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pothetical catalytic transition state. The location of this active site, nestled in the central groove between C_{1a} and C_2 (like forskolin but at a site farther from $G_{s\alpha}$·GTP) would allow ATP or cyclic AMP to diffuse easily from or into the cytoplasm. Site-directed mutations had suggested catalytic roles for several amino acids close to the site. Mutation of one of these, arginine-1029 of the C_2 domain, sharply reduces catalytic activity but does not alter the Michaelis constant (K_m) for ATP (*14*, *15*). In the transition state model, arginine-1029 stabilizes the pentacoordinate 5′-α-phosphate intermediate of the cyclase reaction. This arginine's role strongly resembles that of the "arginine fingers" that stabilize transition states of GTP hydrolysis in Ras-like guanosine triphosphatases (GTPases) bound to GTPase-activating proteins and in G protein α subunits (*16*).

So, how does the catalytic site increase cyclic AMP synthesis in response to a $G_{s\alpha}$·GTP molecule bound at a rather distant location (30 Å away)? Because the C_{1a}-C_2 dimer could not be crystallized in the absence of activators, the enzyme's unstimulated conformation was modeled on the previously reported (*17*) 3D structure of a recombinant C_{1a}-C_2 homodimer, a complex that shows negligible catalytic activity even in the presence of forskolin and $G_{s\alpha}$·GTP. Comparison of the putative inactive structure with that of the $G_{s\alpha}$·GTP–C_{1a}-C_2 complex suggests an intriguing scenario for activation [for details, see figure 6 in (*1*)]: The α2 helix of $G_{s\alpha}$·GTP, occupying a cleft between loops at the ends of two helices of C_2, widens the cleft by 3 Å and pushes a loop that contacts the C_{1a} domain. As a result, the domains rotate subtly (by ~7°) in relation to one another, around an axis (vertical in the figure) that passes through the substrate-binding pocket. The rotation puts a gentle squeeze on the catalytic site, persuading key residues of both domains to click into a configuration that promotes the cyclase reaction. Thus, a gossamer web of inference supports a plausible model, which will be rigorously tested with additional mutations, biochemical experiments, and crystal structures.

Gallant knights in other laboratories toil to fit one remaining piece of the Grail into its proper place. Their quest will eventually reveal, in atomic detail, how hormone receptors embrace trimeric G proteins and turn them on.

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TRANSCRIPTION

Getting Around the Nucleosomes

Jonathan Widom

The 2 meters of DNA inside each eukaryotic cell is wrapped locally around tiny spools called nucleosomes, each made of eight proteins called histones, which are in turn packed together to form chromatin. This packaging method efficiently organizes these lengthy molecules, but when it comes time to make another copy of the DNA for a daughter cell or messenger RNA from a gene, they can get in the way. Each strand of DNA in a nucleosome is inaccessible along most of its length in every helical turn (*1*), the higher orders of chromatin folding further restricting accessibility of the DNA (*2*). Nevertheless, the cell somehow solves this problem: Each gene is wrapped in many nucleosomes, yet is successfully transcribed; classic electron micrographs reveal transcriptional elongation occurring predominantly on DNA packaged in nucleosomes in vivo; and in vitro, RNA polymerases can transcribe right through nucleosomal DNA. A new study, published in this week's issue on page 1960 (*3*), shows how the very large polymerases of eukaryotic cells perform this feat: Studitsky *et al.* carefully analyze the mechanism of transcription through nucleosomes by eukaryotic RNA polymerase III (Pol III).

How RNA polymerases are able to transcribe their chromatin substrates has been the subject of intensive study (*4*). Early experiments analyzed the ability of bacteriophage and eukaryotic RNA polymerases to transcribe through reconstituted nucleosomal DNA and showed that, if the promoter is occluded by histone octamer, transcription initiation is essentially blocked. However, if the promoter extends sufficiently far off the nucleosome, polymerases initiate efficiently and readily extend through one or more nucleosomes.

Important new insights have come from recent methodological advances. In one, resolution of nucleosome isomers with distinct electrophoretic mobilities (which reflect different positions of the histone octamer on the DNA) reveals changes in position before and after transcription (*5*, *6*). Thus, transcription can cause repositioning of the histone octamer on DNA or even complete release of free DNA. The balance between these outcomes depends on the nature and concentration of competitor DNA (or nucleosomes) available to trap histones displaced from the transcription templates, and on the rate of transcription. When transcription is slow and sufficient naked DNA is available upstream of the nucleosome, repositioning can occur more frequently than release and is biased such that the histone octamer moves backward along the template, toward the promoter. Under these conditions, the histone octamer appears to step around the elongating polymerase without fully dissociating from the DNA (*3*, *7*).

Another technical innovation allows real-time analysis of the first passages of synchronous polymerases over nucleosomal templates (*3*, *7*–*9*). When transcription is slowed so that a gene is transcribed over the

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course of minutes, the rates of transcription on naked DNA, nucleosomes, and on oxidized nucleosomes (which have their two histone H3 Cys^{110} sulfhydryls linked together, preventing a postulated unfolding) are nearly indistinguishable (*8*). When transcription proceeds more quickly, transcription on the nucleosomal templates lags perceptibly behind that on the naked DNA (*3*, *7*, *9*). This slower progress is due to increased residence times at pause sites that are specified by the DNA sequence itself. The system acts as though there is "friction" between the polymerase and the histone octamer (*8*, *9*). This apparent friction is detected only after transcription has progressed ≈25 base pairs (bp) into the nucleosome, and it is lost after the polymerase passes ≈10 bp beyond the nucleosomal dyad axis (*7*). These observations suggest that whatever structure provides a rate-limiting step for polymerase progression is passed or disassembled as the polymerase moves slightly beyond the nucleosome dyad.

The new results are the most striking obtained to date. The experimental design is similar to that of these authors' previous studies on nucleosome transcription by bacteriophage SP6 RNA polymerase (*5*, *7*). They use a DNA fragment containing a positioned nucleosome, an SP6 promoter, and a short 3′ extension for Pol III initiation. After transcription with yeast Pol III and SP6 polymerase, some of the nucleosomal templates are converted to naked DNA, the fraction depending on the concentration of competitor. Many of the nucleosomal templates yield repositioned nucleosomes, again apparently by forming a loop to accommodate the polymerase, without requiring complete dissociation of the DNA. The actual displacements along the DNA caused by the two polymerases are similar, suggesting that looped intermediates in nucleosome transfer have similar structures for both.

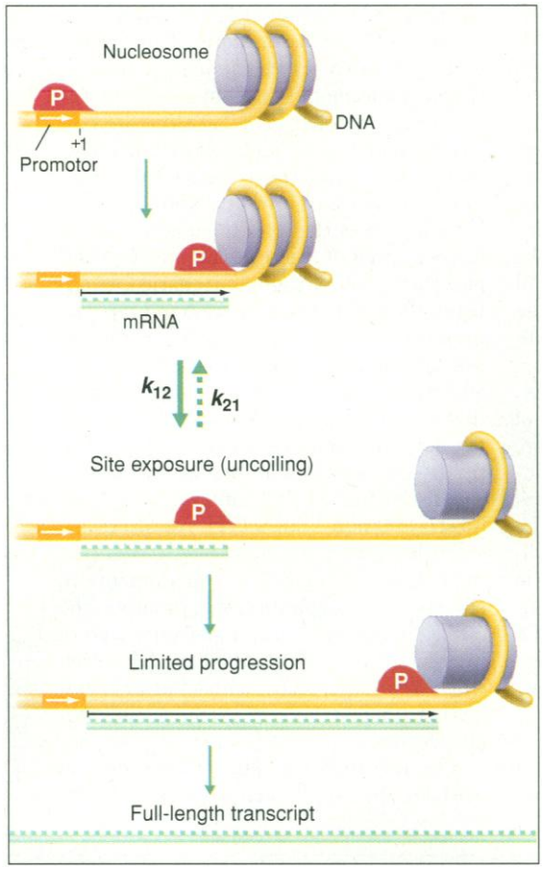

ILLUSTRATION: K. SUTLIFF

Beads on a string: A model for RNA polymerase elongation on nucleosomal DNA. A stretch of DNA is transiently—but nondissociatively—released from the nucleosome surface by spontaneous thermal fluctuations (*11*, *12*), allowing forward progression of the polymerase, as suggested previously (*4*). Posttranslational modifications of the histones (for example, histone acetylation), specific histone gene variants, or the absence of histone H1 could increase the rate of release (or the equilibrium constant for release), thereby facilitating transcriptional elongation. Depending on the availability of competitor DNA and on the rate of transcription, the template DNA may entirely dissociate from the histone octamer or its DNA may be recaptured by the histone octamer at a new location before its complete dissociation, thereby forming a loop and eventually leading to repositioning of the histone octamer.

Transcription by Pol III yields a larger fraction of templates that reposition nucleosomes instead of forming naked DNA than does SP6 polymerase, possibly correlated with the intrinsically slower elongation rate for this enzyme. Pol III exhibits markedly stronger pausing than the SP6 enzyme, and the sites of pausing have a discernible ≈10-bp periodicity. These results may be a consequence of the more constrained motion of the substantially larger Pol III enzyme within a looped transcription intermediate: The polymerase experiences increased friction with the histone surface (or some other nucleosome structure) when it rotates inward toward the histones each helical turn. However, there is also some hint that for Pol III, as for SP6 and T7 polymerases, the sites of pausing in the nucleosome may be coincident with those on naked DNA; so whether the ≈10-bp periodicity of these enhanced pausing sites is really due to nucleosome structure warrants further exploration.

But how do polymerases induce the displacement of DNA from the octamer surface, a necessary precursor to elongation? One might imagine that a molecular motor–like activity of the polymerase could actively pry DNA off the octamer; but this is unlikely to be the case. If the free-energy barrier for release of DNA on a nucleosome is low to fairly large (for example, ≤ΔG of nucleoside triphosphate hydrolysis), then the rate at which the polymerase breaks these bonds will be negligible because the frequency of spontaneous thermal fluctuations having such energies is much greater than the turnover numbers of RNA polymerases. Alternatively, if these bonds are very strong, by "tugging" on one end of a bond a polymerase could augment the energy available from thermal fluctuations to increase the frequency with which sufficiently high energies are reached. But even if all of the work done during a polymerase elongation cycle (*10*) could be redirected to this task, the increase in rate over spontaneous thermal fluctuations would be minor, about threefold at most (*9*). Thus, although polymerase provides a thermodynamic driving force for release of DNA, it contributes insignificantly to the rate.

Rather, forward progression of the polymerase (as well as other processive enzymes) is likely made possible by spontaneous thermal fluctuations in which a stretch of DNA is transiently—but nondissociatively—released from the octamer surface (*4*, *9*). Such "site exposure" events are simplest to imagine if they occur sequentially from an end. Indeed, spontaneous conformational fluctuations allowing proteins access to sites anywhere within a nucleosome do exist (*11*, *12*). This property, intrinsic to nucleosomes, may also allow regulatory proteins to gain access to their DNA target sites in chromatin and the spontaneous movement of nucleosomes along DNA.

Many of the largest questions await further study. Although the proposed site-exposure mechanism can explain the ability of polymerase to elongate through nucleosomes, it has yet to be tested in vivo. Does nucleosome-induced pausing also occur in vivo, and is pausing at these sites regulated? Are histones transferred backward around the polymerase in vivo? How can this process occur for chromatins having very short linker DNA segments? Alternatively, are histones released completely? Some data suggest that at least partial release occurs (*13*). This question has important consequences for how posttranslational modification participates in gene regulation: Complete release implies that any correspondence between a particular modified histone and a particular genetic locus will be lost after one round of transcription. If histones are released, are they able to recombine into nucleosomes at all times in the cell cycle or only during S phase? That is, once released, do histones continue to com-

pete effectively with gene regulatory proteins and polymerase to suppress transcription initiation? The tools needed to attack these questions are now in hand.

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NEUROSCIENCE

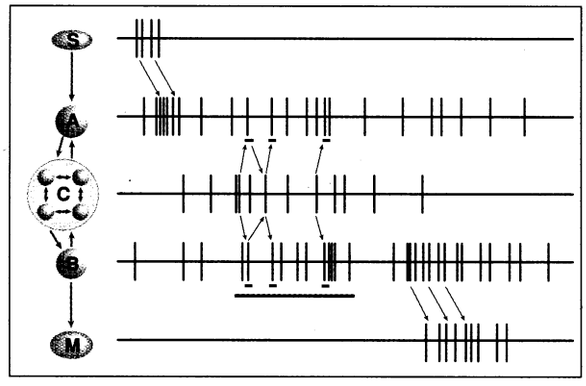
Temporal Coding in Neural Populations?

Eberhard E. Fetz

The waking brain continually performs a remarkable range of neural computations, processing its interactions with the environment as well as performing numerous internal operations. Consider the immediate example of your ability to quickly comprehend the spatial patterns on this page. Scanning successive words and decoding their meaning is a common miracle performed effortlessly by cerebral networks. If you pause to contemplate how neurons could perform these feats, your thoughts are also generated by a complex calculus of neural impulses. Realizing that your entire mental existence depends on neural operations, you can appreciate why neuroscientists would like to understand the underlying mechanisms.

To investigate how neurons process information, researchers typically relate the firing rate of single neurons (the rate at which neurons generate all-or-none action potentials) to a behavioral parameter, such as a sensory stimulus or motor response (*1*–*3*). Neural activity is quite variable, even with precise repetitions of the behavioral conditions, so mean firing rates have been obtained by averaging neural responses over many trials, thereby enhancing the rate-coding "signal." The success of this approach has led to the belief that firing rates of neurons are the necessary and sufficient mechanism underlying information processing, and furthermore, that firing rates not only code peripheral sensory and motor events, but also mediate central cognitive processes.

But in fact, large populations of neurons are involved simultaneously in any behavior (*4*), and the temporal structure of spike activity in neural assemblies provides additional dimensions for coding information. Increasing numbers of researchers are now recording from many neurons simultaneously (*3*, *4*). Indeed, the techniques for multiunit recording have outstripped the development of algorithms for analyzing the dynamics of neural populations. Some investigators have found ways to derive better fits between multiunit activity and behavioral parameters (*3*), but most population-coding schemes still employ functions of firing rates. The possibility that information processing may involve the instantaneous relations between members of neural assemblies has been proposed (*5*, *6*), although such ideas are in dire need of compelling experimental support. In this regard, the report on page 1950 of this issue by Riehle *et al.* (*7*) provides new tools and supporting evidence. Using a simple algorithm for detecting real-time interactions in neural populations, the authors have found that neural activity became transiently synchronized during a purely internal cognitive process—when a monkey expected the presentation of a signal, in the absence of any sensory or motor events.



Neural communication. Schematic representation of interactions of two cortical neurons (A and B). Sensory event S affects A and motor output M is affected by B; in both cases the neuronal firing rate codes a peripheral parameter. The central assembly (C) can also engage A and B in synchronous activity. This can be detected by the increased synchrony in the brief intervals (red) compared to that expected from activity in the longer interval (blue).

These findings relate to a current controversy concerning neural coding: whether neurons process information only by rate coding or whether the brain also exploits "temporal coding," in which the relative timing of spikes can carry information (*1*, *7*–*10*, *14*). Temporal coding can employ the temporal structure of the firing pattern of single neurons (*10*) or, as considered here, the precise relation between firings in multiple neurons of a functional assembly (*5*, *9*, *12*, *14*). If the relative timing of spikes in a neural population can represent information, as it surely does in the auditory system, the "bandwidth" for neural processing could be significantly expanded. As yet, there is no accepted standard for how this increased channel capacity would be used for high-definition neural computation. But a common feature of most temporal codes is the synchronization of spikes in neurons. The report by Riehle *et al.* shows that motor cortex neurons can become significantly more synchronized in relation to expected events, independently of any changes in firing rates.

The figure illustrates the different types of neural interactions. The two representative neurons (A and B) can fire in relation to peripheral events, like a sensory stimulus (S) or movement (M), and can also participate in internal cognitive events, symbolized by the central assembly (C). The proposed signature of the cells' participation in assembly activity is increased synchronization, produced by common input from the assembly. Synchronous activity provides an effective propagating mechanism within the assembly, and can

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