CHEMICAL REVIEWS

Review

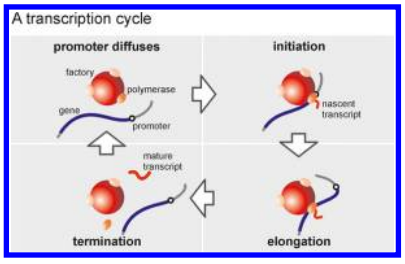
pubs.acs.org/CR

Transcription Factories: Genome Organization and Gene Regulation

Argyris Papantonis[†,‡] and Peter R. Cook*[,†]

[†]Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, United Kingdom

[‡]Center for Molecular Medicine, University of Cologne, 21 Robert-Koch Street, 50931 Cologne, Germany



CONTENTS

1. INTRODUCTION

All eukaryotic cells contain four RNA polymerases originally defined by their sensitivity to different drugs, and now by the sets of genes they transcribe.[1−4] Polymerase I produces 45S rRNA (a precursor of 18S and 28S rRNA), polymerase II transcribes most protein-coding genes, polymerase III makes various small RNAs (including tRNAs), and the mitochondrial enzyme transcribes the small genome of this organelle. Active polymerases I, II, and III are found in nuclei, and the last in the cytoplasm. Plant cells contain additional activities: polymerases IV, V,[5] and the chloroplast enzyme.[6] Of course, there are always exceptions in biology: in African trypanosomes, RNA polymerase I makes rRNA in nucleoli but also copies genes encoding certain surface (glyco-)proteins in a different (but nucleolar-like) structure in the nucleoplasm,[7] and human *RPPH1* is transcribed by both polymerases II and III.[8]

The traditional model for transcription sees the active form of the polymerase tracking along the DNA template as it makes its transcript.[9] Here, we review evidence for an alternative where the active enzyme is concentrated with others engaged on different templates in discrete sites called "factories". These factories contain high local concentrations of the machinery

Special Issue: 2013 Gene Expression

Received: December 18, 2012
Published: April 18, 2013

   dx.doi.org/10.1021/cr300513p | *Chem. Rev.* 2013, 113, 8683−8705

required to make RNA.[10,11] One corollary of this model is that a polymerase is attached to a factory, and immobilized when active; then, it works by reeling in the template as the transcript is extruded. In other words, the DNA moves relative to the polymerization site (Figure 1; for a movie, see Cook[12]). Note

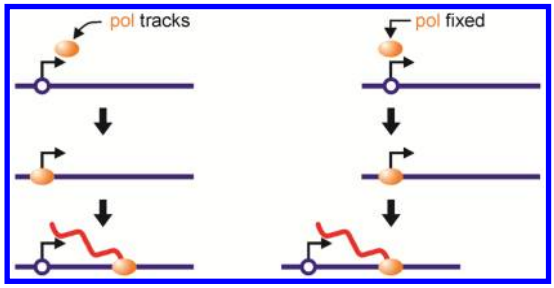


Figure 1. Models for transcription involving a tracking (left) or fixed (right) RNA polymerase.

that time-lapse imaging of many genetic loci (in living yeast, fly, and human nuclei) tagged with fluorescent proteins shows that DNA can diffuse freely throughout a local nuclear volume with a diameter of 0.5−1 μm within a minute or so,[13] during which a locus can visit several different factories that are typically spaced ~500 nm apart (see Papantonis et al.[14] and Larkin et al.[15] for recent estimates of interfactory spacing). A second corollary is that the active form of the enzyme becomes a critical molecular tie that loops the genome (Figure 2).

1.1. Definition of a Transcription Factory

Factories take their name from a related field. When a mammalian cell is infected with a single *Vaccinia* virion and grown in the DNA precursor, [^{3}H]thymidine, autoradiography reveals one cytoplasmic focus of viral DNA synthesis that enlarges to contain hundreds of genomes.[16] In 1968, the term "factory" was applied to such foci,[17] and it is now used to describe analogous sites where other viruses are produced. In the 1990s, the term was applied to places where endogenous genes are replicated,[18] transcribed,[19] and repaired.[20] In each case, the term seems appropriate, as all of these sites contain high local concentrations of the relevant machinery and raw materials that act through the law of mass action to drive efficient production. For example, HeLa nuclei contain a 1-μM pool of RNA polymerase II, but essentially all transcripts are made in factories where the local concentration is ~1000-fold higher.[21]

We will use the term "transcription factory" to describe a site containing at least two RNA polymerases (plus associated machinery) active on at least two different templates. This compares with the definition of a "factory" in The Oxford English Dictionary as "a building or range of buildings with plant for the manufacture of goods" that includes no restriction on the scale of a factory or the number of machines in it. The purpose behind our definition is to differentiate our use from two other cases. First, the term transcription "factory" has been applied to the various machines involved in the production of only one mature message (e.g., those involved in capping, splicing, and polyadenylation).[23−26] Second, we wish to distinguish our factories from cases where two polymerases are active on the same template. However, our restriction to two or more polymerases and templates is arbitrary, and nature will surely not recognize it! As we shall see, factories share properties with "active chromatin hubs"[27] and "chroperons"

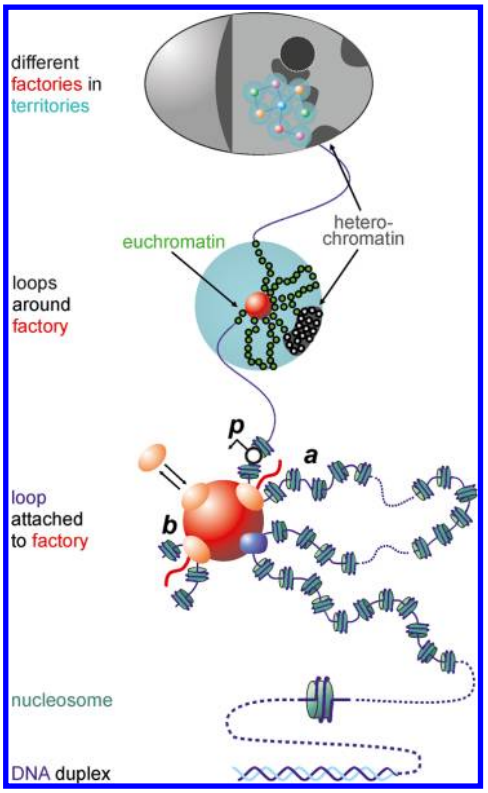


Figure 2. A model for the organization of chromatin. DNA is coiled into nucleosomes, and runs of nucleosomes form a string looped by attachment to a factory (red sphere) through transcription factors (blue) and engaged polymerases (orange). A promoter (*p*) has initiated, and a fixed polymerase is reeling in its template and is about to transcribe *a*; another polymerase is transcribing *b*. Components in a factory exchange continually with the soluble pool, and ~16 loops (only a few are shown) are attached to the factory. Distal nucleosomes in long loops tend to acquire a heterochromatic histone code that spreads down the fiber; they often aggregate around the lamina, nucleoli, and centromeres. Different factories (different colors) specialize in transcribing different sets of genes. Modified and reprinted with permission from ref 22. Copyright 2001 John Wiley & Sons.

(chromatin-based clusters of "operons" or multigene interaction complexes).[28]

1.2. Markers for Transcription Factories

Factories can be localized in various ways; all have shortcomings. Arguably the best focus on activity and involve incorporation of modified precursors into nascent RNA (defined as transcripts still associated with the polymerase). The traditional approach involves growing cells for short periods in [^{3}H]uridine, then localizing the resulting [^{3}H]RNA by autoradiography; however, this gives poor resolution, as silver grains can lie >100 nm away from the tritium source.[29] Newer ways involve fluorescence microscopy after incubation with tagged precursors like (i) bromo-uridine (BrU), bromo-uridine triphosphate (BrUTP; Figure 3), or biotin-cytidine triphosphate, when the tagged RNA is detected by indirect immuno-labeling,[19,30−32] or (ii) 5-ethynyl uridine (EU), which is detected after attaching a fluor using "click" chemistry.[33] [Sporadic reports use fluors conjugated directly to UTP,[34−36] but these are not widely applied because of concerns whether RNA polymerases can incorporate these precursors in vivo (although many do so in vitro).] Cells are grown in

dx.doi.org/10.1021/cr300513p | *Chem. Rev.* 2013, 113, 8683−8705

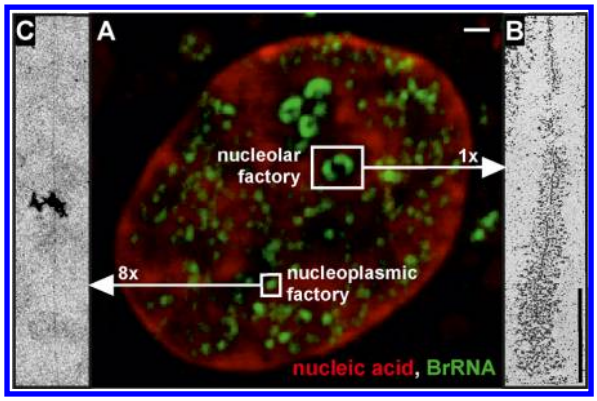


Figure 3. Transcription factories imaged using a "confocal" microscope. Bars: 1 μm. (A) HeLa cells were permeabilized, engaged polymerases allowed to extend their transcripts by ~40 nucleotides in BrUTP, and fixed; after cutting 100-nm cryo-sections, BrRNA was immuno-labeled with fluorescein (green), nucleic acids counterstained with TOTO-3 (red), and images collected. Nascent BrRNA is found in factories in mitochondria (where the respective polymerase is active),[41,42] in nucleoli (where polymerase I is active), and nucleoplasm (where polymerases II or III are active). Image courtesy of A. Pombo, reprinted with permission from ref 43. Copyright 1999 American Association for the Advancement of Science. (B) Stripping off and spreading one of the crescents from the nucleolar factory yields the iconic (electron microscope) image of a "Christmas tree" with ~125 closely packed polymerases. As a nucleolar factory typically contains four crescents on the surface of a "fibrillar center" (only two are seen here), ~500 polymerases are active on 4 genes. Reprinted with permission from ref 44. Copyright 1972 Society of the European Journal of Endocrinology. (C) Stripping off and spreading one of ~8 active transcription units in a nucleoplasmic factory yields this (electron microscope) image with one polymerase engaged on its template. Reprinted with permission from ref 32. Copyright 1998 American Society for Cell Biology.

unphosphorylated precursors for tens of minutes to allow entry into nuclei, equilibration with internal pools, and incorporation of enough label to allow detection. However, this time is long compared to the ~3 min it takes a human RNA polymerase II to make (at ~50 nucleotides/s)[37,38] the 8400 nucleotides in a typical transcript,[31] and the ~1 min for the yeast enzyme to terminate.[39] These problems become more acute with the short transcripts of ~100 nucleotides made by RNA polymerase III. As a result, some labeled transcripts will leave synthetic sites during labeling, and this inevitably results in mis-localization. Use of the immediate precursors, tagged triphosphates, requires permeabilization to permit entry into cells. This has advantages, despite the obvious disadvantage that structure may be distorted in the process: internal pools are lost so labeling becomes more efficient, the rate of polymerization can be controlled by manipulating precursor concentrations, pulse-chase experiments show that little BrRNA or biotin-RNA leave the incorporation site,[30,40] and immuno-labeling of the many tags incorporated into one transcript provides increased sensitivity.

Factories can also be detected by immuno-labeling the molecules they contain. Unfortunately, only ~25% RNA polymerase II in a mammalian cell is engaged,[45] and <10% of many transcription factors is bound to factories,[46] so the majority is a poor marker for the active fraction. Fortunately, (human) RNA polymerase II becomes differentially phosphorylated during the transcription cycle at specific residues in many of the 52 heptad repeats in the C-terminal domain (CTD) of the largest catalytic subunit,[47−49] so antibodies targeting the relevant epitopes are often used to immuno-localize initiating and elongating fractions.[50,51] This again provides increased sensitivity as more than one antibody molecule can bind to the many cognate epitopes in one CTD.[52]

RNA fluorescence in situ hybridization (FISH) is also used. As <1% of a message can be at the transcription site,[53] and as introns are usually excised and degraded cotranscriptionally,[54] intronic RNA is generally targeted. However, little is known about where introns are degraded, and, as their half-lives (~5 min in mammals)[55] are roughly the time taken to complete a transcript, it is possible that a fraction of intronic RNA might lie distant from the polymerase that produced it.

Many approaches described above involve immuno-labeling coupled to fluorescence microscopy, but the resolution afforded by conventional light microscopy is (at best) ~200 nm in the x- and y-axes and ~500 nm in the z-axis.[56] Even (indirect) immuno-gold labeling coupled to electron microscopy has the drawback that the antibodies used are large (length ~9 nm); the center of a labeling gold particle with a 5-nm radius then might lie ~23 nm away (i.e., 9 + 9 + 5 nm) from the antigen it marks, a significant fraction of the ~87-nm diameter of a typical nucleoplasmic factory.[57]

Use of a tag like the green fluorescent protein (GFP; diameter ~5 nm) fused to a transcription factor,[58,59] or a subunit of RNA polymerase I[60] or II,[37,45,61−63] allows both tighter localization and live-cell imaging. However, one question inevitably arises in any study using GFP-tagging: to what extent does the tagged protein behave like its natural counterpart? The best way of ensuring normal behavior is to replace the endogenous gene with one encoding the hybrid protein, establish a stable cell-line expressing the modified gene, and confirm that doubling times remain unchanged. This is rarely done in mammalian cells, as precise gene replacement is so difficult. In the case of the GFP-tagged polymerase II described above, the next best approach was used. An extra (tagged) gene was integrated into the genome of a mutant (Chinese hamster) cell that possessed a (lethal) temperature-sensitive mutation in the largest catalytic subunit; a stable cell line then was established that doubled at the same rate as the wild-type cells at the nonpermissive temperature.[64] The tagged polymerase could be seen in numerous overlapping foci (factories) throughout the nucleoplasm in living cells, but individual ones were too numerous to be resolved one from another (even using a confocal microscope). [In Figure 3, individual foci are resolved because the cell has been sectioned.] In the case of the GFP-tagged polymerase I described above, no equivalent mutation was available, so a "transient" transfection was used.[60] Even so, the tagged enzyme was distributed in nucleoli much like the untagged enzyme.

GFP is also used to tag nascent transcripts at (or close to) transcription sites. The approach requires two steps: a binding site for the RNA-binding protein, MS2, is inserted into an intron in the gene of interest, and GFP-MS2 is expressed in the cell. The GFP-MS2 then binds to the corresponding transcript, allowing its localization.[61,65,66] Yet, again, a fraction of intronic RNA could lie distant from the polymerase that made it.

Given that each approach described above has its drawbacks, it is only prudent to use a number of different ones when localizing factories.

dx.doi.org/10.1021/cr300513p | Chem. Rev. 2013, 113, 8683−8705

1.3. What Is Covered in This Review

We provide comprehensive coverage of papers mentioning transcription factories up to January 2013. We concentrate discussion on factories containing the nuclear polymerases of mammals, because they have been analyzed in most detail. They have been discussed in many reviews.[10,11,36,61,67−114] We also focus attention on studies using "physiological" buffers, as active enzymes are known to aggregate in abnormal salt concentrations.[115,116] Necessarily, we also address the two corollaries, that the polymerase attached to a factory is inevitably immobilized when active, and that the active enzyme is one of the major molecular ties that organizes the genome. In addition, we present a simple, unified model for the way factories are involved in regulating gene expression.

Although this Review will focus on eukaryotes, there is some evidence that transcription factories are also found in bacteria. This includes the following: (i) The bacterial nucleoid provides the prototypic example of loops attached to a core rich in RNA polymerase.[117] (ii) The molecular ties maintaining loops have been mapped in *Salmonella typhimurium* (using site-specific recombination), and most attached sequences turn out to be active genes,[118] which would be required if factories existed. (iii) Most RNA polymerase in rapidly growing *E. coli* is engaged on the ribosomal cistrons, and GFP-tagging shows it to be concentrated in foci reminiscent of nucleolar factories.[119] (iv) The DNA-binding protein, H-NS, drives clustering of the genes it regulates into discrete foci in living *E. coli*, which could well be factories.[120]

2. HISTORY

The idea that both DNA and RNA polymerases move along their templates as they polymerize so pervades our thinking that it is difficult to establish why and when the idea first arose. It seems not to stem from experimental results, but from a perception of relative size; it would be the smallest component that had to move. This perception is embodied in the statement made by Arthur Kornberg in 1987 that "The primosome... moves like a locomotive down the template track."[121] Only now do we know that polymerases are huge structures that dwarf the template; for example, a complex containing at least 60 proteins assembles at a promoter during initiation by a mammalian RNA polymerase.[3]

2.1. Are Active DNA Polymerases Immobilized?

Despite the prevailing view, there was early evidence that active DNA polymerases could be immobilized. For example, Jacob and colleagues speculated in 1963 that DNA polymerases might be attached to the bacterial membrane to facilitate regulated initiation of replication and precise distribution of duplicated templates to daughter cells.[122] In the mid-1970s, Dingman then proposed a model of how fixed DNA polymerases might work,[123] and Berezney and Coffey[124] showed that nascent DNA was tightly associated with a nuclear "matrix", an observation that led to a huge literature.[125] For example, the DNA polymerases involved in repairing damage in the template are also attached to an analogous "cage".[126] However, results obtained using matrixes and cages were dogged by the criticism that the structures were artifacts; perhaps nascent DNA and polymerases aggregated during extraction in the extreme salt concentrations used during preparation.[127]

The critical experiment that changed the prevailing view involved growing rat fibroblasts briefly in the DNA precursor, bromo-deoxyuridine, and immuno-labeling sites containing the incorporated label; S-phase cells contained discrete nuclear foci that each contained many active polymerases.[128] Concurrently, and using isotonic buffers during fractionation, essentially all DNA polymerizing activity in human cells was shown to be attached to the nuclear substructure.[129] Soon, clusters containing many polymerases were extracted from nuclei,[130] and imaged both in the electron microscope[18] and in living cells expressing a GFP-tagged marker, proliferating cell nuclear antigen, where they were closely associated with active polymerases.[131] As a result, it is now accepted that DNA polymerases are immobilized when active.[43,132] However, whether or not active RNA polymerases are also immobilized remains controversial, despite (as we shall see) evidence similar to that described above.

2.2. Evidence That Active RNA Polymerases Track

There seems to be only two general kinds of evidence supporting the idea that active RNA polymerases might track. The first is exemplified by the iconic images of "genes in action" taken by Miller and colleagues.[133] The most striking of these depict the "Christmas tree" seen in textbooks, where the trunk is often a ribosomal cistron packed with polymerases, and the branches are nascent RNAs (Figure 3B). Significantly, no immobilizing factory is seen. Analogous images are obtained with "lampbrush" chromosomes, which can be prepared from oocytes of many species (but conditions have not yet been developed that allow us to prepare them from mammals) at the stage during meiosis when parental homologues pair.[134] Unlike transcriptionally-inert mitotic chromosomes, these are hyperactive and produce many of the transcripts that sustain the developing embryo; for example, they are 100-fold more active than interphase chromatin from embryonic cells.[135] Nascent transcripts can be seen attached to long chromatin loops that extend away from the chromomeric axis. Again, no immobilizing structures are seen.

When looking at such images with a traditional eye, it is easy to imagine that polymerases are frozen in the act of tracking along the template. However, these static images tell us nothing about relative movement. Moreover, they are obtained by disruptive spreading, reflected by the "trunk" of the "Christmas tree" in Figure 3B being 10-fold longer than the compact crescent from which it is derived. The images are also highly selected; we are generally shown the well-spread examples, but in others nascent RNA (and active polymerase) remains associated with clumps of partially unfolded chromatin (which probably represent intermediates in the deconstruction).[32,136−138] A hypotonic buffer is also used during preparation, and results obtained contrast with those found after spreading human DNA in a hypertonic buffer: essentially all nascent RNA remains associated with a central cluster, and none is seen in loops.[139] Why then should one believe results obtained using one set of destructive conditions and not the other? In summary, this evidence might be photogenic, but it is hardly compelling.

The second kind of evidence is based on the successful reconstruction of transcription in vitro using soluble components;[1−4] the argument then runs, if soluble enzymes work, there is no need to postulate any role for larger structures like factories. However, this kind of evidence is compromised. First, such reactions are inefficient. For example, the synthetic "super core promoter" is one of the strongest available, but template usage in a typical reaction is still only ~40%, despite the very high protein concentrations and incubations lasting

many tens of minutes.[140] Powerful viral promoters are used with efficiencies of only 5−15%,[141,142] natural cellular promoters even less,[143] and natural promoters covered with nucleosomes hardly at all.[144] Second, transcription reactions require lengthy preincubations, during which the transcriptionally competent fraction forms into complexes large enough to be pelleted by a 5-min spin in a microcentrifuge.[145] Reactions may start with soluble components, but the evidence shows that a minority of larger complexes constitute the active fraction. Note also that transcription reactions are generally carried out in about one-tenth the natural salt concentration.[140−144]

2.3. First Evidence That Active RNA Polymerases Might Be Fixed

Treating *Escherichia coli* with lysozyme, a detergent, and 1 M NaCl releases "nucleoids" containing rosettes of naked (supercoiled) DNA attached to a cluster of engaged polymerases.[117] Transcription maintains the structure, as pretreatment with rifampicin (a polymerase inhibitor) or post-treatment with ribonuclease releases the supercoils and disperses DNA. In tune with the prevailing view in the 1970s, there was no suggestion that equivalent structures might exist in vivo, and it was assumed that tracking polymerases and their sticky transcripts were aggregating artifactually to generate the structure.

Analogous "nucleoids" were soon obtained by lysing human cells in a detergent and 2 M NaCl;[146] they also contained loops of supercoiled DNA confined in a residual nuclear lamina or "cage".[147] The logical next step was to see which DNA sequences might tether loops to the substructure. Nucleoids were treated exhaustively with nucleases, and it was assumed that this would detach the remaining DNA and its associated nascent RNA; only DNA sequences tethering loops to DNA-binding proteins would be left. Moreover, these DNA sequences would be repeated and highly conserved, as supercoiled loops had been seen in yeasts, flies, chickens, and man.[148−150] Yet contrary to expectation, the residual DNA was transcribed, and it remained associated with essentially all nascent RNA (labeled with a 1-min pulse of [^{3}H]uridine). This prompted the suggestion that active RNA polymerases were the molecular ties that attached loops to the substructure, with the corollary that the enzyme was immobilized when active.[139] [We now know that the imagined conserved and repeated DNA sequences do not exist, as the genome projects would surely have uncovered them.] Analogous experiments soon showed that genes attached and detached as they became active and inactive,[151] and that a different RNA polymerase, that of influenza virus, was also immobilized when active.[152]

These results were rightly criticized on the grounds that extraction in 2 M NaCl might induce tracking transcription complexes to aggregate artifactually, and this provoked the development of "gentle" methods for permeabilizing cells in a "physiological" buffer. Using such a buffer, RNA (and DNA) polymerases were found to "run-on" at rates found in living cells.[153,154] If polymerases aggregated during extraction, they still worked more efficiently than those isolated using conventional buffers! Nonetheless, the decisive experiment showing that active RNA polymerases were attached to the substructure involved encapsulating cells in agarose microbeads (to protect cells during washes), permeabilizing in a "physiological" buffer, and combining nuclease treatment with electro-elution to remove most chromatin; essentially all nascent RNA and run-on activity then remained (Figure 4).[155]

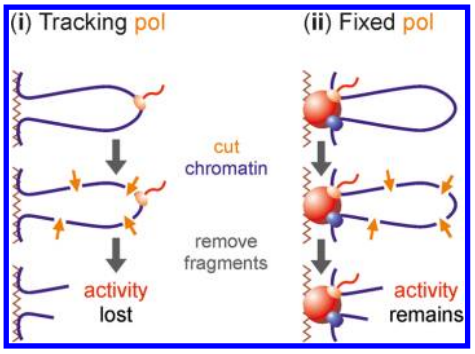


Figure 4. Distinguishing whether active RNA polymerases are attached to the underlying structure or not. Cells were permeabilized, chromatin cut with a restriction enzyme, and electro-eluted to remove detached fragments; all steps were carried out in a "physiological" buffer. (i) A polymerase (orange oval) tracks along chromatin (which might be attached to the substructure; brown zigzag line) as it makes a transcript (red line). After cutting chromatin into ~10 kbp pieces with a restriction enzyme, chromatin should electro-elute with associated polymerases and be lost. (ii) The polymerase in a factory (red sphere), which is attached to the substructure, reels in the template, as the transcript is extruded. Despite cutting and electro-eluting to remove ~75% chromatin, essentially all polymerizing activity remains.[155,156] This experiment was also used to (i) map which DNA sequences attach loops to the substructure (after exhaustive digestion, residual sequences turned out to be transcribed),[155−158] (ii) measure the contour length of loops (in HeLa, the average is ~86 kbp),[159] and (iii) determine whether transcription factors tended to bind mainly to factories or out in the loop (many are bound to factories).[46]

2.4. First Evidence That Active RNA Polymerases Might Be Clustered

As in the case of replication, the critical experiments that challenged the prevailing view involved visualizing sites of activity; seeing is believing. In one experiment, HeLa cells (again encapsulated in microbeads) were permeabilized in a "physiological" buffer, incubated in BrUTP, and sites containing BrRNA immuno-labeled; after extending nascent RNA chains by <400 nucleotides, ~300−500 focal sites, factories, were seen in nuclei, and these remained despite nucleolytic detachment of ~90% chromatin.[19] In another, BrUTP was microinjected into human fibroblasts, which were then grown for 15 min; after immuno-labeling, discrete foci were again seen.[30] These two results neatly complement each other: in the first, the possibility that the foci were aggregation artifacts cannot be excluded (despite the use of isotonic buffers), while in the second, the labeling time is so long that many completed transcripts could have left synthetic sites. The combination makes it likely that both sets of foci reflect the synthetic sites. The important questions were: Does a focus mark many polymerases active on one gene or a cluster of many active genes, and are the active polymerases immobilized?

2.5. Theory: Side-Stepping the Untwining Problem

An RNA polymerase utilizes the energy derived from the hydrolysis of nucleotide triphosphates to allow each successive base in the template to occupy the polymerization site. The template must move relative to the polymerization site (for movies, see Cheung and Cramer[160] and Cook[12]); theory suggests it must be the template that moves.

Two topological problems arise when a tracking polymerase transcribes a double helix. One, the generation of torsional stress, has been widely discussed and is solved by topoisomerase action on each side of the polymerase; it arises whether or

dx.doi.org/10.1021/cr300513p | Chem. Rev. 2013, 113, 8683−8705

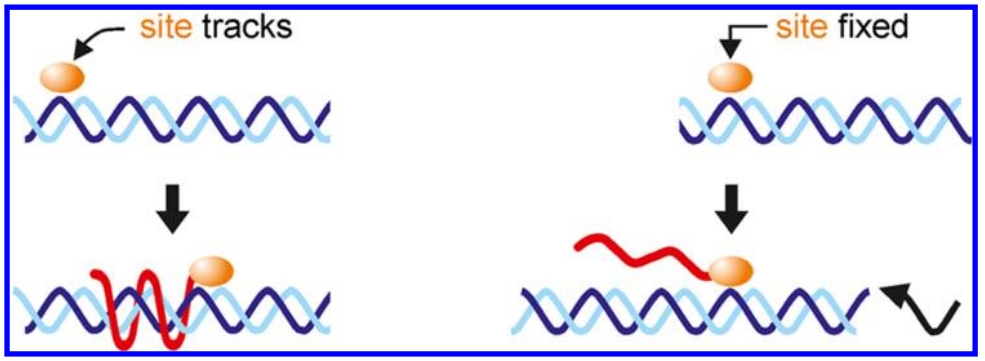


Figure 5. The "untwining problem". Left: If the polymerization site (orange) tracks, the transcript (red) becomes entangled about the double helix. Right: If it is fixed, there is no entanglement (the helix then rotates, indicated by the curved black arrow).

not the polymerase tracks.[161] [For example, topoisomerase I activity seems to be tightly coupled to transcriptional on the *c-fos* gene.[162]] The other, the "untwining problem",[163] awaits solution. Consider the relative movements of an active polymerase and template, around and along the helical axis. As each of the two components can either move or remain still, there are four formal possibilities. In one, the polymerase moves both laterally and rotationally, as in our textbooks (Figure 5, left). Next, as each helical turn is transcribed, the polymerase plus nascent transcript must rotate around the template so the transcript becomes entwined about the template, once for every 10 bp transcribed. Even with a short gene of 1000 bp, the transcript becomes entwined ∼100 times, and some mechanism must be found to untwine it to allow escape to the cytoplasm; no such mechanism has been uncovered. Should one exist, it must be precise, as untwining once too few times (or once too many) would still leave an entangled transcript. One way of side-stepping this problem is for the transcript to ride piggy-back on the polymerase. If so, that polymerase would also have to carry ∼10 engaged ribosomes in prokaryotes or a spliceosome in eukaryotes (as translation and splicing occur cotranscriptionally). As this seems unlikely, this model, and one that also involves a rotating polymerase and moving template, probably do not apply.

Now consider the case where the enzyme translocates laterally but its rotation is restricted, perhaps by the frictional drag of the transcript; DNA rotates instead. Yet even one accidental rotation, which is likely when the transcript is short and frictional drag limited, would yield an entwined transcript. Imagining any mechanism that might prevent such accidental rotation without immobilizing the polymerase is difficult. In Figure 5 (right), the untwining problem is side-stepped because the enzyme is static; DNA both translocates and rotates. If one believes in the textbook model, the onus is on believers to uncover some solution to the untwining problem; alternatively, if the polymerase is fixed, the problem does not arise.

2.6. Attached Polymerases Can Work in Vitro

Two experiments showed that immobilized enzymes can work. One involved adsorbing the bacterial RNA polymerase on to a glass slide, and adding a template with a promoter at one end and a gold particle at the other;[164] two kinds of particle then could be seen in the light microscope. One moved with Brownian motion, the other was restricted to a small hemisphere on the surface of the slide; presumably some templates were free, others were tethered through the promoter to an enzyme attached to the surface. On initiation, tethered particles become even more restricted in their movement as they were reeled in by the attached polymerase. The elongation rate, deduced from the rate the hemisphere shrank, was the same as that given by the soluble enzyme. A second experiment measured activity directly.[163] A hybrid protein containing the T7 polymerase was tethered to a large plastic bead through a peptide linker containing a site for a specific protease; after incubation without (or with) protease, the bound (and free) polymerases were found to elongate equally well (but the attached one initiated more slowly, as might be expected).

Force measurements on single polymerase molecules are now routinely made using immobilized enzymes,[165] and RNA polymerases turn out to be more powerful molecular motors than kinesin or myosin. Between 10% and 20% of the free energy available from one cycle of ribonucleotide addition is converted into mechanical energy, the efficiency stemming from the low gearing (the step length of the polymerase is the short distance between nucleotides, and is ∼1/10th that of kinesin).

3. ISOLATING FACTORIES

We have seen that two factors make purification of mammalian polymerases engaged on endogenous templates difficult.[32,46] First, active enzymes represent a small fraction of the total population; most are part of a rapidly-diffusing soluble pool. Second, engaged polymerases are tightly bound to the underlying nuclear substructure. Recently, large fragments of factories were partially purified from HeLa cells; caspases were used to detach them (in a "physiological buffer").[166] Caspases are a family of cellular proteases that cut their targets at specific sites and were chosen because they deconstruct nuclei during apoptosis; the ones selected did not cut any subunits of the three nuclear RNA polymerases, except RPB9. Nuclei were isolated, most chromatin detached with DNase, and fragments of factories released with caspases and retreated with DNase; this left ∼50% nascent RNA and endogenous elongating capacity in a soluble form. Electrophoresis in "blue native gels" then allows resolution of three partially overlapping complexes (named complex I, II, or III after the polymerases they contain); all migrate slower than the largest (8 MDa) marker available. Finally, mass spectrometry shows that all complexes share proteins like RNPs, while each possesses a characteristic set of others. For example, 83% proteins in complex I are also in the nucleolar proteome, while complex II uniquely contains five polymerase II subunits plus various transcription factors (e.g., AP-2, C/EBPβ, CTCF) and epigenetic modifiers (e.g., histone-lysine *N*-methyl transferases EZH2, SUV39H1/2). Each complex also contains the expected RNAs (e.g., complex

dx.doi.org/10.1021/cr300513p | *Chem. Rev.* 2013, 113, 8683−8705

I has ~33-fold more nascent 45S rRNA, while complex II is richer in nascent protein-coding RNAs).

4. THE NUCLEOLUS: THE PROTOTYPIC FACTORY

Human loci encoding 45S rRNA are carried on chromosomes 13, 14, 15, 21, and 22; each locus consists of ~50 tandem 43-kbp repeats containing the 45S rRNA gene and an untranscribed spacer.[167,168] Each locus appears as a "secondary constriction" in the mitotic chromosome, and is known as a nucleolar organizing region (NOR). UBF (upstream binding factor), the main transcription factor for RNA polymerase I, is bound to some NORs, and, on exit from mitosis, these NORs (plus nucleolus-associated chromatin domains containing satellite repeats)[169] fuse into one or more nucleoli. NORs lacking bound UBF remain inactive and are not initially incorporated into functional nucleoli.[170−172] The resulting nucleolus is the most prominent cytological feature within the nucleus (Figure 6A);[167,168] it contains high concentrations of RNA and protein, but little DNA.

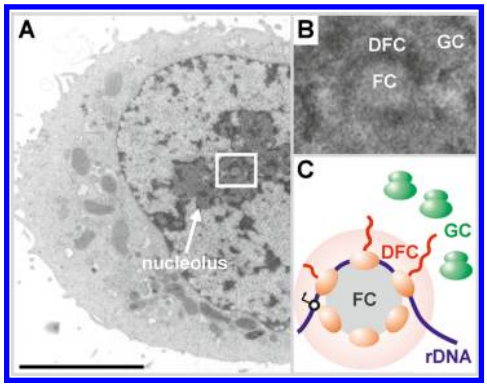


Figure 6. The nucleolar factory. (A) Electron micrograph of a HeLa cell with the nucleolar region indicated. Bar: 5 μm. Originally published in ref 153. Copyright 1985 Nature Publishing Group. (B) Magnification of inset in (A) illustrating the three zones in a nucleolar factory, the central fibrillar center (FC) with associated dense fibrillar component (DFC), and surrounding granular component (GC). (C) As the FC is rich in polymerase I and UBF, a promoter is likely to initiate there, and, once extruded by a polymerase (oval), it lies near another on the surface and so is likely to reinitiate. Successive initiations then occur as the promoter snakes over the surface of the FC. Extruded transcripts (red) are found in the DFC, and, on completion, these assemble into ribosomes (green) in the GC.

4.1. The Nucleolar Assembly Line

A yeast ribosome contains ~70 structural proteins associated with one copy of the 28S, 18S, 5.8S, and 5S rRNA species; an additional ~170 nonribosomal proteins and ~70 small nucleolar rRNAs (most of which are essential) are involved in ribosome biogenesis.[173] These ancillary proteins include helicases, GTPases, AAA-ATPases, chaperones, and enzymes involved in modifying rRNAs (mainly through 2′ *O*-methylation and pseudouridylation). Mammalian ribosomes are even more complex, and most of the processing involved in their manufacture occurs in the nucleolus, which can truly be likened to an assembly line.

The nucleolus has three distinct zones recognized by classical electron microscopists (Figure 6B).[167,168] The "fibrillar center" contains high concentrations of RNA polymerase I and UBF. It appears as the "black hole" in the upper inset in Figure 3A where it is surrounded by two crescents, each a "dense fibrillar component". Transcription by RNA polymerase I of its sole target gene (encoding 45S rRNA, which is then processed to 28S, 18S, and 5.8S rRNA) takes place on the surface of the fibrillar center.[174] Each gene is tightly packed with ~120 engaged polymerases, and neither the active gene nor nascent RNA can be detached with nucleases in an experiment like that in Figure 4.[156] As nascent RNA emerges from the polymerase, it begins its assembly into ribosomes in the associated dense fibrillar component.[174] Newly-made transcripts are then processed further in the surrounding "granular component" to emerge into the nucleoplasm as mature ribosomal subunits. Quantitative analysis shows that a typical nucleolar factory in a HeLa cell (i.e., a fibrillar center plus 4 associated dense fibrillar components) contains ~500 polymerases engaged on ~4 templates.[32] Figure 7C illustrates how this assembly line might work.

The above discussion gives the impression that one nucleolus is much like another. While this is true of some cell types, nucleoli in others can be highly polymorphic. For example, the ~234 fibrillar centers in a human fibroblast fall to ~156 on serum-starvation,[175] and the ~9 in a peripheral blood lymphocyte rise to ~80 as it is stimulated to divide.[176] These results are consistent with the idea that the surface area of the fibrillar center, and so the number of polymerases accessible to promoters, determines the transcription rate.

4.2. Nucleolar Factories: General Principles

Despite such variations in nucleolar number and structure, some principles emerge. (i) Transcription occurs on the surface of a core rich in polymerases and cognate transcription factors. (ii) The number of factories (each with a fibrillar center at the core) is directly related to transcription rate. (iii) Two or more transcription units are generally associated with one factory (with the structures induced by the inhibitor, 5,6-dichloro-1-β-D-ribo-furanosyl-benzimidazole, DRB, being an exception).[176] (iv) These units are usually encoded by one chromosome, but occasionally they can be from different chromosomes. (v) On entry into mitosis, active units are "bookmarked" by bound transcription factors to become active in daughter cells, as unmarked ones remain inactive. (vi) Just as one car factory might specialize in making Hondas (and not Mercedes), nucleolar factories make just one kind of transcript to the exclusion of others. (vii) The occasional association of NORs on different chromosomes in one fibrillar center provides a precedent for the somatic pairing of homologous genes when they are being transcribed. In the specific example of HeLa, these principles result in ~15 000 polymerase I molecules being active in ~30 factories embedded in several nucleoli, and ~125 enzymes transcribe each of the ~4 active units in one factory.[177]

5. NUCLEOPLASMIC FACTORIES

RNA polymerases II and III are active in the nucleoplasm.[1,2,4] The finding that their nascent transcripts are found in a limited number of discrete foci, factories,[19,30] prompts various interrelated questions including: how many active polymerases and templates might there be in one factory, how big are such factories, and how much transcription occurs outside these hotspots of activity? Superficially, these questions seem easy to answer.

5.1. Number and Diameter

Individual nucleoplasmic factories are so numerous they cannot be resolved one from another using a confocal microscope; the

dx.doi.org/10.1021/cr300513p | *Chem. Rev.* 2013, 113, 8683−8705

optical section is thick enough that a factory in the midplane appears to be overlapped by others lying above and below. However, most can be resolved by confocal imaging of thin cryosections of ~100 nm (as in Figure 3A).[178] Yet some foci in such sections have intensities close to background levels, while others might even lie below it (because labeling and detection are inefficient), and setting the background level bedevils accurate counting. Consider the analogy of counting stars as dusk falls (and background changes); initially one sees only the evening star, but soon millions appear. We can easily put a lower bound on numbers, but how can we be sure all stars are seen?

This general problem was solved as follows.[40] HeLa cells were permeabilized in a "physiological" buffer, engaged

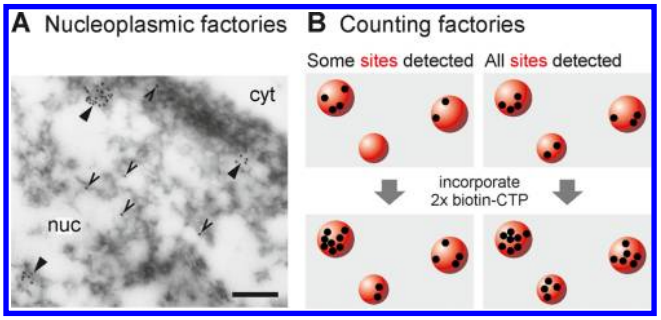


Figure 7. Detecting all transcription sites. HeLa cells were permeabilized, and engaged polymerases allowed to extend their nascent transcripts by up to 2000 nucleotides in biotin-CTP for 0−15 min; after immuno-labeling biotin-RNA with 9-nm gold particles, sections were imaged with an electron microscope. (A) A typical image obtained after incorporation for 15 min. There are three clusters of gold particles in the field (marked by closed arrowheads), and five lone particles (marked by open arrowheads); cyt, cytoplasm; nuc, nucleoplasm. Bar: 250 nm. Originally published in ref 40. Copyright 1996 The Company of Biologists. (B) Left: Only two of the three sites are detected (as they are marked by ≥2 particles), and incorporating more biotin-CTP allows detection of three sites (as the originally unmarked one rises above the level of detection). Right: If conditions allow all sites to be detected (here all marked by ≥2 particles), incorporating more biotin-CTP increases the numbers of particles per site, without affecting site number.

polymerases allowed to extend transcripts in biotin-CTP for 0−15 min, biotin-RNA immuno-labeled with gold particles, and sections imaged in the electron microscope. Clusters of gold particles (marking nascent biotin-RNA) were seen against an inevitable background of lone particles (Figure 7A); this background was so low, it was unlikely that two particles would ever be seen together by chance. Therefore, clusters were selected where two or more particles lay within 40 nm of one another (approximately the maximum distance between two gold particles immuno-labeling one target). If detection were inefficient and only a fraction of factories were marked by clusters after 1 min, then increased incorporation should allow previously undetected factories to be seen (Figure 7B; left). However, no more clusters were detected after 5 or 15 min (so all factories were seen), but the number of particles per cluster increased (as previously detected factories incorporated more biotin; Figure 7B; right). At the same time, the number of lone particles remained the same (so they constituted the background). The total number of clusters (factories) can then be calculated (using standard stereological procedures) from the numbers in a section using nucleoplasmic volume, section thickness, and cluster diameter (which is needed to correct for some factories being missed because sectioning leaves too little to be detected).

Approximately 10 000 nucleoplasmic factories were found in HeLa using this approach. [Faro-Trindade and Cook[177,179] provide numbers corrected using an up-to-date estimate of factory diameter.] Between 2800 and 33 000 are seen in other cells (i.e., aneuploid mouse teratocarcinoma, euploid and totipotent embryonic stem cells, salamander cells), using different precursors (i.e., BrUTP) and imaging methods (i.e., cryosectioning plus light microscopy).[31,40,177−179] [Only ~200 were found in mouse fetal liver, adult thymus, and brain, using antibodies targeting the initiating form of RNA polymerase II;[180,181] however, these are necessarily minimum values, as we have no way of knowing whether all factories were seen.]

Despite large variations in number, factory diameter and density remain similar in different cells.[177,179] For example, mouse embryonic stem (ES) cells can be induced to differentiate into larger or smaller cells; despite a 4-fold difference in nucleoplasmic volume, factory diameter and density remain constant, as the total number of factories increases or decreases. Also, in salamander cells with an 11-fold larger genome than the mouse, diameter and density are again similar, despite large increases in volume and numbers of polymerases.

5.2. Fraction of Transcription in Factories

What fraction of all RNA synthesis takes place in factories? An upper bound can be determined from the experiment described above using biotin-CTP.[40] If we assume the number of gold particles in clusters reflects RNA synthesis occurring in factories, lone particles will reflect any hypothetical nonfactory synthesis plus the inevitable background. After 15 min, there are 10-fold more particles in clusters, and, as no more lone particles appear as more biotin-RNA is made, no lone polymerases seem to mark active sites. In an analogous experiment using BrUTP, lone particles constituted ≤8% of all particles, and, again, most of these were background ones (as they remained when transcription was inhibited).[178] Moreover, sectioning cuts through some factories to leave just polar caps, and one can estimate how small such caps must be before they go undetected. It turns out that caps containing one-twentieth the nascent RNA in the average factory are detected, so any missed ones can contain ≤5% of the total.[178,182] Clearly, essentially all transcription occurs in factories.

5.3. RNA Polymerases II and III Are Found in Distinct Factories

Three kinds of experiment suggest that active forms of RNA polymerases II and III are each concentrated in their own dedicated factories.[182] All three exploit the greater sensitivity of polymerase II to α-amanitin, a poison from the toadstool *Amanita phalloides*;[2] they involve permeabilizing HeLa cells, BrUTP incorporation, and immuno-detection of BrRNA.

First, if both polymerases are active within the same factories, and if most factories are detected, we would expect the drug to inhibit polymerase II and reduce labeling within each factory without affecting the total number seen. On the other hand, if the two are found in their own dedicated factories, inhibiting polymerase II with α-amanitin should reduce the number of factories seen. Results are consistent with the latter; factory number falls to one-fifth.[182]

The second experiment[182] involved immuno-localizing one or other polymerase and their nascent transcripts: polymerase

dx.doi.org/10.1021/cr300513p | *Chem. Rev.* 2013, 113, 8683−8705

II is found near its own (α-amanitin-sensitive) transcripts but not polymerase III (insensitive) transcripts, while polymerase III is found near its own transcripts but not those made by polymerase II. The third experiment[182] exploits steric hindrance occurring between the large immuno-labeling probes. Thus, an antipolymerase II antibody blocks access of another antibody to BrRNA made by polymerase II, but not to polymerase III protein or the BrRNA it makes. Conversely, an antipolymerase III blocks access to BrRNA made by polymerase III, but not to polymerase II protein or its BrRNA. These results suggest that polymerases II and III, like polymerase I, are found in their own distinct factories.

5.4. Number of Active Polymerases and Genes per Factory

As essentially all RNA synthesis occurs in factories, the number of active polymerases and templates per factory can be calculated from the numbers of (i) active polymerases (or nascent transcripts), (ii) polymerases engaged on each unit, and (iii) factories. We summarize how these three numbers can be derived. Reassuringly, different approaches (which presumably have different thresholds of detection) yield similar numbers.[179] Moreover, some approaches confirm corresponding numbers for polymerase I, which we know reasonably accurately (see section 4).

The numbers of active polymerases can be determined in three general ways. In one, cells are permeabilized, engaged polymerases allowed to extend their transcripts in [^{32}P]UTP for different times (all in a "physiological" buffer), and the resulting [^{32}P]RNAs sized. [In some cases, transcripts are trimmed with ribonuclease A prior to extension to improve the accuracy with which the number of added nucleotides can be measured, and in others drugs (e.g., α-amanitin, actinomycin D, sarkosyl, tagetitoxin) are added to inhibit differentially one or other polymerase.] Next, the number of growing transcripts is calculated from the total number of nucleotides incorporated into all transcripts, and the average increment in length. The second approach involves quantitative immuno-blotting using antibodies targeting hypo- and hyper-phosphorylated forms of polymerase II, and known weights of reference proteins; only a quarter of all molecules in the cell are active.[32,178,179,182] In the third, the numbers of transcription complexes seen in "spreads" made from known numbers of nuclei are counted.[32]

Unlike rDNA genes, a typical (active) polymerase II unit is associated with only one polymerase (Figure 3C).[183−186] For example, analysis of 100 active HeLa units in spreads like that in Figure 3C shows that (at least) two-thirds are associated with only 1 transcript.[32] Even in yeast, <1% genes are transcribed by >1 polymerase.[187,188] Studies on GFP-tagged polymerase II support the idea that transcriptional initiation is rate limiting, so few units ever become loaded with more than one polymerase.[45] In other words, many so-called "active" genes spend most of their time not being transcribed. In the case of RNA polymerase III, transcription units are too short to be simultaneously loaded with more than one polymerase.[189]

5.5. Architecture

The highest resolution images of nucleoplasmic factories have been obtained using a special electron microscope and technique, electron spectroscopic imaging (ESI).[57,181] In conventional electron microscopy, stains that contain heavy metals like uranium enhance contrast by deflecting an electron in the beam so that it fails to pass through the slit to be imaged. In ESI, sections are unstained, and contrast depends on endogenous atoms. When a beam electron interacts with one orbiting a phosphorus or nitrogen nucleus, it loses a characteristic amount of energy (153 or 120 eV, respectively). Scattered electrons now pass through a spectrometer, and images of phosphorus (or nitrogen) in the sample are collected by repositioning the slit.

Relative to other cellular constituents, nucleic acids are rich in phosphorus, and proteins in nitrogen. In Figure 8A,

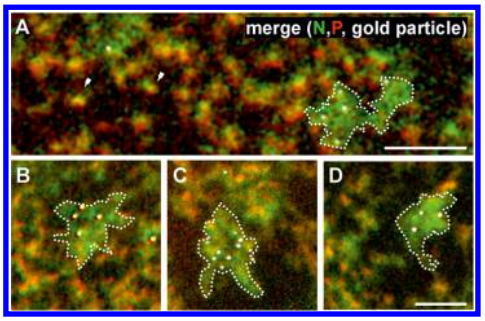


Figure 8. Images of nucleoplasmic factories obtained using electron spectroscopic imaging. HeLa cells were permeabilized, nascent transcripts extended in BrUTP, and resulting BrRNA immuno-labeled with 5-nm gold particles; after sectioning (70 nm), images of endogenous phosphorus (red) and nitrogen (green), plus immuno-labeling gold particles (white), were collected and merged. (A) Five gold particles mark BrRNA in a nitrogen-rich factory (perimeter indicated by a dotted line). Absolute numbers of N and P atoms within this perimeter can be calculated using nearby nucleosomes as references (arrowheads). (B−D) Examples illustrating how polymorphic factories are. Bars: 100 nm. Originally published in ref 57. Copyright 2008 The Company of Biologists.

phosphorus and nitrogen have been pseudocolored red and green, and chromatin, rich in both, appears yellow. Nascent BrRNA is marked by gold particles (pseudocolored white), and these mark a (green) factory.[57] Although factories are polymorphic (Figure 8B−D), they are relatively homogeneous in size. For example, in HeLa, 75% have diameters between 60 and 120 nm, with an average of ~87 nm.[57] In mouse erythroblasts they are slightly larger (i.e., 130 nm), with a fraction rich in the transcription factor KLF1 being larger still (i.e., 174 nm).[181] The number of phosphorus and nitrogen atoms in a factory can be determined by reference to signal from a nucleosome, which has a known atomic constitution. The (green) factory core in HeLa typically has a mass of ~10 MDa, and a density one-tenth that of the nucleosome (so is probably porous like a sponge). It also contains little phosphorus, consistent with templates and nascent transcripts being attached to the surface. As these factories possess such characteristic phosphorus:nitrogen ratios, they can be detected in unpermeabilized HeLa cells (although then one cannot be certain they are transcriptionally active).

The diameter of nucleoplasmic factories has also been measured indirectly using RNA FISH and probes targeting two different transcripts produced in one factory.[15,190,191] Each probe hybridizes to an intronic region in the transcript, which, even if stretched out, spans less than 200 nm (the diffraction limit of the light microscope). If the two transcripts are made in the same factory, the red and green FISH signals inevitably overlap to give a yellow focus. Gaussian curves are fitted to the individual red and green distributions underlying such yellow foci, and the distance between peaks measured with ~15-nm precision. 2D distances range from 7 to 102 nm (mean 62 nm). This distribution fits a model where pairs of red and green

dx.doi.org/10.1021/cr300513p | Chem. Rev. 2013, 113, 8683−8705

points are repeatedly and randomly distributed in a 35-nm shell surrounding an 87-nm diameter sphere. These results are consistent with nascent transcripts copied from the two different genes lying on the surface of one 87-nm factory.

In summary, transcription in nucleoplasmic factories, as in nucleolar ones, occurs on the surface of a protein-rich core, where two or more transcription units are associated with one factory. Unlike nucleolar factories, which vary greatly in size, nucleoplasmic ones generally have diameters of 50−175 nm. In the specific case of polymerase II and a population of dividing (subtetraploid) HeLa nuclei, ∼64 000 molecules are active in ∼8000 factories, each containing ∼8 enzymes active on a different template.[32,40,177−179] If we correct these values using recent estimates of factory diameter (i.e., 90 nm[15,57,190,191] instead of the 46 nm used previously),[178] and the average nucleoplasmic density seen in various cells (i.e., 9.3 factories/μm^3),[177] there would be ∼6000 factories with ∼10 active polymerases per factory. As factory number scales with nucleoplasmic volume,[179] a "normal" diploid human umbilical vein endothelial cell (HUVEC) in the G0 phase of the cell cycle would contain 2200 factories (calculated assuming nucleoplasmic volume represents 80% nuclear volume of ∼300 μm^3, and a density of 9.3 factories/μm^3).[14] In the case of polymerase III and HeLa, ∼10 000 molecules are active in ∼1800 factories.[182]

5.6. The Production Line

The synthesis of a mature mRNA involves cotranscriptional capping, splicing, and polyadenylation.[25,192,193] While we currently lack detailed plans of the production line, it is clear the C-terminal domain (CTD) of the catalytic subunit of the polymerase both interacts with, and regulates, much of the necessary machinery.[47−49] The CTD may even bind to many of the stations on the line simultaneously, simply because the 52 heptad repeats in the human protein could extend ∼200 nm away from the catalytic core.[47]

The presence of one station in the production line, a proofreading unit containing a translating ribosome that first detects incorrectly positioned stop codons in a (faulty) transcript and then triggers "nonsense mediated decay" (NMD),[194] is controversial.[195−203] Nevertheless, (i) ribosomal proteins do associate with nascent RNA,[195,196,200,201] (ii) translational initiation factors (i.e., EEF1D, EIF1AY, EIF2S1/2, EIF3A/C/D/I) copurify with polymerase II factories, but not those containing other polymerases,[166] and (iii) the CTD interacts with translation initiation factors (e.g., eIF4E, eIF4G), ribosomal subunits (e.g., S6, ribosomal P site antigen), NMD proteins (e.g., UPF1, 2, 3a),[200] components involved in destroying unwanted transcripts,[204−206] and the proteasome.[207−209] Whatever the outcome of this controversy, the inclusion of so many other stations in this production line ensures that the organization is complicated, and Figure 9 illustrates a model for it.

The phenomenon known as "*trans*-splicing", where an exon in one gene is unexpectedly joined to an exon in another, occurs extensively in mammals.[210−212] As the DNA templates encoding *trans*-spliced transcripts yield "3C" products (below), it is easy to imagine that the process occurs sporadically in a factory containing a number of closely packed production lines: one intron-containing transcript might mistakenly associate with the splicing machinery in a neighboring line.

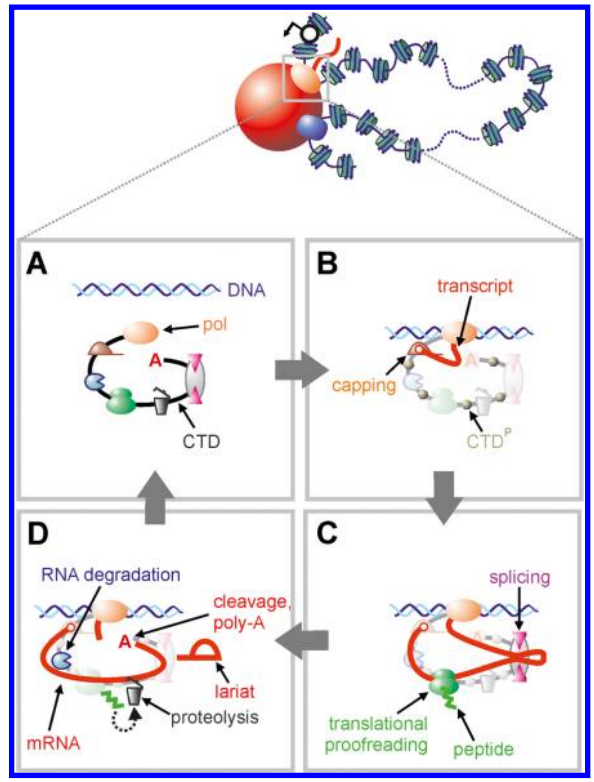


Figure 9. A model for one production line in a polymerase II factory. A loop attached to a factory (top) and the magnification of one polymerizing complex (below) are shown. This complex contains all of the machinery necessary to create a message (positions chosen for artistic convenience), but it is unclear how many different components are simultaneously bound to it. (A) The CTD associates (counterclockwise) with components involved in capping (brown), transcript degradation/NMD (blue), translational proofreading (green), proteolysis (black), splicing (magenta), and polyadenylation (red "A"). (B) Transcription begins as the template binds to the polymerase; the CTD is now hyper-phosphorylated (CTD^P), and a cap now tethers the 5′ end of the nascent transcript to the complex. (C) The transcript is extruded through a splicing complex as the ribosome/NMD machinery proofreads the spliced message; positioning ensures the proofreading machinery cannot read introns that may contain termination codons. (D) Introns are removed (lariat), and the transcript is polyadenylated and ready to be released. If errors are detected by the proofreading machinery, the faulty transcript (and faulty peptide) are degraded by nucleases (and proteasomes). The engaged polymerase will finally terminate, and the mRNA will be exported to the cytoplasm.[213] Adapted from ref 200. Copyright 2004 The Company of Biologists.

6. PRINCIPLES UNDERLYING FACTORY FORMATION

We now discuss some general mechanisms that underlie the formation of large structures like factories that might act in addition to the ones familiar to most biologists such as hydrogen bonding, van der Waals forces, hydrophobic forces, and charge interactions.

6.1. Clustering Driven by DNA-Binding Proteins

Consider two transcription factors, like C/EBPα and β,[214] present at ∼1 nM (a typical concentration in the nucleus), and able to interact with each other with a K_d of 10^{-7} M (again a value typical of a transcription factor); <1% will dimerize (i.e., the equilibrium is well toward the monomers).[215] However, in the presence of a DNA molecule with two cognate binding sites 10 kbp apart, protein binding to these sites creates a local

dx.doi.org/10.1021/cr300513p | *Chem. Rev.* 2013, 113, 8683−8705

concentration that drives two-thirds into the complex; now the equilibrium is well toward the protein:protein complex. This means that bound transcription factors will inevitably cluster, to loop the intervening DNA. However, such clusters/loops are unlikely to persist for long, as GFP-tagging shows the factors typically reside on DNA for <10 s.[13]

6.2. Clustering Driven by the Depletion Attraction

Now consider the crowded nucleus, where many macromolecules continually bombard larger complexes from all sides. When two larger complexes come into contact, the smaller macromolecules are sterically excluded from a volume between the two and so cannot knock the two larger complexes apart; as a result, an entropic "depletion attraction" (equivalent to the osmotic pressure exerted by small macromolecules on opposite sides of the two large complexes) keeps the two large complexes together (Figure 10).[216,217] Theory also suggests

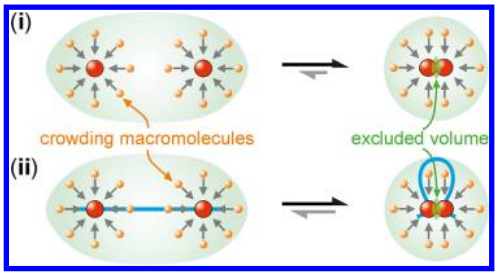


Figure 10. How the nonspecific (entropic) "depletion attraction" drives looping. (i) In a crowded cell, many small soluble macromolecules (orange) bombard large complexes (red) from all sides (arrows). When the two complexes come into contact, small macromolecules are sterically excluded from the green volume between the two and so cannot knock the two large complexes apart; as a result, a "depletion attraction" keeps the large complexes together. (ii) When the large spheres (polymerases) are threaded on a chromatin fiber, this depletion attraction is only partially countered by the entropic cost of looping. It has the strength of a few H-bonds, and will act for as long as polymerases remain engaged. This "attraction" can act in the absence of, but may be supplemented by, forces like H-bonds, van der Waals forces, hydrophobic, and charge interactions. Modified with permission from ref 219. Copyright 2006 Elsevier.

crowding affects the rate by which equilibrium is attained, by speeding looping (by reducing effective loop length and so increasing diffusive encounters) and slowing unlooping (by increasing viscosity).[218] If the larger complexes are mammalian RNA polymerases (with associated transcript and spliceosome) bound 20 kbp apart on one DNA segment, the energy involved in this depletion attraction is roughly equivalent to the entropic cost of looping the DNA. In other words, the two engaged polymerases will often be together.[219] Here, the attraction will act for as long as the polymerases transcribe, which can be many minutes[220] (and even longer as one-third of engaged polymerases are stalled).[221] More open conformations of the chromatin fiber can also drive clustering,[222] and a number of individual loops will themselves cooperatively aggregate into rosettes and more complex structures involving other fibers;[219,223] however, entropic costs increase rapidly as the number of leaves in a rosette increases beyond ~15.[224] [For a model involving the aggregation of multiblock copolymers into microdomains, see Canals-Hamann et al.[225]]

6.3. Disordered Assembly of Preinitiation Complexes and a Role for Kinetic Proofreading

Classical biochemistry shows that active transcription complexes can be assembled in vitro from individual components in a stepwise and rigid temporal order.[2] However, studies using GFP-tagged components suggest that such a pathway is not followed in vivo. Instead, individual components continually collide with each other, and only occasionally do the appropriate ones come together at the same time; usually, the resulting complex disassembles immediately, and only after repeated attempts does a stable, productive, preinitiation complex form.[37,58,60,61]

The formation of the complex involved in nucleotide excision repair is better understood than the ones involved in transcription,[226] and it provides a precedent for the role of "kinetic proofreading" during assembly of a complex biological structure.[227,228] Assembly does not follow a linear pathway; instead, it follows a network of parallel pathways, so inevitably there are many unfavorable paths including some that lead to nonproductive products. Proofreading involves the disassembly of such unwanted products, so the system can have additional goes at making the wanted ones. Naturally, this comes at the cost of unproductive cycling, and so increased reaction time. Importantly, the system also allows assembly with a specificity above the level available from the free energy differences in intermediates, through the input of additional free energy (e.g., by irreversible hydrolysis of ATP). One can imagine this occurring both during the assembly of a factory and transcriptional initiation.

7. CHROMATIN LOOPS

That the chromatin fiber might be looped is an old idea.[21] Initial evidence came from images of lampbrush loops (see section 2.2), the demonstration of supercoiling in linear eukaryotic chromosomes both in vitro[146,148] and in vivo (as looping is required to maintain the supercoils),[229,230] and the rate at which nucleases cut chromatin (fragments were only released when two cuts were made in one loop).[231] Another enduring idea is that some conserved protein would act as the molecular tie that stabilizes the loops,[21] and CCCTC-binding factor (CTCF) is one current favorite.[232] However, the approach used in Figure 4 with a "physiological" buffer showed that (i) transcription units are major players,[155−158] with roughly one-half the attachments being within the body of the units, and one-half from the promoters driving those units,[157] and (ii) loops in HeLa cells had a broad range of contour lengths centered around ~86 kbp.[159] More recently, FISH has been used to measure the physical separation between pairs of human genes in 3D nuclear space; it depends on the number of intervening base-pairs in a way best fit by mixtures of local and giant loops of 0.1−1 Mbp.[233,234] However, decisive evidence for looping awaited the development of new techniques.

7.1. Multiscale Looping Detected Using 3C

Studies on loops were revolutionized by the introduction of chromosome conformation capture (3C).[235] This technique allows detection of two chromatin segments that lie together in 3D nuclear space, and it soon confirmed that a regulatory element often looped back to contact its target promoter.[180,235−237] [A related technique introduced at the same time, RNA TRAP (tagging and recovery of associated proteins), confirmed 3C results.[238]] Subsequently, 3C was coupled to high-throughput read-outs involving microarrays

dx.doi.org/10.1021/cr300513p | Chem. Rev. 2013, 113, 8683−8705

and next-generation sequencing to give a technique known as "4C",[239] while other variants like "5C",[240] "GCC",[241] "Hi-C",[242] and "ChIA-PET" (chromatin interaction analysis coupled to paired-end tagging)[28,243] permit interrogation of genome-wide interactomes at an ever-increasing resolution driven by improvements in sequencing capacity and software.[244−248] All of these techniques confirm that the genome is formed into loops at multiple scales, from a few kbp to many Mbp, and that contacts are often between different chromosomes.

7.2. Contacting Sequences Are Generally Transcribed

The application of 3C shows that both sequences in contact are usually transcribed. Examples at the local level include genes encoding interleukins and their regulatory elements (in CD4+ T lymphocytes),[236] the human pituitary growth hormone gene and its "locus control region" (LCR),[249] globin and its LCR (in erythroid lineages),[180,250−254] various immediate-early genes like *myc* with each other (in stimulated B lymphocytes),[255] V_H with DJ_H regions during V(D)J recombination in the *IgH* locus (in lymphocytes),[256] several *Hox* genes (as digits develop),[257−259] paternally expressed and imprinted murine gene loci,[260] and those involved in mounting the inflammatory response.[14,190] If transcription stabilizes the contacts, inhibiting transcription should eliminate those contacts. However, treatment with DRB (which blocks phosphorylation of Ser2 in the CTD of the largest subunit of the polymerase and so inhibits elongation) eliminates some, but not all, contacts;[190,191,261,262] therefore, DRB-insensitive forms of the polymerase (like the ones phosphorylated at Ser5 of the CTD) must maintain some contacts and/or additional players must be involved.

On a genome-wide scale, active segments of the genome often coassociate, as do inactive ones.[242,243,246] Studies of distinct 0.2−1 Mbp domains on inactive X chromosomes,[263] and of the 1% of the human genome analyzed in the ENCODE project, confirm these general principles.[264] Moreover, reanalysis of the original Hi-C data from human lymphoblasts[242] shows that coexpressed genes and DNase-sensitive sites (which mark active promoters) are frequently found together,[244,245] and this has recently been confirmed in other cell types.[246,265] ChIA-PET applied to various human cells further reinforces the general conclusion; for example, 65% sites binding RNA polymerase II also mediate looping, with many of the binding sites clustering together into "chroperons".[28] As might be expected, similar results are obtained with other eukaryotes. In the fly, inactive (polycomb-occupied) as well as active regions coassociate,[266−268] as do highly transcribed genes in yeast,[247,269] especially coregulated ones and those encoding tRNAs.[270,271]

7.3. Immobilized and Active Polymerases Are Major Molecular Ties Maintaining Loops

A decisive experiment showed that immobilized and active RNA polymerases can act as the molecular ties that maintain loops.[190] It involved two human genes that could be switched on within 10 min by tumor necrosis factor α (TNFα), a cytokine that signals through nuclear factor κB (NFκB) to activate and repress many genes. One gene, 10-kbp *TNFAIP2*, is then transcribed repeatedly; the other, 221-kbp *SAMD4A*, is so long that the polymerase only reaches the terminus after ~75 min. The two genes lie ~50 Mbp apart on chromosome 14. If the conventional model for transcription applies, the short gene should never lie near enough to any part of the long gene to yield a 3C product at any time before or after stimulation (Figure 11A; left). Yet if both responding genes are transcribed by two polymerases immobilized in one "NFκB"

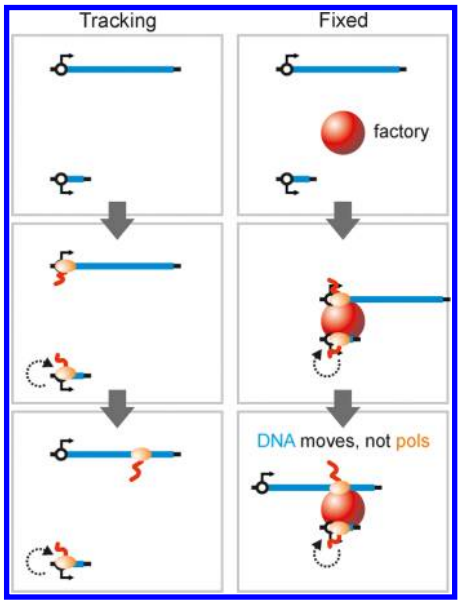


Figure 11. Distinguishing between "tracking" and "fixed" polymerases, after switching on transcription of two genes (blue), one long and one short, lying far apart on the genetic map. (Left) Polymerases (orange ovals) diffuse through the nucleoplasm, bind to promoters, and track along the genes as they produce their transcripts (red lines). As the genes lie far apart in sequence space, the two rarely lie together in 3D space before or after activation. (Right) The two genes diffuse to a factory (red sphere), and initiate there; immobilized polymerases then reel in their templates as they extrude their transcripts. Now, the two promoters are found together. The short gene is repeatedly transcribed as it detaches and reattaches to the factory (dotted arrow). It takes much longer to transcribe the long gene, and when the pioneering polymerase has transcribed two-thirds of the way into the long gene, the segment being transcribed now lies next to the short gene. As a result, contacts (detected by 3C) change in a predictable manner, sweeping down the long gene from promoter to terminus. Results are consistent with the latter model. Modified from ref 190. Copyright 2010 Public Library of Science.

factory, the two promoters should come together when they initiate, and, subsequently, the short gene should contact only the part of the long gene that happened to be transcribed at that particular moment (Figure 11A; right); this is the case. RNA FISH confirms that the respective nascent (intronic) transcripts lie together at the appropriate times. This experiment shows that active polymerases cannot track, and, at the atomic level, we imagine that the template is reeled in through the polymerase just as shown in movies.[12,160] It also indicates that the two polymerases act as the molecular ties that (transiently) maintain a ~50-Mbp chromatin loop. As analogous results are obtained with the same long gene and a short gene lying on a different chromosome, it further shows that the polymerases can mediate both inter- and intrachromosomal contacts.

Analysis of *SAMD4A*, and two other long human genes (312-kbp *EXT1* and 458-kbp *ZFPM2*) that also respond to TNFα, shows that two active polymerases can also fold a gene into a subgene loop. Once a "pioneering" polymerase initiates on each of these genes, it transcribes steadily to reach the terminus >1 h later; additional polymerases ("followers") then repeatedly initiate and abort within ~10 kbp of the promoter (why these

dx.doi.org/10.1021/cr300513p | *Chem. Rev.* 2013, 113, 8683−8705

should abort remains unclear).[38] 3C shows that a subgene loop (tethered by the "pioneer" and one of the "followers" to the same factory) develops within each long gene after stimulation, and that this subgene loop enlarges as the "pioneer" continues to reel in DNA and transcribe it.[15,191] [For a different kind of analysis, see Ohta et al.[272]]

7.4. Transcription Factors Act as Additional Ties

Many other proteins have been suggested to act as the molecular ties that maintain loops in different situations. We suggest most seen to date share one unifying property: they are transcription factors (often bound with active polymerases to genic and nongenic transcription units), and so will probably perform their functions as ties in factories. [Here, we use the term "transcription factor" to include proteins that both up- and down-regulate RNA synthesis. As a result, we consider a protein like HP1, the prototypic marker of heterochromatin, and which is now known to be a transcriptional activator,[273] to be a transcription factor.] Many other transcription factors will be discussed in section 8 (e.g., Oct-1, EKLF, GATA-1, ERα, NFκB), but here we illustrate this point using as examples two very different proteins that are not primarily thought of as transcription factors.

CTCF is a currently considered to be a major molecular tie.[232] It was first characterized as a negative regulator that bound to three direct repeats of the CCCTC-motif in the 5′-flanking sequence of the chicken *c-myc* gene;[274] later it was also shown to be a boundary element, loop organizer, and transcriptional activator.[232] Although some CTCF is bound to regions 3C shows to be in contact, most is found in noncontacting regions;[246] clearly, the majority is not involved in maintaining loops. Moreover, other transcription factors (e.g., E2F4, STAT1, YY1) are usually present both in higher abundance and on more of the contacting partners than CTCF.[275−277] Also, when CTCF is bound, then RNA polymerase II is usually bound too.[28,243−246,250,275] Therefore, in those cases where CTCF functions as a molecular tie, we suggest that current evidence indicates that it acts mainly in conjunction with the polymerase.

Polycomb-group proteins are additional ties that are usually thought to organize inactive (rather than active) genes into (silent) "polycomb" bodies.[78,266] Applying Occam's razor, we speculate that such bodies will prove to be transcriptionally active, simply because polycomb proteins are usually bound to promoters (often in CpG islands) that drive the production of (often noncoding) GC-rich RNAs, which can, in turn, fold into the hairpins that recruit polycomb and associated factors.[278]

In summary, we suggest that current results are consistent with most loops being tied by polymerases and/or transcription factors. Of course, this does not exclude the possibility that some loops will be stabilized by other molecular bridges outside factories. However, we do also suggest that it is only prudent to check whether a protein stabilizing a loop is closely associated with an active polymerase before promoting it as a novel extra-factory tie.

7.5. On Inter- and Intra-chromosomal Contacts

Hi-C experiments clearly demonstrate that a typical point on a chromosome often contacts flanking regions on the same chromosome, and that such *cis* contacts fall off rapidly as the number of intervening base pairs increases.[242,245] In contrast, (*trans*) contacts with other chromosomes are so rare they are often considered part of the background. This may be appropriate for points out in a loop, but not for points attached to a factory. Consider, for example, the *SAMD4A* promoter. When inactive, 4C shows the promoter only makes a few contacts, and essentially all of these are local *cis* ones.[14] This is consistent with the promoter being in relatively empty "outer" space distant from a factory (as in Figure 12B, left); as a result, it behaves like most points in the genome as they are similarly positioned. However, when activated by TNFα, many new contacts appear, and most of these are with other now-active and responsive genes on many different chromosomes, now consistent with the promoter being close to a densely populated factory transcribing responsive genes from around the genome (as in Figure 12B, right).[14] Similarly, ChIA-PET (applied using a pull-down of ERα or the polymerase) allows focus on contacts made just by transcriptionally active sequences; here, two-thirds of the contacts are *trans* ones.[14,243] In summary, a "typical" DNA segment may make few *trans* contacts, but transcribed segments make many, and these do not simply reflect the background!

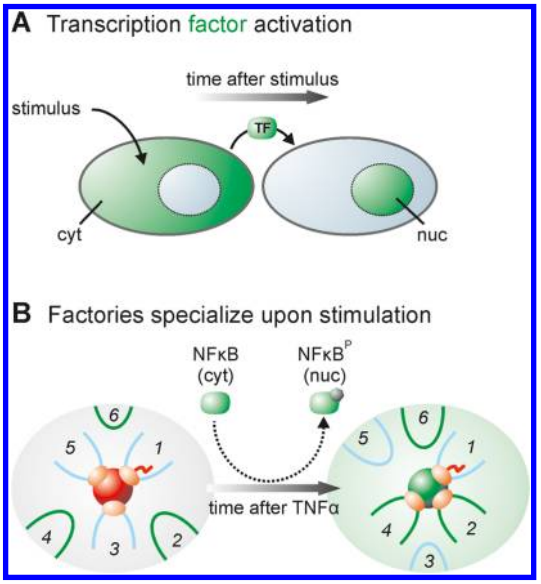


Figure 12. Development of "NFκB" factories on stimulation with TNFα. (A) Before stimulation, the transcription factor (TF; green) is predominantly cytoplasmic (cyt); TNFα induces phosphorylation and translocation into the nucleus (nuc). Modified with permission from ref 301. Copyright 2010 Elsevier. (B) Genes *1*, *3*, and *5* are being transcribed in a factory (red sphere), while TNFα-responsive genes *2*, *4*, and *6* are unattached and inactive. TNFα induces phosphorylation of NFκB (NFκB^P), import into the nucleus, binding to responsive promoters and/or the factory, and, once relevant promoters diffuse through the nucleoplasm and collide with the factory, transcription of responsive genes in what has become a "specialized" factory (green sphere). As a result, gene *2* is now cotranscribed with other responsive NFκB-binding genes. Gene *1* is still attached and transcribed, but may later be replaced by responsive gene *6*. Modified with permission from ref 14. Copyright 2012 Nature Publishing Group.

8. FACTORIES SPECIALIZE IN PRODUCING DIFFERENT TYPES OF TRANSCRIPT

We have seen (section 5.3) that each of the three kinds of nuclear RNA polymerase are concentrated in their own dedicated factories. We now describe how different nucleoplasmic factories further specialize in transcribing different gene subsets.

dx.doi.org/10.1021/cr300513p | *Chem. Rev.* 2013, 113, 8683−8705

8.1. Factories Specializing in Transcribing Protein-Coding Genes

Early work pointed to selected groups of genes associating with a particular nucleoplasmic structure to be transcribed.[94,114] Examples include: (i) genes on human chromosomes 6 and 7 utilizing Oct1 and PTF (with "OPT domains"),[279] (ii) satellite III repeats on human 9q12 that bind heat shock factor 1 (with "stress granules"),[280] (iii) the major histocompatibility locus (with "PML bodies"),[281] (iv) yeast tRNA genes (with the nucleolar surface),[282] (v) infecting viral genes (with concentrations of RNA polymerase II),[283] and (vi) histone genes (with "Cajal bodies"; below). It now seems likely that each of these bodies is a different kind of factory rich in the factors required to promote transcription of selected genes.

Introduction of 3C led to the identification of physical contacts between coregulated genes, and those made by the mouse β-globin gene, *Hbb-b1*, provide the best-characterized examples. *Hbb-b1* lies tens of kbp away on chromosome 7 from its LCR, and ~25 Mbp away from *Eraf* (which encodes an α-globin stabilizing protein); many 3C contacts are seen among the three regions in erythroid nuclei (where all three are transcribed), but not in brain nuclei (where all are inactive).[180,252,253,284−286] DNA and RNA FISH coupled to immuno-labeling, as well as RNA sequencing combined with chromatin immunoprecipitation, confirm that active *Hbb-b1*, the LCR, and *Eraf* are found together in sites rich in polymerase II.[180,254,287,288] These "globin" factories, which are also known as "active chromatin hubs",[284] associate with other highly-expressed genes in the erythroid lineage, and contain many of the required transcription factors (e.g., EKLF, GATA-1, FOG-1).[180,252,253,286,289,290] Genes encoding interleukins,[291] cytochrome c subunits,[292,293] histones,[248] Hox proteins,[258,259] or ERG-driven transcription units[294] also cluster together, presumably in analogous specialized factories.

Although most polymerase II transcripts are spliced, some are not; it seems that different factories make the different sets of transcripts. Thus, early work showed that genes encoding transcripts with characteristic 3′ stem-loops but no introns or poly(A) tails (i.e., histone mRNAs, small nuclear RNAs U1−4, U11, and U12) were all transcribed on the surface of "Cajal bodies".[295,296] Subsequently, a direct test showed that an intron could target a gene to a different factory: two mini-chromosomes carrying identical (intron-less) transcription units were transcribed in the same factories, but inserting into one an intron (or a different promoter) now targets it to a different factory.[297,298] Moreover, ChIA-PET (applied using a "pull-down" of RNA polymerase II) shows that intron-less genes often contact each other,[28] in "non-splicing" factories.

Polymerase II factories can also be categorized by the modifications carried by their polymerases, in particular, by the residues in the heptad repeats in the CTD of the largest catalytic subunit. Thus, "poised" factories contain phospho-Ser5 but not phospho-Ser2, while both residues are phosphorylated in "active" ones.[299] It remains to be seen how many other modifications carried by the CTD[48−51] will prove to be markers for additional types of specialized factories.

8.2. Specialization Induced by Steroids and Cytokines

The way factories become specialized has been analyzed in a few cases. For example, cells encoding both a tandem array of 200 promoters from the mouse mammary tumor virus (MMTV) and a GFP-tagged glucocorticoid receptor (GR) were treated with hormone; this induced binding of the GFP-GR to the hitherto-inactive MMTV promoters, chromatin decondensation, and their incorporation into transcriptionally active foci, factories.[59] Similarly, treatment of MCF-7 fibroblasts with estrogen stimulates binding of the cognate receptor, ERα, to many sites around the human genome; ChIA-PET (applied with a "pull-down" of ERα) then shows that these sites tend to coassociate, presumably in "ERα" factories.[243] Other steroids[300] and cytokines[14] adopt a similar strategy. For example, TNFα stimulates phosphorylation of NFκB and translocation into the nucleus (Figure 12A) where it switches on many genes, including *SAMD4A*. Before stimulation, 4C and ChIA-PET (applied with a "pull-down" of polymerase II) reveal that *SAMD4A* contacts few other genes. However, within 10 min of adding TNFα, it contacts many others that are both up-regulated by the cytokine and bind NFκB (Figure 12B). RNA FISH (using intronic probes) coupled to "super-resolution" localization microscopy confirms that nascent transcripts encoded by *SAMD4A* and some of these other responsive genes lie together on the surface of 90-nm structures, presumably "NFκB" factories. Another cytokine, transforming growth factor β_1 (TGFβ_1), which signals through the SMAD family of transcription factors to activate a different set of genes, induces one responding gene (i.e., *ETS2*) to contact other predominantly responders, presumably in "SMAD" factories.[14]

8.3. Factories Transcribing Noncoding Genes

One can easily imagine that coding and noncoding transcripts might be produced in different factories because they need to be processed in different ways. It seems this is so. For example, some noncoding and conserved elements in K562 cells are transcribed, and 4C shows they tend to contact each other.[302] Similarly, ChIA-PET (applied using a "pull-down" of polymerase II) uncovers many contacts between intergenic elements that are copied into long noncoding RNAs.[28] Furthermore, the nuclease (Drosha) involved in the initial step of micro-RNA (miRNA) processing acts cotranscriptionally[303,304] − and so presumably in a factory; the relevant pre-miRNAs then might also all be produced in "miRNA" factories. This possibility was tested by selecting three genes that both respond to TNFα and encode miRNAs (i.e., *MIR17HG*, *MIR155HG*, *MIR191*); on stimulation, all three associate with other genes that themselves encode miRNAs (and these miRNAs target many mRNAs down-regulated by TNFα).[14] Therefore, the cytokine up-regulates some genes that are transcribed in "NFκB" factories, and represses others through the production of miRNAs made in "NFκB/miRNA" factories.

8.4. Some Speculations on the Formation of Specialized Factories

How might factories become specialized? We can only speculate, but the scenario shown in Figure 12B is attractive.[14] Before stimulation with a cytokine like TNFα, potentially responding genes *2*, *4*, and *6* are "poised"[299] near pre-existing "naïve" factories, which they visit every few minutes as they diffuse through the nucleoplasm. Occasionally, promoters might collide with polymerases in a factory, but few initiate as the concentration of relevant transcription factors is low. On stimulation, the relevant factor is imported into the nucleus where it binds to responsive promoter *2* and stabilizes attachment to a factory. Once productive transcription begins, promoter *2* and any bound transcription factors are now tethered to the factory. A bound factor may soon dissociate, but it is likely to rebind immediately to a nearby binding site in promoter *2*. Alternatively, binding to responsive promoter *4* as

it diffuses by will enhance that promoter's chance of initiating. Once it does, the local concentration of the factor increases further, and a virtuous cycle is established; as responsive promoter *6* is captured, factor concentration increases again, and the factory evolves into one that predominantly, but not exclusively, transcribes responding genes. Now the concentration of the factor in and around the now-specialized factory can be maintained (despite the homogenizing effects of dissociation and diffusion) simply because the local concentration of binding sites is so high (as is seen with the LacI protein in bacteria).[305] Note that this scenario sees factories becoming gradually specialized after stimulation, so any classification into "specialized" and "naïve" becomes an arbitrary (though convenient) one.

How many specialized factories of one type might there be? Again, we can only speculate; one estimate points to *SAMD4A* being able to access ~8 of the 150−250 "NFκB" factories in a HUVEC nucleus, out of a total of ~2200 polymerase II factories.[14] How many different types of specialized factories might there be? If factories evolve as described above, it will be difficult to provide a meaningful answer, especially as some factories transcribing TNFα-responsive genes also transcribe others responding to TGFβ, and such an overlap clearly facilitates the coregulation of different pathways.[14] Even so, the first analyses of the networks of contacts detected by Hi-C point to the existence of many tens of different types of factories.[247,248]

8.5. Modeling Specialized Factories

In a first step toward modeling genes associating with specialized factories,[306] two types of genes (*X* and *Y*) were allowed to diffuse in a volume containing two types of transcription factors and factories (1 and 2); after binding to a factory of appropriate type (i.e., *X* to 1, and *Y* to 2), genes were "transcribed" for a certain time before dissociating. Under conditions where factors were limiting, increasing factor concentration decreased gene colocalization more than increasing factory number or nucleoplasmic volume. As cytokines like TNFα induce cyclic import of their effectors into nuclei,[307] fluctuations in concentration of one transcription factor were also analyzed; genes binding the fluctuating factor colocalize more than those associating with another factor present at a constant (average) concentration.

9. REGULATION

The rate of transcription of the gene encoding rat growth hormone can vary over 8 orders of magnitude,[308] but deleting local elements like promoters and enhancers reduces expression in transient transfection assays by only 3 orders.[309] Clearly, additional factors, which are often described by the term "context", must contribute to the missing 5 orders. Perhaps the most significant part of this "context" involves the position of a gene on a chromosome, as genetic screens have shown that any gene can be completely silenced by translocation close to a breakpoint in heterochromatin.[111,310] The basis for such "position effects" has been mysterious, but intuition suggests that tethering a promoter close to, or distant from, a factory containing the appropriate polymerase and transcription factors will determine whether or not that promoter can diffuse to the factory and so initiate.[31,311−313]

9.1. Modeling a Loop Attached to a Factory

A simple case has been modeled, and it confirms the intuition described above. One (typical) 77-kbp loop attached to a 75-nm factory was allowed to "diffuse" in a computer, and the frequency with which a promoter in the loop occupied a binding zone on the factory was determined.[314] In Figure 13A,

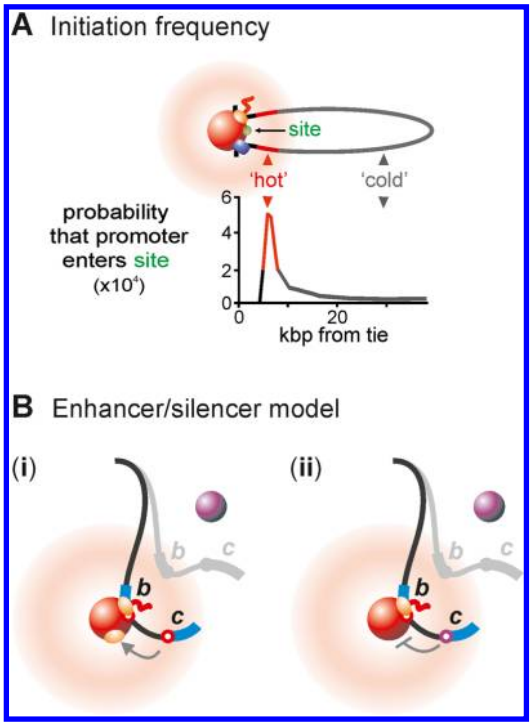


Figure 13. (A) Modeling the initiation frequency of a promoter placed at different positions in a chromatin loop. Monte Carlo simulations indicate a "hot" promoter in a proximal segment (red) in a typical human loop is more likely to collide with a polymerase in the green site on the surface of a factory (and so initiate) than a "cold" one in a more distant segment (gray). Proximal and distal segments would then have eu- and heterochromatic character, respectively. Modified with permission from ref 314. Copyright 2006 Elsevier. (B) A parsimonious model for enhancer/silencer function. (i) Transcription unit *b* binds to the factory (red sphere) and is transcribed; as a result, gene *c* is tethered in the "hot" halo close to the red factory and so is also likely to be transcribed (if "red" transcription factors are present, and if the red factory contains the appropriate factors). In other words, *b* acts as an enhancer of *c*. Another factory (purple) is also shown. (ii) The structure is the same as in (i), but we are at a different stage during development. Unit b has attached as before, but different transcription factors are now bound to *c* (purple), enhancing its affinity for a different transcription factory (also purple). Even though *c* is in the "hot" zone around the red factory, it remains unlikely to be transcribed. In this case, *b* has silenced *c* by distancing it from its favored (purple) factory. Adapted from ref 316. Copyright 2011 Landes Bioscience.

a promoter anywhere in the black segment has too short a tether to reach back and enter the green binding zone on the factory surface, and so can never be active; this may underlie "transcriptional interference", where activity of one gene (which would then be at the tethering point) prevents firing of a neighboring promoter.[312,315] In contrast, a promoter in the "hot" (red) segment can often access the binding zone, and when in the "cold" (gray) segment less so. Clearly, positioning a promoter in a "hot" segment should increase firing. We might also expect "hot" and "cold" segments to represent eu- and heterochromatin, respectively, and we note that increasing loop length, thickness, and rigidity (all typical of heterochromatin) all reduce access to the binding zone.

dx.doi.org/10.1021/cr300513p | *Chem. Rev.* 2013, 113, 8683−8705

At the level of a single gene, transcription occurs sporadically and cyclically, with successive initiations producing a "burst" of transcripts followed by silence.[317−319] Such "bursting" is usually explained by remodeling a permissive chromatin state into a restrictive one.[320] We suggest that if transcription unit *b* in Figure 13B,i is a long one, *c* will remain tethered in the "hot" zone for a long time, and this will drive repeated initiations in the factory and so "bursting".[53,184] This idea is supported by an examination of the changing conformations of *SAMD4A* after stimulation with TNFα.[15] High-resolution RNA FISH was applied to localize (with 30-nm precision) nascent transcripts (used as proxy markers for transcribing polymerases); the promoter fired over and over again to produce a burst, and 3C confirmed the conformations indicated by the RNA FISH. In addition, Monte Carlo simulations yielded results inconsistent with a polymerase binding to the promoter and then tracking down the template; instead, only simulations involving the appropriate distance to adjacent tethering points, a rigidity characteristic of a euchromatic chromatin fiber, and the known interfactory distance gave good fits to the experimental data.[15]

9.2. A Parsimonious Model for Gene Regulation

Motifs like enhancers, silencers, insulators/barriers, and domain borders/boundaries all influence gene expression. Each was seen as different from the others, and each works in a different way. For example, four models have been proposed to describe the molecular mechanism underlying enhancer activity: (i) the "tracking" model involves a protein loading on to the enhancer and then tracking down the fiber to the promoter where it stimulates transcription,[321] (ii) in the "linking" model, a protein loaded on the enhancer drives protein polymerization toward the promoter,[322] (iii) the "relocation" model has the target gene relocating to a nuclear compartment where enhancer−promoter interactions are favored and/or stabilized,[108,323] and (iv) the "looping" model sees direct contact between an enhancer and its target promoter.[324−327] Similarly, insulator function is described by the "roadblock", "sink/decoy", and "topological loop" models.[328−331]

Despite the obvious differences implied by these models, the distinctions between the motifs are becoming ever more blurred. Thus, enhancers were originally characterized by their ability to act positively both in *cis* and in *trans*;[322] however, they are now described just as promoters[193] that fire to generate noncoding enhancer RNAs (eRNAs).[332,333] Like promoters, they are hyper-sensitive to DNase I, and carry the same characteristic histone modifications (e.g., H3K4me1); significantly, they bind RNA polymerase II.[333−336] Silencers, on the other hand, were motifs that prevented gene expression,[328,337] but recent genome-wide analyses fail to distinguish them from enhancers.[246,338] Insulators traditionally subdivided local regions of the genome into functionally autonomous domains,[339] while barriers, boundaries, and borders all demarcate larger domains and act to prevent heterochromatin spreading into euchromatin and/or the other motifs from interacting with their targets.[331] However, all turn out to be promoters marked by bound polymerase.[246,312,330,340−342] For example, Hi-C shows that a human (or mouse) sequence within one domain interacts more with other sequences in the same domain, as compared to those in neighboring domains, much as citizens in one valley rarely cross a watershed into another valley. Yet, on crossing a border (or watershed), the direction of the "average" interaction suddenly changes direction.[246] Remarkably, these borders/boundaries are enriched in promoters (marked by H3K4me1 plus nascent RNA), tRNA genes plus Alu repeats, and CTCF (but only ∼15% CTCF sites are at boundaries), again all markers of active transcription units. Clearly, we need to reevaluate the roles and mechanisms of all of these motifs.

We propose[313] a speculative, unifying model: we suggest all of these motifs are simply transcription units, and each can act as one or other motif depending on the surrounding "context", which is simply proximity to the appropriate factory. Consider, for example, Figure 13B,i where the nongenic transcription unit, *b*, is transcribed in the red factory; as a result, gene *c* is tethered in the "hot" halo close to the same factory, and so is likely to initiate if the appropriate transcription factors are present. So *b* is an enhancer of *c*. But if *c* utilizes a factor concentrated in the purple factory (Figure 13B,ii), it may collide with the red factory, but it will be unlikely to initiate (as the appropriate factors are absent). Now, *b* is a silencer of *c* (Figure 13B,ii). Also, if *a*, *b*, and *c* can all readily access the appropriate factory on the left, but find only inappropriate factories to the right (e.g., polymerase III factories), they will find themselves at a border; even if they diffuse over the watershed, they will be unable to bind stably to a factory on the other side.

The above discussion centers on activity, but can this model be extended to explain the inactivity of whole domains? We suggest it can, but to do so we must dispel the old idea that heterochromatin is transcriptionally inert. First, we now know that ongoing transcription is required to maintain heterochromatin in yeast and plants.[343] Second, even the deepest heterochromatin formed by the centromeric regions of wheat contains a density of transcription factories per unit volume similar to that of euchromatin.[344] Finally, genome-wide profiling of different mammalian cells shows that the large heterochromatic blocks of hundreds of kbp known as LOCKs (large organized chromatin K9 modifications), which carry repressive histone marks like H3K9me2/3 and overlap LADs (lamin associated domains), are nevertheless interspersed with small euchromatic islands. Significantly, these islands are generally hypersensitive to DNase I, bind CTCF, and carry active histone modifications (i.e., H3K4me3, H3K9ac), all characteristic of active transcription units (which are associated with factories).[345] Therefore, we imagine that two adjacent euchromatic islands may be transcribed in the same factory, and that the long intervening segment will then constitute a loop that will acquire repressive chromatin marks (Figures 2 and 13A); in turn, these marks will stabilize aggregation with existing heterochromatin in the interior (to form chromocenters) or at the periphery, driven by the affinity of appropriately modified histones for the lamina,[346] and/or the depletion attraction (Figure 10).[347]

We now describe how the interplay between the number of factories and the contour length of the associated loops might underpin some global changes occurring during differentiation. Here, we discuss results obtained with totipotent (mouse) ES cells (embryonic stem cells) as they are induced by retinoic acid to differentiate into two distinct populations with roughly one-half and double the nucleoplasmic volume.[177,179] Both the total numbers of factories and molecules of active polymerase II track the changes in nucleoplasmic volume, as factory diameter and density remain constant. [A similar trend is seen when ES nuclei are compared to salamander nuclei possessing an 11-fold larger genome.] As the different mouse cells contain the same amount of DNA, and as active polymerases are major molecular

dx.doi.org/10.1021/cr300513p | *Chem. Rev.* 2013, 113, 8683−8705

ties, it then follows that loop length must rise as polymerase numbers fall. We suggest that the nucleoplasm then shrinks spontaneously; doubling loop length increases the radius, *r*, of the volume occupied by one randomly folded loop ~1.5-fold,[314] and *r* will become close to unity if the "extra" DNA out in the loop is now packed into heterochromatin. The system then is self-regulatory, with changes in loop length having little effect on factory density (despite changes in nucleoplasmic volume).

If proximity to the appropriate factory determines gene activity, what are the major determinants of a gene's position in 3D nuclear space? Consider, for example, one allele of the human gene, *SAMD4A* (Figure 14). As the nucleus is self-organizing, statements about its position will necessarily be probabilistic (not absolute), and, as the structure has so many components, no two cells will ever possess identical structures (even in sister cells). Moreover, position changes from moment to moment: in less than a minute, the promoter can move ~1 μm passively by diffusion,[13] or actively as a fixed polymerase transcribes (at ~3 kbp/min). [As one allele diffuses, the other might be transcribed.[37,38]] The position of *SAMD4A* will then depend on the resolution of various conflicting forces. In some, but not all, cells in the population, the chromosome encoding one allele might associate with polymerase I factories in a nucleolus (as it carries a NOR), and different (heterochromatic) G bands will tend to bind to different parts of the lamina or different internal chromocenters. At the local level, distance from domain boundaries and the appropriate factory will depend on stochastic variation and the past history of that particular cell with its unique concentration of different transcription factors. Finally, we would like to stress that the model described above should not be viewed as deterministic; rather we imagine that the system integrates the conflicting forces (which include the effects of transcriptional noise[348,349]) in much the same way that a spider's web integrates the struggles of any trapped flies to inform activity.

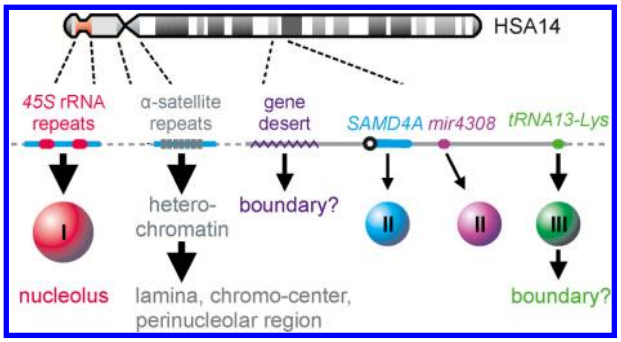


Figure 14. Major determinants of the position of a typical inducible gene in 3D nuclear space. *SAMD4A* lies on human chromosome 14 (ideogram shown) with a NOR encoding *45S* rDNA repeats (red), the centromere (with *α*-satellite repeats; gray), a *SAMD4A*-proximal gene desert (purple), and downstream genes encoding a miRNA (pink) and tRNA (green). The nuclear position of *SAMD4A* depends on the resolution of various conflicting forces that drive attachment (arrows reflect relative strengths) of the NOR to nucleolar factories, centromeric repeats to heterochromatin (and so to the lamina, chromocenter, or perinucleolar region), *SAMD4A* to a "NF*κ*B" factory (when NF*κ*B is present), and nearby genes to their cognate (specialized) factories. Boundaries flanking *SAMD4A* represent zones that fail to nucleate the formation of the appropriate kinds of factory for its transcription.

10. CONCLUSIONS AND PERSPECTIVES

Different cellular regions specialize in performing different biochemical functions so that high local concentrations can drive productive interaction, and there are two ways of maintaining such concentrations, enclosure in a membrane (as in mitochondria, where energy production depends upon membrane integrity) and clustering (as in the case discussed here).[43,81,88,350] The attachment of an active RNA polymerase to a cluster, the factory, has several important consequences. First, the polymerase is inevitably immobilized, and this means we must reevaluate how this vital machine works. By analogy, we should also reconsider whether all polymerases, including those involved in replication (section 2.1), repairing damage in DNA,[20,351] reverse transcription,[352] and synthesizing telomeres,[22] are also immobilized when active. Second, RNA polymerase becomes the major structural component of the chromatin loop by acting as the critical molecular tie; consequently, it also becomes the major determinant of genome organization. It then seems likely that pre-existing transcription factories will nucleate the formation of new factories that work on DNA in different ways, including those involved in replication,[353] repair,[351] and recombination.[81] This organization also has obvious implications for genome rearrangements and cancer.[255,354−358] Moreover, we should also look to the polymerase as the major effector of the genome reorganization that occurs during, for example, mitosis[359] and chromosome pairing.[360−362] Third, proximity to the appropriate factory then becomes the critical determinant of whether or not a gene is transcribed. As a result, analysis of the expression of a gene best begins with a complete inventory of all flanking transcription units, coexpressed genes, and the 3D distances between them and relevant factories. Moreover, we would expect coregulated genes to be so positioned in 2D sequence space on the chromosome that they might visit the same specialized factories, and they are.[363−366]

While the evidence for the existence of factories in mammalian cells, and the immobilization of active RNA polymerases within them, is convincing, it is nevertheless indirect. Therefore, we now need to visualize factories in living cells, and watch individual polymerases and promoters as they diffuse through the nucleus to come together with others in a factory to be transcribed. Fortunately, tagging with fluorescent proteins and "super-resolution" techniques now allow us to monitor such interactions in ever-sharper detail.[367] We also need to isolate the different kinds of factories and characterize their molecular contents. Unfortunately, biochemical techniques for analyzing such large and polymorphic structures have yet to be developed. Perhaps the greatest challenge of all is the development of ways to analyze and visualize a functional genome, a 3D network of transcription units (both coding and noncoding) tethered to ever-changing factories through transient attachments. We have argued it is this network that represents the "context" that underlies gene expression.

AUTHOR INFORMATION

Corresponding Author

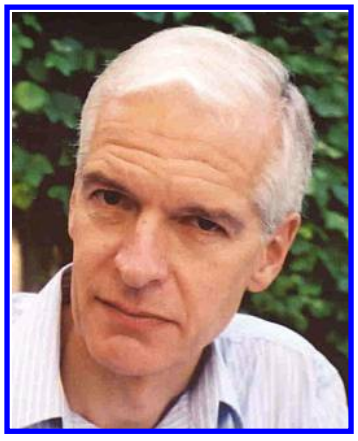
*Telephone: +44 1865 275528. Fax: +44 1865 275515. E-mail: peter.cook@path.ox.ac.uk.

Notes

The authors declare no competing financial interest.

Biographies

Argyris Papantonis received his Diploma (in 2002) and Ph.D. (in 2008, under the supervision of Prof. Rena Lecanidou) in Biology at the National and Kapodistrian University of Athens. Since 2008 he has been working with Prof. Peter Cook at the University of Oxford. In 2013 he was appointed a Junior Group Leader at the University of Cologne.



Peter R. Cook obtained a B.A. in Biochemistry at the University of Oxford, and a D.Phil. (in 1971) at the same university under the supervision of Prof. Henry Harris. Since then, he has worked in Oxford in the general field of nuclear structure and function.

ACKNOWLEDGMENTS

We thank the Biotechnology and Biological Sciences Research Council for financial support via the ERASysBio+/FP7 initiative, Ana Pombo for permission to reproduce the image in Figure 3A, and Jon Bartlett for his help over the years. A.P. holds a Junior Group Leader position at the University of Cologne; P.R.C. holds the E.P. Abraham Chair of Cell Biology and a Professorial Fellowship at Lincoln College, Oxford.

REFERENCES

(1) Chambon, P. *Annu. Rev. Biochem.* **1975**, *44*, 613.
(2) Roeder, R. G. *Nat. Med.* **2003**, *9*, 1239.
(3) Kornberg, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 12955.
(4) Cramer, P.; Armache, K. J.; Baumli, S.; Benkert, S.; Brueckner, E.; Buchen, C.; Damsma, G. E.; Dengl, S.; Geiger, S. R.; Jaslak, A. J.; Jawhari, A.; Jennebach, S.; Kamenski, T.; Kettenberger, H.; Kuhn, C. D.; Lehmann, E.; Leike, K.; Sydow, J. E.; Vannini, A. *Annu. Rev. Biophys.* **2008**, *37*, 337.
(5) Haag, J. R.; Pikaard, C. S. *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 483.
(6) Liere, K.; Weihe, A.; Borner, T. *J. Plant Physiol.* **2011**, *168*, 1345.
(7) Navarro, M.; Gull, K. *Nature* **2001**, *414*, 759.
(8) James Faresse, N.; Canella, D.; Praz, V.; Michaud, J.; Romascano, D.; Hernandez, N. *PLoS Genet.* **2012**, *8*, e1003028.
(9) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*, 4th ed.; Garland Science Taylor & Francis Group: New York, 2002.
(10) Cook, P. R. *J. Mol. Biol.* **2010**, *395*, 1.
(11) Chakalova, L.; Fraser, P. *Csh Perspect. Biol.* **2010**, 2.
(12) Cook, P. R., http://youtu.be/Naa-JwGU6PM.
(13) Wachsmuth, M.; Caudron-Herger, M.; Rippe, K. *Biochim. Biophys. Acta, Mol. Cell Res.* **2008**, *1783*, 2061.
(14) Papantonis, A.; Kohro, T.; Baboo, S.; Larkin, J. D.; Deng, B.; Short, P.; Tsutsumi, S.; Taylor, S.; Kanki, Y.; Kobayashi, M.; Li, G.; Poh, H.-M.; Ruan, X.; Aburatani, H.; Ruan, Y.; Kodama, T.; Wada, Y.; Cook, P. R. *EMBO J.* **2012**, *31*, 4404.
(15) Larkin, J. D.; Papantonis, A.; Cook, P. R.; Marenduzzo, D. *Nucleic Acids Res.* **2013**, *41*, 2216.
(16) Cairns, J. *Virology* **1960**, *11*, 603.
(17) Joklik, W. K. *Annu. Rev. Microbiol.* **1968**, *22*, 359.
(18) Hozak, P.; Hassan, A. B.; Jackson, D. A.; Cook, P. R. *Cell* **1993**, *73*, 361.
(19) Jackson, D. A.; Hassan, A. B.; Errington, R. J.; Cook, P. R. *EMBO J.* **1993**, *12*, 1059.
(20) Jackson, D. A.; Balajee, A. S.; Mullenders, L.; Cook, P. R. *J. Cell Sci.* **1994**, *107*, 1745.
(21) Cook, P. R. *Nat. Genet.* **2002**, *32*, 347.
(22) Cook, P. R. *Principles of Nuclear Structure and Function*; Wiley: New York, 2001.
(23) Bentley, D. *Curr. Opin. Cell Biol.* **2002**, *14*, 336.
(24) Perales, R.; Bentley, D. *Mol. Cell* **2009**, *36*, 178.
(25) Maniatis, T.; Reed, R. *Nature* **2002**, *416*, 499.
(26) Hagiwara, M.; Nojima, T. *J. Biochem.* **2007**, *142*, 11.
(27) de Laat, W.; Grosveld, F. *Chromosome Res.* **2003**, *11*, 447.
(28) Li, G. L.; Ruan, X. A.; Auerbach, R. K.; Sandhu, K. S.; Zheng, M. Z.; Wang, P.; Poh, H. M.; Goh, Y.; Lim, J.; Zhang, J. Y.; Sim, H. S.; Peh, S. Q.; Mulawadi, F. H.; Ong, C. T.; Orlov, Y. L.; Hong, S. Z.; Zhang, Z. Z.; Landt, S.; Raha, D.; Euskirchen, G.; Wei, C. L.; Ge, W. H.; Wang, H. E.; Davis, C.; Fisher-Aylor, K. I.; Mortazavi, A.; Gerstein, M.; Gingeras, T.; Wold, B.; Sun, Y.; Fullwood, M. J.; Cheung, E.; Liu, E.; Sung, W. K.; Snyder, M.; Ruan, Y. J. *Cell* **2012**, *148*, 84.
(29) Rogers, A. W. *Techniques of Autoradiography*, 3rd ed.; Elsevier: Amsterdam; New York, 1979.
(30) Wansink, D. G.; Schul, W.; Vanderkraan, I.; Vansteensel, B.; Vandriel, R.; Dejong, L. *J. Cell Biol.* **1993**, *122*, 283.
(31) Iborra, F. J.; Pombo, A.; McManus, J.; Jackson, D. A.; Cook, P. R. *Exp. Cell Res.* **1996**, *229*, 167.
(32) Jackson, D. A.; Iborra, F. J.; Manders, E. M. M.; Cook, P. R. *Mol. Biol. Cell* **1998**, *9*, 2698.
(33) Jao, C. Y.; Salic, A. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 15779.
(34) Hanna, M. M.; Yuriev, E.; Zhang, J.; Riggs, D. L. *Nucleic Acids Res.* **1999**, *27*, 1369.
(35) LaMorte, V. J.; Dyck, J. A.; Ochs, R. L.; Evans, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4991.
(36) Sinha, B.; Bhattacharya, D.; Sinha, D. K.; Talwar, S.; Maharana, S.; Gupta, S.; Shivashankar, G. V. *Methods Cell Biol.* **2010**, *98*, 57.
(37) Darzacq, X.; Shav-Tal, Y.; de Turris, V.; Brody, Y.; Shenoy, S. M.; Phair, R. D.; Singer, R. H. *Nat. Struct. Mol. Biol.* **2007**, *14*, 796.
(38) Wada, Y.; Ohta, Y.; Xu, M.; Tsutsumi, S.; Minami, T.; Inoue, K.; Komura, D.; Kitakami, J.; Oshida, N.; Papantonis, A.; Izumi, A.; Kobayashi, M.; Meguro, H.; Kanki, Y.; Mimura, I.; Yamamoto, K.; Mataki, C.; Hamakubo, T.; Shirahige, K.; Aburatani, H.; Kimura, H.; Kodama, T.; Cook, P. R.; Ihara, S. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 18357.
(39) Larson, D. R.; Zenklusen, D.; Wu, B.; Chao, J. A.; Singer, R. H. *Science* **2011**, *332*, 475.
(40) Iborra, F. J.; Pombo, A.; Jackson, D. A.; Cook, P. R. *J. Cell Sci.* **1996**, *109*, 1427.
(41) Iborra, F. J.; Kimura, H.; Cook, P. R. *BMC Biol.* **2004**, *2*, 9.
(42) Kukat, C.; Wurm, C. A.; Spahr, H.; Falkenberg, M.; Larsson, N. G.; Jakobs, S. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 13534.

(43) Cook, P. R. *Science* **1999**, *284*, 1790.
(44) Miller, O. L.; Bakken, A. H. *Acta Endocrinol.-Cop* **1972**, 155.
(45) Kimura, H.; Sugaya, K.; Cook, P. R. *J. Cell Biol.* **2002**, *159*, 777.
(46) Kimura, H.; Tao, Y.; Roeder, R. G.; Cook, P. R. *Mol. Cell. Biol.* **1999**, *19*, 5383.
(47) Meinhart, A.; Kamenski, T.; Hoeppner, S.; Baumli, S.; Cramer, P. *Gene Dev.* **2005**, *19*, 1401.
(48) Buratowski, S. *Mol. Cell* **2009**, *36*, 541.
(49) Heidemann, M.; Hintermair, C.; Voß, K.; Eick, D. *Biochim. Biophys. Acta* **2012**, *1829*, 55.
(50) Egloff, S.; Dienstbier, M.; Murphy, S. *Trends Genet.* **2012**, *28*, 333.
(51) Mayer, A.; Heidemann, M.; Lidschreiber, M.; Schreieck, A.; Sun, M.; Hintermair, C.; Kremmer, E.; Eick, D.; Cramer, P. *Science* **2012**, *336*, 1723.
(52) Markaki, Y.; Smeets, D.; Fiedler, S.; Schmid, V. J.; Schermelleh, L.; Cremer, T.; Cremer, M. *BioEssays* **2012**, *34*, 412.
(53) Lionnet, T.; Singer, R. H. *EMBO Rep.* **2012**, *13*, 313.
(54) Han, J. H.; Xiong, J.; Wang, D.; Fu, X. D. *Trends Cell Biol.* **2011**, *21*, 336.
(55) Singh, J.; Padgett, R. A. *Nat. Struct. Mol. Biol.* **2009**, *16*, 1128.
(56) Pawley, J. B. *Handbook of Biological Confocal Microscopy*, 2nd ed.; Plenum Press: New York, 1995.
(57) Eskiw, C. H.; Rapp, A.; Carter, D. R.; Cook, P. R. *J. Cell Sci.* **2008**, *121*, 1999.
(58) McNally, J. G.; Muller, W. G.; Walker, D.; Wolford, R.; Hager, G. L. *Science* **2000**, *287*, 1262.
(59) Muller, W. G.; Rieder, D.; Karpova, T. S.; John, S.; Trajanoski, Z.; McNally, J. G. *J. Cell Biol.* **2007**, *177*, 957.
(60) Dundr, M.; Hoffmann-Rohrer, U.; Hu, Q. Y.; Grummt, I.; Rothblum, L. I.; Phair, R. D.; Misteli, T. *Science* **2002**, *298*, 1623.
(61) Darzacq, X.; Yao, J.; Larson, D. R.; Causse, S. Z.; Bosanac, L.; de Turris, V.; Ruda, V. M.; Lionnet, T.; Zenklusen, D.; Guglielmi, B.; Tjian, R.; Singer, R. H. *Annu. Rev. Biophys.* **2009**, *38*, 173.
(62) Hieda, M.; Winstanley, H.; Maini, P.; Iborra, F. J.; Cook, P. R. *Chromosome Res.* **2005**, *13*, 135.
(63) Fromaget, M.; Cook, P. R. *Exp. Cell Res.* **2007**, *313*, 3026.
(64) Sugaya, K.; Vigneron, M.; Cook, P. R. *J. Cell Sci.* **2000**, *113*, 2679.
(65) Bertrand, E.; Chartrand, P.; Schaefer, M.; Shenoy, S. M.; Singer, R. H.; Long, R. M. *Mol. Cell* **1998**, *2*, 437.
(66) Zenklusen, D.; Wells, A. L.; Condeelis, J. S.; Singer, R. H. *CSH Protoc.* **2007**, *2007*, pdb.prot4870.
(67) Dorier, J.; Stasiak, A. *Nucleic Acids Res.* **2010**, *38*, 7410.
(68) Eskiw, C. H.; Cope, N. F.; Clay, I.; Schoenfelder, S.; Nagano, T.; Fraser, P. *Cold Spring Harbor Symp.* **2010**, *75*, 501.
(69) Razin, S. V.; Gavrilov, A. A.; Pichugin, A.; Lipinski, M.; Iarovaia, O. V.; Vassetzky, Y. S. *Nucleic Acids Res.* **2011**, *39*, 9085.
(70) Deng, B.; Melnik, S.; Cook, P. R. *Semin. Cancer Biol.* **2012**, *23*, 65.
(71) Edelman, L. B.; Fraser, P. *Curr. Opin. Genet. Dev.* **2012**, *22*, 110.
(72) Van Bortle, K.; Corces, V. G. *Annu. Rev. Cell Dev. Biol.* **2012**.
(73) Papantonis, A.; Cook, P. R. *Curr. Opin. Cell Biol.* **2010**, *22*, 271.
(74) Cook, P. R. *J. Cell. Biochem.* **1995**, 116.
(75) Jackson, D. A.; Cook, P. R. *Int. Rev. Cytol.* **1995**, *162a*, 125.
(76) Ethier, S. D.; Miura, H.; Dostie, J. *Biochim. Biophys. Acta* **2012**, *1819*, 401.
(77) Rieder, D.; Trajanoski, Z.; McNally, J. G. *Front. Genet.* **2012**, *3*, 221.
(78) Pirrotta, V.; Li, H. B. *Curr. Opin. Genet. Dev.* **2012**, *22*, 101.
(79) Egecioglu, D.; Brickner, J. H. *Curr. Opin. Cell Biol.* **2011**, *23*, 338.
(80) Meldi, L.; Brickner, J. H. *Trends Cell Biol.* **2011**, *21*, 701.
(81) Lucas, J. S.; Bossen, C.; Murre, C. *Curr. Opin. Cell Biol.* **2011**, *23*, 318.
(82) Rajapakse, I.; Groudine, M. *J. Cell Biol.* **2011**, *192*, 711.
(83) Hubner, M. R.; Spector, D. L. *Annu. Rev. Biophys.* **2010**, *39*, 471.
(84) Cope, N. F.; Fraser, P.; Eskiw, C. H. *Genome Biol.* **2010**, *11*, 204.
(85) Schoenfelder, S.; Clay, I.; Fraser, P. *Curr. Opin. Genet. Dev.* **2010**, *20*, 127.
(86) Jackson, D. A. *Essays Biochem.* **2010**, *48*, 25.
(87) Cremer, T.; Cremer, M. *Cold Spring Harbor Perspect. Biol.* **2010**, *2*, a003889.
(88) Malyavantham, K. S.; Bhattacharya, S.; Berezney, R. *Adv. Enzyme Regul.* **2010**, *50*, 126.
(89) van Steensel, B.; Dekker, J. *Nat. Biotechnol.* **2010**, *28*, 1089.
(90) Ferrai, C.; de Castro, I. J.; Lavitas, L.; Chotalia, M.; Pombo, A. *Cold Spring Harbor Perspect. Biol.* **2010**, *2*, a000588.
(91) Gondor, A.; Ohlsson, R. *Nature* **2009**, *461*, 212.
(92) Osborne, C. S.; Eskiw, C. H. *J. Cell. Biochem.* **2008**, *104*, 1553.
(93) Carter, D. R. F.; Eskiw, C.; Cook, P. R. *Biochem. Soc. Trans.* **2008**, *36*, 585.
(94) Bartlett, J.; Blagojevic, J.; Carter, D.; Eskiw, C.; Fromaget, M.; Job, C.; Shamsher, M.; Trindade, I. F.; Xu, M.; Cook, P. R. *Biochem. Soc. Symp.* **2006**, 67.
(95) Iborra, F. J.; Cook, P. R. *Curr. Opin. Cell Biol.* **2002**, *14*, 780.
(96) Babu, M. M.; Janga, S. C.; de Santiago, I.; Pombo, A. *Curr. Opin. Genet. Dev.* **2008**, *18*, 571.
(97) Fedorova, E.; Zink, D. *Biochim. Biophys. Acta* **2008**, *1783*, 2174.
(98) Rippe, K. *Curr. Opin. Genet. Dev.* **2007**, *17*, 373.
(99) Higgs, D. R.; Vernimmen, D.; Hughes, J.; Gibbons, R. *Annu. Rev. Genomics Hum. Genet.* **2007**, *8*, 299.
(100) Sexton, T.; Schober, H.; Fraser, P.; Gasser, S. M. *Nat. Struct. Mol. Biol.* **2007**, *14*, 1049.
(101) Sexton, T.; Umlauf, D.; Kurukuti, S.; Fraser, P. *Semin. Cell Dev. Biol.* **2007**, *18*, 691.
(102) de Laat, W. *Curr. Opin. Cell Biol.* **2007**, *19*, 317.
(103) Misteli, T. *Cell* **2007**, *128*, 787.
(104) Pombo, A.; Branco, M. R. *Curr. Opin. Genet. Dev.* **2007**, *17*, 451.
(105) Fraser, P.; Bickmore, W. *Nature* **2007**, *447*, 413.
(106) Sutherland, H.; Bickmore, W. A. *Nat. Rev. Genet.* **2009**, *10*, 457.
(107) Dillon, N. *Chromosome Res.* **2006**, *14*, 117.
(108) Lanctot, C.; Cheutin, T.; Cremer, M.; Cavalli, G.; Cremer, T. *Nat. Rev. Genet.* **2007**, *8*, 104.
(109) Chakalova, L.; Debrand, E.; Mitchell, J. A.; Osborne, C. S.; Fraser, P. *Nat. Rev. Genet.* **2005**, *6*, 669.
(110) Chuang, C. H.; Belmont, A. S. *Genome Biol.* **2005**, *6*, 237.
(111) Spector, D. L. *Annu. Rev. Biochem.* **2003**, *72*, 573.
(112) Martin, S.; Pombo, A. *Chromosome Res.* **2003**, *11*, 461.
(113) Brown, K. *Crit. Rev. Eukaryotic Gene Expression* **1999**, *9*, 203.
(114) Pombo, A.; Jones, E.; Iborra, F. J.; Kimura, H.; Sugaya, K.; Cook, P. R.; Jackson, D. A. *Crit. Rev. Eukaryotic Gene Expression* **2000**, *10*, 21.
(115) Cook, P. R. *J. Cell Sci.* **1988**, *90*, 1.
(116) Jackson, D. A.; Dickinson, P.; Cook, P. R. *Nucleic Acids Res.* **1990**, *18*, 4385.
(117) Pettijohn, D. E. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*; Neidhardt, F. C., Ingraham, J. L., Lin, E. C. C., Brooks Low, K., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., Umbarger, H. E., Eds.; American Society for Microbiology: Washington, DC, 1996.
(118) Deng, S.; Stein, R. A.; Higgins, N. P. *Mol. Microbiol.* **2005**, *57*, 1511.
(119) Jin, D. J.; Cabrera, J. E. *J. Struct. Biol.* **2006**, *156*, 284.
(120) Wang, W.; Li, G. W.; Chen, C.; Xie, X. S.; Zhuang, X. *Science* **2011**, *333*, 1445.
(121) Kornberg, A. *J. Cell Sci.* **1987**, 1.
(122) Jacob, F.; Cuzin, F.; Brenner, S. *Cold Spring Harbor Symp.* **1963**, *28*, 329.
(123) Dingman, C. W. *J. Theor. Biol.* **1974**, *43*, 187.
(124) Berezney, R.; Coffey, D. S. *Science* **1975**, *189*, 291.
(125) Nickerson, J. A. *J. Cell Sci.* **2001**, *114*, 463.
(126) McCready, S. J.; Cook, P. R. *J. Cell Sci.* **1984**, *70*, 189.
(127) Pederson, T. *Mol. Biol. Cell* **2000**, *11*, 799.
(128) Nakamura, H.; Morita, T.; Sato, C. *Exp. Cell Res.* **1986**, *165*, 291.

(129) Jackson, D. A.; Cook, P. R. *EMBO J.* **1986**, *5*, 1403.
(130) Tubo, R. A.; Berezney, R. *J. Biol. Chem.* **1987**, *262*, 5857.
(131) Leonhardt, H.; Rahn, H. P.; Weinzierl, P.; Sporbert, A.; Cremer, T.; Zink, D.; Cardoso, M. C. *J. Cell Biol.* **2000**, *149*, 271.
(132) Natsume, T.; Tanaka, T. U. *Chromosome Res.* **2010**, *18*, 7.
(133) Miller, O. L.; Hamkalo, B. A.; Thomas, C. A. *Science* **1970**, *169*, 392.
(134) Morgan, G. T. *Chromosome Res.* **2002**, *10*, 177.
(135) Anderson, D. M.; Smith, L. D. *Dev. Biol.* **1978**, *67*, 274.
(136) Gall, J. G.; Murphy, C. *Mol. Biol. Cell* **1998**, *9*, 733.
(137) Snow, M. H.; Callan, H. G. *J. Cell Sci.* **1969**, *5*, 1.
(138) Mott, M. R.; Callen, H. G. *J. Cell Sci.* **1975**, *17*, 241.
(139) Jackson, D. A.; McCready, S. J.; Cook, P. R. *Nature* **1981**, *292*, 552.
(140) Juven-Gershon, T.; Cheng, S.; Kadonaga, J. T. *Nat. Methods* **2006**, *3*, 917.
(141) Dignam, J. D.; Lebovitz, R. M.; Roeder, R. G. *Nucleic Acids Res.* **1983**, *11*, 1475.
(142) Hawley, D. K.; Roeder, R. G. *J. Biol. Chem.* **1987**, *262*, 3452.
(143) Revyakin, A.; Zhang, Z. J.; Coleman, R. A.; Li, Y.; Inouye, C.; Lucas, J. K.; Park, S. R.; Chu, S.; Tjian, R. *Gene Dev.* **2012**, *26*, 1691.
(144) Li, G. H.; Margueron, R.; Hu, G. B.; Stokes, D.; Wang, Y. H.; Reinberg, D. *Mol. Cell* **2010**, *38*, 41.
(145) Culotta, V. C.; Wides, R. J.; Sollner-Webb, B. *Mol. Cell. Biol.* **1985**, *5*, 1582.
(146) Cook, P. R.; Brazell, I. A. *J. Cell Sci.* **1975**, *19*, 261.
(147) McCready, S. J.; Akrigg, A.; Cook, P. R. *J. Cell Sci.* **1979**, *39*, 53.
(148) Benyajati, C.; Worcel, A. *Cell* **1976**, *9*, 393.
(149) Cook, P. R.; Brazell, I. A. *J. Cell Sci.* **1976**, *22*, 287.
(150) Pinon, R.; Salts, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 2850.
(151) Cook, P. R.; Lang, J.; Hayday, A.; Lania, L.; Fried, M.; Chiswell, D. J.; Wyke, J. A. *EMBO J.* **1982**, *1*, 447.
(152) Jackson, D. A.; Caton, A. J.; McCready, S. J.; Cook, P. R. *Nature* **1982**, *296*, 366.
(153) Jackson, D. A.; Cook, P. R. *EMBO J.* **1985**, *4*, 913.
(154) Jackson, D. A.; Yuan, J.; Cook, P. R. *J. Cell Sci.* **1988**, *90*, 365.
(155) Jackson, D. A.; Cook, P. R. *EMBO J.* **1985**, *4*, 919.
(156) Dickinson, P.; Cook, P. R.; Jackson, D. A. *EMBO J.* **1990**, *9*, 2207.
(157) Jackson, D. A.; Cook, P. R. *J. Cell Sci.* **1993**, *105*, 1143.
(158) Jackson, D. A.; Bartlett, J.; Cook, P. R. *Nucleic Acids Res.* **1996**, *24*, 1212.
(159) Jackson, D. A.; Dickinson, P.; Cook, P. R. *EMBO J.* **1990**, *9*, 567.
(160) Cheung, A. C. M.; Cramer, P. *Cell* **2012**, *149*, 1431.
(161) Liu, L. F.; Wang, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7024.
(162) Stewart, A. F.; Herrera, R. E.; Nordheim, A. *Cell* **1990**, *60*, 141.
(163) Cook, P. R.; Gove, F. *Nucleic Acids Res.* **1992**, *20*, 3591.
(164) Schafer, D. A.; Gelles, J.; Sheetz, M. P.; Landick, R. *Nature* **1991**, *352*, 444.
(165) Herbert, K. M.; Greenleaf, W. J.; Block, S. M. *Annu. Rev. Biochem.* **2008**, *77*, 149.
(166) Melnik, S.; Deng, B. W.; Papantonis, A.; Baboo, S.; Carr, I. M.; Cook, P. R. *Nat. Methods* **2011**, *8*, 963.
(167) Boisvert, F. M.; van Koningsbruggen, S.; Navascues, J.; Lamond, A. I. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 574.
(168) McKeown, P. C.; Shaw, P. J. *Chromosoma* **2009**, *118*, 11.
(169) Nemeth, A.; Conesa, A.; Santoyo-Lopez, J.; Medina, I.; Montaner, D.; Peterfia, B.; Solovei, I.; Cremer, T.; Dopazo, J.; Langst, G. *PLoS Genet.* **2010**, *6*, e1000889.
(170) Dousset, T.; Wang, C.; Verheggen, C.; Chen, D. Y.; Hernandez-Verdun, D.; Huang, S. *Mol. Biol. Cell* **2000**, *11*, 2705.
(171) Roussel, P.; Andre, C.; Comai, L.; HernandezVerdun, D. *J. Cell Biol.* **1996**, *133*, 235.
(172) Leung, A. K. L.; Gerlich, D.; Miller, G.; Lyon, C.; Lam, Y. W.; Lleres, D.; Daigle, N.; Zomerdijk, J.; Ellenberg, J.; Lamond, A. I. *J. Cell Biol.* **2004**, *166*, 787.
(173) Dinman, J. D. *J. Biol. Chem.* **2009**, *284*, 11761.
(174) Hozak, P.; Cook, P. R.; Schofer, C.; Mosgoller, W.; Wachtler, F. *J. Cell Sci.* **1994**, *107*, 639.
(175) Jordan, E. G.; Mcgovern, J. H. *J. Cell Sci.* **1981**, *52*, 373.
(176) Haaf, T.; Hayman, D. L.; Schmid, M. *Exp. Cell Res.* **1991**, *193*, 78.
(177) Faro-Trindade, I.; Cook, P. R. *Biochem. Soc. Trans.* **2006**, *34*, 1133.
(178) Pombo, A.; Hollinshead, M.; Cook, P. R. *J. Histochem. Cytochem.* **1999**, *47*, 471.
(179) Faro-Trindade, I.; Cook, P. R. *Mol. Biol. Cell* **2006**, *17*, 2910.
(180) Osborne, C. S.; Chakalova, L.; Brown, K. E.; Carter, D.; Horton, A.; Debrand, E.; Goyenechea, B.; Mitchell, J. A.; Lopes, S.; Reik, W.; Fraser, P. *Nat. Genet.* **2004**, *36*, 1065.
(181) Eskiw, C. H.; Fraser, P. *J. Cell Sci.* **2011**, *124*, 3676.
(182) Pombo, A.; Jackson, D. A.; Hollinshead, M.; Wang, Z. X.; Roeder, R. G.; Cook, P. R. *EMBO J.* **1999**, *18*, 2241.
(183) Jackson, D. A.; Pombo, A.; Iborra, F. *FASEB J.* **2000**, *14*, 242.
(184) Finan, K.; Cook, P. R. *Genome Organization and Function in the Cell Nucleus*; Wiley-VCH Verlag GmbH & Co. KGaA: New York, 2011; p 235.
(185) Miller, C.; Schwalb, B.; Maier, K.; Schulz, D.; Dumcke, S.; Zacher, B.; Mayer, A.; Sydow, J.; Marcinowski, L.; Dolken, L.; Martin, D. E.; Tresch, A.; Cramer, P. *Mol. Syst. Biol.* **2011**, *7*, 458.
(186) Schwanhausser, B.; Busse, D.; Li, N.; Dittmar, G.; Schuchhardt, J.; Wolf, J.; Chen, W.; Selbach, M. *Nature* **2011**, *473*, 337.
(187) Bon, M.; McGowan, S. J.; Cook, P. R. *FASEB J.* **2006**, *20*, 1721.
(188) Pelechano, V.; Chavez, S.; Perez-Ortin, J. E. *PLoS One* **2010**, *5*, e15442.
(189) Bartholomew, B.; Durkovich, D.; Kassavetis, G. A.; Geiduschek, E. P. *Mol. Cell. Biol.* **1993**, *13*, 942.
(190) Papantonis, A.; Larkin, J. D.; Wada, Y.; Ohta, Y.; Ihara, S.; Kodama, T.; Cook, P. R. *PLoS Biol.* **2010**, *8*, e1000419.
(191) Larkin, J. D.; Cook, P. R.; Papantonis, A. *Mol. Cell. Biol.* **2012**, *32*, 2738.
(192) Moore, M. J.; Proudfoot, N. J. *Cell* **2009**, *136*, 688.
(193) Djebali, S.; Davis, C. A.; Merkel, A.; Dobin, A.; Lassmann, T.; Mortazavi, A.; Tanzer, A.; Lagarde, J.; Lin, W.; Schlesinger, F.; Xue, C.; Marinov, G. K.; Khatun, J.; Williams, B. A.; Zaleski, C.; Rozowsky, J.; Roder, M.; Kokocinski, F.; Abdelhamid, R. F.; Alioto, T.; Antoshechkin, I.; Baer, M. T.; Bar, N. S.; Batut, P.; Bell, K.; Bell, I.; Chakrabortty, S.; Chen, X.; Chrast, J.; Curado, J.; Derrien, T.; Drenkow, J.; Dumais, E.; Dumais, J.; Duttagupta, R.; Falconnet, E.; Fastuca, M.; Fejes-Toth, K.; Ferreira, P.; Foissac, S.; Fullwood, M. J.; Gao, H.; Gonzalez, D.; Gordon, A.; Gunawardena, H.; Howald, C.; Jha, S.; Johnson, R.; Kapranov, P.; King, B.; Kingswood, C.; Luo, O. J.; Park, E.; Persaud, K.; Preall, J. B.; Ribeca, P.; Risk, B.; Robyr, D.; Sammeth, M.; Schaffer, L.; See, L. H.; Shahab, A.; Skancke, J.; Suzuki, A. M.; Takahashi, H.; Tilgner, H.; Trout, D.; Walters, N.; Wang, H.; Wrobel, J.; Yu, Y.; Ruan, X.; Hayashizaki, Y.; Harrow, J.; Gerstein, M.; Hubbard, T.; Reymond, A.; Antonarakis, S. E.; Hannon, G.; Giddings, M. C.; Ruan, Y.; Wold, B.; Carninci, P.; Guigo, R.; Gingeras, T. R. *Nature* **2012**, *489*, 101.
(194) Maquat, L. E. *J. Cell Sci.* **2005**, *118*, 3213.
(195) Iborra, F. J.; Jackson, D. A.; Cook, P. R. *Science* **2001**, *293*, 1139.
(196) Brogna, S.; Sato, T. A.; Rosbash, M. *Mol. Cell* **2002**, *10*, 93.
(197) Dahlberg, J. E.; Lund, E.; Goodwin, E. B. *RNA* **2003**, *9*, 1.
(198) Nathanson, L.; Xia, T. L.; Deutscher, M. P. *RNA* **2003**, *9*, 9.
(199) Dahlberg, J. E.; Lund, E. *Curr. Opin. Cell Biol.* **2004**, *16*, 335.
(200) Iborra, F. J.; Escargueil, A. E.; Kwek, K. Y.; Akoulitchev, A.; Cook, P. R. *J. Cell Sci.* **2004**, *117*, 899.
(201) Schroder, P. A.; Moore, M. J. *RNA* **2005**, *11*, 1521.
(202) Dolan, B. P.; Knowlton, J. J.; David, A.; Bennink, J. R.; Yewdell, J. W. *J. Immunol.* **2010**, *185*, 6728.
(203) David, A.; Dolan, B. P.; Hickman, H. D.; Knowlton, J. J.; Clavarino, G.; Pierre, P.; Bennink, J. R.; Yewdell, J. W. *J. Cell Biol.* **2012**, *197*, 45.

(204) Andrulis, E. D.; Werner, J.; Nazarian, A.; Erdjument-Bromage, H.; Tempst, P.; Lis, J. T. *Nature* **2002**, *420*, 837.
(205) Libri, D.; Dower, K.; Boulay, J.; Thomsen, R.; Rosbash, M.; Jensen, T. H. *Mol. Cell. Biol.* **2002**, *22*, 8254.
(206) Lykke-Andersen, J. *Mol. Cell. Biol.* **2002**, *22*, 8114.
(207) Verma, R.; Chen, S.; Feldman, R.; Schieltz, D.; Yates, J.; Dohmen, T.; Deshaies, R. J. *Mol. Biol. Cell* **2000**, *11*, 3425.
(208) Ferdous, A.; Kodadek, T.; Johnston, S. A. *Biochemistry* **2002**, *41*, 12798.
(209) Gillette, T. G.; Gonzalez, F.; Delahodde, A.; Johnston, S. A.; Kodadek, T. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5904.
(210) Djebali, S.; Lagarde, J.; Kapranov, P.; Lacroix, V.; Borel, C.; Mudge, J. M.; Howald, C.; Foissac, S.; Ucla, C.; Chrast, J.; Ribeca, P.; Martin, D.; Murray, R. R.; Yang, X.; Ghamsari, L.; Lin, C.; Bell, I.; Dumais, E.; Drenkow, J.; Tress, M. L.; Gelpi, J. L.; Orozco, M.; Valencia, A.; van Berkum, N. L.; Lajoie, B. R.; Vidal, M.; Stamatoyannopoulos, J.; Batut, P.; Dobin, A.; Harrow, J.; Hubbard, T.; Dekker, J.; Frankish, A.; Salehi-Ashtiani, K.; Reymond, A.; Antonarakis, S. E.; Guigo, R.; Gingeras, T. R. *PLoS One* **2012**, *7*, e28213.
(211) Gingeras, T. R. *Nature* **2009**, *461*, 206.
(212) Li, S.; Heermann, D. W. *DNA Res.* **2012**, *20*, 45.
(213) Iborra, F. J.; Jackson, D. A.; Cook, P. R. *J. Cell Sci.* **1998**, *111*, 2269.
(214) Tsukada, J.; Yoshida, Y.; Kominato, Y.; Auron, P. E. *Cytokine* **2011**, *54*, 6.
(215) Rippe, K. *Trends Biochem. Sci.* **2001**, *26*, 733.
(216) Asakura, S.; Oosawa, F. *J. Polym. Sci.* **1958**, *33*, 183.
(217) Marenduzzo, D.; Finan, K.; Cook, P. R. *J. Cell Biol.* **2006**, *175*, 681.
(218) Toan, N. M.; Marenduzzo, D.; Cook, P. R.; Micheletti, C. *Phys. Rev. Lett.* **2006**, *97*, 178302.
(219) Marenduzzo, D.; Micheletti, C.; Cook, P. R. *Biophys. J.* **2006**, *90*, 3712.
(220) Margaritis, T.; Holstege, F. C. P. *Cell* **2008**, *133*, 581.
(221) Nechaev, S.; Fargo, D. C.; dos Santos, G.; Liu, L.; Gao, Y.; Adelman, K. *Science* **2010**, *327*, 335.
(222) St-Jean, P.; Vaillant, C.; Audit, B.; Arneodo, A. *Phys. Rev. E* **2008**, *77*, 061923.
(223) Junier, I.; Martin, O.; Kepes, F. *PLoS Comput. Biol.* **2010**, *6*, e1000678.
(224) Marenduzzo, D.; Orlandini, E. *J. Stat. Mech.: Theory Exp.* **2009**, L09002.
(225) Canals-Hamann, A. Z.; das Neves, R. P.; Reittie, J. E.; Iniguez, C.; Soneji, S.; Enver, T.; Buckle, V. J.; Iborra, F. J. *BMC Biophys.* **2013**, *6*, 2.
(226) Dinant, C.; Luijsterburg, M. S.; Hofer, T.; von Bornstaedt, G.; Vermeulen, W.; Houtsmuller, A. B.; van Driel, R. *J. Cell Biol.* **2009**, *185*, 21.
(227) Hopfield, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4135.
(228) Murugan, A.; Huse, D. A.; Leibler, S. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 12034.
(229) Sinden, R. R.; Carlson, J. O.; Pettijohn, D. E. *Cell* **1980**, *21*, 773.
(230) Matsumoto, K.; Hirose, S. *J. Cell Sci.* **2004**, *117*, 3797.
(231) Igo-Kemenes, T.; Zachau, H. G. *Cold Spring Harbor Symp.* **1978**, *42*, 109.
(232) Ohlsson, R.; Bartkuhn, M.; Renkawitz, R. *Chromosoma* **2010**, *119*, 351.
(233) Jhunjhunwala, S.; van Zelm, M. C.; Peak, M. M.; Cutchin, S.; Riblet, R.; van Dongen, J. J. M.; Grosveld, F. G.; Knoch, T. A.; Murre, C. *Cell* **2008**, *133*, 265.
(234) Mateos-Langerak, J.; Bohn, M.; de Leeuw, W.; Giromus, O.; Manders, E. M. M.; Verschure, P. J.; Indemans, M. H. G.; Gierman, H. J.; Heermann, D. W.; Van Driel, R.; Goetze, S. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 3812.
(235) Dekker, J.; Rippe, K.; Dekker, M.; Kleckner, N. *Science* **2002**, *295*, 1306.
(236) Spilianakis, C. G.; Lalioti, M. D.; Town, T.; Lee, G. R.; Flavell, R. A. *Nature* **2005**, *435*, 637.
(237) Lomvardas, S.; Barnea, G.; Pisapia, D. J.; Mendelsohn, M.; Kirkland, J.; Axel, R. *Cell* **2006**, *126*, 403.
(238) Carter, D.; Chakalova, L.; Osborne, C. S.; Dai, Y. F.; Fraser, P. *Nat. Genet.* **2002**, *32*, 623.
(239) de Wit, E.; de Laat, W. *Gene Dev.* **2012**, *26*, 11.
(240) Dostie, J.; Richmond, T. A.; Arnaout, R. A.; Selzer, R. R.; Lee, W. L.; Honan, T. A.; Rubio, E. D.; Krumm, A.; Lamb, J.; Nusbaum, C.; Green, R. D.; Dekker, J. *Genome Res.* **2006**, *16*, 1299.
(241) Rodley, C. D.; Bertels, F.; Jones, B.; O'Sullivan, J. M. *Fungal. Genet. Biol.* **2009**, *46*, 879.
(242) Lieberman-Aiden, E.; van Berkum, N. L.; Williams, L.; Imakaev, M.; Ragoczy, T.; Telling, A.; Amit, I.; Lajoie, B. R.; Sabo, P. J.; Dorschner, M. O.; Sandstrom, R.; Bernstein, B.; Bender, M. A.; Groudine, M.; Gnirke, A.; Stamatoyannopoulos, J.; Mirny, L. A.; Lander, E. S.; Dekker, J. *Science* **2009**, *326*, 289.
(243) Fullwood, M. J.; Liu, M. H.; Pan, Y. F.; Liu, J.; Xu, H.; Bin Mohamed, Y.; Orlov, Y. L.; Velkov, S.; Ho, A.; Mei, P. H.; Chew, E. G. Y.; Huang, P. Y. H.; Welboren, W. J.; Han, Y. Y.; Ooi, H. S.; Ariyaratne, P. N.; Vega, V. B.; Luo, Y. Q.; Tan, P. Y.; Choy, P. Y.; Wansa, K. D. S. A.; Zhao, B.; Lim, K. S.; Leow, S. C.; Yow, J. S.; Joseph, R.; Li, H. X.; Desai, K. V.; Thomsen, J. S.; Lee, Y. K.; Karuturi, R. K. M.; Herve, T.; Bourque, G.; Stunnenberg, H. G.; Ruan, X. L.; Cacheux-Rataboul, V.; Sung, W. K.; Liu, E. T.; Wei, C. L.; Cheung, E.; Ruan, Y. J. *Nature* **2009**, *462*, 58.
(244) Dong, X.; Li, C.; Chen, Y. Q.; Ding, G. H.; Li, Y. *BMC Genomics* **2010**, *11*, 704.
(245) Yaffe, E.; Tanay, A. *Nat. Genet.* **2011**, *43*, 1059.
(246) Dixon, J. R.; Selvaraj, S.; Yue, F.; Kim, A.; Li, Y.; Shen, Y.; Hu, M.; Liu, J. S.; Ren, B. *Nature* **2012**, *485*, 376.
(247) Ben-Elazar, S.; Yakhini, Z.; Yanai, I. *Nucleic Acids Res.* **2013**, *41*, 2191.
(248) Sandhu, K. S.; Li, G.; Poh, H. M.; Quek, Y. L.; Sia, Y. Y.; Peh, S. Q.; Mulawadi, F. H.; Lim, J.; Sikic, M.; Menghi, F.; Thalamuthu, A.; Sung, W. K.; Ruan, X.; Fullwood, M. J.; Liu, E.; Csermely, P.; Ruan, Y. *Cell Rep.* **2012**, *2*, 1207.
(249) Ho, Y.; Shewchuk, B. M.; Liebhaber, S. A.; Cooke, N. E. *Mol. Cell. Biol.* **2013**, *33*, 1723.
(250) Simonis, M.; Klous, P.; Splinter, E.; Moshkin, Y.; Willemsen, R.; de Wit, E.; van Steensel, B.; de Laat, W. *Nat. Genet.* **2006**, *38*, 1348.
(251) Zhou, G. L.; Xin, L.; Song, W.; Di, L. J.; Liu, G.; Wu, X. S.; Liu, D. P.; Liang, C. C. *Mol. Cell. Biol.* **2006**, *26*, 5096.
(252) Schoenfelder, S.; Sexton, T.; Chakalova, L.; Cope, N. F.; Horton, A.; Andrews, S.; Kurukuti, S.; Mitchell, J. A.; Umlauf, D.; Dimitrova, D. S.; Eskiw, C. H.; Luo, Y. Q.; Wei, C. L.; Ruan, Y. J.; Bieker, J. J.; Fraser, P. *Nat. Genet.* **2010**, *42*, 53.
(253) Noordermeer, D.; de Wit, E.; Klous, P.; van de Werken, H.; Simonis, M.; Lopez-Jones, M.; Eussen, B.; de Klein, A.; Singer, R. H.; de Laat, W. *Nat. Cell Biol.* **2011**, *13*, 944.
(254) Mitchell, J. A.; Clay, I.; Umlauf, D.; Chen, C. Y.; Moir, C. A.; Eskiw, C. H.; Schoenfelder, S.; Chakalova, L.; Nagano, T.; Fraser, P. *PLoS One* **2012**, *7*, e49274.
(255) Osborne, C. S.; Chakalova, L.; Mitchell, J. A.; Horton, A.; Wood, A. L.; Bolland, D. J.; Corcoran, A. E.; Fraser, P. *PLoS Biol.* **2007**, *5*, e192.
(256) Verma-Gaur, J.; Torkamani, A.; Schaffer, L.; Head, S. R.; Schork, N. J.; Feeney, A. J. *Proc. Natl. Acad. Sc.i U.S.A.* **2012**, *109*, 17004.
(257) Wurtele, H.; Chartrand, P. *Chromosome Res.* **2006**, *14*, 477.
(258) Noordermeer, D.; Leleu, M.; Splinter, E.; Rougemont, J.; De Laat, W.; Duboule, D. *Science* **2011**, *334*, 222.
(259) Montavon, T.; Soshnikova, N.; Mascrez, B.; Joye, E.; Thevenet, L.; Splinter, E.; de Laat, W.; Spitz, F.; Duboule, D. *Cell* **2011**, *147*, 1132.
(260) Rodriguez-Jato, S.; Shan, J.; Khadake, J.; Heggestad, A. D.; Ma, X.; Johnstone, K. A.; Resnick, J. L.; Yang, T. P. *PLoS One* **2013**, *8*, e52390.
(261) Mitchell, J. A.; Fraser, P. *Gene Dev.* **2008**, *22*, 20.

(262) Palstra, R. J.; Simonis, M.; Klous, P.; Brasset, E.; Eijkelkamp, B.; de Laat, W. *PLoS One* **2008**, *3*, e1661.
(263) Nora, E. P.; Lajoie, B. R.; Schulz, E. G.; Giorgetti, L.; Okamoto, I.; Servant, N.; Piolot, T.; van Berkum, N. L.; Meisig, J.; Sedat, J.; Gribnau, J.; Barillot, E.; Bluthgen, N.; Dekker, J.; Heard, E. *Nature* **2012**, *485*, 381.
(264) Sanyal, A.; Lajoie, B. R.; Jain, G.; Dekker, J. *Nature* **2012**, *489*, 109.
(265) Kalhor, R.; Tjong, H.; Jayathilaka, N.; Alber, F.; Chen, L. *Nat. Biotechnol.* **2012**, *30*, 90.
(266) Tolhuis, B.; Blom, M.; Kerkhoven, R. M.; Pagie, L.; Teunissen, H.; Nieuwland, M.; Simonis, M.; de Laat, W.; van Lohuizen, M.; van Steensel, B. *PLoS Genet.* **2011**, *7*, e1001343.
(267) Sexton, T.; Yaffe, E.; Kenigsberg, E.; Bantignies, F.; Leblanc, B.; Hoichman, M.; Parrinello, H.; Tanay, A.; Cavalli, G. *Cell* **2012**, *148*, 458.
(268) Moorman, C.; Sun, L. V.; Wang, J.; de Wit, E.; Talhout, W.; Ward, L. D.; Greil, F.; Lu, X. J.; White, K. P.; Bussemaker, H. J.; van Steensel, B. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12027.
(269) Tanizawa, H.; Iwasaki, O.; Tanaka, A.; Capizzi, J. R.; Wickramasinghe, P.; Lee, M.; Fu, Z. Y.; Noma, K. *Nucleic Acids Res.* **2010**, *38*, 8164.
(270) Duan, Z.; Andronescu, M.; Schutz, K.; McIlwain, S.; Kim, Y. J.; Lee, C.; Shendure, J.; Fields, S.; Blau, C. A.; Noble, W. S. *Nature* **2010**, *465*, 363.
(271) Tokuda, N.; Terada, T. P.; Sasai, M. *Biophys. J.* **2012**, *102*, 296.
(272) Ohta, Y.; Nishiyama, A.; Wada, Y.; Ruan, Y.; Kodama, T.; Tsuboi, T.; Tokihiro, T.; Ihara, S. *Phys. Rev. E* **2012**, *86*, 021918.
(273) Kwon, S. H.; Workman, J. L. *BioEssays* **2011**, *33*, 280.
(274) Lobanenkov, V. V.; Nicolas, R. H.; Adler, V. V.; Paterson, H.; Klenova, E. M.; Polotskaja, A. V.; Goodwin, G. H. *Oncogene* **1990**, *5*, 1743.
(275) Handoko, L.; Xu, H.; Li, G.; Ngan, C. Y.; Chew, E.; Schnapp, M.; Lee, C. W.; Ye, C.; Ping, J. L.; Mulawadi, F.; Wong, E.; Sheng, J.; Zhang, Y.; Poh, T.; Chan, C. S.; Kunarso, G.; Shahab, A.; Bourque, G.; Cacheux-Rataboul, V.; Sung, W. K.; Ruan, Y.; Wei, C. L. *Nat. Genet.* **2011**, *43*, 630.
(276) Wang, H.; Maurano, M. T.; Qu, H.; Varley, K. E.; Gertz, J.; Pauli, F.; Lee, K.; Canfield, T.; Weaver, M.; Sandstrom, R.; Thurman, R. E.; Kaul, R.; Myers, R. M.; Stamatoyannopoulos, J. A. *Genome Res.* **2012**, *22*, 1680.
(277) Wang, J.; Lunyak, V. V.; Jordan, I. K. *Nucleic Acids Res.* **2012**, *40*, 511.
(278) Orlando, D. A.; Guenther, M. G.; Frampton, G. M.; Young, R. A. *Genomics* **2012**, *100*, 320.
(279) Pombo, A.; Cuello, P.; Schul, W.; Yoon, J. B.; Roeder, R. G.; Cook, P. R.; Murphy, S. *EMBO J.* **1998**, *17*, 1768.
(280) Jolly, C.; Metz, A.; Govin, J.; Vigneron, M.; Turner, B. M.; Khochbin, S.; Vourc'h, C. *J. Cell Biol.* **2004**, *164*, 25.
(281) Wang, J.; Shiels, C.; Sasieni, P.; Wu, P. J.; Islam, S. A.; Freemont, P. S.; Sheer, D. *J. Cell Biol.* **2004**, *164*, 515.
(282) Thompson, M.; Haeusler, R. A.; Good, P. D.; Engelke, D. R. *Science* **2003**, *302*, 1399.
(283) Puvion-Dutilleul, F.; Besse, S.; Diaz, J. J.; Kindbeiter, K.; Vigneron, M.; Warren, S. L.; Kedinger, C.; Madjar, J. J.; Puvion, E. *Gene Expression* **1997**, *6*, 315.
(284) Patrinos, G. P.; de Krom, M.; de Boer, E.; Langeveld, A.; Imam, A. M.; Strouboulis, J.; de Laat, W.; Grosveld, F. G. *Genes Dev.* **2004**, *18*, 1495.
(285) Brown, J. M.; Green, J.; das Neves, R. P.; Wallace, H. A. C.; Smith, A. J. H.; Hughes, J.; Gray, N.; Taylor, S.; Wood, W. G.; Higgs, D. R.; Iborra, F. J.; Buckle, V. J. *J. Cell Biol.* **2008**, *182*, 1083.
(286) Soler, E.; Andrieu-Soler, C.; de Boer, E.; Bryne, J. C.; Thongjuea, S.; Stadhouders, R.; Palstra, R. J.; Stevens, M.; Kockx, C.; van Ijcken, W.; Hou, J.; Steinhoff, C.; Rijkers, E.; Lenhard, B.; Grosveld, F. *Genes Dev.* **2010**, *24*, 277.
(287) Bender, M. A.; Ragoczy, T.; Lee, J.; Byron, R.; Telling, A.; Dean, A.; Groudine, M. *Blood* **2012**, *119*, 3820.
(288) Ragoczy, T.; Bender, M. A.; Telling, A.; Byron, R.; Groudine, M. *Genes Dev.* **2006**, *20*, 1447.
(289) Song, S. H.; Kim, A.; Ragoczy, T.; Bender, M. A.; Groudine, M.; Dean, A. *Blood* **2010**, *116*, 2356.
(290) Yien, Y. Y.; Bieker, J. J. *Mol. Cell. Biol.* **2013**, *33*, 4.
(291) Cai, S. T.; Lee, C. C.; Kohwi-Shigematsu, T. *Nat. Genet.* **2006**, *38*, 1278.
(292) Dhar, S. S.; Wong-Riley, M. T. T. *J. Neurochem.* **2010**, *115*, 676.
(293) Wong-Riley, M. T. *Adv. Exp. Med. Biol.* **2012**, *748*, 283.
(294) Rickman, D. S.; Soong, T. D.; Moss, B.; Mosquera, J. M.; Dlabal, J.; Terry, S.; MacDonald, T. Y.; Tripodi, J.; Bunting, K.; Najfeld, V.; Demichelis, F.; Melnick, A. M.; Elemento, O.; Rubin, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 9083.
(295) Callan, H. G.; Gall, J. G.; Murphy, C. *Chromosoma* **1991**, *101*, 245.
(296) Jacobs, E. Y.; Frey, M. R.; Wu, W.; Ingledue, T. C.; Gebuhr, T. C.; Gao, L.; Marzluff, W. F.; Matera, A. G. *Mol. Biol. Cell* **1999**, *10*, 1653.
(297) Larkin, J. D.; Papantonis, A.; Cook, P. R. *J. Cell Sci.* **2013**, DOI: 10.1242/jcs.123653.
(298) Xu, M.; Cook, P. R. *J. Cell Biol.* **2008**, *181*, 615.
(299) Ferrai, C.; Xie, S. Q.; Luraghi, P.; Munari, D.; Ramirez, F.; Branco, M. R.; Pombo, A.; Crippa, M. P. *PLoS Biol.* **2010**, *8*, e1000270.
(300) Grontved, L.; Hager, G. L. *Mol. Cell. Endocrinol.* **2012**, *357*, 30.
(301) Papantonis, A.; Cook, P. R. *Curr. Opin. Cell Biol.* **2010**, *22*, 271.
(302) Robyr, D.; Friedli, M.; Gehrig, C.; Arcangeli, M.; Marin, M.; Guipponi, M.; Farinelli, L.; Barde, I.; Verp, S.; Trono, D.; Antonarakis, S. E. *PLoS One* **2011**, *6*, e17634.
(303) Morlando, M.; Ballarino, M.; Gromak, N.; Pagano, F.; Bozzoni, I.; Proudfoot, N. J. *Nat. Struct. Mol. Biol.* **2008**, *15*, 902.
(304) Pawlicki, J. M.; Steitz, J. A. *J. Cell Biol.* **2008**, *182*, 61.
(305) Kuhlman, T. E.; Cox, E. C. *Mol. Syst. Biol.* **2012**, *8*, 610.
(306) Kang, J.; Xu, B.; Yao, Y.; Lin, W.; Hennessy, C.; Fraser, P.; Feng, J. F. *PLoS Comput. Biol.* **2011**, *7*, e1002094.
(307) Ashall, L.; Horton, C. A.; Nelson, D. E.; Paszek, P.; Harper, C. V.; Sillitoe, K.; Ryan, S.; Spiller, D. G.; Unitt, J. F.; Broomhead, D. S.; Kell, D. B.; Rand, D. A.; See, V.; White, M. R. *Science* **2009**, *324*, 242.
(308) Ivarie, R. D.; Schacter, B. S.; O'Farrell, P. H. *Mol. Cell. Biol.* **1983**, *3*, 1460.
(309) Banerji, J.; Rusconi, S.; Schaffner, W. *Cell* **1981**, *27*, 299.
(310) Baker, W. K. *Adv. Genet.* **1968**, *14*, 133.
(311) Cook, P. R. *BioEssays* **1994**, *16*, 425.
(312) Cook, P. R. *J. Cell Sci.* **2003**, *116*, 4483.
(313) Kolovos, P.; Knoch, T. A.; Grosveld, F. G.; Cook, P. R.; Papantonis, A. *Epigenetics Chromatin* **2012**.
(314) Bon, M.; Marenduzzo, D.; Cook, P. R. *Structure* **2006**, *14*, 197.
(315) Emerman, M.; Temin, H. M. *Mol. Cell. Biol.* **1986**, *6*, 792.
(316) Papantonis, A.; Cook, P. R. *Transcription* **2011**, *2*, 41.
(317) Chubb, J. R.; Trcek, T.; Shenoy, S. M.; Singer, R. H. *Curr. Biol.* **2006**, *16*, 1018.
(318) Raj, A.; Peskin, C. S.; Tranchina, D.; Vargas, D. Y.; Tyagi, S. *PLoS Biol.* **2006**, *4*, e309.
(319) Zenklusen, D.; Larson, D. R.; Singer, R. H. *Nat. Struct. Mol. Biol.* **2008**, *15*, 1263.
(320) Raj, A.; van Oudenaarden, A. *Cell* **2008**, *135*, 216.
(321) Kolesky, S. E.; Ouhammouch, M.; Geiduschek, E. P. *J. Mol. Biol.* **2002**, *321*, 767.
(322) Bulger, M.; Groudine, M. *Cell* **2011**, *144*, 327.
(323) Zobeck, K. L.; Buckley, M. S.; Zipfel, W. R.; Lis, J. T. *Mol. Cell* **2010**, *40*, 965.
(324) Merika, M.; Thanos, D. *Curr. Opin. Genet. Dev.* **2001**, *11*, 205.
(325) Ptashne, M.; Gann, A. *Nature* **1997**, *386*, 569.
(326) Spitz, F.; Furlong, E. E. M. *Nat. Rev. Genet.* **2012**, *13*, 613.
(327) Kulaeva, O. I.; Nizovtseva, E. V.; Polikanov, Y. S.; Ulianov, S. V.; Studitsky, V. M. *Mol. Cell. Biol.* **2012**, *32*, 4892.
(328) Gohl, D.; Aoki, T.; Blanton, J.; Shanower, G.; Kappes, G.; Schedl, P. *Genetics* **2011**, *187*, 731.
(329) Bantignies, F.; Cavalli, G. *Trends Genet.* **2011**, *27*, 454.

(330) Yang, J.; Corces, V. G. *Curr. Opin. Genet. Dev.* **2012**, *22*, 86.
(331) Barkess, G.; West, A. G. *Epigenomics* **2012**, *4*, 67.
(332) Wang, D.; Garcia-Bassets, I.; Benner, C.; Li, W.; Su, X.; Zhou, Y.; Qiu, J.; Liu, W.; Kaikkonen, M. U.; Ohgi, K. A.; Glass, C. K.; Rosenfeld, M. G.; Fu, X. D. *Nature* **2011**, *474*, 390.
(333) De Santa, F.; Barozzi, I.; Mietton, F.; Ghisletti, S.; Polletti, S.; Tusi, B. K.; Muller, H.; Ragoussis, J.; Wei, C. L.; Natoli, G. *PLoS Biol.* **2010**, *8*, e1000384.
(334) He, H. H.; Meyer, C. A.; Shin, H.; Bailey, S. T.; Wei, G.; Wang, Q.; Zhang, Y.; Xu, K.; Ni, M.; Lupien, M.; Mieczkowski, P.; Lieb, J. D.; Zhao, K.; Brown, M.; Liu, X. S. *Nat. Genet.* **2010**, *42*, 343.
(335) Heintzman, N. D.; Hon, G. C.; Hawkins, R. D.; Kheradpour, P.; Stark, A.; Harp, L. F.; Ye, Z.; Lee, L. K.; Stuart, R. K.; Ching, C. W.; Ching, K. A.; Antosiewicz-Bourget, J. E.; Liu, H.; Zhang, X.; Green, R. D.; Lobanenkov, V. V.; Stewart, R.; Thomson, J. A.; Crawford, G. E.; Kellis, M.; Ren, B. *Nature* **2009**, *459*, 108.
(336) Rada-Iglesias, A.; Bajpai, R.; Swigut, T.; Brugmann, S. A.; Flynn, R. A.; Wysocka, J. *Nature* **2011**, *470*, 279.
(337) Li, L. M.; Arnosti, D. N. *Curr. Biol.* **2011**, *21*, 406.
(338) Raab, J. R.; Kamakaka, R. T. *Nat. Rev. Genet.* **2010**, *11*, 439.
(339) Gaszner, M.; Felsenfeld, G. *Nat. Rev. Genet.* **2006**, *7*, 703.
(340) Chopra, V. S.; Cande, J.; Hong, J. W.; Levine, M. *Genes Dev.* **2009**, *23*, 1505.
(341) Valenzuela, L.; Kamakaka, R. T. *Annu. Rev. Genet.* **2006**, *40*, 107.
(342) Raab, J. R.; Chiu, J.; Zhu, J.; Katzman, S.; Kurukuti, S.; Wade, P. A.; Haussler, D.; Kamakaka, R. T. *EMBO J* **2012**, *31*, 330.
(343) Volpe, T.; Martienssen, R. A. *Cold Spring Harbor Perspect. Biol.* **2011**, *3*, a003731.
(344) Abranches, R.; Beven, A. F.; Aragon-Alcaide, L.; Shaw, P. J. *J. Cell Biol.* **1998**, *143*, 5.
(345) Wen, B.; Wu, H.; Loh, Y. H.; Briem, E.; Daley, G. Q.; Feinberg, A. P. *BMC Genomics* **2012**, *13*, 566.
(346) Hirano, Y.; Hizume, K.; Kimura, H.; Takeyasu, K.; Haraguchi, T.; Hiraoka, Y. *J. Biol. Chem.* **2012**.
(347) Cook, P. R.; Marenduzzo, D. *J. Cell Biol.* **2009**, *186*, 825.
(348) Munsky, B.; Neuert, G.; van Oudenaarden, A. *Science* **2012**, *336*, 183.
(349) Ladbury, J. E.; Arold, S. T. *Trends Biochem. Sci.* **2012**, *37*, 173.
(350) Guillou, E.; Ibarra, A.; Coulon, V.; Casado-Vela, J.; Rico, D.; Casal, I.; Schwob, E.; Losada, A.; Mendez, J. *Gene Dev.* **2010**, *24*, 2812.
(351) Jackson, D. A.; Hassan, A. B.; Errington, R. J.; Cook, P. R. *J. Cell Sci.* **1994**, *107*, 1753.
(352) Cook, P. R. *J. Gen. Virol.* **1993**, *74*, 691.
(353) Hassan, A. B.; Errington, R. J.; White, N. S.; Jackson, D. A.; Cook, P. R. *J. Cell Sci.* **1994**, *107*, 425.
(354) Zhang, Y.; McCord, R. P.; Ho, Y. J.; Lajoie, B. R.; Hildebrand, D. G.; Simon, A. C.; Becker, M. S.; Alt, F. W.; Dekker, J. *Cell* **2012**, *148*, 908.
(355) Simonis, M.; Klous, P.; Homminga, I.; Galjaard, R. J.; Rijkers, E. J.; Grosveld, F.; Meijerink, J. P.; de Laat, W. *Nat. Methods* **2009**, *6*, 837.
(356) Engreitz, J. M.; Agarwala, V.; Mirny, L. A. *PLoS One* **2012**, *7*, e44196.
(357) Cowell, I. G.; Sondka, Z.; Smith, K.; Lee, K. C.; Manville, C. M.; Sidorczuk-Lesthuruge, M.; Rance, H. A.; Padget, K.; Jackson, G. H.; Adachi, N.; Austin, C. A. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 8989.
(358) Cowell, I. G.; Austin, C. A. *Cell Cycle* **2012**, *11*, 3143.
(359) Cook, P. R. *J. Cell Sci.* **1995**, *108*, 2927.
(360) Cook, P. R. *J. Cell Sci.* **1997**, *110*, 1033.
(361) Xu, M.; Cook, P. R. *Biochim. Biophys. Acta* **2008**, *1783*, 2155.
(362) Xu, N.; Donohoe, M. E.; Silva, S. S.; Lee, J. T. *Nat. Genet.* **2007**, *39*, 1390.
(363) Cohen, B. A.; Mitra, R. D.; Hughes, J. D.; Church, G. M. *Nat. Genet.* **2000**, *26*, 183.
(364) Spellman, P. T.; Rubin, G. M. *J. Biol.* **2002**, *1*, 5.
(365) Singer, G. A.; Lloyd, A. T.; Huminiecki, L. B.; Wolfe, K. H. *Mol. Biol. Evol.* **2005**, *22*, 767.
(366) Janga, S. C.; Collado-Vides, J.; Babu, M. M. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 15761.
(367) Galbraith, C. G.; Galbraith, J. A. *J. Cell Sci.* **2011**, *124*, 1607.