Cells respond to environmental stimuli by fine tuned regulation of gene expression. In this thesis we investigate the dose dependent modulation of the genetic response upon nutrient and stress signals in yeast. A destabilized version of firefly luciferase was used in living yeast cells as a real-time reporter for gene expression. This highly sensitive and non-invasive system can be simultaneously used upon many different experimental conditions in small culture aliquots. This allows the dose–response behaviour of gene expression driven by any yeast promoter to be reported and can be used to quantify important parameters, such as the threshold, sensitivity, response time, maximal activity and synthesis rate for a given stimulus.

We applied the luciferase assay to the nutrient-regulated GAL1 promoter and the stress-responsive GRE2 promoter. We find that luciferase expression driven by the GAL1 promoter responds dynamically to growing galactose concentrations, with increasing synthesis rates determined by the light increment in the initial linear phase of activation. The GAL1 gene is activated with continuously increasing synthesis rates in a well defined range of galactose concentrations, correlating with a dynamic increase of histone remodeling and subsequent association of the RNAPII complex. Dose dependent chromatin remodeling appears to be the basis for the dynamic GAL1 expression since mutants with impaired histone dynamics show severely truncated dose response profiles.

In the case of the GRE2 promoter, we demonstrate that the very short-lived version of luciferase used here is an excellent tool to quantitatively describe transient transcriptional activation. The luciferase expression controlled by the GRE2 promoter responds dynamically to a gradual increase of osmotic or oxidative stress stimuli, which is mainly based on the progressive increase of the time the promoter remains active. In contrast, the GRE2 promoter operates like an off/on switch in response to increasing osmotic stress with almost constant synthesis rates and exclusively temporal regulation of histone remodeling and RNAPII occupancy. The Gal3 inducer and the Hog1 MAP kinase seem to determine the different dose response strategies at the two promoters. Our analysis reveals important differences in the way dynamic signals create dose sensitive gene expression outputs. Taken together, the luciferase assay described here
is an attractive tool to rapidly and precisely determine and compare kinetic parameters of gene expression. Additionally, the function of the specific transcriptor factor Smp1 involved in the yeast osmostress response was investigated. Location analyses upon osmotic stress reveal that Smp1 associates preferentially with the whole transcribed regions (ORFs) upon stress as opposed to other transcriptional activators involved in the osmostress response. However, Smp1 seems to be important for stress-activated gene expression only in the presence of the natural induced gene and not of artificial promoter fusions. This highlights the possible role of Smp1 in regulating gene expression from ORF sequences rather than promoter regions.