PEPTIDE CHAIN INITIATION IN A SPECIES OF *NOSTOC* AND IN CHLOROPLASTS OF *EUGLENA GRACILIS*

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1. Introduction

Peptide chain initiation in *Escherichia coli* requires the coordinate interaction of at least three protein factors (initiation factors). Through a sequence of partially understood reaction, *N*-formyl-methionyl-tRNA (fMet-tRNA), selected by the initiator codon AUG, is bound to the ribosome and becomes available for the formation of the first peptide bond [1–5]. A number of reports have demonstrated the presence of fMet-tRNA in other bacteria [6], in mitochondria [7–8] and in chloroplasts [9]. Furthermore, mitochondria [8] and chloroplasts [10] incorporate *N*-formyl-methionine into the *N*-terminal position of polypeptides. These findings raise the question of whether the reactions for peptide chain initiation are similar in all prokaryotic organisms and in organelles endowed with bacterial type ribosome (70 S), as already demonstrated in the case of the reactions for peptide chain elongation [11]. The similarity in the initiation reactions may be ascertained *in vitro* by testing the functional interchangeability of ribosomes and initiation factors prepared from different sources. This approach has already shown that ribosomes prepared from *Bacillus subtilis* and from mitochondria of *Neurospora crassa* may carry out the same initiation reactions catalyzed by *E. coli* ribosomes. In addition *E. coli* initiation factors are active on ribosomes from *B. subtilis* and from mitochondria of *N. crassa* [12].

In the present investigation we provide evidence that this is the case also for a species of a blue-green alga (*Nostoc* sp.), and for chloroplasts of *Euglena gracilis*, both containing ribosomes of the bacterial type [13, 14].

2. Methods

E. gracilis strain Z was grown in the light at 27° in an heterotrophic acidic medium [15]. The cells were collected by centrifugation, washed twice with water and used immediately. Chloroplasts were prepared according to Eisenstadt and Brawerman [16]. "Unwashed" chloroplast ribosomes were prepared by resuspending the chloroplasts in a buffer containing 0.01 M tris-HCl (pH 7.8), 0.01 M $MgCl_2$, 0.05 M NH_4Cl, 0.006 M β-mercaptoethanol and 2% Triton-X-100 (BDH-Chemicals). For the preparation of "washed" chloroplast ribosomes, the concentration of NH_4Cl was raised to 0.5 M. After clarification at 30,000 *g* for 30 min, the ribosomes were pelleted by centrifugation at 105,000 *g* for 3 hr and resuspended in the low NH_4Cl buffer without Triton-X-100.

Nostoc sp., a strain isolated in our laboratory, was grown in the light at 27° in an autotrophic chelated medium [17]. Cells were harvested during the growth phase by centrifugation, washed twice with water and frozen at −70° until used. "Washed" ribosomes were prepared as described for *E. gracilis*, except that cell disruption was achieved by grinding in a mortar with sand (BDH-Chemicals; 40–100 mesh).

All ribosomal preparations were kept at −70° and used only once after thawing.

The preparation of *E. coli* ribosomes, initiation factors, and *N*-formyl-^{14}C-methionyl-tRNA (f-^{14}C-Met-tRNA) as well as the analytical methods and the assay for fMet-puromycin are described elsewhere [12]. *E. coli* ^{14}C-phenylalanyl-tRNA (^{14}C-Phe-tRNA) was prepared as described by Kaji, Kaji and Novelli [18].

3. Results and discussion

E. coli ribosomal preparations contain initiation factors, which can be washed off by treatment with high concentrations of NH_4Cl [19]. The results of table 1 show that this appears to be the case also for ribosomes prepared from chloroplasts of *E. gracilis*. Indeed, "unwashed" ribosomal preparations may carry out the synthesis of fMet-puromycin while "washed" preparations are almost totally dependent on added *E. coli* factors. We were unable to perform the same experiments on "unwashed" *Nostoc* ribosomes, due to difficulties encountered in the preparation of active ribosomes from this organism. However, washed ribosomes of *Nostoc* sp., as in the case of "washed" chloroplast ribosomes, are able to catalyze the synthesis of fMet-puromycin in heterologous mixtures containing *E. coli* initiation factors. In every case the properties of the reaction, i.e. dependence on the initiator codon AUG, GTP and fMet-tRNA are similar to those determined for the initiation reactions in *E. coli* (table 2).

Table 1
Synthesis of fMet-puromycin by "washed" and "unwashed" ribosomal preparations from *Euglena gracilis* chloroplasts, *Nostoc* sp. and *Escherichia coli*.

Ribosomes from		fMet-puromycin (pmoles per mg ribosome)	
		–Initiation factors	+*E. coli* initiation factors
E. coli	"washed"	0.4	81.1
	"unwashed"	31.6	40.0
E. gracilis	"washed"	0.9	49.4
	"unwashed"	15.3	23.7
Nostoc sp.	"washed"	0.8	44.6

The 50 μl assay mixtures contained 100 μmoles/ml tris-HCl pH 7.2, 50 μmoles/ml KCl, 5 μmoles/ml magnesium acetate, 1 μmole/ml GTP, 10 μmoles/ml β-mercaptoethanol, 0.8 absorbance units/ml AUG and 422 pmoles/ml f-^{14}C-Met-tRNA (sp. act. 233 μCi/μmole). Where indicated, the reaction mixtures contained 1.75 mg/ml of *E. coli* "washed" ribosomes, 1.47 mg/ml of *E. coli* "unwashed" ribosomes, 1.40 mg/ml of chloroplast "washed" ribosomes, 1.55 mg/ml of chloroplast "unwashed" ribosomes, 2.0 mg/ml "washed" *Nostoc* sp. ribosomes. When present, 0.50 mg/ml of *E. coli* initiation factors were added. The extent of synthesis of fMet-puromycin was assayed with the method of Leder and Bursztyn [20] after incubation for 15 min at 24° followed by a 20 min incubation at the same temperature in the presence of 1 μmole of puromycin.

Table 2
Characteristics of fMet-puromycin synthesis by "washed" ribosomes prepared from *Nostoc* sp., chloroplasts of *Euglena gracilis* and *Escherichia coli*.

Reaction mixtures	% Activity of ribosomes from		
	Nostoc sp.	Chloroplasts of *E. gracilis*	*E. coli*
Complete	100	100	100
–*E. coli* factors	10	11	2.2
–AUG	10	19	18
–GTP	32	44	23
–fMet-tRNA, +Phe-tRNA	1.5	0.7	0.6

The reaction mixtures and the assay conditions were as described in table 1. The complete mixtures containing ribosomes of *Nostoc* sp., of chloroplasts and of *E. coli* synthesized 44.6, 25.4 and 62.8 pmoles fMet-puromycin per mg ribosomes, respectively.

No attempt was made to isolate initiation factors from the NH_4Cl-wash of chloroplast and *Nostoc* sp. ribosomes, owing to quantitative limitations in the material under study.

The above results give further support to the idea that in prokaryotic organisms (bacteria and blue-green algae) and in cellular organelles (chloroplasts and mitochondria) protein synthesis initiates by a mechanism which is similar if not identical to that outlined for *E. coli*. Such a concept is strengthened by this and previous reports that *E. coli* initiation factors and *E. coli* fMet-tRNA are both utilized for peptide chain initiation by the ribosomes prepared from *B. subtilis* [12], *N. crassa* mitochondria [12], *Nostoc* sp. and *E. gracilis* chloroplasts. It should be added that while such results argue in favor of an exchangeability of initiation factors F_1 and F_2, nothing may be concluded in the case of factor F_3, since such a factor is required for peptide chain initiation *in vitro* with natural mRNA, but not with the trinucleotide AUG [1, 3]. The striking structural and functional similarities in the complex

mechanism for peptide chain initiation gives further support to the hypothesis of a close phylogenetic relationship between bacteria, blue-green algae, chloroplasts and mitochondria.

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