The Detection of Messenger Ribonucleic Acid Sequences in Heterogeneous Nuclear Ribonucleic Acid Fractions of the Oestrogen-Stimulated Rat Uterus

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cDNA (complementary DNA) complementary to the abundant sequences of mRNA isolated from oestrogen-stimulated uterus was hybridized to polyadenylated and non-polyadenylated uterine hnRNA (heterogeneous nuclear RNA). High-molecular-weight polyadenylated hnRNA, isolated under denaturing conditions, was able to saturate the cDNA, but complementary sequences were in low abundance when compared with homologous mRNA. The mRNA sequence content of the polyadenylated hnRNA increased considerably during oestrogen-induced growth of the uterus. Non-polyadenylated hnRNA also contained sequences complementary to the cDNA.

Previous reports from this laboratory have shown that an early action of oestrogen in the immature rat uterus is a dramatic stimulation of the synthesis of three classes of hnRNA (Knowler & Smellie, 1973; Aziz & Knowler, 1978). Subsequently, mRNA, which is presumably derived from hnRNA, accumulates in the cytoplasm and brings about the aggregation of pre-existing ribosomes into polyribosomes (Teng & Hamilton, 1967; Merryweather & Knowler, 1977, 1980). This early response, and the translation of the mRNA into a small number of ill-characterized proteins (Garland *et al.*, 1978), appears to be a prerequisite for the subsequent striking increase in the synthesis of rRNA, the accumulation of new ribosomes and the overall growth response of the uterus (Knowler & Smellie, 1971; Luck & Hamilton, 1972; Borthwick & Smellie, 1975).

We have shown that, during these early changes, the population of uterine mRNA alters dramatically such that, of the 8000 diverse sequences expressed in an immature uterus responding to 4 h oestrogen treatment, very few were present at the same concentration in unstimulated rats (Aziz *et al.*, 1979a,b). The present paper attempts to relate the changes in mRNA population to the changes in hnRNA synthesis and shows that mRNA sequences can be detected in the hnRNA population.

Abbreviations used: hnRNA, heterogeneous nuclear RNA; cDNA, complementary DNA.

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Materials and Methods

The reagents, experimental animals, preparation of uterine polyribosomal mRNA, preparation and characterization of cDNA, conditions for mRNA · cDNA hybridization and generation of computer-fitted hybridization curves have been previously described (Aziz *et al.*, 1979a). The fractionation of uterine messenger cDNA and preparation of abundant-sequence cDNA have also been previously described (Aziz *et al.*, 1979b).

Uterine high-molecular-weight hnRNA was purified and fractionated on poly(U)–Sepharose as described by Aziz & Knowler (1978). For further purification, the high-molecular-weight species, sedimenting at greater than 45S on aqueous sucrose density gradients (Aziz & Knowler, 1978), were collected by ethanol precipitation in the presence of 0.14 M-NaCl and resedimented in denaturing gradients as described by McNaughton *et al.* (1974). Briefly, the RNA was denatured in 90% (v/v) formamide in a boiling-water bath for 3 min and layered over a 13 ml 5–20% (w/v) sucrose density gradient containing 85% formamide, 0.002 M-EDTA and 0.01 M-Tris/HCl, pH 7.5. Sedimentation was at 28000 rev./min at 30°C for 20 h in the SW40 rotor of a Beckman L2-65B ultracentrifuge. hnRNA species maintaining sedimentation values of 45S and greater, relative to HeLa-cell nucleolar 45S and 32S markers run on parallel gradients (Aziz & Knowler, 1978), were pooled from two separate preparations. They were then subjected to fractionation on poly(U)–Sepharose as previously described (Aziz & Knowler, 1978).

Results and Discussion

The preparation of high-molecular-weight uterine hnRNA used in this laboratory (Aziz & Knowler, 1978) employs sedimentation of RNA through sucrose density gradients and collection of RNA larger than 45S. This high-molecular-weight hnRNA is then further fractionated on poly(U)-Sepharose into those species containing poly(A), those containing oligo(A) and those not polyadenylated (Aziz & Knowler, 1978). Any attempt to demonstrate sequences coding for mRNA in hnRNA can be frustrated by the possibility that the hnRNA itself may be contaminated with mRNA. For this reason, all experiments here reported show duplicate analyses. In the first of these, hnRNA was prepared as described by Aziz & Knowler (1978), but in repeats the hnRNA of sedimentation value greater than 45S was collected and resedimented in denaturing sucrose gradients as described in the Materials and Methods section. Only RNA which retained sedimentation values of 45S or greater under denaturing conditions was then fractionated on poly(U)-Sepharose and used in hybridization studies with messenger cDNA. Such a rigorous selection of high-molecular-weight hnRNA resulted in very low yields and insufficient material was recovered from complete R_0t -curve analysis. The hybridization studies described therefore concentrate on attempting to demonstrate complementarity between the hnRNA and abundant messenger cDNA.

Fig. 1 illustrates the hybridization of poly(A)-containing high-molecular-weight hnRNA to cDNA complementary to the abundant species of mRNA from the uteri of the rats treated with oestradiol-17 β [oestra-1,3,5(10)-triene-3,17 β -diol] 4 h before death. This 4 h mRNA population represents the peak of a dramatic population change which occurs in immature animals responding to a single oestrogen administration (Aziz *et al.*, 1979b). The hnRNA is prepared from two earlier times in the oestrogen-induced response. A 30 min time point is taken to represent the time at which hormone-stimulated hnRNA synthesis is first observed, and is most dramatic in polyadenylated species (Knowler & Smellie, 1973; Aziz & Knowler, 1978). A 2 h time point is taken because the stimulation of hnRNA synthesis reaches a peak at this time (Aziz & Knowler, 1978) and because the most profound changes in the abundant uterine mRNA populations occur in the first 2 h of the oestrogenic response (Aziz *et al.*, 1979b). It would also be desirable to investigate hnRNA from unstimulated animals, but yields are so small as to make this impractical.

Fig. 1 shows that both preparations of polyadenylated high-molecular-weight hnRNA are fully able to saturate the abundant cDNA preparation in that they hybridize with it to the same extent

Fig. 1. Hybridization of cDNA of abundant uterine mRNA against uterine polyadenylated hnRNA

Each point represents 2000 c.p.m. of [3 H]cDNA and 1 mg of RNA/ml. The solid curve (O—O) represents hybridization of the abundant cDNA to its own template, namely polyribosomal mRNA from rats receiving 1.0 μ g of oestradiol-17 β 4 h before death. The remaining curves illustrate heterologous hybridization between the cDNA and polyadenylated hnRNA: —, hnRNA recovered from 2h-oestradiol-treated rats by using non-denaturing gradients; ●, hnRNA recovered from 2h-treated rats by using non-denaturing gradients, followed by denaturation in gradients containing 85% formamide; ..., hnRNA recovered from 30 min-oestradiol-treated rats by using non-denaturing gradients; Δ , hnRNA recovered from 30 min-oestradiol-treated rats by using non-denaturing followed by denaturing gradients. The curves are all computer-fitted to the data; the points of the curves for heterologous hybridization are omitted for reasons of clarity.

as the homologous mRNA. It is noticeable that the rate of hybridization differs between the two hnRNA preparations. As expected, they both hybridize much slower than mRNA, reflecting a greater dilution of complementary sequences in hnRNA as compared with mRNA. However, the preparation from rats treated with oestradiol for 30 min hybridizes much slower than that from rats receiving 2 h oestrogen treatment. The former has a $R_0t_{\frac{1}{2}}$ of 60 mol $\cdot$ s $\cdot$ litre $^{-1}$ and the latter has a $R_0t_{\frac{1}{2}}$ of 1.5 mol $\cdot$ s $\cdot$ litre $^{-1}$. The abundant mRNA content of the two high-molecular-weight hnRNA preparations could be estimated by comparing the above values with the corrected $R_0t_{\frac{1}{2}}$ value of 0.051 mol $\cdot$ s $\cdot$ litre $^{-1}$ obtained with homologous-mRNA $\cdot$ abundant-cDNA hybridization (Aziz *et al.*, 1979b). Thus the content of 4h-oestrogen-treated uterine abundant mRNA in the hnRNA from 30 min- and 2h-treated rats was 0.085% and 3.4% respectively.

Fig. 1 also shows that the results obtained were essentially identical whether the hnRNA was isolated as a high-molecular-weight preparation from non-denaturing gradients or from non-denaturing gradients followed by denaturing gradients.

Fig. 2 shows the hybridization of the cDNA to non-polyadenylated hnRNA purified from the uteri of rats given oestradiol- 17β 2h before death. Again, sequences complementary to the cDNA are present, but on this occasion hybridization is incomplete at a R_0t of 20000, indicating that some abundant mRNA sequences may be absent from the non-polyadenylated hnRNA. The $R_0t_{1/2}$ of $70 \text{ mol} \cdot \text{s} \cdot \text{litre}^{-1}$, compared with that of $1.5 \text{ mol} \cdot \text{s} \cdot \text{litre}^{-1}$ for polyadenylated hnRNA from the same 2h-oestrogen-treated rats, indicates a much lower content (0.073%) of abundant messenger sequences in the non-polyadenylated species. It was again

noted that there was no difference between the results obtained with high-molecular-weight hnRNA isolated from non-denaturing gradients and that obtained from non-denaturing followed by denaturing gradients.

The results show that two uterine hnRNA components, a polyadenylated fraction and a non-polyadenylated fraction, each contain sequences complementary to abundant messenger cDNA from 4h-oestrogen-treated rats. The two hnRNA fractions must therefore have sequences in common. The polyadenylated fraction contains complementary sequences 30min and 2h after oestrogen administration to the rats, but such sequences are most abundant in the preparation from 2h-treated rats. Thus the changes in the population of mRNA species in the uterus responding to oestrogen are reflected in changing populations of polyadenylated hnRNA.

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Fig. 2. Hybridization of cDNA of abundant uterine mRNA against uterine non-polyadenylated hnRNA. Each point represents 2000 c.p.m. of $[^3\text{H}]$ cDNA. The solid curve (O—O) represents hybridization of the abundant cDNA to its own template and is reproduced from Fig. 1. ---, Heterologous hybridization between the cDNA and non-polyadenylated hnRNA recovered from 2h-oestradiol-treated rats by using non-denaturing gradients. The curve is a computer fit of data, the points of which are omitted for reasons of clarity. The symbols represent similar heterologous hybridization in which the high-molecular-weight hnRNA from rats treated with oestradiol 2h before death was isolated from non-denaturing gradients followed by denaturing gradients: ●, 1 mg of RNA/ml; ▲, 5 mg of RNA/ml.