Supplementary Data

Distinct GDP/GTP Bound States of the Tandem G-Domains of EngA Regulate Ribosome Binding

Sushil Kumar Tomar, Neha Dhimole, Moon Chatterjee and Balaji Prakash



Fig.S1. Nucleotide binding to wt EngA (*E.coli*). Nucleotide binding was assayed by recording emission spectra (λ_{ex} 355 nm) upon incubating 8 μ M of *E.coli* EngA with 0.4 μ M fluorescent mant-nucleotides (mant-GDP or mant-XDP). Spectra corresponding to various nucleotide bound forms of the protein are indicated in different colors and numbers, Emission spectra indicate that wt EngA binds preferentially to mant-GDP.

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EngA	MRCLMIYKNEALNMVPVVALVGRPNVGKSTLFNRLTRTRDALVADFPGLTRDRKYGRAEI	60
YphC	MGKPVVAIVGRPNVGKSTIFNRIAGERISIVEDTPGVTRDRIYSBAEW	48
Der	MATVLIVGRPNVGKSTLFNKLVKKKAIVEDEEGVTRDPVQDTVEW	46
	110	
EngA	EGREFICIDTGG-IDGTEDGVETRMAEOSLLAIEEADVVLFMVDARAGLMPADEAIAKHL	119
YphC	LNYDFNLIDTGG-IDIGDEPFLAQIRQQAEIAMDEADVIIFMVNGREGVTAADEEVAKIL	107
Der	YGKTFKLVDTCGVFDNPQDIISQKMKEVTLNMIREADLVLFVVDGKRGITKEDESLADFL	106
	1	
Engl	DEPENDENT ANY THOUGH DE - DAVUE PYELCI CETY DTA ASHCOCULET, BUILT, DUME	177
YphC	YPTKE DVULAUNE LDNTEM - BANTYDFYSLGFGEDY DISCTHGLGLGDLLDAV	159
Der	RKSTVDTILVANKAENLREFEREVKPELYSLGFGEPIPVSAEHNINLDTMLETIIK	162
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Enga	DLAPOEEVDEDAEIWAQPEAEENGEEEEEBDDFDPOELPIKLAIVGEVOKSTLINKILG	237
Der		202
Der		
EngA	EERVVVYDMPGTTRDSIYIPMERDGREYVLIDTAGVRKRGKIT-DAVEKPSVIKTLQAIE	296
YphC	EERVIVSNVAGTTRDAVDTSFTVNQQEFVIVDTAGMRKKGKVY-ETTEKYSVLRALKAID	256
Der	KERALVSPIPGTTRDPVDDEVFIDGRKYVPVDTAGLRRKSRVEPRTVEKYBNYRVVDSIE	262
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Engl		354
YphC	ESEVVAVVLDGEEGIIEODKEIAGVAHEAGKAVVIVVNKWDAVDKDESTMKEFEENIEDH	316
Der	KADVVVIVLDATOGITRODORMAGLMERRGRASVVVPNKWDLVVHREKRYDEPTKLPREK	322
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EngA	LGFIDFARVHFISALHGSGVGNLFESVREAYDSSTRRVGTSMLTRIMTMAVEDHQPPLVR	414
YphC	POPLDYAPILFMSALTKKRIHTLMPAIIKASENHSLRVQTNVLNDVIMDAVAMNPTPTHN	376
Der	LYFIDYSPLIPTSADKGWNIDRMIDAMNLAYASYTTKVPSSAINSALQKVLAFTNLP	379
EngA	GREVELKVAHAGGVNPPIVVIHGNOVKDLPDSVKRVLMNVPRK-SLDVMGSPIRIOPKEG	473
YphC	GSRLKIYYATOVSVKPPSFVVFVNDPELMHFSYERFLENRIRD-AFGFEGTPIKIFAR	433
Der	-RGLKIFFGVQVDIKPPTFLFFVNSIEKVKNPQKIFLRKLIRDYVPPFEGSPIFLKFK	436
EngA	ENPYANKENTLTPTOMEKEKELMKHIKKNK 503	
YphC	ARK 436	
Der	RSR 439	

Fig.S2. Multiple sequence alignment of EngA (*E. coli*), YphC (*B. subtilis*) and Der (*T. maritima*). Sequences were aligned using Clustalx (1). The residues mutated in this study are indicated by arrows. Residues are 1 - 207 and 217 - 385 constitute the domains GD1 and GD2 of EngA.

References:

1. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.*, **25**, 4876-4882.