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KINETICS OF PULSE-LABELING OF RIBONUCLEIC ACID IN HeLa CELLS

by

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SUMMARY

HeLa cells have been submitted to short tritiated nucleoside pulses, and their radioactivity has been followed in the nucleus minus nucleolus (N), in the nucleolus (n) and in the cytoplasm (C) after various incubation times in unlabeled media. The independent uptake of these nucleosides into the nucleus *minus* nucleolus and the nucleolus has been confirmed — and part of this radioactivity is transferred from both these cell parts into the cytoplasm. However, the cytoplasmic gain is greater than the nuclear loss of grains. Cytoplasmic ribosomal RNA is probably formed from precursors synthesized in the nucleus and nucleolus, but it is not known whether this RNA undergoes any rearrangement during its transfer to the cytoplasm. Messenger RNA constitutes probably also an important fraction of the RNA label transferred from the nucleus to the cytoplasm.

INTRODUCTION

The kinetics of labeling of RNA in a variety of biological materials has led to the concept that cytoplasmic RNA originates in the nucleus[1-4]. It has been demonstrated in microbeam experiments that more than 60% of cytoplasmic RNA is dependent on the integrity of the nucleolus, and most of the remaining RNA, if not all, is dependent on the rest of the nucleus[1].

There is however as yet no unequivocal evidence demonstrating the passage of macromolecular RNA from the nucleus to the cytoplasm and pulse experiments by HARRIS[5] have led this author to conclude that only a minor fraction of nuclear RNA migrates to the cytoplasm in cultured macrophage or connective tissue cells. However, PERRY *et al.*[3] have argued that cell geometry considerations may have obscured these results. In a recent communication HARRIS[5] supports the view that nuclear RNA is first broken down before its constituent nucleotides become reincorporated into cytoplasmic RNA.

In the course of preliminary studies on the migration of pulse-labeled RNA of

Abbreviations: n,nucleolus; N,non-nucleolar part of the nucleus; C,cytoplasm.

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HeLa cells it was observed that only 25–30% of the label migrated from the nucleus to the cytoplasm. Since the pulse-labeling was carried out by exposure to tritiated precursors for a period varying from 20 min to 1 h, it was felt that the label remaining in the nucleus could be due either to non-specific incorporation or to the synthesis of RNA required for the functions of the nucleus. The non-specific incorporation may be reduced to a minimum if the pulse-labeling is carried out for a very short time. With this in view the migration kinetics of pulse-labeled RNA has been examined after appropriate corrections for cell geometry[3].

HeLa cells were exposed for 2, 5 and 10 min, respectively, to tritiated nucleosides and further incubated in non-radioactive medium. Although the loss of nuclear (N and n) grains cannot account quantitatively for all of the cytoplasmic gain (there is usually a slightly greater increase of cytoplasmic grains as compared to the nuclear loss), these experiments are compatible with the idea that a high proportion of the cytoplasmic RNA labeled after short pulses of precursor receives this label from the nucleus.

MATERIAL AND METHODS

HeLa cells were cultivated[3] on a medium (Φ_{10}) consisting of the following components: Hank's balanced salt solution, yeast extract, lactalbumin hydrolysate and 10% calf serum. For the kinetic experiments the cells were grown on coverslips for 48 h in Φ_{10} medium. The medium was removed and the coverslips were incubated for 2 h in Eagle's medium before transferring them to cuvettes containing the radioactive precursor in Eagle's medium. They were exposed to the tritiated precursor for 2, 5 and 10 min as indicated in the figures. At the end of this incubation some of the coverslips were fixed immediately in cold (2°) alcohol–acetic acid followed by 70% alcohol. The other coverslips were rapidly rinsed in Eagle's medium containing a large excess of the unlabeled precursor and transferred to Eagle's medium containing the unlabeled nucleoside (15 μg/ml) for varying lengths of time ranging from 5 min to 4 h before fixing them in alcohol–acetic acid followed by 70% alcohol. All the incorporation and chasing experiments were carried out at 37° in a CO_2 incubator. The method employed for the preparation of the coverslips for autoradiography was essentially similar to that described by PERRY *et al.*[2,3].

The grains over the various parts of the cell were counted. In order to correct for errors introduced by the geometry of the cells, the nucleolar (n) values were multiplied by 2.3 and the "nuclear" (N) values by 1.7 (see ref. 3). Since the radioactivities of the precursors were different for each experiment the corrected cell counts for n, N and C were summed up and the average radioactivity over n, N and C was computed for a total grain count of 1000. This facilitates a direct comparison of the grain counts in the different experiments.

The tritiated precursors employed in these experiments were obtained from commercial sources. The activity of the stock solutions were as follows: [^{3}H]adenosine, 1 mC/0.52 mg/ml; [^{3}H]guanosine, 1 mC/0.7 mg/2 ml; [^{3}H]cytidine, 1 mC/0.81 mg/ml. The stock solutions were suitably diluted with Eagle's medium to obtain the activities indicated in the legends for the figures.

RESULTS

The kinetics of migration of pulse-labeled RNA obtained by exposing the cells to

[^{3}H]adenosine, [^{3}H]cytidine and [^{3}H]guanosine for 2-, 5- and 10-min periods is illustrated in Figs. 1, 2 and 3, respectively.

Total uptake

After 2 min of incubation (Fig. 1) in the radioactive medium, about 20% of the label is already found in acid-insoluble components. 10 min later (Fig. 3) about twice this amount is acid-insoluble. This indicates an extremely rapid uptake of nucleosides into the soluble pool and the fraction of radioactivity still acid-soluble gives an indication of the magnitude of this pool. The fact that large amounts of non-radioactive precursors are incapable of diluting the labeled compounds is in

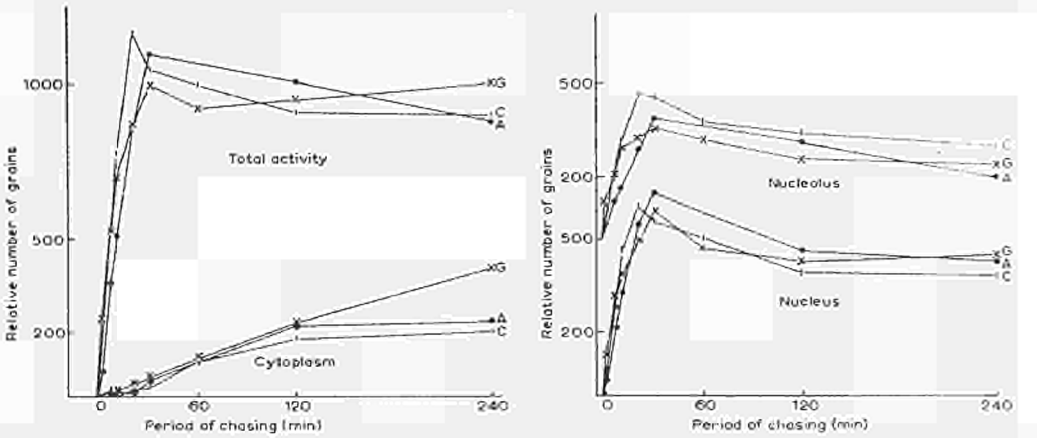

Fig. 1. Kinetics of migration of 2-min pulse-labeled RNA from the nucleus and nucleolus to the cytoplasm. The activity of [^{3}H]adenosine, [^{3}H]guanosine and [^{3}H]cytidine used were 8 μC, 10 μC and 8 μC per ml, respectively. The total radioactivity (A + G + C) is also included in the figure for comparison. A, adenosine; G, guanosine; C, cytidine. Ordinates, number of grains; Abcissae: left of origin, time in labeled nucleoside; right of origin, time in unlabeled nucleoside (chase).

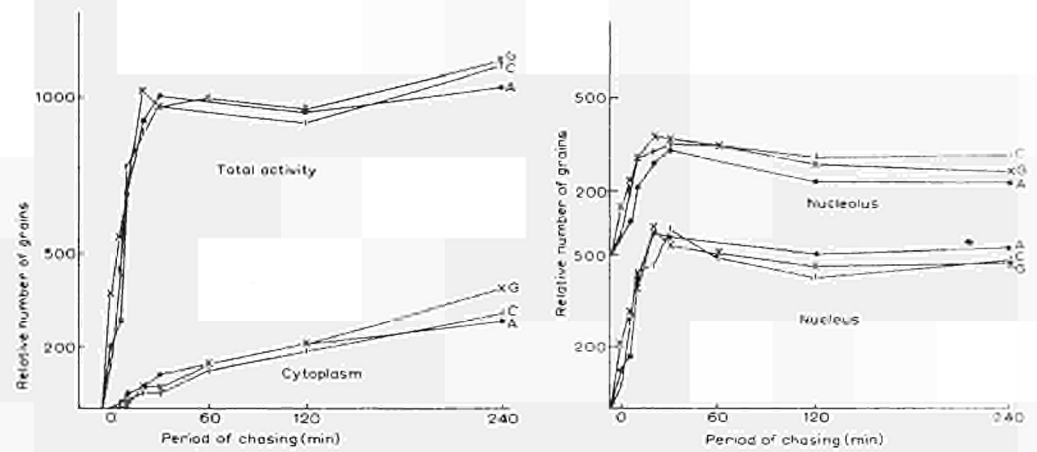

Fig. 2. Kinetics of migration of 5-min pulse-labeled RNA. The experimental conditions and coordinates were similar to those described in Fig. 1.

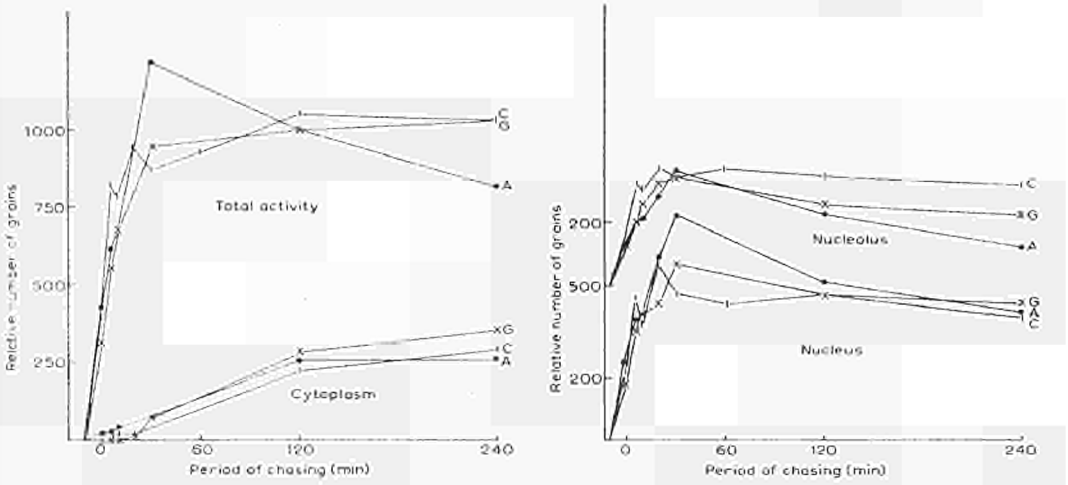

Fig. 3. Kinetics of migration of 10-min pulse-labeled RNA. The activity of [^{3}H]adenosine, [^{3}H]-guanosine and [^{3}H]cytidine used were 4 μC, 5 μC and 4 μC per ml, respectively. The coordinates are similar to those used in Fig. 1.

favor of the view that even after 2 min most of the label has already been converted to nucleotides and other derivatives. In all three pulse experiments (*i.e.*, 2, 5 and 10 min) all the label becomes acid-insoluble within 20–30 min of transfer to the chasing medium and by this time the cytoplasm contains no more than 15% of the total grain count of the cell. During the subsequent 210 min there does not appear to be any loss of label from the cells (Table I). Earlier, PERRY *et al.*[3] had shown by the use of RNAase that 80–85% of the label is found in RNA after 1 or 2 h incubation with a labeled nucleoside. As the DNA-synthesizing period of these cells is of the order of 6–8 h one can expect that, for a cell cycle of 24 h, about 30% of the cells will have taken up a minor fraction of the label into DNA. Therefore, under the conditions of short exposures the primary labeling occurs in the RNA of the cells.

Nuclear uptake

After 2 min of exposure to the radioactive precursor about 20% of the radioactivity is already found in nucleolar (n) and in other nuclear (N) RNA's. There is no demonstrable lag in the uptake of label into one of the compartments as compared to the other. This signifies that both n and N are synthesizing RNA independently of each other. Careful study of the radioautographs has given the impression that after very short periods of incubation there was a greater density of silver grains at the periphery of the nucleoli but only fine sectioning can confirm this observation. PERRY *et al.*[3] have also advanced similar ideas, *i.e.*, that nucleolar RNA is probably synthesized in close association with nucleolar-associated DNA. In this connection it is interesting to point out that RHO AND BONNER[7] find that chromatin becomes labeled before the nucleoli in plant tissues. However, in HeLa cells the nucleolar RNA is probably synthesized independently of the chromatin which is not closely associated with it[3]. If the contrary were true, the maximum activity in the nucleus would occur earlier than in the nucleolus.

TABLE I

BALANCE OF GRAIN DISTRIBUTION DURING PULSE EXPERIMENTS

Max., highest number of grains observed (after about 20–30 min); Loss, difference between number of grains at maximum and at end of chase; %, per cent of grains lost during chase.

Precursor	*Pulse (min)*	*n*			*N*			*n + N loss between 30 and 240 min*	*Cytoplasm*			
		Max.	*Loss*	*%*	*Max.*	*Loss*	*%*		*n + N loss at 30 min*	*Increase between 30 and 240 min*	*Grains not accounted for by N + n loss*	*Grains not accounted for by N + n loss expressed as per cent of cytoplasmic count*
Cytidine	2*	475	160	33	580	210	36	370	(45)	(175)	—	—
	5	350	40	11	575	120	21	160	(70)	230	70	23
	10	380	50	13	510	100	20	150	(30)	270	120	40
Guanosine	2	350	100	29	595	150	25	250	(60)	350	100	24
	5	385	120	31	580	110	19	230	(75)	305	75	20
	10	360	130	36	515	70	14	200	(70)	280	80	23
Adenosine	2*	390	180	46	650	220	34	400	(50)	(200)	—	—
	5	335	100	30	565	80	14	180	(100)	190	10	33
	10*	370	230	62	700	290	41	520	75	(285)	—	—

* In these experiments a loss of total count has occurred and correct balance of radioactivity cannot be obtained.

Nucleolar and other nuclear RNA's become maximally labeled at the same time (20–30 min) and both lose some of their label to the cytoplasm during the first 90 min after reaching the maximum, further losses being small. Although the radioautography data may lack the precision needed for computing the base composition of the migrating RNA, the results in Table I indicate that the nucleolus loses less than 30% RNA cytidylic acid and about 30% RNA guanylic acid and more than 30% RNA adenylic acid. In the case of the rest of the nucleus (N), the percentage loss of RNA adenylic acid is also probably greater than RNA guanylic acid or cytidylic acid. This would suggest that the RNA's leaving the nucleus probably contain more adenine than cytosine. A comparison of the base composition of the pulse-labeled [^{32}P]RNA of the nucleus before and after chasing also supports these findings[7]. HARRIS[5] had also concluded from his experiments that nucleolar RNA has a low adenine and a high cytosine content — this would correspond to the RNA remaining in the nucleolus. The nucleolar RNA of starfish oocytes is similarly enriched with respect to cytidylic acid (24%) as compared to adenylic acid (19%)[9]. Therefore our results can be taken as an indication that there exist at least two types of nucleolar RNA's as has already been suspected, one remaining and one leaving the nucleolus.

Cytoplasm

In the presence of the labeled nucleosides (2–10 min) no significant radioactivity is taken up into the cytoplasmic RNA. However, the time lag before cytoplasmic radioactivity actually begins to appear is difficult to measure accurately but it does not seem to be greater than a few minutes (less than 5). When nuclear radioactivity begins to decline, the cytoplasmic activity is of the order of 10–15% of the final activity reached by the cytoplasm. The cytoplasmic RNA appears to be relatively richer in guanylic acid but the greater loss of nucleolar adenine is not reflected in an increase of adenine in cytoplasmic RNA. This could be attributed to some independent cytoplasmic RNA synthesis.

Finally, we have attempted to compare the total nuclear grain loss to the cytoplasmic gain. In most experiments (those in which no total grain loss has been observed) the cytoplasmic gain is somewhat greater than the nuclear loss of grains. It is difficult to discuss this excess of cytoplasmic grains because it often amounts to less than 10% of the total grain count of the cell. However, this excess is of the order of 30% of the cytoplasmic count and could very well represent some independent cytoplasmic incorporation of the nucleoside. The remaining 70% of the cytoplasmic labeling undoubtedly comes from the nucleus.

DISCUSSION

The results obtained favor the conclusion that the synthesis of pulse-labeled RNA in HeLa cells occurs independently in the nucleolus (n) and in the rest of the nucleus (N). A portion of the label thus rapidly incorporated is transferred to the cytoplasm and accounts for at least 70–75% of the cytoplasmic RNA labeled during these experiments. Preliminary centrifugation studies[9] have shown that after a relatively short incubation period (1 h) the RNA with the highest specific radioactivity is found either in the nucleus (N + n) or in the cytoplasmic fraction non-sedimentable at

100 000 × *g* for 90 min. If the pulse-labeled cells are then incubated in non-radioactive medium, the heavier cytoplasmic particles are now found to acquire the label. CHENG[11] has also demonstrated that after very short periods of incubation (15 min) most of the radioactivity is present in an RNA fraction sedimenting at 4–8 S and after 40 min incubation the activity is also found in the heavier RNA fraction. These observations are in agreement with those of GEORGIEV AND SAMARINA[12] who found that ^{32}P uptake starts in the nucleus either as chromosomal bound material or as ultramicrosomes or microsomes of the nuclear sap. In recent experiments the characterization of pulse-labeled RNA has been carried out in the presence of inhibitors of RNAase. With such inhibitors SCHERRER AND DARNELL[13] have observed that the early labeled RNA of HeLa cells (5–60 min) was chiefly 45 S and 33 S and probably represents the RNA located in the cell nuclei. HARRIS AND WATTS[6] have also reported the early labeled nuclear RNA to be 28 S and 16 S but its first appearance in the cytoplasm is in a light component (3 S). In liver cells[14] the label first appears in a variety of nuclear RNA's (some of which are heavier than 30 S). However in the cytoplasm of these cells the label can first be detected in a lighter RNA (4 S). It therefore appears plausible that the cytoplasmic RNA of mammalian cells originates as relatively small molecular species which are then subsequently built into the larger ribonucleoproteins (larger ribosomes) similar to the ideas advanced by MCCARTHY AND ARONSON[15] and BRITTEN *et al.*[16,17], to explain their findings in bacteria.

The state of the labeled compounds migrating from the nucleus to the cytoplasm and their exact nature remains to be established. The experiments of HARRIS AND WATTS[6] and HIATT[14] indicate that there might be a partial degradation of nuclear labeled RNA before it reaches the cytoplasm. Until the fractions are further characterized with regard to their lipid content, secondary structure, metal ions, *etc.*, it may be premature to equate sedimentation characteristics to molecular weights. However, the breakdown of pulse-labeled nuclear RNA would not be expected to go beyond the stage were the information it contains would be lost, before the label becomes reincorporated in cytoplasmic nucleoproteins.

"Messenger RNA" may be an important fraction of the RNA formed during these short pulse experiments. CHENG[11] has found that this early labeled nuclear RNA has some of the characteristics of messenger RNA (*i.e.*, instability, rapid metabolism, *etc.*) and we have also recently shown that the fraction of pulse-labeled RNA migrating out of the nucleus has a relatively high content of adenine and uracil as compared to guanine and cytosine[8]. However, the only defining characteristic of messenger RNA, that of base complementarity with DNA, has not yet been established for these pulse-labeled fractions of mammalian cells.

The pulse-labeling in the present experiments has been comparatively short in relation to the generation time of these cells. Even under these conditions only about 30% of the nuclear (N + n) label migrates to the cytoplasm. This would suggest that the rest of the non-migratory pulse-labeled RNA is probably required for functions in the nucleus and nucleolus and may be composed of the different types of RNA of the nucleus. This raises the question of the fate of the RNA remaining in the nucleus and nucleolus. According to PRESCOTT, when the nuclear membrane breaks down at the beginning of mitosis, there is a massive loss of nuclear labeled RNA into the cytoplasm. This has also been observed in the present experiments: some of the cells in metaphase have a much higher cytoplasmic to nuclear grain count than the

other cells after 30 or 60 min chase. Observations of cells in anaphase, telophase and of young daughter cells indicate that the cytoplasm of both daughter cells seem to be equally marked, some grains appearing to remain on the chromosomes. Unfortunately, HeLa cells are usually rounded up during mitosis and hence are poor material for such studies. It would be important to ascertain whether any chromosomal RNA (or none) becomes duplicated and transferred intact to the daughter chromosomes.

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