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PROBING THE ERB FAMILY OF RECEPTOR TYROSINE KINASES WITH QUANTUM DOTS AND FRET

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The erbB/HER family of transmembrane receptor tyrosine kinases (RTKs) are responsible for cellular responses to epidermal growth factor (EGF) and other peptide ligands. This family includes four members: erbB1 (the EGF receptor, EGFR), erbB2, erbB3 and erbB4. Activation of these transmembrane proteins initiates signaling cascades regulating numerous processes such as DNA replication and differentiation. Many aspects of the mechanism underlying RTK-mediated signal transduction are poorly understood. The overexpression, particularly of erbB2, and mutation of the erbB family are implicated in many types of cancer1. The fate of the activated receptors is complex: oligomerizartion on the cell surface, endocytosis via coated pits, covalent modification (deactivation by dephosphorylation and ubiquitinylation), and endosomal trafficking leading primarily to proteosomal degradation or recycling to the plasma membrane. We have imaged2 the early stages of RTK-dependent signaling in living cells using: (i) stable expression of erbB1/2/3 fused with visible fluorescent proteins (VFPs); (ii) fluorescent Quantum Dots (QDs) to which biotynilated growth factor have been bound (EGF-QD); and (iii) long-term confocal laser scanning microscopy and flow cytometry. We demonstrate that EGF-QDs are highly specific and potent in the binding and activation of the EGF receptor (erbB1), being rapidly internalized into endosomes that exhibit active trafficking and extensive fusion. EGF-QDs bound to erbB1 expressed on filopodia exhibit a previously unreported retrograde transport process to the cell body. QD-ligands are finding widespread use in basic research with many implications for biotechnological screening and assay strategies.

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THE RHOAD FROM HETEROTRIMERIC G PROTEINS TO THE NUCLEUS

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In the last few years we have witnessed an explosion in the knowledge of how cell surface receptors transmit signals to the nucleus thereby controlling the expression of genes involved in many cellular processes, including normal and aberrant cell growth. We have focused on proliferative signaling through G protein-coupled receptors (GPCRs), and shown that certain GPCRs can behave as potent ligand-dependent oncogenes and that G protein ? subunits of the G?$_{12}$ family, G?$_{12}$ and G?$_{13}$, harbor transforming potential. Interestingly, these G proteins do not appear to activate conventional second messenger generating systems, but instead utilize a unique repertoire of signaling molecules to stimulate a network of MAP kinase cascades, often through the activation of small GTP-binding proteins of the Rho family. These observations prompted the search for the underlying mechanism by which G proteins activate Rho GTPases. That led to the discovery of a family of Rho guanine nucleotide exchange

factors (GEFs) that includes p115RhoGEF, PDZ-RhoGEF and LARG, which exhibits an area of homology to regulators of G protein signaling (RGSs) by which G?$_{12}$ and G?$_{13}$ can bind to these RhoGEFs thereby stimulating Rho. In turn, Rho induces the expression and activity of growth promoting genes, including the c-*jun* proto-oncogene. Recent work on the regulation of RGS-containing RhoGEFs by GPCRs and by the axon-guiding molecule Plexin B, as well the functional consequences of genetically deleting these RhoGEFs in mice will be presented. Current efforts addressing the mechanism(s) by which, in turn, Rho regulates c-*jun* expression through two novel PKN- and ROCK-initiated signal transduction pathways will also be presented.

CHEMICAL-BIOLOGY OF CELL SURFACE RECEPTORS: BETTER UNDERSTANDING OF BIOLOGICAL FUNCTION WITH BETTER CHEMICAL TOOLS

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Chemical Biology approaches the study of signaling cascades and biological processes with the use of refined chemical tools designed to manipulate biological pathways.

In the study of cell surface receptors, a preferred method is to reduce natural ligands (e.g. large polypeptides) into small functional units, preferably organic small molecules called peptidomimetics. Peptidomimetics ligands bind to the target receptor. Functional assays with peptidomimetics allow to characterize their pharmacological and cell biological properties, and unmask otherwise unappreciated details about the target receptor and its signaling properties at the atomic level.

Once lead peptidomimetics are identified it is possible to improve their binding and pharmacological properties through Medicinal Chemistry and analoguing, and through rational design into second generation compounds. Alternatively, the leads can be incorporated into a random «high throughput» screen of chemical libraries, which is a preferred method for the pharmaceutical industry.

Analyses of the Structure-Activity Relationships (SAR) of all active, improved, and even of the highly related but inactive peptidomimetics afford detailed knowledge of what is required to agonize or antagonize a particular receptor.

The evolution and uses of receptor-specific agonistic or antagonistic peptidomimetics will be discussed through several examples of compounds targeting G-coupled receptors and single transmembrane tyrosine kinase receptors; using them for studies of receptor signal transduction and molecular pharmacology.

The drug-like properties of the peptidomimetics (small size, chemical agents, proteolytically stable, inexpensive, amenable to improvements, well-defined mechanism of action) make them possible candidates for «drug leads». Thus, the use of peptidomimetic agents as experimental therapeutics in animal models of neurodegenerative disorders and cancer will be presented.

NUCLEAR G PROTEIN-COUPLED RECEPTORS: A PARADIGM BASED ON COGNATE RECEPTORS OF LIPID MEDIATORS

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Platelet-activating factor (PAF) and lysophosphatidic acid (LPA) are ubiquitous lipid mediators that play important roles in inflammation, cardiovascular homeostasis and immunity and are also known to modulate gene expression of specific pro-inflammatory genes. The mechanism of action of these phospholipids is thought to be primarily dependent on their specific plasma membrane receptors belonging to the superfamily of G protein-coupled receptors (GPCR). However, increasing evidence suggest the existence of a functional intracellular GPCR population. It has been suggested that immediate effects are mediated by cell surface receptors whereas long-term

responses are mediated by intracellular receptors. PAF and LPA1 receptors localize at both the nuclear envelope and inside the nucleus of cerebral microvascular endothelial cells of newborn pig, rat hepatocytes and cells overexpressing each receptor, and stimulation of isolated nuclei reveal biological functions, including transcriptional regulation of major genes, namely cylooxygenase-2 and inducible nitric oxide synthase. We will present evidence on the nuclear localization and signaling of GPCRs recognizing PAF and LPA phospholipids as ligands and discuss possible nuclear localization pathways and functional mechanisms by which nuclear PAF and LPA1 receptors activate gene transcription. Intracrine signaling for lipid mediators uncover novel pathways to elicit their effects; moreover, intracellular GPCRs constitute a distinctive mode of action for gene regulation.