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The role of stable complexes that repress and activate eukaryotic genes

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The state of activity or repression of 5*S* RNA genes is built into the chromatin in the form of stable complexes. The characteristics and composition of these complexes will be described. Stable transcription complexes that activate 5*S* RNA genes consist of multiple factors bound tightly to a control region of the gene. Repressed 5*S* RNA genes do not have these factors bound to them; their repressed state is maintained by nucleosomes.

Two kinds of multi-gene families that encode 5*S* ribosomal RNA are under developmental control in *Xenopus* (see Brown 1982). The smaller family comprising about 400 copies of the gene is called the somatic 5*S* RNA genes (Peterson *et al.* 1980); they encode greater than 95% of the 5*S* RNA synthesized by somatic cells. The larger gene family, the oöcyte 5*S* RNA genes, number about 20000 copies per haploid complement of DNA (and therefore 98% of the animal's 5*S* RNA genes). They are active in growing oöcytes (Ford & Southern 1973; Wegnez *et al.* 1972) but silent in somatic cells. We are trying to understand the molecular basis of this gene control using two kinds of experiments. First, cloned genes are transcribed in extracts from either oöcyte or somatic cells (Birkenmeier *et al.* 1978; Pelham *et al.* 1981). To date, transcription experiments *in vitro* have not been able to account for the thousand-fold discrimination between these two kinds of genes that occurs *in vivo* in somatic cells. The second method uses somatic cell chromatin that is already under developmental control to determine the molecules that repress the oöcyte 5*S* RNA genes and those that activate the somatic 5*S* RNA genes (Bogenhagen *et al.* 1982). The assay for these studies is transcription of 5*S* RNA.

Transcription of cloned genes *in vitro*

The experiments with cloned genes have made us aware of a structure that we call the transcription complex (Bogenhagen *et al.* 1982). A minimum of three different components are needed to program the gene into an active or potentially active state (Segall *et al.* 1980). They form a complex with the gene's internal control region that is remarkably stable. RNA polymerase III accurately initiates transcription on such an activated gene, whereas, the polymerase cannot do so on naked DNA (Cozzarelli *et al.* 1983). The polymerase recognizes the transcription complex but is not an integral part of it. Many rounds of RNA synthesis can occur without disruption of the complex (Bogenhagen *et al.* 1982).

The formation of a transcription complex is inhibited by omitting an essential component (Bogenhagen *et al.* 1982). The partial complex is unstable and formation of a complete complex is blocked by the prior addition of histones (and probably other basic proteins as well). When we compared cloned oöcyte with somatic 5*S* RNA genes by these criteria, we found that both kinds of genes form stable complexes with the same components. One difference between the two genes that we believe has significance is that the somatic 5*S* RNA genes bind one of the

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three essential components about four times more tightly than do oöcyte 5*S* RNA genes (Sakonju & Brown 1982). Two base changes in the centre of the 5*S* RNA gene which is the binding site of this protein (called TFIIIA, factor A or 40 kDa protein in various papers) mediates this difference. The difference is in the right direction, but clearly inadequate to explain the huge developmental discrimination that occurs *in vivo*.

Transcription of chromatin *in vitro*

The states of somatic and oöcyte 5*S* RNA genes in somatic cell chromatin resemble the active and repressed complexes that can be formed *in vitro* on cloned 5*S* RNA genes. Both states are stable. Repressed oöcyte 5*S* RNA genes in somatic cell chromatin are not activated by the addition of transcription factors (Bogenhagen *et al*. 1982; Wormington *et al*. 1982) and active somatic 5*S* RNA genes are not repressed by added histones in reasonable amounts (Schlissel & Brown 1984). The repressed state of oöcyte 5*S* RNA genes in chromatin is mediated by histone H1 (Schlissel & Brown 1984). Any treatment that destroys or removes histone H1 derepresses the oöcyte 5*S* RNA genes, i.e. these genes become available to form active transcription complexes with added transcription factors. No partial supplementation will activate these genes, which leads to the conclusion that the oöcyte 5*S* RNA genes in somatic cell chromatin are not associated with any of the three required components needed to form a functional and stable transcription complex. Since derepressed oöcyte 5*S* RNA genes can be repressed again by adding histone H1 in one copy per 200 base pairs of DNA, we have concluded that the repression involves the other histones presumably in a nucleosome structure.

In contrast, the somatic 5*S* RNA genes in somatic cell chromatin are in active stable transcription complexes. Conditions that derepress the oöcyte 5*S* RNA genes do not affect the activated somatic 5*S* RNA genes. Levels of exogenous histone H1 that repress again the derepressed oöcyte 5*S* RNA genes (one molecule per 200 base pairs of DNA) only slightly inhibit the activated somatic 5*S* RNA genes. The active transcription complex withstands salt concentrations that derepress the oöcyte 5*S* RNA genes. These differences are a function of the kind of complex not the kind of gene. Thus, active complexes formed around oöcyte genes are as stable as those formed around somatic genes (Schlissel & Brown 1984).

These studies have focused our attention on the two kinds of complexes that are involved in the activation and repression of genes. Our experiments suggest that a repressed gene is a gene devoid of transcription factors. Its repressed state is mediated by histone H1, presumably as part of complete nucleosomes. Repression of 5*S* RNA genes is viewed as general in the sense that nucleosomes are pervasive, while activation of the gene is specific since at least one of the factors that activates the gene is specific for 5*S* RNA genes (Engelke *et al*. 1980; Pelham & Brown 1980). In the case of the 5*S* RNA gene, there are a minimum of three such factors that are required to form the complex rendering RNA polymerase III capable of binding to, and initiating at, the start site of the gene, specifically (Segall *et al*. 1980). Repressed and activated states are not in equilibrium. One state must be perturbed in order to establish the other state.

Conclusion

I have proposed that certain features of the determined (or committed) state of a cell can be accounted for by these principles that involve transcription complexes (Brown 1984). If a gene is committed for activity by the formation of an active transcription complex with its

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control region (analogous to prokaryotic promoters), then the stability of the complex can maintain its activity (Bogenhagen *et al.* 1982). Cooperative interactions between multiple components of the complex not only stabilize the complex but can help to reform it should it be perturbed as, for example, must happen at DNA replication. The most difficult events to model are those involving asymmetric determination events. These are programmed events during embryogenesis when two different cell types are formed from a single cell division. Identical genes in sister chromatids must have different functional fates. We presume that the beginning of asymmetry in an embryo must be caused by localization of transcription factors in the egg cytoplasm.

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Discussion

P. N. Schofield (*Department of Biochemistry, University of Oxford, South Parks Road, Oxford, U.K.*). Has Dr Brown measured the nucleosomal repeat length in chromatin to which he has added back H1?

D. D. Brown. The nucleosomal repeat length is always irregular if the chromatin has been derepressed by a salt wash. It is more regular if it has been derepressed at low salt with Bio Rex. The readdition of H1 does not change the previous spacing. Thus, spacing does not correlate with activity.