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Signaling and GPCR biology using cell models

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A wide range of untreatable or difficult to treat disease entities are caused by activating immunoglobulins G (IgG) directed against G-protein coupled receptors (GPCR). Hence, molecular architecture of the binding of these antibodies to the receptors and the functional consequences of changes in specific structural modules are of immense clinical relevance. Our laboratory has focused over the years on the Angiotensin II type 1 receptor (AT1R) and Endothelin-1 type A receptor (ETAR), which signal upon natural ligand Angiotensin II (Ang II) or Endothelin-1 (ET-1)-mediated and AT1R- or ETAR-agonistic IgG (AT1R-/ETAR-IgG)-mediated stimulation. Development of high-resolution methods allows for structural-functional relationship studies of specific receptor modules, including the extracellular domains, in the receptor activation.

To distinguish natural peptide and IgG-mediated activation, we first developed a yeast model where human GPCR activation controls yeasts growth. These yeasts have been modified to express one single human GPCR that couples to a chimera between human and yeast G-protein. Each yeast strain is specific of a different G-protein.

Point mutations can be introduced by site-directed mutagenesis in order to determine the influence of amino acids sequences on the receptor activation. To investigate further the link between conformation and activation of the receptor in a more complicated environment, we performed luciferase reporter assays in Human Microvascular Endothelial Cells (HMEC-1) after transfecting the cells with wild type or mutated receptors and the appropriate reporter plasmids. Comparison between wild-type and mutants, non-stimulated and stimulated cells allowed us to better characterize the influence of the conformation changes on activation of the receptors.

We successfully created models allowing for structural and functional studies of molecular architecture modules appreciating GPCR receptor plasticity, which helped us to define the role of specific extracellular domains. Better understanding of the molecular mechanisms responsible for GPCR activation holds great potential for design of more specific drugs in autoantibody-mediated diseases.