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Structural keys unlock RAS–MAPK signalling

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Activation of the RAF protein is key to the RAS–MAPK signalling pathway, and involves the SMP protein complex. Structures for SMP shed light on the process, and suggest fresh targets for anticancer drug discovery.

Unbridled activation of an intracellular signalling network called the RAS–MAPK pathway is the cause of several cancers and of developmental syndromes known as RASopathies[1]. This well-studied pathway (reviewed in ref. 2) starts with a membrane-bound RAS GTPase enzyme — HRAS, KRAS or NRAS — that, when activated by extracellular growth-factor proteins, binds to the nucleotide molecule GTP. RAS–GTP contacts physically and activates a kinase enzyme called RAF, triggering a signalling cascade that leads to activation of another kinase, MAPK. This enzyme, in turn, modulates the activity of proteins involved in many cellular processes. How RAS activates RAF has been the focus of much research. Writing in *Nature*, Liau *et al.*[3], Kwon *et al.*[4] and Hauseman *et al.*[5] shed light on this crucial step by independently reporting the structure of a protein complex involved in RAF activation.

Multiple regulatory mechanisms act as locks on the RAS–MAPK pathway, reducing the likelihood that the cascade will be inadvertently activated. One such lock involves securing inactive RAF in the cytoplasm through interactions between the dimeric form of a protein called 14-3-3 and two phosphorylated serine amino-acid residues (dubbed the NTpS and the CTpS) in RAF's amino and carboxy termini, respectively[6]. RAS–GTP moves RAF from this secure position by interacting physically with a RAS-binding domain on RAF[7].

Our molecular understanding of what happens next remains largely speculative. The predominant view is that RAS–RAF binding displaces 14-3-3 from the NTpS. The exposed NTpS can then be dephosphorylated, preventing rebinding of 14-3-3. Concomitantly, the ability of RAS to form clusters at the cell membrane induces the dimerization of two RAF molecules — a key step towards RAF's catalytic activation (Fig. 1).

A protein complex known as SMP — composed of the scaffolding protein SHOC2, another RAS GTPase called MRAS and the catalytic subunit of the phosphatase enzyme PP1 (PP1C) — has been reported[8] to be involved in NTpS dephosphorylation. However, several aspects of how this SMP complex functions have remained poorly understood. The three current studies each resolve the structure of the SMP complex, and so offer answers to some lingering questions.

The groups reconstituted the SMP complex from individually purified proteins, and

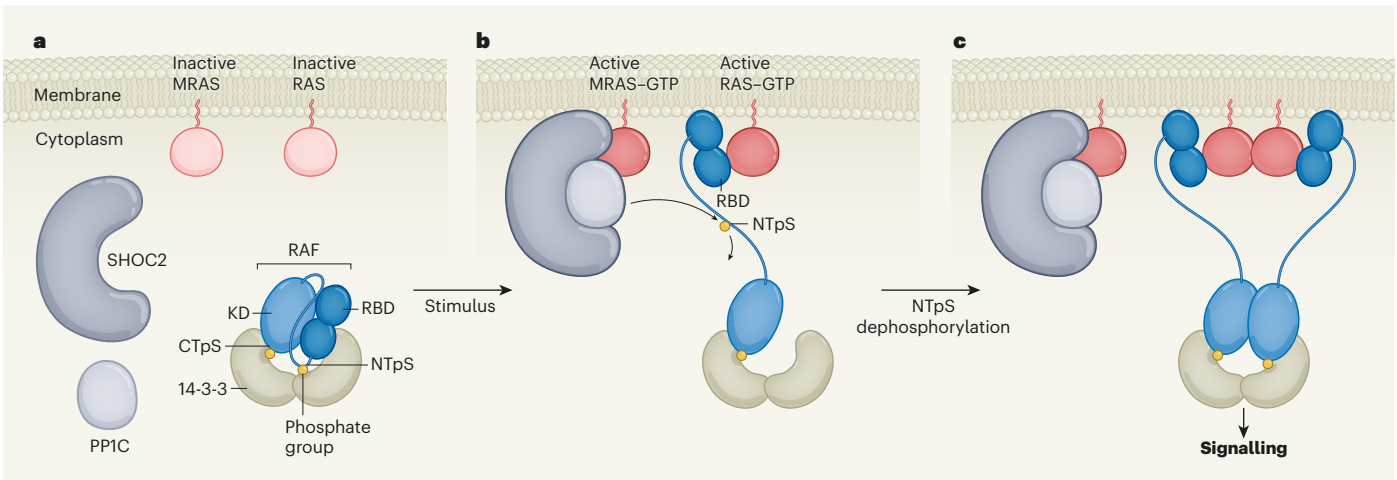

Figure 1 | How the SMP complex helps to activate RAF proteins. An early step in intracellular RAS–MAPK signalling involves activation of RAF by a membrane-bound RAS GTPase enzyme — HRAS, NRAS or KRAS (hereafer referred to collectively as RAS). **a**, RAF proteins contain (among other domains) a RAS-binding domain (RBD), a kinase domain (KD) and two serine residues tagged with phosphate groups, known as NTpS and CTpS. When RAS is inactive, NTpS and CTpS bind to a 14-3-3 protein dimer, restricting RAF to the cytoplasm. A SHOC2–MRAS–PPC1 (SMP) protein complex is thought to be mostly unassembled, owing to inactivity of the enzyme MRAS. **b**, Stimulation by growth factors leads to loading of the nucleotide GTP onto RAS and MRAS. RAS–GTP makes direct contact with the RBD of RAF, perhaps inducing the release of 14-3-3 from NTpS. MRAS–GTP triggers assembly of SMP, bringing PP1C into close proximity to RAF and leading to NTpS dephosphorylation. Three groups[3–5] have resolved high-resolution structures of SMP, providing insights into the structural biology underlying this process. **c**, Following NTpS dephosphorylation, two RAF proteins dimerize through their KDs, driving catalytic activation of RAF and triggering downstream signalling. RAF dimerization is stabilized by a 14-3-3 dimer bound to two CTpS sites, and is aided by the ability of RAS–GTP to cluster at the membrane.

determined the order of assembly and the binding affinities of the interacting partners. The salient feature that emerged is that the binding of GTP to MRAS triggers the assembly of the complex. Consistent with previous observations[9,10], all three studies also showed that HRAS, KRAS or NRAS proteins could substitute for MRAS, albeit with a much reduced affinity. Although it remains to be demonstrated that such complexes exist naturally in cells, these results suggest that multiple RAS GTPases can support the formation of SMP-like complexes.

The teams then analysed the structure of the SMP complex — Liau *et al.* and Kwon *et al.* using cryo-electron microscopy, Hauseman *et al.* using X-ray diffraction. They converged on an identical topology, whereby PP1C and MRAS occupy the large concave surface area in the crescent-shaped SHOC2 (Fig. 1). The topology of the complex exposes PP1C's catalytic site and substrate-binding clefts. MRAS adopts a GTP-bound active conformation in which it can interact extensively with SHOC2 and PP1C, providing a structural explanation for why MRAS requires GTP. The C terminus of MRAS is not involved in these interactions and is therefore available for anchoring the complex to the cell membrane.

An important observation is that the surface of MRAS that is buried in the complex overlaps the one used by RAS proteins to engage RAF. This indicates that a single RAS protein cannot simultaneously engage RAF and the SMP complex — a hypothesis that was verified experimentally by Hauseman *et al.* and Liau and colleagues. It thus seems that two RAS proteins, each with a distinct unlocking task, are required to activate a single RAF molecule. One of them recruits RAF to the membrane to release it from its inhibited conformation; the other recruits the SMP complex for NTpS dephosphorylation.

The NTpS in RAF is, to our knowledge, the only substrate reported to be dephosphorylated by the SMP complex. This suggests a high degree of enzyme–substrate selectivity, but how this is accomplished remains unclear. Is it simply because RAF and PPC1 co-localize at the membrane (in their respective RAS-nucleated complexes), or does the SMP complex confer on PP1C the ability to dephosphorylate the NTpS? In agreement with the second hypothesis, the three studies showed that, *in vitro*, the SMP complex dephosphorylates the NTpS significantly more efficiently than does PP1C alone. Furthermore, Liau *et al.* suggested that the juxtaposition of two adjacent hydrophobic regions in PP1C and SHOC2 in the SMP complex might engage amino-acid residues around the NTpS, and thereby provide selectivity.

Genetic mutations that increase the activity of MRAS, SHOC2 and PP1C are associated with RASopathies[11–13]. The atomic structure of the SMP complex now reveals why. It turns out that most mutations in SHOC2 and PP1C alter amino-acid residues that are direct contact points between the interacting partners. In MRAS, the mutations lead to persistent GTP loading. All the activating mutations tested in the current studies enhance SMP-complex formation. Notably, Kwon *et al.* also identified several putative mutations in SMP members that are recurrently found in cancer databases — although a causative role of these mutations remains to be demonstrated.

As well as answering key questions, the three studies help to frame further ones. For instance, the SHOC2–PP1C surface to which MRAS binds diverges markedly from the fold that RAS-binding domains normally adopt[14]. It will be interesting to investigate whether this unusual binding is unique to the SMP complex, or whether RAS proteins interact with a wider-than-realized repertoire of proteins that lack typical RAS-binding domains.

Another question is whether the SMP complex has other substrates beside RAF proteins, either in the RAS–MAPK pathway or elsewhere. Although genetic studies indicate a tight link between SHOC2 and the RAS–MAPK pathway[3–5,10], they do not exclude a role for SMP in other pathways. For example, there is evidence that SHOC2 might regulate PI3K signalling[15] and mTOR signalling[16], but it is not clear whether this regulation involves dephosphorylation events. SHOC2 might well have other functions that are unrelated to RAS or PP1C.

Lastly, PP2A phosphatases can also dephosphorylate the NTpS site, thus providing an alternative pathway to PP1C-dependent NTpS dephosphorylation[17,18]. How this mechanism operates, and how PP2A coordinates its activity with the SMP complex, is a fascinating area that will warrant further investigation.

Now that the structure of SMP is in hand, the attractiveness of the complex as a drug target for treating cancer and RASopathies is bound to increase. There is little doubt that selective SMP inhibitors will be developed in the coming years. It is to be hoped that some of these will successfully translate to the bedside.

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