INVESTIGATION OF THE *ESCHERICHIA COLI* LAC OPERON ON POPULATION AND ON SINGLE CELL LEVEL

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**Introduction:** The *Escherichia coli* lac operon is the oldest paradigm of gene regulation. But sufficient data for developing or validating mathematical models are still missing or are measured in different or non-characterized strains. With the aim of generating a stochastic model (3) we investigate the lac operon response on population and on single cell level.

**Objectives:**

1. Induction experiments in *E. coli* wild-type and a lacY-mutant.
2. Investigations of single cell behavior after induction with different inducers and in different mutants.

**Methods:** lac operon induction was investigated by measuring β-galactosidase-activity.

To characterize single cell behavior we constructed a strain with a gfp fusion to the lac promoter and observed fluorescence by microscopy.

**Results:**

1. The induction experiments in the *E. coli* wild-type revealed that β-galactosidase activity was detectable at very low IPTG concentrations. By contrast a comparable activity was measured at an approximately 10fold TMG concentration. The comparison with the lacY-deletion strain showed that diffusion of IPTG into the cell plays no significant role at this concentration range.
2. In previous reports about bistability the lac promoter was cloned without operator 2 (1,2). Therefore we generated a PCR-product with all three operator sites and fused it to gfp. We could detect a bistable response at similar TMG concentrations as described (1). Furthermore we recognized an inhibition of growth in dependence of TMG.

**Conclusions:**

1. Through the induction experiments we could characterize the uptake of TMG and IPTG and elaborate the role of the diffusion of these inducers.
2. With the help of the Plac-gfp reporter fusion we were able to detect the bistable response of the *E. coli* lac operon. On this basis we plan to analyze single cell behavior in different *E. coli* mutant backgrounds.

**References:**

1 Ozbudak et al. (2004), Nature, 427, 737-740.
2 Mettetal et al.(2006), PNAS, 103, 7304-7309.
3 Kremling et al., submission FEMS 2009.