COMMENTARY

The hanging heart: How KRAS lures its prey to the membrane

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KRAS, known as the beating heart of cancer, drives around one in seven of all human cancers. Mutations in KRAS are most frequent in lung adenocarcinomas, colorectal cancers, and pancreatic cancers. KRAS is a small GTPase and the central switch in the MAPK signaling pathway. In cells, most signals are relayed and processed by proteins attached to or embedded in the membrane; KRAS signaling is no exception. However, obtaining insights into biochemical mechanisms at the membranes of cells has proven a significant technical challenge. Van et al. (1) utilize a suite of biophysical methods, capable of probing protein positioning at membranes, to elucidate how KRAS binds to the membrane of cells. They show that KRAS adopts a "hanging heart" conformation distal from the membrane, ready to recruit binding partners like a lure in fly-fishing dangling from the water's surface, waiting to catch a fish.

KRAS functions by cycling through two conformational states. Binding of the nucleotide guanosine diphosphate (GDP) stabilizes the signaling-incompetent state ($KRAS_{OFF}$), while the guanosine triphosphate (GTP) bound form ($KRAS_{ON}$) is capable of binding downstream effectors (e.g., CRAF, PI3K, and RALGDS) and propagating signaling. Point mutations, most commonly at codons 12, 13, and 61, cause an increase in the levels of $KRAS_{ON}$ and lead to oncogenic forms of KRAS. KRAS is attached to the inner leaflet of the cell membrane in a bivalent manner via the C-terminal hypervariable region (HVR) and the CAAX anchor (2, 3). Both KRAS splice variants (KRAS4a and KRAS4b) associate with the membrane via the insertion of a posttranslationally attached farnesyl group, while the second membrane attachment is a posttranslational palmitoyl group in KRAS4a and a polybasic domain for KRAS4b which is responsible for lipid-binding specificity (4).

Not surprisingly, the proteins responsible for activating and inactivating KRAS are also located at the cell membrane. Activation of KRAS through exchange of GDP for GTP is catalyzed by guanine nucleotide exchange factors (GEF) such as SOS1 and SOS2 (5). SOS is also attached to the membrane via two interactions. The pleckstrin homology (PH) domain binds membrane-bound phosphoinositol phosphates, and the positive surface charge of the histone fold interacts with the negative charge of the phosphate head groups in the membrane (5, 6) (Fig. 1). Inactivation of KRAS via hydrolysis of the γ-phosphate of GTP is performed by the weak GTPase activity of KRAS itself but, more importantly, by GTPase-activating proteins (GAPs). GAPs anchor themselves to the cell membrane via a variety of membrane-binding domains like PH and SH2 domains for RASA1 or SEC14 for NF1 (7).

In order to study membrane-associated proteins, systems which mimic the cellular membrane as well as biophysical measurement methods are required. Van et al. (1) utilize anionic sparsely tethered bilayer lipid membranes, also referred to as nanodiscs (8), together with farnesylated and methylated KRAS4b (KRAS4b-FMe) (9) as the model system for the study. Techniques able to study the molecular details of proteins associated with biological membranes have proven notoriously difficult because established solution- and crystal-based approaches are inadequate for membranes. Van et al. (1)apply neutron reflectometry (NR), fast photochemical oxidation of proteins (FPOP), and NMR to successfully elucidate KRAS positioning at the membrane.

Previous studies have shed some light on KRAS behavior at the membrane, but the positioning of the G domain has remained an open question until now. Fluorescence anisotropy has demonstrated free rotation of the G domain and high lateral movement when KRAS is anchored in a membrane (10). Earlier NMR paramagnetic relaxation enhancement (NMR-PRE) studies of KRAS4b covalently tethered to nanodiscs (8, 11) have also indicated a hanging heart conformation for KRAS. In contrast to the biophysical evidence reported by Van et al. (1), the bulk of historical computational approaches have found the G domain to be mostly associated with the membrane.

NR is a surface-sensitive scattering technique which can determine the penetration depth into and

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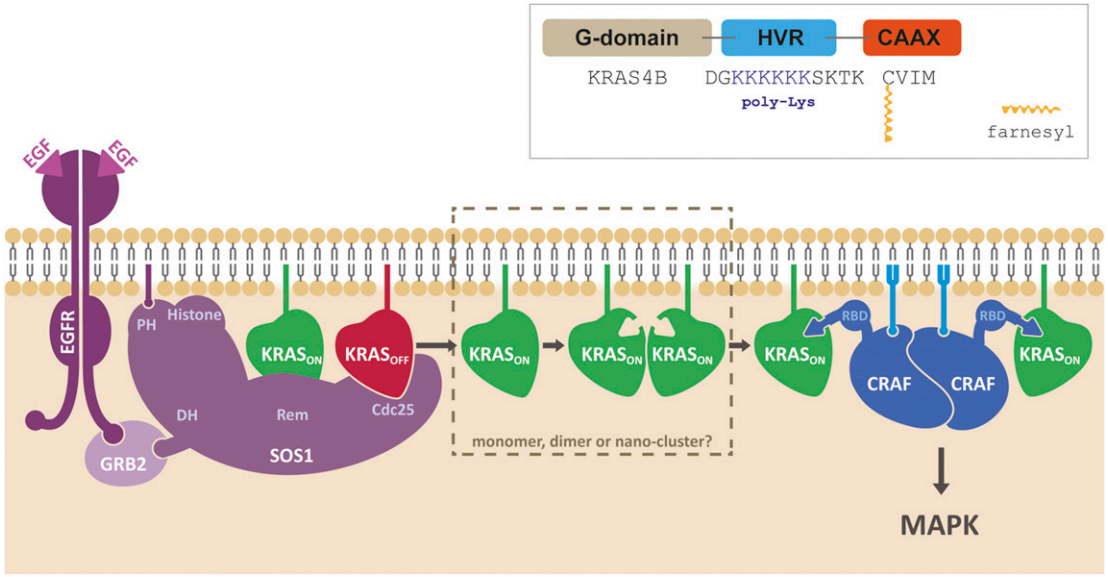

Fig. 1. KRAS4B domain organization and RAS signaling pathway at the cell membrane. Image credit: Biolution GmbH, licensed under CC BY-NC-ND 4.0.

orientation on a lipid membrane for membrane-associated proteins (12). NR experiments indicated that the G domain of KRAS4b is situated 36 ± 4 Å from the lipid head groups of the membrane. FPOP footprinting, a method analogous to hydrogen/deuterium exchange (H/D exchange), uses hydroxy radicals to oxidize exposed amino acids while those protected (by the membrane in this case) remain unoxidized (13). Recently, the incorporation of control peptides into FPOP footprinting has allowed the comparison of data over time and in the presence of radical-reactive species such as lipids (14). FPOP experiments with KRAS4b-FMe showed that only amino acids of the HVR, in particular, lysine residues of the polybasic region, were protected from oxidation and thus interacted with the membrane. The absence of any FPOP signal for the G domain supports the membrane distal position determined by NR.

NMR-PRE is an established method for providing information regarding the proximity of a protein surface and a paramagnetic nuclei through the enhancement of relaxation rates (15). Van et al. (1) incorporated the paramagnetic gadolinium (Gd^{3+}) into the membranes of the nanodiscs and measured changes in signal intensity of ^{15}N-KRAS4b-FMe NMR cross-peaks. The NMR-PRE experiments showed complete loss of the residues around the polybasic region, indicating that they are very close to the membrane. The NMR-PRE experiment also showed reduced signal intensities for portions of β-strands 1 to 3 and regions of α-helices 2 to 5, indicating that KRAS4b is orientated in a hanging heart conformation distal from the membrane (Fig. 1).

Interestingly, no difference between $KRAS_{ON}$ and $KRAS_{OFF}$ was observed, and, upon binding with the RAS binding domain of CRAF, no change in KRAS4b positioning was seen. It should be noted that, upon binding full length CRAF, KRAS could be forced to adopt a membrane-proximal conformation. While Van et al. (1) highlight the current limitations of the method, using the obtained biophysical data as constraints, MD simulations identified the membrane distal conformation as the most populated state.

Given the complexity of the plasma membrane, mechanistic studies using simplified artificial membranes will have limitations. However, a number of further mechanistic insights into RAS function at the membrane could be gained by further study: KRAS4a versus KRAS4b and SOS–KRAS interplay. Impairing KRAS dimerization and oligomerization is emerging as a potential therapeutic concept to target KRAS-driven cancers. Such an approach as described by Van et al. (1) would also provide an ideal approach to study molecules such as the recently disclosed dimeric KRAS protein–protein interaction stabilizers (16).

Acknowledgments

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