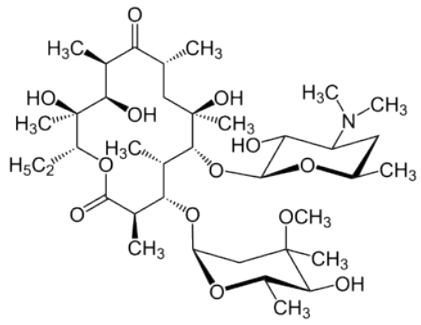
Frameshifting in a leader ORF (uORF) induces antibiotic resistance

Original title: Regulation of Gene Expression by Macrolide-Induced Ribosomal Frameshifting.
Gupta et al., Mol Cell 52, 1-14, 2013

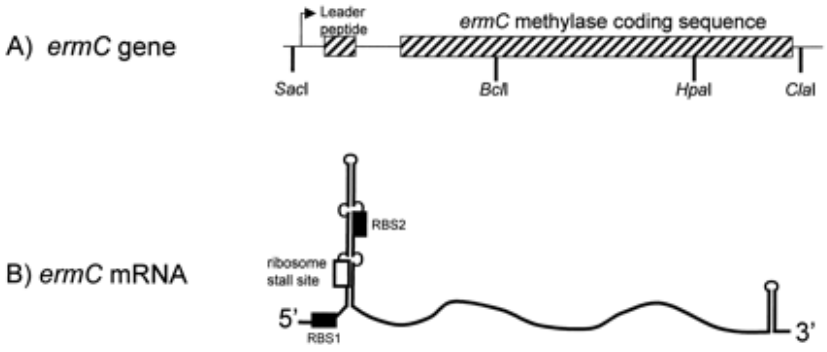


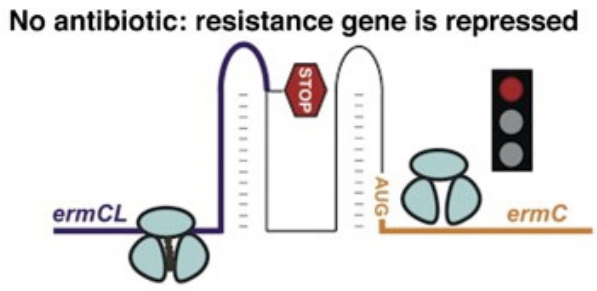
Erythromycin belongs to the **macrolide antibiotics** that have several sugars, cladinose or desosamine, attached to a macrolide ring (Fig. http://en.wikipedia.org/wiki/Erythromycin). Erythromycin was first isolated in 1949 from the bacterium *Streptomyces erythreus*. **Macrolide antibiotics bind at the Nascent Peptide Exit Channel and thereby block translation**.

Resistance to erythromycin in *E. coli* is obtained **by di-methylation of adenine 2058 of the 23S rRNA** by plasmid or chromosome encoded methylases. The resistance gene (e.g., *ermC*) is not constitutively expressed, but is induced in presence of the antibiotic.

The induction of the resistance by and to erythromycin resides in stalling the ribosome movement by the antibiotic in an upstream open reading frame of the resistance gene, similar to well known tryptophan synthesis control discovered by Charles Yanofsky. Whereas in the case of the tryptophan synthetase the stalling of the ribosome, due to the absence of tryptophan, leads to alternative mRNA secondary structures that prohibit the formation of a secondary structure for Rho dependent termination, the **stalling of the ribosome at codon 9 of the uORF of the *ermC* mRNA opens a secondary structure to allow ribosome entry at the Shine Dalgarno sequence (SD or RBS) on the downstream ORF**.

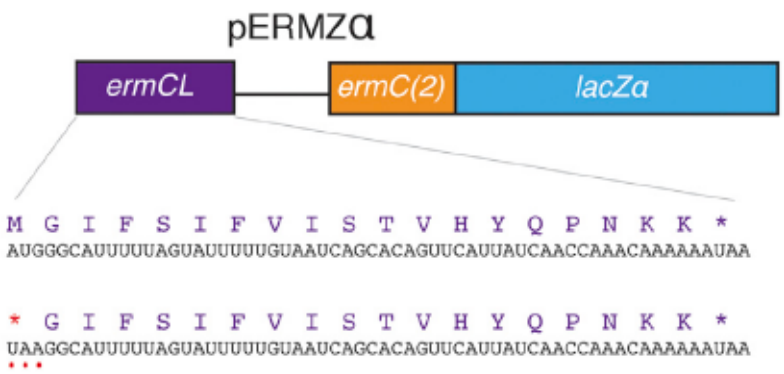
The *ErmC* mRNA with its secondary structure that will be opened by stalling ribosomes to allow access to the 2nd ribosome binding site

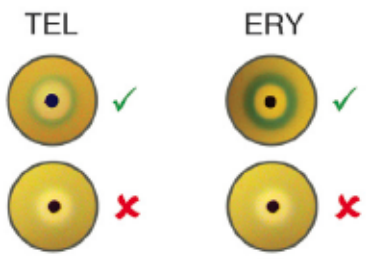




J. Bacteriol. November 1998 vol. 180 no. 22 5968-5977

Ketolides (e.g., telithromycin, TEL) are a new generation of macrolides that have a sugar residue of the macrolide replaced by a keto group. These antibiotics bind to two sites on the ribosome, but allow a residual amount of translation to occur (up to 25%). Ketolides do not introduce translational arrest in the ErmC leader peptide, but still induce translation of the ErmC reading frame. In the presented paper, Gupta and coworkers used an *rmC-lacZ* fusion in *E. coli* to follow induction of the *ermC* gene in presence of macrolides and ketolides.



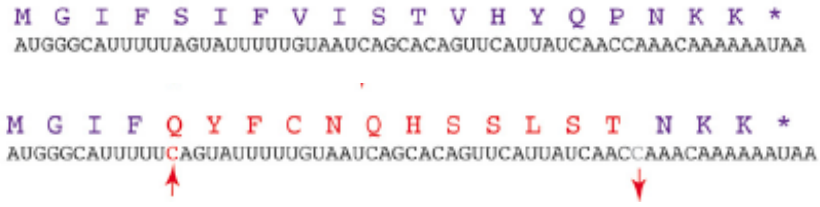


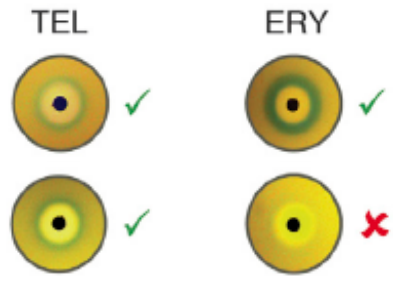
Induction of β-galactosidase in presence of a telithromycin or erythromycin discs
In presence of the uORF, the β-galactosidase is induced

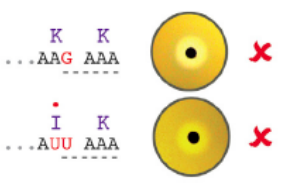
Inactivation of the AUG of the uORF abolishes *ermC-lacZ* induction

If ketolides do not cause premature arrest of ribosomes in the uORF, how does it induce translation at the down stream AUG?

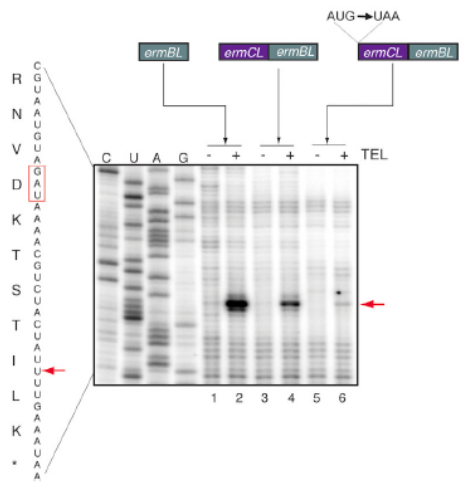
The peptide sequence Ile_6-Phe_7-Val_8-**Ile_9**, required for erythromycin induced stalling, is **not** required for ErmC induction by ketolides



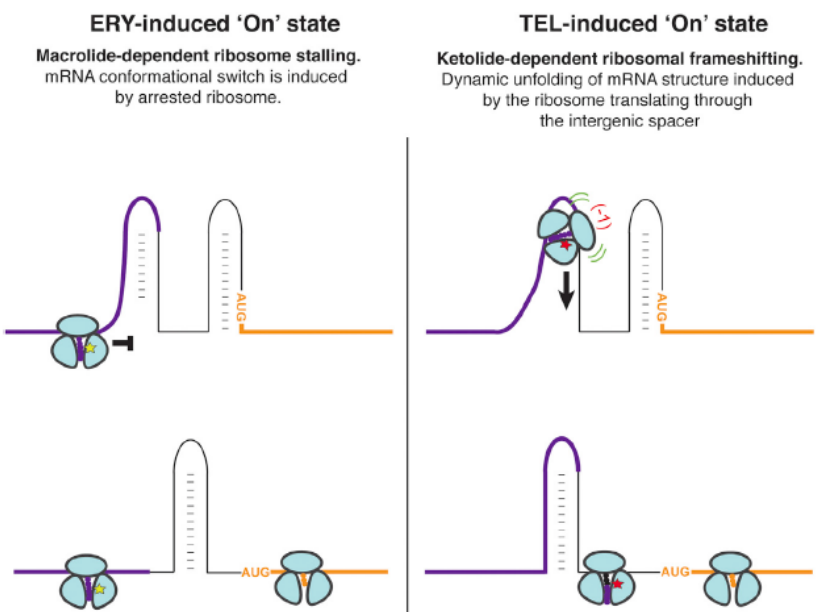




In contrast to the ERY induced stalling at codon 9, ketolide requires a slippery sequence AAA AAA coding for two lysines at the end of the uORF. Replacement by synonymous codons abolishes expression of ErmC expression.



Translation of the leader peptide does not displace the ketolide out of the peptide channel. Ketolides do induce ribosome stalling at the ErmB leader peptide. A fusion of the ermC-leader with ermB-leader leads to a ribosome stalling at the ermB-leader sequence, as shown by ribosome toe-printing.



Frameshift mutants in the intergenic region showed that the ribosome does not translate the leader and ErmC peptide as fusion protein. Furthermore, protein sequencing showed that translation of the main ORF starts at the cognate AUG of ErmC.

Altogether, the report shows that sublethal concentration of ketolides induce frameshifting to unwind a secondary structure of ErmC 5'UTR to allow ribosome binding and translation of the main ORF encoding the dimethylase to confer macrolide resistance.