TRANSCRIPTION FROM UNIQUE AND REDUNDANT DNA SEQUENCES IN SEA URCHIN EMBRYOS

R. Stephen McColl and Arthur I. Aronson
Department of Biological Sciences
Purdue University, Lafayette, Ind. 47907

Received November 1,1973

SUMMARY

Heterogeneous nuclear RNA is transcribed from both unique and moderately redundant DNA sequences in blastula stage sea urchins. Extremely rapidly reassociating DNA sequences are apparently not transcribed. In contrast, messenger RNA is transcribed almost entirely from unique DNA sequences. These differences may reflect the selective processing of mRNA in the nucleus or selective transport of mRNA molecules.

INTRODUCTION

The synthesis of DNA-like RNA in the nucleus of the eukaryotic cell is characterized by a rapid initial incorporation of labelled precursor into a class of large, heterogeneous RNA molecules (HnRNA). Most HnRNA (85% or more) is confined to the nucleus and turns over rapidly with a half-life of 7-15 min in the sea urchin (1,2). Only a small fraction of the labelled molecules (5-15%) is exported to the cytoplasm as newly synthesized mRNA (3). The function of HnRNA and its relationship to the synthesis of mRNA remains obscure. There is some evidence that mRNA is produced by selective conservation and export to the cytoplasm of specific sequences present within the HnRNA (4).

The genome of eukaryotic organisms contains both unique DNA sequences and a variety of repeated DNA sequences with frequencies ranging from $< 10^1$ to $> 10^6$ (5). By hybridization of a small amount of labelled RNA with a great excess of unlabelled DNA it is possible to determine within a given RNA population the proportion of transcripts derived from any frequency class of DNA (6). We have compared the relative abundance of transcripts from the unique and repetitive DNA classes in HnRNA and mRNA synthesized by hatched blastula embryos of the sea urchin Strongylocentrotus purpuratus. We report here differences between the two populations which may be a consequence of the selective processing of mRNA from HnRNA or of the selective transport of mRNA molecules.

METHODS

Suspensions of Strongylocentrotus purpuratus eggs were fertilized and

cultured as previously described (1,7). Labelled DNA or RNA was obtained by incubation with 0.05 μCi/ml ^{14}C-thymidine (50 mCi/mmol) or 0.150 μCi/ml ^{3}H- guanosine (234 mCi/mmol) respectively for 3 hr prior to harvesting. All embryos were harvested at 24 hr after fertilization and washed 3 times with 1.5 M dextrose.

DNA was prepared by the urea-phosphate method and sheared to lengths of 400-500 nucleotides in a high pressure cell at 50,000 psi (8). DNA:DNA re-annealing experiments were performed as described by Britten and Kohne (5).

Cells were fractionated by a modification of the method of Brandhorst and Humphreys (3). Washed, pelleted embryos were resuspended in KMT buffer (0.24 M KCl, 5mM $MgCl_2$, 10 mM tris pH 7.4) with 1 mg/ml bentonite plus 0.5% Triton X-100, and homogenized 7-9 strokes in a Dounce homogenizer with a B pestle The lysate was layered over 1 M sucrose / 5mM $MgCl_2$ / 10 mM tris pH 7.4 and centrifuged 20 min at 3000 G. RNA extracted from the pellet is referred to as HnRNA. The supernatant above the sucrose was clarified by centrifuging at 10,000 G for 20 min and the polysomes pelleted by centrifuging through a discontinuous sucrose gradient (equal volumes of 15%, 25%, and 35% sucrose in KMT) for 10 hr at 25,000 rpm at 4°C in a SW 25.1 rotor. The resulting pellet was resuspended in KMT buffer, brought to 0.1 M EDTA to dissociate polysomes, and centrifuged through a 10-35% linear sucrose gradient (with 50 mM KCl, 10 mM tris pH 7.4) at 25,000 rpm for 10 hr at 4°C in a SW 25.1 rotor. RNA was extracted from the top 2/3 of the gradient and is referred to as polysomal RNA (pRNA).

Both the nuclear and polysomal preparations were adjusted to 100 mM tris pH 9.0, 0.5% SDS, 0.5% DOC, 100 μg/ml bentonite, and extracted several times with chloroform:phenol (1:1). Two volumes of absolute ethanol were added to the aqueous phase and the pellet collected by centrifugation at 5,000 G. The pellet was dissolved in 0.1 M tris pH 7.4 / 1 mM $MgCl_2$ / 1 mg/ml bentonite and incubated with DNase (Worthington DPFF) at 20 μg/ml at 20°C for 60 min followed by pronase (Calbiochem, nuclease free B grade), 100 μg/ml at 37°C for 30 min. The RNA was then extracted with an equal volume of chloroform:phenol (1:1) and further purified by successive precipitations with ethanol, 2 M LiCl, and 2 mM cetyltrimethylammonium bromide. The RNA was finally dissolved in a small volume of 0.01 M EDTA pH 7.0.

RESULTS AND DISCUSSION

The kinetics of hybridization of HnRNA to a great excess of sheared, denatured DNA is presented in Fig. 1. Using a value of $C_o t$ 20 as the boundary between the redundant and unique frequency classes*, it can be seen

*The $C_o t$ kinetics in these experiments are similar to those reported by Britten et al. (9) and C. Miller [reported in (10)]. Three major classes of DNA may be arbitrarily defined by their reassociation kinetics and relative abundance:

Extremely redundant DNA	$C_o t < 10^{-4}$, 5% of genome
Moderately redundant DNA	$C_o t$ 10^{-2} to 2×10^{1}, 25% of genome
Unique DNA	$C_o t > 2 \times 10^{1}$, 70% of genome

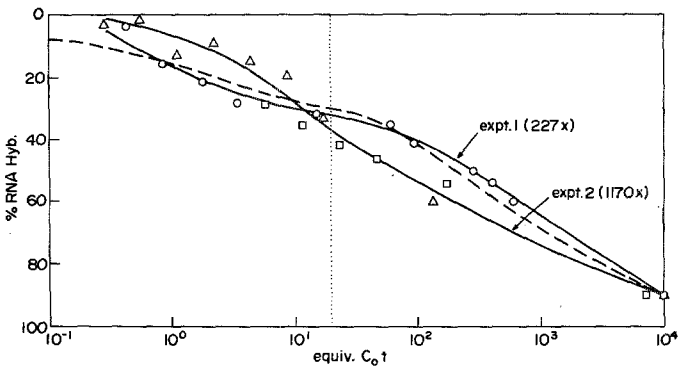

Fig. 1. Kinetics of hybridization of HnRNA with a great excess of DNA. Input DNA:RNA ratios for two separate experiments are shown. All points represent the average value of duplicate samples. All reactions were carried out at 60°C in 0.12 M Na phosphate / 0.25% SDS / .0025 M EDTA / .002 M TES pH 6.7. For prolonged incubations, the Na phosphate concentration was raised to 0.40 M. The reaction was terminated by rapid cooling at a specified $C_o t$ and the extent of hybridization was assayed by RNase treatment (5 μg/ml RNase A, 2 U/ml RNase T_1 15 min at 25°C) followed by separation of hybrids by hydroxyapatite chromatography. Since DNA is 90% reassociated by $C_o t$ 10^4, all values for % RNA hybridized are normalized such that 90% of the hybridizable RNA counts are reacted by $C_o t$ 10^4. Actual % RNA counts finally hybridized was 37.5% (expt. 1) and 45% (expt. 2).

- - - - - - - DNA:DNA reassociation. ———— DNA:RNA hybridization. (ratios shown)

that approximately 30-35% of the HnRNA hybridizes to redundant DNA and about 70% to unique DNA. There is no detectable hybridization before $C_o t$ 10^{-1} and therefore only moderately redundant and not extremely redundant DNA is transcribed at this stage of development. In two separate experiments using DNA:RNA ratios of 227 and 1170 (assuming all A_{260} absorbing material is HnRNA) the hybridization curve is similar to the curve for reassociation of the moderately redundant and unique DNA. Thus, in HnRNA any frequency class of DNA except the extremely redundant is represented by RNA transcripts whose abundance is approximately proportionate to the abundance of that class in the genome. There appears to be no greatly disproportionate transcription of any one frequency class of genes, but rather a representative spectrum of RNA transcribed from many frequency classes.

The kinetics of hybridization of pRNA are presented in Fig. 2. Labelled pRNA hybridizes almost exclusively to unique DNA. In two separate experiments with DNA:RNA ratios of 486 and 923, only 8% and 3% respectively of the RNA hybridized before $C_o t$ 20. It should be noted that the stated ratios are based on the calculation that only about 2% of the total input RNA consists of labelled, hybridizable, messenger-like RNA sequences (3). Most of the remaining 98% consists of unlabelled rRNA which is essentially unreactive at the

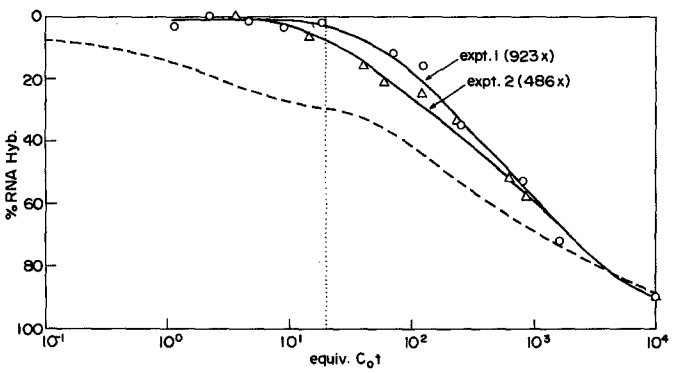

Fig. 2. Kinetics of hybridization of pRNA with a great excess of DNA. Details are as in Fig. 1. Actual % RNA counts hybridized was 51.4% (expt. 1) and 54.9% (expt. 2).

- - - - - - - DNA:DNA reassociation. ———— DNA:RNA hybridization. (ratios shown)

DNA:RNA ratios used here. We therefore consider the labelled pRNA which hybridizes in Fig. 2 to actually be equivalent to functional mRNA especially since it is released from polysomes by EDTA treatment (see Methods). Moreover, very little of the EDTA-insensitive labelled ribonucleoprotein characteristic of nuclear RNA contamination is found in the polysome region of the cytoplasmic extracts. The use of Triton X-100 during homogenization, which permits isolation of "nuclei" without cytoplasmic tags by removing most of the nuclear membrane material, releases very little pulse-labelled RNA from the nucleus (11) About 2% of the unique DNA will form hybrids before C_0t 20. The remaining hybrids which form before C_0t 20 may represent either small traces of contaminating HnRNA or the presence of a small percentage of redundant sequence transcripts in mRNA.

The extent of RNA hybridizing in the experiments reported here (see legends Figs. 1 and 2) is similar to other values in the literature but somewhat less than that reported by Goldberg et al. (12) for very high DNA:RNA ratios. The fraction of RNA hybridizing will depend on (1) the extent of DNA excess and C_0t, (2) the relative frequency of various RNA molecules in each population and (3) experimental artifacts due to RNA and DNA shearing and imperfect base pairing in the hybrids. Any generalizations about the data are of course limited by these restrictions.

Such differences between nuclear and mRNA populations have been reported for Dictyostelium (13), cells in culture (14), and for sea urchin embryos at the gastrula stage (12). While there are quantitative differences in terms of the percent repetitive DNA transcripts found in the mRNA fraction, a relative reduction was found in all cases.

The reduced frequency of repetitive transcripts in mRNA relative to that in HnRNA has different implications depending on the model adopted. If mRNA's are excised from HnRNA sequences, there would have to be selective removal of repetitive sequences. A rather extensive processing is implied since most of the repetitive sequences in sea urchin DNA are interspersed with unique sequences (10). Perhaps the repetitive sequences in the HnRNA have a prominent role in this processing via structural alterations of the HnRNA or by some other property of their primary sequence. It is also important to note that Glisin et al. (15) have evidence for changes in repetitive sequence transcripts during development of sea urchin embryos.

If on the other hand, mRNA's are synthesized and transported to the cytoplasm as unique transcripts without processing, then HnRNA molecules containing sequences complementary to repetitive DNA would have a completely separate function in the nucleus. Since only 5-15% of the nuclear RNA synthesized at any time is transported to the cytoplasm, this possibility must be seriously considered.

In either case, it is clear that whatever the function of repetitive DNA sequences, they are transcribed and presumably have primarily a nuclear function

ACKNOWLEDGEMENTS

This research was supported by the National Science Foundation and a David Ross Fellowship from the Purdue Research Foundation.

REFERENCES

1. Aronson, A. I., and Wilt, F. W., Proc. Nat. Acad. Sci. 62, 186 (1969).
2. Brandhorst, B. P., and Humphreys, T., Biochemistry 10, 877 (1971).
3. Brandhorst, B. P., and Humphreys, T., J. Cell Biol. 53, 471 (1972).
4. Darnell, J. E., Jelinek, W. R., and Molloy, G. R., Science 181, 1215 (1973).
5. Britten, R. J., and Kohne, K. E., Science 161, 529 (1968).
6. Melli, M., Whitfield, C., Rao, K. V., Richardson, M., and Bishop, J. O. Nature New Biology 231, 8 (1971).
7. Hinegardner, R. T., in Methods in Developmental Biology, F. H. Wilt and N. K. Wessells, eds., T. Y. Crowell, New York, 1967, p. 139.
8. Britten, R. J., Pavich, M., and Smith, J., Carn. Inst. Wash. Yearb. 68, 400 (1970).
9. Britten, R. J., Graham, D. E., and Henery, M., Carn. Inst. Wash. Yearb. 71, 270 (1972).
10. Britten, R. J. Brookhaven Symp. 23, 80 (1972).
11. Aronson, A. I., Wilt, F. H., and Wartiovaara, J., Exp. Cell Res. 72, 309 (1972).
12. Goldberg, R. B., Galau, G. A., Britten, R. J., and Davidson, E. H., in press (1973).
13. Firtel, R. A., Jacobson, A., and Lodish, H. F., Nature New Biology 239, 225 (1972).
14. Greenberg, J. R., and Perry, R. P., J. Cell Biol. 50, 774 (1971).
15. Glisin, V. R., Glisin, M. V., and Doty, P., Proc. Nat. Acad. Sci. 56, 285 (1966).