Relationship between polyamine accumulation and RNA biosynthesis and content during the cell cycle[1]

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Summary. Polyamine accumulation was related to RNA biosynthesis and content during the cell cycle of CHO-K1 and HeLa cells. The highest correlations were observed between polyamine accumulation and RNA content. Feeding, serum-starved cultures of embryonic chick fibroblasts, which exhibit a 3-fold increase in RNA content, demonstrated that RNA content was most closely paralleled by spermidine content.

There is considerable evidence that the polyamines are involved in the maintenance of cell growth[3]. Although the roles that the polyamines play in the cell are not fully understood at present, we now have good indications as to their most probable sites of action. These molecules have high affinities for nucleic acids[4] and very high correlations have been observed between polyamine and RNA contents in many animal systems[5–7]. From these findings it has been suggested that polyamines bind to RNA in the cell to stabilize ribosomes and possible tRNAs[3]. However, studies of cell-free chromatin-primed or DNA-primed RNA synthesis have demonstrated that addition of polyamine has a pronounced stimulatory effect[8–10]. Also, addition of polyamine increases [^{3}H]-uridine incorporation in *Drosophila* larvae[11], sea urchin embryos[12] and perfused rabbit heart[13]. These observations have suggested, on the contrary, that polyamine accumulation in the cell is important in stimulating RNA synthesis.

It is therefore of interest to examine how polyamine accumulation relates to changes in the rate of RNA synthesis and RNA content during the fundamental unit of cell proliferation, the cell cycle. For this reason I have studied these changes during the cell cycles of hamster CHO-K1 and human HeLa cells. These are highly synchronized and morphologically homogeneous cultures, and as such overcome problems of interpreting data from systems such as regenerating rodent liver[14].

Materials and methods. CHO-K1 cells were grown in suspension cutlure in F12 medium[15], supplemented with 7.5% (v/v) horse serum. The cells had been conditioned to horse serum for at least 4 weeks continuous culture before being used in this study. CHO-K1 cells were synchronized by colcemid arrest. 6×10^6 cells were seeded into 140-mm petri dishes and incubated for 12 h at 37 °C. The dishes were shaken and the medium aspirated to remove floating dead cells. The dishes were washed with prewarmed medium, and then 15 ml prewarmed medium containing 0.1 µg/ml colcemid was added to each dish before incubating at 37 °C for 2.5 h. After this time the dishes were shaken gently to dislodge the mitotic cells. HeLa cells were grown in suspension culture in Eagles minimal essential medium[16], supplemented with 5% (v/v) foetal calf serum. The HeLa cells were prepared for synchronization in the same way as the CHO-K1 cells; the only difference was that 4×10^6 cells were initially seeded. The cells were synchronized by incubating the dishes in an atmosphere of N_2O/CO_2 (95:5, v/v) under 5×10^5 Pa pressure at 37 °C for 3.5 h[17]. The mitotic cells were removed by gently shaking the dishes. Fibroblasts were isolated from 12-day chick embryos from which the head, limbs and viscera had been removed. The cells were passaged at least 2 times before use in the experiments. The cells were grown in monolayer in Eagles minimal essential medium[16], supplemented with 10% (v/v) foetal calf serum. To synchronize the fibroblasts, 2×10^6 cells were seeded into 90-mm petri dishes and incubated for 3 days in the absence of serum. The cultures were stimulated by changing the medium to medium containg 10% (v/v) serum.

Polyamine content was determined by the dansylation method[18] and RNA content assayed by the orcinol method[19], with yeast RNA as standard. The rate of RNA synthesis was estimated by labelling the cells with [^{3}H]-uridine (0.3 µCi/ml; 29.2 Ci/mmole) for 30 min, during which time incorporation was linear. The RNA was then precipitated by ice-cold 10% (w/v) trichloroacetic acid, filtered onto a glass-fibre 2.5-cm-diameter GF/C disc (Whatman), immersed in 1 ml scintillant [4 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-(5-phenyloxazol-2-yl) benzene in 1 l of toluene] and counted for radioactivity in a Packard Tri-Carb liquid-scintillation counter.

Results and discussion. The cells were synchronized by techniques which produce minimal perturbation of polyamine content[20]. Under these conditions both CHO-K1 and HeLa exhibited similar cell cycles as measured by the time-course of [^{3}H]-thymidine incorporation and by the occurrence of a 2nd wave of mitotic cells. In both systems spermidine content increased mainly during S and G_2 phases, whereas spermine and putrescine exhibited their main increase slightly later during late-S and G_2. Both spermidine and spermine almost doubled during the cell cycle but putrescine increased only 1.4 times.

The rate of RNA synthesis, as measured by [^{3}H]-uridine incorporation into acid-precipitable material, increased throughout G_1 and S-phase. In CHO-K1 cells, [^{3}H]-uridine incorporation increased 5 times to peak during S-phase and thereafter steadily declined. In HeLa cells, [^{3}H]-uridine incorporation increased 12 times to a peak early in G_2 and then decreased slightly. The relationship of polyamine content to [^{3}H]-uridine incorporation is shown in figure 1. A high correlation coefficient ($r=0.95$) was observed only

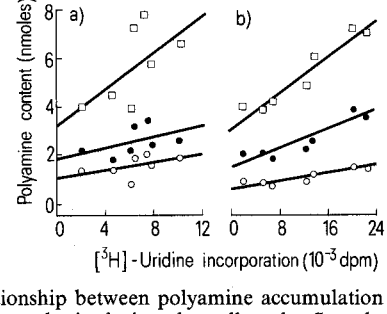

Fig. 1. Relationship between polyamine accumulation and the rate of RNA biosynthesis during the cell cycle. Samples were taken every 3 h during the cell cycle and analyzed for polyamine content and [^{3}H]-uridine incorporation as described in the 'materials and methods' section. The polyamine content is nmoles per million mitotic cells seeded after release from the synchronization block. [^{3}H]-uridine incorporation is dpm per 30 min per 10^5 mitotic cells seeded. ○—○, Putrescine; □—□, spermidine; ●—●, spermine. *a* CHO-K1; *b* HeLa. The lines for all sets of data were calculated by linear regression analysis. Pearsons correlation coefficients for parametric data were calculated and are as follows: *a* Putrescine, $r=0.47$ ($p>0.1$); spermidine, $r=0.66$ ($p>0.1$); spermine, $r=0.43$ ($p>0.1$). *b* Putrescine, $r=0.87$ ($p<0.02$); spermidine, $r=0.95$ ($p<0.002$); spermine, $r=0.89$ ($p<0.01$).

between spermidine and [^{3}H]-uridine incorporation in HeLa cells. Generally there appeared to be a poor to good correlation ($r < 0.9$) between these parameters. These data indicate that the observation that polyamine addition stimulates RNA synthesis in cell-free systems[8-10] may not be relevant to the actual situation in the cell. Additions of polyamines to animal systems have also been found to increase [^{3}H]-uridine incorporation into RNA[11-13]. However, this can be explained by postulating that exogenous polyamine does not increase RNA synthesis but instead reduces RNA degradation, and there is evidence that this is the case from studies of perfused rat liver[21].

RNA content exhibited a similar time-course of increase to that of spermidine and increased from late G_1 to G_2. In both systems the RNA content almost doubled during the cell cycle and its relationship with the polyamines is summarized in figure 2. Good to very high correlation coefficients were observed between RNA and the polyamines. However, these data may not be of significance as both RNA and the polyamines might be expected to double during the cycle of proliferating cells.

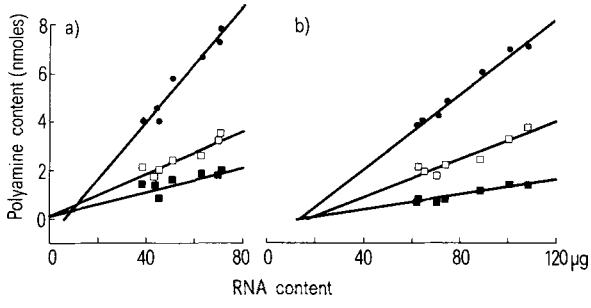

Fig. 2. Relationship between polyamine accumulation and RNA content during the cell cycle. Samples were taken every 3 h during the cell cycle and analyzed for polyamine and RNA contents as described in the 'materials and methods' section. The polyamine and RNA contents are per million mitotic cells seeded after release from the synchronization block. ■—■, Putrescine; ●—●, spermidine; □—□, spermine. *a* CHO-K1; *b* HeLa. The lines for all sets of data were calculated by linear regression analysis. Pearsons correlation coefficients for parametric data were calculated and are as follows: *a* Putrescine, $r = 0.83$ ($p < 0.05$); spermidine, $r = 0.97$ ($p < 0.001$); spermine, $r = 0.93$ ($p < 0.002$). *b* Putrescine, $r = 0.94$ ($p < 0.002$); spermidine, $r = 0.99$ ($p < 0.001$); spermine, $r = 0.94$ ($p < 0.002$).

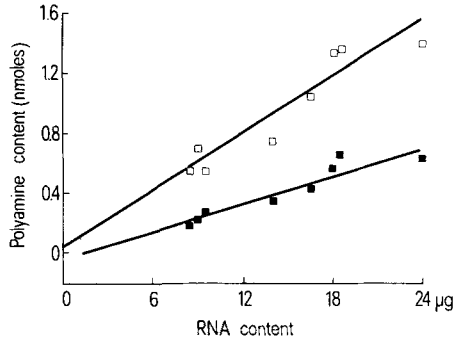

Fig. 3. Relationship between polyamine accumulation and RNA content after feeding serum-starved cultures of embryonic chick fibroblasts. ■—■, Spermidine; □—□, spermine. The lines for all sets of data were calculated by linear regression analysis. Pearsons correlation coefficients for parametric data were calculated and are as follows: spermidine, $r = 0.94$ ($p < 0.002$); spermine, $r = 0.93$ ($p < 0.002$).

In an attempt to clarify this situation the embryonic chick fibroblast system was studied. It has been found that in many situations in which quiescent cells have been stimulated to grow, the RNA content increases significantly more than 2 times during the 1st cycle after stimulation [22,23], and therefore such a system could be of value in assessing the significance of polyamine accumulation.

The situation in chick fibroblasts is particularly interesting as these cells have a very low putrescine content. The putrescine content was difficult to determine accurately, but at no time after feeding did it increase above 15 pmoles per million cells. This suggests that putrescine does not have an important role in binding to RNA. 2ndly, the RNA content trebles during the 1st cycle of growth after stimulation. The time-course of accumulation of RNA was similar to that of spermidine and spermine (figure 3). However, spermidine content likewise trebled during the cycle but spermine only doubled (the latter being similar to the situation observed during the cycles of proliferating cells).

These observations support the concept of spermidine binding to RNA in the cell. The situation with spermine appears to be more complex. Spermine may bind to RNA but it is probable that it has some additional function(s) in the cell, and recent evidence has indicated that it may be involved in chromatin condensation[5,24]. As the time-courses of spermidine and RNA accumulation are closely related, it is possible that an increase in one of these will induce increased biosynthesis of the other. Recent evidence supports this idea[25], for studies of a temperature-sensitive animal cell mutant have demonstrated the causal link between RNA and polyamine biosynthesis. Therefore, as polyamine accumulation is more closely correlated with RNA content than with the rate of RNA synthesis, I suggest that the accumulation of RNA during the cell cycle is responsible for the increase in spermidine biosynthesis.

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