

# Supplemental Materials

*Molecular Biology of the Cell*

Noree et al.

Figure S1. Frequency and length of Ura7p-GFP structures (WT and mutants) are not correlated with their expression levels

A) Western blots of wild type (WT) and mutant Ura7p-GFP. B) Expression level versus frequency of structure formation of mutant Ura7p-GFP. C) Expression level versus median length of mutant Ura7p-GFP structures. Fold-change is calculated, relative to expression level, frequency, and length of wild type (WT) Ura7p-GFP.

Figure S2. Alignment of amino acid sequences translated from genes encoding CTP synthases in *H. sapiens*, *E. coli*, *D. Malanogaster*, *S. cerevisiae*, *L. lactis*, *T. thermophilus* (HB8).

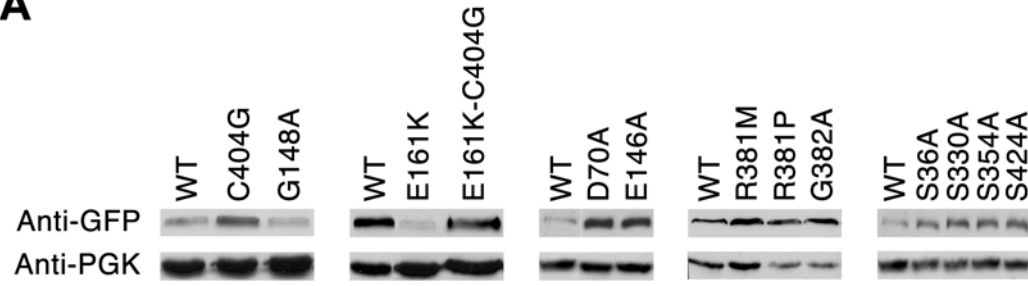
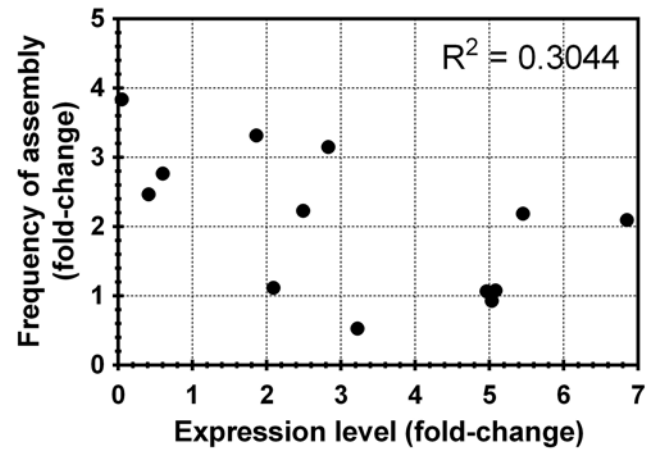
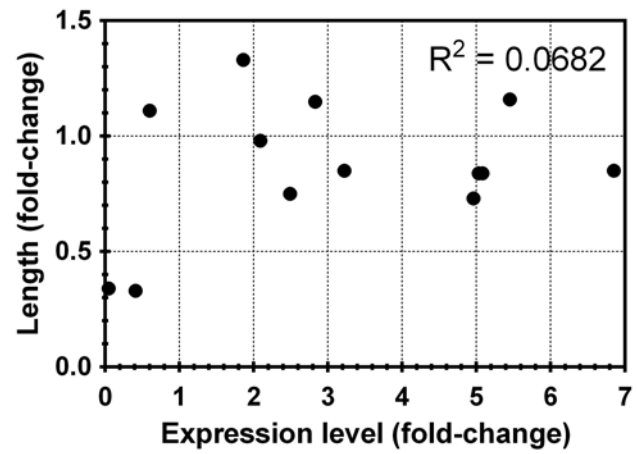
The alignment shows that their sequences share 128 identical amino acids (19% identity, marked with stars), and 151 similar amino acids (marked with dots). Amino acids highlighted in grey are mutagenized in this study; ATP binding sites (D70A and E146A), CTP binding site (E161K), GTP binding sites (R381M/P and G382A), tetramerization site (G148A), catalytic site (C404G), phosphorylation sites (S36A, S330A, S354A, and S424A). Analysis was performed using program 'CLUSTAL O 1.2.1' ([www.uniprot.org](http://www.uniprot.org)).

Figure S3. Expression levels are not related to types of structures of E161K Ura7p-GFP

A) Western blots of wild type (WT), E161K (made in this study), and E161K Ura7p-GFP (integrated plasmid pRS403; described in Noree et al., 2010). B) Representative images of yeast expressing E161K Ura7p-GFP (this study versus our previous study).

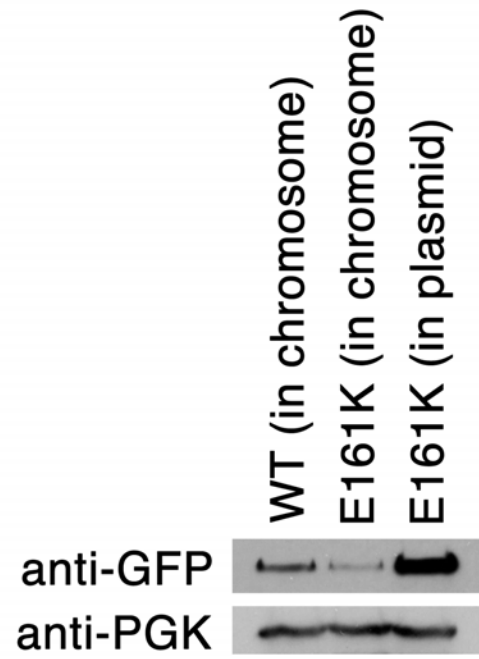
**Supplementary Table 1. Summary for frequency of assembly, median length, and protein expression level of each mutant relative to those of WT (fold-change)**

Mutants	Fold change relative to WT		
	Frequency of assembly	Median Length	Expression level
WT			
E161K	3.84	0.34	0.05
E161K-C404G	2.47	0.33	0.41
E146A	2.19	1.16	6.85
D70A	2.10	0.85	5.45
R381M	3.32	1.33	1.86
R381P	3.15	1.15	2.83
G382A	2.23	0.75	2.49
C404G	1.12	0.98	2.09
G148A	2.77	1.11	0.60
S330A	1.08	0.84	5.08
S354A	1.07	0.73	4.96
S424A	0.93	0.84	5.03
S36A	0.50	0.85	3.22

**A****B****C**

Position	Amino Acid Sequence	Position	Accession no.	Gene/Organism
1	-----MKYILVTGGVIGSGIGKGIASSVGTILKSCGLHVTSIKIDPYINIDAGTFSPYEHGEVFLDDGGVEVLDLGNVYERFLDIRLTKDNNLT	89	<a href="#">P17812</a>	PYRG1_HUMAN
1	-----MKYILVTGGVIGSGIGKGIASSVGTILKSCGLRVTAIKIDPYINIDAGTFSPYEHGEVFLDDGGVEVLDLGNVYERFLDIRLTKDNNIT	89	<a href="#">Q9NRF8</a>	PYRG2_HUMAN
1	-----MTNYIFVTGGVSSLGKIAAASLAALEARGLAVTIMKLDPIYINVDPTGMSPIQHGVEVVTEDGAEATDLDLGHYERFDIRTKMRRNNFT	91	<a href="#">P0A7E5</a>	PYRG_ECOLI
1	-----MKYILVTGGVIGSGIGKGIASSVGTILKSCGLDVTSIKIDPYINIDAGTFSPYEHGEVFLDDGGVEVLDLGNVYERFLDIRLTKDNNIT	89	<a href="#">Q9VUL1</a>	PYRG_DROME
1	-----MKYVVVSGGVIIGIGKGLVASSGTMMLKTLGLKVTIKIDPYMNIIDAGTMSPLHGEVFLDDGGVEVLDLGNVYERFLDIRLTKDNNIT	89	<a href="#">P28274</a>	URA7_YEAST
1	-----MKYVVVSGGVIIGIGKGLVASSGTMMLKTLGLKVTIKIDPYMNIIDAGTMSPLHGEVFLDDGGVEVLDLGNVYERFLDIRLTKDNNIT	89	<a href="#">P38627</a>	URA8_YEAST
1	-----MSTKYIFVTGGVSSMGKIVAAASLGRLLKNRGLKVTIQKFDPIYINVDPTGMSPIQHGVEVVTEDGAEATDLDLGHYERFDIRTKMRRNNFT	91	<a href="#">O87761</a>	PYRG_LAACLAM
1	MNGSADAGPRPKYVFIITGGVSSLGKILTSGLGALLRARGYVTAIKIDPYVNVVAGTMRPYEHGEVFLDDGGVEVLDLGNVYERFLDIRLTKDNNLT	100	<a href="#">Q5S1A8</a>	PYRG_THET8
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90	TGKIYQVINKERKGDYLGKTVQVPHITDAIQEWVVRQALIPVDEGLEPQVCVIELGGTVGDIEMSPFIEAFRQFQFKVRENFNCNHHVSLVQPSSST	189	<a href="#">P17812</a>	PYRG1_HUMAN
90	TGKIYQVINKERKGDYLGKTVQVPHITDAIQEWVVRQALIPVDEGLEPQVCVIELGGTVGDIEMSPFIEAFRQFQFKVRENFNCNHHVSLVQPSAT	189	<a href="#">Q9NRF8</a>	PYRG2_HUMAN
92	TGRIVSDVLRKERRGDYLGKTVQVPHITDAIQEWVVRQALIPVDEGLEPQVCVIELGGTVGDIEMSPFIEAFRQFQFKVRENFNCNHHVSLVQPSAT	183	<a href="#">P0A7E5</a>	PYRG_ECOLI
90	TGKIYKLVIEKERTGEYLGKTVQVPHITDAIQEWVVRQALIPVDEGLEPQVCVIELGGTVGDIEMSPFIEAFRQFQFKVRENFNCNHHVSLVQPSAT	188	<a href="#">Q9VUL1</a>	PYRG_DROME
90	TGKIYSHVIAKERKGDYLGKTVQVPHITDAIQEWVVRQALIPVDEGLEPQVCVIELGGTVGDIEMSPFIEAFRQFQFKVRENFNCNHHVSLVQPSAT	187	<a href="#">P28274</a>	URA7_YEAST
90	TGKIYSHVIAKERKGDYLGKTVQVPHITDAIQEWVVRQALIPVDEGLEPQVCVIELGGTVGDIEMSPFIEAFRQFQFKVRENFNCNHHVSLVQPSAT	187	<a href="#">P38627</a>	URA8_YEAST
92	SGKVVSEILRKRKKEGELGTVQVPHITDAIQEWVVRQALIPVDEGLEPQVCVIELGGTVGDIEMSPFIEAFRQFQFKVRENFNCNHHVSLVQPSAT	185	<a href="#">O87761</a>	PYRG_LAACLAM
101	TGQVLSVIQKERRGEYLGKTVQVPHITDAIQEWVVRQALIPVDEGLEPQVCVIELGGTVGDIEMSPFIEAFRQFQFKVRENFNCNHHVSLVQPSAT	194	<a href="#">Q5S1A8</a>	PYRG_THET8
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190	GEQTKTQNSVRELRLGLSPDLVRCRNSPLDTSVKEIKSMPFCHVEPEQVICVHDVSSVYRPLLEEQQVVDYFLRLDLPI-----ERQPR-KML	282	<a href="#">P17812</a>	PYRG1_HUMAN
190	GEQTKTQNSVRELRLGLSPDLVRCRNSPLDTSVKEIKSMPFCHVNPQVICVHDVSSVYRPLLEEQQVVDYFLRLDLPI-----GDSAS-NLL	282	<a href="#">Q9NRF8</a>	PYRG2_HUMAN
184	GEVTKTKTQHSVVELLISGIDPDIICRSDDRAVNERAKIALFCNVPEKAVISLKDVSDEYIKIPLGLKSGQLDDYICKRFSLNCP-----EANL	273	<a href="#">P0A7E5</a>	PYRG_ECOLI
189	GEFKTKTQSSVRELRLGLSPDLVRCRNSPLDTSVKEIKSMPFCHVNPQVICVHDVSSVYRPLLEEQQVVDYFLRLDLPI-----DMSKRTKCL	282	<a href="#">Q9VUL1</a>	PYRG_DROME
188	GEQTKTQNSVRELRLGLSPDLVRCRNSPLDTSVKEIKSMPFCHVNPQVICVHDVSSVYRPLLEEQQVVDYFLRLDLPI-----LHARLKLDEISLTEEEKQGLLEL	287	<a href="#">P28274</a>	URA7_YEAST
188	GEQTKTQNSVRELRLGLSPDLVRCRNSPLDTSVKEIKSMPFCHVNPQVICVHDVSSVYRPLLEEQQVVDYFLRLDLPI-----LHARLKLDEISLTEEEKQGLLEL	287	<a href="#">P38627</a>	URA8_YEAST
186	GELTKTQNSVRELRLGLSPDLVRCRNSPLDTSVKEIKSMPFCHVNPQVICVHDVSSVYRPLLEEQQVVDYFLRLDLPI-----KADM	275	<a href="#">O87761</a>	PYRG_LAACLAM
195	EEFKTKTQNSVRELRLGLSPDLVRCRNSPLDTSVKEIKSMPFCHVNPQVICVHDVSSVYRPLLEEQQVVDYFLRLDLPI-----IPNL	284	<a href="#">Q5S1A8</a>	PYRG_THET8
	:*: :* :*			
283	MKWKEMADRYDRLLTETSIALVGVKTYKSDSYASVIAKALEHSALAINHKLKIKYIDSADLEPITSQEEPVRYHEAWQKCSAHGVLVFGGFGVGRGTEGI	382	<a href="#">P17812</a>	PYRG1_HUMAN
283	FKWNRMDRYDRLLTETSIALVGVKTYKSDSYASVIAKALEHSALAINHKLKIKYIDSADLEPITSQEEPVRYHEAWQKCSAHGVLVFGGFGVGRGTEGI	382	<a href="#">Q9NRF8</a>	PYRG2_HUMAN
274	SEHQVIFEEANVSEVTTIGMVGKVIELPDYAKSVIEALKHGGLKNRVSVNLIKIDSDQVTRGV-----EILKGLDAIILVFGGFGYGRGVEGMI	362	<a href="#">P0A7E5</a>	PYRG_ECOLI
283	QQRDLARTEVTRVEICVAVGVKTYKSDSYASVIAKALEHSALAINHKLKIKYIDSADLEPITSQEEPVRYHEAWQKCSAHGVLVFGGFGVGRGTEGI	382	<a href="#">Q9VUL1</a>	PYRG_DROME
288	SKWKATTGFDNEMETKIALVGVKTYKSDSYASVIAKALEHSALAINHKLKIKYIDSADLEPITSQEEPVRYHEAWQKCSAHGVLVFGGFGVGRGTEGI	387	<a href="#">P28274</a>	URA7_YEAST
288	TNWNMTKNDSDSDVVKIALVGVKTYKSDSYASVIAKALEHSALAINHKLKIKYIDSADLEPITSQEEPVRYHEAWQKCSAHGVLVFGGFGVGRGTEGI	387	<a href="#">P38627</a>	URA8_YEAST
276	AEWSAMVDHVMNMLKVKYIALVGVKTYKSDSYASVIAKALEHSALAINHKLKIKYIDSADLEPITSQEEPVRYHEAWQKCSAHGVLVFGGFGVGRGTEGI	365	<a href="#">O87761</a>	PYRG_LAACLAM
285	SPWQEAERVLLKHPERTKIALVGVKTYKSDSYASVIAKALEHSALAINHKLKIKYIDSADLEPITSQEEPVRYHEAWQKCSAHGVLVFGGFGVGRGTEGI	374	<a href="#">Q5S1A8</a>	PYRG_THET8
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383	QAIWARNQKPKPFLGVLGMQLAVVFSRNVLWGDANSTEFDPDT-----SHPVVVDMPEH-----NPGQMGTMRLGKRRTLFQTK--NSVMR	465	<a href="#">P17812</a>	PYRG1_HUMAN
383	QAIWARNQKPKPFLGVLGMQLAVVFSRNVLWGDANSTEFDPDT-----PVPLVIDMPEH-----NPNLGGTMRLGKRRTLFQTK--NSILR	465	<a href="#">Q9NRF8</a>	PYRG2_HUMAN
363	TTARFARENNIPYVLGICLGMQVALIDYARHVNMMENANSTEFVDPD-----KYPVVALITFWRDENGNEVRSSEKSDLGTMRLGQCCQLVDD--SLVR	455	<a href="#">P0A7E5</a>	PYRG_ECOLI
383	RACQWARENQKPKPFLGVLGMQLAVVFSRNVLWGDANSTEFDPDT-----ANALVIDMPEH-----HTQQLGTMRLGKRRTLFQTK--PSVIR	465	<a href="#">Q9VUL1</a>	PYRG_DROME
388	LAARWARENHIPPFLGVLGMQVALIDYARHVNMMENANSTEFVDPD-----IDKHNHVVFMPEI-----DKETMGSMRLGLRPTFPQNETEWSQIK	474	<a href="#">P28274</a>	URA7_YEAST
388	LAARWARENHIPPFLGVLGMQVALIDYARHVNMMENANSTEFVDPD-----DKEHMGTMRLGKRRTLFQTK--PQNSWENIR	476	<a href="#">P38627</a>	URA8_YEAST
366	AAIKYARENVDVPMGLGICLGMQVALIDYARHVNMMENANSTEFVDPD-----KYPVIDIMRQV-----DVEDMGTMRLGKRLGKRTLFQTK--SRAK	448	<a href="#">O87761</a>	PYRG_LAACLAM
375	RAAQYAREKIPYVLGICLGMQVALIDYARHVNMMENANSTEFDPDT-----PHPVIDIMPEQL-----EVEGLGTMRLGKRWPMRIFKPG--TLRH	457	<a href="#">Q5S1A8</a>	PYRG_THET8
	:*: :* :*			
466	KLYGDADYLEERHRRFVFNPNVWKKCLEEQGLKFGVQ-----DVEGERMEI VELEDHPFFVGVQYHPEFLSRPIKPSPPYFGLLLASVGRLSHYLQKGR	560	<a href="#">P17812</a>	PYRG1_HUMAN
466	KLYGDADYLEERHRRFVFNPNVWKKCLEEQGLKFGVQ-----DVGDRMEI IELANHPYFVGVQYHPEFLSRPIKPSPPYFGLLLASVGRLSHYLQKGR	560	<a href="#">Q9NRF8</a>	PYRG2_HUMAN
456	QLYNAPTIV-ERHRRHRYEVNPNMLLQIEDAGLRVAGR-----SGDDQVLEI I EVPNHPFVACQHPPEPTSTPRDGHPLFAGFVKAASEFQKQKAK----	545	<a href="#">P0A7E5</a>	PYRG_ECOLI
466	QLYGPKPSQERHRRHRYEVNPNVWKKCLEEQGLKFGVQ-----DVKTRMEI I ELSGHPYFVATQYHPEFLSRPIKPSPPYFGLLLASVGRLSHYLQKGR	560	<a href="#">Q9VUL1</a>	PYRG_DROME
475	KLYGDVSEVHERHRRHRYEVNPNVWKKCLEEQGLKFGVQ-----DDTKRCEI I ELKHPHYFVATQYHPEFLSRPIKPSPPYFGLLLASVGRLSHYLQKGR	569	<a href="#">P28274</a>	URA7_YEAST
477	KLYGEVNEVHERHRRHRYEVNPNVWKKCLEEQGLKFGVQ-----DETQRCCEI FELKHPHYFVATQYHPEFLSRPIKPSPPYFGLLLASVGRLSHYLQKGR	571	<a href="#">P38627</a>	URA8_YEAST
449	AAYNDAEVVQRHRRHRYEVNPNVWKKCLEEQGLKFGVQ-----SPDNRLVEI I VELSGLKFFVACQYHPEFLSRPIKPSPPYFGLLLASVGRLSHYLQKGR	535	<a href="#">O87761</a>	PYRG_LAACLAM
458	RLYGKEEVL-ERHRRHRYEVNPNVWKKCLEEQGLKFGVQ-----TPGMRGRGAGLVEAIELKDHPPFLGQLQSHPEKSRPMRSPPPYFGLLLASVGRLSHYLQKGR	550	<a href="#">Q5S1A8</a>	PYRG_THET8
	:*: :* :*			
561	LSPRDYSRSGSSSPDS-----EITELKFPNSINH-----	591	<a href="#">P17812</a>	PYRG1_HUMAN
561	LSSSDRYSDASDDSFSEP-----RIALELIS-----	586	<a href="#">Q9NRF8</a>	PYRG2_HUMAN
546	-----	545	<a href="#">P0A7E5</a>	PYRG_ECOLI
561	LSPRQLDASDDEEDSVVGLAGATKLSLSPKIPITPTNGISKSCNGSISTSDSEGACGVDPPTNGHK	627	<a href="#">Q9VUL1</a>	PYRG_DROME
570	LEAGENK-----FNF-----	579	<a href="#">P28274</a>	URA7_YEAST
572	SEGGENE-----	578	<a href="#">P38627</a>	URA8_YEAST
536	-----	535	<a href="#">O87761</a>	PYRG_LAACLAM
551	-----	550	<a href="#">Q5S1A8</a>	PYRG_THET8

A



B

