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Plant protein biosynthesis : methionyl-tRNA synthetases, a new example of the cooperative activity of nucleocytoplasmic and organellar systems

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Summary. The cooperative activity of nucleoplasmic and organellar systems appears to be of paramount importance for plant protein biosynthesis. Methionyl-tRNA synthetases from wheat were encoded in nuclear DNA, synthesized in cytoplasm and segregated into different compartments following post-translational events.

Introduction.

Plant protein biosynthesis is located in both the cytoplasm and the organelles, especially in chloroplasts whose proteins account for approximately 50 p. 100 of the cell protein content (Ellis, Blair and Hartley, 1973).

Based on a genome size of about 1.10^8 , the theoretical number of proteins which can be synthesized in the chloroplast is 120 units ; each protein has a mean molecular weight of 5.10^4 (Cifferi, 1978). This is not enough for an autonomous living status, and thus many chloroplast proteins in the soluble fraction as well as the membrane-bound ones are coded in the nuclear genome, translated in the cell cytoplasm and imported into the organelle.

Moreover, at least one soluble chloroplast protein (ribulose diphosphate carboxylase) and one membrane-bound (coupling factor CF1) are composed of polypeptides synthesized in different cell compartments (nucleus and chloroplast) (Mendola-Morgenthaler, Morgenthaler and Price, 1976). Thus, the cooperative activity of the nucleocytoplasmic and organellar systems appears to be of paramount importance for chloroplast development and function.

The most detailed reports of the interaction between the nuclear genetic system and that in the chloroplast concern the synthesis of ribulose diphosphate carboxylase. This enzyme, representing up to 50 p. 100 of the soluble chloroplast protein, is composed of large (MW : 54 000) and small (MW : 14 000) sub-units with probably 8 of each of these sub-units per enzyme molecule ((Kawashima and Wildman, 1970). The large sub-unit is coded in the chloroplast DNA (Chan and Wildman, 1972) and synthesized in the organelle (Blair and Ellis, 1973), while the small sub-unit is coded (Kawashima

and Wildman, 1972) and synthesized in the nucleocytoplasm (Dobberstein, Blobel and Chua, 1977 ; Highfield and Ellis, 1978).

The small sub-unit precursor (MW : 20 000) from isolated pea and spinach chloroplasts is synthesized to completion in the cytoplasm and then transported into the chloroplast. It has an additional sequence at its N-terminal but this is removed by a processing enzyme, which is not membrane-associated but soluble, located in the stroma fraction of the chloroplast (Ellis, 1979). It appears that this model can be demonstrated for many polypeptides synthesized in cell-free systems programmed with cytoplasmic mRNA, and there are signs that a similar mechanism operates for mitochondria and peroxysomes (Chua and Schmidt, 1979).

Preliminary results on plant aminoacyl-tRNA synthetases indicate that both the chloroplastic and the cytoplasmic enzymes are encoded in nuclear DNA, synthesized in cytoplasm and segregated into different compartments after a post-translational event (Parthier, Krauspe and Müller-Uri, 1976).

Materials and methods.

Quiescent wheat embryos and etiolated or green wheat seedlings were obtained from commercial caryops.

Crude and purified methionyl-tRNA synthetases and tRNAs^{Met} from wheat were prepared according to Chazal *et al.* (1975, 1977) and Quintard *et al.* (1978). The chloroplastic enzymes, extracted from purified chloroplasts, were all analyzed by PAG electrophoresis and electrofocalization (pH range : 3.5-9) to determine their molecular weight, quaternary structure and pHi. Assays for ability to methionylate tRNA^{Met} enzymatically were performed according to Rosa and Sigler (1977) and Quintard *et al.* (1978).

Results and discussion.

Plant cells contain several iso-tRNAs^{Met} which can be separated into two main species : (i) prokaryotic tRNAs^{Met} composed of mitochondrial and chloroplastic tRNA_f^{Met} and tRNA_m^{Met} and (ii) eukaryotic tRNAs^{Met}, including cytoplasmic tRNA_i^{Met} and tRNA_m^{Met} (Ghosh *et al.*, 1975). These molecules are methionylated *in vivo* by several enzymatic species (Weil *et al.*, 1977). The data summarized in table 1 show that :

- high-molecular cytoplasmic pre-enzymatic complexes from wheat can aminoacylate all the cytoplasmic tRNAs^{Met} ;
- cytoplasmic free enzyme (MW : 165 000, β_2) can also aminoacylate all cytoplasmic tRNAs^{Met}, except the organellar ones ;
- cytoplasmic proteolysed enzyme (MW : 70 000, α), a product of the two precedent species (fig. 1), only aminoacylates cytoplasmic tRNA_i^{Met} ;
- a chloroplastic enzyme (MW : 70 000, α), imported from the cytoplasm and segregated into the prokaryotic compartment, can aminoacylate prokaryotic tRNAs^{Met}, including chloroplastic tRNA^{Met} and cytoplasmic tRNA_i^{Met} (fig. 2).

TABLE 1

Physical and functional properties of some bacterial and plant methionyl-tRNA synthetases

Bacteria or plant	Subcellular localisation	M.W.	Q.S.	tRNA ^{Met} substrate	Bibliographical reference
<i>E. coli</i>	Cytoplasmic	170 000	β_2	Pro. tRNA ^{Met}	Cassio and Waller, 1971
<i>E. coli</i>	Cytoplasmic (trypsinized enzyme)	60 000	α	Pro. tRNA ^{Met}	Cassio and Waller, 1971
Lupine	Cytoplasmic	165 000	β_2	tRNA ^{Met}	Joachimiak <i>et al.</i> , 1978
Quiescent wheat embryo	Microsomal	> 3.10 ⁶	β_2	tRNA ^{Met} (1)	Quintard <i>et al.</i> , 1978
Quiescent wheat embryo	Cytosol	165 000	β_2	tRNA ^{Met} (1)	Chazal, 1975
Wheat embryo	Cytoplasmic	70 000 (proteolyzed enzyme)	α	Cyto. tRNA ^{Met}	Thomes and Julien, unpublished data (fig 1)
		105 000	β	tRNA ^{Met} (1)	Rosa and Siegler, 1977
		70 000	α	tRNA _r ^{Met} and tRNA _i ^{Met}	Rosa and Siegler, 1977
Etiolated and green wheat seedlings	Cytoplasmic	165 000	β_2	tRNA ^{Met}	Quintard and Julien unpublished data.
		70 000	α	Cyto. tRNA _i ^{Met} Pro. tRNA ^{Met}	Quintard and Julien, unpublished data
Green wheat seedlings	Chloroplastic	70 000	α	Pro. tRNA ^{Met} Cyto. tRNA _i ^{Met}	Carias and Julien, unpublished data (fig. 2)

(1) Except organellar tRNA^{Met}; Pro. : procaryotic; Cyto. : cytoplasmic.
M.W. : molecular weight; Q.S. : quaternary structure.

From this data, it appears that the organellar enzyme encoded in the nucleus was produced in the cytoplasm by endogenous proteolysis of native cytoplasmic enzymes and then probably imported into the prokaryotic compartment. It is interesting that the only apparent functional property which was conserved during the transformation process was the ability to aminoacylate the cytoplasmic tRNA^{Met} initiator. This molecule has two unique features : (i) unlike other eukaryotic initiator tRNA^{Met} species, wheat germ tRNA_i^{Met} is not recognized by *E. coli* transformylase, although it is charged with *E. coli* synthetase, and (ii) it cannot insert methionine in the internal position of the peptide chain, whereas other initiator tRNA^{Met} species can donate methionine in internal positions (Ghosh *et al.*, 1974).

It can be concluded that, although cytoplasmic tRNA_i^{Met} was methionylated by the chloroplastic enzyme, it could not initiate protein synthesis in the organellar system since it was not recognized by the transformylase.

Cytoplasmic wheat germ tRNA_i^{Met} should play a regulatory role, but this would only be possible if there is import across the membrane.

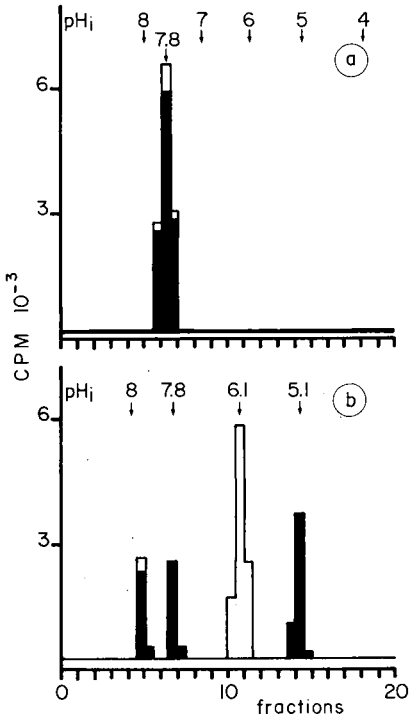


FIG. 1

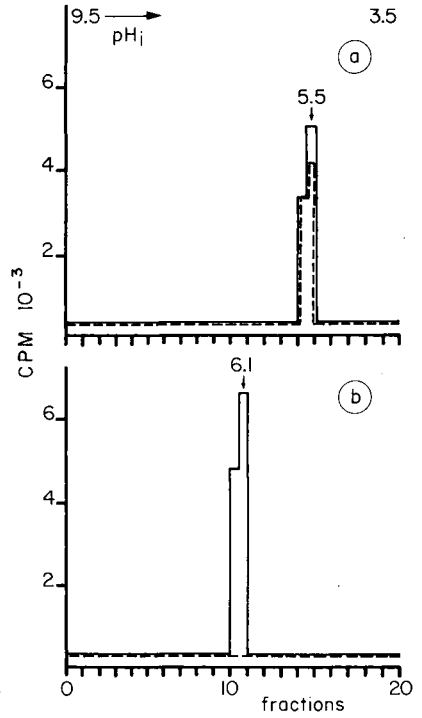


FIG. 2

FIG. 1. — PAG electrofocalisation of high-molecular weight pre-enzymatic complexes and membrane-bound cytoplasmic Met-tRNA synthetase.

Assays were performed with : ■ prokaryotic-tRNA ; □ cytoplasmic-tRNA^{Met} (see Materials and methods).
a) before incubation ; b) after incubation (37 °C, 1 h).

FIG. 2. — PAG electrofocalisation of chloroplastic enzyme and cytoplasmic proteolysed enzyme.

Assays were performed with : - - - - chloro-tRNA ; □ cyto-tRNA^{Met} (see Materials and methods).
a) chloroplastic enzyme ; b) cytoplasmic proteolysed enzyme.

The possible post-translational events characterizing the transformation of the cytoplasmic enzyme into organellar enzymes are : dissociation of the non-covalent β_2 structure, specific proteolysis and the chemical modifications necessary for import across the membrane, and ability to aminoacylate organellar tRNA^{Met}.

As proteins, plant methionyl-tRNA synthetases are no more interesting than numerous other discrete proteins synthesized in the cytoplasm before importation into the organelle. However, as enzymes, capable of producing iso-Met-tRNAs^{Met}, especially those used for cytoplasmic and organellar protein biosynthesis initiation, their functional properties are of paramount importance.

Résumé. La biosynthèse des protéines dans une cellule végétale fait appel à une coopération développée des systèmes localisés dans différents compartiments. Dans le cas des méthionyl-tRNA synthétases, ces enzymes codées par des gènes nucléaires sont fabriquées dans le cytoplasme et importées finalement, à la suite de modifications, dans les différents compartiments cellulaires.

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