A centrosomal scaffold shows some self-control

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The scaffolding protein AKAP350A is known to localize to the centrosome and the Golgi, but the molecular details of its function at the centrosome remain elusive. Using structure–function analyses, protein interaction assays, and super-resolution microscopy, Kolobova et al. now identify AKAP350A’s specific location and protein partners at the centrosome. The authors further define an autoregulatory mechanism that likely controls AKAP350A’s ability to nucleate microtubule growth.

Protein kinase A–anchoring proteins (AKAPs) constitute a large family of scaffolding proteins that bind protein kinase A and mediate cAMP signaling. One member of this family, AKAP350 (AKAP9/AKAP450/C-G-NAP/iperion), is thought to regulate microtubule (MT) nucleation from the surface of two organelles—the Golgi and the centrosome (1, 2). AKAP350 is related to pericentrin in that these are the only mammalian proteins to contain the pericentrin-AKAP450 centrosome–targeting (PACT) domain (3), and pericentrin has garnered significant attention for its role in MT nucleation at the centrosome. However, AKAP350’s role remains largely unexplored, which could limit our overall understanding of MT nucleation at centrosomes. A new study by Kolobova et al. (4) provides significant insight into the molecular architecture of the longest AKAP350 isoform, AKAP350A, and its binding partners at the centrosome. It also identifies an intriguing autoinhibitory mechanism that may regulate multiple functions of AKAP350A.

The centrosome is a non-membrane–bound organelle composed of a core pair of centrioles surrounded by pericentriolar material (PCM), a complex protein assembly responsible for nucleating and anchoring MTs. Pioneering work by several labs in 2012 used structured illumination microscopy (SIM) to investigate the spatial distribution of many centrosome proteins (5). These studies showed that pericentrin is an extended molecule with its C terminus (containing the PACT domain) near the centriole wall and its N terminus extending outward. Surprisingly, when Kolobova et al. (4) performed SIM on centrosomal AKAP350A, they found that it does not assemble into an extended molecule like pericentrin. Rather, AKAP350A localizes to the intercentriolar linker (Fig. 1A), thus establishing a distinct localization for AKAP350A from pericentrin, likely driven by unique PACT domain–mediated protein interactions. In support of such a mechanism, the authors show that AKAP350A interacts with Cep68, a known intercentriolar linker component required for centriole cohesion. This result raises the possibility that AKAP350A may regulate centriole linkage (Fig. 1B, Q1), perhaps complementing the recently-identified role for pericentrin (6).

To investigate AKAP350A’s function further, Kolobova et al. (4) expressed the 4000-amino acid AKAP350A as three more technically manageable fragments: an N-terminal F1 fragment, a central F2 fragment, and a C-terminal F3 fragment. Overexpression of F3 led to an unexpected result: ectopic assembly of multiple MT-nucleating centers (MTNCs) that contained known PCM proteins such as Cdk5RAP2. Although these MTNCs are not normally present in cells, this observation led the authors to wonder if AKAP350A might play a significant and unappreciated MT nucleation function at centrosomes. However, when Kolobova et al. (4) tested the role of full-length AKAP350A at centrosomes, they observed that AKAP350A depletion resulted in a reduction of Cep68 and Cep170 but did not impact levels of the PCM proteins Cdk5RAP2. Although these MTNCs are not normally present in cells, this observation led the authors to wonder if AKAP350A might play a significant and unappreciated MT nucleation function at centrosomes. However, when Kolobova et al. (4) tested the role of full-length AKAP350A at centrosomes, they observed that AKAP350A depletion resulted in a reduction of Cep68 and Cep170 but did not impact levels of the PCM proteins Cdk5RAP2 and γ-tubulin. Thus, although AKAP350A can initiate MTNCs, its role in MT nucleation at the centrosome remains unclear (Fig. 1B, Q2). Combinatorial loss–of–function experiments may help elucidate AKAP350’s PCM recruitment role at the centrosome, which may be masked by the presence of pericentrin.

The authors next mapped a “promoting” domain within F3 to amino acids 2762–3458 that is required to induce MTNC formation and interacts directly with Cdk5RAP2 based on yeast two-hybrid analysis. Together, these data suggest that AKAP350A’s C terminus not only targets the full-length protein to the centriole linker but also promotes centrosome MT nucleation by directly recruiting Cdk5RAP2 upstream of γ-tubulin. This centrosome-based MT nucleation pathway differs from the Golgi-based pathway, which relies on AKAP350’s N terminus for both Golgi targeting and the recruitment of GCP2/3, a component of the γ-tubulin ring complex (2, 7). Of note, a second Golgi–targeting domain within AKAP350A has been identified just upstream of the PACT domain (8). It would seem, therefore, that AKAP350A can utilize organelle-specific targeting domains to promote spatially distinct MT nucleation pathways.

Based on the differences in behavior between the truncated and full-length AKAP350 sequences, the authors suspected that full-length AKAP350 contains a sequence that prevents unchecked MTNC formation. Indeed, mapping efforts identi-
fied an “inhibitory” domain (amino acids 1881–2183) that suppresses MTNC formation. Thus, AKAP350A cannot only promote PCM recruitment, it can also suppress this recruitment via an autoinhibition mechanism. Consistent with this idea, yeast two-hybrid analyses, biochemical experiments, and proteomics analysis performed by Kolobova et al. (4) showed that the interaction landscape for AKAP350A changes in the presence of the inhibitory domain. Specifically, the authors show that the presence of the inhibitory domain reduced binding of Cep68, Cep170, and Cdk2RAP5. The inhibitory domain may be sensitive to cell state based on overlap with a previously identified site that interacts with RIIα (see text for details (9)). The precise role of AKAP350 at the intercentriole linker (Q1) and the centrosome (Q2) remains unclear.

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Figure 1. AKAP350 is controlled by an autoregulatory mechanism. A, the distinct localizations of AKAP350 and pericentrin (both of which contain PACT domains (black dots) at the centrosome. Specifically, AKAP350 (blue) localizes to the intercentriole linkage (orange), while pericentrin (pink) localizes to the proximal end of centrioles (olive). Cdk5RAP2 (green) is anchored to the centrosome by pericentrin; it remains unknown if AKAP350 also positions Cdk5RAP2 at the centrosome. B, a rough sequence map captures AKAP350’s autoregulatory mechanism as identified by Kolobova et al. (4). The gray-shaded area, which was the focus of the authors’ work, contains the newly discovered inhibitory domain (Inh, red), which regulates the ability of the newly discovered promoting domain (green) to interact with Cep68, Cep170, and Cdk2RAP5. The inhibitory domain may be sensitive to cell state based on overlap with a previously identified site that interacts with RIIα (see text for details (9)). The precise role of AKAP350 at the intercentriole linker (Q1) and the centrosome (Q2) remains unclear.

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