SUPPLEMENTARY INFORMATION

# RNase III initiates rapid degradation of *proU* mRNA upon hypo-osmotic stress in *Escherichia coli*

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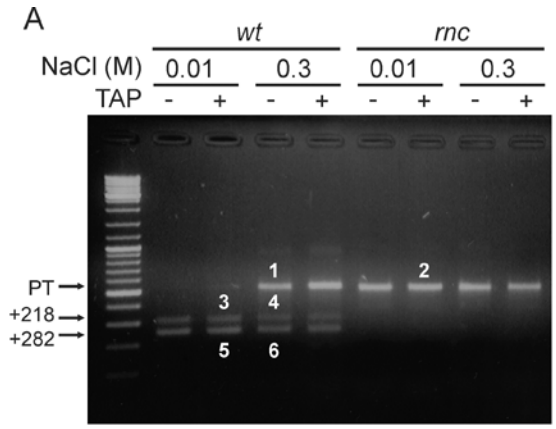


B

| Band | Clones sequenced | Position of 5´ end (number of clones) |
|---|---|---|
| 1 | 6 | +1, +7, +15, +22, +24, +26 |
| 2 | 6 | +1, +15 (3), +22, +25 |
| 3 | 5 | +218 (4), +229 |
| 4 | 6 | +218 (6) |
| 5 | 5 | +281 (5), +291 |
| 6 | 5 | +281 (5) |

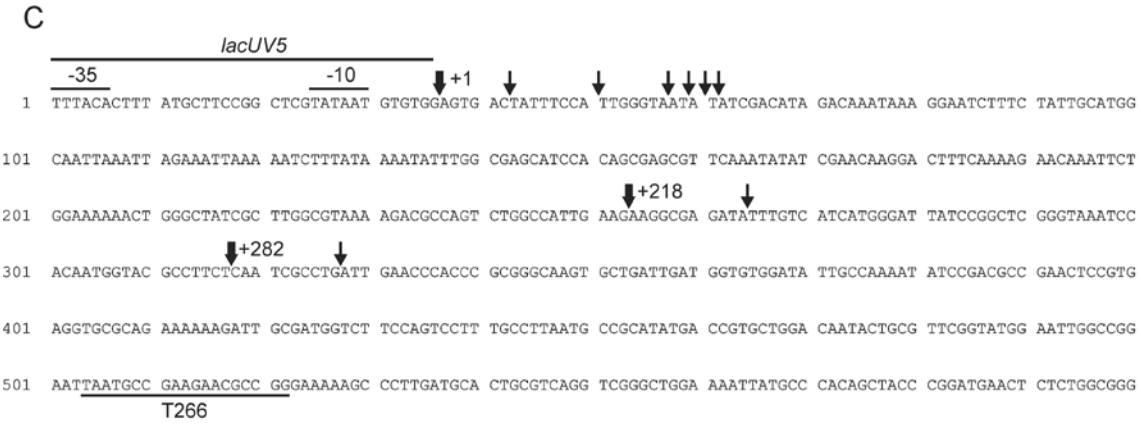


**Fig. S1 5´ RACE analysis of *proU* mRNA processing by RNase III.** (A) Gel electrophoresis of amplification products of cDNA. Analysis of processing of *proU* mRNA in wild type cells and in *rnc105* mutant cells is shown. Each DNA band on a gel corresponds to the separate RNA species, such as primary *proU* transcript (PT) or processing product. Expression of *proU* (+1 to +1260) encoded by low-copy-number plasmid pKEKK12 was directed by the *lacUV5* promoter. Transformants of the wild-type (S3460) and *rnc* mutant (S4023) with pKEKK12 were grown in LB supplemented with either 0.01 M or 0.3 M NaCl. RNA was extracted and further analyzed by 5' RACE. (+) and (-) refers to TAP treatment prior to ligation of the RNA adapter. Numbers indicate the DNA bands that were subsequently purified from the gel, cloned into pUC12, and analyzed by sequencing. (B) and (C) mapping of primary transcripts and RNase III processing sites by sequencing. (B) The band numbers in the table correspond to the band numbers in (A). Most DNA bands are a mixture of fragments with different 5' ends. Therefore, five to six clones were sequenced per cloning of each band. The number of clones sequenced, the positions of 5' ends

determined by sequencing, and the number of clones with corresponding 5' ends (in brackets) are shown. (C) Sequence of the plasmid pKEKK12 used for 5´ RACE analysis with RNase III processing sites. Sequence includes the *lacUV5* promoter and upstream part of *proU* analyzed by 5´ RACE. Primer T266 was used for PCR amplification of cDNA, and therefore corresponds to the 3´ end of the analyzed *proU* sequence. The location of 5´ ends of clones determined by sequencing are taken from Fig. S1B and are indicated by arrows. For each cloning, the most upstream position was depicted as a bold arrow.

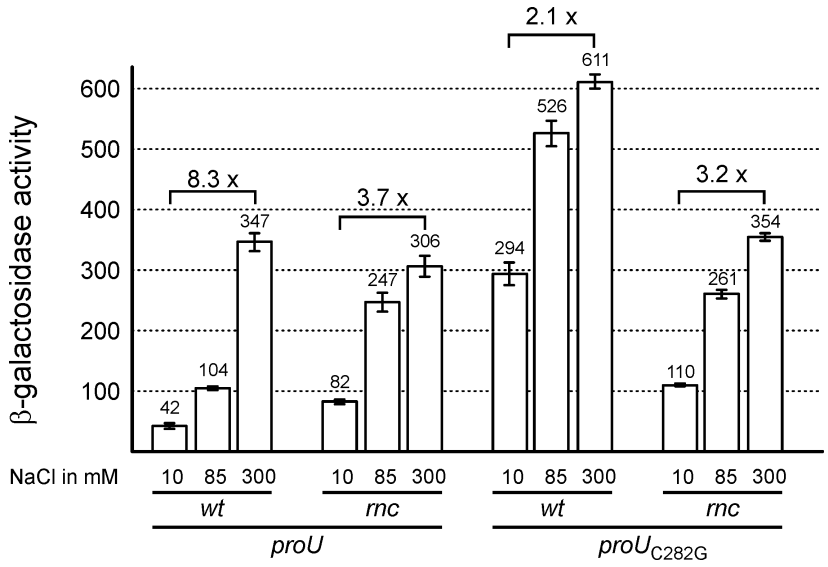


**Fig. S2 Osmoregulation of *proU* in an *rnc* mutant.** Steady-state expression levels of the translational *PlacUV5* Φ*proU-lacZ* (*proU*) and its C282G mutant (*proU*$_{C282G}$) were determined in the wild-type (wt) and *rnc* mutant. β-galactosidase values are indicated as bars, and the fold osmoregulation is indicated above the horizontal brackets. Strains used: *PlacUV5* Φ*proU-lacZ* in the wt (strain S4070), *PlacUV5* Φ*proU*$_{C282G}$*-lacZ* fusion in wt (strain T811), *PlacUV5* Φ*proU-lacZ* in the *rnc* mutant (strain T100), *PlacUV5* Φ*proU*$_{C282G}$*-lacZ* in the *rnc* mutant (strain T825).

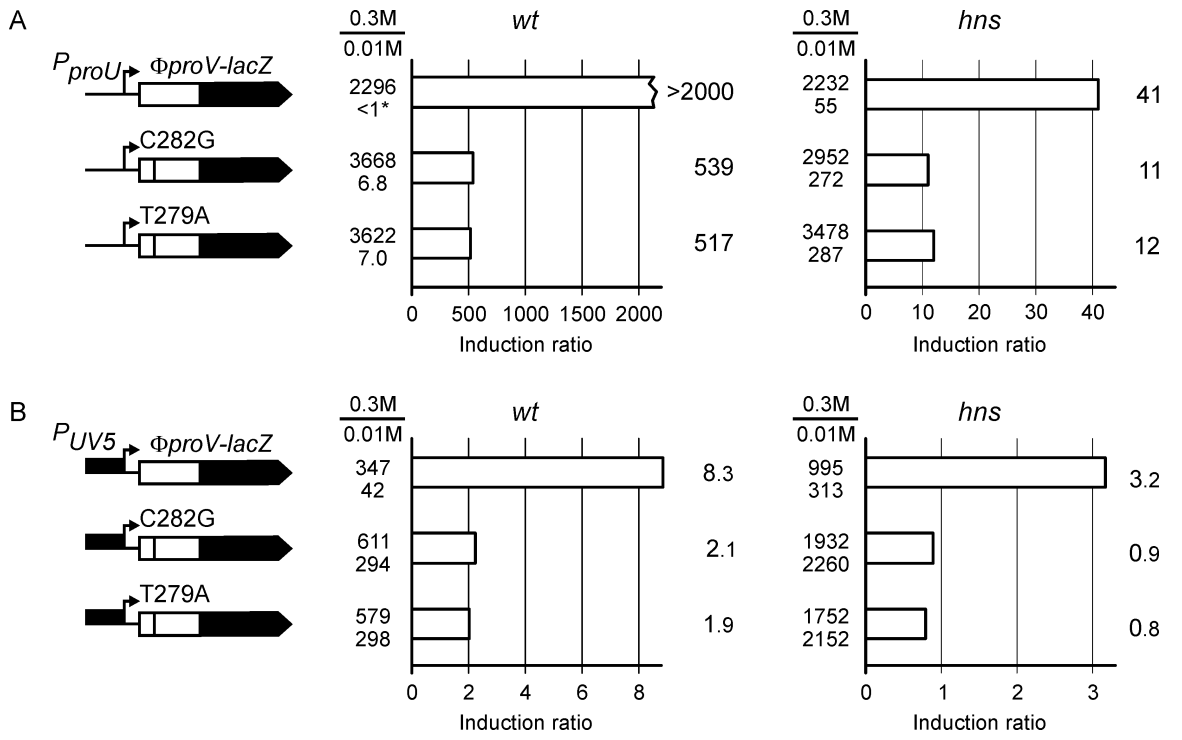


**Fig. S3 Effect of mutation of the RNase III processing site on osmoregulation of *proU*.** Steady-state expression levels of translational *proU-lacZ* reporter fusions were determined of cultures grown in low and high osmolarity LB medium. Expression levels of fusions whose expression is directed by the *proU* promoter (A) and the *lacUV5* promoter (B) were determined in the wild-type (*wt*) (A and B left panel) and *hns* mutants (A and B right panel). The values of the expression levels are given as numbers in Miller units, and the fold difference in expression at high and low osmolarity conditions is indicated by bars. Note that different scales are used. * indicates that the β-galactosidase activity was below the detection threshold of 1 unit. Mutation of the RNase III processing sites results in the same reduction of osmoregulation, independent of the promoter and of H-NS. Strains used: A: wild-type *proU* (strain S4068), $proU_{C282G}$ (T881), $proU_{T279A}$ (T879); B: *proU* (S4115), $proU_{C282G}$ (T893), $proU_{T279A}$ (T891); C, *PlacUV5-proU* (S4070), $proU_{C282G}$ (T811), $proU_{T279A}$ (T887); and D: *proU* (S4117), $proU_{C282G}$ (T833), and $proU_{T279A}$ (T909).

Table S1: Oligonucleotides

| primer | Sequence | Description |
|---|---|---|
| S372 | cggaattcAGTGACTATTTCCATTGGGTAATATATCG | Cloning of proU(+1 to +1260)<br>EcoRI-site underlined, proU(+1 to +29) in capital letters |
| S779 | ttccggctcgtataatgtgtggAGTGACTATTTCCATTGGGTAATATATCG | Cloning of PlacUV5 proU(+1 to +1260)<br>PlacUV5 sequence in lower case letters, proU in upper case letters |
| S696 | AATATCACTACCCGCAGCAGGGAAATAATTCCCGCCAAATAGCgtgtaggctggagctgcttcg | deletion of proU<br>proU(-277 to -235) in upper case letters, sequence matching pKD3 in lower case letters |
| S697 | GGATGCGGCAAAAACGCCTTATCCGCCCGAATAAAAATTAcatatgaatatcctccttagttcctattcc | deletion of proU<br>proU sequence in upper case letters with proX stop codon underlined, sequence matching pKD3 in lower case letters |
| T005 | cgagctcgagAGATGAAGACTGGAATTTCTGAGGG | Cloning of proU(-315 to +1260)<br>XhoI site underlined, proU(-315 to -291) in upper case letters |
| T008 | cgactctagaTTAAGCGTAATCTGGAACATCGTATGGGTAGCCATTATTTACCCCCTCACGA | Cloning of proU(-315 to +1260) with HA-tag<br>proU(+1239 to +1260) in capital letters, HA-coding sequence in capital letters underlined, XbaI site underlined |
| T265 | gcgcgaattcCTGTAGAACGA | Forward primer for RACE homologous to RNA adapter T268, EcoRI-site underlined |
| T266 | gactgtcgacCCGGCGTTCTTCGGCATTA | Reverse primer for RACE homologous to proU(+468 to +486), SalI site underlined |
| T267 | TCCGGGTAGCTGTGGGCATAA | Primer for reverse transcription of the proU mRNA for 5´RACE, homologous to proU(+528 to +548) |
| T268 | AUAUGCGCGAAUUCCUGUAGAACGAACACUAGAAGAAA | RNA adapter for 5´RACE |
| T376 | GGTAAATCCACAATGGTACGCCTACTCAATCGCCTGA | Mutation of T279 to A, proU(+262 to +299) |
| T378 | GGTAAATCCACAATGGTACGCCTTCTGAATCGCCTGATT | Mutation of C282 to G, proU(+262 to +301) |
| T520 | AACTGGGCTATCGCTTGGCGTA | qRT-PCR proU(+171 to +192) |
| T521 | GCAATATCCACACCATCAATCAGCA | qRT-PCR proU(+314 to +338) |
| T522 | CGAAATGATGCCGACATACTGTTGG | qRT-PCR proU(+1188 to +1212) |
| T523 | AGGTCTTGATGCGGCGCTGA | qRT-PCR proU(+1071 to +1090) |
| T524 | ATCCGGCTCGGGTAAATCCACAATGGTACGCCTTCTGAATCGCCTGATTGAACCCTAGGGATAACAGGGTAATgtgtaggctggagctgcttcg | Chromosomal mutagenesis of proU C282 to G<br>proU(+246 to +300) in capital letters,I-SceI site underlined, sequence matching pKD4 in lower case letters |
| T525 | CAGCACTTGCCCGCGGGTGGGTTCAATCAGGCGATTCAGAAGGCGTACCATTGTGcatatgaatatcctccttagttcctattcc | Reverse primer for chromosomal mutagenesis of proU C282 to G proU(+264 to +318) in capital letters, sequence matching pKD4 in lower case letters |
| T528 | GGTGTAGCGGTGAAATGCGTAGAG | qRT-PCR, for amplication of 16S rRNA from position 682nt to 846nt (rrsA, -B, -C, -E, -H) or 697nt to 861nt (rrsG, -D) |
| T529 | CTCAAGGGCACAACCTCCAAGTC | qRT-PCR, for amplication of 16S rRNA from position 682nt to 846nt (rrsA, -B, -C, -E, -H) or 697nt to 861nt (rrsG, -D) |