Engaging the ribosome: universal IFs of translation

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Eukaryotic initiation factor 1A (eIF1A) and the GTPase IF2/eIF5B are the only universally conserved translation initiation factors. Recent structural, biochemical and genetic data indicate that these two factors form an evolutionarily conserved structural and functional unit in translation initiation. Based on insights gathered from studies of the translation elongation factor GTPases, we propose that these factors occupy the aminoacyl-tRNA site (A site) on the ribosome, and promote initiator tRNA binding and ribosomal subunit joining. These processes yield a translationally competent ribosome with Met-tRNA in the ribosomal peptidyl-tRNA site (P site), base-paired to the AUG start codon of a mRNA.

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The landmark structures of the ribosome and its small and large subunits, generated by X-ray crystallography [1–4] and electron microscopy [5], have provided a collection of direct insights into the protein synthesis machinery. When combined with the results of other structural studies published over the past decade, these data yield a highly informative picture of the mechanisms underpinning translation elongation in prokaryotes. Given the marked similarity of elongation factors between eukaryotes and bacteria, many of the architectural and biochemical principles unveiled in the bacterial system will almost certainly be applicable to eukaryotes. A full structural understanding of translation initiation and termination has been somewhat slower to emerge. Recently determined structures of two evolutionarily conserved translation initiation factors, eukaryotic initiation factor 1A (eIF1A)/IF1 and IF2/eIF5B, provide important new insights into crucial early steps responsible for preparing the ribosome for translation initiation.

Although the mechanisms of translation initiation are fundamentally similar in all organisms, the requirements for non-ribosomal protein components differ substantially between bacteria and eukaryotes. Translation initiation in eubacteria requires only three initiation factors for assembly of the ribosome–mRNA–initiator tRNA complex: IF1, IF2 and IF3. The principal player is IF2, which promotes binding and correct positioning of fMet-tRNA^{fMet} in the P site and also facilitates ribosomal subunit joining. In contrast to bacteria, eukaryotes use >12 initiation factors (together comprising 27 polypeptide chains) to assemble an 80S ribosome that is competent for protein synthesis. In addition, GTP is an essential requirement for translation initiation in both prokaryotes and eukaryotes. Amino acid sequence comparisons of the translation initiation factors in eubacteria, archaea and eukaryotes [6–8] revealed the conservation of two translation initiation factors throughout evolution: IF1/eIF1A and IF2/eIF5B (Fig. 1).

These observations were surprising because, at the time, the general belief was that bacterial and eukaryotic translation initiation factors would be wholly unrelated. IF1/eIF1A and IF2/eIF5B were subsequently shown to interact with one another, both on and off the ribosome in eukaryotes [8], and on the ribosome in eubacteria [9], suggesting that they form an evolutionary conserved functional unit in translation initiation. Solution nuclear magnetic resonance structures of *Escherichia coli* IF1 [10], human eIF1A [11] and the C-terminal domain of *Bacillus stearothermophilus* IF2 [12], plus X-ray structures of full-length IF2/eIF5B from *Methanobacterium thermoautotrophicum* in three states (apo, GDP-bound and bound to the non-hydrolyzable GTP analog, GDPNP) [13], are now available for comparison and analysis (Fig. 2). In this article, we briefly review the structures of these two conserved translation initiation factors, and propose a model for IF2/eIF5B function with IF1/eIF1A.

IF1/eIF1A: A site unseen

The three-dimensional structures of IF1 and eIF1A reveal that they are both members of the S1 family of oligonucleotide-binding (OB)-fold proteins, comprising a five-stranded β-barrel (Fig. 2a) [10,11]. Human eIF1A possesses an additional segment consisting of two α helices and two extended β strands. Earlier RNA protection experiments revealed that IF1 protects some of the same nucleotides as the A site aminoacyl-tRNA [14,15], and it was even proposed that IF1 might mimic the A site tRNA. Binding of IF1 to the ribosomal A site was subsequently confirmed by the X-ray crystal structure of a complex of IF1 bound to the 30S ribosomal subunit from *Thermus thermophilus* [16]. IF1 does indeed bind at the base of the A site, adjacent to the mRNA. However, the charge distribution on the surface of IF1, and the way it interacts with the small ribosomal subunit, suggest that it does not mimic the A site tRNA. Binding of IF1 to the ribosomal A site is thought to play a role in helping direct the initiator methionyl-tRNA to the ribosomal P site [16].

The structure of the 30S subunit–IF1 complex also shows that binding of IF1 distorts the conformation of rRNA helix H44, which plays a crucial role in inter-subunit interactions. This observation is particularly interesting because IF1 is thought to accelerate the kinetics of ribosomal subunit interactions. Alone, IF1 increases the rate of ribosomal subunit association. In the presence of additional initiation factors such as IF3, IF1 enhances dissociation of the subunits [17].

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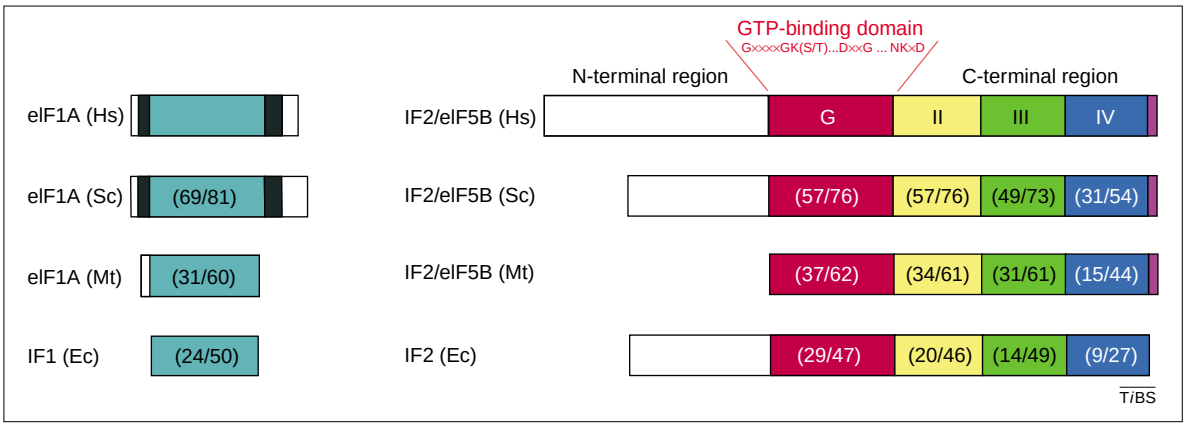

Fig. 1. Schematic alignments of selected IF1, eIF1A and IF2/eIF5B homologs from eubacteria, archaea and eukaryotes. Sequence identities and similarities of the conserved domains are given as percentages (identity/similarity). The GTP-binding motifs in IF2/eIF5B are shown in dark red. Abbreviations: Ec, *Escherichia coli*; eIF1A, eukaryotic initiation factor 1A; eIF5B, eukaryotic initiation factor 5B; Hs, *Homo sapiens*; IF1, initiation factor 1; IF2, initiation factor 2; Mt, *Methanobacterium thermoautotrophicum*; Sc, *Saccharomyces cerevisiae*.

IF2/eIF5B: a wholly molecular 'grail' of translation?

IF2/eIF5B is a member of a large family of translation enzymes that catalyze GTP hydrolysis during mRNA translation. These include elongation factor 1A (EF1A, previously known as EF-Tu), EF2 (previously known as EF-G) and release factor 3 (RF3). It is thought that all members of this family interact with the same GTPase-activating center of the ribosome. To date, X-ray crystal structures have been determined for EF1A(GDP) and EF1A(GTP) [18,19], aminoacyl-tRNA–EF1A(GTP) [20], and free EF2 and EF2(GDP) [21,22]; these structures demonstrate significant structural similarities (Fig. 3). Moreover, the crystal structure of the aminoacyl-tRNA–EF1A(GTP) ternary complex resembles that of EF2(GDP), suggesting that molecular mimicry is at play during the elongation cycle. This hypothesis was confirmed by electron microscopy, which demonstrated that the aminoacyl-tRNA–EF1A(GTP) ternary complex and EF2(GDP) bound to overlapping sites on the ribosome [23,24].

Mapping of the X-ray structures of EF1A and EF2 onto these higher-order structures revealed that their GTP binding (G) domains interact with the base of the L7/L12 stalk, which is believed to serve as the GTPase-activating center of the ribosome and probably contains ribosomal proteins L11 and L10 (bacterial numbering scheme). The G domains of EF1A and EF2 also bind to a fragment in the 23S RNA, which is referred to as the α-sarcin/ricin stem loop because it interacts with the toxins α-sarcin and ricin [25–27]. In the case of EF2, ribosomal proteins L7/L12 alone were reported to weakly stimulate the GTPase activity of this factor [28]. Cryoelectron microscopy studies revealed that the second domains of EF2 and EF1A (Fig. 3) both interact with the shoulder of the small subunit, whereas the anticodon stem of the aminoacyl-tRNA in the tRNA–EF1A(GTP) ternary complex, as well as the apical domain of EF2, are found in the A site of the small subunit, reaching into the decoding center.

The X-ray crystal structure of IF2/eIF5B revealed a novel protein architecture, consisting of four domains (G, II, III and IV) arranged in the shape of a molecular 'chalice' (Fig. 2b). Domains G, II and III form the cup, connecting downwards through a long α helix to domain IV, which forms the base. Conservation levels among IF2/eIF5B homologs from eubacteria, archaea and eukaryotes (pairwise amino acid sequence identities >27%) document that they share the same

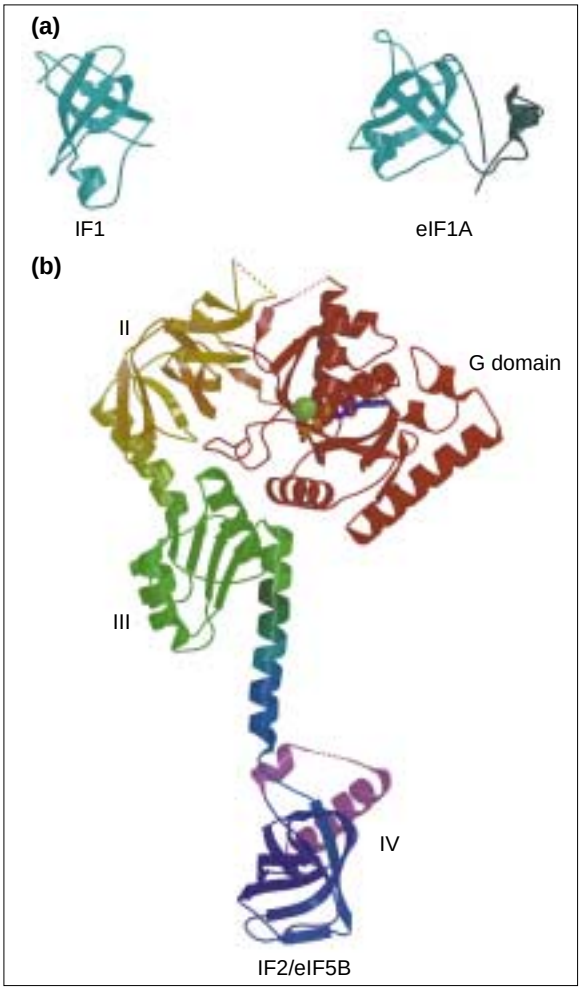

Fig. 2. High-resolution structures of IF1, eIF1A and IF2/eIF5B(GTP). (a) Ribbon diagrams of *Escherichia coli* IF1 (left) and human eIF1A (right). The oligonucleotide-binding (OB)-fold domains of IF1 and eIF1A are shown in cyan. Portions of the N- and C-termini of eIF1A lacking defined structure are not shown. (b) Ribbon diagram showing the nucleotide-binding site view of *Methanobacterium thermoautotrophicum* IF2/eIF5B. Domain color coding is as follows: G domain, dark red; domain II, yellow; domain III, green; domain IV, blue; C-terminal α helices, magenta. GTP is shown as a ball and stick model, and the Mg^{2+} ion is denoted by a labeled green sphere. Abbreviations: IF1, initiation factor 1; eIF1A, eukaryotic initiation factor 1A; IF2, initiation factor 2; eIF5B, eukaryotic initiation factor 5B; G domain, GTP binding/GTPase domain. Adapted, with permission, from Ref. [13].

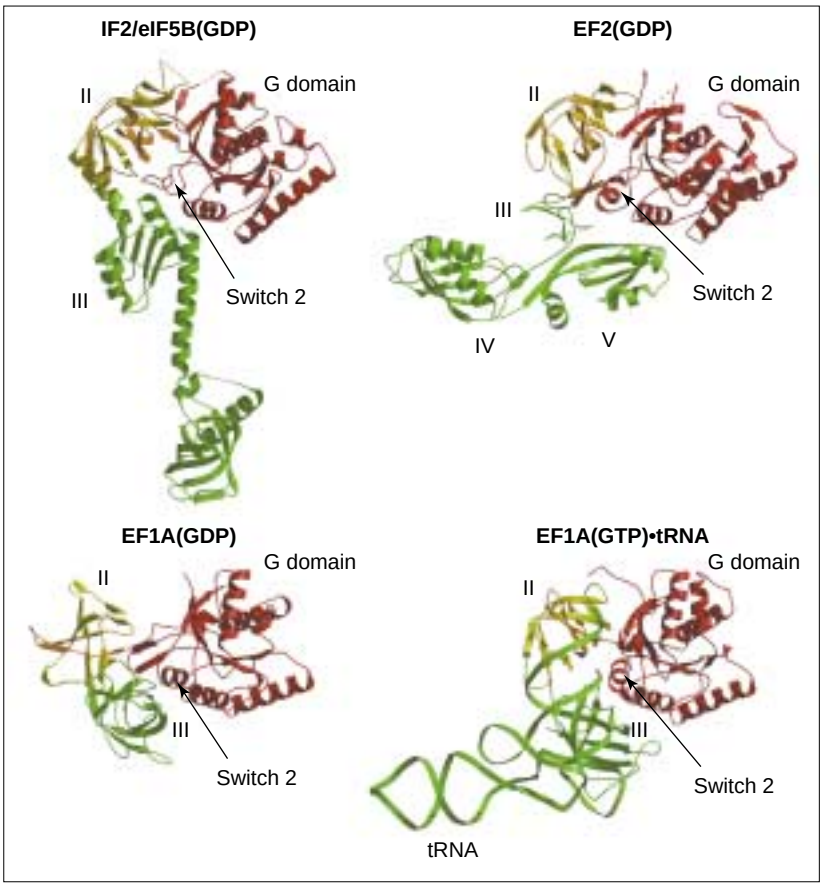

Fig. 3. Comparison of translation GTPase structures. Ribbon diagrams of IF2/eIF5B(GDP), EF2(GDP) [22], EF1A(GDP) [18] and Phe-tRNA^{Phe}–EF1A(GTP) [20]. IF2/eIF5B is shown as in Fig. 2b, with the eubacterial GTPases in the same orientation. Domain color coding: G domain, red; domain II, yellow; domains III and IV of IF2/eIF5B, green; domains III, IV and V of EF2, green; domain III of EF1A and the Phe-tRNA^{Phe}, green. Abbreviations: EF1A, elongation factor 1A; EF2, elongation factor 2; eIF5B, eukaryotic initiation factor 5B; IF2, initiation factor 2. Adapted, with permission, from Ref. [13].

overall three-dimensional structure (Figs 1;2b). The conserved N-terminal G domain and the β-barrel domain II of IF2/eIF5B are structurally similar to the G domain and domain II of EF1A (and EF2), respectively (Fig. 3). They also display a similar relative spatial arrangement, suggesting that these two domains form an integral substructure common to all translation GTPases that participates in similar interactions with both eukaryotic and bacterial ribosomes. The C-terminal half of IF2/eIF5B is composed of a novel α/β/α sandwich (domain III), connected to a second β-barrel (domain IV) by a 40 Å-long α helix. The β-barrel domain IV resembles the corresponding domain from *B. stearothermophilus* IF2, which binds to the 3′-end of fMet-tRNA^{fMet} [12].

The two β-barrels present in IF2/eIF5B almost certainly support distinct biochemical functions: domain II constitutes part of the ribosome-binding platform and domain IV binds the 3′-end of the P-site tRNA, at least in eubacteria [12]. Biochemical studies in *E. coli* have shown that the 3′-terminal fMet-adenosine of the initiator tRNA is primarily responsible for fMet-tRNA^{fMet} binding and recognition by IF2 [29]. Conserved residues that participate in fMet-tRNA^{fMet} binding are not preserved in IF2/eIF5Bs from archaea and eukaryotes, which is consistent with the specificity of eubacterial IF2 for formylated Met-tRNA, and it is thought unlikely that domain IV of IF2/eIF5B binds the initiator tRNA off the ribosome in either archaea or eukaryotes. Instead, a heterotrimeric GTPase, eIF2, delivers the initiator Met-tRNA$_i^{Met}$ to the small ribosomal subunit and is then released upon GTP hydrolysis. The requirement in eukaryotes for both IF2/eIF5B and eIF2 suggests that two molecules of GTP are hydrolyzed during each round of translation initiation, which provides an additional entry point for regulation of eukaryotic gene expression that is not present in eubacteria.

Model for IF1–IF2 (eIF1A–eIF5B) function: ribosomes join the fun

The GTP-bound form of IF2/eIF5B promotes ribosomal subunit joining without hydrolyzing GTP, which occurs only after 80S ribosome assembly [30]. GTP hydrolysis is required for release of this factor from the ribosome [30,31], and might also be required to induce conformational changes in the ribosome to render it competent for translation elongation. Similar to other GTPases, IF2/eIF5B undergoes a conformational switch between its active GTP-bound and inactive GDP-bound states. The nucleotide-binding site in all G proteins is flanked by two structural elements, referred to as switch 1 and switch 2. These switch motifs help communicate the identity of the bound nucleotide (GTP versus GDP) to the rest of the molecule. Comparison of the X-ray structures of IF2/eIF5B(GDPNP) and IF2/eIF5B(GDP) reveals *en bloc* rearrangements of domains II–IV that originate from modest conformational changes in the switch 2 region of the G domain, induced by Mg^{2+}/GTP binding (Fig. 4). The enzyme uses an articulated lever mechanism to amplify the structural consequences of GTP binding in the enzyme active center over a distance of >90 Å to domain IV.

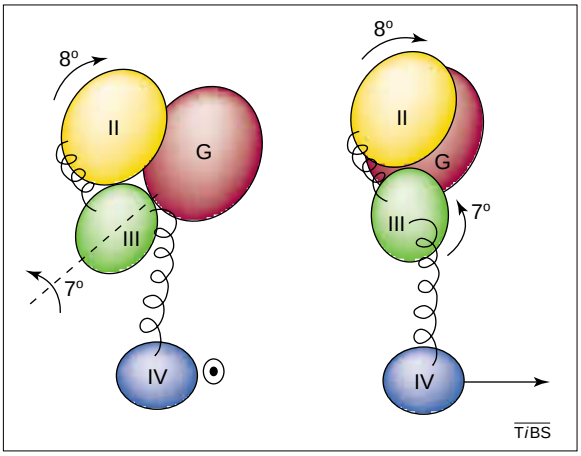

Fig. 4. Domain movements in IF2/eIF5B induced by GTP binding. Schematic views of IF2/eIF5B (left: nucleotide-binding site view; right: rotated by 90°), showing the domain movement induced by exchange of GDP for GTP. Domain color coding is as follows: G domain, red; domain II, yellow; domain III, green; domain IV, blue. Movements are indicated by → and ⊙, which denotes movement towards the reader. Abbreviations: eIF5B, eukaryotic initiation factor 5B; IF2, initiation factor 2.

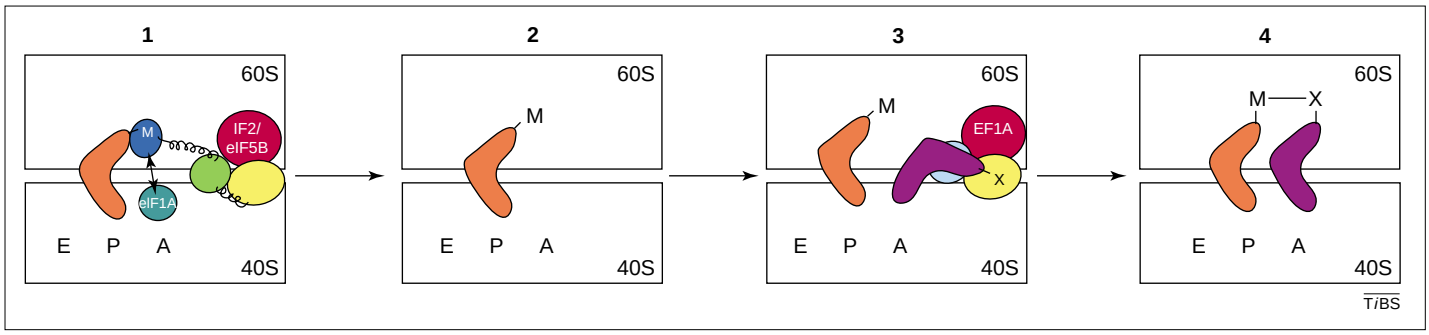

Fig. 5. Schematic drawing of IF2/eIF5B, EF1A, and P and A site tRNAs on the ribosome. Panels 1–4 depict the steps involved in positioning the initiator tRNA in the P site by IF2/eIF5B and IF1/eIF1A (1 and 2), followed by recruitment and docking of an elongator tRNA in the A site by EF1A (3 and 4). IF2/eIF5B domains are color coded as follows: G domain, dark red; domain II, yellow; domain III, light green; domain IV, blue. EF1A domains are color coded: G domain, dark red; domain II, yellow; domain III, light blue. P and A site tRNAs are shown in bright red and purple, respectively. The tRNA binding sites (A, P and E) are indicated on the rectangles representing the small (40S) and large (60S) ribosomal subunits. Panel 1 shows Met-tRNA$_i^{Met}$ bound to the P site. IF2/eIF5B binds to eIF1A through domain IV, which can also interact with the aminoacyl end of the initiator Met-tRNA$_i^{Met}$ and/or with ribosomal constituents of the P site. Hydrolysis of GTP by IF2/eIF5B following subunit joining triggers the release of IF2/eIF5B and possibly eIF1A (2). Release of IF2/eIF5B and eIF1A yields a vacant A site on the 80S ribosome that can be occupied by the first elongating tRNA as part of the EF1A(GTP)–aminoacyl-tRNA ternary complex (3). GTP hydrolysis by EF1A releases this factor from the ribosome, leaving the elongating tRNA in the A site, enabling formation of the first peptide bond (4). Abbreviations: A site, aminoacyl-tRNA site; E site, exit site; EF1A, elongation factor 1A; eIF1A, eukaryotic initiation factor 1A; eIF5B, eukaryotic initiation factor 5B; IF2, initiation factor 2; P site, peptidyl-tRNA site; M, methionine; X, any amino acid.

We believe that these GTP-induced conformational changes enable IF2/eIF5B to bind simultaneously to both ribosomal subunits, thereby facilitating ribosomal subunit joining (Fig. 5, Panel 1). The G domain almost certainly interacts with the base of the L7/L12 stalk on the large subunit, as seen for the corresponding domains of EF2 and EF1A. Although domain IV could contact the small ribosomal subunit directly, it is most likely to interact with the 30S/40S subunit via IF1/eIF1A [32]. It is also possible that IF1/eIF1A prevents premature subunit joining by distorting the structure of rRNA helix H44. IF2/eIF5B binding and/or GTP hydrolysis could trigger IF1/eIF1A release, and restore the appropriate positioning of H44 to permit subunit joining.

IF2/eIF5B is markedly elongated with dimensions similar to those of EF2 and the EF1A ternary complex (length 110 Å, maximum diameter 66 Å). As discussed earlier, the G domain and domain II of IF2/eIF5B are structurally similar to those of EF2 and EF1A, and appear to serve as a functional unit common to all translation GTPases. The architectural similarity between IF2/eIF5B and both EF2 and the EF1A ternary complex, breaks down in the distal portions of the molecules. The position of the apical domain (domain IV) with respect to domains I and II in IF2/eIF5B differs dramatically from the dispositions of domain IV of EF2 or of the tRNA anticodon loop in the EF1A ternary complex (Fig. 3). In addition, the β-barrel domain IV of IF2/eIF5B bears no resemblance to domain IV of EF2. Thus, IF2/eIF5B is almost certainly not a tRNA mimic.

Domain IV of IF2/eIF5B is responsible for binding eIF1A in eukaryotes [32]. In eubacteria, domain IV of IF2 binds to the 3′-end of the initiator tRNA and interacts with IF1 on the ribosome. These documented interactions suggest that domain IV of IF2/eIF5B is located close to the top of the A site, where it can bind the 3′-end of P-site tRNA and, together with IF1/eIF1A, block unproductive binding of initiator tRNA to the A site (Fig. 5, Panel 1). Steric hindrance by a complex of IF2/eIF5B and IF1/eIF1A might be required to ensure proper engagement of the initiator tRNA by the P site, despite the fact that the P site has a higher affinity for tRNA than does the A site (Fig. 5, Panel 2). A stable interaction between archaeal and eukaryotic IF2/eIF5B and Met-tRNA$_i^{Met}$ has not been observed in solution. However, overexpression of the gene encoding tRNA$_i^{Met}$ partially suppresses the severe slow-growth phenotype of yeast strains lacking IF2/eIF5B [8]. It remains to be determined whether or not IF2/eIF5B makes direct contact(s) with Met-tRNA$_i^{Met}$ on the ribosome. It is possible that domain IV of IF2/eIF5B acts indirectly via ribosomal constituents to stabilize binding of Met-tRNA$_i^{Met}$.

Whereas domain IV of EF2 and the anticodon loop of tRNA from the EF1A ternary complex reach into the decoding center (Fig. 5, Panels 3 and 4), domain IV of IF2/eIF5B is positioned at the top of the A site, close to the peptidyl-transferase center, where it can bind the 3′ end of the P-site initiator tRNA. However, one cannot rule out that while dissociating from the ribosome, IF2/eIF5B(GDP) changes its conformation, leaving a stereochemically appropriate molecular imprint of a ribosome-binding site suitable for the capture of the EF1A ternary complex. It is also possible that when IF2/eIF5B(GTP) is on the ribosome, the initiator tRNA is not yet correctly positioned in the P site and the final positioning requires GTP hydrolysis by IF2/eIF5B. These possibilities remain to be tested through further footprinting studies, electron microscopic and crystallographic analyses.

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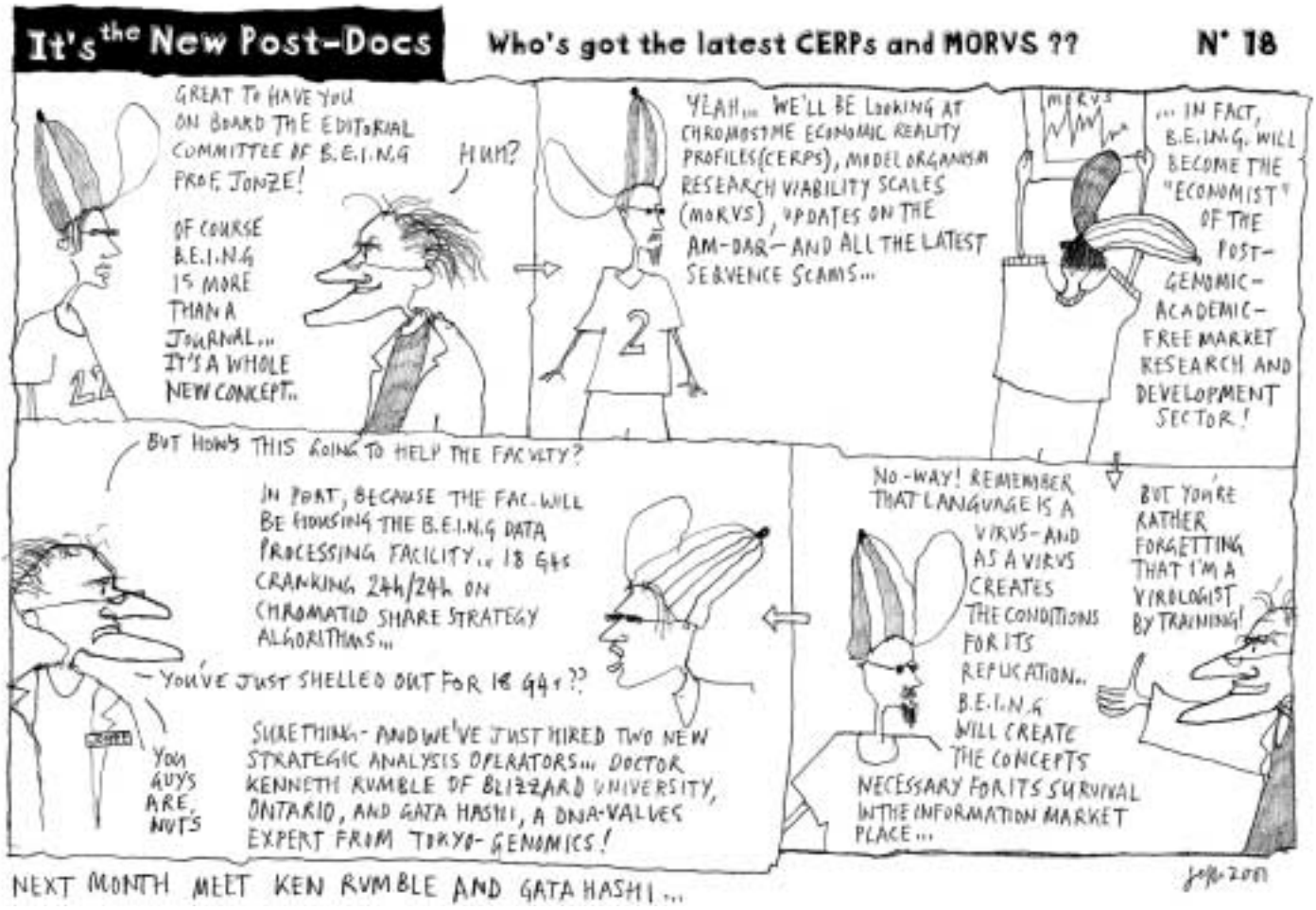

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