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Signal Transduction through the Met Receptor Studied at the Single-Molecule Level
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The human receptor tyrosine kinase Met and its ligand hepatocyte growth factor (HGF) are essential during embryonic development and play an important role during cancer metastasis and tissue regeneration [1]. In addition, it was found that Met is also relevant for infectious diseases and is the target of different bacteria, amongst them *Listeria monocytogenes* that induces bacterial uptake through the surface protein internalin B (InlB) and causes human listeriosis [2]. Many of the mechanistic steps of Met activation, however, are still unclear. Here, we use single-molecule fluorescence microscopy techniques [3, 4] to unravel association and activation of Met receptor through its ligands, HGF and InlB. In particular, we use techniques which provide high temporal and spatial resolution and excellent statistics to study both the spatial distribution as well as the dynamics of Met receptor in eukaryotic cells. In addition, we investigate interactions with membrane microdomains.
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Spatiotemporal Regulation of cAMP-Dependent Protein Kinase (PKA) in Cardiac Myocytes
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Activation of the cAMP/PKA pathway has short term beneficial effects on cardiac function, but is deleterious on the long term, leading to hypertrophic remodelling and heart failure. Here we characterized the regulatory mechanisms governing PKA activation in the cytoplasm and in the nucleus of adult rat ventricular myocytes by using the FRET-based A-kinase activity reporter (AKAR) targeted to these compartments. We show that in response to maintained stimulation of beta-adrenergic receptors (beta-AR) by isoprenaline (Iso, 1 μM), PKA activation is ten times faster in the cytoplasm than in the nucleus. Consequently, pulse stimulation with Iso (15 s, 100 nM) strongly activates PKA in the cytoplasm but marginally in the nucleus. Similar results were obtained when measuring the phosphorylation of endogenous PKA substrates located in these compartments, myosin-binding protein C and the transcription factor CREB. Inhibition of PP1 by inhibitor 1 (I-1) prolonged the AKAR response to Iso pulse in the cytoplasm (t1/2 off =150.2 ± 11.6 s, n=14 versus 78.6 ± 5.7 s, n=13, for Iso alone). A similar effect was also observed with okadaïc acid (100 nM), an inhibitor of PP2A, and with cyclosporin A (5 μM), an inhibitor of PP2B. Simultaneous inhibition of PP1 and PP2A, or of PP1 and PP2B further slowed down cytosolic AKAR dephosphorylation (t1/2 off = 411.1 ± 30.3 s, n=6 and 447.5 ± 43.1 s, n=18, respectively). I-1 also potentiated nuclear PKA activation (FRET ratio increased from 2.5 ± 0.5%, n=31, to 16.1 ± 2.3%, n=25) and increased CREB phosphorylation by about 40% (n=7) in response to Iso pulse. These results indicate that the major cardiac Ser/Thr phosphatases (PP1, PP2A and PP2B) contribute to the termination of the beta-AR response in the cytoplasm and PP1 plays an important role in the regulation of PKA activity in the nucleus.

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Spatial Sensitivity of the Map Kinase Signaling Pathway in the Cellular Cytoplasm
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The MAP kinase signaling cascade plays a crucial role in regulating cellular growth, differentiation and mitosis. The signaling patterns and their sensitivities have been found to be significantly different depending on whether signaling emanates from the plasma membrane (Harding et al. 2005, Cur. Biol., 15:869-873), the Golgi apparatus or endosomes (Blvona et al. 2003, Nature, 424:694-698), implying that the same signaling cascades operate in dissimilar ways depending on the location of activators and/or deactivators in the cell. In order to fundamentally understand this phenomenon, we consider a reaction-diffusion model of MAP kinase signaling involving a four-step cascade as proposed by several recent studies (Kholodenko 2006, Nat. Rev. Mol. Cell Biol., 7:165-176). By applying the calculus of variation to this model, we compute the spatial sensitivity of MAP kinase signaling to small perturbations in a number of physiological parameters such as phosphorylated substrate protein concentration, kinase/phosphatase concentration, and cytoplasmic streaming. The spatial sensitivity analysis reveals that each physiological perturbation leads to its own intracellular spatial localization that maximizes the concentration of phosphorylated proteins directly involved in gene regulation. For instance, the cytoplasmic region close to the nucleus is found to be the region that is most sensitive to perturbations in phosphorylated substrate protein and kinase concentrations. On the other hand, for perturbations in phosphatase concentration and cytoplasmic streaming, the spatial location that exhibits the highest sensitivity depends on where the perturbations are imposed: as the location where the perturbations are imposed is moved downstream, the cytoplasmic region of highest sensitivity to changes in phosphatase concentration and cytoplasmic streaming moves towards the nucleus. The present findings raise intriguing and experimentally testable ideas of spatial organization strategies that cells use to effectively control intracellular signaling.

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Human Epidermis as a Model System for the Investigation of Intracellular Signaling Pathways, In Situ
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The epidermis forms the outermost layer of the skin, and plays a critical role in maintaining organism homeostasis. It is primarily comprised of interfollicular keratinocytes that detach from the underlying basement membrane and execute a terminal differentiation program. This establishes a spatiotemporal differentiation gradient across the depth of the epidermis allowing us to examine intracellular signalling pathwaysusing cells within their native tissue.
The ERK-MAPK signalling cascade has been implicated in regulating keratinocyte differentiation. Due to the importance of this pathway a wealth of information is available on the biochemical nature of its molecular interactions. Thus, fourteen proteins and four phospho-proteins were selected within and around the ERK-MAPK signalling cascade, and their abundance was measured within human epidermis using immunofluorescence labelling with confocal microscopy. The field of view within the resulting image data is large enough to observe keratinocytes at all stages of differentiation, with multiple cells at each relative stage (depth). Furthermore, this was achieved while retaining sufficient resolution to identify the major sub-cellular compartments: cytoplasm; nucleus; and plasma-membrane; allowing localised protein abundances to be measured. These data were subsequently conditioned upon a normalised distance coordinate, allowing boundaries between epidermal layers to be intrinsically defined.As a consequence of this transformation, concomitant changes in protein abundance could be examined along the gradient of keratinocyte terminal differentiation. Measures of statistical association (Pearson correlation and mutual information) were calculated between each localised protein pair, and a collection of published network inference techniques were applied (BANJO, ARACNE/TD-ARACNE, and MIKANA).The relationships inferred by these approaches were analysed with respect to known epidermal and cellular biology, and may provide targets for experimental validation.

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Mapping CEACAM1 Dynamics and Interactions in Live Cells by Quantitative Fluorescence Techniques
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Belonging to the immunoglobulin superfamily of membrane glycoproteins, carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) are involved in diverse range of cellular processes and disease mechanisms through both homotypic and heterotypic interactions, In particular, CEACAM1 has been found to suppress tumorigenesis, hepatic lipogenesis, and systemic inflammation, but interestingly promotes tumour metastasis, angiogenesis, insulin clearance, and bacterial invasion. Molecular level insights into CEACAM1's behaviour are therefore essential for understanding its structure-function relationships. To this end, we have exploited high spatial- and temporal-resolution fluorescence imaging to elucidate the molecular dynamics and interactions of YFP-tagged CEACAM1 transfected into wild-type HeLa cells. Total internal