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Keeping in touch with the ER network:

Growth factor receptor internalization occurs at sites of transient membrane contacts

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The epidermal growth factor receptor (EGFR) has fundamental roles in physiology and cancer. It is rapidly internalized (endocytosed) from the cell surface upon ligand binding and activation and can either sustain its signaling by being recycled back to the plasma membrane or get delivered to the lysosome where it is degraded (1). After much debate, it is currently thought that EGFR endocytosis involves clathrin-mediated (CME) and nonclathrin (NCE) mechanisms depending on the ligand concentration and cellular context, with the molecular details of the latter route poorly defined (1, 2). On page 617 of this issue, Caldieri *et al.* (3) report that the NCE pathway involves transient endoplasmic reticulum (ER)–plasma membrane contacts formed close to the sites of EGFR internalization.

Using a proteomic approach, Caldieri *et al.* found more than 100 proteins enriched in purified EGFR-containing NCE vesicles. Further RNA-silencing experiments identified nine of these proteins as EGFR-NCE regulators, including, surprisingly, four ER proteins. Among them, the ER tubulation factor reticulon 3 (RTN3) was found to be an essential regulator. Although RTN3 localized specifically to the ER in all conditions tested, it was found in close proximity to activated EGFR. This suggests the involvement of potential ER–plasma membrane contacts because the ER has frequent contacts with various organelles (4). The authors further demonstrated that RTN3 is required for transient ER–plasma membrane contacts at sites of membrane invagination where EGFR is endocytosed. These membrane contact sites showed increased Ca^{2+} release from the ER, an event that is required for membrane fission, which is the final step of EGFR internalization. Indeed, Ca^{2+} release has been observed at the site of ER contact with other organelles and plays fundamental roles in local trafficking events (5). The findings of Caldieri *et al.* advance our understanding of EGFR-NCE by defining ER–plasma membrane contacts as an essential signaling platform that regulates nonclathrin-mediated invagination, maturation, and fission of EGFR-containing membrane.

Intriguingly, the ER–plasma membrane contacts were selectively observed in EGFR-NCE but not for CME. The NCE pathway is correlated with the lysosomal degradation of activated EGFR, whereas a large percentage of receptor recycling occurs downstream of CME (6). Whether NCE activates specific downstream signaling molecules that are different from those in CME remains to be determined. However, EGFR-NCE, in response to various pathological and therapeutic stress stimuli, results in unusual EGFR functions in many

cancer cells that provide a survival advantage and resistance to therapeutics (7). Given the increasing interest of cancer biology fields in ligand-independent, stress-induced EGFR trafficking and signaling pathways, many of which are based on EGFR-NCE (7), it would be interesting to investigate the roles of ER–plasma membrane contacts in EGFR endocytosis in these situations. Understanding the molecular mechanisms and physiological and pathological consequences of EGFR-NCE could be important for developing approaches to overcome cancer therapeutic resistance.

Is it so surprising to find a role for ER–plasma membrane contacts in EGFR-NCE? Although all cellular functions are ultimately carried out by proteins, whole-cell and compartmental structures are based on lipids and membranes. The physical and functional interactions between proteins and membranes undergoing extensive and highly regulated trafficking lay the ground for most, if not all, cellular processes, including receptor trafficking (8). Transient membrane contacts are one of the best examples of dynamic protein-membrane interplay. They involve specific protein-protein interactions that tether two distinct subcellular compartments, such as ER contacts with the plasma membrane, endosomes, the Golgi complex, mitochondria, and peroxisomes (4). These membrane contacts, transient or constant, dynamically control local cellular events such as receptor sorting and signal transduction.

EGFR regulation by ER membrane contacts actually goes far beyond the plasma membrane (see the figure). The ER contacts most other subcellular membrane compartments, including contacts with endosomes that control endosomal fission (9). Endosomal EGFR frequently co-localizes with ER contacts but is retained at the endosome during the ER contact–mediated endosomal fission (9). During endosomal trafficking, before being sorted onto intraluminal vesicles in the lumen of the multivesicular body (MVB; also called late endosomes), the EGFR is dephosphorylated by protein tyrosine phosphatase 1B (PTP1B), which is localized at the ER surface (10). This process is mediated by the ER–MVB membrane contacts (11) tethered by endosome-localized Annexin A1 and the ER-protein S100A11 (12). ER–EGFR endosome contacts also promote cholesterol transport from the ER to endosomes, which in turn facilitates EGFR intraluminal sorting (12). Stress-induced EGFR function at endosomes may also involve ER–endosome contacts. The role for endosomal inactive EGFR in initiating autophagy (an intracellular self-degradation process) depends on the release of Beclin 1 (a protein involved in autophagy) at the endosomal surface (13). However, autophagy initiation may also require activation of autophagy related 14 (ATG14) at the ER by phosphatidylinositol 4,5-bisphosphate (PIP₂) (14). This lipid is generated by a kinase that associates with a complex at the endosomal surface that contains inactive EGFR and the lysosomal-associated protein transmembrane 4B (LAPTM4B) (13–15). Thus, both active and inactive EGFR are broadly regulated by ER contacts at multiple subcellular membrane sites.

Accumulating studies have demonstrated the importance of ER membrane contacts with various organelles in physiology and in diseases, such as autoimmunity and neurodegeneration. Recent discoveries of molecular details underlying different membrane contact sites include protein tethers that bridge membrane gaps, local Ca²⁺ signaling, lipid metabolism and transport, and actin polymerization (4, 5). The role for ER–plasma

membrane contacts in EGFR-NCE further expands this exciting area and will serve as a new platform for future studies of nonclathrin-mediated receptor endocytosis.

The molecular tether(s) and downstream effectors for the ER–plasma membrane contacts during EGFR-NCE have yet to be identified. It is also unclear whether local lipid transport is required, and how local membrane contacts coordinate the activity of dynamin and actin to drive membrane invagination and fission. Could the mechanism proposed by Caldieri *et al.* apply to EGFR-NCE in response to stress stimuli, such as chemotherapies, radiotherapies, and hypoxic or oxidative stress? Do ER–endosome contacts regulate stress-induced EGFR functions at nondegradative endosomes or mitochondria, and what are the molecular details? Addressing these questions should illuminate the roles and regulation of EGFR in cell and cancer biology.

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Figure. Contact at multiple sites

Epidermal growth factor receptor (EGFR) trafficking and signaling are regulated by ER membrane contacts. Three examples of local membrane contacts in EGFR regulation are shown: ER–PM contacts in EGFR nonclathrin endocytosis; ER–MVB contacts in EGFR dephosphorylation and sorting; and potential ER–MVB contacts in inactive EGFR-mediated autophagy. PIPK γ 5, type Ig phosphatidylinositol 4-phosphate 5-kinase isoform 5; P, phosphate.