Tales from the Crypt: Evidence for Heptahelical Receptor Signaling in the Endocytic Pathway

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The endocytic pathway has traditionally been considered a veritable graveyard for signaling receptors, a place where receptors are silently buried beneath the buzz of signal transduction occurring in the plasma membrane. Nowhere has this view been more tenacious than in the world of heptahelical G protein–coupled receptors (GPCRs). GPCR signaling has been thought for many years to occur exclusively in the plasma membrane, wherein an elegantly defined mechanism mediated by G protein–coupled receptor kinases (GRKs) and arrestins can terminate signal transduction before promoting endocytosis of receptors by clathrin-coated pits (*1*–*3*). The endocytic pathway is seen as a kind of purgatory, in which internalized receptors quietly await their fate. Certain receptors are eternally damned by targeting to lysosomes for proteolytic degradation (down-regulation). Other receptors can escape this fate by undergoing enzymatic dephosphorylation, later recycling to the plasma membrane in a functional state (resensitization). In either case, receptors are thought to be functionally inactivated by the time they get endocytosed. There is an increasing body of evidence challenging this view, and suggesting that the endocytic pathway may not be nearly as silent as previously thought. A study from DeFea *et al*. (*4*) puts a new twist on these "tales from the crypt."

The idea that endocytic membranes might play a role in receptor-mediated signal transduction came originally from studies of certain receptor tyrosine kinases that undergo ligand-induced endocytosis by clathrin-coated pits. For example, ligand-activated EGF receptors (EGFRs) were observed to cofractionate with Shc, Grb2, and SOS in endosomal membranes (*5*). Studies of a dominant-negative mutant form of dynamin, a cytoplasmic guanosine trisphosphatase required for endocytosis of coated pits, provided direct functional evidence that endocytosis of EGFRs may be required for efficient activation of the mitogen-activated protein kinases (MAPKs), $p42^{MAPK}$ and $p44^{MAPK}$ (*6*). Studies of certain other receptor tyrosine kinases, such as the neurotrophin TrkA receptor, suggested that signaling from endocytic vesicles might be a fairly general phenomenon (*7*, *8*).

Initial evidence that the endocytic pathway might play a role in signal transduction through GPCRs came from a study indicating that inhibitors of endocytosis attenuate the ability of the β_2 adrenergic receptor to activate MAPK (*9*). Subsequent investigations by many groups suggested that the ability of endocytic inhibitors to block receptor-mediated activation of MAPK is variable, both among individual GPCRs and among various cell types. Nevertheless, in some cases there does appear to be strong evidence that endocytosis is required for full activation of MAPK by GPCRs. Furthermore, there is evidence that nonvisual arrestins (also called β-arrestins) play a key role in MAPK activation that is distinct from their well-defined role in desensitizing receptor-mediated signaling in the plasma membrane. Thus, it was proposed that arrestins form part of a multiprotein signaling scaffold associated with endocytic membranes. This complex is proposed to mediate a distinct "second wave" of signal transduction by way of MAPK, which occurs after receptor signaling at the cell surface through "classical" pathways (such as signaling via adenylyl cyclase) is already initiated (*10*).

The plot thickened quickly when several studies suggested additional roles for the endocytic pathway in mitogenic signaling. For example, activation of MAPK by the heptahelical μ opioid receptor does not require endocytosis of the heptahelical receptor (*11*), yet MAPK activation by this receptor is strongly inhibited by a dominant-negative mutant dynamin that blocks endocytosis of coated pits (*11*, *12*). The same seems to be true of certain other GPCRs, such as the α_{2A} adrenergic receptor in some cells, although present studies differ in the extent to which receptor-mediated activation of MAPK actually requires endocytosis of the receptor in various cell types (*13*–*15*). These observations suggest that, at least in some cases, dynamin-dependent endocytosis of another molecule may be required for MAPK activation by GPCRs. Recent studies indicate that one such molecule may be a transactivated receptor tyrosine kinase such as the EGFR (*15*). This is an appealing hypothesis because EGFRs have been shown to undergo transactivation by certain heptahelical receptors through a novel, somewhat byzantine mechanism (*16*–*18*). Dynamin-dependent endocytosis may also be required for a distinct, downstream step in the pathway of MAPK activation, the phosphorylation of MAPK itself by the mitogen-activated protein kinase or extracellular signal-regulated protein kinase kinase (MEK) (*19*). Together, these observations support the idea that endocytic membranes may be extremely active participants in signal transduction, perhaps serving multiple functions in MAPK signaling by GPCRs.

At this point in our tale the studies of DeFea *et al*. (*4*) come to the fore. DeFea *et al*. studied activation of MAPK by PAR2, a heptahelical receptor that is proteolytically activated by trypsin or mast cell tryptase. PAR2 couples specifically to G_q and activates phospholipase C (PLC), which in turn leads to the activation of protein kinase C (PKC). In contrast, adrenergic and opioid receptors activate MAPK via G_i. PAR2 undergoes activation-induced endocytosis promoted by receptor interaction with arrestins. These events contribute to inactivating "classical" receptor-mediated signaling through PLC. Previous studies suggested that G_q-coupled receptors do not require endocytosis for activation of MAPK, as they appear to activate Ras directly

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from the plasma membrane through a PKC-dependent mechanism (*20*). This is different from the model proposed for the adrenergic and opioid receptors that are thought to require endocytosis for MAPK activation in certain instances (*9*, *10*, *12*). Indeed, a phosphorylation-defective mutant form of PAR2, which is deficient both in arrestin binding and endocytosis, is fully capable of activating MAPK (*4*). Surprisingly, MAPK activation by the wild-type, internalizing PAR2 receptor—but not the mutant receptor—was exquisitely sensitive to a dominant-negative mutant form of arrestin that blocks endocytosis of the receptor (*4*).

To delve further into this peculiar finding, DeFea *et al*. characterized the signaling pathways involved in MAPK activation by the wild-type and mutant receptors. Wild-type PAR2 appeared to activate MAPK by a pathway that was dependent on arrestins but largely independent of Ras, whereas the opposite was true for activation of MAPK by the phosphorylation-defective mutant of PAR2 (*4*). Investigation into the functional consequences of these distinct modes of MAPK activation led to another surprising discovery. Signaling via the wild-type versus the mutant receptor caused profound differences in the localization of the subsequently activated MAPK. MAPK activated by the wild-type PAR2 remained restricted largely to the cytoplasm and was excluded from the nucleus. In contrast, MAPK activated by the mutant receptor was detected both in the cytoplasm and nucleus.

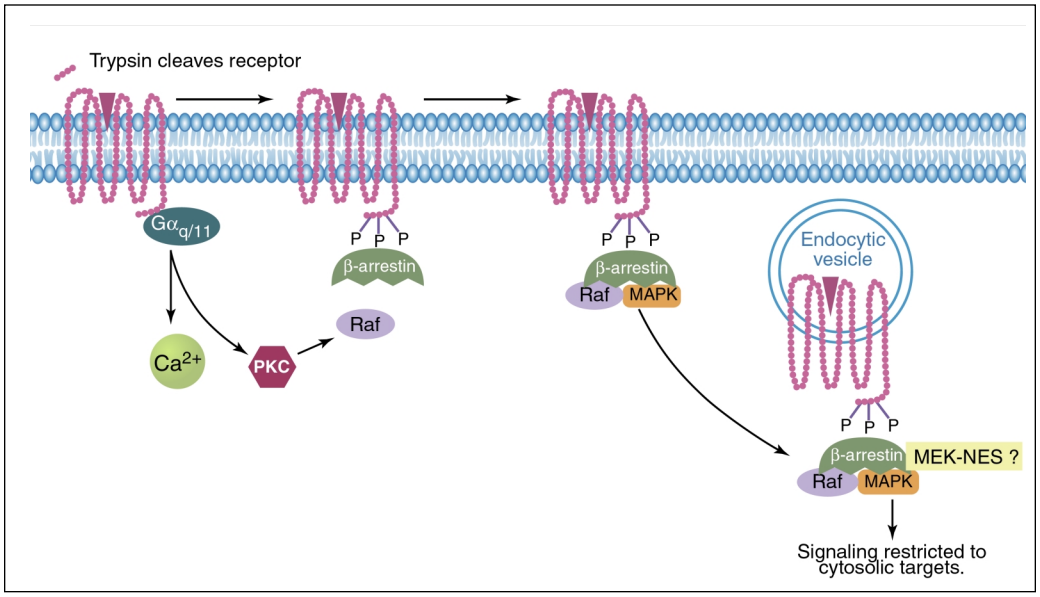

Fig. 1. Proposed structure of the PAR2 endocytic signaling scaffold. DeFea *et al*. provide evidence for an endosome-associated complex containing PAR2, arrestin-2 (β-arrestin-1), Raf, and activated MAPK (ERK 1/2), which they propose controls the subcellular localization of MAPK between nuclear and cytoplasmic compartments (*4*). MEK is included in the diagram, although it was not detected in the complex isolated by DeFea *et al*. (*4*), because MEK activates MAPK and is reported by Fukuda *et al*. (*20*) to contain a nuclear export signal essential for MAPK localization in the cytoplasm.

The molecular basis underlying these different pathways to and consequences of MAPK activation was investigated (*4*). An oligomeric complex present in cells expressing wild-type PAR2 was detected by gel filtration chromatography and coimmunoprecipitation analysis. The formation of this complex was dependent on PAR2 activation, and the complex appeared to contain activated PAR2 along with arrestin-2, Raf, and activated MAPK. Importantly, this complex was not recovered from cells expressing the phosphorylation-defective mutant of PAR2, regardless of whether the mutant receptor was activated. Thus, DeFea *et al*. (*4*) proposed a model in which PAR2 mediates activation of MAPK through an arrestin-containing multiprotein complex that is associated with endocytic membranes, and this signaling complex prevents activated MAPK from entering the nucleus (Fig. 1 and accompanying movie).

These studies are both elegant and provocative. However, the precise role(s) that the endocytic pathway may serve in GPCR signaling are far from clear, and many questions remain. For example, the protein complex isolated by DeFea *et al*. (*4*) has an estimated mass considerably larger than the sum of its known components. What other players are involved? MAPK entry into the nucleus may be controlled by direct protein interaction with MEK, which contains a functional nuclear export signal and may itself associate with endocytic membranes (*21*). Yet MEK was not detected in the arrestin-containing signaling complex (*4*). How does nucleocytoplasmic trafficking of MEK fit into this story, or does the signaling scaffold proposed by DeFea *et al*. (*4*) represent an independent mechanism for controlling the localization of MAPK? Considering the diversity of signaling pathways leading from GPCRs to MAPK cascades in mammals, how generalizable are the results of DeFea *et al*. (*4*) to signaling through other GPCRs (*22*)? Finally, although the results are fully consistent with an important function of a signaling scaffold associated with endocytic membranes, it is still not clear why endocytosis would be necessary for preventing MAPK entry into the nucleus. Does this function of the arrestin-containing scaffold require association with endocytic membranes, or might there be yet another function of the endocytic pathway in this complex signaling pathway that remains to be revealed? While these and many other questions remain to be addressed, the studies of DeFea *et al*. (*4*) provide an intriguing new twist to our understanding of the general importance of intracellular signaling scaffolds (*23*). In particular, these interesting studies will surely stimulate further exploration of the idea that endocytic membrane trafficking mechanisms may serve to guide activated kinases to their appropriate effectors, or may themselves serve as localized "signalosomes" in the cytoplasm. The bottom line is that GPCRs may indeed have an important afterlife in the endocytic pathway. Stay tuned for more tales from the crypt.

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