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Twisting in a Crowd

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FORUM *hypothesis*

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Given our current understanding of nuclear structure, it is difficult to imagine how the nucleus performs its varied functions and controls the traffic of its many components. For example, how can densely packed chromatin be transcribed without the helical nature of the DNA resulting in entangled DNA and RNA and a stalled RNA polymerase? Here, Jacques Dubochet discusses a model of transcription in which DNA rotates around its axis, rather than RNA polymerase rotating around the DNA. Furthermore, he suggests that a view of chromatin as 'liquid' may help in understanding a wide range of nuclear functions.

A cell is a crowded place. With its rich collection of globular macromolecules, filaments and membranes, there is little room for empty space. On average, the distance between globular proteins is of the order of their own size, and they move with respect to one another within the timescale of a nanosecond.

The scenery of the nucleus

The nucleoplasm is no less dense, even though it contains neither membrane nor rigid filament. However, it contains one remarkable type of filament, the DNA, which, for a mammalian cell with a small nucleus, can account for close to 10% (100 mg ml^{-1}) of the nuclear mass (including water). This is a very high concentration – much higher than that generally used for experiments *in vitro* (e.g. 200 μg ml^{-1}). It is close to the concentration at which pure DNA in solution starts to show strong colligative behaviour, resulting in the formation of large organized complexes such as liquid crystals (for example, cholesteric crystals form at 200 mg ml^{-1})[1,2]. The nucleus also contains histones (amounting to roughly the same mass as the DNA), various polymerases, topoisomerases and other enzymes, different kinds of RNAs, and smaller molecules and ions. Altogether, these components account for 20–30% of the dry weight of the nucleus. At a magnification of 100 000 times, the nucleus would appear less than 1 m in diameter, but the DNA it contained would be 0.2 mm thick and 200 km long! Goodsell[3] has drawn a picturesque representation of this crowded environment.

The action

Having set the scene, we must now describe the action. The action is complex and intricate. Thousands of genes are transcribed, in the right place, at the right time and in the right amount. All the necessary ingredients are made available as they are needed, and thousands of products, in particular RNA transcripts, are processed and moved to their destination. The traffic of a big city may provide a relevant analogy to events in the nucleus, despite the fact that city traffic is only two dimensional whereas the more complex cell traffic occurs in three dimensions. There is no need to stress that these nuclear processes must be carefully controlled and tuned, from the molecular level up to the global organization of the whole nucleus. We can admire the result of this organization, but we understand little of how it is achieved except for the fact that nucleosomes seem to be a basic building block of the system.

The double-helical nature of DNA gives an additional twist to the problem. The 200-km-long thread of the 100 000-times-magnified model should in fact be formed by two strands, wrapped around each other three times every millimeter. Altogether there are close to one billion windings, which would accumulate into a gigantic entanglement if we were to try to pull the strands apart. Obviously, the cell must have means to prevent catenanes and knots from forming during replication and recombination. Furthermore, the simple model of transcription in which RNA polymerase follows the helical path of the DNA would result in the formation of a complex in which the transcript becomes hopelessly entangled with the template by hundreds of windings.

Electron microscopy

Electron microscopy can seem the method of choice for understanding how the cell solves its traffic problems. In principle, it provides enough resolution to reveal all the structures down to the molecular level and, indeed, it has contributed greatly to our understanding of nuclear function[4]. However, it suffers one serious limitation: the specimens must be dry before they can be put in the microscope. This is a major drawback when it comes to the study of aqueous solutions. Similarly, it is questionable whether the image of a DNA molecule flattened dry on a supporting film can reveal much about the three-dimensional shape it had when it was still in solution.

Cryoelectron microscopy may solve this problem. It allows the direct observation of fully hydrated specimens at very low temperature (~ −170°C) after they have been vitrified by ultra-rapid cooling (requiring about 10^{-4} s), a procedure that solidifies the specimen, apparently without structural modification. With this method, the particles are seen floating in the immobilized liquid. They are neither stained nor chemically fixed, and there are no artifacts due to adsorption onto the supporting film since the vitrified layer is self-supporting. Delicate biological structures can thus be visualized in an approximately native state[5], and it was recently found that the direct visualization of DNA is possible[6].

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Transcription

While we were observing *Escherichia coli* RNA polymerase attached to the promoters of the naturally supercoiled 2673 bp plasmid pUC9, we noticed that the enzyme was nearly always located at the apical ends of the molecule. This was not very surprising since it is known that the interaction of RNA polymerase with promoter sequences tends to bend DNA[7,8] and since the apexes are the regions of highest curvature in a supercoiled molecule. However, this observation made us wonder how transcription could progress from this starting point – how can RNA polymerase leave the promoter at the apex and move along the superhelical path of the DNA filament, without the trailing RNA transcript becoming entangled? The answer came as a surprise from the direct observation of the vitrified transcribing complex[9] (Fig. 1): even after transcription of a long RNA molecule, the RNA polymerase remains at the apex of the supercoiled plasmid and the RNA transcript seems to grow unhindered from the apparently immobile enzyme. There is one simple explanation for this observation: it is not the enzyme that rotates along the DNA helix during transcription, but the DNA that rotates on itself, while moving like a supercoiled conveyor belt. A schematic representation of the process is shown in Fig. 2.

The idea that DNA rotates during transcription is not new. Theoretical considerations and elegant experimental evidence have suggested it before[10–14]. What had remained unclear was the nature of the force limiting or preventing rotation of the polymerase. Our observation suggests an answer: RNA polymerase does not rotate because it is 'attached' to the geometry of the supercoiled DNA. This 'attachment' originates from the bend (or flexibility) that RNA polymerase induces at its actual transcription site. This, in turn, requires that the enzyme is located at an apex of the supercoiled molecule and that it remains there during transcription. Because RNA polymerase does not rotate, the RNA transcript can grow unhindered and be easily released after termination. On the other hand, the whole DNA molecule must be free to rotate around its axis with minimal hindrance.

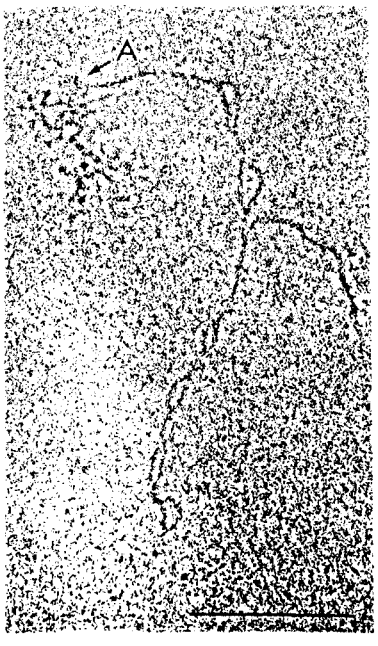

FIGURE 1

Transcription of pUC19 supercoiled DNA by *E. coli* RNA polymerase. After transcription had progressed for 2.5 min, a thin layer of suspension was vitrified and observed by cryoelectron microscopy at –170°C. The interwound supercoiled DNA is branched. The RNA polymerase (A) is located at an apex and the RNA transcript appears as a bushy structure without preferred orientation. Bar, 100 nm. (Reproduced, with permission, from Ref. 9.)

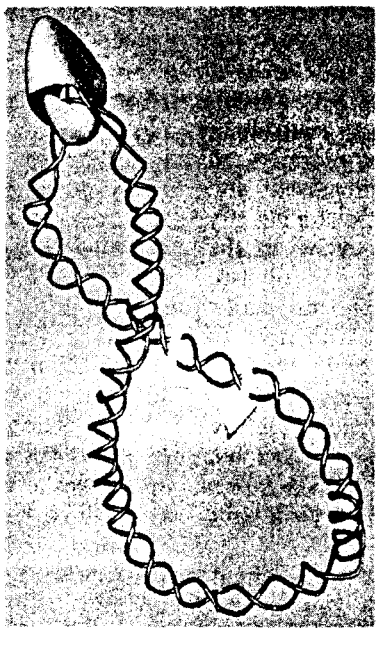

FIGURE 2

Schematic model of the interplay between RNA polymerase and supercoiled DNA during transcription. See the text for more details. (Reproduced, with permission, from Ref. 9.)

For this discussion, we hypothesize that this model also applies to transcription *in vivo*, even in eukaryotic cells. In this case, the transcription unit we are considering is not the whole DNA molecule, as in our *in vitro* model, but the fragment that rotates around its axis during transcription. We call it a topological domain. It must be limited at both ends, by some form of a swivel, which could result from the action of topoisomerase I. We suggest that any number of polymerase molecules can operate at the same time on the same topological domain, provided they are transcribing the same strand at the same speed.

What is the size of a topological domain? This we do not know, but if it is rather large, perhaps as large as the loops seen when histone-depleted chromosomes are delicately spread[14], then our image of chromatin structure and function may be profoundly affected. We would have to imagine large portions of chromatin, with nucleosomes and all their superstructures, in which the DNA becomes free to rotate when transcription starts.

Liquid versus solid

Is it reasonable to consider this is how chromatin works? We think the answer is yes, provided we think in terms of a liquid rather than a solid, and if we state precisely what we understand by these two words.

By 'solid' we mean a structure in which the arrangement of the various components is

strictly defined. Schematically, this means that we can speak unambiguously about nucleosomes, about nucleosomal filaments or about any other level of chromatin organization. Of course, a solid model may have innumerable variations. For example, solid chromatin may have different structures depending on exterior influences, on the change or on the addition of components. In this sense, it can also be dynamic. One feature must remain, however: with a given set of components, the forces holding them together must be strong and specific enough to define the structure uniquely, i.e. the energy holding the system together must be large compared to the thermal energy. Conversely, any change to the structure requires a significant amount of energy to be put into the system. If chromatin is a solid, transcription requiring the free rotation of a large DNA segment would seem to be an expensive procedure.

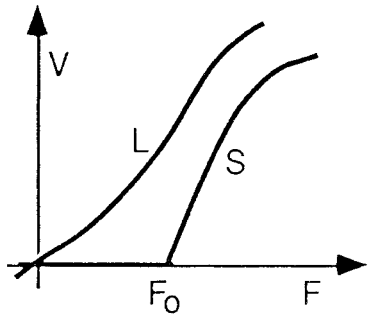

FIGURE 3

Definition of a solid and of a liquid. Equilibrium deformation speed (V) as a function of the force acting on the object (F). The curve for a liquid (L) passes through the origin, whereas for a solid (S), there is a threshold force (F_0) below which no continuous movement takes place.

By contrast, in a 'liquid' there is not one but a large number of possible arrangements of components, and thermal energy is sufficient for a rapid exchange between the various conformations. As a consequence, the components are in constant motion and the liquid can be deformed under the action of any external force. This is in contrast to the case of a solid, in which movement takes place only when the force is larger than some threshold. This difference is expressed in the graphs of Fig. 3.

With the liquid concept of chromatin, the model of DNA rotating during transcription seems to raise no fundamental difficulty. It just requires that among all the exchangeable structures that chromatin can adopt in a topological domain there are some in which DNA can rotate unhindered. Of course, we do not know whether inactive chromatin is mobile or its viscosity is high. Perhaps energy involved in the transcription process induces liquefaction of the transcription domain. The 'smooth-running' liquid model of chromatin may also be valuable in helping us understand other functions of chromatin, such as replication, recombination or trafficking in the nucleus. This advantage, however, has its price: we must look beyond the structure of 'the nucleosome' or 'the nucleosomal filament' to understand how chromatin works. We will have to explore a large range of alternative conformations, and the physical model of working chromatin will become more complicated.

We came to the idea of liquid chromatin through a number of observations. Twisting DNA is one of them. In addition, we have been impressed by the apparent elasticity of the nucleosomes[16] and surprised that chromatin and chromosomes observed in vitrified sections show no apparent higher order structure but appear instead like a 'sea of nucleosomes'[17].

To imagine the liquid model of chromatin, try the following experiment. Prepare a full plate of Italian spaghetti in tomato sauce. The spaghetti must be as long as possible and cooked *al dente*. The experiment consists of finding the extremity of one strand of spaghetti, seizing it delicately between two fingers, and pulling slowly. You will see the apparently unrelated movement of several spaghetti loops appearing at the surface of the plate while all the rest remains immobile. Under optimal experimental conditions, you will observe how the pulling and twisting motions influence each other over long distances. This experiment has the considerable advantage that, if the experimenter dislikes the model, he can at least eat it.

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