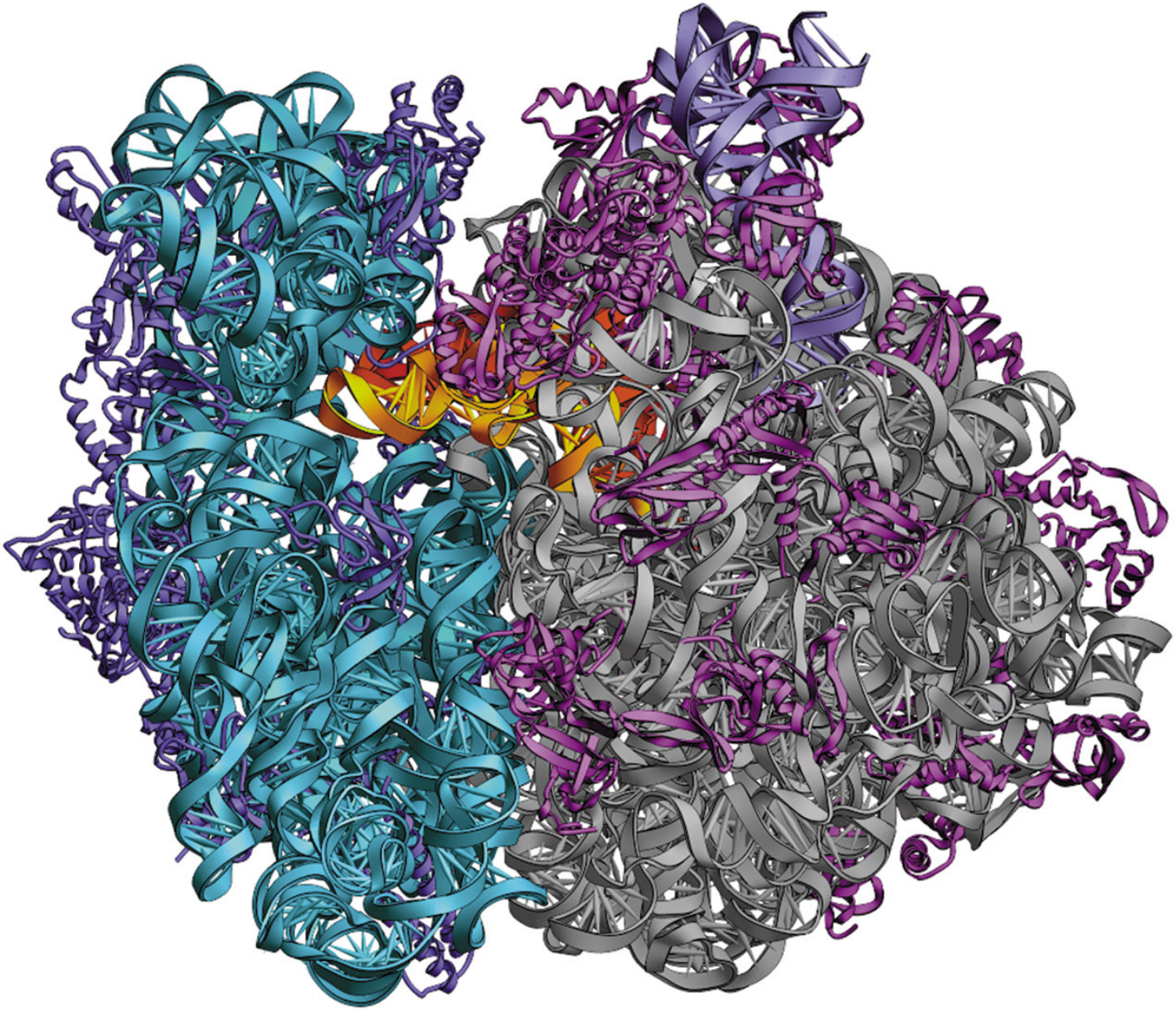
Рибосома как сенсор синтезируемого пептида и малых молекул

Александр Манькин

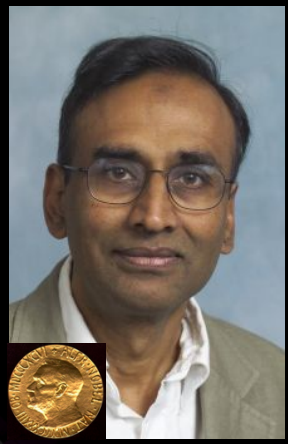
Center for Pharmaceutical Biotechnology
University of Illinois at Chicago

The ribosome is an amazing RNA machine



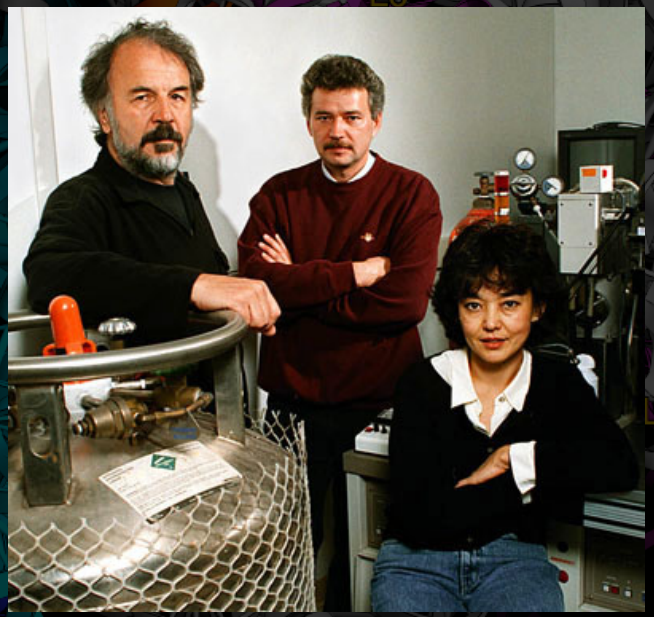
Ada Yonath

Tom Steitz

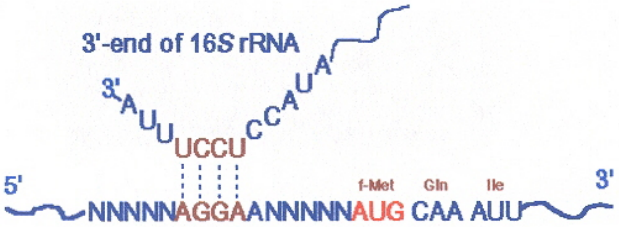


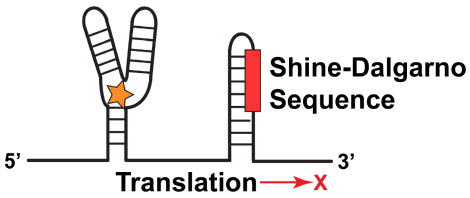
Venki Ramakrishnan

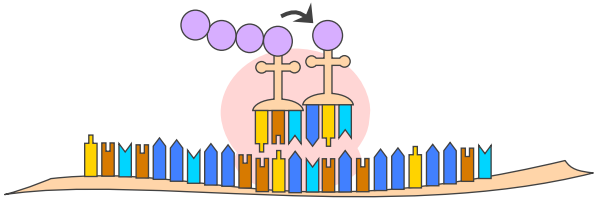
Alexander Spirin

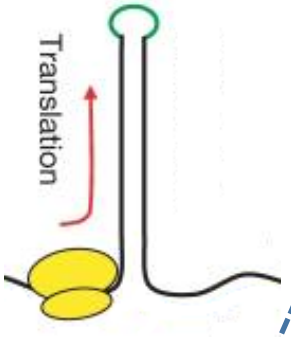


Harry Noller, Marat Yussupov, Gulnara Yussupova



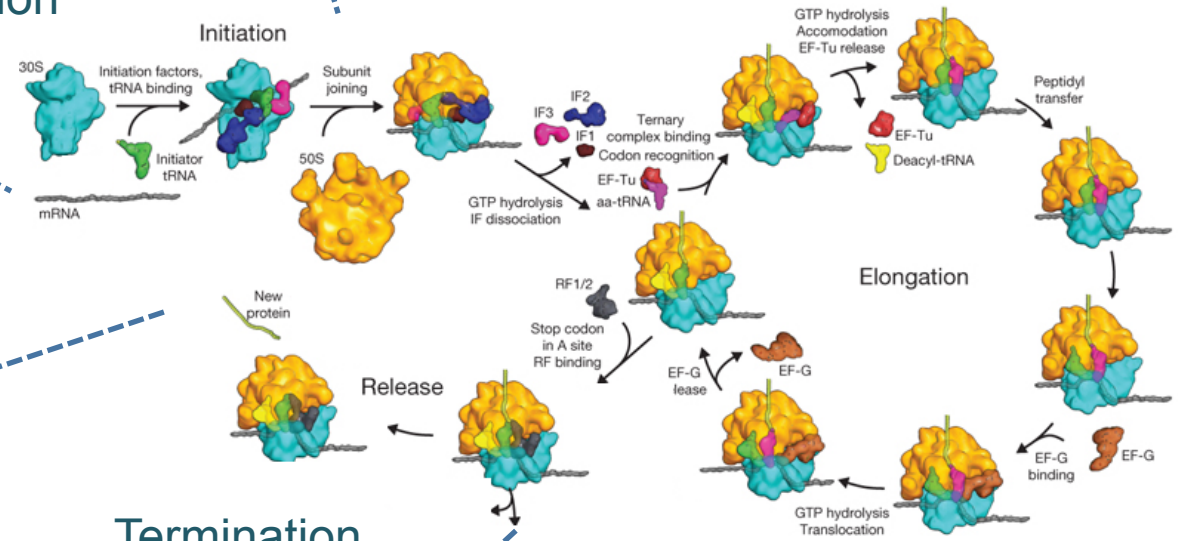




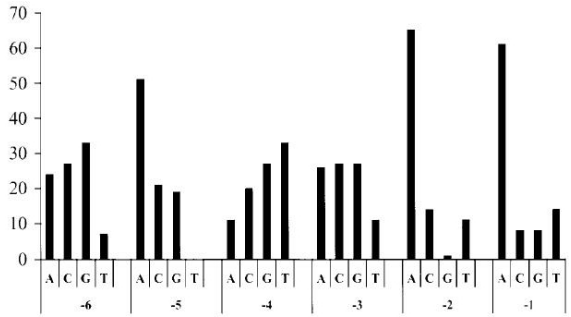


Elongation

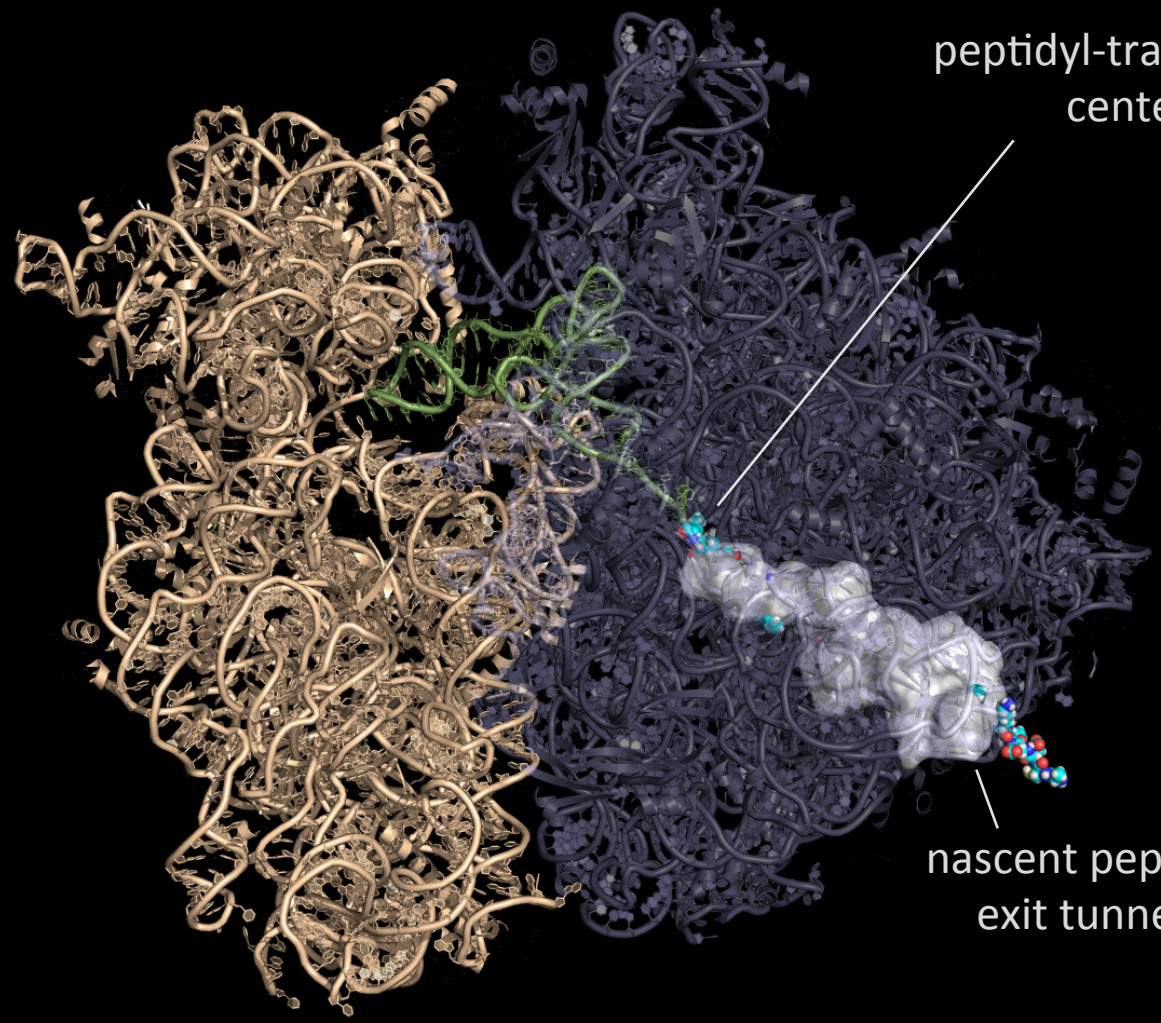
Initiation



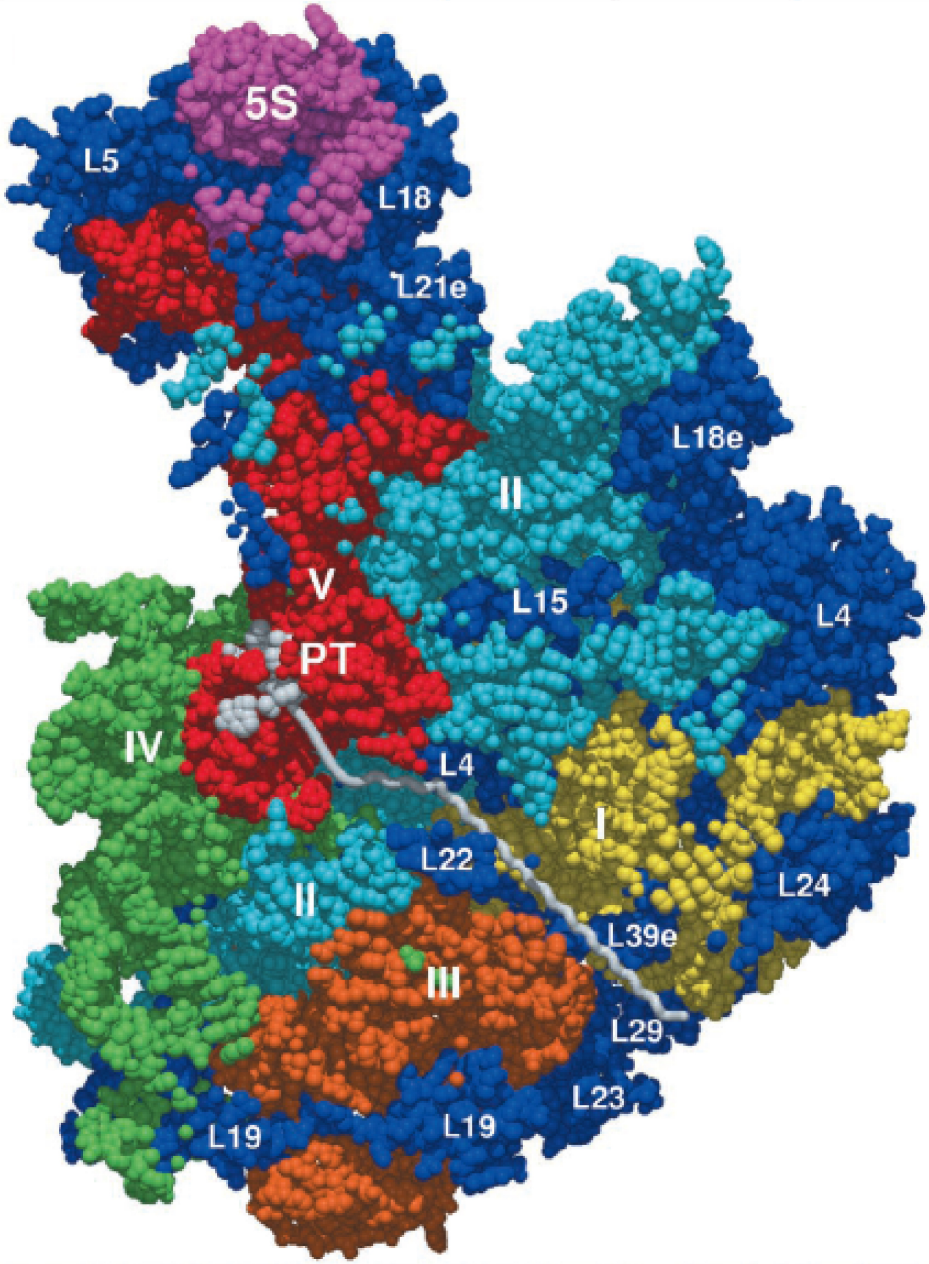
Termination



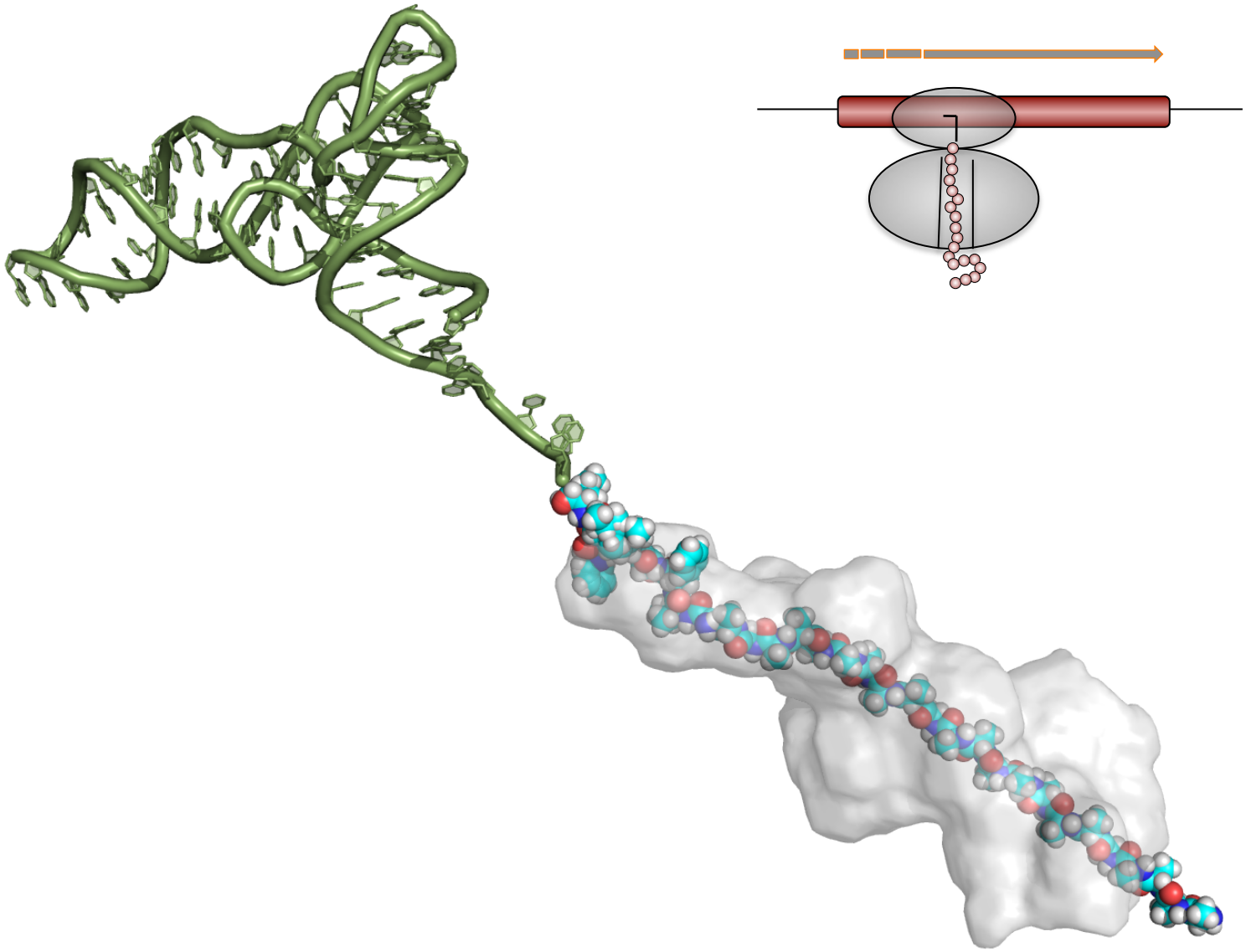
Schmeing & Ramakrishnan (2009) Nature, 461, 1234



Nissen et al., (2000) Science, 289, 920; Voorhees et al., (2009) Nat.Struct.Mol.Biol., 16, 528; Voss et al., (2006) J Mol Biol, 360, 893

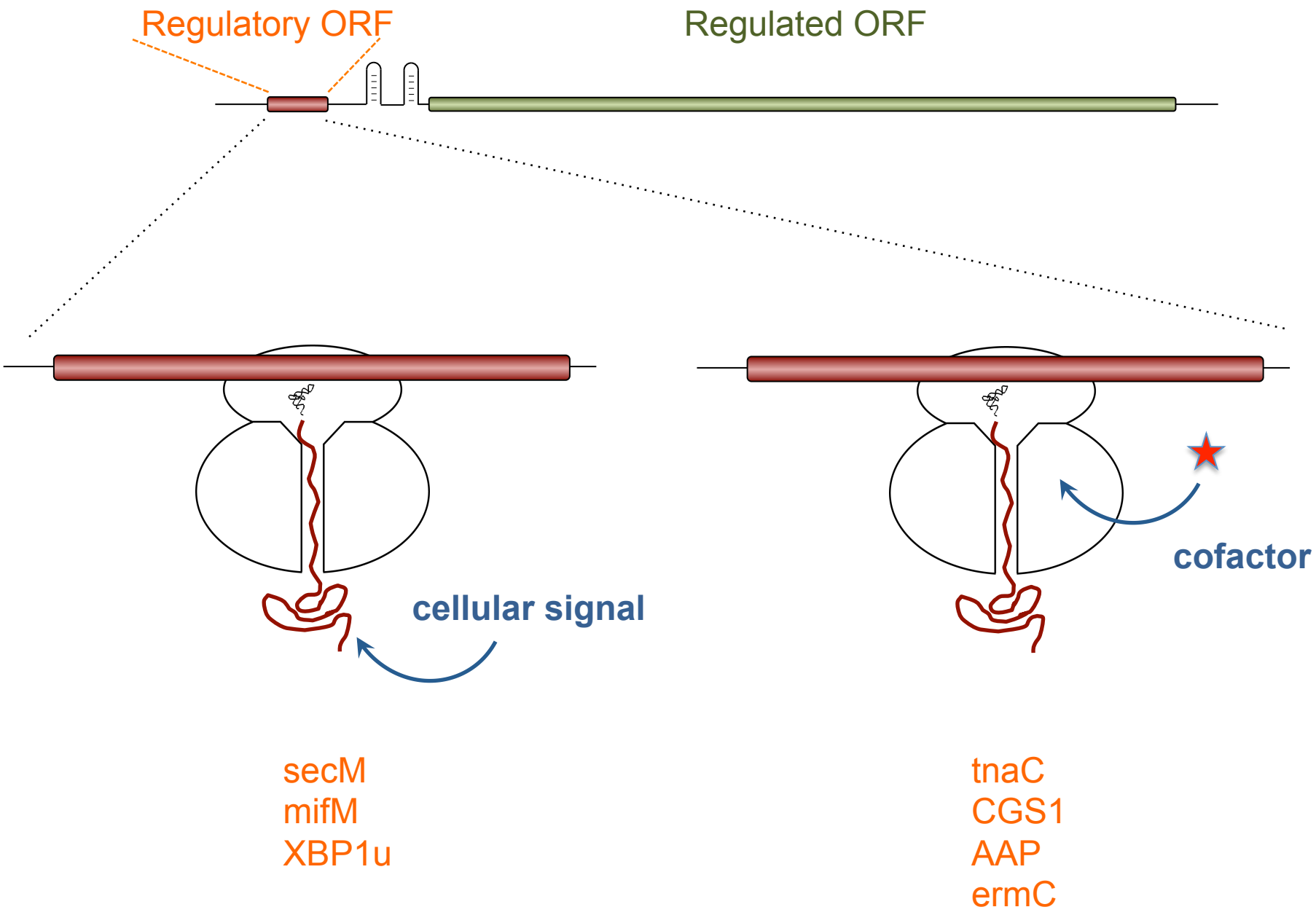


Nissen et al., 2000 Science 289,920



Nakatogawa & Ito, (2002) Cell, 108,629; Gong & Yanofsky, (2002) Science, 297, 1864; Chiba et al., (2009) EMBO J. 28, 3461; Onouchi et al.(2005) Genes Dev. 15, 1799

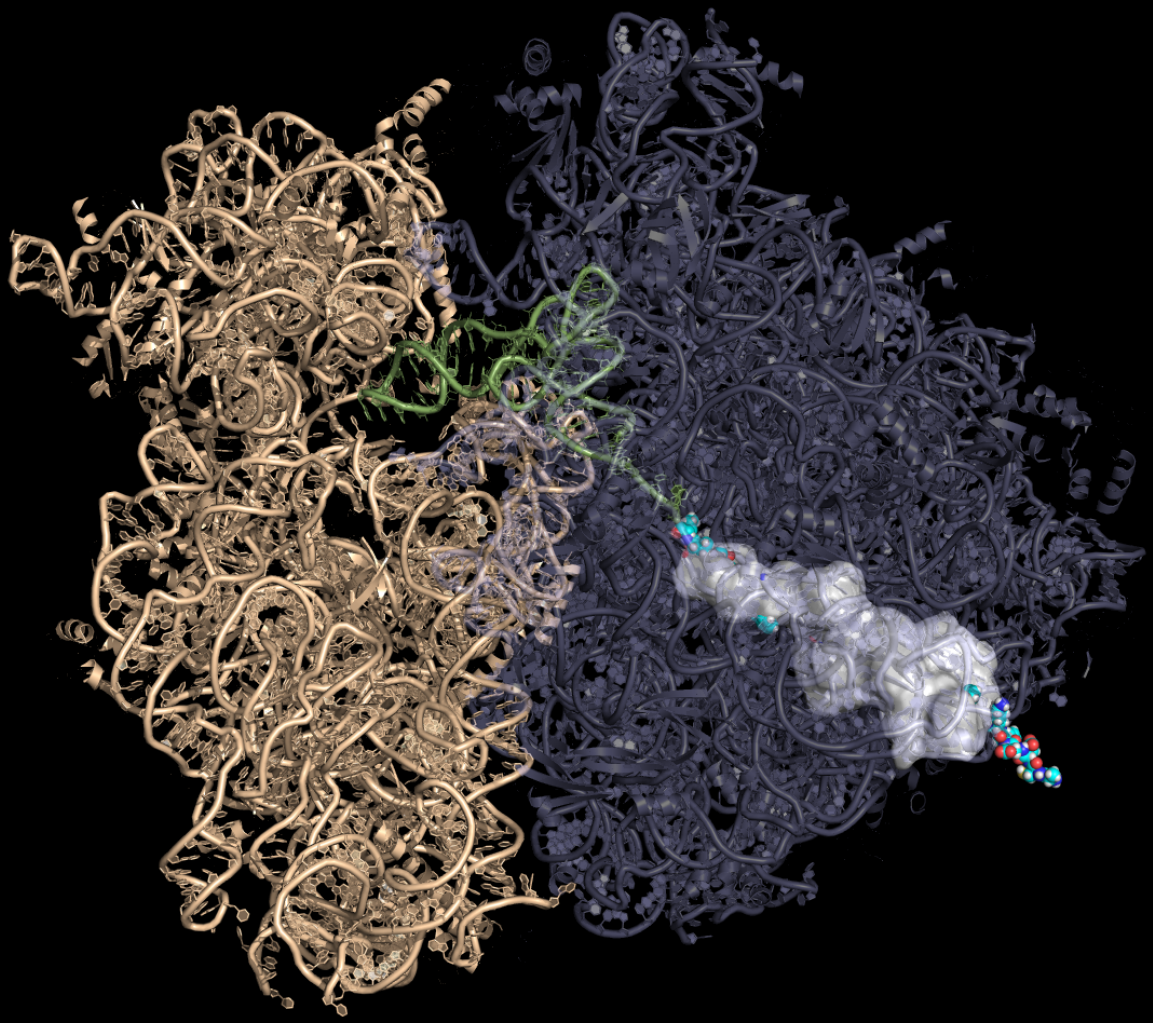
Ribosome stalling controls expression of important bacterial and eukaryotic genes



secM
mifM
XBP1u

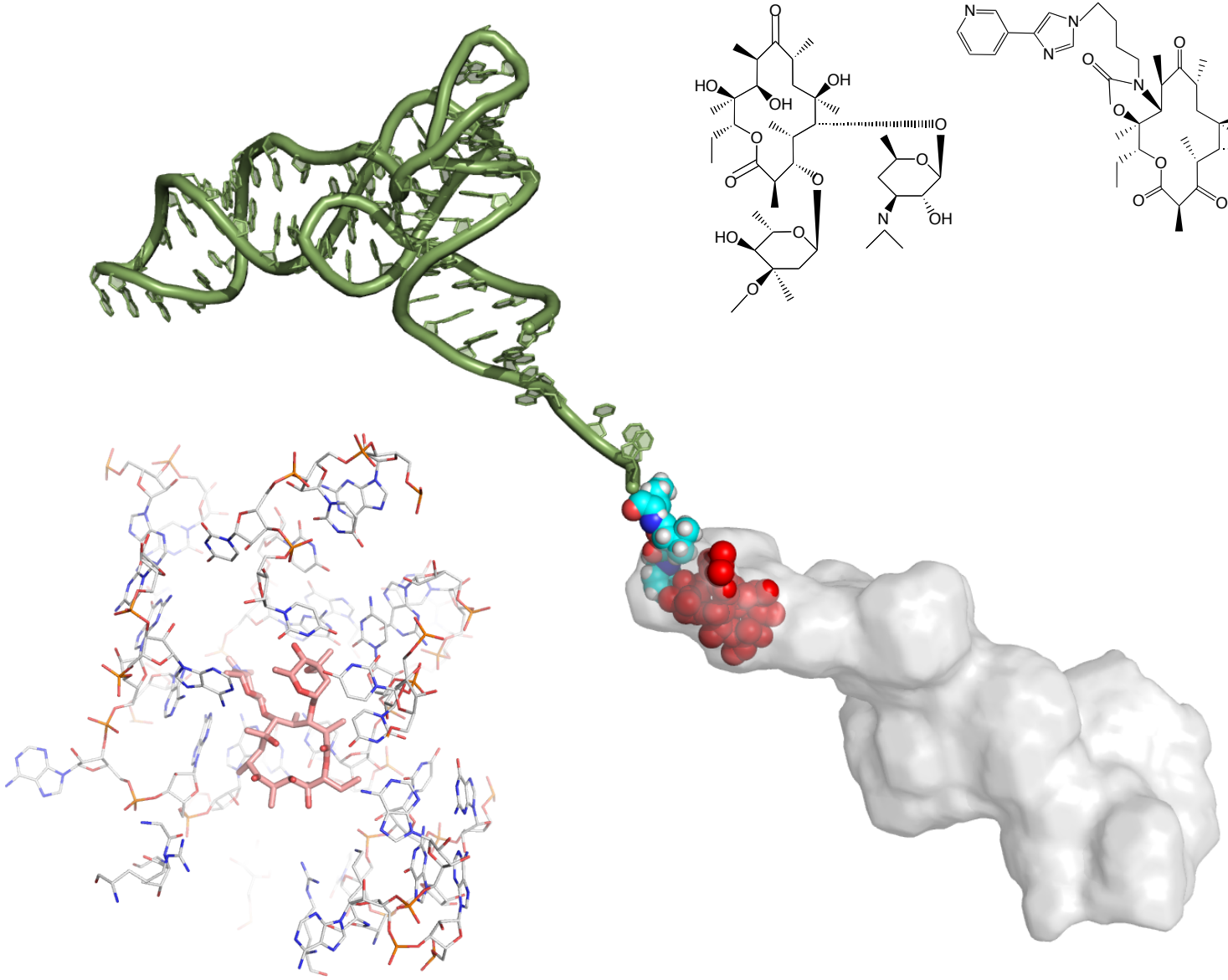
tnaC
CGS1
AAP
ermC

Nakatogawa & Ito, (2002) Cell, 108,629; Gong & Yanofsky, (2002) Science, 297, 1864; Chiba et al., (2009) EMBO J. 28, 3461; Onouchi et al.(2005) Genes Dev. 15, 1799

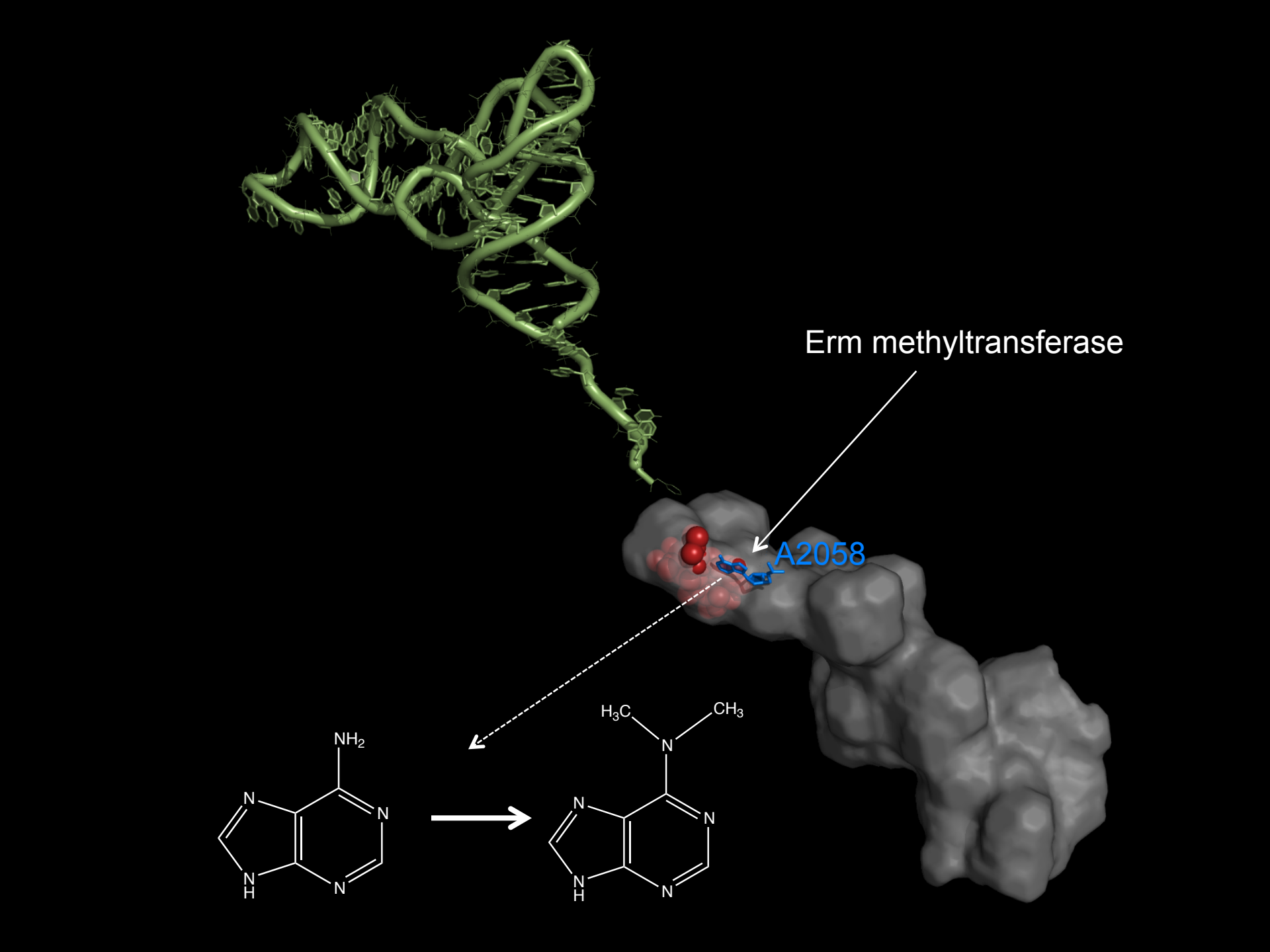


Nissen et al., (2000) Science, 289, 920; Voorhees et al., (2009) Nat.Struct.Mol.Biol., 16, 528; Voss et al., (2006) J Mol Biol, 360, 893

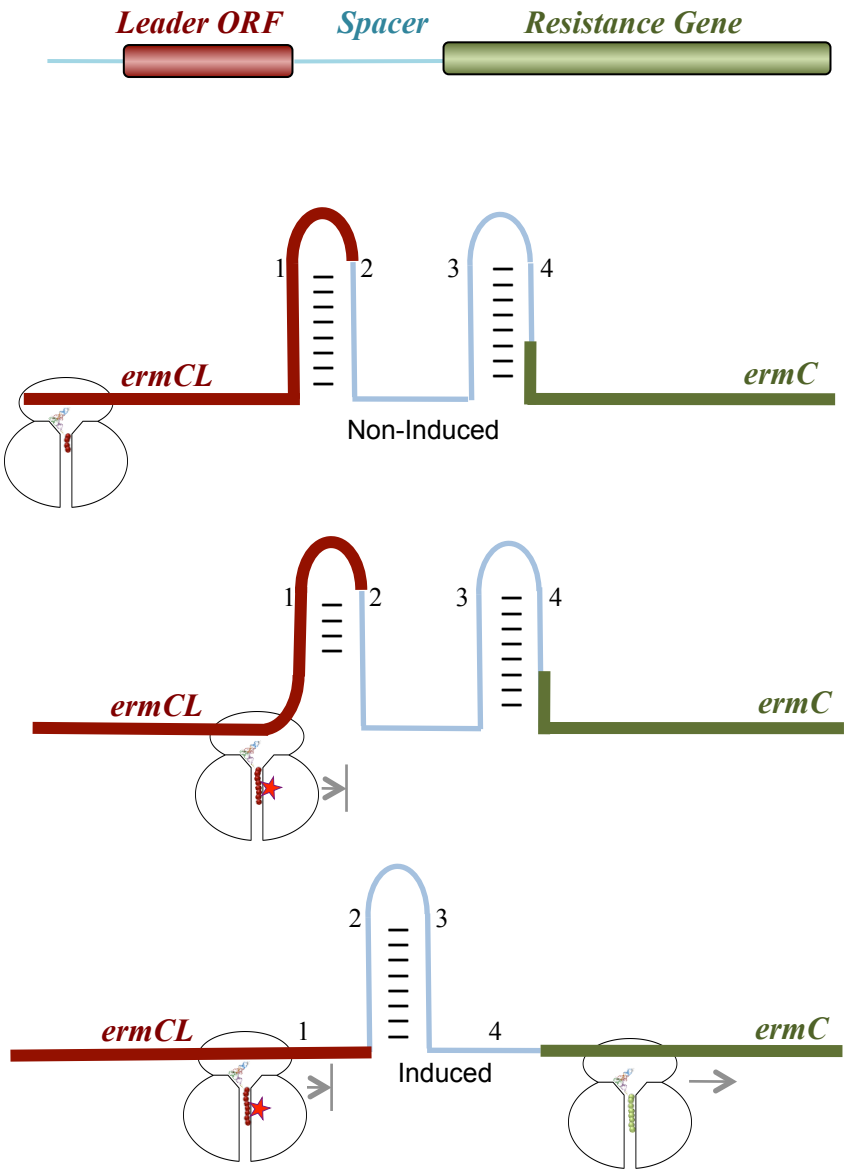
Erythromycin (ERY) Ketolides:Telithromycin (TEL)



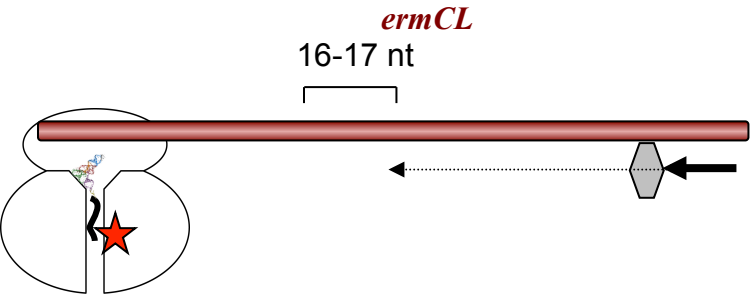
Schlunzen, (2001) Nature, 413,814; Tu et al., (2005) Cell, 121, 257; Bukley et al., (2010). PNAS 107, 17158; Dunkle et al.(2010) PNAS 107, 17152

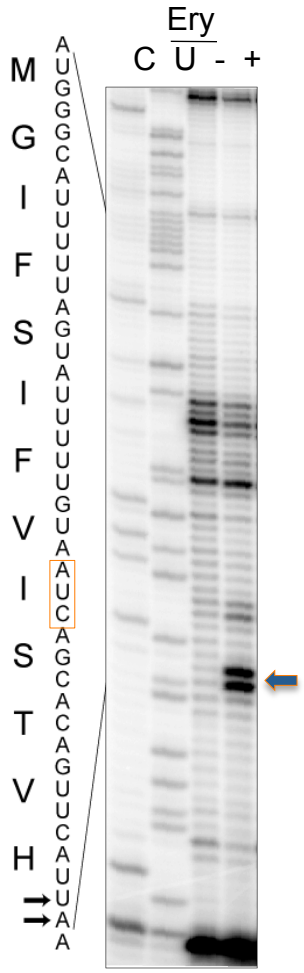

Erm methyltransferase
A2058
NH2
N
N
N
N
H
H3C
CH3
N
N
N
N
N
N
H

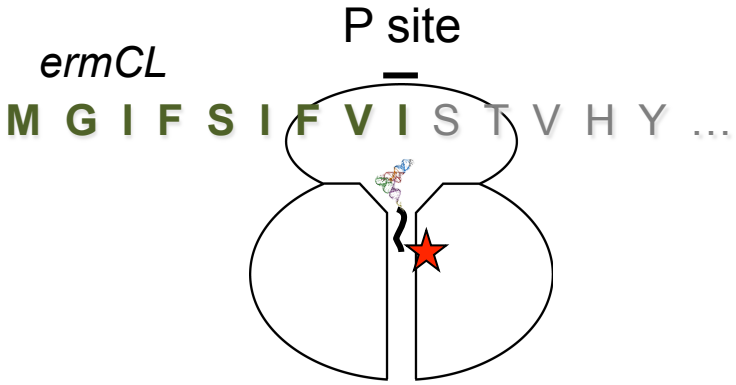
Translation attenuation controls the expression of *erm* genes



Horinouchi & Weisblum (1980) PNAS 77, 7079; Gryczan et al.(1980) Nucl Acids Res 8, 6081

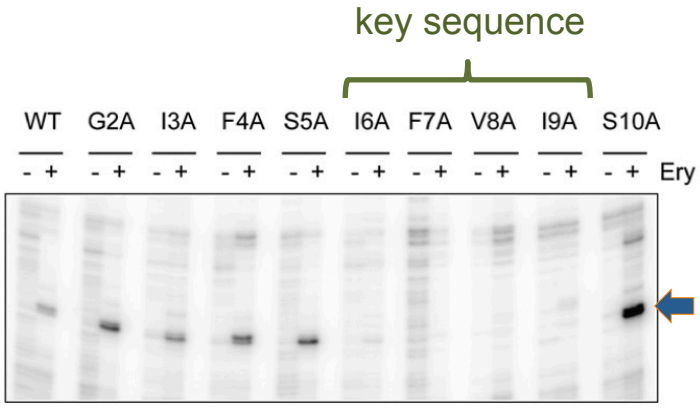


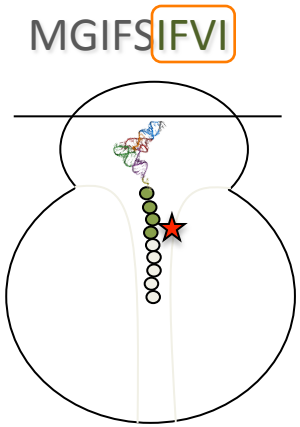




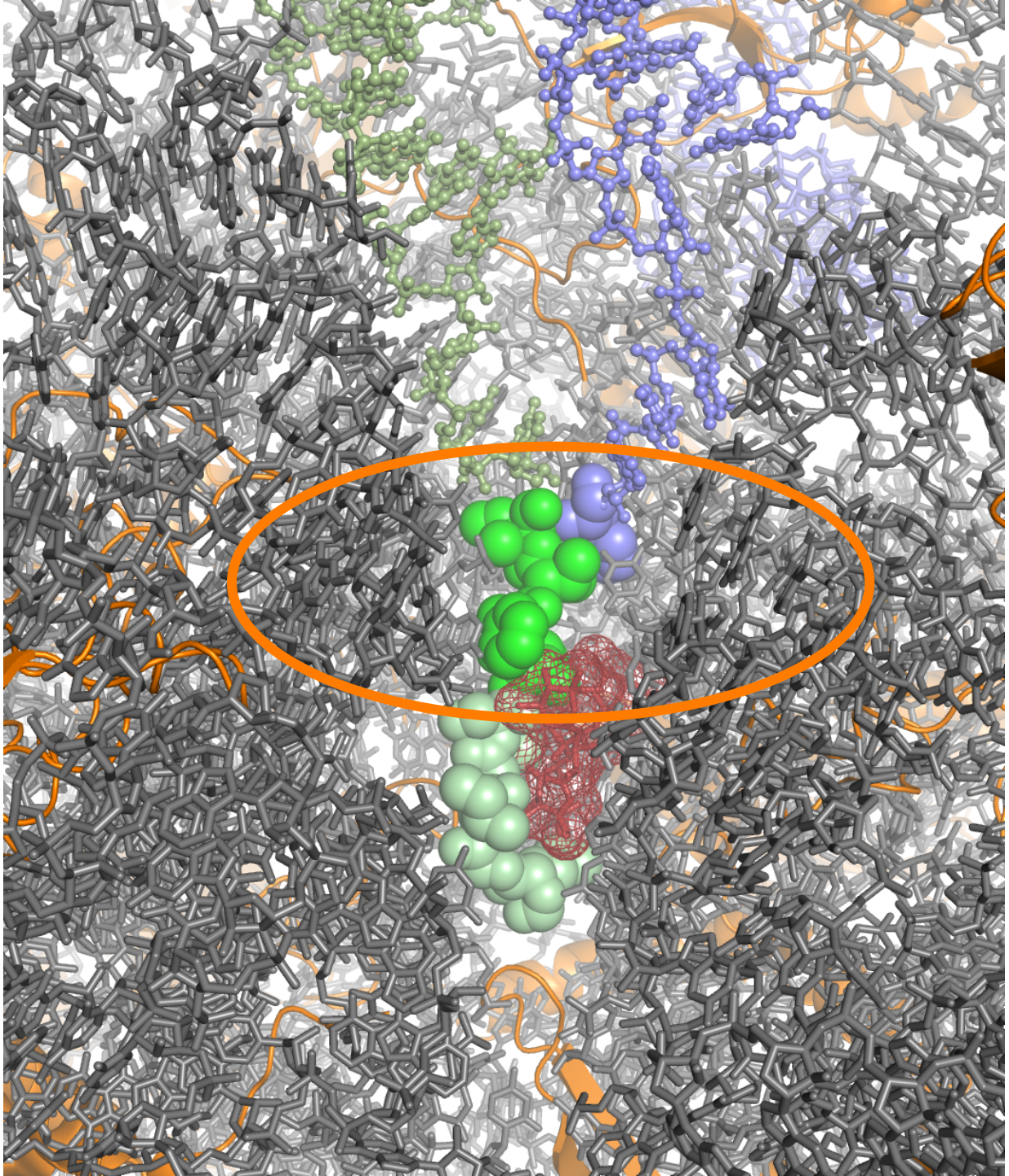
Vazquez-Laslop, et al. (2008) Mol. Cell 30, 190

Specific sequence of the nascent peptide controls stalling

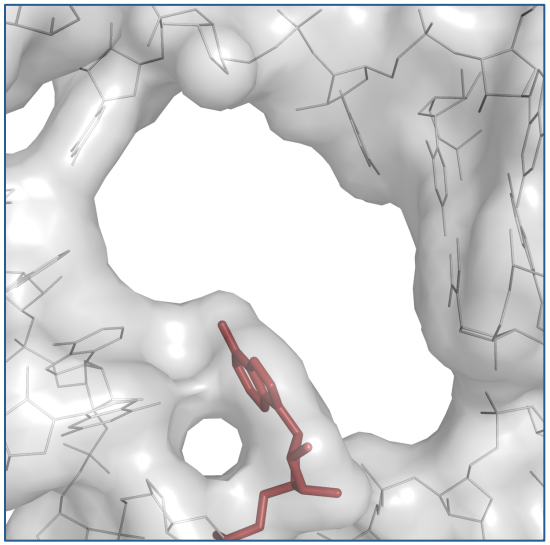


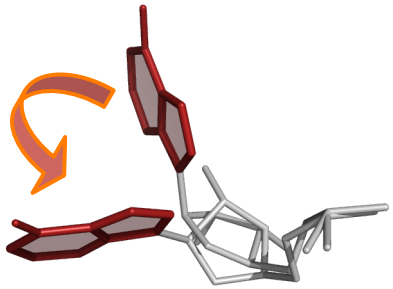


Vazquez-Laslop, et al. (2008) Mol. Cell 30, 190; Ramu et al. (2011) Mol. Cell 41, 321



tunnel

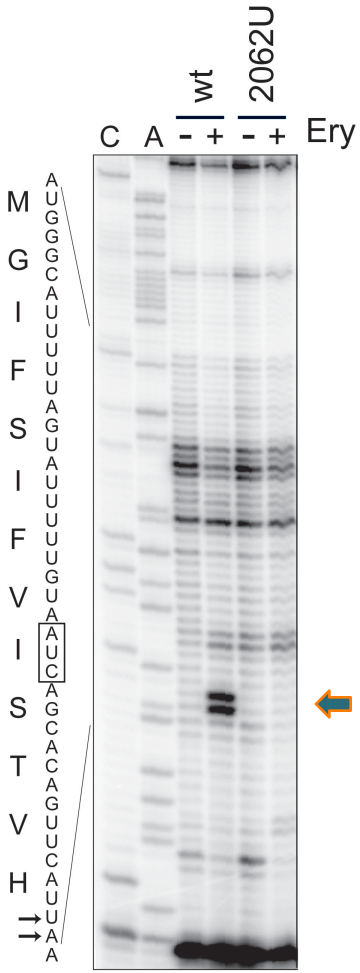




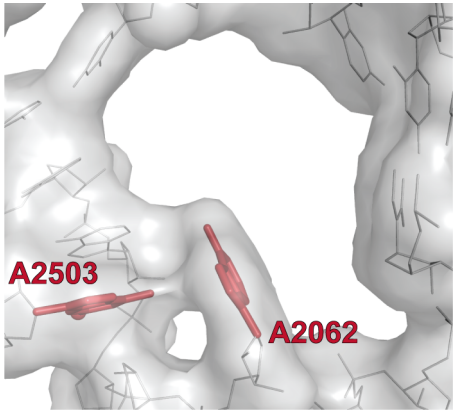
A2062

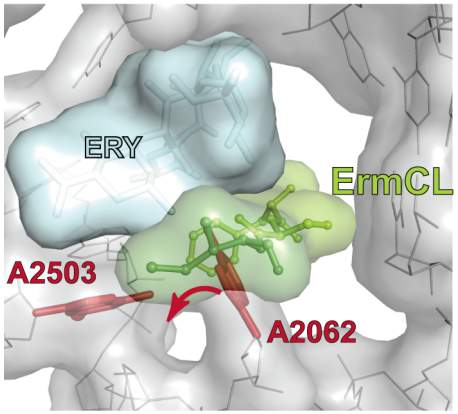
the most flexible residue in the tunnel

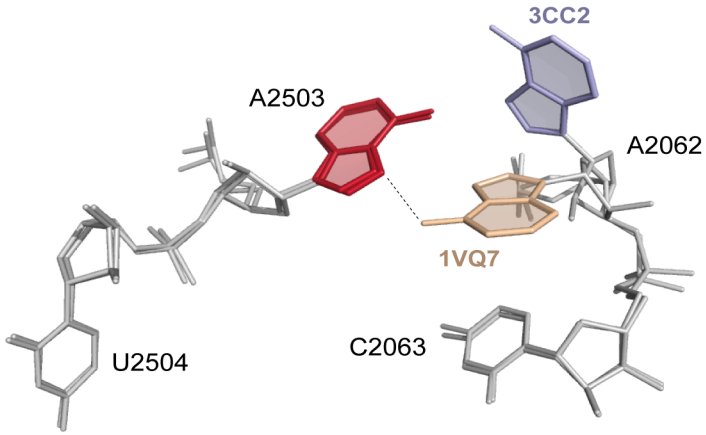
The mutant ribosomes are functionally active, but do not ‘recognize’ the nascent peptide!

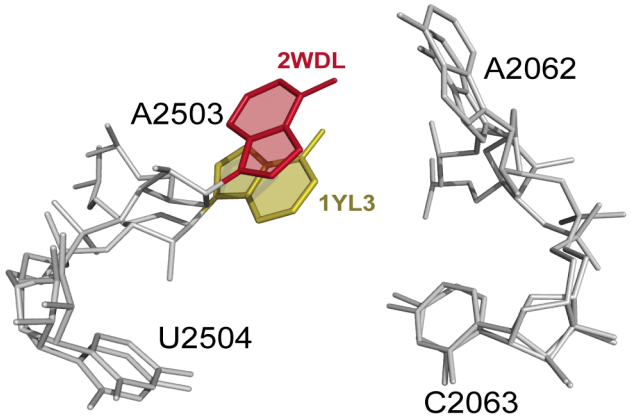


Vazquez-Laslop, et al. (2008) Mol. Cell 30, 190; Vazquez-Laslop, et al. (2010) EMBO J. 29, 3108



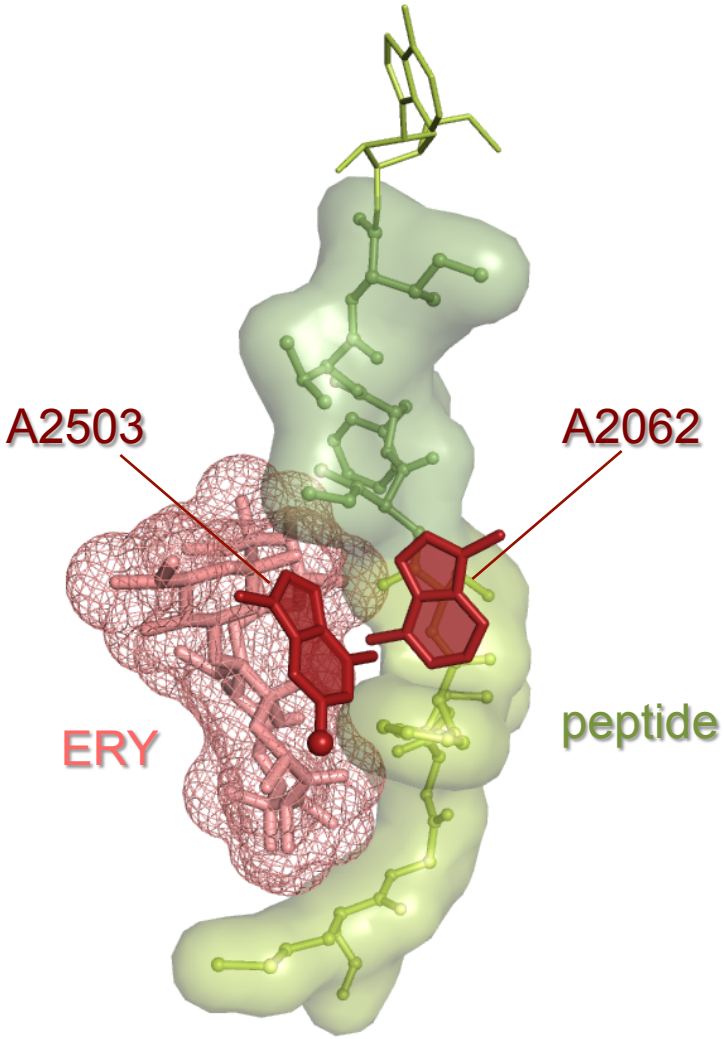




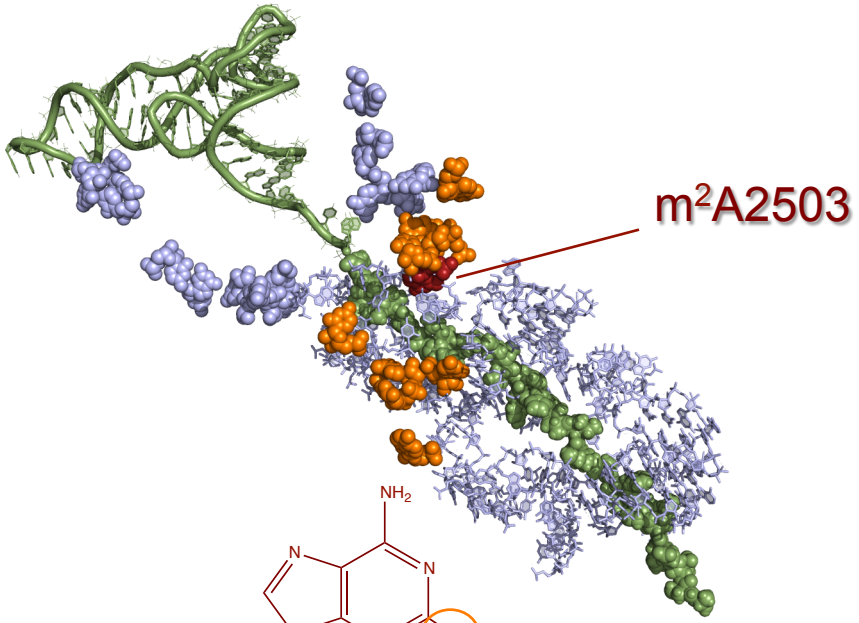


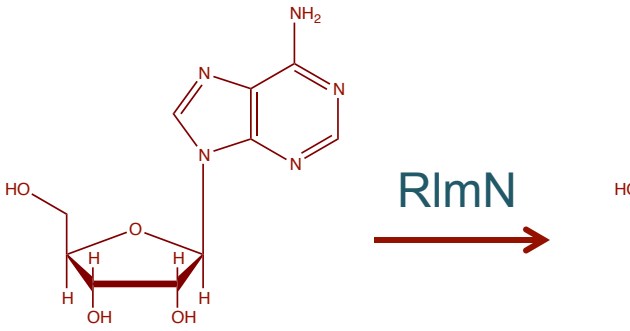
Voorhees et al., (2009) NSMB, 16, 528; Scheming et al. (2005) Nature, 438, 520;

A2062 and A2503 are sensors of the stalling signal in the tunnel

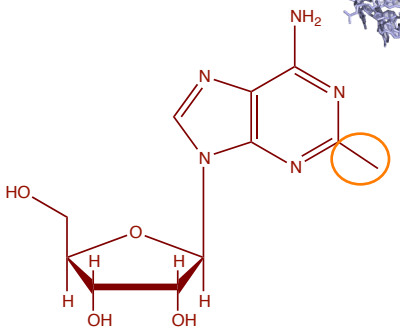


Modified nucleotides in 23S rRNA



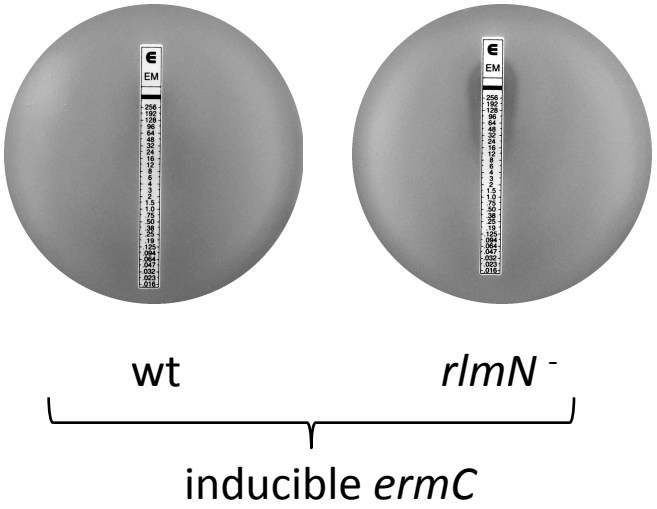


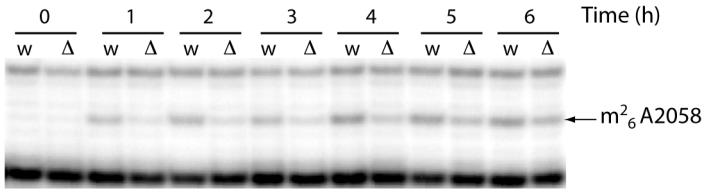
RlmN

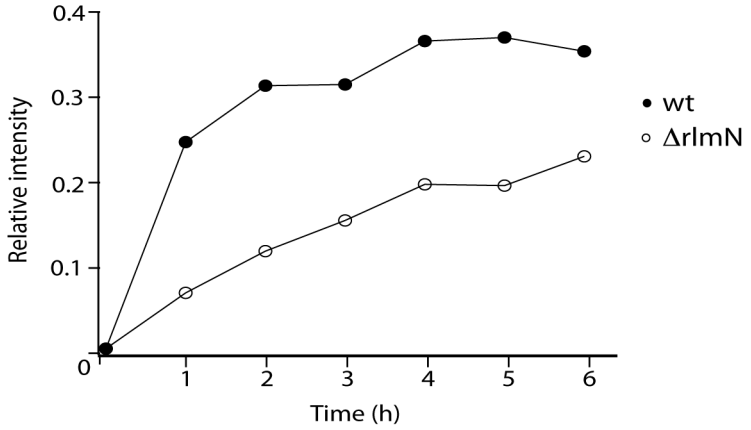


Toh et al. (2008) RNA, 14, 98

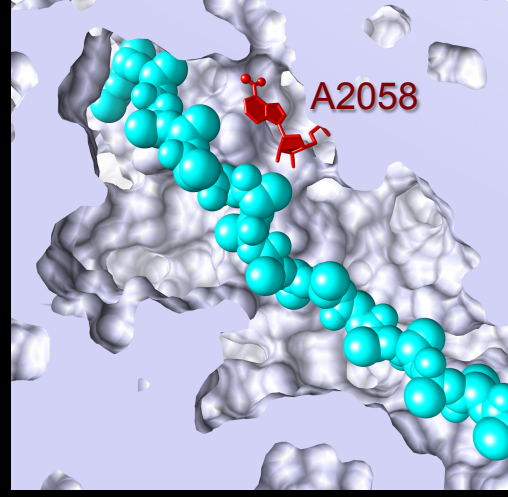
Posttranscriptional modification of the rRNA sensor contributes to the recognition of the stalling signal





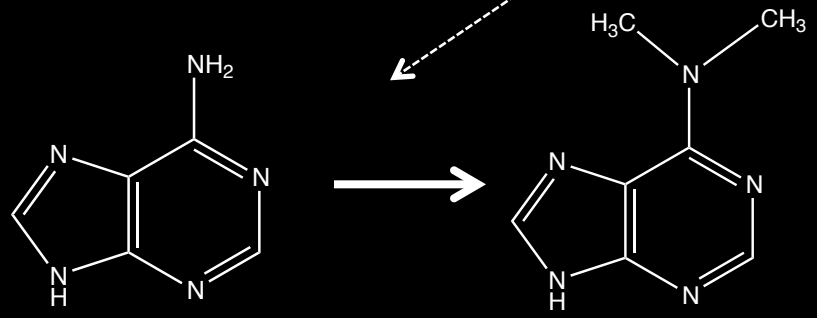


Vazquez-Laslop, et al. (2010) EMBO J. 29, 3108

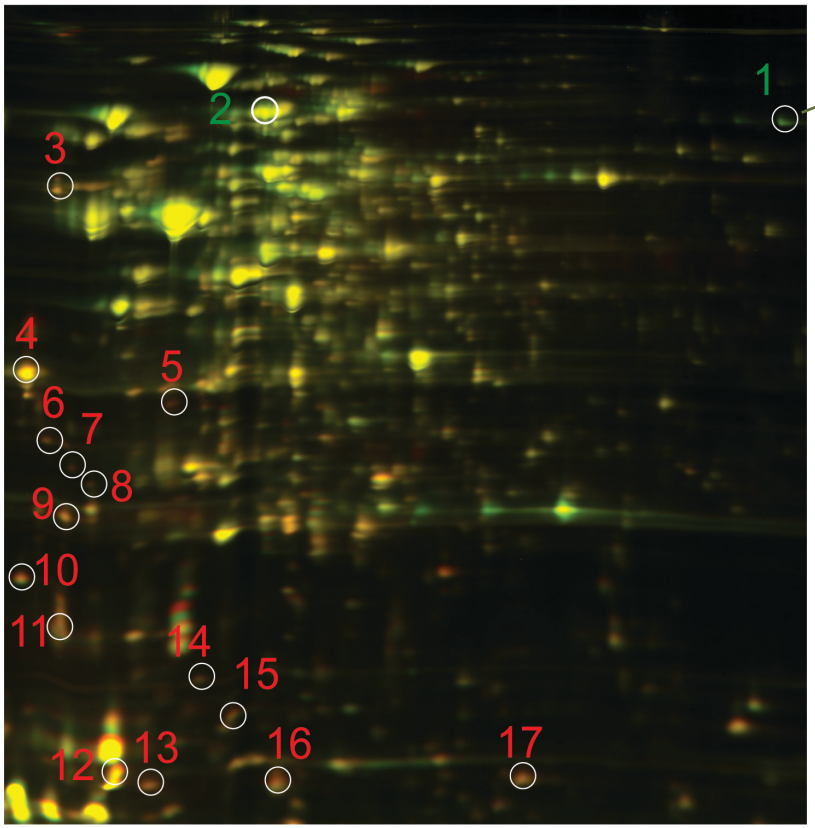


Erm methyltransferase

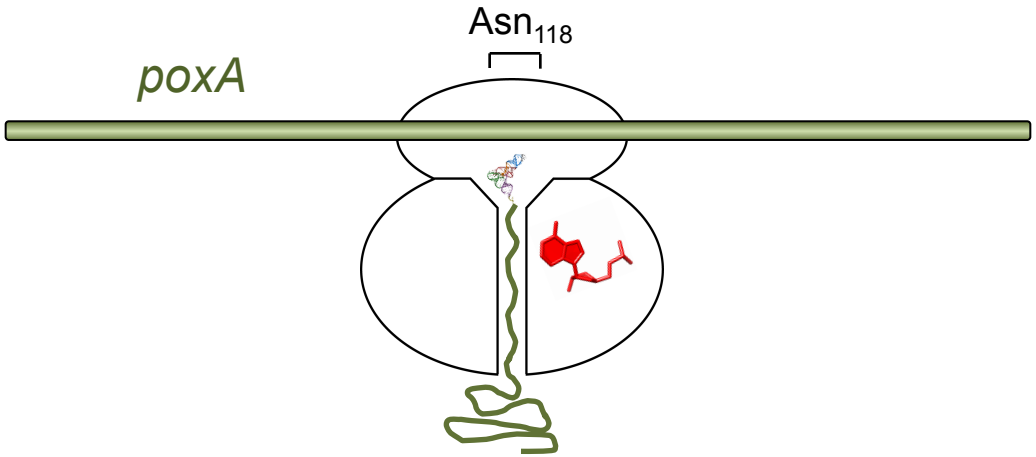
A2058



Modification of A2058 in the tunnel affects translation of several proteins and reduces cell fitness



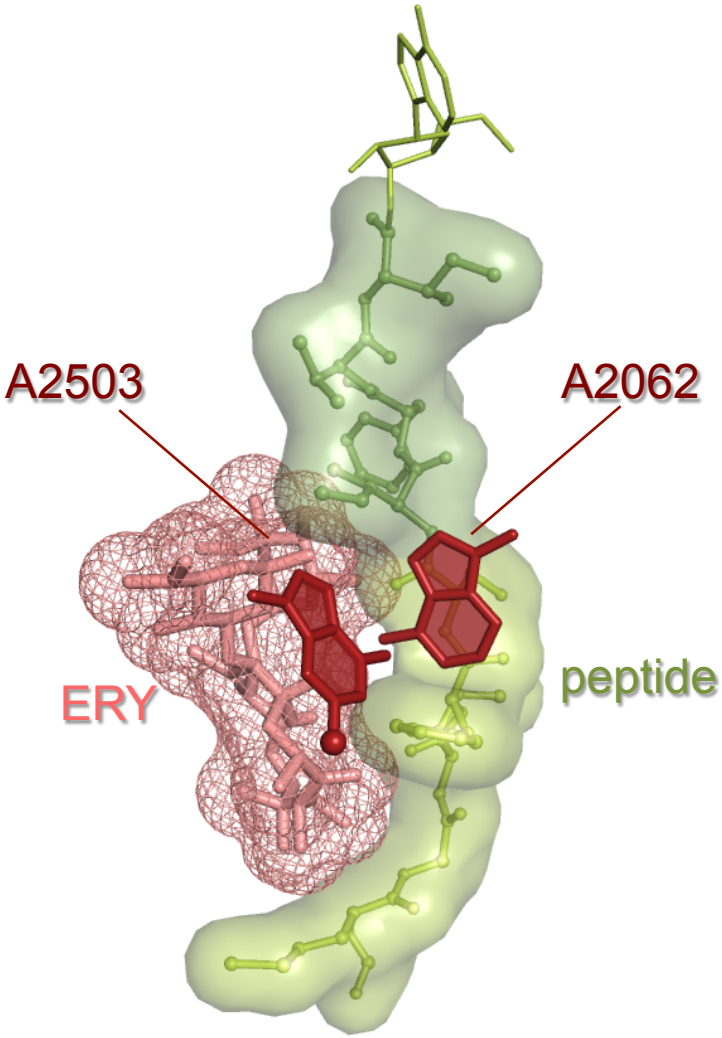
PoxA



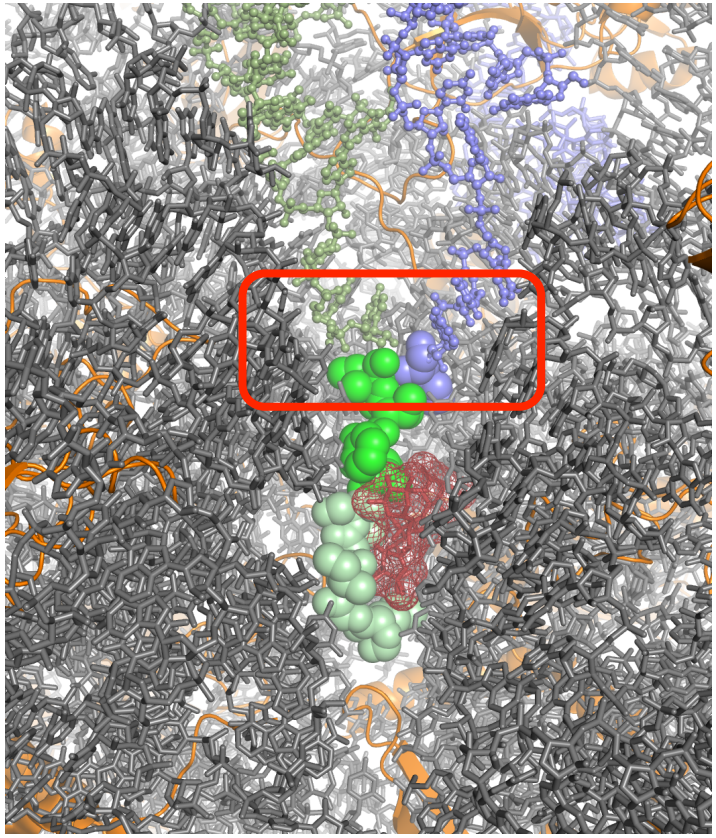
Gupta et al. (2013), Nature Communications, in press

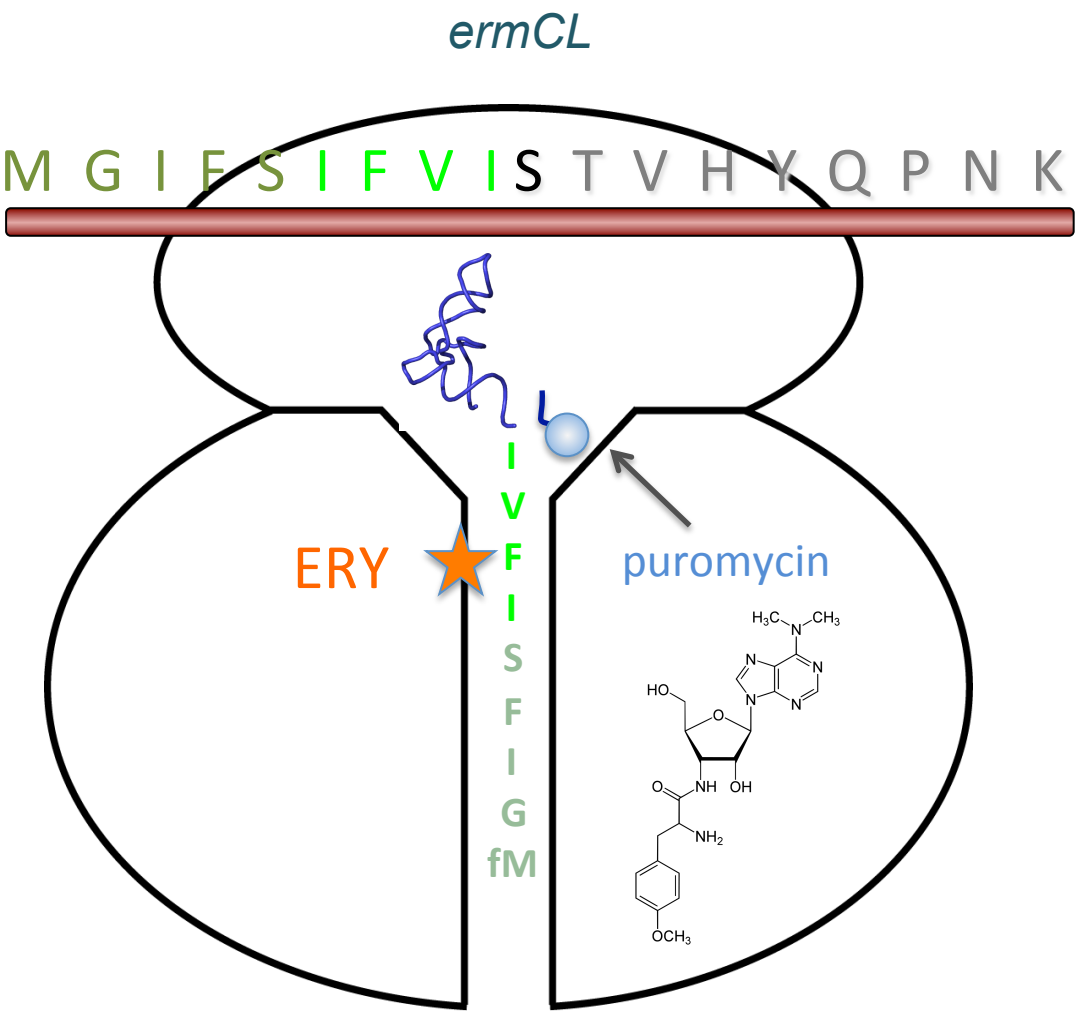
Where do sensors send the signal?

What becomes broken in the stalled ribosome?



peptidyl transferase center

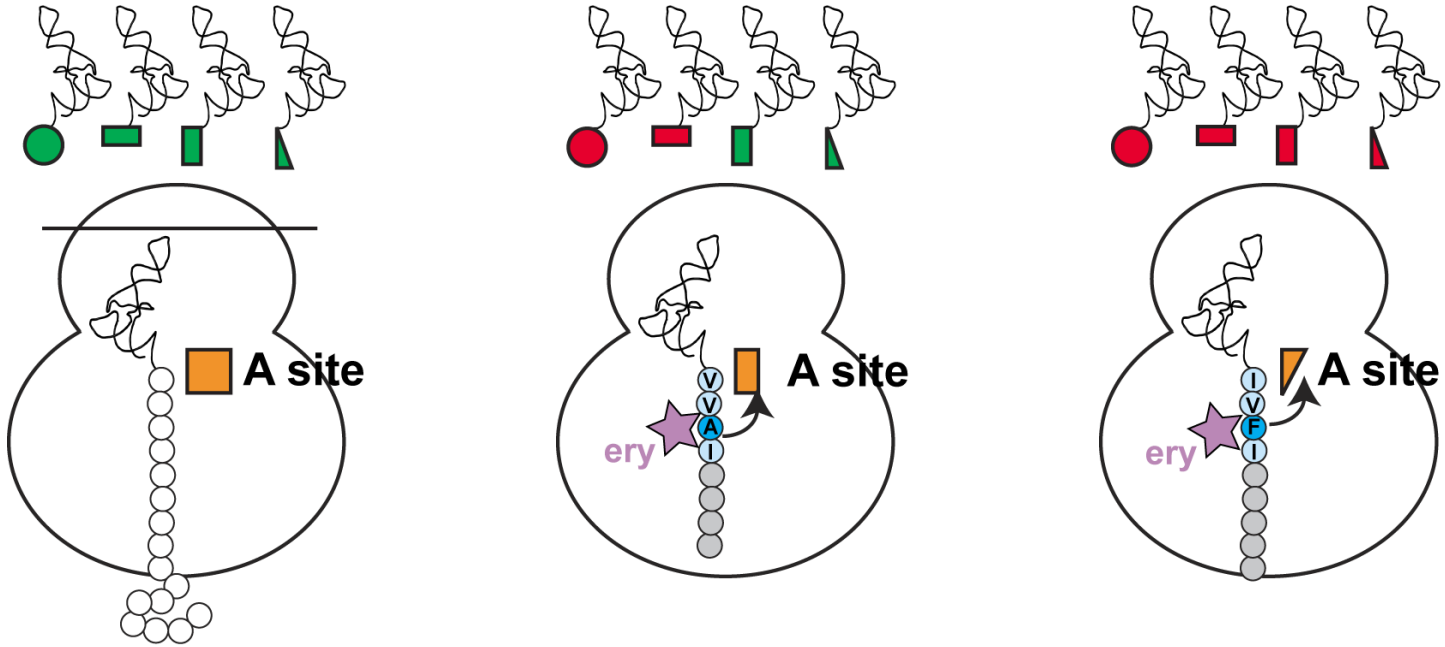




Stalled ribosome is unable to catalyze peptide bond formation

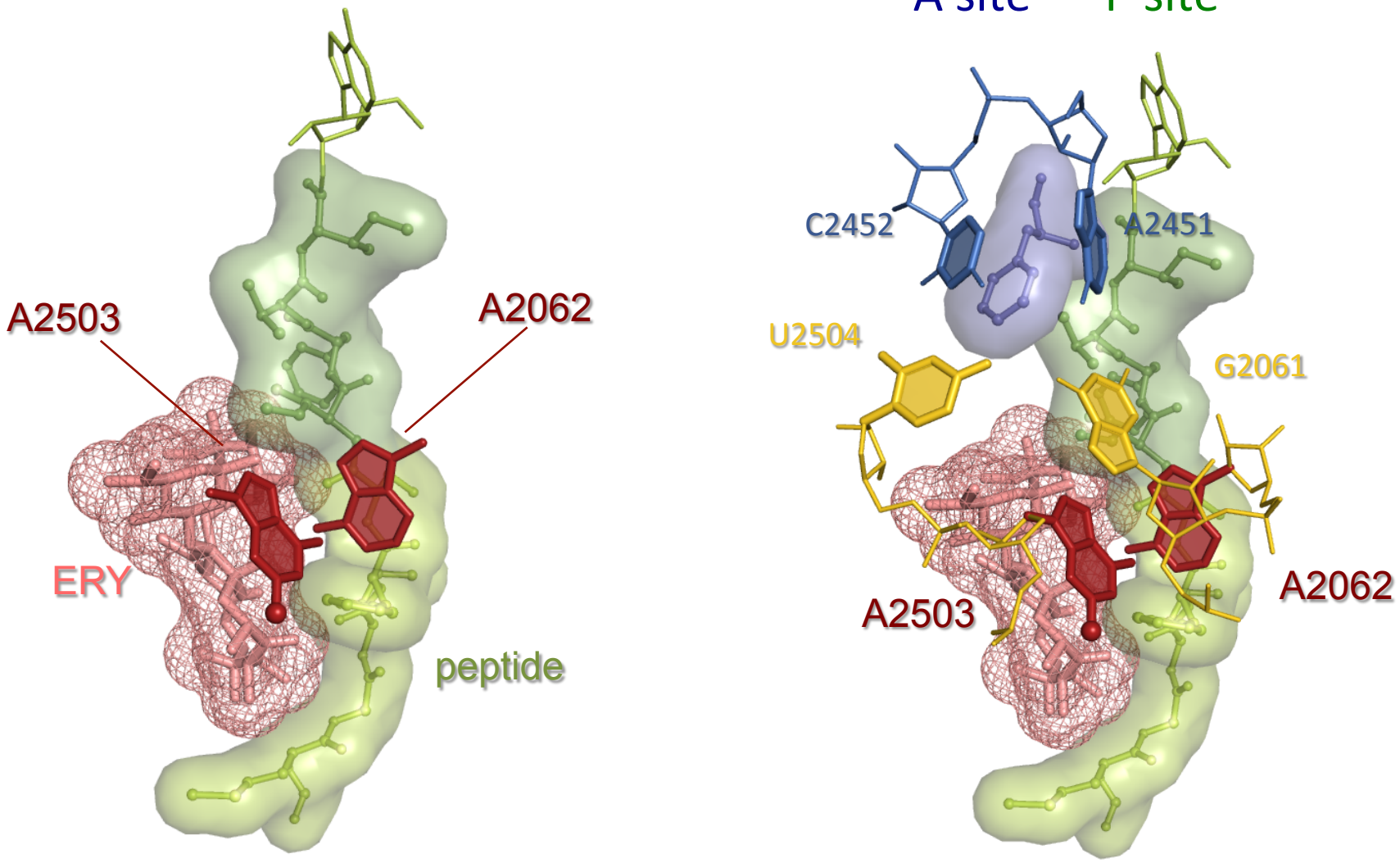
Vazquez-Laslop, et al. (2008) Mol. Cell 30, 190

Peptide and antibiotic affect the peptidyl transferase A-site



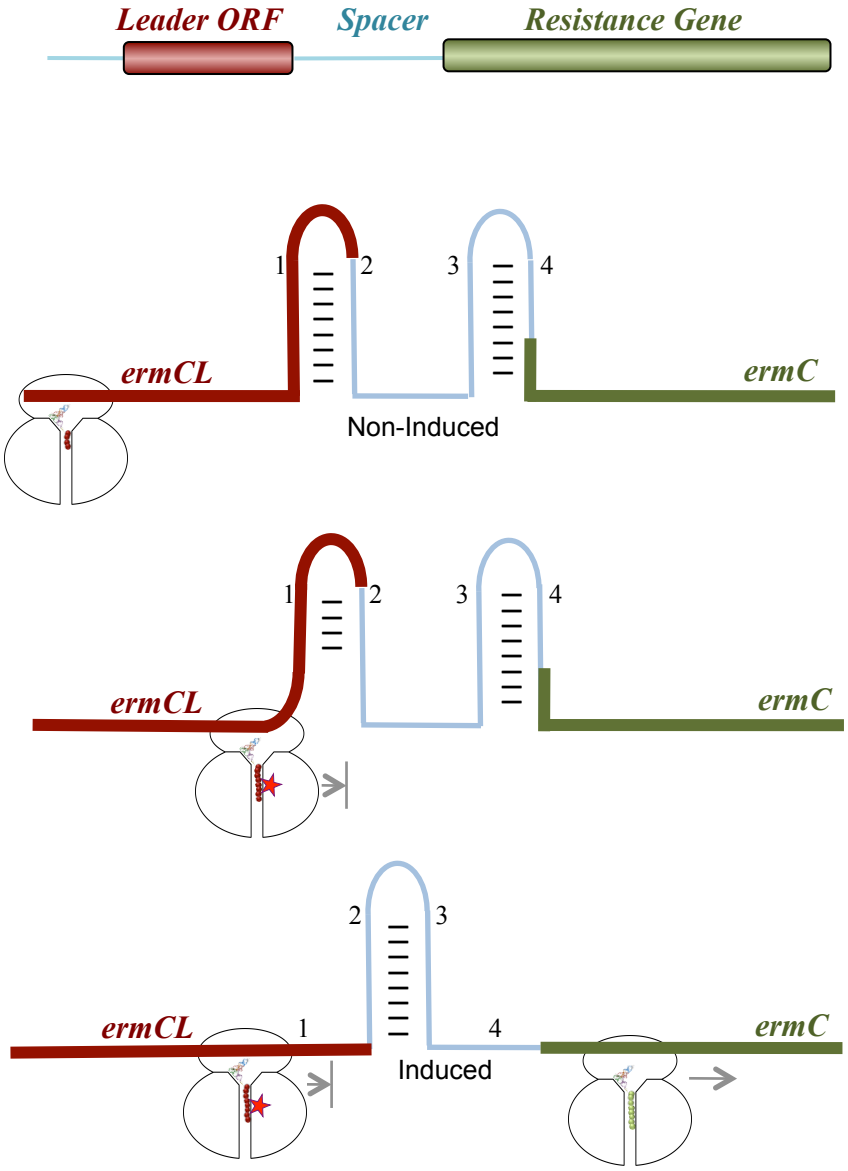
Peptidyl transferase center is the target of the stalling signal

Ramu et al. (2011) Mol. Cell 41, 321



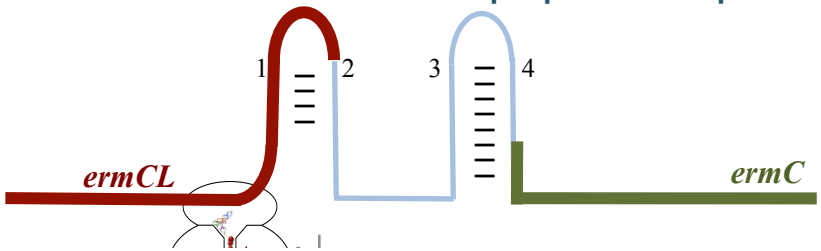
The A2062 and A2503 sensor in the exit tunnel may relay the stalling signal to the A-site of the peptidyl transferase center

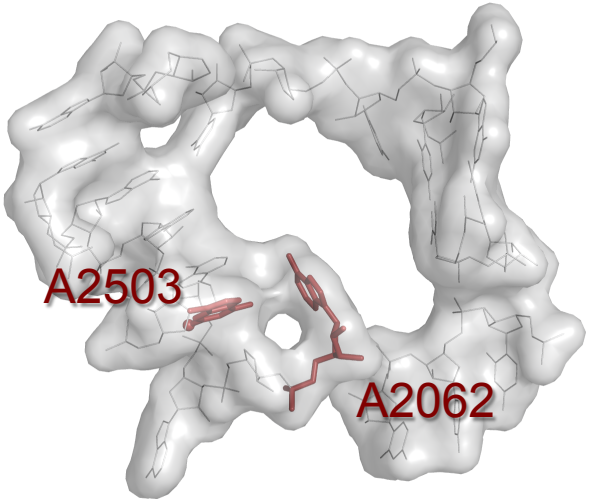
Translation attenuation control requires the antibiotic

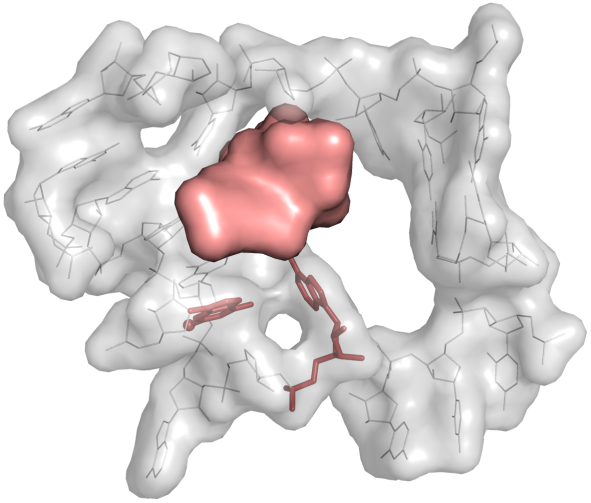


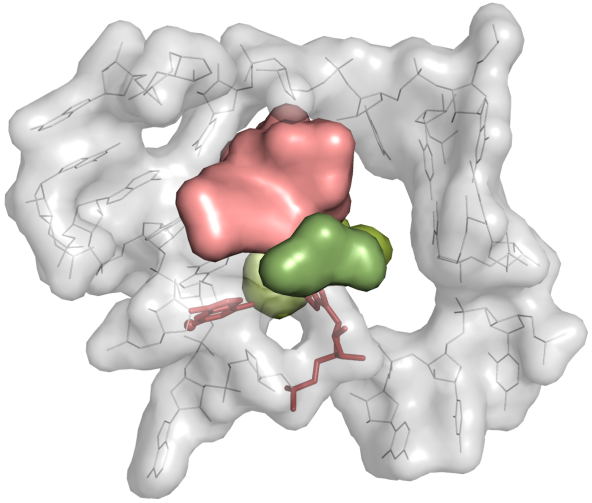
Horinouchi & Weisblum (1980) PNAS 77, 7079; Gryczan et al.(1980) Nucl Acids Res 8, 6081

What is the role of the antibiotic in nascent peptide-dependent ribosome stalling?



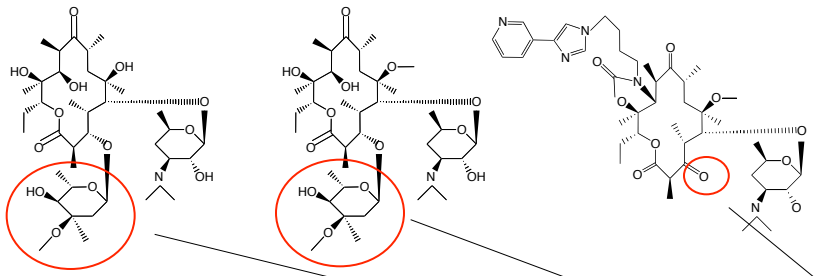


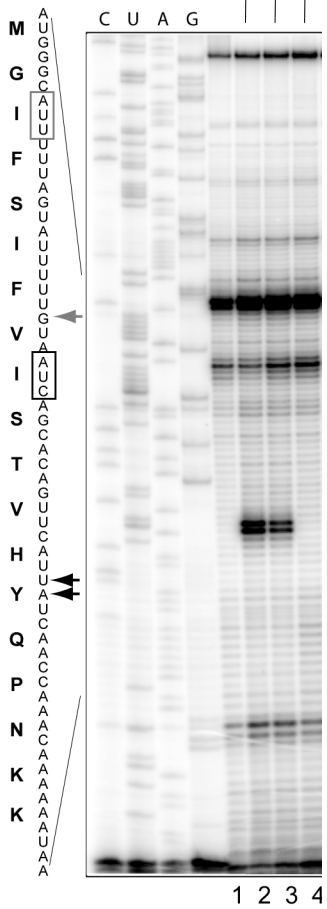


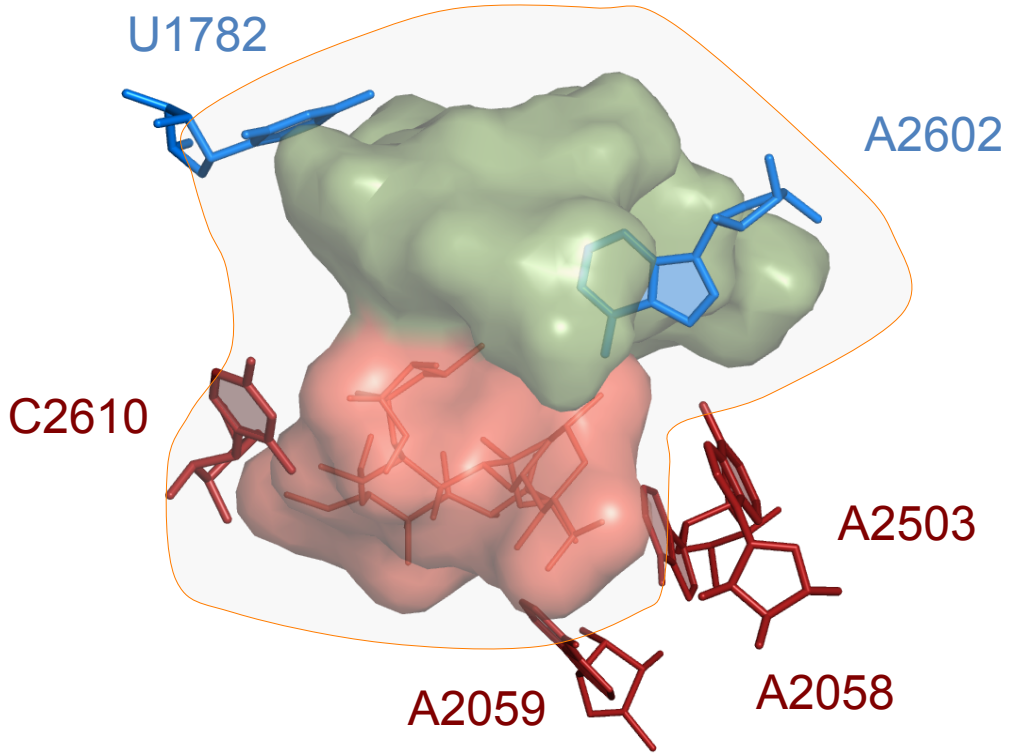


A road-block

ERYTHROMYCIN CLARYTHROMYCIN TELITHROMYCIN

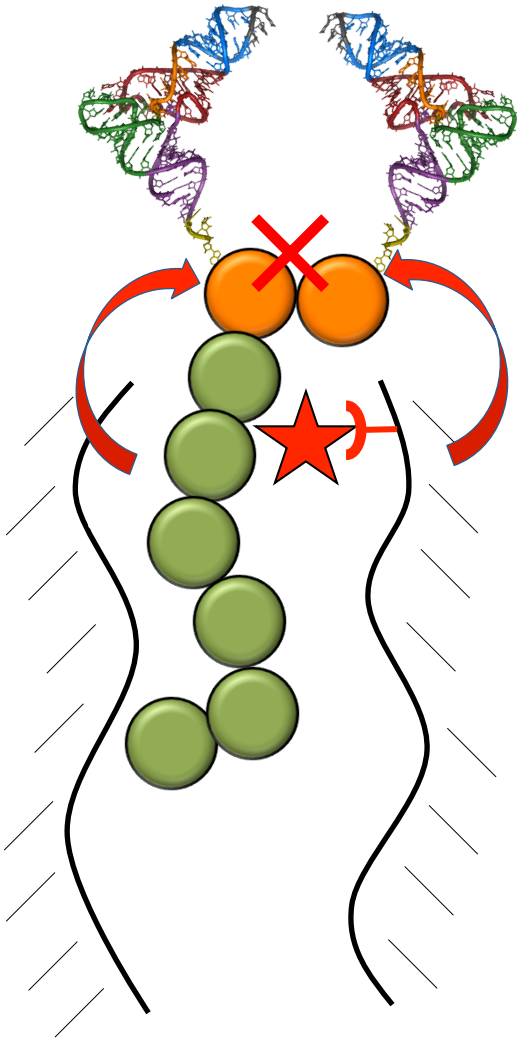






Specific tunnel sensors recognize the presence of the stalling cofactor and its structure

Vázquez-Laslop et al. (2011), PNAS, 108, 10496



Peptidyl transferase

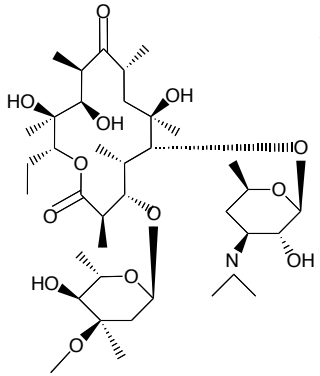
The analog signal from the peptide and small molecular cofactor is integrated in the peptidyl transferase center

Nascent peptide defines the recognition of the small molecular cofactor

MGIFSIFVISTVHYQPNKK

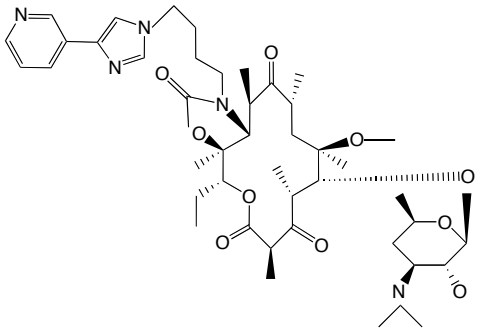
MLVFQMRNVDKTSTI…

✔ Erythromycin (ERY)



✔

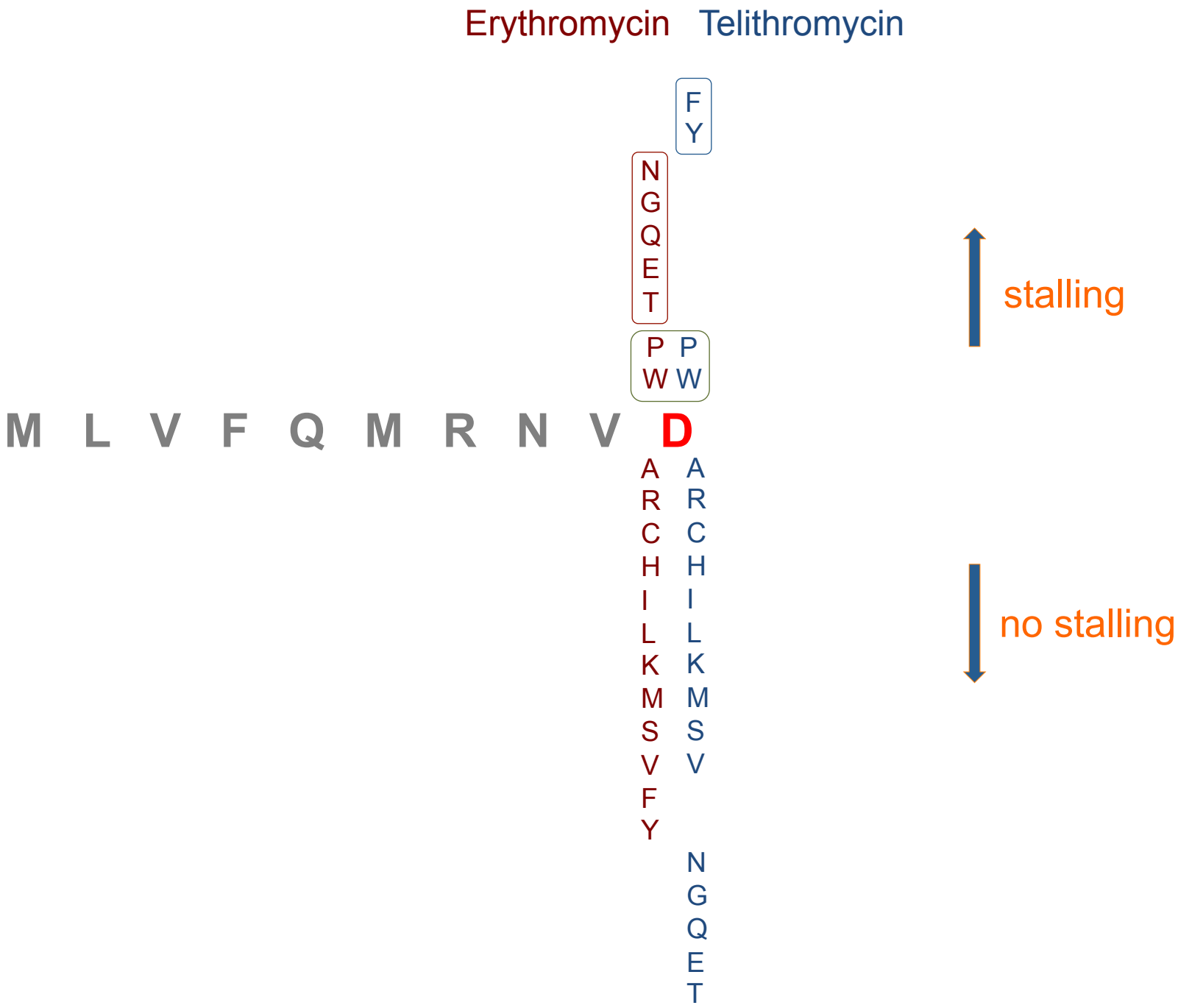
✔ Telithromycin (TEL)



✗

		ERY	TEL
ErmCL:	M G I F S I F V **I**	✔	✗
ErmBL:	M L V F Q M R N V **D**	✔	✔
CL-BL:	M G I F S M R N V **D**	✔	✔

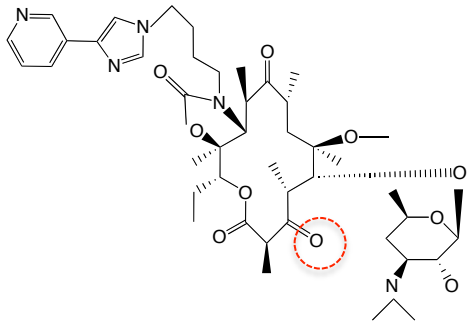
The potential to discriminate between the cofactor structures resides in the C-terminus of the stalling nascent peptide


Erythromycin
Telithromycin
F
Y
N
G
Q
E
T
P
W
P
W
M L V F Q M R N V D
A
R
C
H
I
L
K
M
S
V
F
Y
A
R
C
H
I
L
K
M
S
V
N
G
Q
E
T
stalling
no stalling

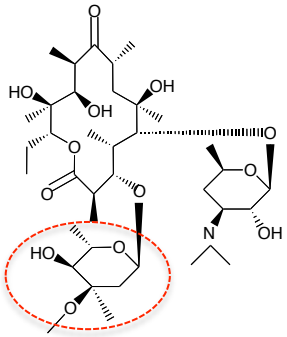
ErmBL: M L V F Q M R N V **D** . . . . .

Stalling peptide	Ery	Tel
R N V D	+	+
R N V E	+	-
R N V Y	-	+
R N V A	-	-

Telithromycin

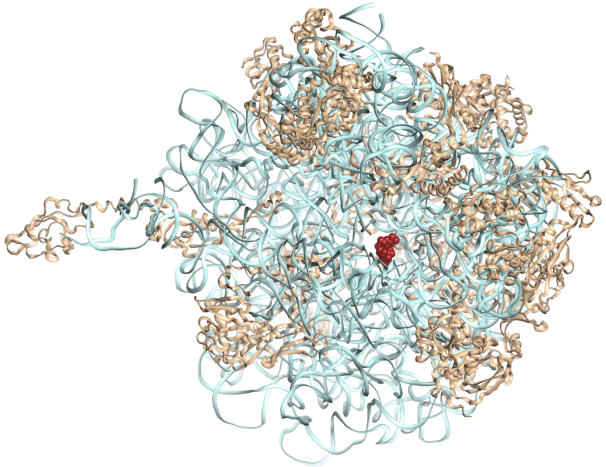


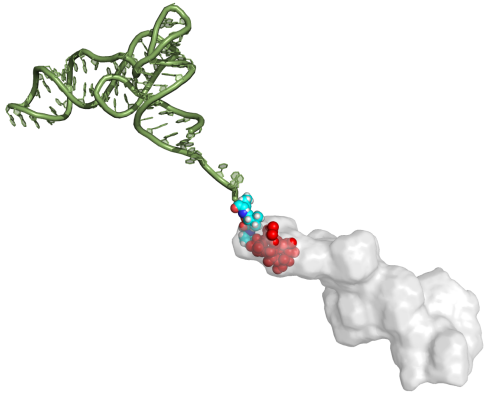
Erythromycin

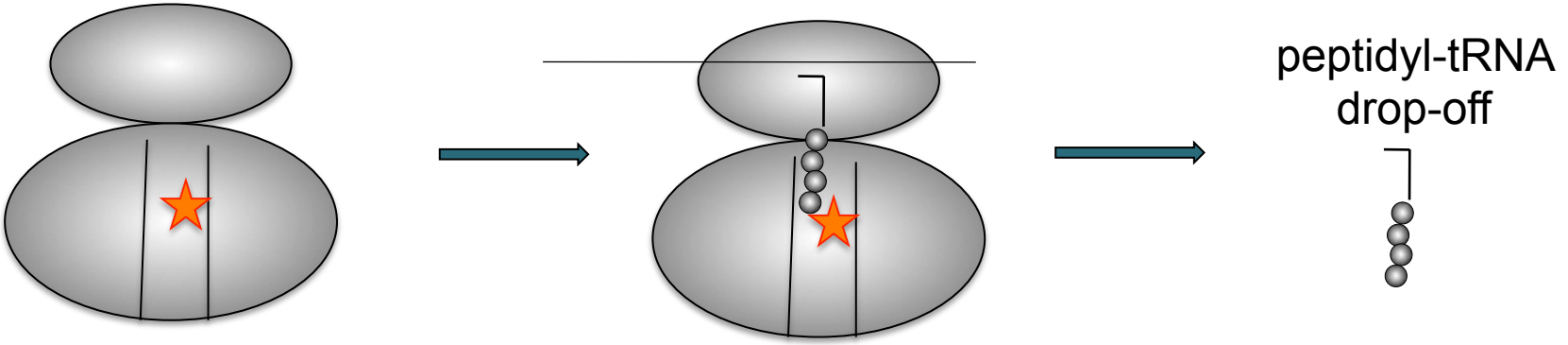


The nature of the P-site amino acid defines what small molecule can be recognized as a stalling cofactor.

Small molecule can affect the discriminating properties of the ribosome



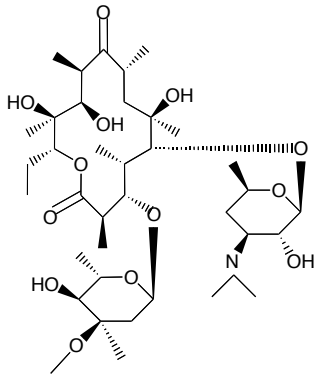




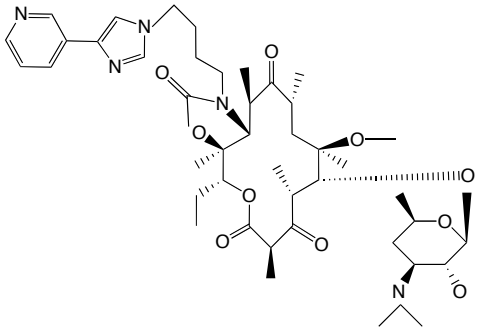
Macrolides stop protein synthesis at the early rounds of translation by promoting drop-off of peptidyl-tRNAs with short nascent peptides

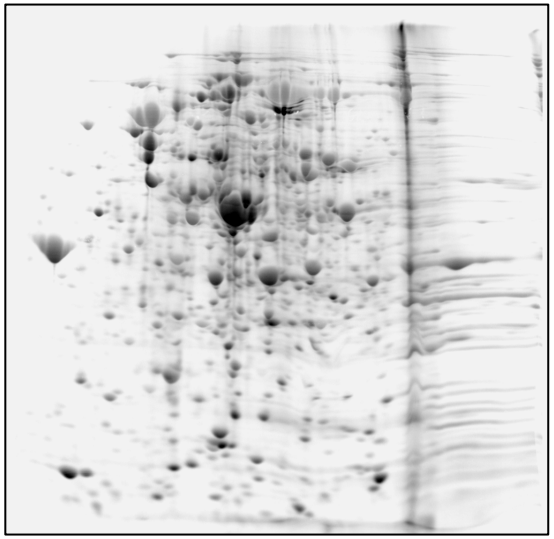
No antibiotic

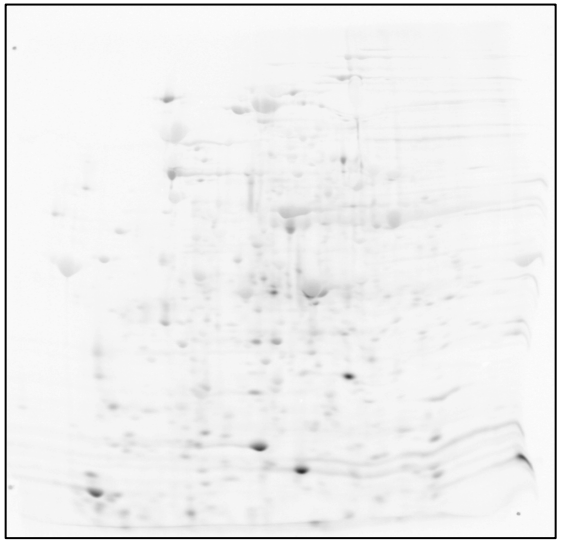
Erythromycin

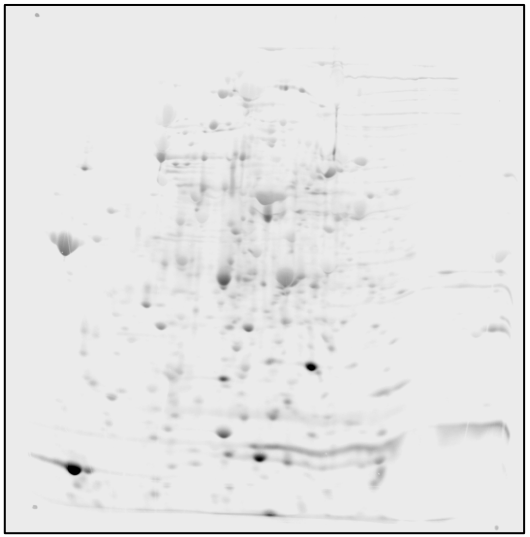


Telithromycin









Specific proteins can be synthesized y the drug-bound ribosome.
The structure of the antibiotic in the tunnel affects the spectrum of synthesized proteins

Kannan et al. (2012), Cell, 151, 508

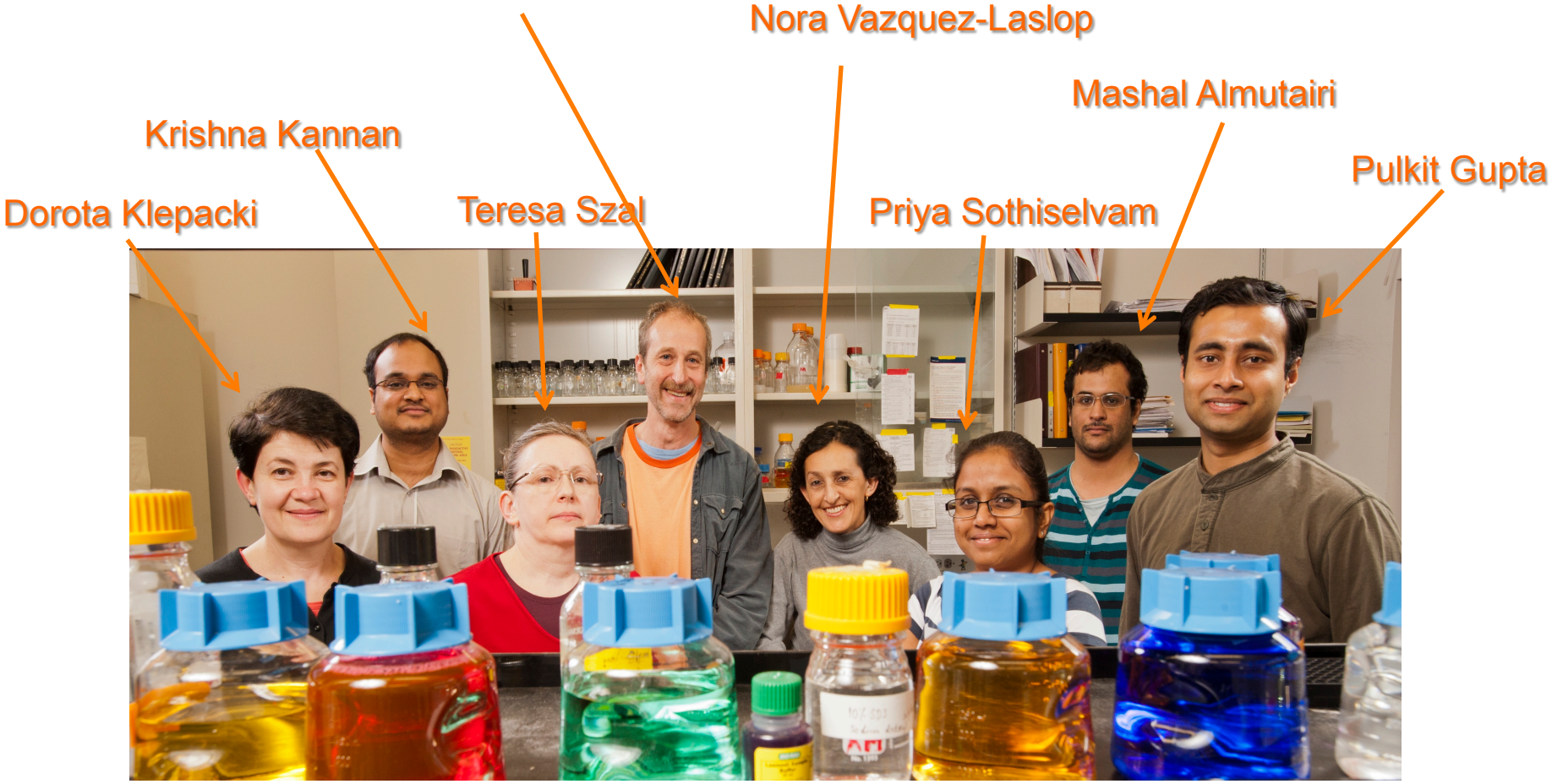
- Специальные нуклеотидные сеносры в туннеле позволяют рибосоме «узнавать» определенные структуры в синтезируемом белке.

- Антибиотик помогает рибосоме распознать такие структуы, но и сам антибиотик распознается как сигнал остановки трансляции.

- Меняя структуру белка, закодированого в мРНА можно научить рибосому узнавать разные низкомолекулярнуе соединения.

- Таким образом рибосома выступает в качестве сенсора и вновь синтесируемого пептида и некоторых малых молекул

shura@uic.edu



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Jonathan Weissman (UCSF)
Tom Steitz (Yale)
Daniel Wilson (U of Munich)
Klaus Schulten (U of Illinois)