SUPPLEMENTARY DATA

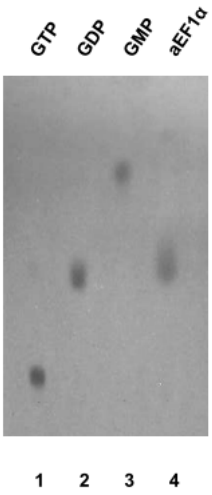
Molecular insights into the interaction of the ribosomal stalk protein with elongation factor 1α

Kosuke Ito[1,*], Takayoshi Honda[1], Takahiro Suzuki[1], Tomohiro Miyoshi[1], Ryo Murakami[1], Min Yao[2], and Toshio Uchiumi[1,*]

[1] Department of Biology, Faculty of Science, Niigata University, 8050 Ikarashi 2-no-cho, Nishi-ku, Niigata 950-2181, Japan
[2] Faculty of Advanced Life Science, Hokkaido University, Kita-ku, Kita-10, Nishi-8, Sapporo 060-0810, Japan

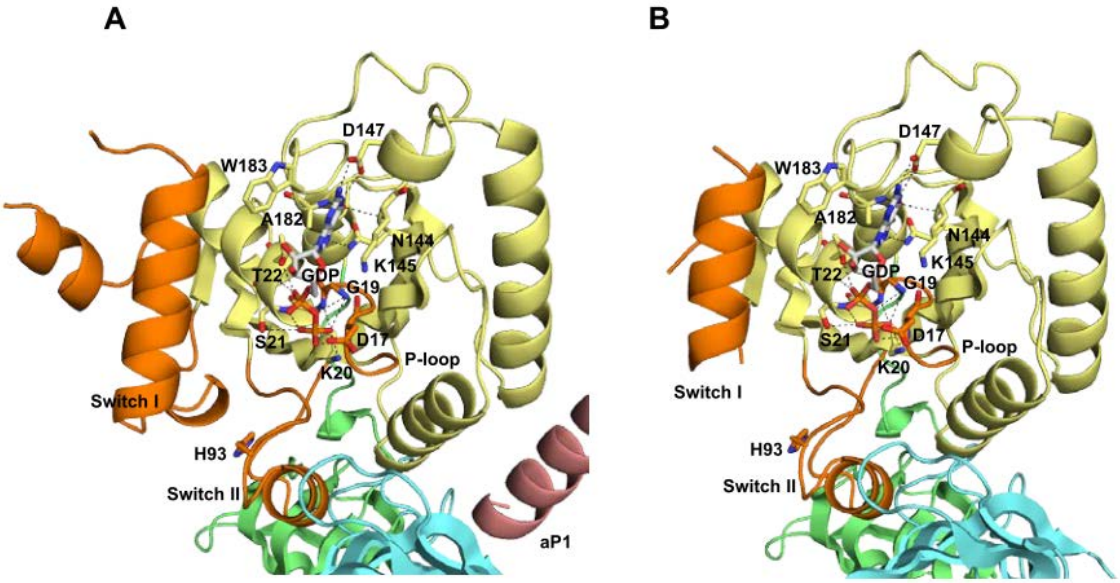
[*]To whom correspondence may be addressed. E-mail: uchiumi@bio.sc.niigata-u.ac.jp; Tel & Fax: 025-262-7792 or k-ito@bio.sc.niigata-u.ac.jp; Tel & Fax: 025-262-7029

SUPPLEMENTARY FIGURES



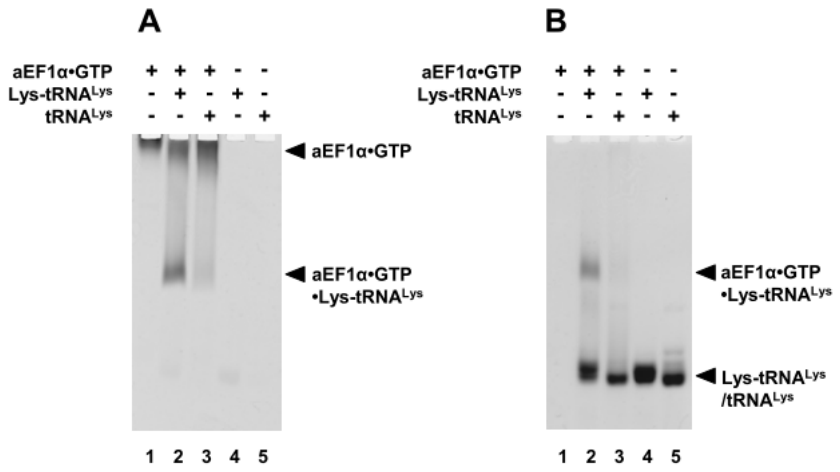
Supplementary Figure S1. Analysis of the bound nucleotide in purified EF1α by thin-layer chromatography.

An aliquot of 4 nmol of purified aEF1α from *E. coli* was treated with phenol/chloroform to extract the bound nucleotide. The aqueous phase was recovered and spotted onto a polyethylenimine cellulose TLC plate. Aliquots of 4 nmol of GTP, GDP, and GMP standards were also spotted onto the TLC plate. A single spot was detected from the purified aEF1α, and the mobility of this spot was consistent with that of the control GDP. Furthermore, the intensity of the spot from the purified aEF1α was comparable to that of the GDP standard, whose amount was equal to that of the phenol/chloroform-treated aEF1α. These facts indicate that the purified aEF1α was predominantly in the GDP-bound form.



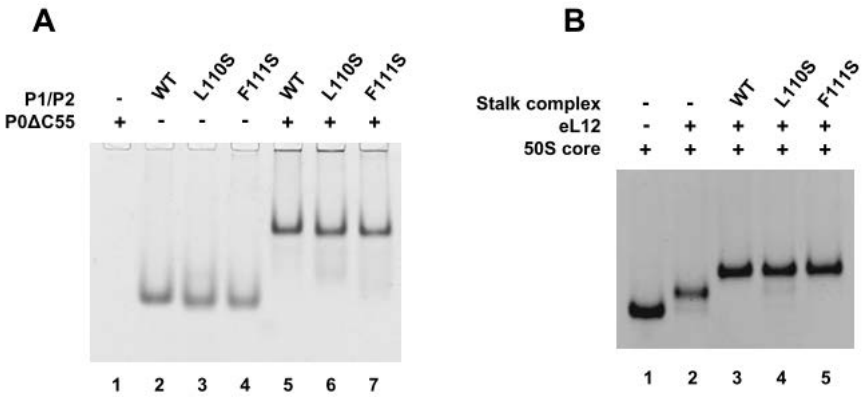
Supplementary Figure S2. Structures of the key regions for GTP hydrolysis.
(**A**) and (**B**) Structures of the key regions for GTP hydrolysis in the aP1(CTD)•aEF1α•GDP complex (A) and in the aEF1α•GDP complex (B). The molecules of aEF1α are represented by a ribbon model, with the same color coding as in Figure. 1. The P-loops and switch I and II regions of aEF1α are highlighted in orange. The bound molecules of GDP, the residues involved in GDP binding, and His93, a general base catalyst candidate, are shown with stick models. Hydrogen bonds are indicated by dashed lines.

Among the key regions for GTP hydrolysis, the GTP/GDP-binding region, the P-loop, and the switch II region exhibit quite similar conformations between the two structures. In contrast, in the switch I region, the disordered residues differ between the two structures. However, this could be the result of different crystal packing, and the conformations of the ordered regions are essentially the same.



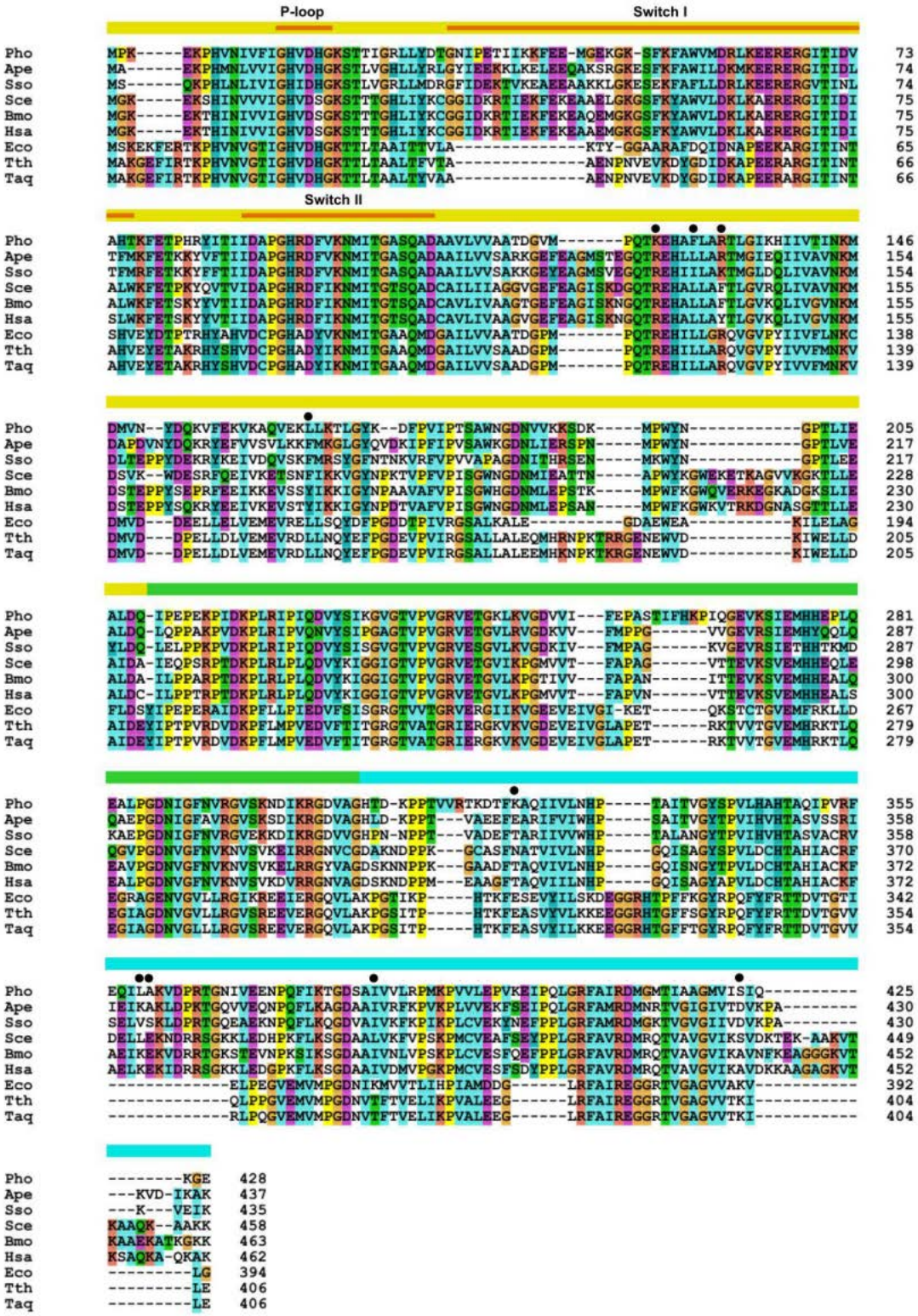
Supplementary Figure S3. Analysis of the interaction between aEF1α and aminoacyl-tRNA.

(**A**) and (**B**) Analysis of the interaction between aEF1α and aminoacyl-tRNA by gel mobility shift assay. An aliquot of 400 pmol of aEF1α•GTP was incubated with an equal amount of Lys-tRNA^{Lys} (lane 2) or deacyl-tRNA^{Lys} (lane 3), and subjected to a gel mobility shift assay as described under Materials and Methods. Aliquots of 400 pmol of aEF1α•GTP (lane 1), Lys-tRNA^{Lys} (lane 4), and deacyl-tRNA^{Lys} (lane 5) were also incubated alone and subjected to the gel mobility shift assay. The gel was stained with CBB (A) or Azur B (B). Note that the amount of tRNA^{Lys} applied to lanes 2 and 4 was 667 pmol because 60.0% lysylated tRNA^{Lys} was used.



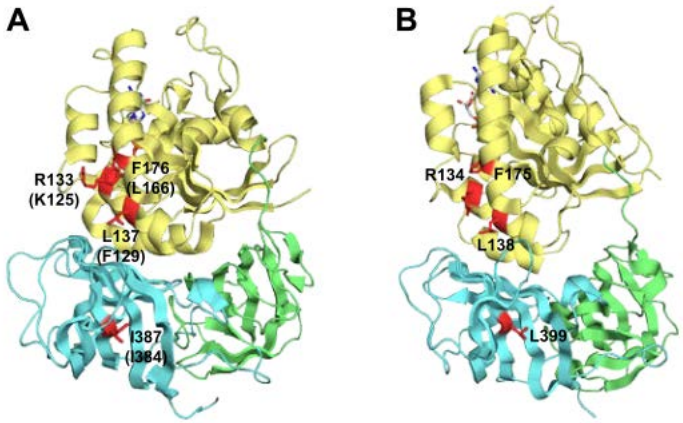
Supplementary Figure S4. Preparation of the hybrid 50S subunits.

(**A**) Formation of the silkworm stalk complex. An aliquot of 100 pmol of silkworm P0 that lacked the C-terminal 55 amino acid residues (P0ΔC55; lane 1) and 200 pmol of each variant of the stalk dimer: P1(WT)•P2(WT) (lane 2), P1(L110S)•P2(L110S) (lane 3), and P1(F111S)•P2(F111S) (lane 4), were incubated together to produce the stalk complexes: P0ΔC55•[P1(WT)•P2(WT)]$_2$ (lane 5), P0ΔC55•[P1(L110S)•P2(L110S)]$_2$ (lane 6), and P0ΔC55•[P1(F111S)•P2(F111S)]$_2$ (lane 7), as described previously (35). Samples were subjected to native gel electrophoresis as described previously (17). (**B**) Formation of hybrid 50 S subunits composed of the *E. coli* 50S core and the silkworm stalk complex variants. An aliquot of 5 pmol of the *E. coli* 50S core that lacked the L10•L7/L12 stalk complex and L11 (lane 1) was incubated with 15 pmol of silkworm eL12 alone (lane 2), or the same amount of silkworm eL12 and 15 pmol of each silkworm stalk complex: P0ΔC55•[P1(WT)•P2(WT)]$_2$ (lane 3), P0ΔC55•[P1(L110S)•P2(L110S)]$_2$ (lane 4), and P0ΔC55•[P1(F111S)•P2(F111S)]$_2$ (lane 5), as described previously (47). These samples were analyzed by acrylamide-agarose composite gel electrophoresis as described previously (47,56). The gel was stained with Azur B.


P-loop
Switch I
Switch II
Pho MPK-----EKPHVNIVFIGHVDHGKSTTIGRLLYDTGNIPETIIKKFEE-MGEKGK-SFKFAWVMDRLKEERERGITIDV 73
Ape MA------EKPHMNLVVIGHVDHGKSTLVGHLLYRLGYIEEKKLKELEEQAKSRGKESFKFAWILDKMKEERERGITIDL 74
Sso MS------QKPHLNLIVIGHIDHGKSTLVGRLLMDRGFIDEKTVKEAEEAAKKLGKESEKFAFLLDRLKEERERGVTINL 74
Sce MGK-----EKSHINVVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAELGKGSFKYAWVLDKLKAERERGITIDI 75
Bmo MGK-----EKTHINIVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAQEMGKGSFKYAWVLDKLKAERERGITIDI 75
Hsa MGK-----EKTHINIVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAEMGKGSFKYAWVLDKLKAERERGITIDI 75
Eco MSKEKFERTKPHVNVGTIGHVDHGKTTLTAAITTVLA-------------KTY-GGAARAFDQIDNAPEEKARGITINT 65
Tth MAKGEFIRTKPHVNVGTIGHVDHGKTTLTAALTFVTA-------------AENPNVEVKDYGDIDKAPEERARGITINT 66
Taq MAKGEFIRTKPHVNVGTIGHVDHGKTTLTAALTYVAA-------------AENPNVEVKDYGDIDKAPEERARGITINT 66
Pho AHTKFETPHRYITIIDAPGHRDFVKNMITGASQADAAVLVVAATDGVM-------PQTKEHAFLARTLGIKHIIVTINKM 146
Ape TFMKFETKKYVFTIIDAPGHRDFVKNMITGASQADAAILVVSARKGEFEAGMSTEGQTREHLLLARTMGIEQIIVAVNKM 154
Sso TFMRFETKKYFFTIIDAPGHRDFVKNMITGASQADAAILVVSAKKGEYEAGMSVEGQTREHIILAKTMGLDQLIVAVNKM 154
Sce ALWKFETPKYQVTVIDAPGHRDFIKNMITGTSQADCAILIIAGGVGEFEAGISKDGQTREHALLAFTLGVRQLIVAVNKM 155
Bmo ALWKFETSKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGTGEFEAGISKNGQTREHALLAFTLGVKQLIVGVNKM 155
Hsa SLWKFETSKYYVTIIDAPGHRDFIKNMITGTSQADCAVLIVAAGVGEFEAGISKNGQTREHALLAYTLGVKQLIVGVNKM 155
Eco SHVEYDTPTRHYAHVDCPGHADYVKNMITGAAQMDGAILVVAATDGPM-------PQTREHILLGRQVGVPYIIVFLNKC 138
Tth AHVEYETAKRHYSHVDCPGHADYIKNMITGAAQMDGAILVVSAADGPM-------PQTREHILLARQVGVPYIVVFMNKV 139
Taq AHVEYETAKRHYSHVDCPGHADYIKNMITGAAQMDGAILVVSAADGPM-------PQTREHILLARQVGVPYIVVFMNKV 139
Pho DMVN--VDQKVFEKVKAQVEKLLKTLGYK--DFPVIPTSAWNGDNVVKKSDK-----MPWYN-----------GPTLIE 205
Ape DAPDVNYDQKRYEFVVSVLKKFMKGLGYQVDKIPFIPVSAWKGDNLIERSPN-----MPWYN-----------GPTLVE 217
Sso DLTEPPYDEKRYKEIVDQVSKFMRSYGFNTNKVRFVPVVAPAGDNITHRSEN-----MKWYN-----------GPTLEE 217
Sce DSVK--WDESRFQEIVKETSNFIKKVGYNPKTVPFVPISGWNGDNMIEATTN-----APWYKGWEKETKAGVVKGKTLLE 228
Bmo DSTEPPYSEPRFEEIKKEVSSYIKKIGYNPAAVAFVPISGWHGDNMLEPSTK-----MPWFKGWQVERKEGKADGKSLIE 230
Hsa DSTEPPYSQKRYEEIVKEVSTYIKKIGYNPDTVAFVPISGWNGDNMLEPSAN-----MPWFKGWKVTRKDGNASGTTLLE 230
Eco DMVD---DEELLELVEMEVRELLSQYDFPGDDTPIVRGSALKALE----------GDAEWEA-----------KILELAG 194
Tth DMVD---DPELLDLVEMEVRDLLNQYEFPGDEVPVIRGSALLALEQMHRNPKTRRGENEWVD-----------KIWELLD 205
Taq DMVD---DPELLDLVEMEVRDLLNQYEFPGDEVPVIRGSALLALEEMHKNPKTKRGENEWVD-----------KIWELLD 205
Pho ALDQ-IPEPEKPIDKPLRIPIQDVYSIKGVGTVPVGRVETGKLKVGDVVI---FEPASTIFHKPIQGEVKSIEMHHEPLQ 281
Ape ALDQ-LQPPAKPVDKPLRIPVQNVYSIPGAGTVPVGRVETGVLRVGDKVV---FMPPG------VVGEVRSIEMHYQQLQ 287
Sso YLDQ-LELPPKPVDKPLRIPIQDVYSISGVGTVPVGRVESGVLKVGDKIV---FMPAG------KVGEVRSIETHHTKMD 287
Sce AIDA-IEQPSRPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVT---FAPAG------VTTEVKSVEMHHEQLE 298
Bmo ALDA-ILPPARPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVLKPGTIVV---FAPAN------ITTEVKSVEMHHEALQ 300
Hsa ALDC-ILPPTRPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVLKPGMVVT---FAPVN------VTTEVKSVEMHHEALS 300
Eco FLDSYIPEPERAIDKPFLLPIEDVFSISGRGTVVTGRVERGIIKVGEEVEIVGI-KET------QKSTCTGVEMFRKLLD 267
Tth AIDEYIPTPVRDVDKPFLMPVEDVFTITGRGTVATGRIERGKVKVGDEVEIVGLAPET------RKTVVTGVEMHRKTLQ 279
Taq AIDEYIPTPVRDVDKPFLMPVEDVFTITGRGTVATGRIERGKVKVGDEVEIVGLAPET------RKTVVTGVEMHRKTLQ 279
Pho EALPGDNIGFNVRGVSKNDIKRGDVAGHTD-KPPTVVRTKDTFKAQIIVLNHP-----TAITVGYSPVLHAHTAQIPVRF 355
Ape QAEPGDNIGFAVRGVSKSDIKRGDVAGHLD-KPPT---VAEEFEARIFVIWHP-----SAITVGYTPVIHVHTASVSSRI 358
Sso KAEPGDNIGFNVRGVEKKDIKRGDVVGHPN-NPPT---VADEFTARIIVVWHP-----TALANGYTPVIHVHTASVACRV 358
Sce QGVPGDNVGFNVKNVSVKEIRRGNVCGDAKNDPPK---GCASFNATVIVLNHP-----GQISAGYSPVLDCHTAHIACRF 370
Bmo EAVPGDNVGFNVKNVSVKELRRGYVAGDSKNNPPK---GAADFTAQVIVLNHP-----GQISNGYTPVLDCHTAHIACKF 372
Hsa EALPGDNVGFNVKNVSVKDVRRGNVAGDSKNDPPM---EAAGFTAQVIILNHP-----GQISAGYAPVLDCHTAHIACKF 372
Eco EGRAGENVGVLLRGIKREEIERGQVLAKPGTIKP-----HTKFESEVYILSKDEGGRHTPFFKGYRPQFYFRTTDVTGTI 342
Tth EGIAGDNVGVLLRGVSREEVERGQVLAKPGSITP-----HTKFEASVYILKKEEGGRHTGFFSGYRPQFYFRTTDVTGVV 354
Taq EGIAGDNVGLLLRGVSREEVERGQVLAKPGSITP-----HTKFEASVYILKKEEGGRHTGFFTGYRPQFYFRTTDVTGVV 354
Pho EQILAKVDPRTGNIVEENPQFIKTGDSAIVVLRPMKPVVLEPVKEIPQLGRFAIRDMGMTIAAGMVISIQ---------- 425
Ape IEIKAKLDPKTGQVVEQNPQFLKAGDAAIVRFKPVKPLVVEKFSEIPQLGRFAMRDMNRTVGIGIVTDVKPA-------- 430
Sso SELVSKLDPRTGQEAEKNPQFLKQGDVAIVKFKPIKPLCVEKYNEFPPLGRFAMRDMGKTVGVGIIVDVKPA-------- 430
Sce DELLEKNDRRSGKKLEDHPKFLKSGDAALVKFVPSKPMCVEAFSEYPPLGRFAVRDMRQTVAVGVIKSVDKTEK-AAKVT 449
Bmo AEIKEKVDRRTGKSTEVNPKSIKSGDAAIVNLVPSKPLCVESFQEFPPLGRFAVRDMRQTVAVGVIKAVNFKEAGGGKVT 452
Hsa AELKEKIDRRSGKKLEDGPKFLKSGDAAIVDMVPGKPMCVESFSDYPPLGRFAVRDMRQTVAVGVIKAVDKKAAGAGKVT 452
Eco ------------ELPEGVEMVMPGDNIKMVVTLIHPIAMDDG------LRFAIREGGRTVGAGVVAKV---------- 392
Tth ------------QLPPGVEMVMPGDNVTFTVELIKPVALEEG------LRFAIREGGRTVGAGVVTKI---------- 404
Taq ------------RLPQGVEMVMPGDNVTFTVELIKPVALEEG------LRFAIREGGRTVGAGVVTKI---------- 404
Pho --------KGE 428
Ape ---KVD-IKAK 437
Sso ---K---VEIK 435
Sce KAAQK--AAKK 458
Bmo KAAEKATKGKK 463
Hsa KSAQKA-QKAK 462
Eco ---------LG 394
Tth ---------LE 406
Taq ---------LE 406

Supplementary Figure S5. Amino acid sequence alignment of archaeal and eukaryotic EF1α and EF-Tu.

The sequences for archaeal aEF1α from *Pyrococcus horikoshii* (Pho), Aeropyrum pernix (Ape), and *Sulfolobus solfataricus* (Sso), eukaryotic eEF1α from *Saccharomyces cerevisiae* (Sce), *Bombyx mori* (Bmo), and *Homo sapiens* (Hsa), and bacterial EF-Tu from *Escherichia coli* (Eco), *Thermus thermophilus* (Tth), and *Thermotoga maritima* (Tma) were aligned and displayed as in Figure 4A. The black dots above the sequences indicate the residues that participated in the interaction with aP1 in *P. horikoshii*. The domain structure is indicated with colored bars, where yellow corresponds to domain 1, green to domain 2, and light blue to domain 3. The P-loop and switch I and II regions are also indicated in orange.



Supplementary Figure S6. Mapping of the conserved amino acid residues of archaeal and eukaryotic EF1α proteins involved in the binding with the stalk protein.

(**A**) and (**B**) Mapping of the conserved amino acid residues on the structures. The amino acid residues of aEF1α that participated in the binding with aP1 and are conserved between archaea and eukaryotes are indicated in red on aEF1α (A). The corresponding amino acid residues in eukaryotes are indicated in red on eEF1α (B). The color coding for the domains is the same as in Figure 1. To clarify the positional conservation of the amino acid residues, archaeal and eukaryotic EF1αs that have a similar domain arrangement are shown as representatives of the respective EF1αs: aEF1α from *Aeropyrum pernixin* in the aEF1α•GTP•Pelota complex (PDB ID: 3AGJ) (54) is shown in (A); eEF1α from *Saccharomyces cerevisiae* in the eEF1A•GDPNP•eEF1Bα complex (PDB ID: 1G7C) (57) is shown in (B). The numbers of the corresponding amino acid residues in *P. horikoshii* aEF1α are indicated in parentheses in (A).

SUPPLEMENTARY TABLE

Table SI. Comprehensive list of the interactions between aP1 and aEF1α

aP1 residue	aP1 atom	aEF1α residue	aEF1α atom	Distance (Å)
Glu96	O	Lys125	NZ	3.37
Leu100	CD1	Phe129	CE1	3.68
Leu100	CD1	Phe129	CZ	3.84
Leu100	CD1	Leu166	CD1	3.88
Leu103	CB	Phe129	CE1	3.61
Leu103	CB	Phe129	CZ	3.76
Leu103	CD1	Lys125	CB	3.85
Leu103	C	Phe129	CE2	3.82
Ser104	CA	Phe129	CE2	3.78
Ala105	O	Lys324	NZ	2.95
Leu106	CD1	Leu359	CB	3.48
Leu106	CD1	Ala360	CB	3.85
Leu106	CD1	Ile384	CG2	3.72
Leu106	O	Lys324	NZ	2.89
Phe107	CB	Phe129	CD2	3.59
Phe107	CG	Phe129	CD2	3.82
Phe107	CD2	Phe129	CB	3.61
Phe107	CD2	Phe129	CG	3.74
Phe107	CD2	Phe129	CD2	3.67
Phe107	CE1	Ile384	CD1	3.77
Phe107	CE2	Phe129	CB	3.77
Phe107	O	Arg132	NH1	3.35
Gly108	O	Lys324	NZ	2.64
Gly108	O	Ser423	OG	2.66
Gly108	OXT	Ser423	OG	3.30

SUPPLEMENTARY REFERENCES

56. Tokimatsu,H., Strycharz,W.A. and Dahlberg,A.E. (1981) Gel electrophoretic studies on ribosomal proteins L7/L12 and the *Escherichia coli* 50 S subunit. *J. Mol. Biol.*, **152**, 397–412.

57. Andersen,G.R., Valente,L., Pedersen,L., Kinzy,T.G. and Nyborg,J. (2001) Crystal structures of nucleotide exchange intermediates in the eEF1A-eEF1Balpha complex. *Nat. Struct. Biol.*, **8**, 531–534.