However, the lethal effect of ROS production is only one side of the coin since the same process is also responsible for providing the mutation-supply for the evolution of antibiotic resistance.By employing series of short term laboratory evolutionary experiments we demonstrated that suppression of antibiotic mediated oxidative mutagenesis by the ROS scavenger thioureasignificantly decreases the capacity of *Escherichia coli* to develop resistance against a wide range of bactericide antibiotics. Additionally we show that this effect applies to mismatch repair deficient mutatorpopulations as well, which are known to be facilitators in the emergence of resistance during clinical infections.

Our work indicates that the mutagenic effect of ROS generated by antibiotics is a critical promoting factor in the evolution of resistance. Hence not the potentiation but on the contrary, the suppression of oxidative damage may afford for a novel therapeutic strategy to arrest the rise of resistant bacteria.

## P-053
## ORIENTATION EFFECT IN THE TRANSPOSITION OF IS30 – ROLE OF SUBTERMINAL SEQUENCES

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The mobile genetic elements (transposons and IS elements) play important role in horizontal gene transfer, in generation and movement of pathogenicity islands and in re-shuffling of genes, finally resulting in the appearance of new bacterial variants. The mobile element IS30 belongs to a growing class of ISs known to transpose through an intermediate formed by abutting the inverted repeats (IR) of the element. These intermediates, minicircles or IS-dimers, carry a joined left and right IR junction with a 2-bp spacer between the ends. The formation of minicircles or dimers can be explained by a site specific deletion, where the IRs of the element or the transposon join to each other. The activity of IS30-based transposons are influenced by the relative orientation of the IS elements (head-head; tail-tail; head-tail). While the head-tail transposons harbouring two IS30 copies in same orientation may transpose as a unit, the head-head and tail-tail transposons of IS30 showed no detectable transposition activity. Moreover, the targeted end in the integration reactions determines the direction of IS30 insertion. The target and the integrating IS30 copies are always attached by their left and right ends leading to a head-to-tail orientation of the elements by joining their left and right inverted repeats. All of these observations suggest, that IS30 able to distinguish between own ends resulting in a so called “orientation effect”.

Here, we demonstrate that the enhancer elements identified previously in the subterminal part of IS30 are the main cause of the orientation effect. These enhancers are located within the 51 bp internal part next to the left IR (AAAC repetitive elements) and within the 17 bp internal part next to the right IR (decanucleotide, 5’-GAGATAATTG-3’). First, it was demonstrated that the nucleotide differences between the 26 bp left and 26 bp right IR sequences do not influence the joining activity. Additionally, both the 26 bp left and right IRs (without the enhancer sequences) are able to form junctions both in vivo and in vitro. However, we can not detect in vivo the connections between the same 68 bp left, nor the same 65 bp right IR ends if they contained the enhancer sequences. On the contrary, the joined left-left and right-right junctions were observed in

"in vitro" experiments. Based on these results, it was predicted that the connection of identical IS*30* ends is significantly inhibited, but not rejected by the presence of the enhancer elements. Moreover the "incorrect" left-left or right-right end connection results in 100-120 bp palindromic sequences in the transposition intermediates, which stimulated deletion formations of the plasmids used. Consecutive reaction of these unstable intermediates (integration into the target sequence, deletions, inversions) may eliminate these palindromic sequences, thus a kind of transposition product (usually deletions) can be isolated.

The role of repetitive sequences was also confirmed by in vivo transposition experiments using a transposon harbouring the minimal 26 bp IR of IS*30* together with an indifferent repetitive DNA sequence. It can concluded, that the joining of identical ends of longer than 65 bp occurs not only at a reduced frequency, but causes serious stability defect.

All of these data indicate that the subterminal sequences have a specific role in the orientation effect.

## P-054
## GENERATION OF GENETIC DIVERSITY IS THE PRIMARY FUNCTION OF THE INDUCIBLE DNA-POLYMERASES IN*E. COLI*

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In response to stress, the level of three minor DNA-polymerases (PolII, PolIV, PolV) increases 10-20-fold in *E. coli*. These polymerases have the ability to synthetize DNA on damaged templates bypassing replication blockages, however, they introduce mutations during translesion synthesis. PolIV and PolV perform low fidelity synthesis, and the generally accurate PolII is also error-prone at certain lesions. While mutagenesis is mostly harmful, it can contribute to the survival of the population by generating beneficial mutations occasionally. It has been a question for a long time, what the main function of inducible polymerases can be: rescuing stalled replication forks or generating genetic diversity under stress. Literature data are controversial: some studies indicate that their primary role is to rescue cells from replication arrest and the mutagenesis is an unavoidable consequence of this function, whereas other data show that they are not essential to replication repair (other DNA repair systems are sufficient to carry out the task) and their only function is the generation of genetic diversity. In order to settle the question, we examined two isogenic strains (MDS42, MDS42pdu) differing only in the presence of the inducible DNA-polymerases. Comparing the growth properties and surviving abilities of the two strains in response to the DNA-damaging agent mitomycin C (mC), it was found that: (1) various mC concentrations cause similar growth inhibition in the two strains, (2) applying low concentrations of mC (which is able to induce polymerases, but causes only moderate growth inhibition), the lack of the polymerases does not influence growth and short-term survival, (3) in the presence of higher concentrations of mC (enhanced stress, increased DNA-damage), the number of living cells decreases more severely in the absence of inducible polymerases, (4) under stressful conditions, stress-resistant mutants arise much more frequently when the polymerases are present. Our results show that under normal growth conditions or under moderate stress damaged DNA sites can be