We study the regulatory mechanisms that control activity-dependent gene expression in excitable cells. Our long-term goal is to develop a global model encompassing the different mechanisms that control gene expression and are regulated directly or indirectly by changes in the nuclear concentration of Ca\(^{2+}\). Along these lines, we aim towards the complete identification of mediator complexes containing DREAM as well as other less well characterized Ca\(^{2+}\)-dependent nuclear cofactors. We also aim to understand changes at the chromatin structure that could be related to changes in nuclear Ca\(^{2+}\), including modifications in the activity of chromatin remodeling enzymes, DNA methylation and post-transcriptional changes at the level of the histones.

Specifically, work with the transgenic regulator DREAM involves several research lines. One research line focus on the functional characterization of transcriptional regulator DREAM in vivo using several transgenic-based models with dominant active mutants of the DREAM protein. These mutants do not bind Ca\(^{2+}\) and do not interact with CREB or CREM proteins. Thus, these mutants will remain bound to DNA after membrane receptor activation and will be used to construct Ca\(^{2+}\) and AMP, and will continue to repress transcription even during membrane depolarizing. By the use of tissue-specific promoters we have targeted these mutants to the brain to obtain constitutive or conditional expression by the use of Tet-Off or Tet-On trans-activating systems.

A second research line tries to characterize putative functional domains within the DREAM protein, in particular those mediating the nuclear translocation, the tetramerization process and the DNA binding domain. Currently, there is strong evidence indicating that the domain responsible for binding to the DREAM sequence is formed upon tetramerization. Sequence analysis of the DREAM protein does not reveal the presence of nuclear import or export sequences in DREAM, thus we are analyzing alternative mechanisms regulating the translocation of DREAM and from the nucleus. We have identified several phospho-residues that modulate the repressor activity of DREAM either affecting its nuclear translocation, its tetramerization and/or its binding to DREAM sites in the DNA. In this regard, we characterized the interaction between DREAM and GRK2 and we showed that DREAM is phosphorylated by GRK2.

Finally, we have used the two hybrid approach to characterize several proteins that are interacting with DREAM, both in Ca\(^{2+}\)- and Ca\(^{2+}\)-independent manner. In this area our main interest is to find those proteins that contribute to, or block the transcriptional function of DREAM, through several interactions without a direct relationship with the nuclear function of DREAM are also under investigation. In addition, we are developing the tools to use proteomic technology to decipher the composition of nuclear complexes containing DREAM as well as the changes in the composition of these complexes upon different experimental and/or physiological conditions.

**FUNCTIONAL ANALYSIS OF TRANSCRIPTIONAL REPRESSOR DREAM**

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**Selected Publications**

