Regulation of rRNA synthesis and processing in animal cells

Effect of nucleoside analogues

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The nucleoside analogues fluorouridine and fluorodeoxyuridine (both at 100 μM) and 8-azaguanine (at 500 μM) inhibit both rRNA transcription and processing in Ehrlich ascites cells. In BHK₂₁ cells fluorodeoxyuridine has no effect on either rRNA maturation or transcription, whereas toyocamycin (at 2 μM) inhibits both processes in BHK₂₁ cells and Ehrlich ascites cells. The drugs inhibit transcription in cells incubated in the complete medium, but have no effect on the decreased transcription in cells incubated in a medium without amino acids. This lack of effect cannot be explained by an altered uptake of the drugs in the amino acid-starved cells, since maturation of the rRNA precursor is affected in cells incubated in media with or without amino acids. The effect of the drugs on rRNA transcription is not the consequence of the inhibition of protein synthesis. The results lend support to the proposal that rRNA processing and transcription are co-ordinately controlled in cells with a high rate of rRNA synthesis.

One of the aspects of the control of ribosome production concerns the degree of stringency with which rRNA transcription and maturation are coupled. In *Tetrahymena*, transcription, processing and translocation of rRNA occur along the nuclear matrix elements in a highly ordered, possibly feed-back-controlled fashion (Herlan *et al.*, 1979). In animal cells in culture, a rather stringent relation between rRNA processing and transcription has been found when protein synthesis is inhibited by cycloheximide (Willems *et al.*, 1969; Craig & Perry, 1970). In this experimental situation it is, however, not clear whether some rate-limiting proteins affect rRNA processing and this inhibits transcription as a secondary effect, or whether newly synthesized proteins are directly involved in the transcription process (Perry, 1973). It has been proposed, for instance, that some rapidly turning-over proteins control the activity of the nucleolar RNA polymerase (Yu & Feigelson, 1972; Franze-Fernández & Fontanive-Sanguesa, 1973; Gross & Pogo, 1974) and the release of nascent rRNA precursor from the DNA template (Craig & Perry, 1970). A means to clarify this point could be to inhibit rRNA processing without altering protein synthesis, and then

Abbreviations used: FUrd, 5-fluorouridine; FUTP, 5-fluorouridine triphosphate; FdUrd, 2'-deoxy-5-fluorouridine.

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determining its effect on transcription. One of the tools that can be used for investigating this problem are the nucleoside analogues. The inhibition of rRNA processing by toyocamycin, FUrd and 8-azaguanine is well documented (Tavittian *et al.*, 1968; Weiss & Pitot, 1974; Wilkinson *et al.*, 1975). We have reported that toyocamycin has, in addition, an inhibitory effect on rRNA transcription in Ehrlich ascites cells cultured in the complete medium, but not in those incubated in a medium lacking amino acids (Iapalucci-Espinoza *et al.*, 1977). Since toyocamycin is an adenosine analogue, the latter effect of the drug could be the consequence of a more general effect on cell growth. Toyocamycin could interfere, for instance, with the synthesis or effect of certain adenosine derivatives involved in cell proliferation (Rapaport & Zamecnik, 1976). Alternatively, the inhibition of rRNA transcription could be related to the effect of toyocamycin on rRNA processing. We have decided to investigate this last possibility by analysing the effect of uracil and guanine analogues on rRNA transcription and processing.

Experimental

Chemicals

[5,6-³H]Uridine 5'-triphosphate (40–60 Ci/mmol), [2-³H]adenosine (25 Ci/mmol), [2-³H]aden-

ine (15–25 Ci/mmol) and L-[4,5-³H]leucine (40–60 Ci/mmol) were from The Radiochemical Centre, Amersham, Bucks., U.K. The α -amanitin was kindly provided by Professor Th. Wieland of the Max Planck Institute, Heidelberg, W. Germany. FUrD and FdUrD were obtained from Dr. W. E. Scott of Hoffmann La Roche, Nutley, NJ, U.S.A.; toyocamycin was provided by Dr. G. Acs, Mount Sinai School of Medicine, New York, NY, U.S.A.; and 8-azaguanine was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. All other reagents were of the highest purity available.

Cells

Ehrlich ascites cells were grown for 5–7 days in the abdominal cavity of mice. Preparation of the cells and the incubation in media with and without amino acids were carried out as described previously (Franze-Fernández & Pogo, 1971). Cells were incubated at a concentration of 2×10^6 /ml medium. BHK₂₁ cells were grown in monolayers in Eagle's minimal essential medium modified by MacPherson & Stoker (1962).

Analytical procedures

Cells were labelled with the [³H]nucleosides and the RNA was extracted from whole cells as described previously (Franze-Fernández & Fontanive-Sanguesa, 1975). The labelled RNA was analysed by electrophoresis in acrylamide gels as indicated (Iapalucci-Espinoza *et al.*, 1977).

Nuclei were isolated as described by Franze-Fernández & Fontanive-Sanguesa (1973).

The DNA-dependent RNA polymerase I (RNA nucleotidyltransferase, EC 2.7.7.6) was assayed as indicated by Iapalucci-Espinoza *et al.* (1977). Reaction mixtures were incubated for 10 min at 30°C. All the assays were performed by triplicate in the presence of 3 μ g of α -amanitin/ml. No further inhibition of the activity was found when 200 μ g of α -amanitin/ml was added, indicating that in our nuclei preparation the α -amanitin-insensitive activity corresponds to RNA polymerase I (Weil & Blatti, 1976). When 400 μ g of heparin/ml was included in the assay, the polymerase I activity was not affected. Since heparin is known to inhibit RNA-chain initiation (Udvardy & Seifart, 1976), this showed that the activity measured in isolated nuclei corresponds to the enzyme bound to the DNA template.

The incorporation of radioactive amino acids into protein was performed as follows. Cells labelled with [³H]leucine were poured on to 10 ml of cold Dulbecco's phosphate-buffered saline and sedimented by centrifugation (800 g for 5 min). The pellet was dissolved in 2 ml of 1 M-KOH, left for 10 min at room temperature, and precipitated with 1.5 ml of 50% (w/v) trichloroacetic acid. The precipitate was collected by centrifugation

(10 min at 2500 g) and washed with 3 $\times$ 7 ml of 5% trichloroacetic acid. The final precipitate was dissolved and the radioactivity counted as described elsewhere (Franze-Fernández & Fontanive-Sanguesa, 1973).

Results and discussion

Effect of FUrD, FdUrD and 8-azaguanine on rRNA processing

The analogues FUrD and 8-azaguanine inhibit rRNA processing in animal cells, probably through their incorporation into RNA (Wilkinson *et al.*, 1975; Weiss & Pitot, 1974). It was reported that FdUrD has no effect on rRNA maturation in Novikoff hepatoma cells (Wilkinson *et al.*, 1975). We obtained a similar result in BHK₂₁ cells (not shown). Since in Ehrlich ascites cells FdUrD is readily transformed to FUTP, which is incorporated into RNA (Harbers *et al.*, 1959), we investigated whether the analogue affects rRNA processing in these cells. As illustrated in Figs. 1(a) and 1(b), when Ehrlich ascites cells incubated in the complete medium are exposed to FdUrD for 2 h, the appearance of 28S and 18S rRNA is prevented. The conversion of 45S into 32S rRNA is also inhibited, but to a lesser extent. In cells incubated in a medium without amino acids, in which rRNA processing is already altered, the drug suppresses the formation of 32S rRNA (Figs. 1c and 1d). Similar results were obtained when the experiment was performed with FUrD instead of FdUrD (not shown).

In the experiment depicted in Fig. 2, it was found that FUrD affects the maturation of the 45S rRNA synthesized within 15 min after adding the drug to the cells. No peaks of radioactivity appeared in the position of the rRNA species in the FUrD-treated cells after a chase with actinomycin D, indicating that the non-processed RNA is degraded during the chase.

In agreement with observations in Novikoff hepatoma cells (Weiss & Pitot, 1974), inhibition of rRNA maturation by 8-azaguanine does not become complete. Some 28S and 18S rRNA appeared in Ehrlich ascites cells incubated with 500 μ M-8-azaguanine (results not shown).

Effect of the nucleoside analogues on rRNA transcription

The use of isolated nuclei for studying transcription of ribosomal genes in a wide variety of experimental systems is well documented (Zylber & Penman, 1971; Franze-Fernández & Pogo, 1971; Green, 1974; Cox, 1976; Campbell & Weissbach, 1980). In a previous report on the effect of toyocamycin on rRNA transcription we demonstrated a close correlation between the rate of rRNA synthesis measured in whole cells and the corres-

Fig. 1. *Effect of FdUr on rRNA maturation*

Ehrlich ascites cells precultured for 40 min in an amino acid-free medium were transferred to: (a) a complete medium; (b) a complete medium plus 100 μ M-FdUr; (c) an amino acid-deprived medium; (d) an amino acid-deprived medium plus 100 μ M-FdUr. All media contained 60 μ Ci of [3 H]adenine/ml. After 2 h of labelling, total cell RNA samples were prepared and the RNA from about 2.5×10^5 cells of each sample was analysed by electrophoresis in 2.6% acrylamide gels at 7 mA/gel for 4.5 h. O, [3 H]Adenine incorporation; ●, 14 C-labelled rRNA marker.

ponding activity of nuclear RNA polymerase I (Iapalucci-Espinoza *et al.*, 1977), thus indicating that isolated nuclei should be a suitable system for measuring rRNA transcription in the studies reported here.

In the experiment depicted in Fig. 3, Ehrlich ascites cells were exposed to different concentrations of the analogues for 75 min. At this time, the nuclei were isolated and the RNA polymerase I activity was measured. The results (Fig. 3a) show that in cells incubated in the complete medium FdUr and FdUr decrease the RNA polymerase I activity to about half the value of the control nuclei. The decreased activity in nuclei from cells incubated in a medium without amino acids is not affected by any of the concentrations of the drugs tested. This lack of effect cannot be explained by an altered uptake of the drugs in the amino acid-starved cells, since rRNA processing is affected in these cells (see Figs. 1c and 1d). Like the fluorinated pyrimidines, 8-azaguanine inhibits transcription in cells incubated in the complete medium, but the effect is less pronounced (Fig. 3b).

The results in Fig. 4 demonstrate that the decreased radioactivity in isolated nuclei from cells treated with FdUr is not the consequence of enhanced RNA degradation. The kinetics of the RNA polymerase I activity in nuclei from control and FdUr-treated cells is similar and the RNA synthesized has the same stability during a 10 min chase with the unlabelled precursor. It should be pointed out that, to prevent non-specific initiations of RNA chains (Iapalucci-Espinoza & Franze-Fernández, 1979), the reactions were performed in the presence of an initiation inhibitor. Thus the lowered RNA synthesis in nuclei from FdUr-treated cells should reflect a decrease either in the number of growing RNA chains or in the rate of RNA chain elongation.

The effect of FdUr on the synthesis of the 45S rRNA precursor in whole cells was also investigated. For that purpose, control and FdUr-treated cells were labelled with a short pulse of [3 H]-adenosine and the labelled RNA was purified and analysed by polyacrylamide-gel electrophoresis. It was found (Fig. 5) that FdUr inhibits the incor-

Fig. 2. Effect of FURd on the maturation of the 45S rRNA synthesized within 15 min after addition of the drug to Ehrlich ascites cells

Cells were resuspended in complete medium, placed in three experimental flasks and incubated for 60 min. At this time, two of the cultures were treated with $100\mu\text{M}$ -FURd. Then 3 min afterwards the three cultures received $[{}^3\text{H}]$ adenosine ($40\mu\text{Ci/ml}$) and were further incubated for 12 min. At this time: (a) the reaction was stopped in one of the FURd-treated cultures and the RNA was extracted and analysed by electrophoresis in acrylamide gels as indicated in Fig. 1; (b) actinomycin D ($5\mu\text{g/ml}$) was added to the remaining flasks. After further incubation for 90 min, the RNA was extracted and analysed by electrophoresis in acrylamide gels as before. ●, RNA from the FURd-treated culture; ○, RNA from the control culture.

poration of $[{}^3\text{H}]$ adenosine into the 45S rRNA in the whole cells. The results in Table 1 indicate that, as previously shown for toyocamycin (Iapalucci-Espinoza *et al.*, 1977), there is a close correlation

Fig. 3. Effect of addition of different concentrations of nucleoside analogues to whole cells on RNA polymerase I activity in isolated nuclei

Ehrlich ascites cells cultured in a complete medium (open symbols) or in amino acid-deprived medium (black symbols) were exposed to different concentrations of the nucleoside analogues for 75 min. At this time cells were harvested, nuclei were isolated and the transcriptional activity was determined. (a) △, ▲, FURd; ○, ●, FdUrd. (b) ○, ●, 8-Azaguanine.

between the rate of rRNA synthesis in whole cells and the corresponding activity of nuclear RNA polymerase I, thus showing that isolated nuclei reflect the transcriptional activity of the intact cell.

Fig. 6 shows that, on addition of FURd to cells incubated in the complete medium, the RNA polymerase I activity in the isolated nuclei declines after a lag of about 20 min. When this result is

compared with that in Fig. 2, it is clear that FUr has an earlier effect on processing than on transcription of rRNA.

Two lines of evidence support the idea that the nucleoside analogues have no direct effect on transcription. First, the analogues do not affect transcription in cells incubated in media lacking amino acids, in spite of the fact that their uptake by the cells, as indicated by the inhibition of rRNA processing, is unimpaired by amino acid deprivation (see Figs. 1c, 1d and 3). Second, when whole cells are exposed to the analogues, the inhibition of

transcription is manifested in the activity of the template-bound RNA polymerase I in the isolated nuclei. Since this assay system *in vitro* depends on the addition of the four ribonucleoside triphosphates for RNA synthesis, it is inferred that nuclei should be almost depleted of the nucleoside analogues and their derivatives. Furthermore, unlike the effect *in vivo*, none of the analogues inhibit RNA poly-

Fig. 4. Kinetics of the RNA polymerase I activity in nuclei from control and FUr-treated Ehrlich ascites cells. Cells were incubated for 90 min in complete medium (○) or in complete medium plus 100 μM-FUr (●). At this time nuclei were isolated and the RNA polymerase I activity was measured in the presence of 400 μg of heparin/ml (—). After 10 min, a 30-fold excess of unlabelled UTP was added to one part of the assay, and the incubation was continued for the times indicated (----).

Fig. 5. Effect of FUr on the incorporation of [³H]-adenosine into 45S rRNA. Ehrlich ascites cells were incubated for 90 min in complete medium (○) or in complete medium containing 100 μM-FUr (●). At this time, cells were pulse-labelled with [³H]adenosine (5 μCi/ml) for 10 min. The incorporation was halted by pouring the cells into cold Dulbecco's medium, and a tracer amount of cells labelled with [¹⁴C]uridine was added to correct for small differences in recovery during RNA isolation and analysis. The RNA was extracted by the hot-phenol procedure indicated in the Experimental section and analysed by electrophoresis in acrylamide gels as indicated in Fig. 1. The amount of radioactivity in the 45S peaks was estimated graphically (Girard *et al.*, 1965).

Table 1. Nuclear RNA polymerase I activity and rate of rRNA precursor synthesis in control and drug-treated cells. For results in the first two lines, Ehrlich ascites cells were incubated for 90 min in complete medium or in complete medium plus 100 μM-FUr. At this time, a sample of each culture was removed for pulse-labelling with [³H]adenosine and the samples were processed as indicated in Fig. 5. The data were obtained from the experiment in Fig. 5. From the remaining culture, nuclei were isolated and the RNA polymerase I activity was determined.

Nucleoside analogue	[³ H]Nucleoside incorporation into 45S rRNA (%)	RNA polymerase I activity (%)
—	100	100
FUr (100 μM)	33	40
—	100*	100*
Toyocamycin (2 μM)	59*	58*

* Data from Iapalucci-Espinoza *et al.* (1977). Cells were pulse-labelled with [³H]uridine.

Fig. 6. Effect of FURd on nuclear RNA polymerase I activity in Ehrlich ascites cells cultured in a complete medium

Cells were preincubated in complete medium for 60 min. At zero time FURd was added (final concn. $100\text{ }\mu\text{M}$) to half of the culture and the incubation was continued. Samples of the incubation were taken at the times indicated, from the control (O) and the FURd-treated culture (●), the nuclei were isolated and the RNA polymerase I activity was determined.

Fig. 7. Effect of nucleoside analogues on the incorporation of $[^3\text{H}]$ leucine into proteins

Ehrlich ascites cells were incubated in a complete medium (O), a complete medium plus $100\text{ }\mu\text{M}$ -FdUrd (●), a complete medium plus $100\text{ }\mu\text{M}$ -FURd (Δ), or a complete medium plus $750\text{ }\mu\text{M}$ -8-azaguanine ($\blacktriangle$). All media contained $1\text{ }\mu\text{Ci}$ of $[^3\text{H}]$ -leucine/ml. At the times indicated, samples (about 1.5×10^6 cells) were taken, poured on to cold Dulbecco's saline and processed as described in the Experimental section.

Table 2. Effect of addition of nucleoside analogues to isolated nuclei

Ehrlich-ascites-cell nuclei were isolated and the RNA polymerase I activity was assayed with or without the indicated concentrations of nucleoside analogues.

Nucleoside analogue	RNA polymerase I activity (pmol of UMP/mg of DNA)
—	1389
FURd ($150\text{ }\mu\text{M}$)	1248
FURd ($300\text{ }\mu\text{M}$)	1278
FdUrd ($150\text{ }\mu\text{M}$)	1324
FdUrd ($300\text{ }\mu\text{M}$)	1450
8-Azaguanine ($500\text{ }\mu\text{M}$)	1412
8-Azaguanine ($1000\text{ }\mu\text{M}$)	1335

merase I activity when added to the assay system *in vitro* (Table 2). Thus the drugs appear to affect the mechanism controlling rRNA synthesis.

Effect of the nucleoside analogues on protein synthesis

Since in animal cells in culture the rate of rRNA synthesis is closely coupled to that of protein synthesis (Willems *et al.*, 1969; Craig & Perry,

1970; Franze-Fernández & Fontanive-Sanguesa, 1973; Penman *et al.*, 1976), we have investigated the possibility that the nucleoside analogues affect rRNA transcription through inhibition of protein synthesis. Results in Fig. 7 show that this is not the case, since protein synthesis proceeds normally in the drug-treated cells. In this regard, it is worth noting that the adenosine analogue toyocamycin does not inhibit protein synthesis either (Iapalucci-Espinoza *et al.*, 1977). Furthermore, the 80S ribonucleoprotein particle, which contains 45S rRNA, synthesized in the presence of toyocamycin revealed a full protein complement (Auger-Buendia & Tavittian, 1979).

The effects of the nucleoside analogues on rRNA transcription and maturation are summarized in Table 3. The dose of the drugs that inhibits transcription in Ehrlich ascites cells varies from $2\text{ }\mu\text{M}$ for toyocamycin to $100\text{ }\mu\text{M}$ and $500\text{ }\mu\text{M}$ for the fluorinated pyrimidines and 8-azaguanine respectively. The same doses are required to affect rRNA processing. The guanosine analogue, which does not completely suppress rRNA maturation, has a less pronounced effect on transcription than do the fluorinated pyrimidines and toyocamycin, which abolish the formation of 28S and 18S rRNA.

Table 3. *Effect of nucleoside analogues on rRNA transcription and processing*

Cells incubated in the complete medium were exposed to the drugs for 1 h and transcription was measured in the isolated nuclei as indicated in the Experimental section. Processing of rRNA was investigated by analysing the labelled RNA purified from cells incubated in complete medium and exposed to the drugs for 2 h. Details are indicated in the legend to Fig. 1.

Cells	Analogue	rRNA transcription (% of control)	rRNA processing
Ehrlich ascites cells	Toyocamycin (2 μ M)*	40–50†	No 28S and 18S RNA†
	FdUrd (100 μ M)*	40–50	No 28S and 18S RNA
	FdUrd (100 μ M)*	40–50	No 28S and 18S RNA
	8-Azaguanine (500 μ M)*	70–80	Decreased amount of 28S and 18S RNA
BHK ₂₁ cells	Toyocamycin (2 μ M)*	40	No 28S and 18S RNA
	FdUrd (150 μ M)	100	Normal

* Minimal concentration that exerts the maximal effect on rRNA transcription or processing.

† Data taken from Iapalucci-Espinoza *et al.* (1977).

Another correlation between the effect of the nucleoside analogues on rRNA transcription and processing is observed with FdUrd. In Ehrlich ascites cells FdUrd inhibits both rRNA maturation and transcription, whereas in BHK₂₁ cells neither of these processes is affected. Toyocamycin, on the other hand, inhibits both processes in BHK₂₁ cells.

In conclusion, our results show that the nucleoside analogues affect rRNA transcription and processing in a co-ordinate manner, the latter being affected slightly earlier than transcription. The drugs do not appear to have a direct effect on transcription and their action cannot be explained by the inhibition of protein synthesis. The most likely interpretation of our findings is that the nucleoside analogues, through their incorporation into RNA, inhibit rRNA processing, and this affects transcription as a feed-back effect. Transcription and processing are, however, not coupled at all transcription rates. Even when rRNA maturation is abolished by the analogues, transcription amounts to 40–50% of that in control nuclei. This can explain the lack of effect of the drugs on the decreased extent of transcription in the amino acid-starved cells.

It was reported that in a temperature-sensitive mutant of BHK₂₁ cells, which has a defect in the production of 28S rRNA at 39°C, transcription of rRNA is not affected at the restrictive temperature (Toniolo *et al.*, 1973; Mora *et al.*, 1980). In this mutant, the initial cleavage of the 45S rRNA is not affected at 39°C, and the production of 18S rRNA is normal (Winicov, 1976). The nucleoside analogues, on the other hand, affect the cleavage of the 45S rRNA (Tavitian *et al.*, 1968; Weiss & Pitot, 1974; Wilkinson *et al.*, 1975). These results, together with the present findings, might suggest that, for impairing rRNA transcription, the cleavage of the 45S rRNA should be affected.

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References

- Auger-Buendia, M. A. & Tavitian, A. (1979) *Biochim. Biophys. Acta* **563**, 129–142
- Campbell, J. M. & Weissbach, H. (1980) *J. Biol. Chem.* **255**, 4691–4697
- Cox, R. F. (1976) *Cell* **7**, 455–465
- Craig, N. C. & Perry, R. P. (1970) *J. Cell Biol.* **45**, 554–564
- Franze-Fernández, M. T. & Fontanive-Sanguesa, A. V. (1973) *Biochim. Biophys. Acta* **331**, 71–80
- Franze-Fernández, M. T. & Fontanive-Sanguesa, A. V. (1975) *FEBS Lett.* **54**, 26–29
- Franze-Fernández, M. T. & Pogo, A. O. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 3040–3044
- Girard, M., Latham, H., Penman, S. & Darnell, J. E. (1965) *J. Mol. Biol.* **11**, 187–201
- 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
- Gross, K. J. & Pogo, A. O. (1974) *J. Biol. Chem.* **249**, 568–576
- Harbers, E., Chaudhuri, N. K. & Heidelberg, C. (1959) *J. Biol. Chem.* **234**, 1255–1262
- Herlan, G., Eckert, W. A., Kaffenberger, W. & Wunderlich, F. (1979) *Biochemistry* **18**, 1782–1788
- Iapalucci-Espinoza, S. & Franze-Fernández, M. T. (1979) *FEBS Lett.* **107**, 281–284
- Iapalucci-Espinoza, S., Cereghini, S. & Franze-Fernández, M. T. (1977) *Biochemistry* **16**, 2885–2889
- MacPherson, I. & Stoker, M. (1962) *Virology* **16**, 147–151

- Mora, M., Darzynkiewicz, Z. & Baserga, R. (1980) *Exp. Cell Res.* **125**, 241–249
- Penman, M., Huffman, R. & Kumar, A. (1976) *Biochemistry* **15**, 2661–2668
- Perry, R. P. (1973) *Biochem. Soc. Symp.* **37**, 105–116
- Rapaport, E. & Zamecnik, P. C. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3984–3988
- Tavittian, A., Uretsky, S. C. & Acs, G. (1968) *Biochim. Biophys. Acta* **157**, 33–42
- Toniolo, D., Meiss, H. K. & Basilico, C. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1273–1277
- Udvardy, A. & Seifart, K. H. (1976) *Eur. J. Biochem.* **62**, 353–363
- Weil, P. A. & Blatti, S. P. (1976) *Biochemistry* **15**, 1500–1509
- Weiss, J. W. & Pitot, H. C. (1974) *Arch. Biochem. Biophys.* **160**, 119–129
- Wilkinson, D. S., Tlsty, T. D. & Hanas, R. J. (1975) *Cancer Res.* **35**, 3014–3020
- Willems, M., Penman, M. & Penman, S. (1969) *J. Cell Biol.* **41**, 177–187
- Winicov, I. (1976) *J. Mol. Biol.* **100**, 141–155
- Yu, F. L. & Feigelson, P. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2833–2837
- Zylber, E. A. & Penman, S. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2861–2865