Response of Poly(Adenylic Acid) Polymerase in Rat Liver Nuclei and Mitochondria to Starvation and Re-Feeding with Amino Acids

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Poly(adenylic acid) polymerase was extracted from liver nuclei and mitochondria of rats either fed *ad libitum*, starved overnight or starved and then re-fed with a complete amino acid mixture for 1–3 h. The enzymes were partially purified and assayed by using exogenous primers. Starvation resulted in an 80% decrease in the total activity of the purified nuclear enzyme, and the mitochondrial enzyme activity diminished to almost zero after overnight starvation. Measurements of the protein content of whole nuclei or mitochondria and of the enzyme extracts from these organelles indicated that the decrease in enzyme activity on starvation was not caused by incomplete extraction of the enzyme from the starved animals. Re-feeding the animals with the complete amino acid mixture increased the total activity of poly(A) polymerase from the nuclei and mitochondria by 1.9-fold and 63-fold respectively. Under these conditions, the total protein content of the nuclei and mitochondria increased by only 13 and 32% respectively. These data indicate that poly(A) polymerase is one of the cellular proteins specifically regulated by amino acid supply.

There is abundant evidence that nucleic acid metabolism in animal cells is sensitive to amino acid supply. For example, it has been reported that activities of RNA polymerases I and II of rat liver nuclei undergo meal-related diurnal rhythms (Barbiroli *et al.*, 1973), that rRNA synthesis in cultured cells is dependent on the presence of certain amino acids in the medium (Bölcsföldi & Eliasson, 1972), and that mRNA transfer from liver nuclei to the cytoplasm can be accelerated by tryptophan administration (Murty & Sidransky, 1972). These and other observations suggest that there may be a co-ordinated response of various aspects of nucleic acid metabolism to amino acid supply.

Considerable attention has been given to diet-induced changes in DNA-dependent RNA polymerases (Rickwood & Klemperer, 1970; Henderson, 1970; Clark & Jacob, 1972; Glasser & Spelsberg, 1972; Andersson & von der Decken, 1975). Despite much emphasis on enzymes involved in transcription, little attention has been paid to possible alterations in the activity of RNA-processing enzymes that are responsible for the post-transcriptional modification of RNA molecules. One such enzyme is poly(adenylic acid) polymerase (EC 2.7.7.19), which is involved in the addition of 100–250 adenylic acid residues to the 3'-hydroxyl termini of newly synthesized mRNA

molecules. The presence of poly(A) polymerase in nuclei has been known for some time, and the enzyme has been well characterized by Winters & Edmonds (1973) and also by our group, who extracted poly(A) polymerase from isolated liver nuclei, chromatographically separated it from RNA polymerases (Rose & Jacob, 1974), and further purified this enzyme by phosphocellulose chromatography (Jacob *et al.*, 1975). In addition, a separate poly(A) polymerase has been solubilized from liver and tumour mitochondria (Jacob & Schindler, 1972; Jacob *et al.*, 1972). The liver enzyme has been partially purified (Jacob *et al.*, 1974) and the tumour mitochondrial enzyme has been extensively purified (Rose *et al.*, 1975a). This enzyme appears to be responsible for addition of the poly(A) segment to mitochondrial mRNA, since mitochondrial mRNA is preferred to nuclear mRNA as the primer for the reaction (Rose *et al.*, 1975a). In the present series of experiments, we show that the activities of both nuclear and mitochondrial poly(A) polymerase from rat liver are considerably and rapidly affected by amino acid intake. Although Mn^{2+} - and Mg^{2+} -dependent forms of poly(A) polymerase (Niessing & Sekeris, 1974; Niessing, 1975) have been identified in rat liver nuclei, the present studies were focused on the most predominant Mn^{2+} -dependent nuclear poly(A) polymerase.

Experimental

Materials

[2,8-³H]ATP (34 Ci/mmol) was obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). Pyruvate kinase was a product of Boehringer-Mannheim (New York, NY, U.S.A.). ATP and dithiothreitol were purchased from Calbiochem (La Jolla, CA, U.S.A.). Whatman cellulose phosphate P-11 was purchased from Reeve Angel (Clifton, NJ, U.S.A.) and phosphoenolpyruvate from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Animals

The experiments were performed on Sprague-Dawley male rats (100–125 g body wt.). In each series of experiments, one group of animals consuming Purina chow *ad libitum* was given 3 ml of 0.9% NaCl by stomach tube or by mouth 1 or 3 h before killing [control (fed) group]; another group of animals was starved overnight and was then given 3 ml of 0.9% NaCl by stomach tube (starved group); another group of starved animals received by stomach tube 3 ml of a mixture of 20 amino acids in the proportions described previously (Wunner *et al.*, 1966) (amino acid-re-fed group). For isolation of nuclei, animals were killed at 10:00 h, 1 h after feeding. For isolation of mitochondria, animals were killed at 16:00 h, usually 3 h after feeding. Livers from groups of four rats were pooled and used to isolate the respective organelles.

Isolation of liver-cell nuclei

Nuclei were isolated from the livers of groups of four rats by homogenization in 2.2 M-sucrose containing 15 mM-MgCl₂ and 0.25 mM-spermine (Busch *et al.*, 1967; Jacob *et al.*, 1969), followed by centrifugation at 40000g for 70 min. A portion of the nuclear pellets representing 1–2 g of liver was resuspended in 0.25 M-sucrose containing 0.3% Triton X-100, left for 15 min at 0°C and then centrifuged at 600g for 15 min. This sample was taken for determination of protein and DNA. The remaining pellet was used to extract the enzyme.

Extraction and purification of nuclear poly(A) polymerase

Nuclei were suspended in 50 mM-Tris/HCl (pH 8.9) buffer containing 50 mM-KCl, 2 mM-dithiothreitol, 0.05 mM-EDTA, 1 mM-MgCl₂, 0.05 mM-phenylmethylsulphonyl fluoride, bovine serum albumin (0.12 mg/ml) and 40% (v/v) glycerol and enzymes were extracted as described previously (Rose *et al.*, 1975b). After precipitation with (NH₄)₂SO₄, the proteins were suspended in buffer (0.5 ml/g wet wt liver) containing 50 mM-Tris/HCl (pH 7.9), 25% (v/v) glycerol, 5 mM-MgCl₂, 0.1 mM-EDTA and 0.5 mM-dithiothreitol (TGMED buffer) and dialysed over-

night against the same buffer. After centrifugation (80000g for 40 min) to clarify the extract, the pellet was suspended in a small volume of TGMED buffer, sonicated for 30 s at 10 kHz with a Branson sonifier (model W140) and re-centrifuged at 80000g for 30 min. The supernatants from both centrifugations were pooled and subjected to DEAE-Sephadex chromatography as previously described (Rose & Jacob, 1974). All poly(A) polymerase activity was eluted in the void volume and in the wash fractions of the column. Fractions containing poly(A) polymerase activity were pooled and dialysed against buffer containing 50 mM-potassium phosphate (pH 6.8), 30% (v/v) glycerol, 0.1 mM-EDTA and 0.2 mM-dithiothreitol (PGED buffer). The enzymes from control (fed), starved and amino acid-re-fed groups were then subjected to phosphocellulose chromatography. Enzymes were applied to a column (0.9 cm × 8 cm) previously equilibrated in PGED buffer. After washing the column with 10 ml of PGED buffer, enzymes were eluted with a linear gradient from 0 to 0.8 M-KCl in PGED buffer. Fractions containing the enzyme activity were pooled and dialysed overnight against buffer containing 50 mM-Tris/HCl (pH 7.9) and 50% (v/v) glycerol. After dialysis, fractions containing the enzyme were stored at -70°C.

Assay for nuclear poly(A) polymerase

The enzyme was assayed in a reaction mixture containing 50 mM-Tris/HCl (pH 8), 0.5 mM-MnCl₂, 25 mM-KCl, 350 μM-[2,8-³H]ATP (3.6 × 10⁴ d.p.m./nmol), 400 μg of poly(A)/ml as primer and 5–20 μg of enzyme protein in a final volume of 120 μl. The reaction mixture was incubated at 37°C for 1 h and 100 μl portions were transferred on to DE81 filters. The filters were washed batchwise four times with 5% (w/v) Na₂HPO₄ (200–400 ml/100 filters), twice with glass-distilled water and once with ethanol. The filters were air dried and counted for radioactivity in an Isocap 300 (Amersham-Searle) liquid-scintillation counter, by using a toluene-based fluor (NEN 949).

Isolation of mitochondria

Mitochondria were isolated as described previously (Jacob & Schindler, 1972).

Extraction and purification of mitochondrial poly(A) polymerase

Poly(A) polymerase and its endogenous primer were extracted from isolated mitochondria as described previously (Jacob *et al.*, 1974). Essentially, the method consisted of sonication at 20 kHz in a Branson sonifier for 3 × 15 s in 50 mM-Tris/HCl (pH 9.0) buffer containing 5 mM-MgCl₂, 0.1 mM-EDTA, 0.1 mM-dithiothreitol and 20% (v/v) glycerol (0.3 ml/g of liver), followed by incubation for 30 min

at 37°C and centrifugation at 105000g for 75 min. The supernatant containing the poly(A) polymerase activity was dialysed for 5 h against buffer containing 50 mM-potassium phosphate (pH 6.8), 20% (v/v) glycerol, 5 mM-MgCl₂, 0.1 mM-EDTA, 0.1 mM-dithiothreitol and 30 mM-KCl (PGMEDK buffer). Solubilized enzyme was applied to a column (0.9 cm × 8 cm) of phosphocellulose previously equilibrated in PGMEDK buffer. After washing the column with PGMEDK buffer, the enzyme was eluted with a linear gradient of 0.03–0.5 M-KCl in PGMED buffer. The column fractions containing the enzyme activity (assayed with a constant amount of primer isolated from control animals) were pooled and stored at –70°C. The endogenous primer was eluted in the wash fractions and the poly(A) polymerase at 0.2 M-KCl.

Purification of mitochondrial primer

Column wash fractions containing the bulk of the priming activity (as assayed with a constant amount of purified enzyme from control animals) were pooled and partially purified (Jacob *et al.*, 1974) by heating at 100°C for 2 min, quickly cooling and centrifuging at 8000g for 10 min to remove the denatured protein. In some experiments, the primer was further purified by sucrose-density-gradient fractionation. The ratio E_{260}/E_{280} of the purified primer was 2.

Assay for mitochondrial poly(A) polymerase

The standard assay mixture for mitochondrial poly(A) polymerase contained 30 mM-Tris/HCl (pH 8), 1.5 mM-MgCl₂, 0.06 mM-dithiothreitol, 0.03 mM-EDTA, 10 mM-KCl, 120 μM-[³H]ATP (1.5×10^4 d.p.m./nmol), 6 mM-phosphoenolpyruvate, 2 μg of pyruvate kinase, 0.4 E_{260} unit of primer and 20–100 μg of enzyme protein in 0.35 ml total volume. Reaction mixtures were incubated for 1 h and the reaction was terminated with 1 ml of 10% (w/v) trichloroacetic acid containing 0.04 M-Na₄P₂O₇. The radioactivity in the acid-insoluble material was determined as described previously (Jacob & Schindler, 1972).

Measurement of protein and DNA

Protein was measured by the method of Bennett (1967), and DNA by the procedure described by Burton (1956).

Results

Response of nuclear poly(A) polymerase to starvation and to amino acid re-feeding

Fig. 1 shows the phosphocellulose-column-chromatographic profiles of poly(A) polymerase activity obtained from the nuclei of control (fed), starved, and amino acid-re-fed rats. Despite the use of equal amounts of nuclei harvested from the two groups,

Fig. 1. Phosphocellulose chromatography of nuclear poly(A) polymerase

Enzyme was extracted from nuclei isolated from groups of four rats and subjected to DEAE-Sephadex chromatography as described in the text. The void-volume and column-wash fractions containing activity were pooled and dialysed overnight against PGED buffer. The enzyme fractions were each layered on a column (0.9 cm × 8 cm) of phosphocellulose previously equilibrated in PGED buffer. The columns were washed with 10 ml of PGED buffer and eluted with a gradient (----) from 0 to 0.9 M-KCl in PGED buffer; fractions (1 ml) were collected, and 30 μl portions taken to assay in the standard reaction mixture enzyme activity from control (fed) group (●), starved group (▲) and amino acid-re-fed group (○).

based on DNA content, there was a decrease of about 80% in total enzyme activity as a result of withdrawal of food for 24 h and an increase (twofold relative to the starved group) after re-feeding.

Table 1 provides a more detailed analysis of the amounts of protein recovered during the purification procedure. At each stage from the initial nuclear extract onward, there was less total protein in the preparations from the starved rats. Overnight starvation resulted in 32 and 56% decreases in the protein content of isolated nuclei and the total nuclear extracts respectively. Similarly, the amount of protein in the total enzyme fractions recovered after the final column chromatography was also decreased by 57% on starvation. Since the proportion of the initial nuclear protein extracted from normal and starved animals did not vary significantly, it is unlikely that the differences in the poly(A) polymerase activity between these groups are due to diet-related differential extraction of the enzyme. Re-feeding the animals with the complete amino acid mixture increased the protein content of the isolated nuclei by 13%, whereas the protein con-

Table 1. Effect of amino acid supply on rat liver nuclear protein content and poly(A) polymerase activity

Nuclei were isolated from the livers of groups of four rats which were either fed *ad libitum* (control fed), starved or amino acid-re-fed as described in the text. Total recovery of nuclear DNA was 20 ± 2 mg per group of animals. Enzymes were extracted from each batch of nuclei and purified by chromatography on DEAE-Sephadex and phosphocellulose as described in the text. Enzyme activity was measured with saturating amount of poly(A) as primer and were calculated from the average of several concentrations of each fraction in the linear range of activity. This Table shows the data from a typical experiment. The enzyme assay for each fraction was performed in triplicate.

Measurement	Control (fed)	Starved	Decrease after starvation (%)	Amino acid re-fed	Increase after re-feeding (%)
Protein in isolated nuclei (mg)	57.0	39.0	32	44.0	13
Protein in nuclear extract (mg)	12.6	5.5	56	8.6	56
Protein in pooled nuclear enzyme fractions from phosphocellulose column (mg)	0.7	0.3	57	0.6	100
Total enzyme units (nmol of AMP incorporated/h)	366	74	80	141	91

Fig. 2. Phosphocellulose chromatography of mitochondrial poly(A) polymerase

The enzyme was solubilized from mitochondria isolated from groups of four animals as described in the text. After dialysis against PGMEDK buffer, the enzyme was layered on a column (0.9 cm $\times$ 8 cm) of phosphocellulose previously equilibrated in the same buffer. The column was then washed with 10 ml of PGMEDK buffer and a linear gradient (0.03–0.4 M-KCl in PGMED buffer) was applied; fractions (1 ml) were collected, and 0.1 ml portions were assayed in the standard reaction mixture for the mitochondrial enzyme containing 0.4 E_{260} unit of primer for normal animals. Control (fed) group ($\bullet$); starved group ($\blacktriangle$); amino acid-re-fed group ($\circ$).

tents in the nuclear extracts and in the phosphocellulose fractions containing the enzyme activity increased by about 56 and 100% respectively. Although starvation decreased the nuclear protein by only 32%, the total units of polymerase activity decreased by 80%. Similarly, re-feeding, which caused only a 13% increase in total protein, resulted in a 91% (1.9-fold) increase in total enzyme activity.

Response of mitochondrial poly(A) polymerase to starvation and re-feeding with amino acids

In a parallel series of studies, mitochondria were isolated from animals which were fed (control), starved or amino acid-re-fed. Mitochondrial poly(A) polymerase was solubilized from liver and was partially purified by phosphocellulose chromatography. Starvation for 30 h resulted in almost complete loss of mitochondrial poly(A) polymerase activity (Fig. 2). Re-feeding the animals with a complete amino acid mixture for 3 h restored the poly(A) polymerase activity to normal values. In one set of experiments, animals were killed 1, 3 or 5 h after feeding and the enzyme activity was 65, 98 and 58% of control values (results not shown), suggesting that the maximal response of mitochondrial poly(A) polymerase to the complete amino acid supply occurs within 1–3 h after feeding. Consequently animals were killed as a routine at 3 h after the diet was given.

The effect of amino acid supply on the protein content and poly(A) polymerase activity from mitochondria of normal, starved and amino acid-re-fed groups of animals is shown in Table 2. As with nuclei, no large difference was observed in the percentage recovery of the mitochondrial proteins between control (fed) and starved groups. Starvation, which decreased the amount of protein in the mitochondria by 48%, decreased the total enzyme activity by 99%. Re-feeding the animals with the amino acid diet increased the protein contents of the isolated mitochondria by 32%, whereas the total enzyme activity increased by 6200% (63-fold). To determine whether the difference in enzyme activity between fed and starved animals was influenced by enzyme concentration in the reaction mixture, mitochondrial enzyme fractions from the column were pooled and the activity was determined at different enzyme concentrations. The

Table 2. *Effect of amino acid supply on rat liver mitochondrial protein content and poly(A) polymerase activity*

Mitochondria were isolated from groups of four rats which were either fed *ad libitum* (control fed), starved or amino acid re-fed. Enzymes were extracted and purified by phosphocellulose chromatography as described in the text. Enzyme activity was measured by using saturating quantities of primer obtained from normal animals and was calculated for each fraction from the average of several concentrations in the linear range of activity. This Table shows the data from a typical experiment. The enzyme assay for each fraction was performed in triplicate.

Measurement	Control (fed)	Starved	Decrease after starvation (%)	Amino acid re-fed	Increase after re-feeding (%)
Protein in isolated mitochondria (mg)	84	44	48	58	32
Protein in mitochondrial extract (mg)	45	16	64	25	56
Protein in pooled mitochondrial enzyme fractions from phosphocellulose column (mg)	4.0	0.2	95	3.5	1600
Total enzyme units (nmol of AMP incorporated/h)	8.8	0.1	99	6.3	6200

Fig. 3. *Poly(A) polymerase activity versus protein concentration*

Increasing amounts of mitochondrial poly(A) polymerase partially purified by phosphocellulose chromatography were assayed in the standard reaction mixture by using a constant amount ($0.4E_{260}$ unit) of primer from normal animals. Control (fed) group (●); starved group (▲); amino acid-re-fed group (○). The primer was present in saturating quantities.

enzyme activity in normal and amino acid-fed animals was linear at least up to a protein concentration of $340\mu\text{g/ml}$. At the maximal protein concentration used, the activity of the enzyme from the starved group was not more than 10% of that extracted from the mitochondria of normal or re-fed groups (Fig. 3).

Since the decreases observed in poly(A) poly-

merase activity from both nuclei and mitochondria are greater than the changes in total protein contents of the isolated organelles, it is possible that poly(A) polymerase is one of the cellular proteins specifically depleted on starvation. Further, the increase in enzyme activities after re-feeding was greater than the percentage increase in protein contents of both nuclei and mitochondria, which again suggests that these enzymes can selectively respond to amino acid supply. It should be pointed out that the effects of 16h starvation were most consistent when the animal weight was 100–125 g. Use of larger animals (200–250 g) produced comparable results only when the starvation time was increased to 40h.

Because neither the nuclear nor mitochondrial poly(A) polymerase was purified to homogeneity, poly(A)-degrading nuclease activity was measured. This enzyme is known to occur in liver nuclei (Sporn *et al.*, 1969) and mitochondria (Jacob & Schindler, 1972). No nuclease activity was present as demonstrated by the lack of significant hydrolysis of [^3H]-poly(A) under the assay conditions (not shown). Although there could be other factors that might interfere in the poly(A) polymerase assay, the possible role of the most probable candidate, namely the poly(A)-degrading nuclease, in modifying the amount of poly(A) polymerase in response to amino acid supply, can be eliminated.

Discussion

Our present studies demonstrate that the activity of poly(A) polymerase in the nuclei and mitochondria of rat liver can be affected by amino acid supply. To our knowledge, this is the first demonstration of the response of either of these poly(A) polymerases to nutrient supply, and probably the first demonstration of dietary modification of a mitochondrial enzyme from higher organisms. The most dramatic effect was

observed with the mitochondrial enzyme. Since poly(A) polymerase is probably involved in the post-transcriptional addition of poly(A) sequence to mRNA in the nuclei (for a review, see Brawerman, 1974) as well as in the mitochondria (Perlman *et al.*, 1973; Ojala & Attardi, 1974), physiological modifications of this enzyme have important implications. If it is accepted that polyadenylation of mRNA species in the nucleus is required for processing of at least some mRNA species, the polyribosome disaggregation and the consequent decrease in protein synthesis on amino acid deprivation (Drysdale & Munro, 1967) may be in part related to the non-availability of functional mRNA in the cytoplasm. The accelerated transfer of mRNA to the cytoplasm after administration of tryptophan alone (Murty & Sidransky, 1972) or of dietary protein (Gaetani *et al.*, 1972) to rats or mice may be explained as being due to the increased availability of poly(A) polymerase for the polyadenylation of mRNA. Similarly, the requirement for protein synthesis for the transfer of mRNA to the cytoplasm in yeast spheroplasts (Gross & Pogo, 1974) could also be related to the need for continued synthesis of nuclear poly(A) polymerase.

Published evidence shows that RNA metabolism is sensitive to amino acid supply. Early histological studies (Lagerstedt, 1949; Stenram, 1956) showed nucleolar size changes within 3 h of feeding with protein, and Munro *et al.* (1965) demonstrated changes in liver nuclear RNA content and metabolism within 2 h of feeding protein to starved rats. Subsequent studies have extended these early observations to most aspects of RNA synthesis and processing. It has been shown in HeLa cells (Smulson & Thomas, 1969; Smulson, 1970) that removal of single amino acids from the medium decreases RNA synthesis but not the activity of a crude RNA polymerase preparation made from the cells.

Much attention has been paid to the impact of amino acid supply on the synthesis of the RNA and other components of nucleoli. By using cells in tissue culture, some investigators have found that the synthesis and processing of RNA is retarded by omitting amino acids from the medium (Ellem *et al.*, 1970; Franze-Fernández & Pogo, 1971; Bölcşöldi & Eliasson, 1972; Vaughan, 1972). However, Sköld & Zetterberg (1969) and Shields & Korner (1970) did not observe a decrease in RNA synthesis when amino acids were withdrawn from the culture medium, although the latter group did find retardation of subunit maturation. Altered protein synthesis by ribosomal subunits and maturation of the latter were also noted in amino acid-starved cultures by Maden (1969) and by Pawlowski & Vaughan (1972). In rats, 45S RNA synthesis has been found to be retarded by starvation (Rickwood & Klemperer,

1970) or by omission of tryptophan or methionine from an administered amino acid mixture (S. T. Jacob & H. N. Munro, unpublished work), and subunit maturation was observed to be decreased by feeding rats on a low-protein diet (Wannemacher *et al.*, 1968).

The relationship of these observations to DNA-dependent RNA polymerase activity has also been reported. With intact rats, starvation (Rickwood & Klemperer, 1970) or feeding with a diet lacking leucine or valine (Clark & Jacob, 1972) caused a decrease in the activity of crude RNA polymerase made from liver cells, whereas tryptophan administration restored activity (Henderson, 1970). Such studies suffer from the disadvantage that the RNA-polymerizing system is assayed in the presence of its chromatin template, so that changes in RNA synthesis cannot be attributed to enzyme or template. This has subsequently been clarified by assay of purified polymerases (for reviews, see Jacob, 1973; Chambon, 1974). Both rat liver RNA polymerase I (nucleolar) and polymerase II (nucleoplasmic) activities undergo diet-related diurnal increases in activity (Barbiroli *et al.*, 1973; Glasser & Spelsberg, 1972). The main impact of diet appears to be on the activity of liver RNA polymerase II and is accompanied by increased template activity of the liver nuclear chromatin (Barbiroli *et al.*, 1973, 1975). After longer-term deprivation of dietary protein, both liver polymerases are decreased in activity (Andersson & von der Decken, 1975). With cells in tissue culture, Franze-Fernández & Pogo (1971) noted, however, that RNA polymerase I was sensitive to amino acid supply. Similarly, in yeast preparations, Gross & Pogo (1974) observed that α -amanitin-resistant (polymerase I) activity was decreased somewhat more by tyrosine starvation than was α -amanitin-sensitive (polymerase II) activity.

The various observations published in the literature thus suggest that various components in the formation and processing of both RNA and mRNA and its transport to the cytoplasm are regulated in relation to amino acid supply. Our present studies supplement this by showing that poly(A) polymerase in the nucleus is also sensitive to amino acid supply. It is thus probable that, in response to amino acid influx, there is a co-ordinate increase in different components of the system, resulting in more ribosomes and mRNA becoming available in the cytoplasm. This co-ordination could be achieved if key enzymes and proteins (e.g. ribosome subunit proteins) turn over rapidly and are thus all sensitive to amino acid supply.

Finally, the significance of the response of mitochondrial poly(A) polymerase to amino acid requires comment. If one of the functions of poly(A) is to facilitate transport of mRNA from the nucleus

to the cytoplasm, the role of mitochondrial poly(A) may seem to be obscure. However, studies have demonstrated that poly(A) can protect mRNA against endonuclease activity (Levy *et al.*, 1975), resulting in enhanced stabilization of mRNA *in vivo* (Huez *et al.*, 1974; Marbaix *et al.*, 1975). Thus the role of mitochondrial poly(A) could essentially be to stabilize the mRNA. This would imply that withdrawal of amino acid supply during starvation may decrease the amount of functional mitochondrial mRNA.

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