The interdependence of nuclear structure and function

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2001 was the year of the human genome, but the new information has had little immediate impact on the field of nuclear structure. Rather, functional studies – especially on transcription – are leading us to a better understanding of how genomes might organise themselves into structures we call nuclei.

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Abbreviations
CBP CREB-binding protein
CFP cyan fluorescent protein
GFP green fluorescent protein
GR glucocorticoid receptor
NOR nucleolar organising region
ORC origin recognition complex
SRC-1 steroid receptor co-activator 1
TBP TATA-box binding protein
YFP yellow fluorescent protein

Introduction

2001 will be remembered as the year of the human genome [1••,2••], but how did that draft sequence impact on the field of nuclear structure? One might say, 'Little', because we are still such a long way from being able to predict three-dimensional structure from the primary DNA sequence. Nevertheless, the main principles underpinning nuclear architecture are becoming clearer, and many of those principles are surprising, because they concern function rather than structure. Therefore, in this review we will mainly cover the way nuclear functions influence structure.

The draft genome sequence proved to be important for what it did not contain — a class of repeated sequences obviously underpinning genomic architecture. For example, many models for interphase and mitotic structure involve looping of the chromatin fibre by attachment to a peripheral lamina or internal scaffold, and we might expect the attachment points to be highly conserved. But the various genome projects have failed to point to any of the molecules involved.

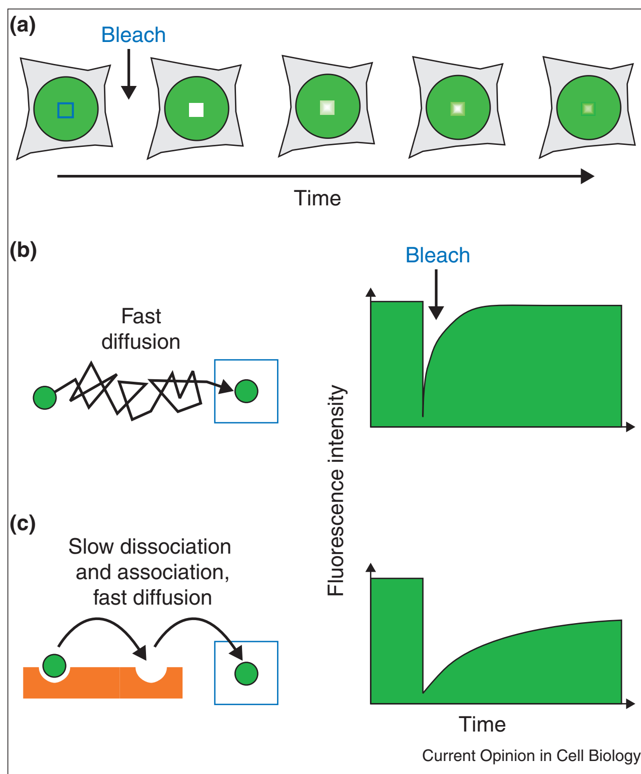
Self-organisation

DNA topoisomerase II has long been a strong candidate for the protein that binds to one of these repeats and organises the genome. It is found in the isolated nuclear matrix and chromosomal scaffold, apparently strategically placed at attachment points. However, a recent study using living cells makes it unlikely that it plays such a role [3••]. Mammals have two isoforms of this topoisomerase (α and β). These were tagged with green fluorescent protein (GFP), and photobleached, revealing that the entire population of both forms exchanges too rapidly with the soluble pool to be a structural component (Figure 1). Moreover, the axial distribution seen in the mitotic scaffold could be generated artefactually; in the living mitotic cell, the α isoform remains fully mobile and uniformly distributed, but it collapses into the axial scaffold on lysis in a hypotonic buffer.

If established models are being eliminated, what are the alternatives? A recent review provides an excellent way of thinking about the possibilities [4••]. Macromolecular structures are generated in two fundamentally different ways. Some virus particles 'self-assemble' to a fixed plan, to attain a true thermodynamic equilibrium. The particles are stable and static, and survive in the absence of a pool of unincorporated subunits once they have been released from the host. Models involving chromatin loops tied permanently to a scaffold are of this type. However, many cellular structures are built using a different — 'self-organising' — principle. For example, cytoskeletons lack a rigid architecture, and they are intrinsically unstable. They persist only by exchanging subunits with others in their surroundings, and they collapse if those subunits are removed or if function is inhibited. Are genomes self-organising structures? If they are, we might guess that transcription would be a candidate for the major function that drives the organisation. It might do so if polymerases scattered along the genome were to cluster into aggregates to form the genome into a surrounding 'cloud' of loops [5]. The nucleolus provides an example of how active transcription units cluster in this way. This structure is assembled under the control of cyclin-dependent kinases [6•] when transcription of the genes encoding rRNA resumes after cell division. It is disassembled when transcription ceases during prophase. Reassembly has been studied using stable lines expressing the nucleolar proteins fibrillarin and Nop52 tagged with GFP [7•]. Competent nucleolar organising regions (NORs) — which might be on different chromosomes — first recruit the two proteins, become active, and eventually aggregate with others to bring whole chromatin territories towards the re-forming nucleolus. The structure of interchromatin granule clusters is similarly dynamic, as overexpression of the serine/arginine cdc2-like kinase (Clk)/STY — but not a catalytically inactive mutant — causes their redistribution throughout the nucleoplasm [8•]. In all these cases, the structure depends on function, and *vice versa*.

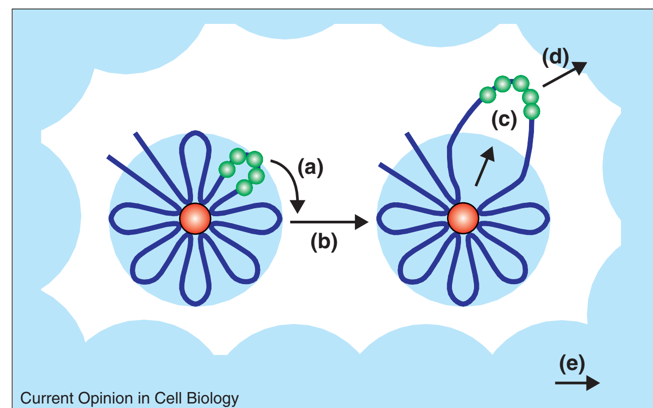
The various genome projects are also facilitating the cataloguing of where different proteins are to be found within nuclei. For example, one recent proteomic analysis combined mass spectrometry and sequence database

Figure 1



Measuring protein binding in living cells using fluorescence recovery after photobleaching (FRAP). **(a)** A protein tagged with GFP is shown distributed throughout the nucleus, and photobleaching the blue area creates a non-fluorescent square that is gradually repopulated by fluorescent molecules from the surroundings. Therefore, the intensity of fluorescence in the blue area first falls on bleaching, and then rises. Information about the diffusion and binding properties of the protein can be extracted from the recovery kinetics. **(b)** An unbleached molecule (green sphere) in the surroundings may diffuse (following a random walk) into the bleached square. Because diffusion is rapid, the intensity recovers quickly. **(c)** A structural protein would be expected to dissociate from that structure slowly; therefore, it enters the bleached rectangle slowly, and this is reflected by a slower increase in intensity after bleaching.

Figure 2



Possible motions of GFP-tagged repressors (green spheres) bound to an array of *lac* operators integrated into a chromatin loop. In this model [5], several loops are attached through engaged polymerases and transcription factors to a transcription 'factory' (red spheres) to form a surrounding 'cloud' (blue). The tagged repressors can move in various ways. **(a)** Although tethered (in this case to a factory), they can diffuse from side to side. **(b)** The whole cloud can move relative to neighbouring clouds, but is nevertheless constrained by them. **(c)** Attachments may be lost/regained (e.g. as tethering polymerases terminate or initiate) to increase or decrease the contour length of loops. **(d)** Nucleosomes in one loop may aggregate with those in another (e.g. when chromatin condenses in G2 phase) to decrease mobility. **(e)** Whole chromatin territories can move relative to others, as the nucleus rotates and tumbles in the cell [43].

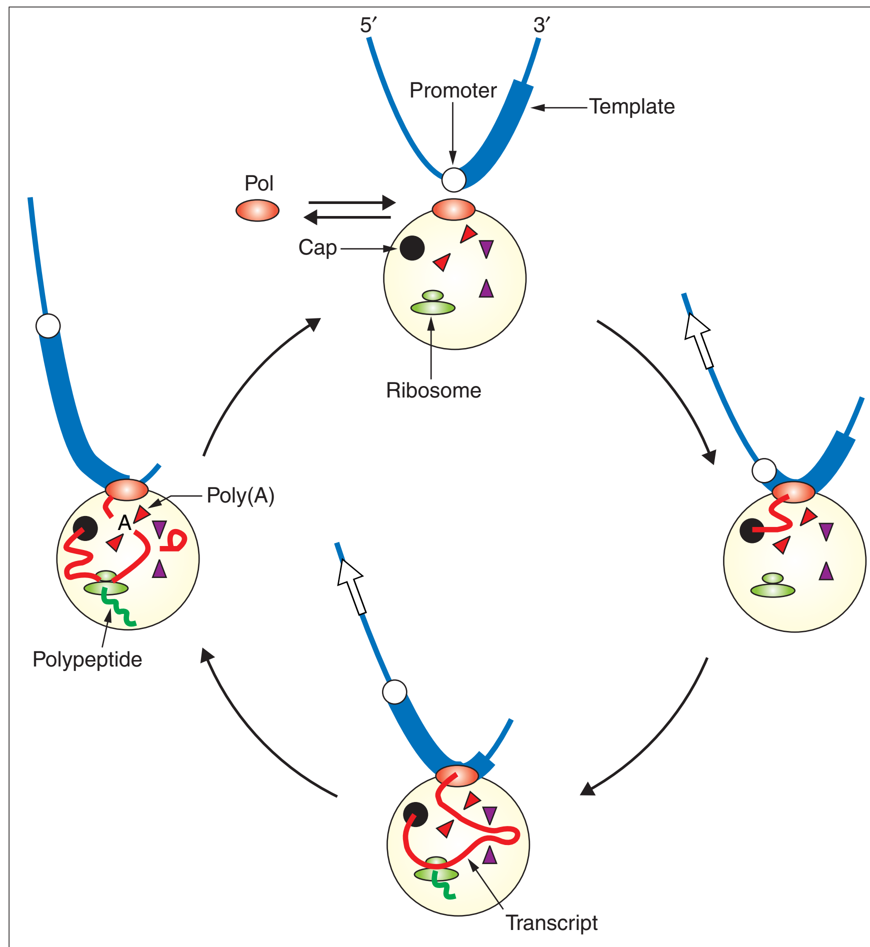
searches to catalogue nucleolar proteins of HeLa nuclei [9•], and more than 100 proteins have been localised to different nuclear compartment in mouse nuclei using a visual screen after inserting GFP randomly into genes [10•]. Unfortunately, the rules that would enable us to deduce a protein's location from the primary DNA sequence still remain elusive [11•].

Chromatin dynamics

It is a truism that nuclear structure changes dramatically during development, and the introduction of GFP tags makes it possible to begin to catalogue these movements in living cells. However, there are considerable technical and analytical problems in interpreting the motions of one region of chromatin relative to others that might be tumbling in the nucleus (Figure 2). The group of John Sedat and David Agard continues to provide the intellectual framework with which to view these movements. For example, they monitored chromatin motion in spermatocyte nuclei of *Drosophila*. DNA repeats encoding the *lac* operator were integrated into different chromosomal regions, and the GFP-tagged repressor expressed in the same cells. As the GFP–repressor binds to the operator, the arrays appear as fluorescent spots that can be followed by time-lapse photography. 'Fast' and 'slow' (random-walk) components were uncovered. Early in G2 phase, a 'fast' motion occurs within a small domain (radius ~0.5 μm), while the 'slow' one is confined to a larger, chromosome-sized domain and this ceases as cells approach meiotic prophase [12••]. Analogous experiments on four chromosomal regions in the yeast genome monitored movements in S phase: early- and late-replicating origins are highly mobile in G1 phase, frequently moving at 0.5 μm/10 s, but this slows when replication begins. By contrast, telomeres and centromeres are both constrained during G1 and S phase [13•]. In higher cells, a particular site moves into the nuclear interior transiently in early G1 and again in early S phase [14•], while loci at or near the nuclear periphery are significantly less mobile than other more nucleoplasmic loci [15•].

Over the years, different studies have led to different ideas about how stably histones are bound to the template; photobleaching studies with GFP-tagged histones are now providing the answers. Thus, it is now known that most histone H1 exchanges within seconds [16], H2B over minutes, and H3 and H4 over hours [17•]. The differing

Figure 3



A model for part of a transcription factory involved in message production. One set of five sites is illustrated: polymerising (red oval); capping (black circle); splicing (purple arrowheads); polyadenylating (red arrowheads); and translating (green ovals). Individual components exchange continually with others, but only the polymerase (Pol) is shown doing so here. A promoter binds to the polymerase, which then reels in the template and extrudes the transcript. The 5′ end of the transcript is soon captured by a capping site, and a cap added. As elongation continues, nascent RNA remains attached at both ends as it loops through sites involved in splicing, translational proofreading and cleavage/poly(A) addition (indicated by 'A'). The transcript also passes through a ribosome, where it is scanned for inappropriately placed stop codons; if found, the transcript and truncated protein are degraded. At the end of the cycle, the template is released, the processed transcript and completed polypeptide move away, and any transcript 3′ of the cleavage/poly(A) site is degraded. Modified from [44]. (Copyright © [2002, J Wiley and Sons]. This material is used by permission of Wiley–Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)

exchange of H2B compared with H3/H4 was surprising, as all form the same nucleosomal structure. Significantly, exchange of a minority of H2B seems to depend on continuing transcription, and this has recently been confirmed *in vitro* [18•]. This suggests that the transcribing polymerase displaces only H2A and H2B from the template, but not H3 or H4.

Replication, recombination and repair

Any DNA molecule seems to be replicated normally when introduced into *Xenopus* eggs or egg extracts. This suggests that the initiation of replication early in development does not involve specific DNA sequences, as it does later. However, initiation at random would generate some inter-origin distances that would be too long to be replicated during the short cycle times found soon after fertilisation. On incorporation of [^{3}H]thymidine or bromodeoxyuridine and analysis of the distribution of labelled DNA in spread fibres, most origins are found to be non-randomly spaced 5–15 kb apart. This spacing is increased by immunodepleting origin recognition complexes (ORCs) from the extract, and can be explained in two general ways: in one, the added DNA is organised into loops, and this organisation then determines where origins fire; in the other, origins are assembled at any point on chromatin, but an 'exclusion zone' around each origin prevents ORCs from binding nearby [19•].

The complexes that work on DNA are very large. Do they pre-exist, or are they only assembled when needed? Various studies indicate that the latter usually applies. For example, recombination between DNA homologues involves Rad51, Rad52 and Rad54, amongst many other proteins. Mammalian cells expressing one or other of these RAD proteins — which were named because of their involvement in the response to radiation — were tagged with GFP and exposed to ionising radiation so that the proteins became concentrated in foci around the induced DNA damage. Each of the three behaved independently of the others, indicating that they were not part of a preformed complex [20••]. An earlier study on the complex involved in nucleotide excision repair led to a similar conclusion [21], and now dissection of that pathway suggests that the active complex is built by the successive recruitment of XPC–hHR23B, TFIIH, XPG, XPA and ERCC1–XPF, before the incisions are made on each side

of the damage [22•]. Executing these DNA transactions through dynamic multiprotein complexes, rather than stable holocomplexes, allows flexibility and crosstalk between the replication, recombination and repair pathways.

Message production and export

This field has been transformed by two recent discoveries. One is the elucidation of the structure — or rather *structures* — of RNA polymerase II, the enzyme responsible for transcribing most genes. The structures are derived from two crystal forms of a ten-subunit polymerase (at 2.8 and 3.1 Å, respectively) [23••], and from an elongation complex containing the nascent transcript (at 3.3 Å) [24••]. The other is the realisation that transcription, capping, splicing, polyadenylation and preparation for export do not occur sequentially but together, with one process influencing another (Figure 3) [25]. So, although we now know the molecular disposition of the subunits within the polymerase, we know little about that polymerase in the context of the larger multifunctional 'factory' that produces the message.

The best view of the relative disposition of the macromolecular complexes associated with a maturing transcript comes from studies on the *Balbiani ring 3* (*BR3*) gene in the giant chromosomes of the salivary gland of *Chironomus tentans*. This gene has 38 introns, of which more than half are co-transcriptionally excised, and its three-dimensional structure was reconstructed using electron tomography [26••]. Each gene is associated with 20–25 nascent transcripts, and each transcript with one RNA polymerase II complex and one splicing complex. These complexes do not have well-defined structures; rather, spliceosomal factors seem to be continuously added to and released from splicing complexes as the transcript continues to be made.

Transcription and chromatin structures

Steroids act quickly to re-programme gene expression, and several studies have examined their effects on higher-order chromatin structure in living cells. In one study, a tandem array encoding the promoter of the mouse mammary tumour virus driving a *ras* reporter gene was inserted into the chromosome, and then a sub-line was derived that stably expressed the glucocorticoid receptor (GR) tagged with GFP. On adding steroid hormone, the GFP–GR bound to the array to give a discrete spot, which later decondensed and recondensed as transcription was successively stimulated and repressed [27•]. In other studies, a cyan fluorescent protein (CFP)-tagged chimera of the *lac* repressor fused with the oestrogen receptor (CFP–LacER) was bound to an array of *lac* operators, and interactions with yellow fluorescent protein (YFP)-tagged steroid receptor co-activator 1 (SRC-1) or CREB-binding protein (CBP) monitored. On adding ligand, nucleoplasmic YFP–SRC-1 and YFP–CBP are rapidly recruited to the arrays, and adding antagonist reverses the process [28•]. The initial binding of the tagged receptor also decondenses the arrays, and adding ligand partially reverses this [29•]. Analogous experiments monitored the effects of targeting the acidic activation domain of VP16 to a specified chromosome site. This activated the target gene, and the site moved from a predominantly peripheral location to a more interior one [14•]. These studies also suggest the polymerase plays an important structural role in regulating chromatin condensation.

Although particular genes do not occupy absolutely specified places within nuclei, they do have preferences, even in mitotic chromosomes [30]. Thus, fluorescence *in situ* hybridisation (FISH) shows that artificial arrays of U2 small nuclear RNA genes often lie close to coiled (Cajal) bodies [31]. Similarly, the gene-rich major histocompatibility complex usually abuts a promyelocytic leukemia body [32], and active immunoglobulin loci are found more often than not in the middle of pro-B nuclei [33•]. The nuclear lamins may even organise some transcription, as disrupting them by overexpressing a dominant-negative lamin mutant inhibits polymerase II activity, but not that of polymerases I and III [34•]. These arrangements are likely to be conserved, as those territories that tend to be found centrally in one primate species are usually found in the same place in others [35•]. As heterochromatin often condenses on to the lamina or nucleolus, and as synteny is so well preserved in primates, it remains to be seen whether differences in amounts of heterochromatin carried by different chromosomes is the main determinant of the nuclear position.

The histones in and around active genes also carry a distinct set of post-translational modifications — a 'histone code' — that regulate access of polymerases to the template [36], and these have been localised relative to active genes. For example, methylation of Lys4 and Lys9 in histone H3 correlates with gene activity and inactivity, respectively; and — as expected — the inactive X chromosome in human cells carries these expected marks [37•,38•]. These marks may be carried through mitosis, so enabling a gene to 'remember' whether it had been active. Photobleaching using the TATA-box binding protein (TBP) tagged with GFP suggests another way of marking a gene: the tagged TBP remains bound to mitotic chromosomes — presumably still on promoters [39] — where it is strategically placed to re-form new polymerising complexes as the cell emerges from mitosis [40•].

Translation

It is widely believed that translation only occurs in the cytoplasm, but some also seems to occur in nuclei [41••]. Cells were permeabilised, incubated with biotin–lysine-tRNA or BODIPY–lysine-tRNA, and any tagged polypeptides localised. Some label was found in the cytoplasm; but, unexpectedly, some was also found in discrete nuclear sites — transcription factories — and a fraction of this nuclear labelling depended on concurrent transcription. The nuclear translation might be used to 'proof-read' new transcripts to see if they have appropriately placed

initiation and termination codons. This idea is supported by the observation that transcripts containing inappropriately placed termination codons do accumulate close to nuclear transcription sites, implying that a checkpoint operates there [42]. Therefore, translation must be added to the other functions occurring in the transcription factory (Figure 3).

Conclusions

If the various functions of DNA drive the self-organisation of the genome into the three-dimensional structure that we call a nucleus, it is clear that the final structure will inevitably depend on how the various conflicting forces generated by those functions are resolved. And as those functions vary in activity throughout the cell cycle, the structure will inevitably change from moment to moment (Figure 2). Despite the difficulties associated with describing an ever-changing structure, we now believe that we have many of the appropriate techniques for analysing such structures.

Acknowledgements

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