Turnover of Nucleic Acids in a Multiplying Animal Cell

2. RETENTION STUDIES

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The work of Schoenheimer and others (see Schoenheimer, 1942) indicated that the protein and RNA of animal tissues are continuously replaced by a process of turnover. A number of studies on homogeneous populations of cells has, however, provided evidence that turnover of this sort does not occur in exponentially multiplying cells (Hogness, Cohn & Monod, 1955; Siminovitch & Graham, 1956; Mandelstam, 1960), although turnover of protein and RNA undoubtedly occurs in non-multiplying cells (Mandelstam, 1958; Harris & Watts, 1958; Watts & Harris, 1959; Harris, 1959). It has been shown in studies on the non-multiplying rabbit macrophage (Watts & Harris, 1959) that, to detect turnover when it occurs, certain criteria of experimental design must be satisfied, and it has been argued that many of the experiments designed to investigate turnover in multiplying cells have not fulfilled these criteria. It was found that the non-multiplying cell, in which no net synthesis of nucleic acid occurs, could reutilize the breakdown products of RNA turnover very efficiently; and it is reasonable to assume that in multiplying cells, where there is net synthesis of nucleic acids, reutilization would be even more efficient. To obtain release of breakdown products of RNA from the non-multiplying cell it was necessary to provide an alternative supply of exogenous precursor that could be used in the resynthesis of RNA. Further, the actual choice of precursor was critical, and different precursors gave widely different observed rates of turnover.

The work on the rabbit macrophage showed that the RNA turned over rapidly and extensively. The nucleus was found to be the major site of this metabolic activity, although it was not possible to decide where in the cell the breakdown actually occurred (Harris, 1959). A similar pattern of RNA metabolism has been observed in exponentially multiplying cells, but the absence of experimental evidence that RNA turnover occurs in multiplying cells has led investigators to assume that this pattern represented synthesis of RNA in the nucleus and subsequent translocation of this RNA to the cytoplasm (Goldstein & Plaut, 1955; Woods & Taylor, 1959; Fitzgerald & Vinijchaikul, 1959; Zalokar, 1959).

The most important single piece of evidence that has been quoted in support of this view is the observation that, when cells that have been grown for a short time in medium containing a radioactive precursor of RNA are transferred to non-radioactive medium containing unlabelled precursors, incorporation of radioactivity into the cytoplasmic RNA continues whereas the amount of radioactivity in the nuclear RNA decreases (Goldstein & Plaut, 1955; Woods & Taylor, 1959; Fitzgerald & Vinijchaikul, 1959; Zalokar, 1959; Harris, 1959). It is known, however, that growing cells accumulate large and persistent pools of radioactive compounds after even short periods of incubation in medium containing labelled precursors of RNA, and these radioactive pools cannot easily be washed out of the cell or readily diluted by unlabelled exogenous precursors (Harris, 1959; Harris & Watts, 1962). Under these conditions, therefore, part at least of the continued rise in the amount of radioactivity in cytoplasmic RNA may be due to incorporation of radioactive compounds from the intracellular pools. Harris & Watts (1962) have also shown that, with exponentially growing HeLa cells, a considerable turnover of RNA takes place. In one experiment in which the RNA was labelled for 10 min., 42 % of the total radioactivity initially present in the RNA was lost during 24 hr. growth in medium containing unlabelled precursors. The simple model that explained these findings by proposing a translocation of RNA from nucleus to cytoplasm is thus based on rather ambiguous evidence.

The idea that cytoplasmic RNA is synthesized in the nucleus is clearly of some importance if it is correct, but the ambiguity of the supporting evidence makes it advisable that the 'chase' experiment should be examined in more detail so as to determine, if possible, the factors that affect the observed kinetics. The experiments described below constitute a general analysis of the 'chase' experiment under conditions that permit the results to be compared directly with those obtained in the study of turnover of RNA in the rabbit macrophage (Watts & Harris, 1959).

EXPERIMENTAL

The cells used in these experiments were grown in suspension culture. The methods of cell culture and analysis of RNA fractions were as described by Watts (1964).

Isolation of purines from the medium. The cells were spun down from the medium at 220g for 3 min., 1 ml. of supernatant was cooled to 0° and made 0.3N with respect to trichloroacetic acid. The precipitate of protein was spun at 2000g for 10 min., and 0.5 ml. of supernatant was taken, acidified with 2 ml. of 0.1N-H₂SO₄ and heated for 1 hr. at 100°. The silver salts of the purines were precipitated with 0.5 ml. of 20% (w/v) AgNO₃ soln., spun down at 500g for 5 min. at 0° and extracted with 1 ml. of N-HCl for 15 min. at 70°. The extract was collected and evaporated to dryness *in vacuo*. Adenine, hypoxanthine and guanine were separated by chromatography on Whatman no. 1 filter paper with first a solvent consisting of butan-1-ol saturated with water and containing 1% (v/v) of aq. NH₃ soln. (sp.gr. 0.880) (MacNutt, 1952), followed in the same direction by a solvent consisting of methanol-ethanol-water-conc. HCl (50:25:19:6, by vol.) (Kirby, 1955). The purines were located and analysed for specific radioactivity as described by Watts (1964).

RESULTS

Choice of unlabelled precursors for use in 'chase' experiments. When cells that had been labelled by growth in medium containing [8-¹⁴C]adenine were transferred into non-radioactive medium containing different adenine and hypoxanthine derivatives, the changes in the specific radioactivity of the adenine in nuclear and cytoplasmic RNA were found to depend on the choice of unlabelled precursor. In the experiments shown in Figs. 1 and 2 the radioactive cells were transferred to a modified 'Eagle's medium' to which 0.25% (w/v) of bovine serum albumin was added instead of whole pig serum, to eliminate serum enzymes that rapidly degrade nucleotides and adenosine derivatives (Watts & Harris, 1959). The unlabelled precursors were added to this medium. The effects of the different precursors were similar to those found in studies on the rabbit macrophage (Watts & Harris, 1959). During the first hour the specific radioactivity of nuclear RNA adenine fell in all samples. This does not normally happen and is probably due to the use of bovine serum albumin instead of pig serum. After the first hour there was no further fall in the specific radioactivity of the nuclear RNA adenine in the control culture that contained no added precursors. A similar result was obtained when the medium contained hypoxanthine, inosine or inosine 5'-phosphate. However, all the adenine derivatives tested produced a steady fall in the specific radioactivity of nuclear RNA adenine, the greatest fall being obtained with adenosine and the adenylic acids. All the corresponding curves for the cytoplasmic RNA adenine showed a large increase in specific radioactivity during the experi-

ment, but the rate and magnitude of the rise varied with the non-radioactive precursor in the medium. Again, the hypoxanthine derivatives had little effect on the rise in the specific radioactivity of the cytoplasmic RNA adenine, but all the adenine

Fig. 1. Retention of [8-¹⁴C]adenine in the nuclear RNA of HeLa cells. The cells were grown for 1 hr. in medium containing 0.01 mM-[8-¹⁴C]adenine (initial specific radioactivity 3.5×10^6 counts/min./μmole) and then transferred to non-radioactive medium containing different purine derivatives at a concentration of 0.2 mM. Samples were taken at intervals and the specific radioactivity of the adenine of the nuclear RNA was determined. ○, Control without added purines in the medium; ●, hypoxanthine in the medium; △, adenine in the medium; ▲, adenosine 3'-phosphate in the medium.

Fig. 2. Retention of [8-¹⁴C]adenine in the cytoplasmic RNA of HeLa cells. The experiment was that described under Fig. 1. The specific radioactivity of the adenine of the cytoplasmic RNA was determined. ○, Control without added purines in the medium; ●, hypoxanthine in the medium; △, adenine in the medium; ▲, adenosine 3'-phosphate in the medium.

derivatives decreased this rise, adenosine and the adenylic acids being the most effective.

This experiment shows clearly that, after transfer to non-radioactive medium, the cells still contained a large intracellular pool of radioactivity. This pool persisted throughout the experiment and could not be washed out. There is about 3–4 times as much cytoplasmic RNA as nuclear RNA (Harris & Watts, 1962). When this was taken into account it was found that the radioactivity in the total RNA of the control culture, which contained no added unlabelled precursors, almost doubled during the experiment. The effect of added unlabelled precursors was to diminish this rise in radioactivity, and the most effective precursors in fact completely eliminated a rise in total radioactivity. Two features of these curves require particular emphasis.

(i) The shapes of the curves are not compatible with a simple precursor-product relationship between nuclear RNA and cytoplasmic RNA. Thus the control cytoplasmic RNA curve is virtually linear but the control nuclear RNA curve is strongly curvilinear. Again, in the presence of unlabelled adenosine or the adenylic acids, the most rapid fall in the specific radioactivity of the nuclear RNA occurred at the time when the rate of incorporation of radioactivity into cytoplasmic RNA was negligible.

Fig. 3. Retention of $[8-^{14}\text{C}]$ adenine in the RNA of HeLa cells in the absence of non-radioactive purine derivatives. The cells were grown for 2 hr. in medium containing $0.02 \text{ mM-}[8-^{14}\text{C}]$ adenine (initial specific radioactivity 3.5×10^5 counts/min./ μmole) and then transferred to non-radioactive medium that did not contain added purine derivatives. Samples were taken at intervals and the specific radioactivity of the adenine in the RNA and DNA was determined. ○, Nuclear RNA adenine; ●, cytoplasmic RNA adenine; △, DNA adenine.

(ii) Those conditions that produced the largest rise in the specific radioactivity of the cytoplasmic RNA produced the smallest fall in the specific radioactivity of the nuclear RNA and vice versa. The simple translocation model predicts the opposite.

'Chase' experiment in the absence of unlabelled precursors. The experiment illustrated in Figs. 1 and 2 was repeated under the normal conditions of cell culture. When cells that had been labelled for 2 hr. in medium containing $[8-^{14}\text{C}]$ adenine were transferred to non-radioactive medium containing no added purine derivatives, radioactivity continued to enter RNA and DNA for some hours (Figs. 3, 4). Since the specific radioactivities of both the adenine and guanine in the DNA continued to rise in an approximately linear fashion throughout the experiment, the intracellular pool of radioactivity must have been retained very efficiently. The specific radioactivity of adenine in nuclear RNA also showed a 'pool effect', i.e. a continued incorporation of radioactivity from the intracellular pool, during the first hour, but after this fell rapidly. The guanine in the nuclear RNA showed a much larger and more protracted 'pool effect' than the adenine, the specific radioactivity rising steeply during the first 3 hr.; but the subsequent fall in specific radioactivity was correspondingly greater, about 40 % in the last 5 hr. of the experiment, compared with 30 % in the last 7 hr. for the nuclear RNA adenine. The specific

Fig. 4. Retention of $[8-^{14}\text{C}]$ guanine in the RNA of HeLa cells in the absence of non-radioactive purine derivatives. The experiment was that described under Fig. 3. The specific radioactivity of the guanine in RNA and DNA was determined. ○, Nuclear RNA guanine; ●, cytoplasmic RNA guanine; △, DNA guanine.

radioactivity of the cytoplasmic RNA rose during the experiment, and the kinetics of incorporation of radioactivity into adenine and guanine showed no detailed correspondence with the fall in nuclear RNA specific radioactivities. When the relative amounts of nuclear and cytoplasmic RNA were taken into account (Harris & Watts, 1962), about three times as much radioactivity appeared in the cytoplasmic RNA as was lost from the nuclear RNA. It is therefore not possible to decide from this experiment whether turnover, or translocation, or both, take place.

'Chase' experiment in the presence of unlabelled adenosine. When cells that had been labelled in medium containing $[8-^{14}\text{C}]$ adenine were transferred to medium containing unlabelled adenosine, the results shown in Fig. 5 were obtained. The unlabelled precursor virtually eliminated the 'pool effect' in adenine in nuclear RNA and increased the observed fall in specific radioactivity to 65 % in 8 hr. The specific radioactivity of the adenine in cytoplasmic RNA continued to rise for about 4 hr., but levelled off after this time. In contrast with the corresponding experiment in the absence of added unlabelled precursors, the increase in the total amount of radioactivity in the adenine in cytoplasmic RNA was about the same as the total loss of radioactivity from the adenine in nuclear RNA. The specific radioactivity curve of the adenine in the total RNA of the cell, as obtained by acid pre-

cipitation without prior treatment of the cells with phenol, is also illustrated. This curve shows that there was a 30 % increase in the amount of radioactivity in the total RNA adenine of the cell during the first 3 hr., so that a large part of the increase in the specific radioactivity of the adenine in the cytoplasmic RNA could have been due to the 'pool effect'. The specific radioactivity of the adenine in the total RNA of the cell fell by 23 % during the last 5 hr. of the experiment. This fall is far greater than can be accounted for by dilution of the radioactive RNA due to growth of the cells (Singh & Koppelman, 1963). It must therefore be assumed that turnover of RNA was taking place during this time. The fall in the specific radioactivity of the adenine in the total RNA of the cells began at about the time that incorporation into the adenine in the cytoplasmic RNA levelled off. This finding supports the view that at least part of the fall in specific radioactivity of the nuclear RNA was the result of degradation of RNA.

'Chase' experiment after different periods of labelling. The study of incorporation of labelled precursors into the RNA of HeLa cells (Watts, 1964) indicated that nuclear RNA contains several fractions that are labelled at different rates. The effect of unlabelled adenosine on the retention of labelled adenine in RNA was therefore examined in cells that had been labelled for a long time (16 hr.) and for a relatively short time (10 min.). When compared with the results obtained after 2 hr. in radioactive medium (Fig. 5), prolonged labelling had little effect on the rate at which radioactivity was lost from the adenine of the nuclear RNA when the cells were subsequently grown in non-radioactive medium containing unlabelled adenosine (Fig. 6). The greatest difference observed was that the specific radioactivity of the adenine in the cytoplasmic RNA showed only a relatively small 'pool effect', presumably because the contribution of the pool was very much smaller relative to the total radioactivity initially present in the cytoplasmic RNA. However, when the period of labelling was decreased to 10 min., the subsequent loss of radioactivity from the adenine in the nuclear RNA was very much faster. In the experiment shown in Fig. 7, 77 % of the radioactivity in the adenine in the nuclear RNA was lost during the last 5 hr. of the experiment. There was virtually no change in the specific radioactivity of the cytoplasmic RNA adenine during the last 2 hr., but the fall in the specific radioactivity of the nuclear RNA adenine was maintained. Even after the most optimistic corrections have been made for growth, the loss of radioactivity from the nuclear RNA adenine greatly exceeds the increase in the amount of radioactivity in the cytoplasmic RNA adenine. This experiment thus demonstrates that appreci-

Fig. 5. Retention of $[8-^{14}\text{C}]$ adenine in the RNA of HeLa cells in the presence of non-radioactive adenosine. The cells were grown for 2 hr. in medium containing 0.02 mM $[8-^{14}\text{C}]$ adenine (initial specific radioactivity 9×10^5 counts/min./ μmole) and then transferred to non-radioactive medium containing 1 mM-adenosine. Samples were taken at intervals and the specific radioactivity of the adenine in RNA was determined. O, Nuclear RNA adenine; ●, cytoplasmic RNA adenine; Δ, total RNA adenine.

able turnover of RNA has occurred. Taken in conjunction with the experiment shown in Fig. 6, it also shows that nuclear RNA contains at least two fractions that turn over at different rates.

'Chase' experiment in cells labelled with $[2-^{14}\text{C}]$ -uridylic acid. When the cells were grown for 10 min. in medium containing $[2-^{14}\text{C}]$ uridylic acid and then transferred to medium containing non-radioactive uridine and cytidine, the results shown in Fig. 8 were obtained. The pattern of labelling was similar to that observed in the experiments with $[8-^{14}\text{C}]$ -adenine. The specific radioactivity curve for the uracil in the total RNA of the cells again showed an initial 'pool effect', whereas the sharp fall in the curve between 15 and 90 min. indicated that turnover of RNA was occurring.

'Chase' experiment in cells labelled with $[^{32}\text{P}]$ -orthophosphate. The experiments on the rabbit macrophage (Watts & Harris, 1959) showed that $[^{32}\text{P}]$ orthophosphate is not a satisfactory precursor for use in studies on turnover of nucleic acids. Since, however, $[^{32}\text{P}]$ orthophosphate has been widely used in this type of work, an experiment was done to determine whether the observations on the macrophage were applicable to the multiplying cell. The cells were labelled for 20 min. in medium containing $[^{32}\text{P}]$ orthophosphate at relatively high concentrations, washed with buffered saline containing orthophosphate, and then resuspended in

Fig. 6. Retention of $[8-^{14}\text{C}]$ adenine in the RNA of HeLa cells after prolonged labelling. The cells were grown for 16 hr. in medium containing 0.15 mM $[8-^{14}\text{C}]$ adenine (initial specific radioactivity $10^5 \text{ counts/min./}\mu\text{mole}$) and then transferred to non-radioactive medium containing 1 mM -adenosine. Samples were taken at intervals and the specific radioactivity of the adenine in the RNA was determined. $\circ$, Nuclear RNA adenine; $\bullet$, cytoplasmic RNA adenine.

Fig. 7. Retention of $[8-^{14}\text{C}]$ adenine in the RNA of HeLa cells after 10 min. labelling. The cells were grown for 10 min. in medium containing 0.005 mM $[8-^{14}\text{C}]$ adenine (initial specific radioactivity $2 \times 10^7 \text{ counts/min./}\mu\text{mole}$) and then transferred to non-radioactive medium containing 1 mM -adenosine. Samples were taken at intervals and the specific radioactivity of the adenine in the RNA was determined. $\circ$, Nuclear RNA adenine; $\bullet$, cytoplasmic RNA adenine.

Fig. 8. Retention of $[2-^{14}\text{C}]$ uridylic acid in the RNA of HeLa cells. The cells were grown for 10 min. in medium containing 0.005 mM $[2-^{14}\text{C}]$ uridylic acid (initial specific radioactivity $8 \times 10^6 \text{ counts/min./}\mu\text{mole}$) and then transferred to non-radioactive medium containing 1 mM -cytidine and 0.5 mM -uridine. Samples were taken at intervals and the specific radioactivity of the uridylic acid in the RNA was determined. $\circ$, Nuclear RNA; $\bullet$, cytoplasmic RNA; Δ , total RNA.

non-radioactive medium. Specimen results are shown in Fig. 9. They are remarkable mainly for the clear-cut demonstration that the cells had accumulated and retained very large pools of ^{32}P -labelled derivatives during the short period of labelling, thus showing that the observations on the macrophage were generally true also for HeLa cells. The presence of these large pools made it impossible to decide whether or not turnover took place, although the kinetics did not suggest any simple precursor-product relationship between nuclear RNA and cytoplasmic RNA.

Recovery of radioactive compounds from the medium. Several attempts were made to determine whether radioactivity appeared in the medium when labelled cells were incubated in medium containing non-radioactive precursors. Radioactivity was in fact found to appear under these conditions, but, even after 24 hr. growth in non-radioactive medium without added precursors, the amount of radioactivity recovered in the medium after the addition of unlabelled precursors was about five-fold greater than could be accounted for by the fall in the specific radioactivity of the nuclear RNA. It was concluded that, even after many hours in non-radioactive medium, the intracellular pools contained considerable amounts of radioactivity. Fig. 10 illustrates one of the recovery experiments. The fall in the specific radioactivity of the adenine in the nuclear RNA mirrored the rise in the amount of radioactivity in the medium, and the point at which both curves flattened off corresponded to the time when all the adenosine in the medium had been deaminated to inosine. It is probable that the curves were related but they do not constitute decisive evidence of RNA turnover.

Discrepancies between the amount of radioactivity in the phenol fractions and in the total ribonucleic acid of the cell. It was shown by Watts (1964) that the amounts of radioactivity in the RNA fractions isolated by the phenol method do not agree with the amount in the total RNA obtained by acid precipitation of whole cells. Consideration of the results shown in Figs. 5 and 8 also reveals a discrepancy of this sort. The specific radioactivity of the adenine in the total RNA of the cell in Fig. 5 did not begin to rise for about 1 hr. Since the curve for nuclear RNA adenine fell steadily during the experiment, the subsequent rise in the curve for total RNA adenine between 1 and 3 hr. implies either that a large and transient 'pool effect' operated during this time, or that a fraction of the total cell RNA rapidly lost its radioactivity during the first hour. In either case it is necessary to assume that a fraction of the RNA was lost during the phenol separation. During the last hour the final specific radioactivity of the total cell RNA adenine in Fig. 5 dropped below both those of the cytoplasmic

RNA and nuclear RNA adenine, so that the total cell RNA at this point contained a fraction of RNA that had a specific radioactivity substantially lower than that of either of the phenol fractions. The simplest explanation of these observations that is also consistent with the results described by

Fig. 9. Retention of $[\text{}^{32}\text{P}]$ orthophosphate in the RNA of HeLa cells. The cells were grown for 20 min. in medium containing 0.03 mM $[\text{}^{32}\text{P}]$ orthophosphate and then transferred to non-radioactive medium containing 1 mM orthophosphate. Samples were taken at intervals and the specific radioactivities of the nucleotides in the RNA were determined. ○, Nuclear RNA cytidylic acid; ●, cytoplasmic RNA cytidylic acid; △, total RNA cytidylic acid.

Fig. 10. Accumulation of radioactivity in the medium when HeLa cells with radioactive RNA were grown in non-radioactive medium. The cells were grown for 6 hr. in medium containing 0.005 mM $[\text{}^{14}\text{C}]$ adenine and then transferred to non-radioactive medium. After growing for a further period of 18 hr. the cells were transferred to non-radioactive medium containing 1 mM adenosine, and samples were taken at intervals and the specific radioactivities of adenine in the RNA and adenosine and inosine in the medium were determined. ●, Nuclear RNA adenine corrected for dilution by growth; ▲, adenosine + inosine in the medium.

Watts (1964) would be that a specific fraction of the nuclear RNA was extensively degraded during phenol extraction. This fraction was turning over very rapidly with a half-life that did not exceed 1 hr. and probably accounted for less than 5 % of the RNA in the cell. It has already been shown that the nuclear RNA isolated by the phenol method contains at least two fractions with different rates of turnover. Thus nuclear RNA apparently consists of at least three fractions that can be distinguished by differing metabolic activities and susceptibility to degradation during isolation.

DISCUSSION

The presence of large intracellular pools of RNA precursors does not permit a simple and unambiguous analysis of the turnover of RNA in the multiplying cell. However, the general pattern of RNA turnover found in the non-multiplying rabbit macrophage (Watts & Harris, 1959; Harris, 1959) occurs also in the HeLa cell. Although the results are somewhat obscured by the high and persistent content of radioactivity in the intracellular pools, there is little doubt that turnover of RNA occurs in exponentially growing HeLa cells. Scott, Taft & Letourneau (1962) have observed a similar process in ascites-tumour cells growing in the peritoneal cavity of mice, and it is probable that turnover of the sort described above is a general phenomenon.

The nuclear RNA apparently consists of at least three fractions that have characteristically different rates of synthesis and breakdown. It is known that phenol does not destroy all ribonuclease activity (Huppert & Pelmont, 1962), but it is remarkable that a specific fraction of RNA should be degraded during the phenol extraction. The present experiments indicate that this fraction is labile and is not a precursor of other, stable, RNA fractions. It is possible that it is located in the cell close to enzyme systems that are able to destroy it and that are not immediately inactivated by cold phenol.

SUMMARY

1. The retention of [8-¹⁴C]adenine, [2-¹⁴C]-uridylic acid and [³²P]orthophosphate in the RNA of multiplying HeLa cells was studied. The results were in general agreement with previous observations that had been made on non-multiplying cells.

2. Turnover of RNA was demonstrated, which appeared to involve mainly nuclear RNA.

3. The cells accumulated large and persistent pools of radioactive precursors of nucleic acids even after brief exposures to radioactive medium. When radioactive cells were transferred to non-radioactive medium, radioactivity continued for several hours to enter DNA and both nuclear RNA and

cytoplasmic RNA. These 'pool effects' were greatest when [³²P]orthophosphate was used to label the cells.

4. The magnitude of the 'pool effect' could be diminished by introducing unlabelled RNA precursors into the non-radioactive medium. When the cells had been labelled with [8-¹⁴C]adenine, the precursors that most effectively diminished the continued rise in radioactivity in RNA were adenosine and the 2'-, 3'- and 5'-phosphates of adenosine. Adenine was less effective, and hypoxanthine, inosine and inosine 5'-phosphate had little effect.

5. When retention of radioactive adenine was studied in medium containing adenosine, the specific radioactivity of adenine in the nuclear RNA fell rapidly, but it was not possible to prevent a rise in the specific radioactivity of adenine in the cytoplasmic RNA.

6. The rate at which the specific radioactivity of adenine in nuclear RNA fell varied with the length of the initial period of growth in radioactive medium. The nuclear RNA contained at least three fractions that had half-lives of turnover between 45 min. and 5 hr.

7. A fraction of nuclear RNA with a fast rate of turnover was degraded to acid-soluble material during the phenol extraction of RNA.

8. The results were not consistent with a simple precursor-product relationship between nuclear and cytoplasmic RNA.

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