INSTITUT PASTEUR

Research in Microbiology 160 (2009) 493–501

www.elsevier.com/locate/resmic

Begin at the beginning: evolution of translational initiation

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Received 29 April 2009; accepted 23 June 2009
Available online 2 July 2009

Abstract

Initiation of protein synthesis, entailing ribosomal recognition of the mRNA start codon and setting of the correct reading frame, is the rate-limiting step in translation and the main target of translation regulation in all modern cells. As efficient selection of the translation start site is vital for survival of extant cells, a mechanism for ensuring this may already have been in existence in the last universal common ancestor of present-day cells. This article reviews known features of the molecular machinery for initiation in the primary domains of life, Bacteria, Archaea and Eukarya, and attempts to identify conserved features that may be useful for reconstructing a model of the ancestral initiation apparatus.

Keywords: Universal common ancestor; Domains of life; Translation initiation factors; mRNA structure; Initiator tRNA

1. Introduction

Translation, the link between genotype and phenotype, is a key step in gene expression, but perhaps the hardest to understand in evolutionary terms. Decoding of a nucleic acid into a polypeptide entails evolving a dictionary (the genetic code) and specific machinery (tRNA, ribosomes) to actually read the code. The modern translational apparatus is of staggering complexity, and the biosynthesis of its components takes up a substantial amount of the cell's metabolic energy. Life in its modern sense is not conceivable without an efficient and accurate translational apparatus. Yet a fairly complex machinery for protein synthesis must have already been present in the last universal common ancestor (LUCA) of extant life forms. A complete understanding of evolution of the translational apparatus will probably never be attained. However, ever-increasing knowledge of the structure and role of its components in many organisms has provided numerous clues on the possible emergence of several of its features. We are fairly certain of the most conserved and divergent characteristics of translation: on the basis of these data, inferences on the evolutionary history of this most central of the cell's basic processes can be drawn.

The process of translation comprises several steps, each in turn subdivided into a number of different phases. Although the general mechanism is well conserved across all life forms (in fact, translation is one of the most conserved cellular processes), each of the three primary domains (Archaea, Bacteria and Eukarya) has elaborated specific variants of some steps of translation. While the elongation phase is essentially invariant in all cells, translation initiation, and in part termination and ribosome recycling, have specific features in each of the three domains [1].

Translational initiation occurs when the small ribosomal subunits land on the initiation codon of an mRNA and establish the correct reading frame for successful decoding. This is a key step in the gene expression process: indeed, initiation is the main rate-limiting step in translation, and efficiency of initiation largely dictates efficiency for decoding a given mRNA. Therefore, most mechanisms of translational regulation target components of translational initiation machinery.

The setting of a precise start site for reading the message is a central problem in successful decoding that is likely to have been solved early in evolution. One of the aims of research on the translational apparatus is to try to reconstruct its probable

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0923-2508/$ - see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.resmic.2009.06.003

features in the LUCA. The discovery of the Archaea and advancing knowledge on translation in these prokaryotes finally enables overcoming the prokaryotic-eukaryotic dichotomy and distinguishing between primitive and derived characteristic of the translation apparatus. This survey will attempt to do so for the initiation step in translation.

2. Mechanism of translational initiation in the three domains of life

In all cells, the normal route of translational initiation involves the dissociated small ribosomal subunit in the early steps, with the large one entering the game only at a later stage. The task of the small ribosomal subunit is to search for the correct start codon and to ensure its pairing with the anticodon of the initiator tRNA (tRNAi); once this is accomplished, the large subunit will join and the monomeric ribosome will form, allowing elongation to begin. A number of accessory proteins, called translation initiation factors (IFs), assist the process.

In Bacteria (Fig. 1), the small ribosomal subunit binds all the relevant components – mRNA, tRNAi (fmet-tRNA^{fmet}) and the three IFs, (IF1, IF2 and IF3), forming the pre-initiation complex [2]. When the 50S subunit joins the complex, the IFs dissociate and can be recycled; the process entails the hydrolysis of an IF2- bound GTP, whose actual function is still debated.

In Eukarya (Fig. 2), the early steps of initiation separately involve the 40S subunits and the mRNA. The former interact with a number of IFs (eIF1, eIF1A, eIF2, eIF3), forming a pre-initiation complex which also contains the tRNAi (met-tRNA^{met}), carried by eIF2 [3]. Meanwhile, eIF4F, a complex of three proteins, i.e. eIF4A, eIF4E and eIF4G, recognizes the cap at the 5′ end of the mRNA by its component eIF4E and begins to unfold the 5′ UTR by means of the helicase eIF4A. Next, the pre-initiation complex and mRNA are brought together via an interaction between the eIF4G component of eIF4F and eIF3 [3]. The initiation complex so formed ‘‘scans’’ the mRNA, i.e. moves in a 3′ direction until the initiation codon is encountered. Upon codon-anticodon interaction, the GTP bound to eIF2 is hydrolyzed (with the aid of eIF5) and eIF2 dissociates away, along with eIF1 and eIF1A. Finally, the 60S subunit joins the complex, triggering hydrolysis of a second GTP associated with eIF5B [4]. All the factors leave the ribosome and elongation is ready to begin.

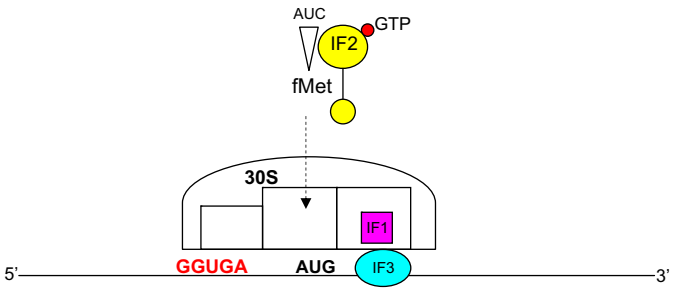

Fig. 1. Bacterial translation initiation. The ternary complex formed by IF2, fmet-tRNA^{fmet} and GTP enters the P site, while the A site is blocked by IF1. IF3, the anti-association factor, hinders premature joining of the 50S subunit. A Shine-Dalgarno motif for ribosome/mRNA recognition is shown in red.

The details of the initiation process in Archaea, and the role played by the various IFs therein, are still unclear. The early steps in translational initiation (Fig. 3) are probably similar to the bacterial ones: the small (30S) ribosomal subunit forms an initiation complex which includes most of the IFs, the initiator tRNA (met-tRNA^{met}) and mRNA [5]. It is established that, like bacteria but unlike the eukarya, the 30S subunits can interact directly with the mRNA [6]. The 50S subunit then joins and the monomeric 70S ribosome is formed. As in Eukarya, the initiation process entails hydrolysis of two GTP molecules. There are no caps in Archaea, and therefore homologues of the eukaryotic cap-binding and mRNA unfolding factors are lacking. Archaea, however, have more initiation factors than Bacteria (see below) but the precise role in the initiation process of most of them still has to be worked out. Moreover, a special kind of mRNAs particularly abundant in Archaea (the leaderless mRNAs) require a different initiation mechanism whose details are still unclear.

In this diverse scenario, the main task of the evolutionary biologist is to identify the common themes and the conserved features, trying to sketch a plausible model of ancestral initiation. To this aim, it is convenient to separately analyze the principal components of the initiation system – mRNA, tRNAi and initiation factors – trying to unravel similarities and diversities in the three domains of cell descent.

3. Evolution of mRNA structure

Part of current variability in the mechanism of translational initiation is accounted for by mRNA structure, which presents marked differences in eukaryotes and prokaryotes. Extant prokaryotic cells make use of polycistronic mRNAs, which contain information for more than one polypeptide products. In addition, prokaryotic mRNAs are not modified at their 3′ and 5′ ends and may contain the Shine-Dalgarno (SD) motifs that promote the direct binding of the small ribosomal subunit to the mRNA molecule. In fact, prokaryotic 30S subunits are specifically equipped for interacting directly with the mRNA, as the 3′ end of the 16S rRNA contains a very conserved sequence (the anti-SD, or ASD, sequence) complementary to the SD motif in the mRNA.

In contrast, mRNAs in eukaryotes are usually monocistronic, are modified by capping at the 5′ end and by polyadenylation at the 3′ end, and do not contain any SD-type ribosome binding motif. Accordingly, eukaryal small subunit rRNA lacks an ASD sequence. Part of this difference is undoubtedly accounted for by the different structure of eukaryotic and prokaryotic cells. In the latter, which lack a nuclear membrane, transcription and translation are coupled, meaning that the ribosomes bind to, and translate, mRNA while it is still being transcribed. This makes protection of the mRNA ends unimportant and also makes desirable the presence of the SD motifs to enhance ribosome binding. Eukaryotic cells, on the other hand, process the primary transcripts to