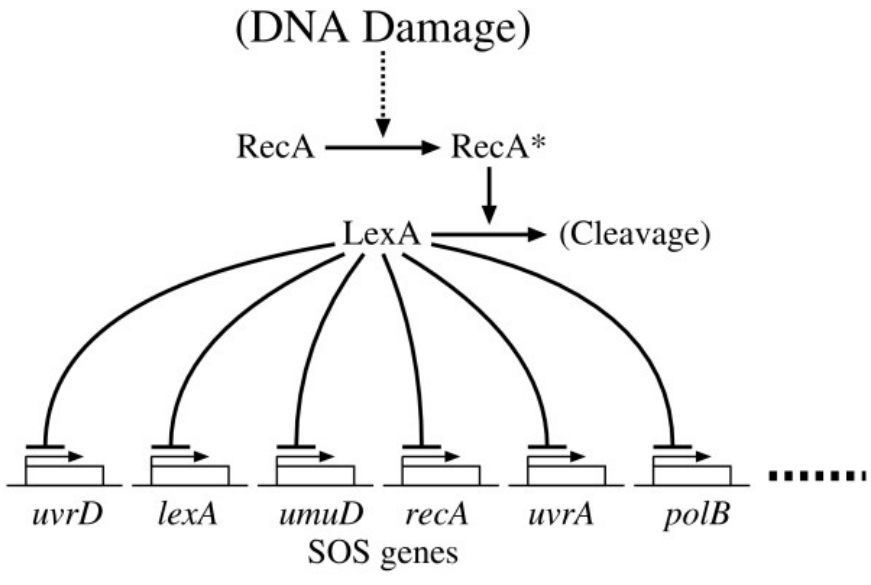
A selection for SOS response deficient mutants

Original title: Identification of genes involved in low aminoglycoside-induced SOS response in *Vibrio cholerae*: a role for transcription stalling and Mfd helicase. Baharoglu Z, Babosan A, Mazel D., Nucleic Acids Res. 42:2366-79, 2014.

The problem: Identification of genes that are involved in antibiotic-induced expression of the SOS response system.
The beauty of the paper: Connect the response to a lethal event to isolate survivors that can no longer induce the system.

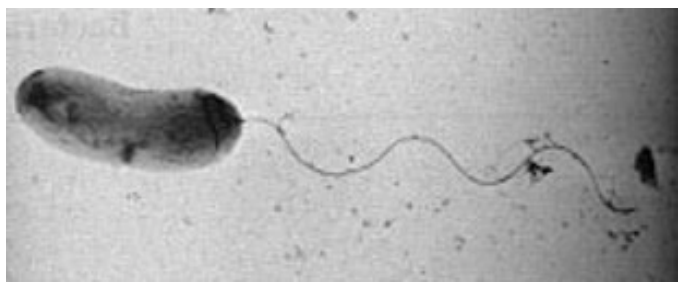


Antibiotica can induce a SOS response:
Damaged DNA results in stalling replication forks, which in turn results in the presence of single stranded DNA. This single stranded DNA is recognized by RecA, and activated RecA protein stimulates the autocatalytic cleavage of LexA, a repressor protein that down regulates a large number of DNA repair genes. Thus single stranded DNA elicits the SOS repair response. In extreme cases, the repair mechanisms are no longer sufficient and error-prone repair, trans-lesion synthesis, is induced.

In addition to known DNA damaging treatments, such as UV or MMS treatment, it has been reported that sub-lethal antibiotic concentrations can induce the SOS response. This in turn may contribute to activation of horizontal gene transfer, contributing to the spreading of antibiotic resistance genes and to the induction of error-prone DNA repair.

Vibrio cholera is a Gram-negative bacterium, causing toxin induced watery diarrhea. It is a slightly curved rod with a single flagellum. Its normal habitat is water and in particular the chitin of crustacean. In the intestine, it produces a type AB toxin, where the A-subunit induces cAMP synthesis, leading to diarrhea.

https://microbewiki.kenyon.edu/index.php/Vibrio_cholerae

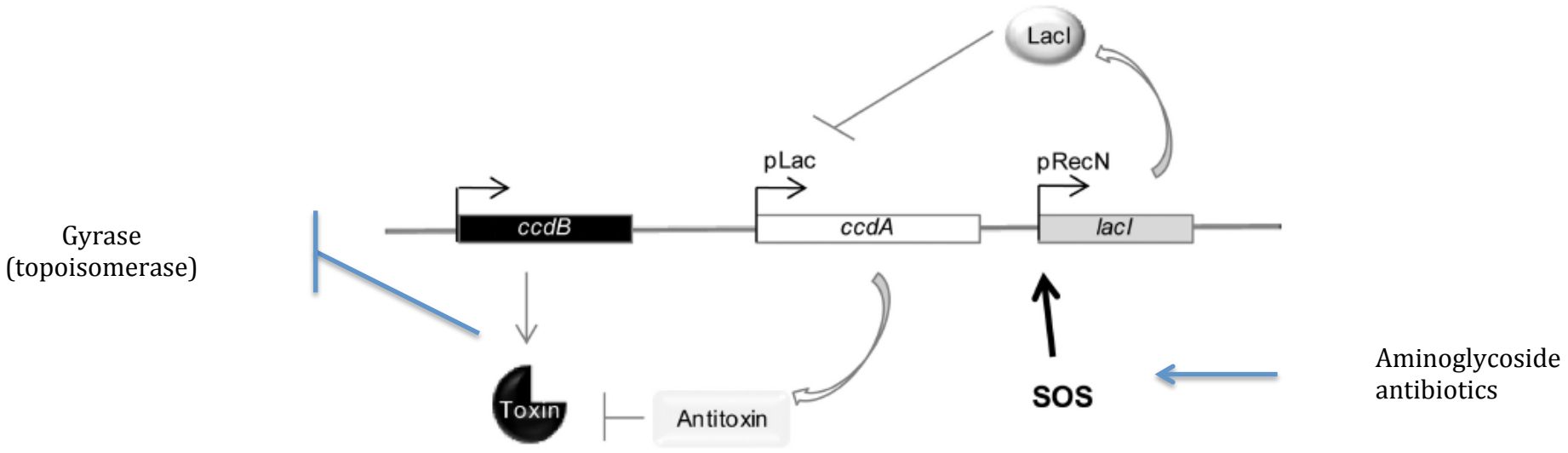


Aminoglycosides induce the SOS response in *Vibrio cholera* (but not in *E. coli*). Since aminoglycosides inhibit translation by binding to the small ribosomal subunit, it is surprising that they have a mutagenic effect in *V. cholera*. Aminoglycosides (Tobramycin, Gentamycin, Amikacin, and the more classical streptomycin and kanamycin) induce misreading and are bactericidal for G-negative bacteria, and in a synergistic effect with β-lactams also to G-positive bacteria. Recently it was shown that sub-lethal concentrations (100x below MIC) induce the SOS response in *Vibrio cholera*, but not so in *E. coli* (Baharoglu and Mazel, 2011). In *E. coli* the stress response, induced by aminoglycosides, is dampened by RpoS (a stress response induced sigma factor, responsible for the transcription of stress related genes). However, the SOS response can be induced in *E. coli*, if RpoS is reduced. It was further shown, that the aminoglycosides induce an oxidative stress and ROS formation.

A genetic screen based on classical modules to learn more about the players involved in the induction of the SOS response by aminoglycosides.

The ingredients:
- A SOS induced promoter RecN
- The lac repressor lacI, which will repress the Lac promoter
- The CcdBA toxin antitoxin system from the F plasmid. The toxin inhibits the gyrase (a topoisomerase) and thereby inhibits growth. If the antitoxin is produced, the toxin is neutralized. (In the natural condition of the F-plasmid, the cells that loose the plasmid can no longer grow, since the antitoxin is more rapidly degraded than the toxin, and therefore only plasmid containing cells will grow: the bacteria are addicted to the plasmid)

If the SOS response is induced, the RecN promoter will be activated and produce the Lac repressor (LacI). This in turn will repress the antitoxin gene that is under control of the Lac repressor. The antitoxin CcdA can therefore no longer neutralize the toxin CcdB and the cells will no longer grow. After SOS induction by aminoglycosides, "only" mutants that can no longer induce the SOS response will be able to grow under these conditions (as do all cells without the induction of the SOS response).



The plasmid carrying this construct was integrated in the genomes of a transposon mutant library in presence of 1% MIC aminoglycoside and surviving candidates were analysed. 30 mutant loci were identified, many of them in replication, repair, and recombination genes. For example two RNA-DNA helicases involved in removal of stalled RNA polymerases were identified, consistent with the fact that aminoglycosides induce oxidative stress that leads to the formation of oxidized nucleotides resulting in RNA polymerase stalling.

Kimura *et al*. *BMC Bioinformatics* 2008 9:23 doi:10.1186/1471-2105-9-23, The SOS DNA repair system in *E. coli*. ,
Kelley WL. Mol Microbiol. (2006) 62:1228-38. Review. Lex marks the spot: the virulent side of SOS and a closer look at the LexA regulon.
Baharoglu Z, Mazel D. Antimicrob Agents Chemother. 201155:2438-41. Vibrio cholerae triggers SOS and mutagenesis in response to a wide range of antibiotics: a route towards multiresistance