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Ribosomal Ribonucleic Acid Synthesis and Function as Influenced by Amino Acid Supply and Stress

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For several years we have been trying to define the possible basic control mechanisms for regulating the rates of protein synthesis in various tissues. As molecular biology evolved, it became obvious that controlling mechanisms were related to the synthesis and utilization of ribosomes and mRNA. Our earlier efforts were directed towards the understanding of how a decrease in supply of dietary amino acids could influence the rate of synthesis of proteins in key tissues such as liver and muscle. Because of its easy availability for the isolation of subcellular particles, liver has received the major research efforts. The effects of a decreased amino acid supply to an experimental animal can be characterized by the following results. When weanling rats were fed on a low-protein diet or adult rats were either hypophysectomized or fed on a protein-free diet for 28 days, there was a marked depression in their hepatic protein and RNA concentrations. Further, there was a marked decrease in hepatic free amino acid concentrations, especially of those known to be essential for protein synthesis. A good correlation was also observed between the rate of hepatic protein synthesis *in vivo* and cellular protein content of the liver. In addition, the rates of protein synthesis, both *in vivo* and *in vitro*, were directly correlated to the hepatic concentration of the essential free amino acids necessary for protein synthesis. A similar correlation was observed between the ribosomal content of the hepatocytes and the hepatic concentration of essential free amino acids. Despite an apparent increase in the rate of RNA synthesis and nuclear RNA polymerase activity, the amount of newly synthesized RNA that appeared in cytoplasmic ribosomes was significantly decreased in rats fed on low-protein diets or in hypophysectomized rats as compared with paired controls. Decreased incorporation of labelled nucleotide into cytoplasmic RNA appeared to be related to a lower rate of release of newly synthesized

ribosomes from the nucleus to the cytoplasm. Thus there appeared to be some regulatory mechanism whereby a diminution in the supply of essential hepatic free amino acids could control the rate of rRNA release and its utilization in the cytoplasm. This diminution in the supply of amino acids to a tissue could represent either dietary effects or hormonal influences on the transport of the amino acids into the cell selected for study.

Since showing that a decrease in amino acid supply will decrease the ribosomal and protein-synthetic ability of a cell, we have recently designed studies to determine whether an increase in amino acid supply would cause increased synthesis of RNA and protein in various tissues. We have used the following three experimental models to study the effects of increased amino acid supply. (a) The feeding of an excess of dietary protein to weanling rats; a maximal rate of gain in body weight was obtained in weanling rats fed on approx. 2g of casein N/day per kg body wt.; higher intakes of casein N resulted in a slight depression in the rate of growth, although the liver continued to increase in RNA and protein content even when the rats consumed as much as 7-8g of casein N/day per kg body wt. With intake up to 4g of casein N/day per kg body wt. there was a good correlation between the essential free amino acids content of the liver and hepatic RNA content, but higher intakes of casein N resulted in increases of RNA content without any change in hepatic free amino acid concentration. Under these conditions a good correlation was observed between rates of hepatic protein synthesis *in vivo* and hepatic free amino acid content in rats fed on 0-4g of casein N/day per kg body wt. (b) Steroid-induced cardiac hypertrophy in the rat; when rats were nephrectomized, injected with deoxycorticosterone acetate and given 1% NaCl in their drinking water for 4 weeks, cardiac hypertrophy developed. This was accompanied by a significant increase in cardiac RNA and protein content, even if the animals were fed on a protein-free diet. Associated with these changes were increased amounts of cardiac free amino acids and rates of protein synthesis *in vivo*. A significant correlation could be observed between total free amino acid content and the rate of protein synthesis within the myocardial tissue. (c) Effects of infection or turpentine-induced abscess on hepatic RNA synthesis and the subsequent synthesis and release of serum proteins; recent studies on rats with an infection or turpentine-induced abscess have provided evidence for an increased flux of amino acids into liver in the absence of a significant accumulation of free hepatic amino acids. The amino acids that move rapidly into the liver during these inflammatory processes were rapidly utilized for synthesis of serum proteins, especially glycoproteins. This whole process was apparently regulated by mediators released from

stimulated phagocytosing cells. The pattern of serum protein synthesis was markedly different from that in normal rats and appeared to be related to an increased synthesis of rRNA moieties. In addition, very early in infection, there appeared to be a de-repression of the chromatin templates of hepatic nuclei, and this may explain the increased rates of RNA synthesis. The changes in chromatin template activity appeared to correlate with the increased flux of amino acids into the liver. Thus it may be postulated that the presence of an excess of free amino acids somehow stimulated increased synthesis of rRNA and perhaps mRNA and the subsequent production and release of specific serum proteins.

From these various experiments it may be concluded that the intracellular availability of certain amino acids can markedly influence the regulatory mechanisms for rRNA synthesis, release and subsequent template activity. With a decrease in amino acid supply, a significant correlation was observed between decreased ribosomal synthesis and activity and the lowered concentration of certain intracellular amino acids; this direct relationship was not as apparent when the amino acid supply was increased.

The Influence of Diet on Ribonucleic Acid Polymerase Activity

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The response of the rat liver cytoplasm to changes in the protein content of the diet has been well documented (see other papers in this Colloquium). The equally profound changes that occur in the nucleus are less well known.

Lagerstedt (1949) was probably first to investigate the histological changes occurring in the rat liver nucleus after changes in the protein content of the diet. He showed that starvation for 24h produced a 50% decrease in the size of the nucleolus and that starvation prolonged beyond this period did not cause further size changes. Feeding of a high-protein diet to these animals led to a very rapid increase (+100%) in nucleolar size within 3h.

Stenram (1956) observed the changes in rat liver nucleolar size for several weeks after feeding with a high-protein diet. Nucleolar size increased for at least 3 days but remained relatively large for a further 7 weeks. A low-protein diet produced very large increases in nucleolar size that took longer to develop than with the high-protein diet.

As the rat liver nucleolus is known to be both the site of synthesis of ribosomal precursor RNA and the location of the Mg^{2+} -activated DNA-dependent RNA polymerase (reviewed by Busch & Smetana, 1970), several investigators have studied the association between this RNA polymerase activity and the diet.

Shaw & Fillios (1968) observed an increase in activity of rat liver RNA polymerase after feeding with a low-protein diet. Rickwood & Klemperer (1970) showed a decrease in activity of the RNA polymerase over 4 days of starvation, and Henderson (1970) obtained an increase in activity of the polymerase within 5–10min of feeding a complete mixture of amino acids to young rats. This increased activity was shown to be short-lived and to be abolished by pretreatment with puromycin or cycloheximide, and it was therefore suggested that a new enzyme was formed after feeding. The omission of tryptophan from the feeding mixture decreased the response of the Mg^{2+} -activated DNA-dependent RNA polymerase to feeding. The activity of the polymerase could be correlated with the liver tryptophan concentrations for both types of feeding mixtures.

With the discovery of multiple forms of RNA polymerase (Roeder & Rutter, 1969) and the extension of these findings by many groups of workers it is expected that a clearer understanding of the influence of diet on nucleolar and extranucleolar RNA polymerases may be gained.

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