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Chromatin transcription with mercurated nucleotides

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Recently, several authors have described the isolation of transcripts synthesized *in vitro* from chromatin templates with *Escherichia coli* RNA polymerase using mercury substituted nucleotide triphosphates (Biessman, Gjerset, Levy & McCarthy 1976; Smith & Huang 1976; Crouse, Fodor & Doty 1976). The advantage of this technique is that the mercurated RNA can be separated from contaminating endogenous RNA sequences present in the isolated chromatin by chromatography on sulphydryl Sepharose. The presence of endogenous RNA has previously presented problems when attempting sequence analysis of the *in vitro* transcripts by cDNA hybridization (Gilmour, Windass, Affara & Paul 1975; Wilson *et al.* 1975).

In the present report mouse embryonic liver chromatin (an erythropoietic tissue, 14 days *in utero*) was incubated with *E. coli* polymerase as previously described (Gilmour & Paul 1973) and with Hg–UTP prepared according to the method of Dale & Ward (1975). Mercurated transcripts were isolated by the use of sulphydryl Sepharose as described in the above paper and the presence of globin mRNA sequences determined by hybridization with [^{3}H]globin cDNA.

In separate experiments it was shown that between 80 and 90 % of the newly synthesized RNA is recovered from the incubation.

Purified transcripts were hybridized to globin complementary DNA (cDNA) at a RNA:cDNA ratio of 4.5×10^4 and hybrids assayed with S_1 nuclease. An $R_0t_{\frac{1}{2}}$ (R_0t, initial RNA concentration multiplied by time) value of about 60 was obtained indicating that one part in 1.5×10^4 of the transcript represented globin mRNA sequences. The hybridization with pure globin mRNA was used as a standard ($R_0t_{\frac{1}{2}} = 4 \times 10^{-3}$) and it was also shown that this value did not alter when the mRNA was mercurated. Where *E. coli* polymerase was omitted only about 5 % of hybridization took place; this was thought to be due to endogenous RNA polymerase activity in the chromatin, since it could be abolished by the addition of α-amanitin (20 μg/ml) to the incubation. In additional experiments, transcripts from adult mouse liver and purified DNA were hybridized to globin cDNA. In neither of these cases was appreciable hybridization seen below $R_0t_{\frac{1}{2}} = 10^3$. These experiments indicate that *E. coli* RNA polymerase transcribes the globin gene *in vitro* in a tissue specific manner.

Previous work has attempted to use chromatin reconstitution to demonstrate that transcriptional specificity is an inherent property of the chromatin (Gilmour *et al.* 1975; Barrett Maryanka, Hamlyn & Gould 1974). In addition to the problem of endogenous RNA sequences there is considerable variation in the methods of reconstitution and the degree of reproducibility achieved.

In this report chromatin was dissociated in either 2 M NaCl or 2 M NaCl containing 5 M urea (all solutions contained TE buffer in addition: 10 mM Tris–HCl, pH 7.5; 1 mM EDTA and 0.1 mM dithiothreitol). Phenylmethylsulphonylfluoride (174 μg/ml) was also included in the

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dissociation buffer to prevent protease activity. The dissociated chromatins were reconstituted in three ways by gradient dialysis:

(*a*) 2 M NaCl 0.6 M NaCl 0.1 M NaCl TE buffer
(*b*) 2 M NaCl, 5 M urea 0.6 M NaCl, 5 M urea 0.1 M NaCl, 5 M urea 5 M urea
(*c*) 2 M NaCl, 5 M urea 0.6 M NaCl, 5 M urea 0.1 M NaCl TE buffer

In all cases more than 95 % of the starting material was recovered as reconstituted chromatin. Chromatins were transcribed with mercurated UTP as before and the transcripts analysed for globin mRNA sequences by hybridization with globin cDNA.

Transcripts from chromatin reconstituted by method (*c*) hybridized with $R_0t_{\frac{1}{2}}$ almost identical to native chromatin, while transcripts from chromatin (*a*) hybridized with kinetics resembling that for DNA transcripts. Method (*b*) yielded chromatin whose transcripts showed properties similar to those prepared by method (*c*). This result shows that the dissociable components of chromatin are responsible for directing tissue specific transcription. The method of reconstitution can, however, influence considerably the result obtained and might explain previous inconsistencies. The significance of these differences will only become clear when the underlying molecular events of reconstitution are known.

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