ONLINE METHODS

Crystallization

E. coli LeuRS with an N-terminal six-histidine tag and *E. coli* $tRNA^{Leu}_{UAA}$ transcript were prepared and purified as previously described[1,2]. Protein was stored in buffer comprising 20 mM Tris-HCl (pH 7.4), 100 mM NaCl, 5 mM $MgCl_2$ and 5 mM 2-β mercaptoethanol. Crystallization was performed at 20°C by the hanging drop vapor diffusion method. For the editing complex, solutions were prepared with 33 μM LeuRS, 40 μM $tRNA^{Leu}$ and 5 mM L-leucine or 1 mM 1-hydroxy-3H-2,1-benzoxaborole (a gift from Anacor Pharmaceuticals, Palo Alto, CA). Crystals were obtained by mixing 2 μL of this solution with 1 μL of reservoir solution containing 0.1 M sodium acetate (pH 5.6), 14-18% (w/v) PEG 6000 and 200 mM NaCl. The crystals were frozen in liquid nitrogen after transfer for a few seconds in the mother liquor containing 22% (v/v) ethylene glycol as cryoprotectant and the corresponding small substrates. In the case of the aminoacylation complex, solutions were prepared with 33 μM LeuRS, 50 μM $tRNA^{Leu}$ and 1 mM leucyl-adenylate analogue (LeuAMS, purchased from RNA-TEC, Leuven, Belgium). Crystals were obtained by mixing 2 μL of this solution with 2 μL of reservoir solution containing 0.1 M bis-TRIS (pH 5.5), 23-25% (w/v) PEG 3350, and 200 mM ammonium acetate. The crystals were frozen in liquid nitrogen prior to X-ray exposure without added cryoprotectant.

Structure determination and refinement

All diffraction data sets were collected at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) using a wavelength of 1.072 Å for the complex with L-leucine, 0.931 and 0.934 Å respectively for the orthorhombic- and monoclinic-crystal forms of the complex with benzoxaborole, and 0.938 Å for the complex with LeuAMS. Data were integrated and scaled with the XDS suite[3]. Further data analysis was performed with the CCP4 suite[4]. The structure of the LeuRS:$tRNA^{Leu}$:L-leucine editing complex was initially solved by molecular replacement with PHASER[5] using the *T. thermophilus* LeuRS structure[6] (PDB 1H3N) without its editing domain and the *E. coli* LeuRS editing domain structure[7] (PDB 2AJG) as models. The model was improved by automatic building using ARP-wARP[8] and manual adjustments were made with COOT[9]. The structure of the aminoacylation complex was solved by molecular replacement with PHASER using the core of the protein

from the *E. coli* editing complex (1-223, 416-568 and 795-860 residues). The resulting map was used to manually position the core of the tRNA and the additional flexibly linked domains using COOT. Residues 159-189 of the Zn domain, not visible in the LeuRSEC editing complex, were built using the LeuRSTT Zn domain as a guide. All models were refined using REFMAC5 with TLS. The orthorhombic ($P2_12_12_1$) and monoclinic ($P2_1$) forms of the LeuRSEC editing complex have only minor differences involving a small coordinated rotation of the C-terminal domain tRNA and editing domain, without changing specific protein-tRNA interactions. In the monoclinic form the C-terminal domain is partially disordered and in general the B-factors are slightly higher. Interfaces were analyzed with the PISA server (http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html) and domain motions with the program DynDom[10]. Structure quality was analyzed with MOLPROBITY[11] (http://molprobity.biochem.duke.edu/) and showed all residues in allowed regions (with 95.1-98.0% of residues in favored regions) for the different models. Figures were drawn with PYMOL (http://www.pymol.org/), and a video showing the conformational changes between the aminoacylation and editing states was generated by CHIMERA (http://www.cgl.ucsf.edu/chimera).

Enzyme assays

A reaction mixture consisting of 60 mM Tris, pH 7.5, 10 mM $MgCl_2$, 1 mM DTT, 4 mM ATP, 21 μM [^{3}H]-leucine (10 μCi nmol $^{-1}$) and 2.5 μM $tRNA^{Leu}$ was initiated by the addition of 25 nM enzyme and quenched[12]. Higher concentrations of 4 μM $tRNA^{Leu}$ and 1 μM enzyme concentrations were used for weak mutant activities (Supplementary Fig. 6c,d). Leucine-dependent PPi-ATP exchange reactions contained 50 mM HEPES pH 8.0, 10 mM $MgCl_2$, 1 mM DTT, 1 mM ATP, 1 mM [^{32}P]-PP_i (10 μCi μmol $^{-1}$), 1 mM leucine and were initiated by 10 nM enzyme. Each reaction was quenched on Baker-Flex poly-ethyleneimine cellulose TLC plates and chromatographed in 750 mM KH_2PO_4 pH 3.5, 4 M urea at 25 ^{o}C.

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