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*Hypothesis*

# The pleiotypic response to amino acid deprivation is the result of interactions between components of the glycolysis and protein synthesis pathways

Marco Rabinovitz

*Laboratory of Molecular Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA*

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Several diverse metabolic events become compromised when mammalian cells are made deficient in essential amino acids or when charging of their tRNA is blocked by amino acid analogs. This rapid general demise of cell function can be due to inhibition of phosphofructokinase (PFK) by uncharged tRNA. It has now been demonstrated that when tRNA is added to PFK in an assay dependent upon the reassociation of inactive, dissociated enzyme subunits, nanomolar concentrations cause complete inhibition. The model for control suggests that charged tRNA becomes associated with EF-1, which is specific for aminoacyl-tRNAs and is present in sufficiently high concentrations in cells to sequester the charged forms from an inhibitory role. Support for this model include: (1) the rapid onset of inhibition of glycolysis and glucose uptake upon amino acid deficiency; (2) the unique role of the product of PFK activity, fructose-1,6-diphosphate, in reactions of peptide chain initiation, particularly its role as a co-factor for purified eIF-2B, the GDP/GTP exchange factor; (3) the correlations of this interaction with the cellular and molecular lesions of insulin insufficiency; (4) the recognition that the anomalous role of high concentrations of cAMP as a stimulant of peptide chain initiation in energy depleted or gel-filtered cell lysates correlates with its stimulatory action on PFK as an analog for the positive effector, adenosine-5′-monophosphate; and (5) the role of fructose-1,6-diphosphate in the formation of glyceraldehyde-3-phosphate, a substrate for synthesis of ribose-5-phosphate via the non-oxidative portion of the pentose phosphate pathway, which, as a precursor of phosphoribosylpyrophosphate, is essential for nucleic acid synthesis.

Amino acid deficiency; tRNA; Aminoacyl-tRNA; Phosphofructokinase; Fructose-1,6-diphosphate; Insulin; Cyclic AMP

## 1. INTRODUCTION

Several investigators [1–3] have observed that depletion of essential amino acids in mammalian cells initiates the rapid inhibition of a series of diverse metabolic events, termed by Hershko et al. [1] the 'Pleiotypic Response'. Processes such as glucose uptake, nucleic acid synthesis, and of course, protein synthesis are rapidly compromised. The latter process, studied intensively for the last 20 years, has yielded a series of consistent observations, specifically, that in cells other than reticulocytes, amino acid deficiency or inhibition of tRNA acylation causes a breakdown of the peptide chain initiation mechanism and a resulting disaggregation of polyribosomes [4]. Data has been presented that this event is the result of inhibition of phosphofructokinase (PFK) by uncharged tRNA [5]. In this communication a hypothesis further supporting this mechanism is described with references to the literature which bring into closer focus the relationship between these two processes.

*Correspondence address:* M. Rabinovitz, Laboratory of Molecular Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.

## 2. ENERGY METABOLISM AND INHIBITION OF PHOSPHOFRUCTOKINASE BY $tRNA^{Phe}$

In the earlier report [5] differences in energy metabolism between several cell types and reticulocytes were presented as the basis for the disparity in the sites of inhibition in protein synthesis found upon amino acid deprivation, at peptide chain initiation and elongation, respectively. It indicated that a major block in glycolysis and glucose uptake by Ehrlich ascites tumor cells in amino acid deficiency had already been reported [6]. Data was presented that this was due to inhibition of PFK by uncharged tRNA, a condition which would cause accumulation of glucose-6-phosphate, a potent inhibitor of hexokinase which promotes glucose uptake by phosphorylation. Inhibition of PFK by uncharged tRNA has now been confirmed with the use of yeast phenylanaline tRNA, ($tRNA^{Phe}$) and rabbit muscle PFK, assayed by following reassociation of inactive dimers to the active tetramer (Fig. 1). Such interaction

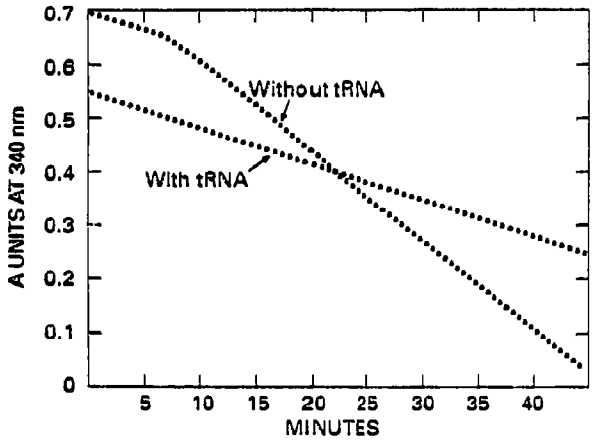


Fig. 1. Inhibition of reassociation dependent activity of dissociated phosphofructokinase (PFK) by $tRNA^{Phe}$. Crystalline rabbit muscle phosphofructokinase obtained from Sigma was dissolved in BESME [5] at a concentration of 200 $\mu$M (estimated as monomers of M.Wt. 85,000) and stored at −20°C. Prior to use it was thawed and diluted 1000-fold in a solution of BES buffer, 0.05 M, pH 7.1 containing 50% glycerol and 5 mM dithiothreitol. This was diluted into an equal volume of water without or with yeast phenylanaline tRNA (Boehringer Mannheim) to yield final concentrations of 30 nM to that illustrated, 100 nM. Aliquots of this interaction mixture were added to an assay system previously described [5] containing 2 mM ATP and now fortified with 5 mM potassium phosphate and ammonium sulfate. The rate of oxidation of NADH was recorded with readings of the cuvettes containing $tRNA^{Phe}$ offset to prevent overlapping of recorded rates. Since complete dissociation of PFK was not achieved during the 10 min interaction period, and irreversible inactivation occurs with prolonged incubation [10] residual active PFK tetramer was present in both control and $tRNA^{Phe}$ interacting systems. Such activity is shown by the initial 6 min rate of NADH oxidation in both control and inhibited systems. In the inhibited system containing 100 nM $tRNA^{Phe}$ the initial rate was preserved, indicating no reassociation dependent activity occurred, thus corresponding to 100% inhibition. A 50% inhibition was estimated for interaction of PFK with 25 nM $tRNA^{Phe}$.

between the oligomeric forms of the enzyme has been proposed to represent one molecular mechanism for physiological control [7–9].

## 3. SEQUESTRATION OF AMINOACYL-tRNA FROM INHIBITION OF PHOSPHOFRUCTOKINASE

As indicated recently by Zhao et al. [10] a wide variety of proteins inactivate PFK by interacting with exposed groups of the inactive enzyme dimer and preventing their reassociation to the active tetramer. It is therefore unlikely that PFK can discriminate between uncharged tRNA (of amino acid deprivation) and aminoacyl-tRNA (of amino acid sufficiency). The only known discriminatory agent with this property is peptide chain elongation factor 1 (EF-1), also described as EF-tu in prokaryotic systems [11]. This factor, when associated with GTP forms a stable ternary complex, aminoacyl-tRNA:EF-1:GTP, whose affinity for the aminoacylated form of tRNA is over 2000 times that for the unacylated variety [12]. Also the quantity of the factor in both prokaryotic [13] and eukaryotic [14] systems is sufficiently abundant for binding available charged tRNA. Thus the high specificity, affinity and capacity of this elongation factor for aminoacyl-tRNA makes it likely that the charged species is complexed within the cell and, when sequenstered as part of the protein synthetic apparatus, is removed from an inhibitory role towards PFK.

## 4. MOLECULAR LESION OF AMINO ACID, FRUCTOSE-1,6-DIPHOSPHATE AND INSULIN INSUFFICIENCIES

The locus of the lesion in peptide chain initiation, a decrease in the ternary complex, GTP-eIF2-initiator methionyl-tRNA bound to the small ribosomal subunit, appears identical under conditions of amino acid deficiency [15] or fructose-1,6-diphosphate omission from gel filtered lysates [16]. The same pattern is observed during insulin insufficiency in diabetic muscle [17]. This hormone had been reported to have a positive pleiotypic effect, stimulating many diverse metabolic functions [1]. One of its earliest activities is to stimulate PFK activity in the absence of enzyme synthesis [18]. Such stimulation results in increases in the cellular concentration of fructose-1,6-diphosphate [19], a specific positive effector of purified eIF-2B [20], the GDP-GTP exchange factor, also termed GEF. This factor is required to remove GDP from its associated factor, eIF-2 [21]. It thus permits the recycling of eIF-2 to form the tertiary iniation complex with GTP and the initiator tRNA, Met-tRNA, and to bind it to the small ribosomal subunit. Harmon et al. [22] have shown that eIF-2 will overcome the biochemical lesion of insulin insufficiency when added to cell-free extracts of diabetic muscle. This suggests that when the initial supply of eIF-2 is prevented from recycling by blocked eIF-2B, additional material can support the peptide chain initiation step. Further support for this molecular mechanism derives from the observations that mixtures of amino acids promote the activity of administered insulin in muscle [23] and adipocytes [24]. They are consistent with the view that uncharged tRNAs limit the activity of PFK, and thereby reduce the full potential of insulin as a stimulant of this enzyme.

## 5. CYCLIC AMP AS A STIMULANT OF PEPTIDE CHAIN INITIATION AND PHOSPHOFRUCTOKINASE

Giloh and Mager [25] recognized that high concentrations of cyclic AMP, in the millimolar range, markedly stimulated initiation of protein synthesis in lysates of rabbit reticulocytes which had been depleted of their ATP content by prolonged anaerobic incubation. They

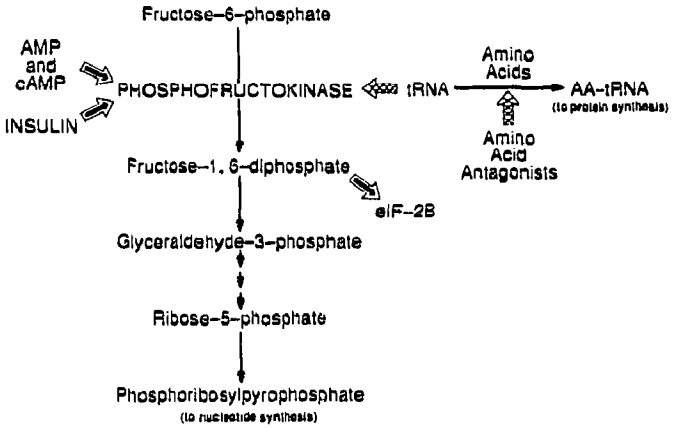


Fig. 2. Diagrammatic outline of interacting effectors. Broad arrows with internal arrows, stimulation. Broad arrows with XX, inhibition.

showed that phosphodiesterase, which converts cyclic AMP to linear adenosine-5'-monophosphate, did not interfere with this activity. Similar stimulations were observed when such high concentrations of cyclic AMP were added to gel filtered lysates [26,27]. The latter authors state 'the high concentrations required (of cyclic AMP) would seem to rule out any direct involvement.... in the normal regulatory process but one might speculate that it is acting as analogue of some small molecule which is directly involved'. This speculation appears to be correct. Cyclic AMP has long been recognized to act as an analog of linear adenosine-5'-monophosphate [28] at the higher concentrations corresponding to physiological levels of the linear compound, a positive effector of phosphofructokinase. The observation of Giloh and Mager, cited above, that added phosphodiesterase did not block the action of cyclic AMP supports this conclusion.

## 6. PHOSPHOFRUCTOKINASE AND NUCLEIC ACID SYNTHESIS

It has been earlier noted that when cells are in the amino acid deficient state they rapidly exhibit inhibition of nucleic acid biosynthesis [1–3,29]. This may occur even before there is a significant decrease in ATP content. The cause for this has been shown by Boss and coworkers [30] to be due to a decrease in the concentration of phosphoribosylpyrophosphate (PRPP) the substrate which introduces ribose into the de novo synthesis of purine and pyrimidine nucleotides. The quantity of the immediate precursor or PRPP, ribose-5-phosphate, is also decreased [31]. This key compound, the source of all ribose and deoxyribose in newly formed RNA and DNA is synthesized in cells from glucose-6-phosphate via the pentose phosphate pathway (for an outline of this pathway see [32]). This route utilizes glyceraldehyde-3-phosphate in its non-oxidative portion, which in tumor cells comprises over 80 percent of the ribose synthetic potential [33]. Since the precursor of glyceraldehyde-3-phosphate in the glycolytic pathway is fructose-1,6-diphosphate, inhibition of PFK by uncharged tRNA would reduce its level in cells, rapidly depleting ribose-5-phosphate, phosphoribosylpyrophosphate, and resulting in nucleic acid synthesis.

Fig. 2 summarizes several of the cellular biochemical and molecular interactions described. Additional analysis and experimentation to challenge or support this wide ranging control system is needed.

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