4. Söder, P.-Ö., Nord, C.-E., Lundblad, G. and Kjellman, O. *Acta Chem. Scand.* **24** (1970) 129.
5. Svensson, H. *Arch. Biochem. Biophys. Suppl.* **1** (1962) 132.
6. Vesterberg, O. and Svensson, H. *Acta Chem. Scand.* **20** (1966) 820.
7. Osserman, E. F. and Lawlor, D. P. *J. Exptl. Med.* **124** (1966) 921.
8. Wiezorek, Z., Czajka, M. and Kowalczyk, H. *Arch. Immunol. Ther. Exp.* **15** (1967) 829.
9. Alderton, G., Ward, W. H. and Fevold, H. L. *J. Biol. Chem.* **157** (1945) 43.
10. Flatmark, T. and Vesterberg, O. *Acta Chem. Scand.* **20** (1966) 1497.
11. Jollés, P. *Proc. Roy. Soc. (London)* **167** (1967)350.
12. Mouton, A. and Jollés, J. *FEBS Letters* **4** (1969) 337.
13. Bernier, I., Van Leemputten, E., Horosberger, M., Bush, D. A. and Jollés, J. *FEBS Letters* **14** (1971) 100.

Received January 5, 1972.

Specificity of RNA-DNA Hybrids in Differentiating Erythroid Cells

RITVA-KAJSA SELANDER and ALBERT DE LA CHAPELLE

The Folkhälsan Institute of Genetics, Apollogatan 1, 00100 Helsingfors 10, Finland

In a previous paper we described molecular hybridization between newly synthesized RNA from differentiating foetal erythroid cells and DNA from adult livers.[1] It was shown that as differentiation proceeded erythroid cell RNA hybridized less efficiently with DNA. We interpreted this to indicate increasing repression of the genome. This view was supported by experiments in which RNAs from cells at different stages of differentiation simultaneously competed for sites on DNA.

The interpretation of RNA-DNA hybridization experiments in mammals is hampered by several difficulties.[1] Firstly, reactions may not display locus specificity.[2] Secondly, the extent of cross reaction among related base sequences is dependent upon reaction conditions such as temperature and salt concentration.[3] Thirdly, repetitious nucleic acid sequences which make up about one third of the mouse genome [4] hybridize at a much faster rate than unique sequences.[5] Therefore, the reaction of such repetitious nucleic acids may completely obscure the contribution from unique or less repetitious sequences.[5] Significant nucleotide mispairing occurs under ordinary reaction conditions when related nucleic acid sequences of repetitious type are used.[6]

The work described here was an attempt to minimize those difficulties of interpretation that stem from the lack of locus specificity. Some apparently fundamental facts relating to the use of RNA-DNA hybridization in our mammalian system were discovered.

Our previously reported reactions were carried out at 67°C. At this temperature chain scission and depurination pose a problem which is only partially overcome by the use of appropriate salt concentrations (in our experiments 4 × SSC; the abbreviation SSC denotes 0.15 M NaCl – 0.015 M sodium citrate). A significant improvement was achieved by using formamide in the reaction mixtures [7,8] because it greatly reduces the thermal stability of nucleic acids so that the reactions can be carried out at low temperatures.[9,10] It has been shown that in formamide at low temperatures rates of reaction between

Table 1. Hybridization of foetal mouse yolk-sac erythroid cell RNA with adult mouse liver DNA. Numbers given under "Hybridization" were calculated as cpm hybridized at saturation level divided by the specific activity (cpm/μg) of the labelled RNA used. Results are means of 5 experiments (without formamide), and 2 experiments (with formamide) ± S.E.

Day of gestation	Hybridization	
	Without formamide	With formamide
11	0.078 ± 0.010	0.010 ± 0.004
12	0.053 ± 0.011	0.006 ± 0.003

mammalian nucleic acids may be several times higher than those obtained at 60°C or 70°C in aqueous solution.[8]

Pulse-labelled RNA from differentiating yolk-sac erythroid cells obtained from mouse foetuses on different days of gestation was hybridized with DNA from adult mouse livers. The methods were as described in detail previously.[1] The conditions of RNA-DNA hybridization were either 4 × SSC at 67°C for 16 h, or, alternatively, 40 % formamide in 4 × SSC at 37°C for 24 h.

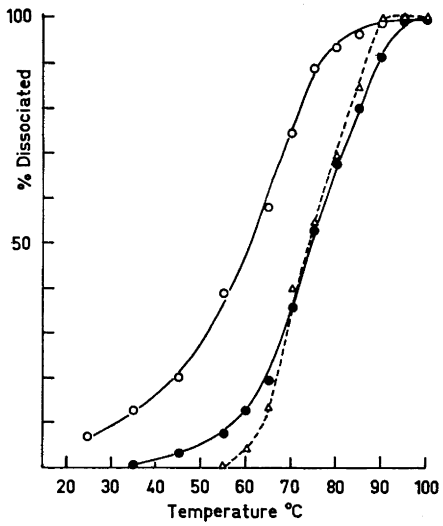

Fig. 1. Thermal dissociation of native DNA in solution (△) and filter-bound RNA-DNA hybrids. RNA-DNA hybridization was carried out as described in the text under two different sets of reaction conditions: ●, 40 % formamide in 4 × SSC at 37°C for 24 h; ○, 4 × SSC at 67°C for 16 h. Adult mouse liver DNA in solution (25 μg/ml in SSC) was incubated for 10 min at temperatures increasing by 5-degree intervals and the optical density was determined after each interval. The numbers on the ordinate signify the cumulative increase in optical density shown as per cent of the total increase. Filters were incubated in 2 ml of SSC for 10 min at temperatures increasing by 5- or 10-degree intervals. After each interval the fluid was drained and its radioactivity determined as described.[1] The numbers on the ordinate indicate the cumulative proportion (per cent) of the total number of cpm dissociated. In both instances, 100 % of the cpm on a control filter were dissociated.

When 11-day and 12-day erythroid cell RNAs were used at saturation level, the cpm bound to the filters fell by a factor of 5 to 10 in the presence of formamide (Table 1). Both with and without formamide there was a decrease in hybridization rate as differentiation proceeded, which confirms our previous findings.[1] However, the rate of hybridization decreased sharply when the reaction was carried out in the presence of formamide.

To explain this unexpected phenomenon thermal denaturation curves of the RNA-DNA hybrids were obtained. From Fig. 1 it is evident that the main slope of dissociation of the hybrids formed in the presence of formamide was practically identical with that of native DNA. The T_m of both was 73°C. By contrast, hybrids formed without formamide had quite a different denaturation profile, in that a high proportion of the hybrids dissociated at low temperatures. This difference in thermal stability is not due to a difference in base composition but indicates that the molecules were only partially complementary, *i.e.* that crossreactions had occurred between nucleic acids of related sequences.[6] Furthermore, decreased thermal stability might indicate decreased chain length.[11] The mismatching is only partial, because the filters containing the hybrids had been subjected to an extensive washing procedure including ribonuclease treatment [1] before the thermal dissociation. Entirely nonspecific binding is thus ruled out.

Table 1 shows that the previously indicated decrease in hybridization with increasing specialization of the foetal erythroid cells was true even under conditions of high stringency. The hypothesis that increasing repression of the genome occurs as differentiation proceeds was tested in experiments on RNA-DNA hybridization competition in which the formamide method was used. The technique of simultaneous competition of pulse-labelled and unlabelled RNA from yolk-sac erythroid cells on different days of gestation was exactly as described,[1] except that the reactions were carried out at 37°C in the presence of 40 % formamide in 4 × SSC. Unlabelled RNA from yolk-sac erythroid cells at 11 days of gestation competed more efficiently than RNA from 12-day foetuses for complementary sites on mouse DNA. These data are interpreted as evidence that fewer different RNA molecules are synthesized at later stages of differentiation, *i.e.* that there is a decrease in transcription.

Our results emphasize a useful rule relating to RNA-DNA hybridization experiments with mammalian nucleic acids. With our source, *viz.* developing foetal erythroid cells, greater stringency of reaction conditions was achieved with formamide and low temperature. Within the range of experimental conditions used by us, the main conclusion drawn from the experiments remained unaltered when mismatching of hybrids was avoided in this way. Transcriptional activity decreased in these cells as the process of specialization proceeded. Hence we conclude that differentiation in these cells is accompanied by increasing repression of the genome.

1. Selander, R.-K. and de la Chapelle, A. *Biochim. Biophys. Acta* **247** (1971) 141.
2. Church, R. B. and McCarthy, B. J. *Biochem. Genet.* **2** (1968) 55.
3. McCarthy, B. J. and McConaughy, B. L. *Biochem. Genet.* **2** (1968) 37.
4. Britten, R. J. and Kohne, D. E. *Science* **161** (1968) 529.
5. Gelderman, A. H., Rake, A. V. and Britten, R. J. *Proc. Natl. Acad. Sci. U.S.* **68** (1971) 172.
6. Shearer, R. W. and McCarthy, B. J. *Biochem. Genet.* **4** (1970) 395.
7. Bonner, J., Kund, G. and Bekhor, I. *Biochemistry* **6** (1967) 3650.
8. McConaughy, B. L., Laird, C. D. and McCarthy, B. J. *Biochemistry* **8** (1969) 3289.
9. Marmur, J. and Ts'o, P.O.P. *Biochim. Biophys. Acta* **51** (1961) 32.
10. Subirana, J. A. and Doty, P. *Biopolymers* **4** (1966) 171.
11. McConaughy, B. L. and McCarthy, B. J. *Biochem. Genet.* **4** (1970) 425.

Received March 17, 1972.

Attempts to Prepare Halogenated Cyclopropanones

KURT ANDERSSON and RAJESH KUMAR

Institute of Chemistry, University of Umeå, S-901 87 Umeå, Sweden

Since 1951, when Loftfield proposed that cyclopropanones are intermediates in the Favorsky rearrangement, these ring compounds have been of special interest to organic chemists.[1] Several attempts have been made to synthesize them, but until very recently all these experiments have failed. In 1966 Hammond and Turro succeeded in preparing cyclopropanone and 2,2-dimethylcyclopropanone by the action of diazomethane on ketene and dimethylketene. These compounds were reported to be highly unstable, and reacted at low temperatures with acids, bases and alcohols.[2,3] In 1968 Crandall and Machleder reported that the stable 2,2-di-*t*-butylcyclopropanone (IIIa) could be isolated from the peracid oxidation of 1,1-di-*t*-butylallene (Ia).[4] The authors consider