SIGNAL TRANSDUCTION

Building insights into KRAS signaling complexes

The critical event in KRAS signaling and oncogenic transformation is activation of the RAF–MEK–MAPK cascade. This requires assembly of a multiprotein–lipid complex on the plasma membrane. In a tour de force of modeling, Mysore et al. now provide the first glimpse of what this structure may actually look like.

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KRAS is a membrane-localized GTPase that oscillates between active GTP-bound and inactive GDP-bound states and functions as a critical node in growth factor receptor signaling pathways. To generate an output signal, KRAS must recruit effector proteins from the cytosol for activation. One example is the MAPK cascade, whereby KRAS recruits RAF to the plasma membrane for activation, in turn triggering the activation of MEK and ERK. In this issue of *Nature Structural & Molecular Biology*, Mysore et al.[1] use molecular dynamics (MD) simulations to devise a fascinating model of this activation complex, which they term a signalosome, that accommodates the membrane; KRAS; the core kinases CRAF, MEK and ERK; and the ancillary regulatory components GAL3 and 14-3-3.

KRAS is organized on the plasma membrane into nanoclusters comprising 6–8 molecules[2]. These RAS assemblies are probably constructed from RAS dimers and monomers, since dimerization appears to be essential for nanocluster formation[3,4]. KRAS has a membrane anchor comprising a farnesyl lipid and a polylysine sequence, which together exhibit exquisite binding specificity for phosphatidylserine[5]. KRAS nanoclustering results in nanoscale lipid remodeling of the membrane to generate a lipid domain that is highly enriched in phosphatidylserine. Nanoclusters are the primary site of RAF activation on the plasma membrane. In the model proposed by Mysore et al., KRAS nanoclusters therefore form the core of the signalosome.

Multiple distinct RAS dimer conformations have been modeled by MD simulations and other methods over the past few years[6–8]. The most common are symmetric or semisymmetric and involve residues at helices α4/α5 or α3/α4 in the C-terminal lobe of the KRAS catalytic G-domain. Other models have N-terminal lobe residues from α2 and switch 2 participating in dimer formation[8]. Mysore et al. now propose an asymmetric dimer that shares features with previous models, including a dimerization interface that involves the α4/α5 helices, but differs in other aspects —most notably, in advocating a role for the KRAS-bound GTP in dimer formation. This is a critical feature because it provides a molecular basis for GTP-dependent lateral segregation, in which GTP-loaded KRAS proteins form nanoclusters that are spatially non-overlapping with GDP-loaded KRAS proteins[2].

As in previous dimer models, the GTP-mediated dimer (GMA) interface is almost exclusively polar, suggesting that the GMA dimer is weak. Extensive MD simulations indicate that KRAS dimerization via this interface is a relatively rare event, since the GMA dimer formed in only 5% of simulations[1]. It may be argued that the GMA dimer interface is therefore only one among a diverse set of interfaces that KRAS uses to organize itself into dynamic nanoclusters. Although this is possible[3], the GMA dimer is nevertheless important because it explains the nucleotide dependence of KRAS nanoclustering and favors the signaling-competent membrane orientation of the KRAS G-domain[9,10]. The GMA dimer stabilizes the α-orientation, wherein helices α3–α4/α5 face the membrane, and disfavors the alternative β-orientation, in which the KRAS effector-binding region is occluded by the membrane[1].

Mysore et al. add KRAS proteins to an initiating GMA dimer head-to-tail to form a helical structure of two tiers or turns. The base tier sees four KRAS proteins attached to the membrane by their C-terminal anchors. The next tier of another four KRAS proteins is intriguing, in that their anchors are not embedded in the membrane. To mitigate what would be an energetically unfavorable event, in the model, the farnesyl chains are protected from solvent by binding to a hydrophobic pocket on GAL3. The authors favor GAL3 for this role over the more conventional prenyl-binding protein PDEδ because of previous work showing that GAL3 facilitates KRAS nanoclustering[11,12]. Prior to capping the KRAS anchors with GAL3, the authors add a RAF-RBD (RAS binding domain) to each of the KRAS proteins in the helical structure, which is possible because of the α-orientation of each KRAS G-domain. This is followed by addition of a RAF-CRD (cysteine-rich domain) and RAF kinase domain dimers plus the missing linkers between the constituent parts to generate full-length contiguous RAF proteins. 14-3-3 proteins and MEK are included subsequently.

The resulting structure has many remarkable features. First, there is the simple fact that all of the constituent proteins can actually be docked together without spatial clashes. Second, new interactions, largely polar, between multiple side chains of the CRD, RBD and RAF kinase domains and between side chains and KRAS monomers within the signalosome add stability. Third, there is at least some evidence that phosphatidylserine molecules of the lipid membrane, which were previously shown to be critical for KRAS-mediated RAF activation[13], engage with the KRAS anchors and RAF-CRD. Fourth, most of the residues previously characterized as participating in dimer formation are involved in either primary, secondary or tertiary RAS–RAS interactions in the proposed complex structure. Nevertheless, some structural inconsistencies remain. Most notable is that the signalosome model is consistent with some but not all of the features of recent structures of KRAS–RAF complexes[14–16]. This may reflect the nature of the modeling, the multiple proteins included and the membrane constraints.

The model is compatible with many oncogenic KRAS mutations. It is also supported by extensive mutational analysis to validate key predictions about the structure of the GMA dimer, although some of these results may also be explained by alternative oligomer models[3]. Reconstitution experiments on lipid nanodiscs and negative-stain cryo-EM images, at a higher resolution than that of previous work with model membranes imaged by AFM[1,17], provide intriguing

evidence for the core KRAS structure and for the assembly of KRAS monomers into higher-order oligomers. The final signalosome model is consistent with much of the previous literature, providing, for example, a structural rationale for the role of GAL3 and RAF dimers in enhancing KRAS nanoclustering. The large size of the complex also increases the plasma membrane area that is receptive to RAF recruitment and hence involved in MAPK activation, a key feature of nanoclusters in the context of high-fidelity signal transmission[18]. Nevertheless, the model as configured poses questions that remain to be addressed. The authors advocate a role for GAPs and GEFs, and perhaps KRAS-GDP, in regulating assembly and disassembly. This seems unlikely, since oncogenic KRAS is constitutively GTP-bound and insensitive to GAP-stimulated hydrolysis. The dynamics of nanocluster assembly and turnover, as well as RAF recruitment, as visualized by single-particle tracking suggest, however, similar dynamics for GTP-loaded wild-type and oncogenic mutant KRAS, indicating that turnover is not linked to GTP hydrolysis[19,20]. Moreover, additional protein components of the MAPK cascade, such as KSR, are not included in the model. KSR operates as a scaffold for MEK and ERK to facilitate switch-like signaling in the module. Since MEK is the most peripheral of the components, KSR could be incorporated in future refinements.

In summary, Mysore et al. combine a large set of atomically detailed MD simulations and cell assays to propose an exquisite structural model of the RAS–RAF signalosome. The authors rightly claim that their model can account for many observations reported over the last 15 years, including the much-debated role of RAS dimerization in the assembly of higher-order RAS nanoclusters on the plasma membrane and the activation of ERK by RAS–RAF–MEK complexes. The model as constructed, however, also raises important questions related to the mechanism and timescales of signalosome assembly and disassembly relative to the rate of GTP hydrolysis and GEF activity. The model is specific to KRAS, since HRAS and NRAS form spatially distinct nanoclusters, each with distinct lipid compositions that may support assembly of different signalosome complexes[2]. Finally, as the authors acknowledge, and we agree, the model may not be applicable to all KRAS-mediated signaling pathways. As such, alternative dimer models and signalosome structures should be investigated; this study provides an important roadmap for how this may be accomplished. ❐

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References
1. Mysore, V. P. et al. *Nat. Struct. Mol. Biol.* https://doi.org/10.1038/s41594-021-00667-6 (2021).
2. Zhou, Y., Prakash, P., Gorfe, A. A. & Hancock, J. F. *Cold Spring Harb. Perspect. Med.* **8**, a031831 (2018).
3. Sarkar-Banerjee, S. et al. *J. Am. Chem. Soc.* **139**, 13466–13475 (2017).
4. Spencer-Smith, R. et al. *Nat. Chem. Biol.* **13**, 62–68 (2017).
5. Zhou, Y. et al. *Cell* **168**, 239–251.e16 (2017).
6. Güldenhaupt, J. et al. *Biophys. J.* **103**, 1585–1593 (2012).
7. Muratcioglu, S. et al. *Structure* **23**, 1325–1335 (2015).
8. Prakash, P. et al. *Sci. Rep.* **7**, 40109 (2017).
9. Mazhab-Jafari, M. T. et al. *Proc. Natl Acad. Sci. USA* **112**, 6625–6630 (2015).
10. Prakash, P., Zhou, Y., Liang, H., Hancock, J. F. & Gorfe, A. A. *Biophys. J.* **110**, 1125–1138 (2016).
11. Elad-Sfadia, G., Haklai, R., Balan, E. & Kloog, Y. *J. Biol. Chem.* **279**, 34922–34930 (2004).
12. Shalom-Feuerstein, R. et al. *Cancer Res.* **68**, 6608–6616 (2008).
13. Winkler, D. G. et al. *J. Biol. Chem.* **273**, 21578–21584 (1998).
14. Fang, Z. et al. *Proc. Natl Acad. Sci. USA* **117**, 12101–12108 (2020).
15. Tran, T. H. et al. *Nat. Commun.* **12**, 1176 (2021).
16. Van, Q. N. et al. *Proc. Natl Acad. Sci. USA* **117**, 24258–24268 (2020).
17. Weise, K. et al. *J. Am. Chem. Soc.* **133**, 880–887 (2011).
18. Tian, T. et al. *Nat. Cell Biol.* **9**, 905–914 (2007).
19. Hancock, J. F. & Parton, R. G. *Biochem. J.* **389**, 1–11 (2005).
20. Murakoshi, H. et al. *Proc. Natl Acad. Sci. USA* **101**, 7317–7322 (2004).

Competing interests
The authors declare no competing interests.

CHROMATIN

Embryogenesis without CTCF in flies and vertebrates

CTCF is a conserved DNA- and RNA-binding protein with roles in genome folding and transcriptional regulation. Two recent studies investigated how CTCF knockout perturbs genome biology and derails embryogenesis in zebrafish and *Drosophila melanogaster*, revealing contrasting effects across species.

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Since its discovery over 30 years ago[1], the CCCTC-binding factor (CTCF) has remained an enigmatic molecule. CTCF is conserved across bilaterian animals[2], contains 11 zinc fingers that recognize a long DNA motif, and can also bind RNA[3,4]. In the human genome, CTCF binding most often occurs outside transcription start sites or enhancers (~70% of chromatin immunoprecipitation–sequencing (ChIP–seq) peaks). What makes CTCF an especially unusual transcription factor is how it controls interphase genome folding in vertebrates, by interfering with DNA loop extrusion by cohesin proteins[5]. In addition to roles in chromosome architecture, a substantial body of work using mammalian cell culture put forward functions for CTCF in activating or repressing promoters, modulating enhancer activities, controlling RNA splicing, DNA repair and many other processes[3,4]. But what happens to the development of an organism when CTCF is mutated? Two new studies