Supplementary Material

C21orf57 is a human homologue of bacterial YbeY proteins

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Running title: Characterization of Human YbeY

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Materials and methods

Bacterial strains, plasmids, cloning, and bacterial growth. Cloning and plasmid manipulations were performed in *E. coli* XL1 blue (Stratagene); stress assays were performed in *E. coli* MC4100 wild-type or MC4100 Δ*ybeY* [1]. Plasmid pBR322 was used to clone the gene of interest as described before [1]; point mutations were created using the QuikChange II Site-Directed Mutagenesis Kit (Agilent Technologies) according to the manufacturer's instructions. Strains carrying either pBR322 (control) or recombinant plasmids were grown in Luria-Bertani (LB) medium supplemented with 100 µg/ml of ampicillin at 37^0C. *E. coli* BL21(DE3) plysS Δ*rna* Δ*pnp* was generated by using P1vir transduction method. Δ*pnp::kan* allele was transferred from the Keio knockout strain to *E. coli* BL21(DE3) plysS Δ*rna* strain [2].

Stress assays. For assessment of stress tolerance, overnight cultures of the respective *E. coli* MC4100 wild-type or Δ*ybeY* transformants were subcultured into fresh medium. Exponentially growing cultures were diluted in a 1/10-dilution series and spotted onto LB-agar plates containing antibiotics kasugamycin (200 µg/ml) or cefotaxime (15 ng/ml). For UV stress, bacterial cultures were spotted onto LB plates and exposed to radiation at 100 J/m^2 for 70 seconds using a 280 nm lamp. All plates were incubated overnight at 37^0C. For heat stress, plates were incubated overnight at 45^0C after spotting. A control plate without antibiotics was prepared in parallel and incubated overnight at 37^0C. Experiments were performed at least in triplicates; a representative experiment is shown.

Purification of HuYbeY. The *huYBEY* gene (wild-type or mutants R55E D90R and R55E H118A) was cloned into plasmid pET28A downstream of the T7 promoter (Addgene database) for expression of HuYbeY with an N-terminal maltose-binding-protein (MBP) tag.

E. coli BL21(DE3) plysS Δ*rna* [2] was co-transformed with the respective recombinant pET28A constructs and plasmid pRARE2, which carries genes for co-expression of various rare tRNAs in *E. coli* [3] to compensate for unfavorable codon usage. Transformants were plated onto LB-agar containing 50 µg/ml of kanamycin and 25 µg/ml of chloramphenicol and incubated overnight at 37^0C. 50 ml of LB

containing antibiotics and 0.8% of glucose was inoculated with a single bacterial colony and grown overnight at 30^0C. This overnight culture was diluted to an OD_{600} of 0.05 in 4 liters of LB containing antibiotics and 0.2% of glucose and grown at 30^0C to an OD_{600} of ~0.45. 0.5 mM IPTG was added to the culture and bacteria were allowed to grow at 30^0C for 90 minutes. Cells were washed once with HuYbeY buffer (10 mM Tris pH 7.5, 200 mM potassium acetate, 5 mM beta-mercaptoethanol and 5% glycerol) and sonicated after treating with 1 mg/ml of lysozyme for 45 minutes. cOmplete™, EDTA-free Protease Inhibitor Cocktail (Roche) was added to the lysis buffer. Recombinant MBP-HuYbeY protein was first affinity purified using amylose resin (NEB) and then by size exclusion. Before loading the protein sample, the amylose column (3 ml) was washed three times with 20 ml of HuYbeY buffer. The column was washed three times with 20 ml of HuYbeY buffer supplemented with protease inhibitors and then proteins were eluted with 2 column volume of HuYbeY buffer supplemented with 10 mM maltose and protease inhibitors. Eluates were concentrated to 2 ml using Vivaspin 6.5 kDa column (GE Healthcare). After washing the Hiload™ 16/60 Superdex 75 (GE Healthcare) with 2 column volumes (110 ml) of HuYbeY buffer, concentrated eluates from the amylose column were loaded. Proteins were eluted by passing 110 ml of HuYbeY buffer through the size exclusion column, and eluates were collected in 1 ml fractions. Pooled protein fractions were concentrated and stored in small aliquots at -80^0C in HuYbeY buffer. Protein concentration was measured using Bio-Rad Protein Assay Dye Reagent Concentrate. Approx. 0.5 mg of purified protein were obtained from a 4 liters of culture. The purity of recombinant proteins was assessed by SDS-PAGE and mass-spectral analysis (Massachusetts Institute of Technology Biopolymers & Proteomics Core Facility). All the steps, starting from the induction of the protein production to protein purification using amylose resin, were performed within 24-36 hours at 4^0C unless indicated otherwise.

Alternatively, wild-type HuYbeY was overexpressed in *E. coli* BL21(DE3) plysS Δ*rna* Δ*pnp* as described above, except all bacterial cultivations prior to induction with IPTG were performed at 37^0C

instead of 30^0C. Instead of pooling all MBP-HuYbeY containing fractions, individual protein fractions were concentrated and stored at -80^0C. Purity of the protein fractions was assessed by running protein sample on SDS-PAGE.

RNA extraction, RNase assay, and agarose gel analysis. Total RNA was extracted from HEK293T cells using Quick-RNA MiniPrep Kit (Zymo Research) according to the manufacturer instructions. 125 ng of total RNA, 1 - 5 µM of wild-type or mutant HuYbeY, 50 mM HEPES pH7.5 and 50 mM NaCl were mixed in a total reaction volume of 20 µl. The control reaction mixture contained buffer instead of enzyme. All reaction mixtures were kept at 37^0C for two hours. Reactions were stopped by addition of gel-loading dye. Total RNA was analyzed by agarose gel electrophoresis as described in Jacob *et al*. [2].

Expression of HuYbeY in yeast. The *huYBEY* gene was cloned into the shuttle vector pESC-Leu (Invitrogen) under control of the GAL1 promoter. *Saccharomyces cerevisiae* CKY473 cells (*ura3-52 leu2-3,112, Gal$^+$;* C. Kaiser, Massachusetts Institute of Technology) were transformed using a standard lithium acetate protocol [4]. Transformants were selected throughout by growth in synthetic minimal medium lacking leucine. The initial selection and liquid precultures (5 ml, ~24 h) were carried out at 30^0C in synthetic minimal medium supplemented with 2% glucose. Aliquots of cells were then spread onto plates prepared from synthetic minimal medium containing 2% glucose/0.5% galactose, 2% raffinose/0.5% galactose or 3% glycerol/0.5% galactose and incubated at 30^0C for 2 days, followed by a 7-day incubation at room temperature.

Supplemental Figures

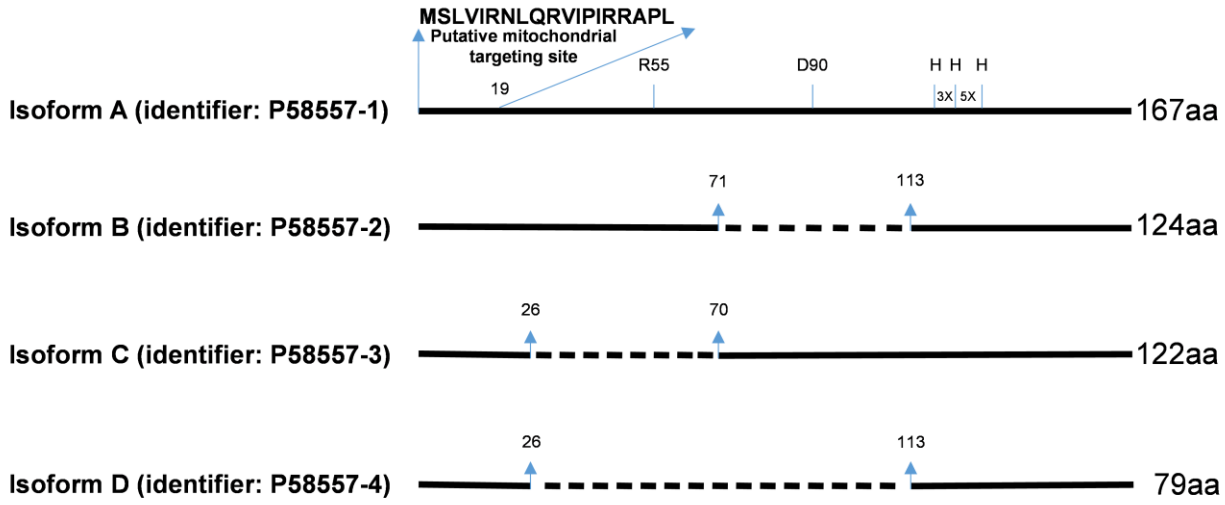
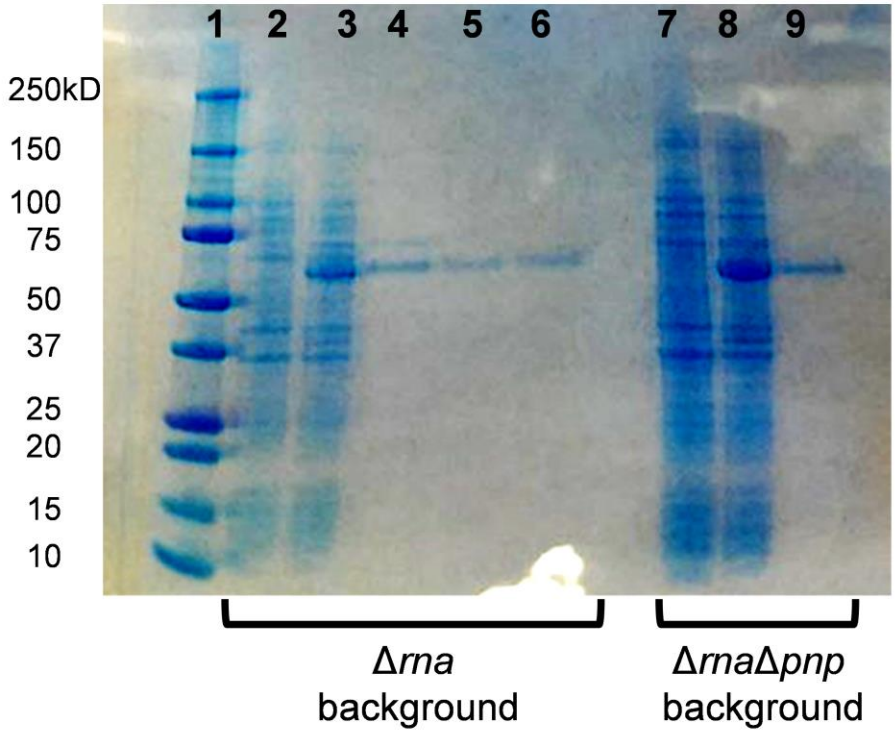


Fig S1. Schematic presentation of isoforms of HuYbeY. Based on cDNA analysis, HuYbeY has three additional isoforms that are smaller in size than the full length protein, each missing a portion of the canonical protein sequence; the regions that are missing in the isoforms of HuYbeY are indicated as dashed lines [5]. The length of the HuYbeY protein variants is indicated (aa). Relevant amino acids are indicated.

A



B

	HuYbeY	R55E_D90R	R55E_H118A
PNPase	0.2%	4.2%	2.6%
RNase E	-	1.17%	0.59%

Fig S2. Analysis of HuYbeY overexpression and purification. (A) Coomassie Blue stained gel of protein samples. Samples are ladder, total protein (uninduced), total protein (induced), purified MBP-HuYbeY, purified MBP- HuYbeY R55E_D90R, purified MBP-HuYbeY R55E_H118A, total protein (uninduced), total protein (induced) and purified MBP-HuYbeY (wells 1-9). Protein samples loaded into wells 2 to 6 and 7 to 9 are extracted from Δ*rna* and Δ*rna* Δ*pnp* background strains of *E. coli*, respectively. 250 ng of protein was loaded into wells 4, 5, 6 and 9. (B) Mass spectrometry analysis shows the amount of PNPase and RNase E in the HuYbeY protein samples isolated from Δ*rna* background strain.

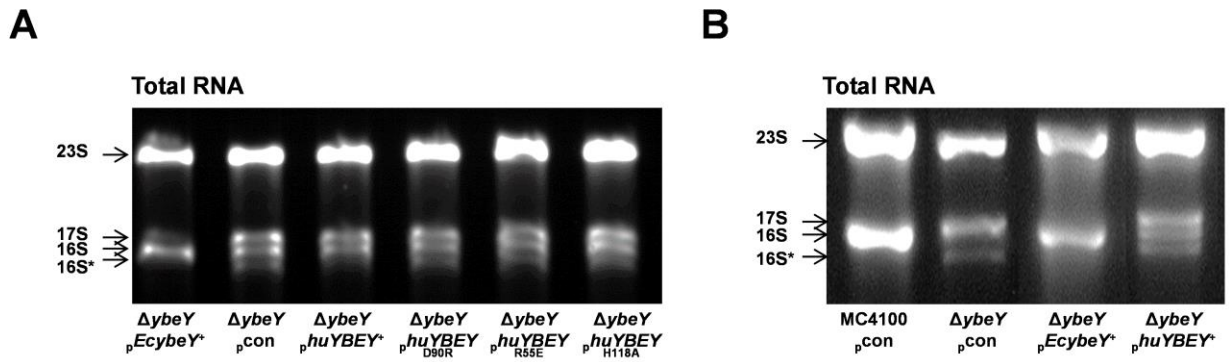


Fig S3. Overproduction of HuYbeY alters the processing of 16S rRNA

(A) The rRNA profile of Δ*ybeY* strains carrying *huYBEY* overexpression plasmid grown at 37^0C. (B) The rRNA profile of Δ*ybeY* strains after heat stress (2 hours at 45^0C). Δ*ybeY*$_p$*huYBEY*$^+$ strain shows partial conversion of precursor 17S rRNA to mature 16S rRNA in comparison to Δ*ybeY*$_p$con strain.

Supplementary References

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[5] Uniprot. http://www.uniprot.org/uniprot/P58557, 2016 (accessed 12.14.2016).