fetal liver, where *Hbb-b1* is transcribed; 80% are with other active units (compared with 13% seen in fetal brain). (**B**) A model for LCR function. (i) In the erythroid stem cell, the globin gene is tethered to a factory (pink sphere). As it is in a long loop far from the factory and embedded in heterochromatin, it is unlikely to diffuse to the factory and initiate. The LCR is closer to the factory, and collides with it more frequently. During erythropoiesis, the concentration of some critical transcription factor increases; this factor increases the chances that the promoter in the LCR will initiate when it collides with a polymerase in the factory. (ii) The LCR has now initiated, and the polymerase in the factory is reeling in its template (grey arrow); this movement has brought the globin gene out of the 'cold' heterochromatin into a 'hot' region close to the factory where it has a much higher chance of colliding with a polymerase in the factory and so initiating. In practice, a number of different transcription factors and promoters are probably involved in bringing the globin gene close to the factory. Modified from Experimental Cell Research, **229**, Iborra, F. J., Pombo, A., McManus, J., Jackson, D.A. and Cook, P.R., The topology of transcription by immobilized polymerases, pp. 167–173, © 1996, with permission from Elsevier.



The nucleolus provides the best example of a specialized factory; it is dedicated to 45S rRNA synthesis and ribosome production. Human rDNA loci are carried on chromosomes 13, 14, 15, 21 and 22; each locus encodes approx. 80 tandem repeats of the 45S rRNA gene, and forms a secondary constriction, or NOR (nucleolar organizing region), in the mitotic chromosome. Inactive RNA polymerase I and its transcription factor UBF (upstream binding factor) are bound to some NORs, and, on exit from mitosis, these NORs fuse to form one or more nucleoli [32]. NORs lacking bound UBF and polymerase remain inactive and do not fuse [33]. During interphase, nascent rRNA is found in the 'dense fibrillar component' (i.e. in the crescent in the box in Figure 1A) on the surface of a 'fibrillar centre' containing polymerase I and UBF, and newly completed transcripts are processed in a surrounding 'granular component' to emerge as mature ribosomal subunits into the nucleoplasm [34]. Here, several active transcription units cluster into one dedicated factory that contains the machinery necessary to make a ribosome. Stripping one crescent off the surface of the fibrillar centre then yields the iconic image of a 'Christmas tree' (Figure 1B).

Is this kind of specialization carried further, so that 'polymerase II factories' only transcribe class II units, and 'polymerase III factories' only class III units? Various studies indicate they do, and the most convincing one exploits the steric hindrance that occurs between immunolabelling probes [35]. Anti-polymerase II blocks access to RNA being made by polymerase II, but not to polymerase III protein or its transcripts; conversely, anti-polymerase III blocks access to RNA being made by polymerase III, but not to polymerase II protein or its transcripts. Do polymerase II factories specialize further still? Again, it seems they do. For example, some factories contain higher concentrations of transcription factors than others [36,37], and we have recently developed an assay that allows us to establish the extent of specialization. Pairs of plasmids encoding different promoters are introduced into cells, and their nascent transcripts localized by RNA FISH; different polymerase II promoters (and the presence of an intron) target plasmids to different host factories (Figure 2; [38]).

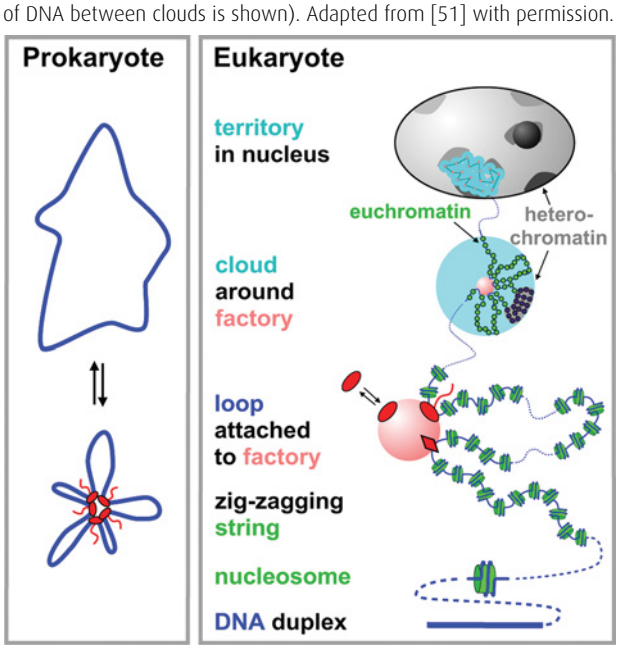
Given the evidence that factories can specialize in the transcription of class I, II or III units, and into class II units with or without introns, is it possible that they can further specialize to transcribe genes that share transcription factors in their regulation? We must now go on to establish how many different types of factory there might be. Note that active globin genes and their LCRs do not invariably share the same factory [17], perhaps because the stochastic nature of transcription [39–41] ensures that a gene associates with a factory only when it is transcribed, and because a gene might be transcribed in any one of a number of different factories.

The structure of nucleoplasmic factories

Imaging nucleoplasmic factories remains difficult, largely because they are small enough to lie below the resolution of most light microscopes. In the electron microscope, nascent RNA is found in clusters approx. 50 nm in diameter spread throughout the nucleoplasm [35]. The density and diameter of these nucleoplasmic clusters is very similar in nuclei in human, mouse and newt and in mouse embryonic stem cells as they differentiate [42]. We have recently imaged nucleoplasmic factories using ESI (electron spectroscopic imaging), a powerful ultrastructural method that can be used to map atomic distributions in unstained preparations [43]. Nascent RNA is almost invariably found on the surface of polymorphic nitrogen-rich (but phosphorus-poor) structures with minimum diameter of approx. 87 nm and a mass of 10 MDa

Figure 3 | Models for genome organization

In both prokaryotes and eukaryotes, structure determines function (and vice versa); genes tethered close to a factory are more likely to initiate than distant ones. In bacteria, transcription of the circular chromosome (top), followed by aggregation of polymerases (ovals) and transcripts (red lines), generates a looped structure (bottom) that is self-sustaining (as promoters in active genes now lie close to polymerases). Reproduced from [50] with permission. In eukaryotes (and specifically in a HeLa cell), DNA is coiled around the histone octamer, and runs of nucleosomes form a zigzag string. At the intermediate level in the hierarchy, this string is organized into loops (average contour length 86 kb; range 5–200 kb) by attachment to transcription factors (diamond) and engaged RNA polymerases (ovals). (There are other ties, in addition to these major ones.) A total of 10–20 such loops (only a few are shown) form a cloud around the factory, to give a structure equivalent to that of the bacterial nucleoid. [Active transcription units that are nearest neighbours are shown bound to one factory here, but the structure is more complex; units distant on the genetic map (perhaps on different chromosomes) will sometimes bind to the factory.] Active polymerases do not track along their templates; they are bound to a transcription factory and act both as motors that reel in their templates and as one of the critical structural ties that maintain the loops. Loops inevitably appear and disappear as polymerases initiate and terminate, and the factors bind and dissociate. Nucleosomes in long loops are static and acquire a (heterochromatic) histone code that spreads down the fibre; they also aggregate on to the lamina, nucleoli and chromocentres. Each transcription factory contains one type of RNA polymerase (i.e. I, II or III) to the exclusion of others, and some factories are richer in certain transcription factors than others (and so are involved in the transcription of specific sets of genes). Individual components in the factor exchange continually with others in the soluble pool. A total of 50–200 successive clouds strung along the chromosome form a territory (the general path of DNA between clouds is shown). Adapted from [51] with permission.



(calculated by reference to nucleosomes with known numbers of phosphorus and nitrogen atoms). Stripping an active transcription unit off the surface of the factory followed by electron microscopy yields the image shown in Figure 1(C). Then, it seems that the organization of nucleolar (polymerase I) and nucleoplasmic (polymerase II) factories is similar, with active polymerases and their templates on the surface of a dense core.

A model for all genomes

The results described above support a model for genome organization (Figure 3) in which the central architectural feature is the clustering of active units into factories, with engaged polymerases and transcription factors acting as the ties that organize the loops [2]. In eukaryotes, strings of factories would underlie the structure of chromosome territories, with chromatin in long loops aggregating into heterochromatin. This organization is supported by results from a 3C derivative, known as 4C (3C-on-chip), which show that active units cluster together, away from inactive chromatin [25]. Interactions between different chromosomes can also be detected by 4C [18,21,45]; presumably, fibres from the different chromosomes intermingle [44] because they share the same factory. This model also explains the finding that transcriptional inhibition alters the size and morphology of chromosome territories and regions of chromosome intermingling [44,46].

This model is easily extended to bacteria [4], and it can explain how the interphase organization is converted into a mitotic chromosome [2], how chromosomes might pair [47] and how regulatory motifs (e.g. LCRs, enhancers, barriers and silencers) might work [4,24].

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