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# EGF receptor: which way to go?

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## 1. Introduction

Not so long ago, everything looked very simple, as the EGF receptor (EGFR) was thought to sit on the cell surface and wait for the incoming ligand to bind. Upon binding of EGF, the receptors dimerized and the cytoplasmic tails, containing the kinase domains, activated each other by transphosphorylation. The phosphorylated tyrosines, usually six, then served as docking sites for the effector molecules, normally other kinases or enzymatically sterile adaptor proteins. They further conveyed the message to the machinery involved in cell proliferation and differentiation. The specificity of the signaling was seen to be dictated primarily by the nature of the ligand and the receptor with further electiveness built into the interactions between the cytoplasmic 'business' component of the receptor and its effectors. The command lines were viewed as separate rows of arrows from the cell surface to the interior. The cessation of signaling was marked by the uptake of the receptor by the endocytic system and by its demise in the lysosomes.

It was soon apparent that something else was taking place as well. One of the curious observations in need of clarification was the capacity of EGFR to carry on its mitogenic functions even when its major phosphorylatable tyrosines were eliminated [1]. There was also the unresolved question of what the receptor was doing during transit to endosomes and lysosomes; after all, during this trip in the cytosolic vesicles, the receptor exposes its activated cytoplasmic tail into a sea of putatively free-floating effector molecules [2]. In some cells, moreover, tickling with the receptor implied the tolling of apoptotic death bells, quite unexpected of a protein thought to be dedicated to coaching its effectors into promoting cell proliferation and differentiation [3]. And still looming in the background was the question of how the specific outcomes in various types of cells are generated, given the apparently limited number of options inherent from the structure of the receptor and receptor/effector complexes. In other words, where are the 'missing' determinants of specificity?

In this issue of FEBS Letters, three articles explore some of these lesser known facets of EGFR. First, relating to determinants of specificity from EGFR signaling, Jang et al. [4] show that PLCγ1 (one of the downstream effectors of EGFR) colocalizes with the receptor in caveolae. These are the sites where PIP2, the substrate of PLCγ1, is also concentrated and this colocalization is critical for the signaling from PLCγ1. Secondly, Högnason et al. [5] show that an elevated level of EGFR in certain cells leads to apoptosis in a manner that depends on the kinase activity of the receptor and which is enhanced by blocking the Ras pathway. In the third paper, Bae et al. [6] show that EGFR is a substrate for caspases and suggest that proteolytic inactivation of the receptor is a crucial part of a general apoptotic signaling cascade.

## 2. EGFR and caveolae

Caveolae are currently viewed as specialized membrane domains in which signaling pathways, or at least their very proximal components, sit preorganized waiting for the ligand to call them to action. As to the EGFR, it has already been known for some time that in unstimulated cells EGFRs are concentrated in the caveolae [7,8], in which also EGFRs' downstream adaptors and effectors are enriched. It has also become clear that many of the critical steps in the EGFR pathway (signaling, activation of the receptor itself, phosphorylation of its substrates, recruitment of the adaptors and kinases, and activation of MAPKs) take place in the caveolae [9].

The exit of the EGFR from the caveolae and the subsequent engulfment in the coated pits and endosomes is not only a prelude to the down-regulation of the signal but also part of active signaling. Mineo et al. [8] have put forward a scheme in which the activated receptor in transit could, in its various locations, induce different sets of signals. In this view, the question of the 'missing' determinants of specificity is partially resolved by envisioning a migratory receptor (in complex with effectors) that arrives at various stations (caveolae, bulk membrane, coated pits, endosomes) in which different types of signaling machineries are activated depending on the local 'ecology'. This new model is in concert with the idea proposed by Vieira et al. [10], which is based on studies indicating that only part of the signaling effects of an activated EGFR become manifested in endocytosis-deficient cells. In this vision, ligands have evolved to regulate their signals by EGFR trafficking [11]. Just recently, the first components of the molecular machinery that integrate EGFR signaling and receptor trafficking have come to light [12].

A new piece of evidence in support of the scheme underlining the importance of the spatial aspects of the signaling pathway is now provided by Jang et al. [4]. They demonstrate that, in response to EGF, PLCγ is moved to and becomes phosphorylated in caveolae. Pretreatment with MβCD (a cholesterol-binding compound that delocalizes PIP2 from the caveolae) led to inhibition of PtdIns turnover. However, it did not diminish phosphorylation of PLCγ. Thus, colocalization of the substrate and the enzyme in the caveolae is a prereq-


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uisite for PLCγ-mediated PtdIns turnover, one of the major signaling events induced by EGFR. Interestingly and fitting with the results of Jang et al., Haugh et al. [13] have recently shown that EGFR signaling through the PLC pathway at the cell surface is spatially restricted at a point between PLCγ1 phosphorylation and PIP2 hydrolysis. Obviously, there is still a lot of uncharted territory here. According to a recent suggestion, EGF stimulates redistribution of an entire signaling complex involving Ras, Raf-1, Mek and MAPK from the caveolae to the endosomes in which the rest of the signaling events could take place [14].

## 3. EGFR and apoptosis

EGFR is mostly referred to as a regulator of cell proliferation, migration and differentiation. For a long time it has been known, however, that in some cell types EGFR can also, 'paradoxically', mediate apoptotic signals. It is seen especially in cells overexpressing EGFR (for instance A431) and in some tumor cells. However, it also occurs in normal epithelial cells and organisms. Its mechanism is poorly understood. EGF-induced attenuation of cell adhesion [15], EGF acting as a dedifferentiation factor for some cells [16] and down-regulation of EGFR in response to EGF [17] have all been suggested as possible mechanisms.

In this issue, Högnason et al. [5] have studied the mechanism of the 'paradoxical' apoptosis-inducing effect of EGF stimulation. They show that experimental increases in the level of EGFR expression predictably lead to apoptosis in a variety of cell types. This effect is dependent on tyrosine kinase but does not require autophosphorylation of the receptor. Blocking of the Ras signaling augments the apoptotic effect, most likely by impairing Akt activation. As a mechanism, it is proposed that in EGFR-overexpressing cells a threshold may be reached, beyond which the balance is tipped from growth gain to apoptosis due to misregulation of the signaling circuitry. Clearly, a role for EGF in anti-proliferative signaling and apoptosis is gaining ground. One of the important still unresolved questions is whether EGF-induced apoptosis is due to anoikis [18] or is independent of cell adhesion, as proposed by Högnason et al. Further studies are needed to delineate the stoichiometries of EGFR and its effectors as determinants of proliferation vs. apoptosis.

It is noteworthy that EGF-induced apoptosis also occurs in normal cells and is part of 'normal' physiology. In these cases, excessive signaling may not be as credible an explanation as in tumor cells. Could it be that in these cells, similar to the scheme proposed by Mineo et al. [8], the receptor stops by 'stations' in which everything is geared (unlike in other cells) to induce apoptosis instead of e.g. differentiation? Such a predisposition to apoptosis upon EGF-binding could be due to either hardwiring that is inherent in the differentiated phenotype of the cell, or to coordinated cross-talk between different signaling pathways.

Another window to view the fates and ways of EGFR and its relation to apoptosis is the report by Bae et al. [6], in which EGFR is shown to be a target of an incapacitating cleavage by apoptotic caspases 1, 3 and 7. The authors suggest that this may be the way the anti-apoptotic signals, normally emanating from the EGFR and including possible activation of PI3K and PKC-α, are abrogated. It remains to be explored, however, whether the inactivation of the anti-apoptotic mechanisms plays only a minor role in the play of apoptosis or whether incapacitating the mitotic receptor in a way analogous to growth factor withdrawal could be a more proximate factor in the initiation of the death cascade.

Curiously, the growth inhibitory effect of EGF on A431 cells is one of the early observations on the effects of EGF on cultured cells [19]. Apart from the current results there are also some other recent data that help us to make sense of this 'anomaly'. Namely, EGF, together with interferon γ, induces (in a STAT dependent manner) the expression of the caspase 1 enzyme and the cell cycle inhibitor P21WAF1/CIP1, which are involved in apoptosis and growth inhibition, respectively [20,21].

## 4. Location definitely plays a role

It is becoming increasingly clear that not only the intrinsic properties of the EGFR prior, upon, and subsequent to its activation by the ligand-binding, but also its position relative to the various locations that are available to it are of importance as to what its impact will be on the cell phenotype. One aspect of this differential effect of the location on the receptor function is already well established, i.e. differential response in different cell types. It is evident that, in the future, more discriminating approaches (á la mode functional topography) are needed in which the differential functioning of not only EGFR but also other receptors in various cell locations and under various conditions is scrutinized.

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