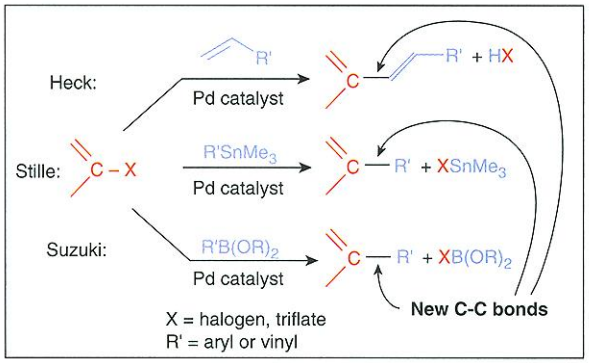

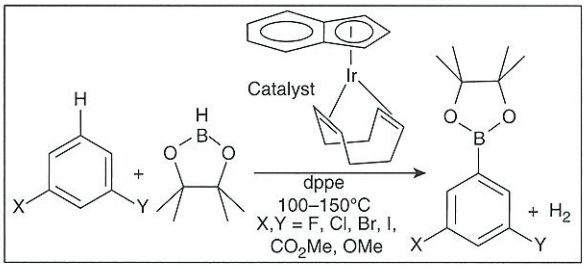

A versatile route to carbon-carbon bonds. (Left) Catalytic formation of carbon-carbon bonds can be accomplished with palladium (Pd) catalysts, but prior methodologies require the existence of a functionalized carbon center. **(Right)** The new chemistry reported by Cho *et al.* (*1*) allows direct conversion of aromatic C–H bonds to aryl-boronate products, which can be used in a variety of carbon-carbon bond-forming reactions without the need for functionalization.

mains intact. Esters and ethers also do not interfere with the reaction. It is remarkable (and fortunate) that no aryl halide-aryl boronate coupling is observed even though both species are present in the reaction mixture.

Second, "tandem reactivity" has been demonstrated by performing the iridium-based borylation of aryl halides in the presence of a palladium catalyst. The iridium catalyst first borylates the aryl halide to give a halo- and boryl-substituted arene. This difunctional arene then undergoes palladium-catalyzed Suzuki coupling with itself, resulting in the formation of a polyphenylene material. This method is likely to increase further the scope for industrial applications of Suzuki coupling.

Third, a further iridium catalyst, derived from the first, can perform borylation of the highly reactive aryl iodides while leaving the aryl-iodine bond intact. The ability to selectively produce aryl boronates with iodide functionality should greatly extend the range of applicability of the Suzuki reaction.

Finally, the authors make a strong case for the presence of Ir(III)/Ir(V) species as intermediates. Further studies will be required, however, to elucidate the detailed mechanism of the arene borylation reaction.

Cho *et al.* describe an important new route to aromatic precursors to carbon-carbon bond formation. The reactions are quite general and selective and are likely to find widespread application in a variety of synthetic processes. The catalysts permit the direct conversion of arene C–H bonds to reactive C-BX_2 intermediates without the necessity of initial functionalization of the C–H bond and represent an efficient means for building up organic frameworks in complex molecules.

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PERSPECTIVES: CELL BIOLOGY

Slick Recruitment to the Golgi

Vytas A. Bankaitis

The Golgi complex is the central protein sorting station of the cell. Here, proteins destined for secretion are segregated into vesicles and are transported to the cell surface. As part of its trafficking duties, this organelle must execute a complicated program of vesicle formation and recycling. Vesicle biogenesis first requires the coordinated recruitment to Golgi membranes of the proteins that direct vesicle formation. Complexes of these proteins form vesicle budding machines that transform Golgi membranes with low surface curvatures into highly curved structures. Subsequently, the closely apposed Golgi membrane lipid bilayers positioned at the necks of nascent vesicles resolve in a fission reaction that liberates cargo-laden vesicles from the organelle. The intricacies of this process become particularly pronounced at the trans-Golgi network (TGN) from which are formed distinct vesicle populations destined for the plasma membrane, secretory vesicles, or lysosomes.

How are the TGN membrane domains that are involved in the formation of different vesicle populations segregated? What are the cues for recruiting particular proteins to specific areas of the TGN? What other components must be brought to these domains so that vesicle assembly can be initiated? How are vesiculation reactions limited so that Golgi integrity is preserved? The incisive work of Baron and Malhotra (*1*) on page 325 of this issue yields some fascinating insights. These investigators make a compelling case that diacylglycerol (DAG), a signal-transducing lipid, is essential for recruitment of a vesicle biogenesis factor, protein kinase D (PKD), to mammalian cell TGN membranes so that a specific class of transport vesicle can be formed. These findings uniquely connect a lipid with a protein kinase that participates directly in the budding of TGN-derived vesicles.

PKD consists of two cysteine-rich domains (C1a and C1b), a pleckstrin homology (PH) domain, and a catalytic domain (see the figure). The connection between PKD and the Golgi arose from analyses of how ilimaquinone (a natural product of marine sponges) affects wholesale vesiculation of the Golgi in mammalian cells. Baron and Malhotra discovered that ilimaquinone hyperstimulates PKD, resulting in the inappropriate elevation of Golgi-derived vesicle formation (*2*). In contrast, a reduction in PKD activity results in failure of Golgi tubules to undergo fission and resolution into transport vesicles (*3*). Thus, PKD regulates TGN vesicle formation.

How does cytosolic PKD engage the TGN? Although PKD has a PH domain, this module is not involved in its recruitment by the TGN. Rather, Baron and Malhotra present several lines of biochemical evidence supporting the notion that the C1a domain of PKD binds to DAG and that this binding is a prerequisite for PKD's recruitment to the Golgi. Moreover, they show that compromising DAG production by treating cells with pharmacological agents such as fumonisin B1 or propanolol

The author is in the Department of Cell and Developmental Biology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. E-mail: vytas_bankaitis@med.unc.edu

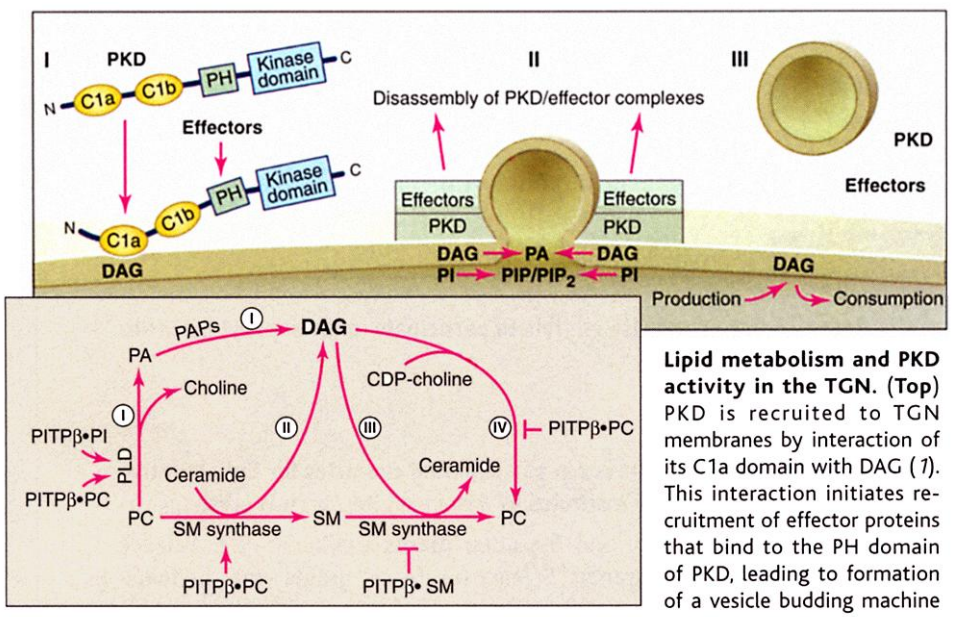
prevents PKD recruitment to the TGN and blocks protein trafficking from the TGN to the cell surface. This secretory block is specific and it occurs without morphological alteration of the TGN. Finally, the authors show that incorporation of a synthetic short-chain form of DAG into the plasma membrane results in acute mistargeting of PKD to that organelle (*1*). Taken together, the data show that DAG is the entity that recruits PKD to target TGN membranes, and that this recruitment is an early event in the vesicle biogenesis program.

It remains unclear how PKD promotes tubule fission. The PH domain of PKD—which binds to phosphatidylinositol (PI) 4-kinase, PI-4-P 5-kinase, the η and ε isoforms of protein kinase C, 14-3-3 proteins, Bruton's tyrosine kinase, and heterotrimeric G protein β and γ subunits—is poised to interface with a variety of signaling pathways. Indeed, PKD activates PI kinases whose products may contribute to the fission reaction. Phosphatidic acid (PA) is also implicated as a fission-promoting lipid (*4*). Perhaps the DAG pool that recruits PKD to the TGN becomes a substrate for DAG kinases. In this two-stage scenario, the DAG pool forms a recruitment/assembly platform for a vesicle-budding machine and is subsequently converted into a pool of PA, which endows nascent vesicle membranes with physical properties compatible with fission (see the figure).

The Baron and Malhotra work raises the issue of which signaling pathways generate and consume the TGN pool of DAG that recruits PKD. Specifically, their work implies an intimate connection between lipid metabolic pathways and Golgi activity. There are two lipid metabolic pathways that set up the Golgi for DAG production. In the first pathway, phospholipase D hydrolyzes phosphatidylcholine (PC) to PA and choline. PA is then dephosphorylated to DAG by PA phosphatases (PAPs). Because PC is the most abundant lipid in mammalian membranes, this pathway potentially serves as a robust vehicle for DAG production (see the figure). Indeed, the finding that propanolol-mediated PAP inhibition blocks PKD recruitment to Golgi membranes in vivo supports the notion that PAP contributes to the Golgi DAG pool (*1*). Reports that phospholipase D becomes localized in the Golgi are also consistent with this model (*5*).

The second pathway involves sphingomyelin (SM) synthase, a Golgi enzyme that interconverts PC and SM. Both PC and SM share a phosphocholine head group, but differ in their backbones, DAG and ceramide, respectively. Thus, SM synthase engaged in SM production consumes PC and ceramide and liberates SM and DAG (see the figure). This reaction is compromised by the ceramide synthase inhibitor fumonisin B1 used by Baron and Malhotra (*1*). Because the SM content of mammalian cell Golgi membranes is ~12 mol % of total lipid, a large DAG pool must be generated therein as a result of SM synthesis (*6*). The potent capability of Golgi membranes to produce DAG must be countered by efficient pathways for DAG clearance—and here "household" pathways for PC biosynthesis may be involved. The conversion of SM to PC by SM synthase consumes SM and DAG, generating ceramide and PC. Thus, regulating the direction of SM synthase activity may be critical for the homeostasis of the Golgi DAG pool. The robustly active cytidine diphosphate–choline pathway for PC biosynthesis is also likely to contribute to DAG clearance as a result of the stoichiometric consumption of DAG for every PC molecule synthesized.

How might the TGN maintain an essential balance between DAG-generating and DAG-consuming pathways such that an environment permissive for vesicle formation is created? Studies in yeast, where the first clear linkage between the TGN and lipid metabolism was discovered, suggest possibilities. A PI-PC transfer protein, Sec14p, is essential for the transport of protein vesicles from the yeast TGN (*7*). Sec14p may, at least in part, regulate the homeostasis of the Golgi DAG pool (*8*). Consistent with this idea, Sec14p inhibits metabolic flux and therefore DAG consumption, through the cytidine diphosphate–choline pathway for PC biosynthesis. Ablation of the cytidine diphosphate–choline pathway fully restores TGN secretory activity to Sec14p-deficient yeast (*8*, *9*). Although the protein targets of DAG action in yeast Golgi remain mysterious, mammalian cells express a Sec14p ortholog (PITPβ) that becomes localized on the Golgi complex and binds to PI, PC, and SM lipids. PITPβ is an important Golgi housekeeping protein—mice carrying mutations in PITPβ die early in embryogenesis, and PITPβ may be essential for stem cell viability (*10*). The lipid-binding substrates of PITPβ identify this protein as a potential regulator of pathways for DAG production and consumption in mammalian TGN (see the figure). It is possible that PITPβ controls PKD recruitment to mammalian TGN membranes and thereby regulates TGN secretory competence.



Lipid metabolism and PKD activity in the TGN. (Top) PKD is recruited to TGN membranes by interaction of its C1a domain with DAG (*1*). This interaction initiates recruitment of effector proteins that bind to the PH domain of PKD, leading to formation of a vesicle budding machine that deforms TGN membranes into short tubules. This process is limited by the catalytic activity of PKD, the conversion of PI to phosphoinositides (PIPs), and the conversion of DAG to the fission-promoting lipid PA (*2*). This results in timed disassembly of the vesicle budding machine (*2*), making its components available for another round of budding (*3*). **(Bottom)** TGN DAG is produced by the phospholipase D (PLD)/PAP pathway (*1*) and by SM synthase–mediated conversion of PC to SM (*2*). DAG is consumed by PC biosynthetic reactions that involve SM synthase (*3*) or the cytidine diphosphate (CDP)–choline pathway (*4*). Golgi-associated PITPβ binds to the lipids PI, PC, and SM. The bound form of each lipid may regulate DAG homeostasis by promoting production pathways and down-regulating consumption pathways.

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