Biochem. J. (1976) **154**, 529–539
Printed in Great Britain

Nucleo–Cytoplasmic Relationships of High-Molecular-Weight Ribonucleic Acid, including Polyadenylated Species, in the Developing Rat Brain

By WOLFGANG BERTHOLD and LOUIS LIM*
Miriam Marks Department of Neurochemistry, Institute of Neurology, The National Hospital, Queen Square, London WC1N 3BG, U.K.

(*Received* 28 *August* 1975)

The metabolism of high-molecular-weight RNA in the nuclear and cytoplasmic fractions of newborn and adult rat brain was investigated after the intracranial administration of [^{32}P]P_i. In young brain, a considerable proportion of the newly synthesized radioactive RNA is transferred to the cytoplasm, in contrast with the adult brain, where there appears to be a high intranuclear turnover. Electrophoretic analysis of the newly synthesized RNA showed that processing of rRNA precursor to yield the 28 S and 18 S rRNA may be more rapid in the adult than in the young, although most of the adult rRNA in the nucleus is not transferred to the cytoplasm. In young brain, processing is probably tightly coupled to transport of rRNA into the cytoplasm, so that 28 S and 18 S rRNA are not subjected to possible degradation within the nucleus. Polyadenylated RNA turns over in concert with high-molecular-weight RNA in the nuclei of the adult rat brain. In the cytoplasm the polyadenylated RNA has a higher turnover rate relative to rRNA. In the young brain the polyadenylated RNA is transferred to the cytoplasm along with rRNA, although polyadenylated RNA is transported into the cytoplasm at a faster rate. The nuclear and cytoplasmic polyadenylated RNA species of young brain are larger than their corresponding adult counterparts. These results suggest that there are considerable changes in the regulation of the nucleo–cytoplasmic relationship of rRNA and polyadenylated RNA during the transition of the brain from a developing replicative phase to an adult differentiated and non-dividing state.

It is generally accepted that the bulk of cytoplasmic high-molecular-weight RNA is derived from RNA synthesized within the nucleus. The nuclear precursors are synthesized as rather large molecules, which are first processed before they are transported into the nucleus. For example, the nuclear 45 S rRNA precursor is cleaved in several successive steps to yield the 28 S and 18 S rRNA. These rRNA molecules are then transported to the cytoplasm as 40 S and 60 S ribosomal subunits respectively, with the small subunit appearing first (Darnell, 1968). For most mRNA, the nuclear precursor is believed to be a giant transcript, referred to as heterogeneous nuclear RNA, which is also further processed by various cleavage steps. The discovery of poly(A) residues in cytoplasmic mRNA (Lim & Canellakis, 1970; Kates, 1970), followed by reports (Brawerman, 1974) that poly(A) was added post-transcriptionally to heterogeneous nuclear RNA, has established that at least some of the nuclear precursor to mRNA is polyadenylated. Since we have found that the synthesis of polyadenylated RNA is decreased during development of the rat cerebral cortex (Berthold & Lim, 1976), we were interested in determining whether other changes occurred in the metabolism of both polyadenylated RNA and rRNA. In view of the known precursor–product relationship between RNA in the cytoplasm and in the nucleus we have studied the metabolism of brain high-molecular-weight RNA in these two compartments. The results are discussed in the context of the change in the brain from a proliferating state in the newborn to a non-dividing finally differentiated state in the adult.

* To whom reprint requests should be addressed.

Materials and Methods

Chemicals

A.R.-grade chemicals were used throughout. Sarkosyl (laurylsarcosine) was obtained from Ciba–Geigy Co., Duxford, Cambs., U.K. Formamide (99.9% purity) was a product of Merck and Co., Poole, Dorset, U.K. Solutions were all made up in glassware previously treated with 0.05 M-NaOH and rinsed with water, to minimize contamination with

extraneous ribonuclease. In experiments involving binding of polyadenylated RNA to oligo(dT)-cellulose all the solutions were subjected to treatment with diethyl pyrocarbonate to destroy ribonuclease (Williamson *et al.*, 1971).

Animals

[^{32}P]P_i (The Radiochemical Centre, Amersham, Bucks., U.K.) was administered to 3-day-old and 150-day-old rats by intracranial injections at a dosage of 1mCi/g wet wt. of brain as previously described (Berthold & Lim, 1976). At appropriate times after the injection, the rats were decapitated and the skull was cut away to expose the brain. The cerebellum was removed *in situ* and the brain transected at the level of the pons–medulla. The forebrain was removed, the olfactory lobes were dissected out, and meninges and remaining blood vessels teased out. The forebrains were then immediately homogenized at 4°C in medium containing 0.25M-sucrose, 40mM-Tris/HCl, pH7.6, 25mM-KCl and 1mM-$MgCl_2$ by using ten strokes of a loose-fitting Teflon–glass tissue grinder (Thomas and Co., Philadelphia, Pa., U.S.A.) to disperse the tissue, present as a 10% (w/v) suspension.

Subcellular fractionation

The subcellular fractions were obtained by subjecting the homogenate to a modification of the combined centrifugation–phenol treatment by the method of Markov & Arion (1973) based on that of Georgiev (1967), except that all operations with brain tissue was performed at 4°C. An initial cytoplasmic fraction (C) was obtained by centrifuging the homogenate at 1000*g* for 10min in the MSE Mistral 4L centrifuge to sediment the nuclei. This cytoplasmic supernatant was immediately subjected to the phenol treatment previously described for preparation of high-molecular-weight RNA (Berthold & Lim, 1976). The crude nuclear fraction was resuspended in the original volume of fresh homogenization medium or, occasionally, 0.9% NaCl. An equal volume of ice-cold phenol, previously equilibrated with water and titrated with 0.1M-NaOH to adjust the pH to 6.0, was added to the resuspension. The mixture was carefully shaken and then left at 4°C for 20min. The phases were separated by centrifugation in the Mistral 4L centrifuge at 3000*g* for 20min. The supernatant was removed and designated the first wash fraction (W1). The residue was washed as above with water and this second wash was removed and corresponded to fraction W2 of Table 1. The final phenol phase contained as a white flocculent precipitate the nuclear fraction (N). High-molecular-weight RNA from the different fractions was obtained by a combined phenol extraction–LiCl precipitation procedure as previously described (Berthold & Lim, 1976).

Electrophoretic analyses

^{32}P-labelled high-molecular-weight RNA was analysed on dilute polyacrylamide gels as previously described (Berthold & Lim, 1976). More recent analyses have been performed on composite gels containing 1.5–1.75% (w/v) polyacrylamide in a matrix of 0.5% purified agarose (Koch–Light Ltd., Colnbrook, Bucks., U.K.; Summers, 1969; Peacock & Dingman, 1968). The molecular weights of the RNA species were determined by using 28 S (mol.wt. 1.7×10^6) and 18 S (mol.wt. 0.7×10^6) rRNA standards (Loening, 1968).

Oligo(dT)-cellulose chromatography

The isolation of polyadenylated RNA was performed by a modification of the batchwise method of Edmonds (1971) by using formamide to elute polyadenylated RNA from the oligo(dT)-cellulose (Lindberg & Persson, 1972). ^{32}P-labelled high-molecular-weight RNA, in 0.5ml of a high-salt solution, containing 0.4M-NaCl, 10mM-Tris/HCl, pH7.4, 0.1% sodium dodecyl sulphate, 0.1% laurylsarcosine, 1mM-EDTA and 10% (v/v) formamide, was mixed thoroughly with 15mg of the oligo(dT)-cellulose (previously washed with 0.1M-NaOH, and later with the high-salt solution). The mixture was left to react for 5min and a further 1.5ml of the high-salt solution was then added to the mixture with constant shaking. The cellulose-bound polynucleotide was sedimented by centrifugation at 3000*g* for 5min either at 4°C or at room temperature (20°C), since this made no difference to the elution pattern. The 2ml high-salt fraction was removed from the sediment, and the cellulose, containing polyadenylated RNA, was subjected to further washes, each time involving 2ml of the high-salt solution. Complete elution of the RNA not containing polyadenylate was monitored by Cerenkov-radiation measurements of these washes (Berthold & Lim, 1976). The polyadenylated RNA was eluted by similar 2ml washes with a low-salt solution, which was the high-salt solution from which 0.4M-NaCl was omitted and replaced by formamide, to give a final concentration of 90% (v/v). Complete elution of the labelled polyadenylated RNA, again monitored by measurements of Cerenkov radiation, was achieved with 10ml of the low-salt/formamide solution. RNA in the combined first two washes of the high-salt solutions was precipitated directly by the addition of 2vol. of ethanol. The polyadenylated RNA solution in the low-salt fraction was adjusted to contain 0.2M-sodium acetate, pH6.0, and carrier yeast RNA was added before precipitation with 3vol. of ethanol. The samples were left at −20°C for at least 16h for complete precipitation. The RNA precipitates were sedimented by centrifugation at 30000*g* for 20min in a refrigerated MSE 18 centrifuge. The RNA species

were then subjected to electrophoretic analyses as described above for determination of their molecular-weight distribution. The results obtained by the batchwise method were similar to those obtained by column chromatography (Berthold & Lim, 1976) and the method proved to be more convenient for isolating polyadenylated RNA.

Results

Developmental differences in the incorporation of ^{32}P into high-molecular-weight RNA

Relative-specific-radioactivity measurements. High-molecular-weight RNA was isolated from subcellular fractions derived from the forebrains of newborn or adult rats, at different times after the intracranial administration of [^{32}P]P_i. About 40% of the total cellular high-molecular-weight RNA was present in the cytoplasmic fraction (C) after an initial centrifugation step to sediment the nuclei (Table 1). The first nuclear wash with a phenolic solvent released almost as much RNA into the supernatant (W1). This fraction contained no DNA at all and u.v. analyses of the high-molecular-weight RNA showed it to be similar to the C fraction. These two cytoplasmic fractions combined represented about 75% of the total cellular high-molecular-weight RNA in both young and adult brains. The second phenolic wash of the nucleus (W2) always contained a small proportion of the high-molecular-weight RNA, and in the case of nuclear lysis, which occurred occasionally, also some DNA. The residual nuclear pellet contained 16–20% of the total cellular high-molecular-weight RNA in both young and adult rat brain preparations.

The nuclear fraction is considered together with fraction W2 as a combined nuclear fraction Nc in the extended analysis on Table 2, which shows the relative specific radioactivity of the different fractions, 4h and 24h after the administration of [^{32}P]P_i, calculated from the relative proportions of both radioactivity and absorbance in the fractions. In the adult brain, there was proportionately more radioactivity in the nucleus than in the cytoplasm, on the basis of nucleic acid content, even after 24h of labelling. This was shown by the high nuclear/

Table 1. *Content of high-molecular-weight RNA in different fractions of forebrains of newborn and adult rats*

High-molecular-weight RNA was isolated from subcellular fractions of the forebrains of 3- and 150-day-old rats as described in the Materials and Methods section. W1 and W2 represent the first and second washes of the crude nuclear fraction respectively. The values shown are the various proportions of high-molecular-weight RNA in the fraction relative to the total high-molecular-weight RNA recovered (based on E_{260} measurements). The results of a typical experiment are shown.

	E_{260} recovered (%)	
Fraction	Adult	Newborn
Cytoplasm (C)	44.8	38.3
1st wash (W1)	32.0	35.7
2nd wash (W2)	7.0	5.7
Nuclei (N)	16.2	20.3

Table 2. *Developmental changes in the relative accumulation of ^{32}P-labelled high-molecular-weight RNA in the various fractions of rat forebrain*

^{32}P-labelled high-molecular-weight RNA was isolated from the different fractions of the forebrains of 3- and 150-day-old rats injected intracranially with 1 mCi of [^{32}P]P_i/g of brain as described in the Materials and Methods section. The rats were killed 4 or 24 h after the injections. The combined nuclear fraction (Nc) consisted of the purified nuclei and W2 (second wash) shown in Table 1. Radioactivity and E_{260} of the high-molecular-weight RNA are expressed as the recovery of each in the various fractions compared with the total recovery. These values are from a typical experiment.

			Recovery (%)		Relative sp. radioactivity (R.S.A.) $\left(\frac{\text{radioactivity recovery}}{E_{260}\text{ recovery}}\right)$	R.S.A. (Nc) / R.S.A. (C)
Age	Labelling period (h)	Fraction	Radioactivity	E_{260}		
150 days (adult)	4	Cytoplasm (C)	14.6	44.8	0.33	
		Wash (W1)	36.6	32.0	1.14	
		Nuclear (Nc)	48.9	23.2	2.10	6.3
	24	Cytoplasm	23.7	36.4	0.66	
		Wash	24.7	39.9	0.62	
		Nuclear	51.5	23.7	2.18	3.3
3 days	4	Cytoplasm	24.8	38.2	0.65	
		Wash	39.1	35.7	1.09	
		Nuclear	36.1	26.0	1.39	2.1
	24	Cytoplasm	45.7	42.3	1.08	
		Wash	30.7	33.6	0.92	
		Nuclear	23.6	24.1	0.98	1.0

cytoplasmic ratio of 3, which, although lower than the value of 6 at 4h of labelling, represents a considerable differential.

In contrast, the corresponding nuclear/cytoplasmic relative-specific-radioactivity ratio in the young brain fell from 2 at 4h of labelling to 1 by 24h of labelling. Thus at this latter time, both fractions are equivalently labelled. The relative specific radioactivity for the W1 fraction in early labelling studies was intermediate between those of the combined nuclear and the cytoplasmic fractions in both young and old, approaching the cytoplasmic values by 24h of labelling. All subsequent studies on the nucleo–cytoplasmic relationships of high-molecular-weight RNA were based on analyses of the purified nuclear fraction (N) and the cytoplasmic fraction (C).

Specific-radioactivity measurements. The incorporation of [^{32}P]P_i into high-molecular-weight RNA of the total cellular extract as well as into the nuclear and cytoplasmic fractions was then studied over a 48h period in both newborn and adult rats. The results are expressed as specific radioactivities (radioactivity/E_{260}; Fig. 1) and showed that at both ages, incorporation of the precursor into total cellular high-molecular-weight RNA continued well into the second day after a single intracranial administration of [^{32}P]P_i.

In the adult (Fig. 1*a*), at all times after the administration of precursors, the specific radioactivity of the nuclear fraction exceeded that of the cytoplasmic fraction and the increase in the cytoplasmic fraction paralleled that in the nucleus. In the young rat brain (Fig. 1*b*), however, the specific radioactivity of the nuclear RNA after 5h of labelling was similar to that after 24h, with a decrease from 24h to 48h. After an initially slow start, the specific radioactivity of the cytoplasmic RNA increased continuously.

Electrophoretic analyses of the labelling of high-molecular-weight RNA

Adult rat brain. The high-molecular-weight RNA was subjected to electrophoretic analyses on polyacrylamide gels to determine if further differences in the nucleo–cytoplasmic relationships of the high-molecular-weight RNA occurred during development that could be characterized as changes in the nature of the transcribed product. In the adult brain, 2.5h after [^{32}P]P_i administration, despite the low radioactivity in the total high-molecular-weight RNA, the presence of discrete 28S and 18S rRNA could be detected along with labelled RNA of much larger molecular weights (Fig. 2*a*). By 5h of labelling, most of the radioactivity incorporated was in the 28S and 18S RNA, although there were still significant amounts in the larger RNA molecules (Fig. 2*b*). Within 24h (Fig. 2*c*) the labelling of these larger forms was considerably decreased in relation to that of the rRNA.

Within the nucleus, the 2.5h-labelled RNA consisted largely of the heavier-than-28S RNA species, in addition to the 28S and 18S rRNA. The contribution of the larger RNA to the labelling of the high-molecular-weight RNA decreased progressively with increasing times of labelling. In the cytoplasm radioactivity was predominantly in the rRNA.

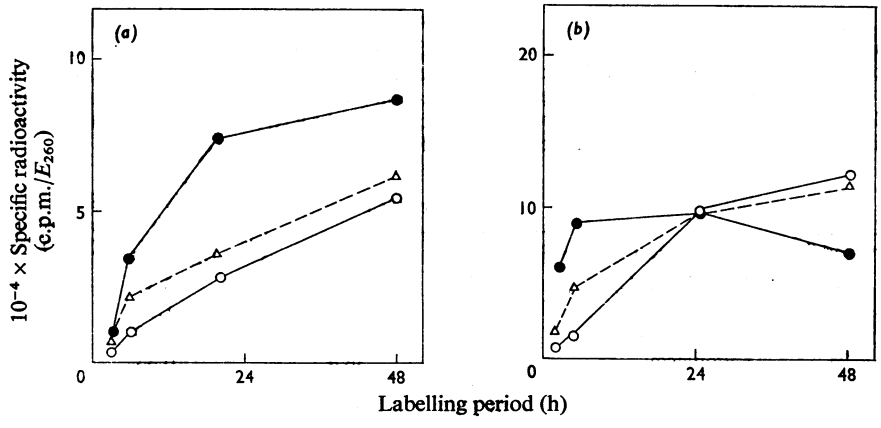

Fig. 1. *Incorporation of* [^{32}P]*orthophosphate into brain high-molecular-weight rRNA*

(*a*) Adult rats (150 days old) and (*b*) 3-day-old rats were each injected intracranially with 1mCi of [^{32}P]P_i/g of brain. High-molecular-weight RNA was isolated from unfractionated brain homogenates and cytoplasmic and purified nuclear fractions as described in the Materials and Methods section. Radioactivity and E_{260} measurements were made of the high-molecular-weight RNA isolated from brains of rats killed at the various times after the injection. ●, Nucleus; ○, cytoplasm; △, total unfractionated homogenates.

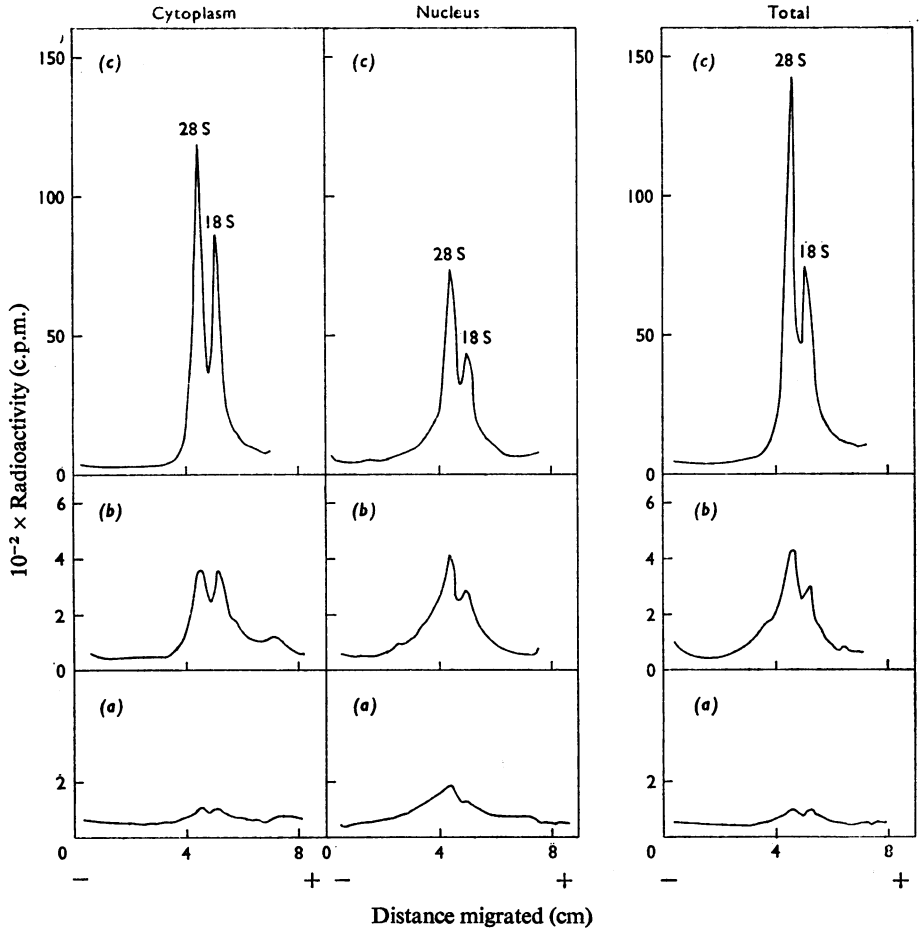

Fig. 2. *Electrophoretic analyses of* ^{32}*P-labelled high-molecular-weight RNA from the different fractions of adult rat brain*
^{32}P-labelled high-molecular-weight RNA from the different adult brain fractions derived from the preparations shown in Fig. 1 were electrophoresed at (*a*) 2.5h, (*b*) 5h or (*c*) 24h after the injections. For details see the Materials and Methods section. Electrophoresis was at 5mA for 1.5h.

Newborn-rat brain. In the newborn-rat brain, the majority of RNA labelled after 2.5h was much heavier than the 28S rRNA and, although the presence of 28S and 18S rRNA could be detected (Fig. 3*a*), the labelling of these rRNA species was less pronounced than the corresponding preparations from the adult brain. By 5h of labelling, the incorporation of radioactivity into the rRNA had increased relative to the other heavier species (Fig. 3*b*).

Within the nucleus the RNA labelled after 2.5h consisted mainly of the larger RNA species, although labelling of the 28S and 18S rRNA, as well as their precursor forms, 20S, 32S and 41S RNA, also occurred. After 5h the contribution of the 28S and 18S RNA to the radioactivity of the nuclear high-molecular-weight RNA had increased, although, as for the 2.5h nuclear sample, the relative amounts of labelled 28S and 18S RNA were markedly lower than in the corresponding nuclear RNA samples from the adult brain. The labelling of the cytoplasmic RNA at these short periods after ^{32}P administration, as well as at longer periods, was not unlike that in the corresponding samples from adult brains, with most of the radioactivity being in 28S and 18S rRNA.

Metabolism of polyadenylated RNA

The contribution of polyadenylated RNA to the overall pattern of high-molecular-weight RNA synthesis was next examined. In the adult forebrain (Fig. 4*a*), the polyadenylated RNA comprised about 28–30% of the ^{32}P-labelled high-molecular-weight RNA in the nucleus at all time-periods investigated. The corresponding cytoplasmic content of labelled

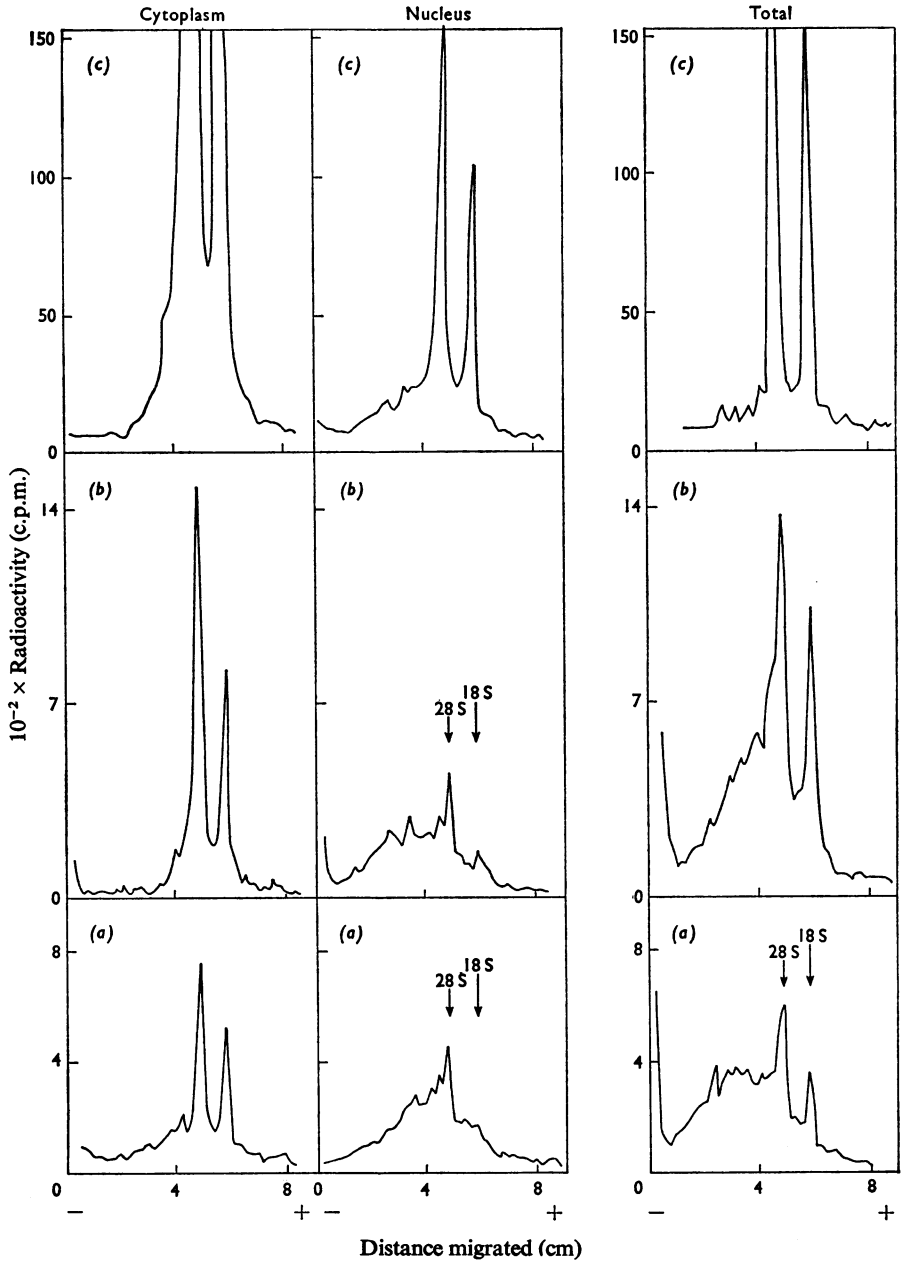

Fig. 3. *Electrophoretic analysis of* ^{32}*P-labelled high-molecular-weight RNA from forebrain of newborn rats*

^{32}P-labelled high-molecular-weight RNA from the different fractions of the 3-day-old rat brain was prepared as described in Fig. 1 and characterized as in Fig. 2. The positions of marker 28 S and 18 S RNA are shown.

polyadenylated RNA fell from 21% at 2.5h of labelling to about 5% within 48h.

In the young brain (Fig. 4*b*) the content of radioactive polyadenylated RNA in the nucleus was also 28–30% up to 24h of labelling. This value had fallen to 14% by 48h of labelling. In the cytoplasm, the content of radioactive polyadenylated RNA at 2.5h of labelling was similar to the nuclear content (approx. 30%). By 5h the cytoplasmic content was lower than the nuclear content, finally falling to about 10% by 48h of labelling. In both the young and adult brains, the content of radioactive polyadenyl-

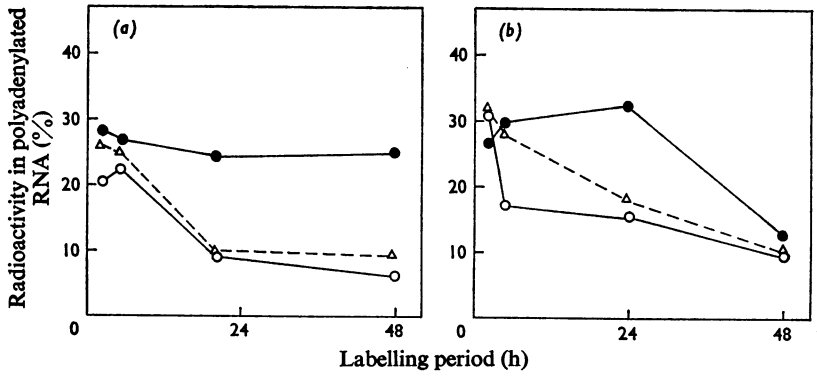

Fig. 4. *Metabolism of ^{32}P-labelled polyadenylated RNA*

^{32}P-labelled high-molecular-weight RNA from the different fractions of forebrains of (*a*) adult and (*b*) newborn rats prepared as shown in Fig. 1 were subjected to oligo(dT)-cellulose chromatography to isolate polyadenylated RNA as described in the Materials and Methods section. The proportion of radioactivity present in the polyadenylated RNA fraction was then determined at different times. ●, Nucleus; ○, cytoplasm; △, total unfractionated homogenate.

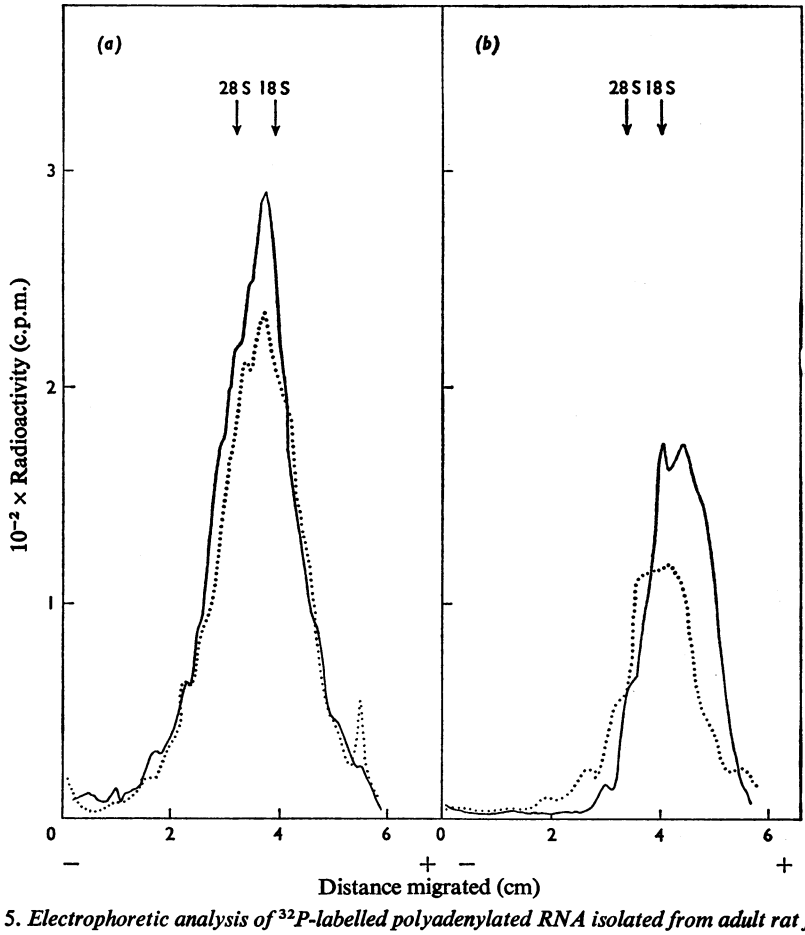

Fig. 5. *Electrophoretic analysis of ^{32}P-labelled polyadenylated RNA isolated from adult rat forebrain*

Polyadenylated RNA was isolated from purified high-molecular-weight RNA derived from nuclear (*a*) and cytoplasmic (*b*) fractions of the brains of 150-day-old rats. The rats had been previously injected with [^{32}P]P_i either 3h or 48h before being killed. Conditions for electrophoresis are as in Fig. 2. The positions of marker 28S and 18S RNA are shown. ····, 3h labelling; ——, 48h labelling.

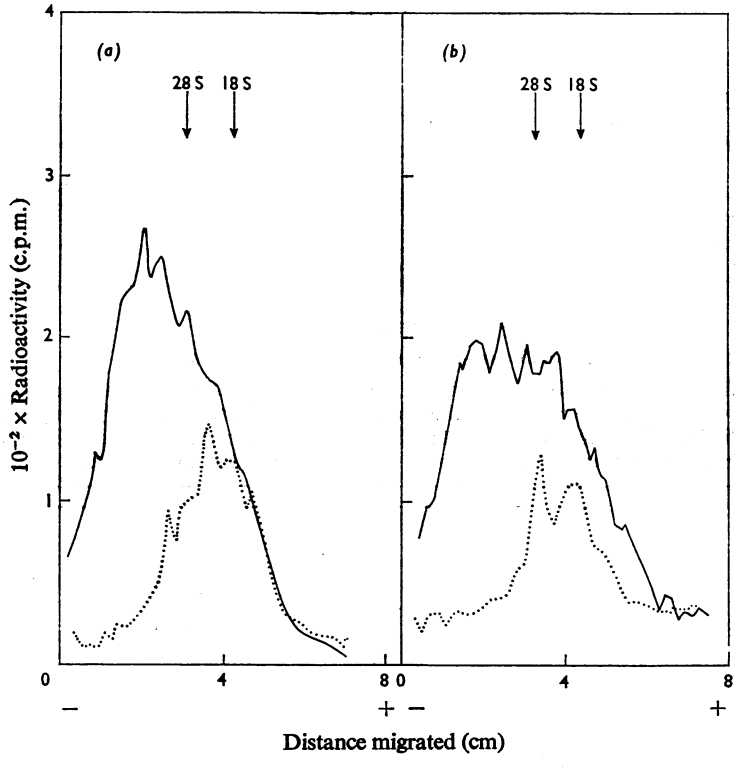

Fig. 6. *Electrophoretic analysis of ^{32}P-labelled polyadenylated RNA isolated from newborn-rat forebrain*

Polyadenylated RNA was isolated from 3-day-old rats (*a*) 2.5h or (*b*) 5h after the intracranial administrations of [^{32}P]P_i as described for Fig. 5. The polyadenylated RNA was electrophoresed as described in Fig. 2. The positions of marker 28S and 18S are shown. ····, Cytoplasm; ——, nucleus.

ated RNA in the total cellular high-molecular-weight RNA was intermediate between the nuclear and cytoplasmic values, except for an anomalously high value in the preparation from the young rat after labelling for 2.5h. This high value suggests that initially there was transient labelling of polyadenylated RNA in a fraction other than the nucleus and cytoplasmic fractions used as a routine. Although this point was not pursued further, it is likely that this fraction corresponds to W1, i.e. the first phenolic wash of the initial sediment after centrifugation at 1000*g* for 10min, which may include membranes involved in transport of mRNA (Cornudella *et al.*, 1973).

Molecular-weight distribution of adenylated RNA

In the adult (Fig. 5*a*) the mean molecular weight of the labelled polyadenylated RNA in the nucleus was found to be about 1.3×10^6 after either a 3h or 48h labelling period. In both cases, the value ranged from 0.2×10^5 to 10×10^6. In the cytoplasm (Fig. 5*b*), there was also marked heterogeneity in the molecular-weight distribution of the polyadenylated RNA. There appeared to be a decrease in the mean molecular weight of the polyadenylated RNA from 0.6×10^6 after 3h of labelling to about 0.5×10^6 after 48h of labelling. In the young rat, the mean molecular weight of the labelled polyadenylated RNA in the nucleus (Fig. 6*a*) was about 2.5×10^6 after 2.5h of labelling, a value much larger than for the adult and, accordingly, with a molecular-weight distribution containing more of the larger species than the adult. In the corresponding cytoplasmic preparation, the mean molecular weight was 0.8×10^6, which was again larger than the value for the adult cytoplasmic preparation. It was difficult to ascertain whether any change in the size of the cytoplasmic polyadenylated RNA had occurred after 24h of labelling, since after this time the contribution of contaminating rRNA of high specific radioactivity (the result of continued accumulation of RNA in young rats) obscured the radioactivity of the polyadenylated RNA. This masking effect could be seen even after 5h of labelling (Fig. 6*b*), when it was extremely difficult to separate the

radioactivity of labelled 28S and 18S rRNA peaks from the labelled polyadenylated RNA peak. All these values of molecular-weight distributions have to be tempered by the possibility of aggregation, which seems particularly to affect those RNA species containing polyadenylate residues (White *et al.*, 1975).

Discussion

In order to study the nucleo–cytoplasmic relationship of brain high-molecular-weight RNA during development we chose to use the combined centrifugation–phenol procedure [see Georgiev (1967) and the Materials and Methods section] for obtaining nuclear fractions essentially free of cytoplasmic contamination. The use of alternative methods involving purification of brain nuclei by sedimentation in 2.2M-sucrose (Lovtrup-Rein & McEwen, 1966) resulted in considerable breakdown of rapidly labelled nuclear RNA, possibly because of nuclease activity during the extended centrifugation. Phenol treatment of crude brain nuclear fractions (i.e. the 'phenolic nuclei' of Georgiev, 1967) prevented breakdown of labelled high-molecular-weight RNA. Electrophoresis of high-molecular-weight RNA from these nuclear fractions gave consistent RNA patterns without evidence of degradation (e.g. Fig. 2). Significant contamination of these nuclear fractions by cytoplasmic RNA could be ruled out by comparing the content of radioactive polyadenylated RNA in the nuclear and cytoplasmic fractions (e.g. after 24h of labelling). Thus in the adult brain preparations the content of radioactive polyadenylated RNA in the nucleus remained above 25%, whereas the corresponding value in the cytoplasm was 10% (Fig. 4). In the young rat the relevant values were 32% in the nucleus against 12% in the cytoplasm. If cross-contamination had occurred, especially after this extended period of labelling with very high incorporation of radioactivity into RNA in both fractions, there would not be this great disparity in the polyadenylated RNA content. In addition, in both young and adult brains, the mean molecular weight of nuclear polyadenylated RNA was far greater than that of the corresponding cytoplasmic polyadenylated RNA (Figs. 5 and 6), ruling out gross contamination of the nuclear RNA with the cytoplasmic RNA. In considering the cytoplasmic fraction we have chosen to include the contribution of mitochondrial RNA rather than subject the nuclear supernatant to further centrifugation. This contribution is small compared with that of microsomal RNA, which forms the bulk of the cytoplasmic RNA (Balázs & Cocks, 1967).

Changing relationships of nuclear high-molecular-weight RNA to cytoplasmic high-molecular-weight RNA

In the adult the relative specific radioactivity in the cytoplasm increases from 0.33 at 4h to 0.66 at 24h after the administration of precursor, whereas the values for nuclei remain at 2 at these times, indicating a disproportionate retention of radioactive RNA in the nucleus (Table 2). This result is consistent with the view that in adult brain much of the RNA synthesized in the nucleus turns over and that therefore only a fraction of the synthesized RNA is transferred to the cytoplasm (see Harris, 1974). In the young rat brain by 4h the relative specific radioactivity of the cytoplasm approaches that of the nucleus. After 24h of labelling the values are equivalent in both fractions. These results indicate that there was a continuous accumulation of newly synthesized high-molecular-weight RNA in the cytoplasm. This accumulation, mainly of rRNA (Fig. 2*c*), occurred at the expense of nuclear high-molecular-weight RNA.

The changing relationships of nuclear high-molecular-weight RNA to cytoplasmic high-molecular-weight RNA during development are also illustrated by the results shown in Fig. 1, which are expressed as a ratio of cytoplasmic to nuclear values (C/N) in Fig. 7. After 48h of labelling the C/N ratio in the adult rat brain was not substantially increased over the ratio at 2.5h. Thus most of the synthesized RNA, which included both rRNA and polyadenylated RNA, remains within the nucleus. In contrast, in the young rat brain the C/N ratio increased continuously from 2.5 to 48h after administration of label. The high C/N ratio of 1.65 at 48h indicates again that the continuous transfer of RNA from the nucleus to the cytoplasm has depleted the nucleus of its content of radioactive newly synthesized RNA.

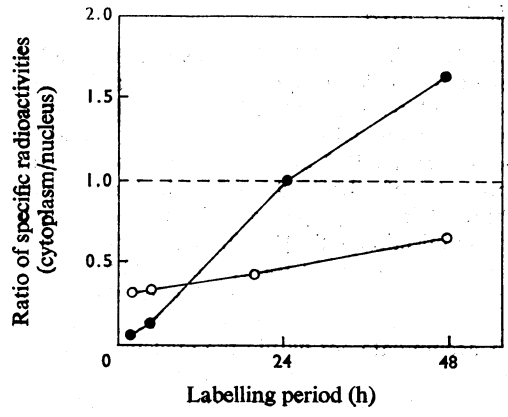

Fig. 7. *Nucleo–cytoplasmic relationships of high-molecular-weight RNA in adult and newborn-rat forebrain*

Data from Fig. 1 are plotted as a ratio of the specific radioactivity of ^{32}P-labelled high-molecular-weight RNA in the cytoplasm to that in the nucleus at various times. Results are shown for both adult (○) and newborn (●) rats.

Synthesis of rRNA

At early periods of labelling 28S and 18S rRNA represent a smaller proportion of the radioactive high-molecular-weight RNA in the nuclei of young brains compared with the adult (Figs. 2 and 3). At both ages the 18S and 28S rRNA were major components of the radioactive high-molecular-weight RNA in the cytoplasm during periods of up to 2.5h after injection of the [^{32}P]P_i. It appears therefore that in the young brain transfer of rRNA into the cytoplasm is coupled to processing of the rRNA precursor, so that at any time processed 28S and 18S rRNA accumulate not within the nucleus but in the cytoplasm. In contrast, in the adult, processing of rRNA precursor without quantitative transfer of the processed rRNA into the cytoplasm leads to accumulation of the 28S and 18S rRNA within the nucleus. These results indicate that the processing of rRNA precursor is faster in the adult than in newborn brain and that most of the processed rRNA in the adult nuclei must undergo turnover within the nucleus as part of the general pattern of high-molecular-weight RNA turnover.

Metabolism of polyadenylated RNA

Adult rat brain. In the adult the proportion of radioactive RNA in the nucleus that was polyadenylated remained at about 25% at all times after the administration of [^{32}P]P_i (Fig. 4*a*). This synthesis of poly(A) in concert with the rest of the nuclear RNA has been previously observed much earlier in liver (Lim *et al.*, 1969, 1970). Since nuclear RNA turns over, this maintenance of a constant percentage of polyadenylated RNA indicates that it turns over in a co-ordinated manner with the rest of the nuclear RNA. Therefore polyadenylation of any RNA transcript does not necessarily lead to its transport into the cytoplasm, in agreement with the findings of Perry *et al.* (1974), who also reported the intranuclear turnover of poly(A) residues.

The report that HeLa-cell nuclear RNA contained short internal poly(A) sequences of 20–40 nucleotides, which did not appear in the cytoplasm (Nakazato *et al.*, 1974), does not interfere with the conclusions of Perry *et al.* (1974), whose studies were conducted on terminal poly(A) sequences containing above 60 nucleotides and averaging 200 nucleotides. In brain the poly(A) nucleotides range from 100 to 200 nucleotides (Lim *et al.*, 1974; DeLarco *et al.*, 1975), and there is as yet no evidence that short poly(A) strands found internally in nuclear RNA are common to all mammalian tissues.

The fall in the proportion of radioactive polyadenylated RNA in total cellular high-molecular-weight RNA indicates that this polyadenylated RNA turns over at a much faster rate than the rest of the high-molecular-weight RNA. Since in the nuclei the polyadenylated RNA component turns over in concert with non-polyadenylated RNA, this fall must be due to the cytoplasmic component. Cytoplasmic high-molecular-weight RNA is present at 4–5 times the nuclear values. This more rapid turnover of cytoplasmic polyadenylated RNA is in keeping with observations in other systems (Brawerman, 1974) that mRNA turns over at a faster rate than rRNA. A direct examination of the cytoplasmic value indicates that the proportion of radioactive polyadenylated RNA falls over a 48h period from 22 to 5%. This could have been partly explained by a more rapid appearance of mRNA in the cytoplasm if the total cellular metabolism of polyadenylated RNA had not been investigated.

Young rat brain. In the young rat brain the proportion of radioactive polyadenylated RNA in the total high-molecular-weight RNA also falls over the 48h period, as in the adult, indicating a more rapid turnover of the polyadenylated RNA (Fig. 4*b*). Continued transport of high-molecular-weight RNA into the cytoplasm leads to depletion of radioactive nuclear RNA, which will thus have a correspondingly decreased polyadenylated RNA content with time, since the polyadenylated RNA component is transferred out faster than is rRNA. Because of the continued accumulation of rRNA in the cytoplasm, measurements of the contribution of the polyadenylated RNA to the radioactivity of the cytoplasmic high-molecular-weight RNA after labelling for longer than 5h will be unduly affected by the presence of very large amounts of radioactive rRNA.

The cytoplasmic polyadenylated RNA is smaller than nuclear polyadenylated RNA at all times and is consistent with the view that there is a precursor–product relationship between the two forms (Figs. 5 and 6). In the adult cytoplasm, the polyadenylated RNA appears to be decreased in size with increasing times of labelling, indicating a further processing of mRNA (Fig. 5*b*). This may involve changes in the length of the poly(A) segment (Lim & Canellakis, 1970; Brawerman, 1974). Although the change in size of the poly(A) segments concerns at most 200 nucleotides and would appear to be too small to explain the apparent decrease in size in cytoplasmic polyadenylated RNA (Fig. 5*b*), large apparent shifts in size can occur because of the anomalous behaviour of poly(A)-containing species (Pinder *et al.*, 1974).

Developmental changes in the nucleo–cytoplasmic relationships of high-molecular-weight RNA in the brain

Our investigations reported in this and in the previous paper (Berthold & Lim, 1976) have led us to

the following conclusions. (1) More polyadenylated RNA is synthesized in young brain than in adult. (2) This increased synthesis may be necessary because of increased turnover of mRNA in young brain, perhaps as a result of changing requirements in the nature of proteins during development. (3) In the adult brain nuclei, high-molecular-weight RNA, including polyadenylated RNA, turns over, whereas in the young brain this turnover, if it occurs, is superimposed on a pattern of RNA metabolism geared to accumulation of rRNA in the cytoplasm. (4) In the young brain there is a greater transfer of rRNA and polyadenylated RNA into the cytoplasm. (5) Processing of rRNA precursors, relative to transcription, is lower in the young brain compared with the adult brain. This may be a protective device against accumulation of rRNA in the nuclei of young brain, where it would be degraded. (6) Subject to the possibility of extensive aggregation, both the initial transcripts that are polyadenylated and the cytoplasmic mRNA are larger in size in young brains than in adult.

Thus there are changes in the regulation of the various metabolic processes involving mRNA and rRNA which must occur in a co-ordinated manner during development. We have previously shown that neonatal-rat brain polyribosomes are completely dissociated into ribosomal subunits on exposure to 0.5M-KCl, whereas adult brain polyribosomes are more resistant (Lim & White, 1974). This dissociability of neonatal-rat polyribosomes may be related to the wide disparity between the properties of mRNA (which is unstable) and rRNA (which is only stable in dividing tissues; Green, 1974) and may be a means of ensuring that the mRNA is more easily released from ribosomes for degradation, making the ribosome more responsive to continued influx of new and changing populations of mRNA.

We thank Professor A. N. Davison for his help and encouragement. This research was supported by grants from The Nuffield Foundation and the Medical Research Council.

References

Balázs, R. & Cocks, W. A. (1967) *J. Neurochem.* **14**, 1035–1055
Berthold, W. & Lim, L. (1976) *Biochem. J.* **154**, 517–527
Brawerman, G. (1974) *Annu. Rev. Biochem.* **43**, 621–642
Cornudella, L., Faiferman, I. & Pogo, A. O. (1973) *Biochim. Biophys. Acta* **294**, 541–546
Darnell, J. E. (1968) *Bacteriol. Rev.* **32**, 262–290
DeLarco, J., Abramovitz, A., Bromwell, K. & Guroff, G. (1975) *J. Neurochem.* **24**, 215–222
Edmonds, M. (1971) in *Procedures in Nucleic Acid Research* (Cantoni, G. L. & Davies, D. R., eds.), vol. 2, pp. 629–640, Harper and Row, New York
Georgiev, G. P. (1967) *Prog. Nucl. Acid Res.* **6**, 259–351
Green, H. (1974) in *Control of Proliferation in Animal Cells* (Clarkson, B. & Baserga, R., eds.), pp. 743–755, Cold Spring Harbor Laboratory, Cold Spring Harbor
Harris, H. (1974) *Nucleus and Cytoplasm*, Clarendon Press, Oxford
Kates, J. R. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35**, 743–752
Lim, L. & Canellakis, E. S. (1970) *Nature (London)* **227**, 710–712
Lim, L. & White, J. O. (1974) *Biochim. Biophys. Acta* **366**, 358–363
Lim, L., Canellakis, Z. N. & Canellakis, E. S. (1969) *Biochem. Biophys. Res. Commun.* **34**, 536–540
Lim, L., Canellakis, Z. N. & Canellakis, E. S. (1970) *Biochim. Biophys. Acta* **209**, 112–127
Lim, L., White, J. O., Hall, C., Berthold, W. & Davison, A. N. (1974) *Biochim. Biophys. Acta* **361**, 241–247
Lindberg, U. & Persson, T. (1972) *Eur. J. Biochem.* **31**, 246–254
Loening, U. E. (1968) *J. Mol. Biol.* **38**, 355–365
Lovtrup-Rein, H. & McEwen, B. S. (1966) *J. Cell Biol.* **30**, 405–415
Markov, G. G. & Arion, V. J. (1973) *Eur. J. Biochem.* **35**, 186–200
Nakazato, H., Edmonds, M. & Kopp, D. W. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 200–204
Peacock, A. C. & Dingman, C. W. (1968) *Biochemistry* **7**, 668–674
Perry, R. P., Kelley, D. E. & LaTorre, J. (1974) *J. Mol. Biol.* **82**, 315–331
Pinder, J. C., Staynov, D. Z. & Gratzer, W. B. (1974) *Biochemistry* **13**, 5373–5378
Summers, W. C. (1969) *Virology* **39**, 152–182
White, J. O., Hall, C., Lim, L. & Davison, A. N. (1975) *Biochem. Soc. Trans.* **3**, 94–95
Williamson, R., Morrison, M., Lanyon, G., Eason, R. & Paul, J. (1971) *Biochemistry* **10**, 3014–3021