Three critical regions of the erythromycin resistance methyltransferase, ErmE, are required for function supporting a model for the interaction of Erm family enzymes with substrate rRNA

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Supporting Information

Table S1. Oligonucleotides used in this study.

Name	Use	F/R	Sequence
prRJM005	*ermE* Y134A	F	GCGCTGGTAATCCCGGCGGGAATCGCACCCAC
prRJM006	*ermE* Y134A	R	GTGGGTGCGATTCCCGCCGGGATTACCAGCGC
prJBH101	*ermE* E160A	F	GTTTGCGAGCAAACGCCAGCTGCGTCACC
prJBH102	*ermE* E160A	R	GGTGACGCAGCTGGCGTTTGCTCGCAAAC
prJBH103	*ermE* E160Y	F	GTGCGTTTGCGAGCAAAATACAGCTGCGTCACCATGG
prJBH104	*ermE* E160Y	R	CCATGGTGACGCAGCTGTATTTTGCTCGCAAACGCAC
prJBH105	*ermE* E160W	F	GCGTTTGCGAGCAAACCACAGCTGCGTCACCATG
prJBH106	*ermE* E160W	R	CATGGTGACGCAGCTGTGGTTTGCTCGCAAACGC
prRCT310	*ermE* K164A	F	ATAGTCACCCGTGCGTGCGCGAGCAAACTCCAGC
prRCT311	*ermE* K164A	R	GCTGGAGTTTGCTCGCGCACGCACGGGTGACTAT
prJAD308	*ermE* R171A*	F	GTGACTATGGTNNKTGGAGTCGCCTTACGGTCATGACCTGGCC
prJAD309	*ermE* R171A*	R	GTAAGGCGACTCCAMNNACCATAGTCACCCGTGCGTTTGCGAGC
prJAD348	*ermE* F196A QC	F	TTCGGGACGGGTTTAGCGAGGCGCCGATCCAC
prJAD349	*ermE* F196A QC	R	GTGGATCGGCGCCTCGCTAAACCCGTCCCGAA
prRES009	*ermE* F196Y QC	F	CGGGACGGGTTTATAGAGGCGCCGATC
prRES010	*ermE* F196Y QC	R	GATCGGCGCCTCTATAAACCCGTCCCG
prJAD350	*ermE* P198A QC	F	TTCGGGACGGCTTTAAAGAGGCGCCGATCC
prJAD351	*ermE* P198A QC	R	GGATCGGCGCCTCTTTAAAGCCGTCCCGAA
TAC_S138_F	*ermE* S138A QC	F	CAGTCCACAATCGCGGCGGTAATCCCGTAGGG
TAC_S138_R	*ermE* S138A QC	R	CCCTACGGGATTACCGCCGCGATTGTGGACTG
prRES001	*ermE* S138V QC	F	CCAGTCCACAATCGCGACGGTAATCCCGTAGGGA
prRES002	*ermE* S138V QC	R	TCCCTACGGGATTACCGTCGCGATTGTGGACTGG
prJAD342	*ermE* R165A QC	F	ATAGTCACCCGTGGCTTTGCGAGCAAACTCCAGC
prJAD343	*ermE* R165A QC	R	GCTGGAGTTTGCTCGCAAAGCCACGGGTGACTAT
prJAD344	*ermE* R165E QC	F	ATAGTCACCCGTCTCTTTGCGAGCAAACTCCAGCTGCG
prJAD345	*ermE* R165E QC	R	CGCAGCTGGAGTTTGCTCGCAAAGAGACGGGTGACTAT
prJAD347	*ermE* S173A QC	R	TGACTATGGTCGGTGGGCTCGCCTTACGGTCATG
prRES007	*ermE* S173V QC	F	TCATGACCGTAAGGCGAACCCACCGACCATAGTCAC
prRES008	*ermE* S173V QC	R	GTGACTATGGTCGGTGGGTTCGCCTTACGGTCATGA
TAC_R163A_F	*ermE* R163A QC	F	TCACCCGTGCGTTTGGCAGCAAACTCCAGCTG
TAC_R163A_R	*ermE* R163A QC	R	CAGCTGGAGTTTGCTGCCAAACGCACGGGTGA
prRES106	*ermE* R163E IP	F	GAGAAACGCACGGGTGACTATG
prRES107	*ermE* R163E IP	R	AGCAAACTCCAGCTGCGTC
prRES003	*ermE* R163K QC	F	CATAGTCACCCGTGCGTTTCTTAGCAAACTCCAGCTGCGTC
prRES004	*ermE* R163K QC	R	GACGCAGCTGGAGTTTGCTAAGAAACGCACGGGTGACTATG
RM_K164_F	*ermE* K164A SSM	F	AGCTGGAGTTTGCTCGCNNKCGCACGGGTGACTATGG
V48	Erm substrate	N/A	GGCCUGCGGCAAGACGGAAAGACCAUGGUCCCUAUCUGCCGCAGGAUC

QC, QuikChange. IP, inverse PCR. SSM, site-saturation mutagenesis using QuickChange Multi. F/R forward or reverse primer.

**ermE* R171A was obtained as a clone during a site-saturation mutagenesis reaction of *ermE* R171 previously reported in DOI:https://doi.org/10.1074/jbc.RA120.014280.

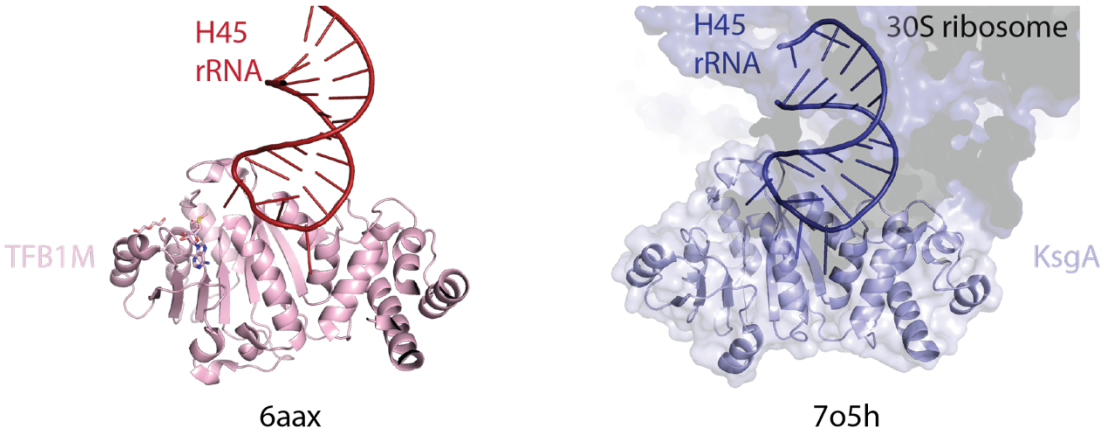
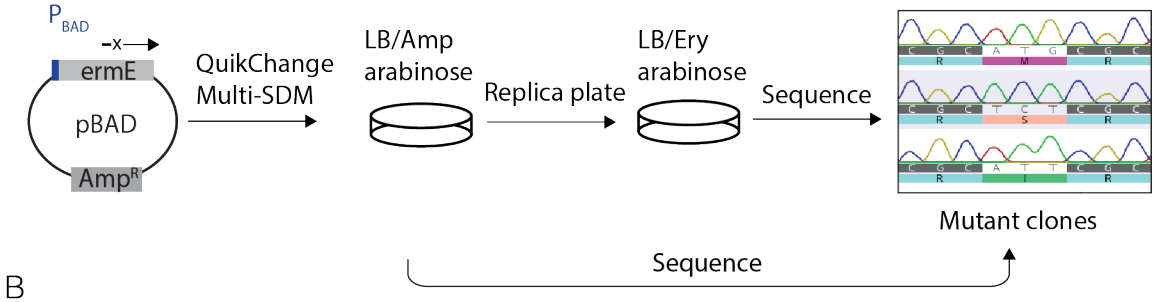


Figure S1. Available structures indicate ribosomal RNA adenine dimethylase (RRAD) family members TFB1M and KsgA bind helix 45 (H45) rRNA in a similar manner. The crystal structure given by pdb code 6aax is shown on the left. A model derived from the cryo-EM structure of KsgA (also known as RsmA) bound to the 30S ribosome is given by pdb code 7o5h. On the right the KsgA and H45 components of the structure are emphasized.

A



B

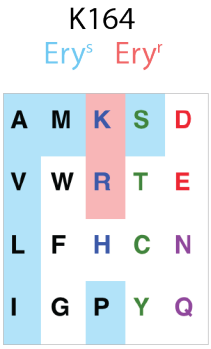


Figure S2. Site-saturation mutagenesis of ErmE K164. (A) pBAD-ermE was modified by a Quikchange Multi Site-Directed Mutagenesis reaction carried out with an oligo containing a degenerate codon (5' NNG/T 3') in the position of the K164 codon. The resulting transformants were characterized as erythromycin sensitive (Ery^s) or erythromycin resistant (Ery^r) based on their ability to grow on a LB/Ery plate. (B) A table depicts the correlation between the Ery resistance phenotype and the amino acid at position K164. Amino acids not highlighted in red or blue were not captured during mutagenesis.

Fig. S3. Sequence alignment of ErmC and ErmE. An alignment of S. erythraea ErmE (Uniprot code P07287) is shown aligned with *B. subtilis* ErmC (P13956). The sequence of ErmE is shown from the N-terminus up to R299 after which a low complexity, Gly-rich tail occurs.