genuinely anapsid-grade reptile which has convergently evolved several carapace-like traits (Figure 1C). These scenarios could be investigated by applying a genomic scaffold to the phylogenetic analyses: enforcing relationships among living taxa to conform to the molecular evidence (e.g. turtles as sister-group to archosaurs), and then using morphological data to best place all fossil taxa within this framework.

Whether or not the affinities of *Eunotosaurus* with turtles are eventually confirmed, the novel similarities identified in recent studies [3,15,16] will ensure that this enigmatic taxon occupies a pivotal position in future investigations. The resurrection of *Eunotosaurus* from obscurity highlights how preconceived relationships can hinder phylogenetic analyses (taxa cannot be inferred to be related if they are never simultaneously considered), and how development, genomics and the fossil record are mutually relevant.

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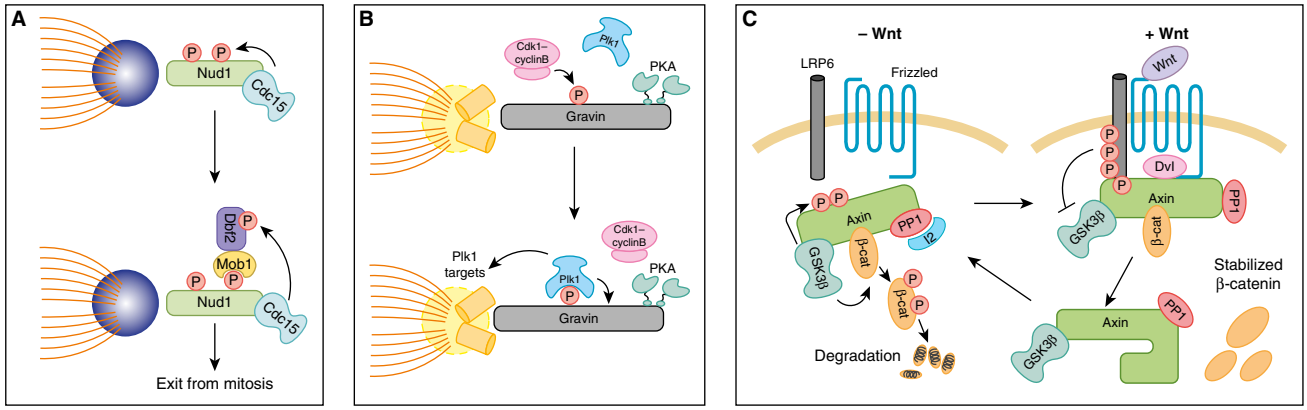
Scaffolding Proteins: Not Such Innocent Bystanders

Sequential transfer of information from one enzyme to the next within the confines of a protein kinase scaffold enhances signal transduction. Though frequently considered to be inert organizational elements, two recent reports implicate kinase-scaffolding proteins as active participants in signal relay.

F. Donelson Smith and John D. Scott

Signaling networks are exquisitely organized to respond efficiently to external stimuli. Scaffolding and anchoring proteins provide a molecular framework for the integration, processing and dissemination of intracellular signals. Not surprisingly, the concept of enzyme scaffolding has profoundly influenced our thinking about how particular signaling events occur within precise intracellular environments and are insulated from promiscuous crosstalk. Early work identified scaffolds that consolidate kinase-signaling cascades. For example, Ste5 in yeast, and the mammalian proteins KSR (kinase suppressor of Ras) and JIP (JNK-interacting protein) organize multi-enzyme MAP kinase assemblies that relay phosphorylation-dependent signals to potentiate activation of the terminal 'transduction' enzyme [1,2]. A variation on this theme is the family of A-kinase anchoring proteins (AKAPs) that compartmentalize combinations of signaling enzymes that respond to distinct inputs. AKAPs nucleate multimeric protein complexes that cluster signal activation components, such as G-protein-coupled receptors and protein kinases, with signal termination enzymes, including protein phosphatases and cyclic nucleotide phosphodiesterases [3]. This permits local and reversible control of signal-dependent responses. In addition, sophisticated mathematical modeling has derived algorithms to simulate how scaffolding and anchoring proteins shape signaling events [4,5]. A common denominator has been the notion that scaffolding and anchoring proteins are passive participants that simply hold their enzyme binding partners in place. Two papers recently published in *Science* challenge this concept by demonstrating that certain 'scaffolding' proteins are actually active elements in the enzyme complexes that they organize [6,7].

In the first of these papers, Rock *et al.* [6] present exciting work on the yeast mitotic exit network (MEN) scaffold protein Nud1. This protein is an important hub in the signaling network that controls exit from mitosis during the cell cycle. Components of the



Current Biology

Figure 1. Scaffolding proteins offer additional modes of regulation in signaling cascades.

(A) In yeast, mitotic exit requires Nud1 phosphorylation by Cdc15. This essential step causes recruitment of the Dbf2–Mob1 kinase complex to spindle pole bodies, where it is activated by Cdc15 (adapted from [6]). (B) In mammalian cells, Cdk1–cyclinB phosphorylates the AKAP gravin during the cell cycle, and allows phosphorylation-dependent binding of Plk1. Active Plk1 recruitment in the complex is then able to locally phosphorylate targets that are crucial for cell-cycle progression. (C) A third example of phosphorylation-dependent interactions comes from scaffolding in the Wnt signaling pathway. Axin is a scaffolding protein that participates in both β-catenin 'stabilization complexes' and β-catenin 'destruction' complexes. The formation of these complexes is dependent on phosphorylation–dephosphorylation cycles that are based on the balance between GSK3β and PP1cγ (PP1) activities (adapted from [7]).

yeast MEN pathway are conserved in metazoans where they function in Hippo signaling [8,9]. Nud1 is a mitotic phosphoprotein, and mutation of a set of 42 high-probability mitotic phosphorylation sites causes anaphase arrest, much like a complete loss of Nud1 [6]. This mutant Nud1 protein is correctly localized to spindle pole bodies (SPBs), along with several other pathway members, including Tem1, Bfa1 and Cdc15. However, the Dbf2–Mob1 kinase complex is mislocalized and absent from SPBs, rendering Dbf2 unable to be activated by Cdc15. Therefore, Nud1 phosphorylation seems to be essential for recruiting Dbf1–Mob1 to SPBs.

Elegant genetic and biochemical approaches narrowed down the critical phosphorylation site for the interaction between Nud1 and Mob1 to threonine 78 (T78) on Nud1 [6]. Furthermore, Mob1 binds Nud1 in a *CDC15*-dependent manner and this association is necessary for Dbf2 localization to SPBs, suggesting that Mob1 binds specifically to phospho-T78-Nud1. Peptide arrays and library screening confirmed that this interaction is dependent on Nud1 phosphorylation, and the structural details of this interaction were explored by X-ray co-crystallography of Mob1 in complex with an optimal phosphopeptide [6]. Together, these data suggest that Nud1 is a critical regulator of signal relay through the yeast Hippo pathway. Mechanistically, the data support a model in which Cdc15 must first phosphorylate the Nud1 scaffold, causing recruitment of Dbf2–Mob1 for Cdc15-dependent phosphorylation and activation. Finally, activated Dbf2 then switches on the phosphatase Cdc14 to promote exit from mitosis (Figure 1A).

These findings are reminiscent of recent work by Canton *et al.* [10] showing that the AKAP gravin serves as a scaffold for active Polo-like kinase 1 (Plk1) at mitotic spindle poles and centrosomes during mitosis (Figure 1B) [11]. Gravin is heavily phosphorylated during the cell cycle, and phosphorylation of threonine 766 by cyclin-dependent kinase 1 (CDK1) generates a Plk1 Polo-box binding site and recruits Plk1 into the gravin complex [10]. Loss of gravin, or overexpression of a Plk1-binding-deficient mutant, results in defects in progression through the cell cycle and reduced proliferation. A common theme in this study and the work by Rock *et al.* [6] is the phosphorylation-dependent recruitment of signaling elements. Although we are just beginning to understand the impact of phosphoserine and phosphothreonine recognition motifs, it is important to recognize that this work echoes classic studies by Pawson, Cantley and Hanafusa [12–14], who elegantly demonstrated the utility of phosphotyrosine-binding (PTB), Src homology 2 (SH2) domains and related protein interaction modules. Nonetheless, signaling through phosphoserine and phosphothreonine recognition motifs on scaffolding proteins such as Nud1 may be a more ubiquitous mechanism, since eukaryotic cells have higher levels of phosphoserine and phosphothreonine [15].

So, why would nature organize signaling pathways in this manner? One possibility is that covalent modification of the scaffold protein introduces an additional layer of control that protects against spurious signaling. Moreover a requirement for two molecular events to advance the signaling process suggests that some scaffolding proteins act as coincidence detectors. This synergistic arrangement offers an additional means to modulate the recruitment of enzymes by the scaffold proteins and should abrogate any concentration-dependent sequestering of signaling components. The latter property is evident in the situation of Nud1- or gravin-mediated scaffolding, where phosphorylation is necessary to recruit the terminal kinase (Dbf2 or Plk1, respectively) to the complex, and the non-phosphorylated scaffold protein will not bind and titrate away the output kinase. This mechanism could also allow the scaffolding protein to serve

multiple functions, where it can be 'constitutively active' for certain functions (i.e. static binding and localization of one member, as in the MEK1–KSR1 interaction) but not participate in other pathways until appropriately activated.

In the second of the recent papers in *Science*, Kim *et al.* [7] describe an even more complicated mode of regulation of the scaffolding protein Axin, a critical regulator of Wnt–β-catenin signaling. β-catenin translates Wnt signals at the plasma membrane into changes in gene expression that are required for many aspects of cell survival and proliferation, differentiation, and development [16]. Levels of β-catenin are tightly regulated by phosphorylation, which is dependent on the formation of a 'destruction complex' centered around Axin [7]. In the absence of Wnt stimulation, Axin binds β-catenin, glycogen synthase kinase 3β (GSK3β) and adenomatous polyposis coli (APC) and promotes continuous phosphorylation and proteosome-mediated degradation of β-catenin. Engagement of Frizzled and its co-receptor LRP6 by Wnt initiates a series of protein–protein interactions and phosphorylation–dephosphorylation cycles that lead to stabilization of β-catenin levels, movement of this transcription factor into the nucleus and induction of gene expression [7]. Kim *et al.* [7] provide new insights into how the scaffolding protein Axin is dynamically regulated to facilitate these events. Axin is phosphorylated as part of the 'destruction complex', but an additional step in this process is the transient dephosphorylation of Axin by protein phosphatase 1cγ (PP1cγ), which itself is regulated by the PP1 inhibitor I2 [7].

Wnt stimulation re-orders these protein complexes into an alternative 'signaling complex', primarily through LRP6 phosphorylation and subsequent recruitment of Axin, resulting in β-catenin stabilization through phospho-LRP6-mediated inhibition of GSK3 activity. These complexes are dynamic and are assembled in the immediate aftermath of Wnt stimulation, but dissociate after long-term Wnt stimulation as Axin is dephosphorylated and released from LRP6. As the Axin–β-catenin interaction is also weakened by Wnt stimulation, it is likely that both sets of interactions have some degree of dependence on phosphorylation, possibly due to phosphorylation-induced conformational switching. Pertinent to this proposed mechanism is the finding that Axin undergoes intramolecular protein–protein interactions with itself that may be regulated by GSK3β phosphorylation [7]. Interestingly, PP1cγ appears not to dephosphorylate LRP6 at the same time, leaving phospho-LRP6 available for binding to a new pool of activated Axin. The authors propose that phosphorylated LRP6 may "inactivate Axin in a 'catalytic' manner", such that a continuous cycle of Axin activation, recruitment and disassembly could proceed through a small number of activated LRP6 receptors to promote β-catenin stabilization [7]. In this model, as β-catenin levels peak, Axin binding and formation of the 'destruction complex' is favored, perhaps in coordination with I2-mediated PP1cγ inhibition. This would essentially autoregulate β-catenin levels to prevent inappropriate β-catenin activity. The prospect of cycling activation of signaling enzymes through a scaffold-based complex is an exciting idea that clearly merits further investigation (Figure 1C).

A recurring theme in each of the examples described herein is that scaffolding proteins also receive input from signaling machinery and must undergo some modification themselves in order to encourage the assembly of 'functional' complexes. Such mechanisms may protect cells from spurious signaling due to unwanted protein–protein interactions. Furthermore, these more elaborate modes of signal relay would also guard against the tonic relay of signals through an enzyme scaffold just because all the players are in the right place at the right time. Thus, when the Rock *et al.* [6] and Kim *et al.* [7] articles are considered in the light of other recent reports of scaffold modification [10,17,18], a strong case can be made for scaffolding proteins actively participating in the signaling processes they coordinate. This view is in sharp contrast to the original concept of scaffolding proteins as the 'molecular glue' that facilitates the action of their binding partners [19]. Irrespective of whether scaffolding proteins are active or passive signaling elements, these discoveries provide a framework for future studies on this important class of signal organizers.

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