[Communicative & Integrative Biology 2:1, 35-36; January/February 2009]; ©2009 Landes Bioscience

Article Addendum

Remodeling of the Golgi structure by ERK signaling

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Key words: Golgi, centrosome, migration, ERK signaling, GRASP65

Emerging evidence suggests that the Golgi functions as a regulatory node for various signaling cascades. Modules of the MAPK pathway are targeted to the Golgi upon stimulation of cells with mitogens. The target for activated ERK on the Golgi membranes is GRASP65, a peripheral membrane protein required for Golgi cisternal stacking. Phosphorylation of GRASP65 at Serine 277 results in a loss of its oligomerization and causes unstacking of Golgi cisternae. This reorganization of the Golgi structure is required for the polarization of the Golgi and the centrosomes towards the leading edge in migrating cells. Preventing GRASP65 phosphorylation with mutants lacking the phosphorylation site blocks Golgi and centrosome orientation. This demonstrates a mechanism for cell polarization involving dynamic remodeling of the Golgi mediated by local phosphorylation of a Golgi protein induced by mitogen signaling.

Several signaling transducers involved in the regulation of secretion, cell motility and cell proliferation have been localized on the Golgi, including Src family kinases (SFKs),[1] protein kinase D (PKD),[2] trimeric G proteins,[3] Cdc42,[4] and modules of the mitogen-activated protein kinase (MAPK) pathway such as Ras and ERK.[5,6] The recruitment of signaling molecules to the Golgi increases the complexity and specificity of signaling pathways.[7,8] On the other hand, signaling on the Golgi also regulates its function. The flux of cargo proteins through the Golgi is fine-tuned by signaling cascades to respond to varying growth conditions. An increased load of cargo arriving from the ER activates SFKs on the Golgi membranes, which accelerates transport through the Golgi and therefore secretion.[1] Another signaling circuit that may cause an increase in cargo output from the Golgi is the MAPK cascade, one of the essential contributors to cell proliferation and differentiation. While the SFK pathway senses the intracellular change in cargo load, the MAPK cascade responds to the extracellular cues.

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Submitted: 11/14/08; Accepted: 11/17/08

Previously published online as a *Communicative & Integrative Biology* E-publication: http://www.landesbioscience.com/journals/cib/article/7421

Addendum to: Bisel B, Wang Y, Wei JH, Xiang Y, Tang D, Miron-Mendoza M, Yoshimura S, Nakamura N, Seemann J. ERK regulates Golgi and centrosome orientation towards the leading edge through GRASP65. J Cell Biol 2008; 182:837–43; PMID: 18762583; DOI: 10.1083/jcb.200805045.

Binding of the epidermal growth factor (EGF) to its cognate receptor activates ERK, which then translocates into the nucleus to stimulate the transcription of genes involved in cell proliferation and differentiation. The specificity and the strength of the response can be modulated by directing and/or by restricting active ERK to distinct subcellular compartments via different sets of scaffold proteins.[9,10] Among these scaffolds, Sef recruits active MEK/ERK to the Golgi, which competes with ERK translocation into the nucleus and thereby fine-tunes the transcriptional response.[11] In addition to the role as a spatial regulator, Sef might facilitate to selectively activate downstream ERK targets on the Golgi. GRASP65, a peripheral Golgi membrane protein, is phosphorylated by ERK and might therefore participate in mitogen-induced signaling on the Golgi.[12] Whether Sef is required for the phosphorylation of GRASP65 by ERK needs to be established.

Our recent study showed that the phosphorylation of GRASP65 by ERK causes remodeling of the Golgi structure.[13] Stimulation of cells with mitogens (EGF, LPA or serum) activates ERK, which then phosphorylates GRASP65 at Ser277. The same residue is also phosphorylated in mitosis, but by cdk1/cyclinB1.[12,14] GRASP65 phosphorylation by either kinase triggers similar structural changes of the Golgi. Upon mitotic phosphorylation of GRASP65, the Golgi cisternae unstack, which facilitates the extensive fragmentation and vesiculation of the Golgi in preparation for its subsequent partitioning.[15-18] Similarly, GRASP65 phosphorylation by ERK causes the cisternae to come apart. Unstacked cisternae provide an increased surface area from which vesicles can bud, thereby resulting in a boost of protein transport through the Golgi to the plasma membrane.[17] An increased rate of secretion triggered by ERK activation may account for the higher secretory demands during cell proliferation and differentiation.

Although phosphorylation of GRASP65 during interphase and mitosis leads to similar structural changes of the Golgi, it is induced by different kinases and for distinct processes. While the remodeling of the Golgi is required for mitotic progression and Golgi division,[15,19] reorganization of the Golgi structure by ERK in interphase is essential for the establishment of cell polarity preceding cell migration.[13] In the initial step of cell migration, the centrosomes and the Golgi reposition towards the direction of migration, so that membranes and proteins can be delivered to the leading edge.[20,21] We showed that the orientation of the Golgi is induced by mitogens that activate ERK signaling on the Golgi and induce Golgi reorganization. The target for ERK is GRASP65, which forms homo-oligomers in trans and thereby links adjacent cisternae together into stacks.[12,16]

Phosphorylation of GRASP65 by ERK causes the loss of oligomerization and Golgi cisternal unstacking. Suppression of ERK activity by chemical inhibitors or expression of GRASP65 mutants lacking the phosphorylation site inhibits Golgi orientation. Furthermore, the centrosomes also fail to orient when Golgi reorganization is inhibited, which is rescued by pharmacological disassembly of the Golgi with Brefeldin A. This demonstrates that the movements of the Golgi and centrosomes are highly coordinated during cell polarization. Our results further suggest that the widely-accepted mechanism of centrosome orientation during cell migration, regulated by Cdc42 and driven by local actin polymerization,[22,23] is sufficient only with concurrent Golgi remodeling.

The remaining question involves the mechanism(s) responsible for the observed inhibition of centrosome orientation. One possibility is the mechanical resistance from an intact Golgi. The Golgi may be too large to be moved by the polarization forces acting on the centrosome and microtubules. Breaking the Golgi by unstacking reduces the size and therefore allows the mobilization of the Golgi. Another possibility is a feedback mechanism that couples the orientation of centrosomes and the Golgi. Cues for centrosome orientation may be generated or activated by molecules that regulate Golgi reorganization. Upon unstacking, these molecules could be released or exposed and then exert effects on centrosomes. In any case, our results demonstrate that centrosome polarization cannot be achieved without Golgi reorganization. Given this evidence, GRASP65 acts as a negative regulator of Golgi remodeling and cell polarization in a closely integrated mechanism that regulates centrosome orientation.

In line with this study, a role for Golgi-localized signaling that is directly involved in Golgi polarization has been described.[24] The Golgi matrix protein GM130 acts as a scaffold for the kinases YSK1 and MST4, which regulate the orientation of the centrosomes and the Golgi in migrating fibroblasts. Upon downregulation of either YSK1 or its receptor GM130, Golgi and centrosome polarization are lost.[24,25] We have demonstrated another aspect of Golgi-localized kinase activity that is required for cell polarization, emphasizing the Golgi as an organelle not only important for serving as a signaling platform but also capable of exerting its function such as secretion and cell polarization in response to stimuli.

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