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Introductory Remarks

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III. TRANSCRIPTION

Introductory remarks

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This discussion meeting is entitled 'Structure of eukaryotic chromosomes and chromatin'. Now, some scientists are interested in the study of structure for its own sake; they are the purists, the *Ars gratia artis* school, as it were. Others, including myself, are interested in structure mainly because this knowledge may help to elucidate the way in which chromosomes function. By chromosomal function we mean the rôle of chromosomes in gene expression, mainly the control of transcription. This session is devoted to consideration of the proposition that the transcriptionally active part of chromatin is structurally different from the inactive part. Indeed, there is a good deal of evidence for this.

The notion is inferred from morphological studies of chromosomes, notably lampbrush chromosomes in amphibians and giant chromosomes in dipterans. Lampbrush loops have long been suspected and more recently have been shown to be sites of active transcription and quite distinct from the transcriptionally inactive structures within the chromomeres. Similarly, evidence is now conclusive that transcription occurs in the puff structures of giant chromosomes which are structurally and biochemically distinct from the unpuffed regions. The puffs contain not only DNA and histones but also large concentrations of RNA and non-histone proteins which are absent or nearly absent from the unpuffed regions. These observations have been greatly extended by electron microscopic studies, some of which will be presented in this session.

A second major approach to this problem has involved the use of enzymes as probes of chromatin structure. The enzyme which has been mainly used is DNA-dependent RNA polymerase, usually from *Escherichia coli*. One might not expect a bacterial enzyme to initiate and terminate in precisely the same way as a eukaryotic enzyme on eukaryotic chromatin. On the other hand, the bacterial enzyme is about the same size as the eukaryotic enzyme. Hence, if the active component of chromatin were more accessible to this enzyme, this might be revealed by attempting to transcribe chromatin. Studies of this kind were initiated 15 years ago and all showed that chromatin can be used as a template for bacterial RNA polymerase but that only about 10% of the RNA in chromatin is accessible to the enzyme. We, and others, extended these studies by introducing the use of nucleic acid hybridization to investigate the kind of RNA transcribed. We were able to show that this RNA was extensively similar to the RNA found in the nuclei of the cells from which chromatin was derived. The implication is that the DNA normally transcribed *in vivo* is more readily accessible to the polymerase in chromatin *in vitro*.

Other enzymes have also been used as probes. For example, it has been shown that only a small percentage of the DNA in chromatin is accessible to DNA polymerase. Recently interest has been mainly concentrated on the behaviour of chromatin towards nucleases, largely arising

out of the work on nucleosomes which was the subject of the previous sessions. It has been observed by a number of workers that a fraction of the DNA in chromatin is very rapidly digested by both double-stranded nucleases and single-stranded nuclease. It has been claimed too that the rapidly degraded DNA contains the genes characteristic of the specific function of the tissue; for example, globin genes are claimed to be selectively digested by DNase I in chromatin from erythroid tissue.

In view of the gross structural differences seen between active and inactive regions in certain chromosomes and the differences in accessibility to different enzymes, it might seem reasonable to suppose that it would be easy to fractionate chromatin into active and inactive components. Indeed, during the past 15 years very many attempts have been made to do this and it has been found relatively easy to obtain different fractions by a variety of techniques. However, when these are subjected to critical analysis with a view to determining whether one fraction represents the euchromatin of the cells and another the heterochromatin, the results have almost invariably been disappointing in that they have shown no real differences in the nucleotide sequences in the fractions. Nevertheless, some experiments have been more successful than others and we will hear of one of the more successful approaches to fractionation this morning.

If the proposition is correct that transcriptionally active chromatin has a different structure from inactive chromatin, what differences would we expect to find between them? One of the major problems arising from the models which we now have of nucleosomes, is how a polymerase molecule can move along DNA associated with nucleosomes. If one considers these models only briefly, appreciating that an RNA polymerase molecule is about the same size as a nucleosome, then one realizes that an immediate topological and mechanical problem is encountered in transcription in the presence of nucleosomes. It seems necessary to propose some special mechanisms to circumvent this. The simplest possibility is that nucleosomes are simply removed from transcriptionally active regions. This could be achieved by modifying the histones of which the nucleosomes are composed. A second possibility is that the nucleosomes might be displaced. As discussed in an earlier session, in the absence of histone H1, it is possible for the nucleosomes to slide along DNA, at least in solutions of high ionic strength. It might be possible, therefore, for RNA polymerase simply to displace nucleosomes along to beyond the transcriptional active region. A third possibility, which is the subject of some current models, is that the nucleosome may be capable of unfolding. First the two halves, which are possibly symmetrical, may open up as on a hinge, then the entire nucleosome might unfold, with the histones remaining attached by their basic amino-terminal 'tails' located in the greater groove of the DNA helix. I should like to point out that if this did happen then it is possible that these 'tails' could associate entirely with one strand of DNA, so that the other strand could separate from it for transcription.

These are mainly speculations at the moment and the first question to which we must address ourselves is whether intact nucleosomes are present in transcriptionally active regions or not. Some of the evidence we have about this will be presented in this session.

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