ROLE OF NON-HISTONE COMPONENTS IN DETERMINING ORGAN SPECIFICITY OF RABBIT CHROMATINS

R.S. GILMOUR* and John PAUL

Beatson Institute for Cancer Research, 132 Hill Street, Glasgow, C.3, Scotland

Received 4 July 1970

1. Introduction

In previous publications [1,2] we have shown that, in isolated mammalian chromatin, RNA transcription is restricted to a small percentage of the DNA. This restriction is thought to be characteristic for each tissue; it is conferred by the proteins of chromatin, histones having a non-specific inhibiting role and non-histone proteins a more specific regulatory function [1]. Here we describe experiments in which various rabbit chromatins were fractionated into DNA, histone and non-histone components and then reconstituted under conditions in which only the source of the non-histone fraction differed. Hybridization analysis of the RNAs transcribed from these chromatins provides further evidence that the non-histone fraction can interact with DNA and modify transcription in a manner characteristic of the tissue from which it was derived.

2. Materials and methods

Rabbit bone marrow, thymus, liver and kidney chromatins were prepared as previously described [3], dissolved in 2 M NaCl and 5 M urea – 0.01 M tris pH 8.3 at 0.5 mg DNA/ml and centrifuged in an MSE 8 × 25 ml head at 39,000 rpm for 36 hr at 4°. The pelleted DNA was further purified according to Marmur [4]. The supernatant, which contained the ionically bound proteins of chromatin, was saturated with solid ammonium sulphate at 0° and the precipitate which formed on standing was collected by centrifugation at 48,000 *g*. The pellet was dissolved in 5 M urea – 0.01 M tris pH 8.3 and dialysed against the buffer to remove residual ammonium sulphate. QAE Sephadex A-50 (previously equilibrated against the same buffer) was added to form a slurry (2 g/50 ml dialysate) which, after 10 min, was filtered under suction. The filtrate contained the histone fraction. The Sephadex was washed thoroughly with buffer and then extracted with a small volume of 2 M NaCl-5 M urea-0.01 M tris, pH 8.3. This extract contained the non-histone fraction.

3. Results and discussion

First, homologous DNA, histone and non-histone fractions were reassociated by gradient dialysis. After mixing in 2 M NaCl-5 M urea-0.01 M tris, pH 8.3 (DNA: total protein ratio 1:4 w/w) the samples were dialysed overnight against the same buffer containing 0.6 M NaCl and then for 3 hr each against 0.4 M and 0.2 M NaCl. The reconstituted nucleoproteins were collected by centrifugation and used as primers in an *in vitro* RNA synthesising system with *Micrococcus lysodeikticus* RNA polymerase. The ^{3}H-labelled RNA products were hybridized to rabbit thymus DNA using the method of Gillespie and Spiegelmen [5] as shown in fig. 1. Hybridization to DNA of the RNAs transcribed from the reconstituted nucleoproteins from rabbit kidney, bone marrow or liver gave identical saturation values, determined by double reciprocal plots to be 6.8% for bone marrow chromatin, 4% for liver and thymus chromatin and 3% for kidney chromatin.

* Present address: Dept. of Biochemistry, University of British Columbia, Vancouver, Canada.

North-Holland Publishing Company – Amsterdam

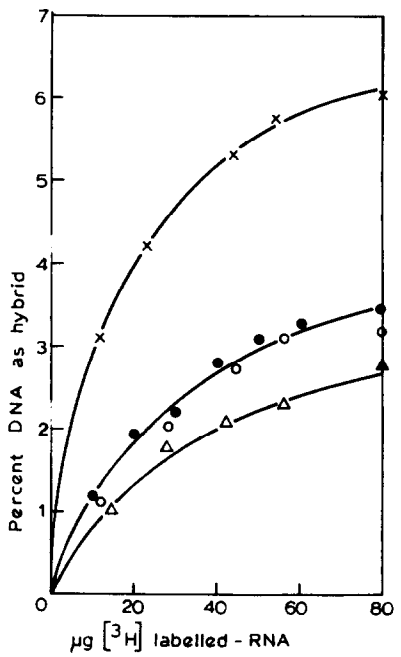

Fig. 1. Kinetics of hybridization to rabbit thymus DNA of ^{3}H-RNAs made by *M. lysodeikticus* polymerase in the presence of reconstituted rabbit bone marrow chromatin x—x; rabbit thymus chromatin •—•; rabbit liver chromatin ○—○; or rabbit kidney chromatin △—△. Filters were loaded with 5 μg denatured rabbit thymus DNA and the ^{3}H-RNA preparation incubated for 18 hr at 67° at the concentrations shown.

To investigate the role of the non-histone fraction, the DNA and histone fractions of bone marrow and thymus were pooled; reconstituted chromatins were then prepared by combining these with either marrow or thymus non-histone fractions. ^{3}H-labelled RNAs were transcribed from them and hybridized to DNA in the presence of unlabelled RNA prepared *in vitro* from natural bone marrow or thymus chromatins. It has been shown previously that RNA transcribed from natural chromatin *in vitro*, is very similar to the RNA of the tissue from which it was prepared [1,2]. The degree of competition observed gives an index of the similarity between the RNAs from the reconstituted chromatins and the *in vivo* synthesized RNAs for bone marrow or thymus.

Fig. 2a shows that natural thymus RNA competes more effectively with ^{3}H-RNA synthesized from chromatin reconstituted with thymus non-histone fraction than that reconstituted with marrow non-histone fraction. Less difference is observed when the ^{3}H-labelled RNAs are challenged with unlabelled marrow (fig. 2b); this is consistent with the previous observation [2], which was interpreted as indicating that marrow RNA may contain most of the thymus specific species plus some which are unique to marrow cells.

When liver and kidney non-histone fractions were

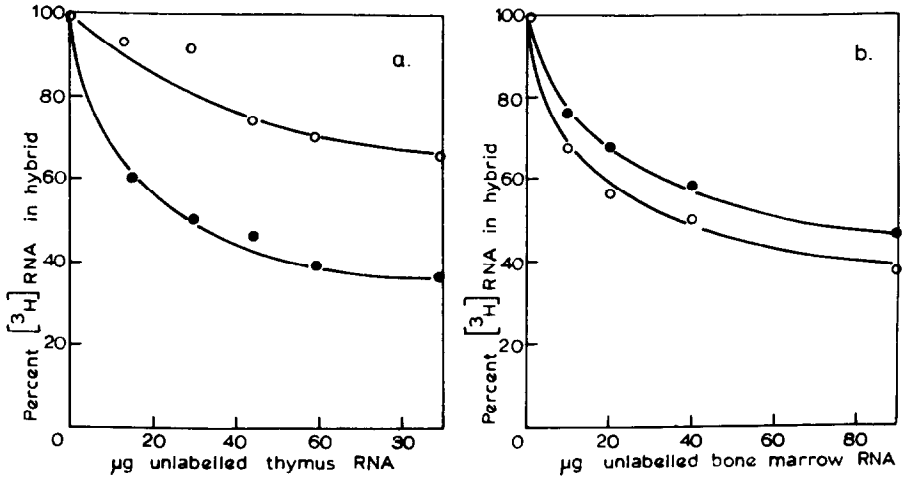

Fig. 2. Competitive hybridization between ^{3}H-labelled RNAs synthesized from chromatins reconstituted with bone marrow non-histone fraction ○—○; or thymus non-histone fraction •—•, and unlabelled RNAs synthesized from natural thymus chromatin, *a*, or natural bone marrow chromatin, *b*. In all cases near saturating levels of labelled RNAs were hybridized to 5 μg denatured DNA in the presence of increasing amounts of unlabelled RNA.

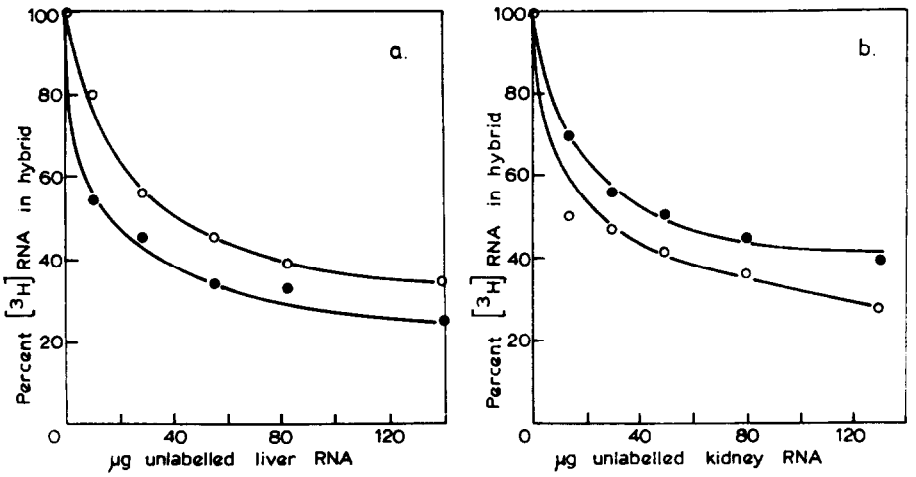

Fig. 3. Competitive hybridization between ^{3}H-labelled RNAs synthesized from chromatins reconstituted with liver non-histone fraction, •—•, or kidney non-histone fraction ○—○, and unlabelled RNAs synthesized from natural liver RNA, *a*, or natural kidney chromatin, *b*.

compared by the same procedure (fig. 3 a,b) the differences were not so pronounced. However, as in the previous experiment, in both instances more effective competition was observed when the competing RNA and the reconstituted non-histone fraction came from the same tissue.

In conducting competition experiments it is theoretically desirable to work in conditions in which complementary DNA sites are almost saturated by ^{3}H-RNA. In practice this is not possible and therefore changes in ratios of different RNA species in the RNA preparation may affect the experimental results as well as absolute differences in RNA species. Since the ^{3}H-RNA was made *in vitro* with the same *M. lysodeikticus* preparation, differences in relative transcription rates or turnover of RNA are less likely. Competition curves provide a useful criterion of comparison between different RNA preparations. Our experiments indicate that ^{3}H-RNA transcribed from reconstituted chromatin resembles more closely RNA from the tissue providing the non-histone component than RNA from another tissue.

In summary, the results of these cross-over experiments suggest that tissue specific restriction of transcription in chromatin is directed by the non-histone fraction. The effect of crossover is most pronounced when tissues with marked qualitative differences in restriction are compared (as with rabbit bone marrow and thymus). The non-histone fractions prepared by QAE Sephadex A-50 are known to contain both acidic proteins and varying amounts of RNA depending on tissue source. It has not yet proved possible to determine which is responsible for conferring the properties described.

Acknowledgements

This work was supported by grants from the Medical Research Council and Cancer Research Campaign. We are grateful to Dr. A. Hell for the illustrations and to Drs. A.J. MacGillivray and J. Ingles for useful discussions.

References

[1] J. Paul and R.S. Gilmour, J. Mol. Biol. 34 (1968) 305.
[2] J. Paul and R.S. Gilmour, Nature 210 (1966) 992.
[3] R.S. Gilmour and J. Paul, J. Mol. Biol. 40 (1969) 137.
[4] J. Marmur, J. Mol. Biol. 3 (1961) 208.
[5] D. Gillespie and S. Spiegelman, J. Mol. Biol. 12 (1965) 829.