UNIVERSITEIT VAN AMSTERDAM

Design, Construction and Testing of a Photoactivatable and Diffusive Protein Network in Saccharomyces Cerevisiae

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The primary goal of the work presented in this thesis was to provide a unique tool that may increase our understanding of the mechanism of cytoplasmic diffusion in signal transduction (networks) in eukaryotic cells, in the form of a chimeric, light-dependent, cytoplasmic signal transduction device in the yeast Saccharomyces cerevisiae.

Chapter 1 gives a general introduction to the optogenetics field, as well an insight into the issues connected to signal diffusion in the spatially differentiated eukaryotic cells.

In chapter 2 we further characterize the in vivo redox transitions of a flavin-containing photo-sensory receptor domain for such a chimeric device. We provide further evidence for the overlap of the ranges of the redox midpoint potential of the flavin in a specific photoreceptor protein and the redox potential of key intracellular redox-active metabolites, and demonstrate that the redox state and photochemical activity of LOV domains can be recorded in vivo in Escherichia coli. Significantly, so far in vivo reduction of LOV domains under physiological conditions was not observed.

Chapter 3 describes the design and functional characterization of the intended chimera: A light-sensitive histidine protein kinase, derived from the Sln1 kinase of S. cerevisiae, translationally fused in a coiled-coil motif with the LOV domain of the stressosome component YtvA from Bacillus subtilis. In most chimeras exposure to blue light decreased the rate of autophosphorylation, but rational engineering also allowed the construction of a light-stimulated histidine protein fusion kinase.

In chapter 4 we describe tests that show the in vivo functionality of one of the light-sensitive histidine protein kinases, C9, in which exposure to blue light leads to a decrease of its autophosphorylation activity. This orthogonal photo-transduction system can be used to both activate and repress gene expression in S. cerevisiae, depending on the specific promoter that is targeted. Furthermore, the device can also be used to initiate nuclear accumulation of a selected signal transduction protein with incident blue light.

In chapter 5 we go into detail on one of the methods for functional testing of the phosphorylation of histidine kinases and their cognate response regulator(s), the so-called Phos-tag method. In this chapter it is described that, despite many attempts, we have not been able to use this method for analysis of the level of phosphorylation of the two response regulators of the Sln1 system, presumably because of a lack of separation of their phosphorylated- and non-phosphorylated form and/or the very unstable nature of the phosphorylated form of these response regulators. Screening of the recent literature revealed that until now Phos-tag separation of the two forms, i.e. the phosphorylated and non-phosphorylated form, of a eukaryotic response regulator has not yet been reported.

Finally, in the chapter 6 we discuss a few more relevant points that were not highlighted in the discussions of the earlier chapters, predominantly in the light of recent advances in the field of signal diffusion and the design of the light sensing fusion proteins.