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Structural basis of recognition of farnesylated and methylated KRAS4b by PDE δ

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Farnesylation and carboxymethylation of KRAS4b (Kirsten rat sarcoma isoform 4b) are essential for its interaction with the plasma membrane where KRAS-mediated signaling events occur. Phosphodiesterase- δ (PDE δ) binds to KRAS4b and plays an important role in targeting it to cellular membranes. We solved structures of human farnesylated-methylated KRAS4b in complex with PDE δ in two different crystal forms. In these structures, the interaction is driven by the C-terminal amino acids together with the farnesylated and methylated C185 of KRAS4b that binds tightly in the central hydrophobic pocket present in PDE δ . In crystal form II, we see the full-length structure of farnesylated-methylated KRAS4b, including the hypervariable region. Crystal form I reveals structural details of farnesylated-methylated KRAS4b binding to PDE δ , and crystal form II suggests the potential binding mode of geranylgeranylated-methylated KRAS4b to PDE δ . We identified a 5-aa-long sequence motif (Lys-Ser-Lys-Thr-Lys) in KRAS4b that may enable PDE δ to bind both forms of prenylated KRAS4b. Structure and sequence analysis of various prenylated proteins that have been previously tested for binding to PDE δ provides a rationale for why some prenylated proteins, such as KRAS4a, RalA, RalB, and Rac1, do not bind to PDE δ . Comparison of all four available structures of PDE δ complexed with various prenylated proteins/peptides shows the presence of additional interactions due to a larger protein-protein interaction interface in KRAS4b-PDE δ complex. This interface might be exploited for designing an inhibitor with minimal off-target effects.

prenylation | protein-protein interaction | KRAS4b | KRAS-PDE δ complex | phosphodiesterase- δ

RAS mutations are found in one-third of all human cancers (1). Three RAS genes in humans encode four distinct but highly homologous RAS proteins: HRAS, NRAS, and the splice variants KRAS4a (Kirsten rat sarcoma isoform 4a) and KRAS4b (2). Among the three RAS isoforms, KRAS is the most commonly mutated gene in the RAS family and involved in 95% of pancreatic cancers and a high proportion of colorectal and lung cancers (3). RAS proteins function as molecular switches by alternating between inactive GDP-bound and active GTP-bound states (4–6). The active or inactive state of RAS proteins is regulated by guanine nucleotide exchange factors and GTPase-activating proteins (GAPs) (7–9). In the GTP-bound state, RAS proteins interact with a variety of effector proteins, such as Raf, PI3K, and RalGDS, leading to activation of several signaling cascades within the cell (10, 11). Oncogenic RAS mutations are predominantly found at amino acid positions G12, G13, and Q61 and impair intrinsic and GAP-mediated GTPase function (12).

RAS isoforms contain a highly conserved GTPase domain (G domain) and diverge substantially in the last 20 amino acids at the C-terminal end known as the hypervariable region (HVR) (10, 13). This region contains residues that undergo posttranslational modifications essential for targeting RAS proteins to the cytosolic leaflet of cellular membranes. All RAS proteins are farnesylated and carboxymethylated at the Cys residue present in the C-terminal CaaX motif, where a is an aliphatic amino acid and X is any amino acid. The terminal amino acid (X) determines the nature of the

prenylated lipid chain. A leucine residue as X results in geranylgeranylation, and all other residues result in farnesylation (14). NRAS, HRAS, and KRAS4a are additionally modified by one or two palmitic acids on Cys residues located upstream of the CaaX motif. KRAS4b does not undergo palmitoylation. Instead, it has a polybasic region formed by a stretch of lysines that are believed to interact with negatively charged head groups of plasma membrane lipids (15). RAS proteins are further processed by RCE1 (RAS converting CaaX endopeptidase 1) (16), which removes the -aaX residues, followed by ICMT (isoprenylcysteine carboxyl methyltransferase) (17), which adds a methyl group on the carboxyl group of the C-terminal prenylated Cys. Almost two decades ago, RAS farnesyltransferase inhibitors (FTIs) were intensively explored in clinical trials, but because of alternative prenylation (geranylgeranylation) of KRAS and to a lesser extent, NRAS by geranylgeranyl transferases in the presence of FTIs, their high preclinical anticancer activity could not be translated into clinical efficacy (18, 19). Tumors driven by mutation of HRAS, which can only be farnesylated, are sensitive to FTIs, at least in preclinical models.

Phosphodiesterase- δ (PDE δ ; also known as PDE6 δ , PrBP/ δ , and PDE6D) was initially identified as the noncatalytic δ -subunit of photoreceptor PDE6. It was later implicated in membrane release and localization of prenylated RAS and other prenylated proteins (20–23). PDE δ has been shown to sequester KRAS4b from the cytosol by binding the prenylated HVR, thus preventing it from

Significance

Despite the significant progress made in the last few years toward targeting phosphodiesterase- δ (PDE δ) for KRAS (Kirsten rat sarcoma isoform)-driven cancers, there is no structural information available on posttranslationally modified KRAS4b in complex with PDE δ . The KRAS4b-PDE δ structure reported here provides the structural details of the protein-protein interaction interface and the atomic details of the hypervariable region of KRAS4b. Structural comparison of the two crystal forms allowed identification of a 5-aa-long sequence motif in KRAS4b that could allow PDE δ to bind to both farnesylated and geranylgeranylated KRAS4b. Structural insights obtained from this study could be used to guide the development of improved and more specific inhibitors of the KRAS4b-PDE δ complex.

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Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 5TB5 and 5TAR).

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binding to extensive endomembrane surfaces and thereby, enhancing its diffusion throughout the cell. KRAS4b is then released in perinuclear membranes by the release factors Arl2 and Arl3, where it binds the recycling endosome and is delivered to the plasma membrane via vesicular transport (24, 25). Binding of either GTP-bound Arl2/3 or prenylated RAS to PDE δ has been suggested to be mutually exclusive (26). Biochemical and cellular studies have shown that the interaction between PDE δ and KRAS4b is independent of its nucleotide (GTP/GDP) status (27).

The structure of PDE δ in a complex with fully modified Rheb (a member of the RAS subfamily) has been solved (26). Unlike KRAS4b, Rheb has a smaller HVR with a different amino acid composition and lacks the polybasic region. In this structure, the C-terminal farnesyl chain of Rheb is present in a hydrophobic pocket within the Ig-like β -sandwich fold of PDE δ , with three amino acids upstream of the prenylated cysteine interacting with PDE δ . Based on these studies, a high-throughput screen followed by a rational structure-based design approach resulted in the development of deltarasin as an inhibitor of the KRAS4b–PDE δ interaction (28). Deltarasin binds inside the hydrophobic pocket of PDE δ with low nanomolar affinity in vitro as well as in vivo. Cellular studies with adenopancratic cancer cells showed that addition of 5 μ M deltarasin blocks RAS signaling, reduces proliferation, and causes cell death in KRAS-dependent cells, whereas minimal effects were observed in KRAS-independent cells (28). Additionally, the administration of deltarasin to nude mice bearing s.c. xenografts of human Panc-Tu-I tumors caused a dose-dependent reduction of tumor growth. Overall, these results showed that PDE δ could be a potential target, and deltarasin and other small molecules represent a platform for finding better and more specific inhibitors of the KRAS4b–PDE δ complex.

Despite significant progress made in the last few years in identifying and targeting PDE δ for KRAS-driven cancers, there is no structural information available on fully processed KRAS4b in complex with PDE δ . In this paper, we describe structures of farnesylated–methylated KRAS4b (KRAS4b-FMe) in complex with PDE δ in two different crystal forms. Our structures provide atomic details of the HVR of KRAS4b in complex with PDE δ . Comparison of the two crystal forms describes not only how KRAS4b-FMe binds to PDE δ but also, how binding of prenylated KRAS4b and PDE δ is likely to be facilitated by the presence of a 5-aa-long sequence motif in the HVR of KRAS4b. The conserved sequence of this motif may account for the binding of PDE δ to both forms of prenylated KRAS4b. Finally, structure-based mutational studies suggest key roles played by various amino acids involved in the KRAS4b–PDE δ interaction.

Results

The Role of Farnesyl and Methyl Groups in Interactions Between KRAS4b and PDE δ . To understand the role of farnesylation and carboxymethylation of C185 of KRAS4b (Fig. 1A) in its interaction with PDE δ , we characterized the interaction of farnesylated KRAS4b (KRAS4b-Far) and KRAS4b-FMe with PDE δ using sedimentation velocity analytical ultracentrifugation (SV-AUC) and isothermal titration calorimetry (ITC). Sedimentation velocity studies on PDE δ , KRAS4b-FMe, and KRAS4b-Far showed the presence of a single species with sedimentation coefficients of 1.90 S, 2.25 S, and 2.24 S, respectively (Fig. 1B). The best fit molar masses of 18 ± 1 kDa for PDE δ , 22.9 ± 0.5 kDa for KRAS4b-FMe, and 22.9 ± 0.6 kDa for KRAS4b-Far show that these species are monomers in solution. To characterize the interaction of PDE δ with KRAS4b-FMe, solutions containing approximately equimolar amounts of these two species were studied at different concentrations. The $c(s)$ profiles (sedimentation coefficient distributions) obtained indicate the formation of a high-affinity 1:1 complex (Fig. S1A). At the highest concentration, the predominant species observed has a sedimentation coefficient of 2.92 S and estimated molar mass of 36 ± 3 kDa. As the concentration was reduced, the relative proportion

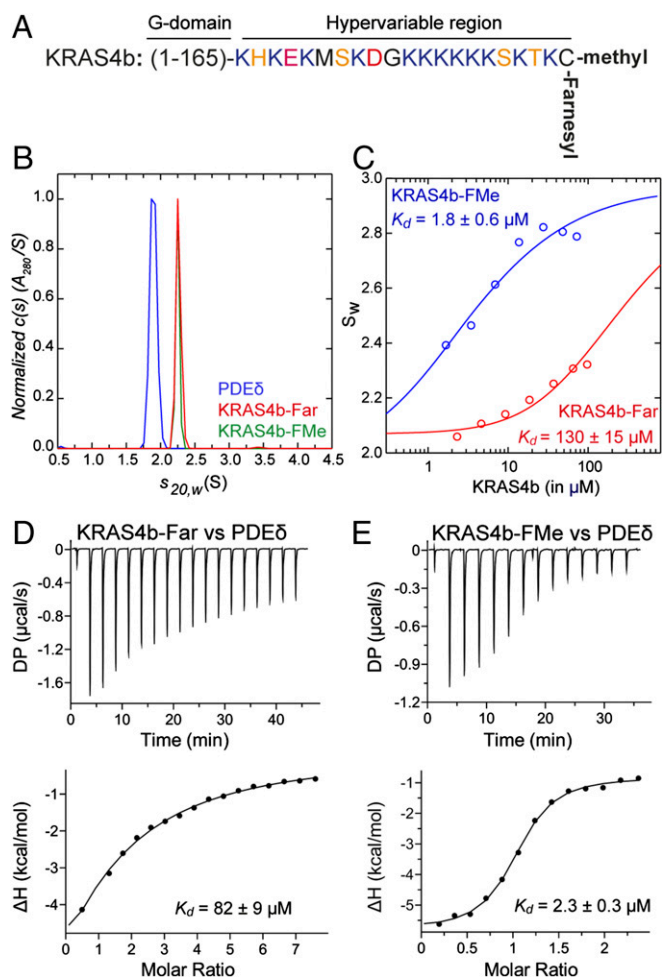


Fig. 1. Carboxymethylation of farnesylated C185 is important for KRAS4b–PDE δ interaction. (A) Amino acid sequence of the HVR of KRAS4b showing the presence of a polylysine patch and farnesylated and carboxymethylated C185 at the C terminus. (B) Normalized absorbance $c(s)$ profiles for PDE δ (15 μ M; blue), KRAS4b-FMe (28 μ M; red), and KRAS4b-Far (40 μ M; green) in SV-AUC experiments show that these three proteins are monomers. Similar profiles were observed using the interference optical detection system. (C) Weighted absorbance sedimentation coefficient isotherms based on $c(s)$ profiles for PDE δ and KRAS4b-FMe (blue) and PDE δ and KRAS4b-Far (red). Data were globally modeled in terms of an $A + B = AB$ heteroassociation model, as described in the text, to yield the best fit shown. (D and E) ITC titration experiments to measure the dissociation constant (D) between KRAS4b-Far and PDE δ and (E) between KRAS4b-FMe and PDE δ . The DP is a measured differential power between the reference cell and the sample cell.

of complex decreased, and based on the $c(s)$ profiles, it seems that the complex is in fast exchange with the free species (Fig. S1A). To determine the affinity of the interaction, a weighted average sedimentation coefficient obtained by integration of the $c(s)$ distribution was used to construct an s_w isotherm and fit to a model describing an $A + B = AB$ interaction (Fig. 1C). The dissociation constant, K_d , was determined to be $1.8 \pm 0.6 \mu$ M. Similar experiments characterizing the interaction of PDE δ with KRAS4b-Far led to $c(s)$ profiles indicative of a much weaker interaction (Fig. S1B), with a significant proportion of uncomplexed material at the highest concentrations studied. The weak association was confirmed in an analysis of the s_w isotherm, which returned an estimated dissociation constant K_d of $130 \pm 15 \mu$ M (Fig. 1C). A similar trend was observed when we measured dissociation constants (K_d) of PDE δ with KRAS4b-Far and KRAS4b-FMe using ITC. ITC measurements showed a weak binding affinity with a K_d of $82 \pm 9 \mu$ M between

KRAS4b-Far and PDE δ (Fig. 1D), whereas the presence of both farnesyl and methyl groups on KRAS4b led to a relatively stronger binding with a K_d of $2.3 \pm 0.3 \mu\text{M}$ (Fig. 1E). These results show that the presence of both the methyl and farnesyl modifications increases the affinity between KRAS4b and PDE δ close to two orders of magnitude compared with only KRAS4b-Far. Previous studies using a fluorescence-polarization (FP) assay showed a 10-fold higher binding affinity ($K_d = 0.3 \mu\text{M}$) between KRAS4b and PDE δ (29). This discrepancy is likely caused by the use of different techniques (analytical ultracentrifugation and ITC vs. FP), different assay conditions, and the use of semisynthetic and refolded KRAS4b vs. recombinant fully processed KRAS4b that we used for this study.

Structures of KRAS4b-FME in Complex with PDE δ in Two Crystal Forms. We attempted to crystallize KRAS4b-Far as well as KRAS4b-FME in complex with PDE δ and were successful in crystallizing only the latter protein in complex with PDE δ in two different crystal forms referred to as crystal forms I and II (Fig. 2 and Table S1). In crystal form I, the asymmetric unit contains two molecules of KRAS4b-PDE δ complex, with PDE δ interacting with the last five residues located upstream of farnesylated-methylated C185 of KRAS4b. In this structure, we did not observe interpretable electron density for three to five amino acids (172–176 or 174–176) in the HVR in two KRAS4b-PDE δ complexes present in the asymmetric unit, indicating flexibility in these residues inside the crystal (Fig. 2A and Fig. S2A). In crystal form II,

the asymmetric unit contained only one molecule of KRAS4b-PDE δ complex, where we see the electron density for the entire HVR of KRAS4b (Fig. S2B). In this crystal form, the C-terminal helix is extended and includes HVR residues up to K180, including six lysine residues (175–180) present in the polybasic region. Unlike crystal form I, in crystal form II, only three terminal residues of KRAS4b located upstream of farnesylated-methylated C185 were involved in complex formation. In crystal form II, most of the residues present in the HVR that were not involved in complex formation formed a long helical secondary structure (Fig. 2B). Secondary structure propensity analysis for residues present in HVR indicates that most of the residues have the tendency to form or favor α -helical structure (Fig. S2C).

In neither crystal form did we observe any specific interaction between the GTPase domain of KRAS4b and PDE δ (Fig. S2D), which provides a rationale for the broad specificity of PDE δ for prenylated proteins and also explains why the KRAS4b-PDE δ interaction is independent of the KRAS4b nucleotide (GDP/GTP) status. Although within the crystal, the GTPase domain of KRAS4b interacts with symmetry-related PDE δ molecules, these interactions are minimal and differ in the two crystal forms, implying that they are nonspecific and limited to crystal contact only. The overall structure and conformation of the switch I and II regions of KRAS4b resemble previously solved structures of KRAS4b-GDP complexes. In crystal form I, no electron density was observed for the switch I region.

Comparison of the interaction between KRAS4b and PDE δ in these two crystal forms suggests two different modes of binding between these two proteins (Fig. 2 and Fig. S2D). Unlike crystal form II, in crystal form I, HVR residues as well as farnesyl and methyl groups go deep inside the PDE δ hydrophobic pocket, presumably resulting in stronger interaction between these two proteins. We will first describe the stronger interaction observed between these two proteins in crystal form I and then, compare it with crystal form II.

Interaction of the HVR Residues of KRAS4b with PDE δ in Crystal Form I.

Residues present in the HVR. In crystal form I, KRAS4b interacts with PDE δ through the C-terminal amino acids S181, K182, T183, K184, and farnesylated-methylated C185 (Fig. 3A and B). Residue K184 forms multiple hydrogen bonds with main chain atoms of PDE δ residues I53, L54, and C56 (Fig. 3B and Fig. S3A). Side chain oxygen and main chain oxygen/nitrogen atoms of T183 form hydrogen bonds with residues E88 and Y149 from PDE δ . The side chain of K182 points toward the surface and does not form any hydrogen bonds with PDE δ residues, but the main chain nitrogen atom of K182 forms a hydrogen bond with E88 from PDE δ . Residue S181 forms three hydrogen bonds with PDE δ via side chain oxygen atom with E88 and via main chain atoms with E110 and A112 from PDE δ . The HVR residue K180 present near the entry gate forms no hydrogen bonds with any PDE δ residues but does form relatively weak van der Waals and hydrophobic interactions with residues W90, E110, A111, and A112 of PDE δ (Fig. 3B and Fig. S3A).

We carried out structure-based mutational studies to examine the role of the HVR residues in the interaction between KRAS4b and PDE δ . A double mutant of KRAS4b, T183A and K184E, was expressed and purified in the farnesylated-methylated form and showed weaker binding by two orders of magnitude, indicating that the composition of residues present upstream of C185 also plays a key role in the interaction between KRAS4b and PDE δ (Fig. 3C). We also mutated S181 to alanine and glutamate and all four terminal residues from S181-K184 to alanine, but these three KRAS4b mutants could only be isolated in the farnesylated form (but not methylated) in our engineered insect cell expression system for reasons that remain to be determined. In these cases, we compared K_d values obtained on mutants with KRAS4b-Far. Farnesylated S181A and SKTK(181–184)AAAA showed slightly weaker binding to PDE δ compared with WT KRAS4b-Far, suggesting

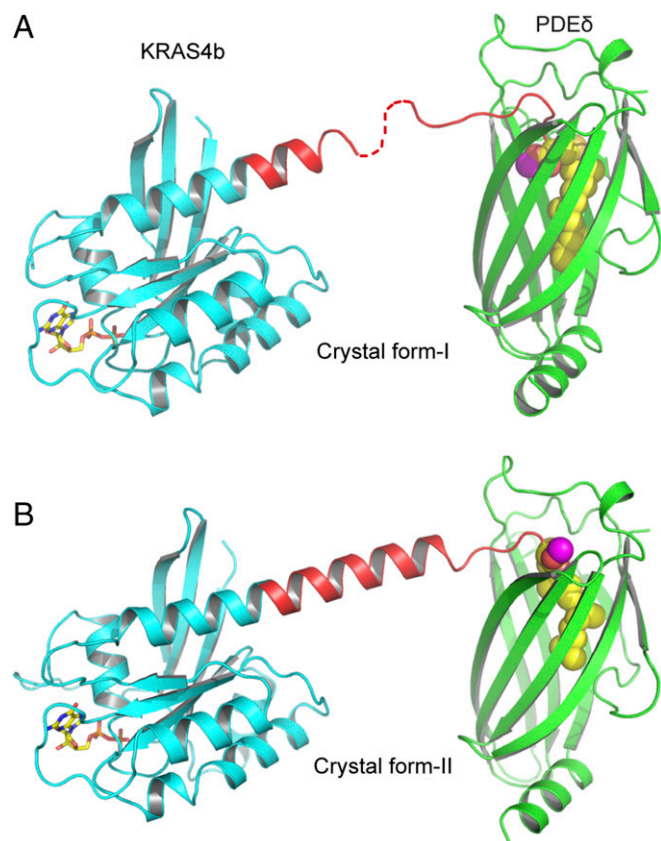


Fig. 2. Overall structure of KRAS4b-PDE δ complex in two different crystal forms. (A) Ribbon representation of GDP-bound KRAS4b-FME in complex with PDE δ in crystal form I. (B) Ribbon representation of GDP-bound KRAS4b-FME in complex with PDE δ in crystal form II. The PDE δ , GTPase domain, and HVR of KRAS4b are shown in green, cyan, and red, respectively. The farnesyl chain is shown as spheres and colored yellow. The carbon and oxygen atoms of carboxymethyl group are colored magenta and red, respectively. GDP is shown as a stick and colored yellow (carbon) and red (oxygen).

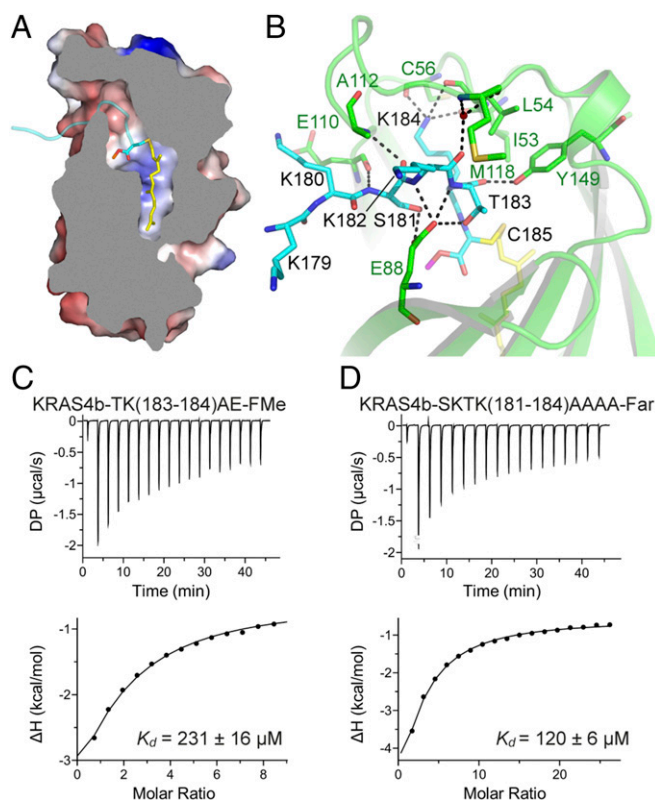


Fig. 3. Details of intermolecular protein-protein interactions in KRAS4b-PDE δ complex and impact of mutation of the HVR residues on KRAS4b-PDE δ interaction. (A) Sliced view of an electrostatic representation of PDE δ showing the region where the HVR residues (colored cyan) bind and a central hydrophobic pocket that accommodates farnesylated (colored yellow) and methylated C185 (colored cyan) residues (shown in stick representation). (B) Intermolecular hydrogen bonding contacts involving residues from the HVR of KRAS4b and PDE δ . Residues from PDE δ and KRAS4b are shown in stick representation and colored green and cyan, respectively. The farnesyl and methyl groups are shown in stick representation and colored yellow and magenta, respectively. Dashed black lines indicate intermolecular hydrogen bonds. (C) ITC titration experiments to measure the dissociation constant between farnesylated-methylated double mutant (T183A-K184E) of KRAS4b and WT PDE δ . (D) ITC titration experiments to measure the dissociation constant between farnesylated quadruple mutant (S181A-K182A-T183A-K184A) of KRAS4b and WT PDE δ . This mutant could only be expressed and purified in farnesylated form in our engineered insect cell expression system. DP, differential power.

that smaller aliphatic residues are tolerated at these positions (Fig. 3D and Fig. S3B). Unlike farnesylated S181A, farnesylated S181E mutant showed much weaker binding with PDE δ , indicating that the presence of a larger and acidic amino acid at residue 181 affects KRAS4b binding to PDE δ (Fig. S3C). Overall, these studies showed that mutations of the HVR residues located upstream of C185 to small aliphatic amino acid, such as alanine, are tolerated but any drastic mutation to bulky and acidic amino acids, such as glutamate, severely affects the interaction between these two proteins. **Farnesylated and methylated C185.** The farnesyl and methyl groups attached to C185 of KRAS4b bind in a hydrophobic pocket formed by \sim 23 PDE δ residues, consisting mainly of Phe, Ile, Leu, Trp, Val, Met, and Ala (Fig. 4A). There are five phenylalanine residues, each present on individual β -strands that line the bottom of the hydrophobic pocket (Fig. 4A). Among these residues, F133 comes closest to the tail end of the farnesyl chain and forms hydrophobic interactions with terminal carbon atoms of the farnesyl chain. Residue W90 of PDE δ is located at the entry point of the pocket and expected to play a role in the entry of the HVR into

the hydrophobic tunnel. Other aliphatic amino acids, such as Ile, Leu, Val, and Ala, of PDE δ position themselves around the aliphatic carbon atoms of the farnesyl chain to provide a hydrophobic environment when KRAS4b is trafficking inside the hydrophilic milieu of the cytoplasm (Fig. 4A). The carboxymethyl group of C185 binds at another pocket formed by PDE δ residues W90, F92, and I109. Residues R61 and Q78 of PDE δ are located close to this pocket and provide a polar environment for the carboxymethyl group of C185 (Fig. 4B).

To understand the role of various PDE δ residues in the interaction with the farnesyl and methyl groups of KRAS4b, we selected eight different PDE δ residues based on the KRAS4b-PDE δ complex structure (F15, M20, W32, E88, W90, Q116, I129, and F133) and mutated those individually to alanine (Fig. 4C and Fig. S4A). We measured the dissociation constants (K_d) of these eight PDE δ mutants with KRAS4b-FMe using ITC. Surprisingly, most of these mutants could bind to KRAS4b-FMe with close to WT affinity, suggesting that single-amino acid substitutions of these amino acids into alanine do not substantially affect the interaction between KRAS4b and PDE δ (Fig. 4C and Fig. S4B-H). Only the E88A mutation showed fivefold weaker binding to KRAS4b-FMe (Fig. 4D). Examination of the interactions mediated by E88 shows formation of multiple hydrogen bonds with side chain atoms of S181 and main chain atoms of T183, supporting the weaker binding for this point mutant (Fig. 4E).

Comparison of Two Crystal Forms Suggests Two Possible Binding Modes Between Prenylated KRAS4b and PDE δ . Structural superposition of the two crystal forms suggests two different binding modes between KRAS4b and PDE δ . Compared with crystal form I, the HVR residues in crystal form II are shifted upstream by two amino acids at the KRAS4b-PDE δ interface, and thus, only the last three amino acids of the HVR and prenylated C185 of KRAS4b interact with PDE δ (Fig. 5A and B). Although it seems surprising at first that, even after shifting two amino acids, the HVR can still form a similar interaction with PDE δ residues, this can be explained by the fact that, in crystal form II, residues K182-T183-K184 occupy the same positions as those of residues K180-S181-K182 in crystal form I (Fig. 5B and C). The methyl group of C185 in crystal form II occupies the same position as that of the side chain atoms of T183 in crystal form I. Consequently, in crystal form II, the farnesyl chain partially occupies the hydrophobic pocket of PDE δ , with the remainder of the empty space packed by conformational changes in the side chain atoms of F133 (Fig. 5B).

Structural superposition of two crystal forms indicates that the hydrophobic pocket of PDE δ in crystal form II can accommodate an additional five carbon atoms if F133 occupies the same rotamer conformation as seen in crystal form I (Figs. 5B and 6A). Previous studies have shown that, when farnesyl transferase inhibitors block farnesylation, KRAS4b undergoes alternative prenylation and is geranylgeranylated by geranylgeranyl transferase (19). The farnesyl chain is 15-carbon atoms long, whereas the geranylgeranyl chain has an additional five carbons attached to it. Structural superposition of the two crystal forms indicates there is space for these additional 5-carbon atoms in the hydrophobic pocket, thus indicating that the 20-carbon-long geranylgeranyl chain would also fit in this pocket (Figs. 5B and 6A).

Crystal Form II Mimics Geranylgeranylated-Methylated KRAS4b Binding to PDE δ . Because crystal form II mimics a possible binding mode of geranylgeranylated-methylated KRAS4b (KRAS4b-GGMe) with PDE δ , we modeled the structure of KRAS4b-GGMe in complex with PDE δ using this structure (Fig. 6B). To allow the geranylgeranyl chain to bind in the hydrophobic pocket, we changed the rotamer conformation of side chain atoms of F133 to the conformation observed in crystal form I. Modeled structures showed an ideal fit for a geranylgeranyl chain in the hydrophobic pocket, with the last three HVR residues mimicking a similar

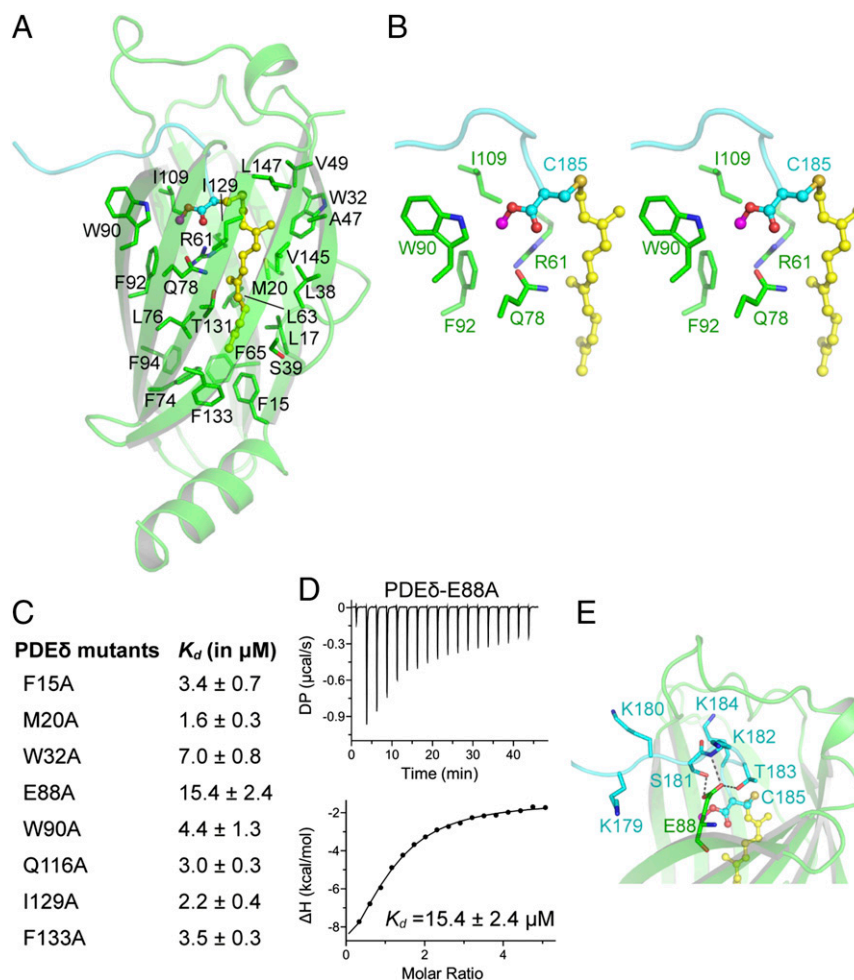


Fig. 4. Recognition of farnesylated and carboxymethylated C185 of KRAS4b by PDE δ and impact of mutation of PDE δ residues on KRAS4b-PDE δ interaction. (A) Nonpolar residues (colored green; stick representation) of PDE δ form a hydrophobic pocket that accommodates the farnesyl chain (colored yellow; ball and stick representation) and carboxymethyl group (magenta and cyan; ball and stick representation). (B) Stereo view of the PDE δ pocket formed by residues (colored green; stick) that accommodates the carboxymethyl group (colored magenta and cyan; ball and stick) of farnesylated C185 (colored yellow; ball and stick). (C) Dissociation constant of PDE δ mutants with KRAS4b-FMe measured using ITC titration experiments. (D) ITC titration experiments to measure the dissociation constant between PDE δ -E88A mutant and KRAS4b-FMe. (E) Intermolecular hydrogen bonds formed by PDE δ residue E88 (colored green; stick) with HVR residues (colored cyan; stick). DP, differential power.

interaction with PDE δ as observed in crystal form II (Fig. 6B). Recently, the structure of PDE δ in complex with geranylgeranylated-methylated peptide from PDE6 α' was reported (30). Comparison of this structure with our modeled structure of KRAS4b-GGMe in complex with PDE δ shows a similar position of the geranylgeranyl chain inside the hydrophobic pocket and similar HVR residues (KSKT in PDE6 α' vs. KSKTK in KRAS4b) upstream of the prenylated Cys, differing mainly in the position of the carboxymethyl group likely because of different penultimate residues in these two proteins (Fig. 6C).

Comparison Between KRAS4b-PDE δ and Rheb-PDE δ Structures. A structural comparison of KRAS4b-PDE δ and Rheb-PDE δ complexes revealed that neither KRAS4b nor Rheb interact with PDE δ via the GTPase domain (26), providing a rationale for the observed broad specificity of PDE δ to other members of the RAS superfamily. Along with this similarity, we observed a few significant differences between these two complexes. Unlike Rheb, the farnesyl and methyl groups in KRAS4b bind deeper inside the hydrophobic pocket of PDE δ in crystal form I (Fig. S5A and B). In addition, the methyl groups present at the farnesylated cysteine in these two structures point in opposite directions with respect to the HVR. In

the Rheb-PDE δ complex, the methyl group occupies the same position as that of K184 in the KRAS4b-PDE δ complex. Furthermore, in the Rheb-PDE δ complex, only three HVR residues and the prenylated cysteine interact with PDE δ (26), whereas in the KRAS4b-PDE δ complex (crystal form I), the last four residues and prenylated cysteine interact with PDE δ (Fig. S5B). Additional interactions in the case of KRAS4b are facilitated by large movements of side chain atoms of W90 at the entry point and F133 at the bottom of the hydrophobic pocket in PDE δ (Fig. S5B). The position of the farnesyl chain in crystal form II matches that of the farnesyl chain in the Rheb-PDE δ structure (Fig. S5C and D). However, the position of the methyl group and interactions between the HVR and PDE δ residues differ in these two structures.

Identification of a Conserved Sequence Motif in KRAS4b and Its Role in Facilitating Binding of Prenylated KRAS4b to PDE δ . The two different modes of binding observed in our crystal structures are possible because of the presence of a unique 5-aa-long sequence motif KSKTK present in KRAS4b (Fig. 6D). This motif maintains many KRAS4b-PDE δ interactions in the crystal form II, even when the HVR residues shift upstream by two amino acids at the protein-protein interaction interface. Sequence analysis of KRAS HVR

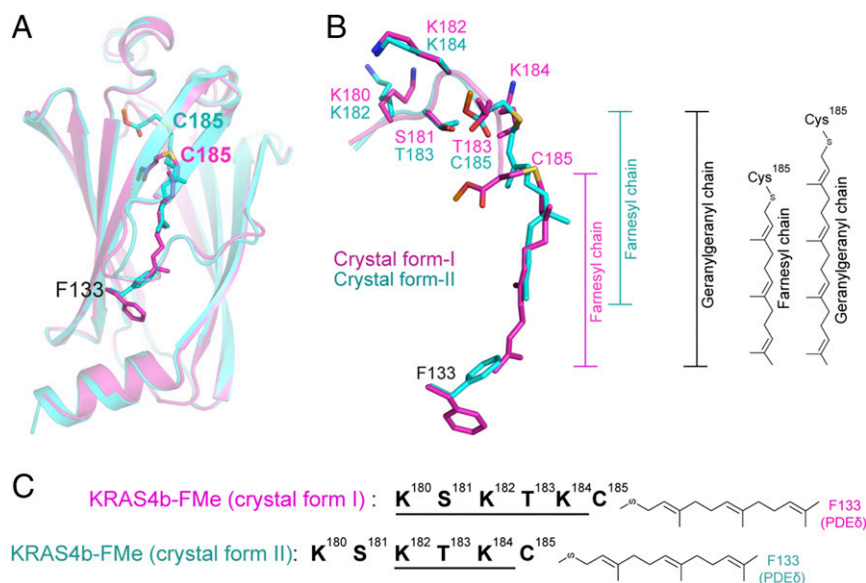


Fig. 5. Structural comparison of two crystal forms of KRAS4b–PDE δ complex shows two possible modes of binding between KRAS4b and PDE δ . (A) Structural superposition of PDE δ residues from two crystal forms showing different positions of farnesyl and methyl groups attached to C185. Crystal forms I and II are colored magenta and cyan, respectively. (B) Enlarged view of structural superposition of HVR residues and farnesylated–methylated C185 from two crystal forms showing upstream shift of the HVR residues by two amino acids at the protein–protein interaction interface in crystal form II, resulting in the empty space in the central hydrophobic pocket of PDE δ . Positions of the farnesyl chains are shown. Structural superposition of the two crystal forms suggests that the empty space inside the hydrophobic pocket of PDE δ in crystal form II would fit an additional five carbons present on the geranylgeranyl chain. (C) Schematic diagram showing a 2-aa shift of the HVR residues in crystal form II. Because of the unique amino acid composition in the region, interaction interface formed by residues K182–T183–K184 residues in crystal form II mimics the interaction interface formed by residues K180–S181–K183 in crystal form I.

residues in other organisms showed the presence of similar motifs that were compatible with binding of prenylated KRAS with PDE δ (Fig. 6D). This “prenylated KRAS recognition” motif contains large basic amino acids (Lys or Arg) at first, third, and fifth positions and smaller polar amino acids (Ser or Thr) at second and fourth positions. Interactions formed at the KRAS–PDE δ interface by the first three amino acids (first, second, and third) of this motif when KRAS is farnesylated would overlap with the last three amino acids (third, fourth, and fifth) present in this motif when KRAS is geranylgeranylated. Thus, conservation of this sequence motif in the KRAS4b sequences of many organisms indicates that PDE δ could bind to KRAS4b in both farnesylated and geranylgeranylated forms.

Structure-Based Sequence Analysis of PDE δ Binding Proteins. Previous studies have shown that a large repertoire of prenyl proteins, such as subunits of PDE6, GRK1, GRK7, Rab13, Rap1a, Rap2b, RhoA, RhoB, Rnd1, Rap2c, RasD2, and Reb-L1, bind to PDE δ (20, 22, 27, 31–33). Among RAS proteins, in addition to KRAS4b, NRAS and HRAS have also been shown to bind to PDE δ (20, 27). However, there have been some discrepancies in the literature on HRAS binding to PDE δ . Nancy et al. (27) showed that HRAS binds to PDE δ , but this observation could not be corroborated by later studies from two different groups (20, 31). Unlike many other prenylated members of RAS superfamily, KRAS4a, RalA, RalB, and Rac1 do not bind to PDE δ (27, 31, 34). To understand how PDE δ binds to a large number of prenylated proteins and why some prenylated proteins do not bind to PDE δ , we carried out structure-based sequence analysis using available structures of PDE δ in complex with prenylated proteins or peptides. Structural superposition of PDE δ in complex with the prenylated proteins KRAS4b and Rheb (26) and prenylated peptides from PDE6 α' (30) and INPP5E (35) showed that prenylated Cys occupies different positions in these complexes and PDE δ interacts with three or four amino acids located upstream of prenylated Cys (Fig. 7A and B). Structure-based sequence alignment of residues interact-

ing with PDE δ is shown in Fig. 7C, which shows that none of the residues located upstream of prenylated Cys in these structures are conserved. Structural superposition shows that, except Thr183, other residues located upstream of prenylated Cys in KRAS4b can be small or large and polar or charged amino acids. In the structural superposition, residue Thr183 in KRAS4b-FMe occupies a position corresponding to Ser, Thr, and Ile in Rheb, PDE6 α' , and INPP5E, respectively (–2 position in KRAS4b-FMe structure and –1 position in other structures). In the crystal form II, this position is occupied by carboxymethyl group of prenylated Cys. Examination of the protein–protein interface at –2 position corresponding to Thr183 in KRAS4b suggests that this position cannot accommodate large amino acids, like Lys, Arg, and Glu (Fig. 7D). Based on this rationale we propose that, for any prenylated protein to be able to bind to PDE δ , it should have small side chain containing residues at –1 and –2 positions.

Sequence alignment of various proteins that have been previously tested for binding to PDE δ shows no conserved amino acids upstream of the prenylated Cys (Fig. 7E). Examination of residues present at –1 and –2 positions in this alignment shows the presence of large and charged amino acids, such as Lys, Arg, and Glu in case of KRAS4a, RalA, RalB, Rac1, and Rheb-L1, suggesting that the presence of these residues would prevent their ability to bind to PDE δ . Although Rheb-L1 has been previously shown to bind to PDE δ (20), our analysis indicates that the two arginines located upstream of prenylated Cys in Rheb-L1 will sterically clash with PDE δ at the protein–protein interface.

Discussion

The prenyl binding protein PDE δ has been shown to modulate signaling through RAS proteins by sustaining their dynamic distribution in cellular membranes (20, 23–25). Impairing KRAS4b localization at the plasma membrane by inhibiting PDE δ provides additional opportunities to target KRAS-driven cancer. Here, we present structures of the fully processed KRAS4b–PDE δ complex, which not only provide atomic details of the protein–protein

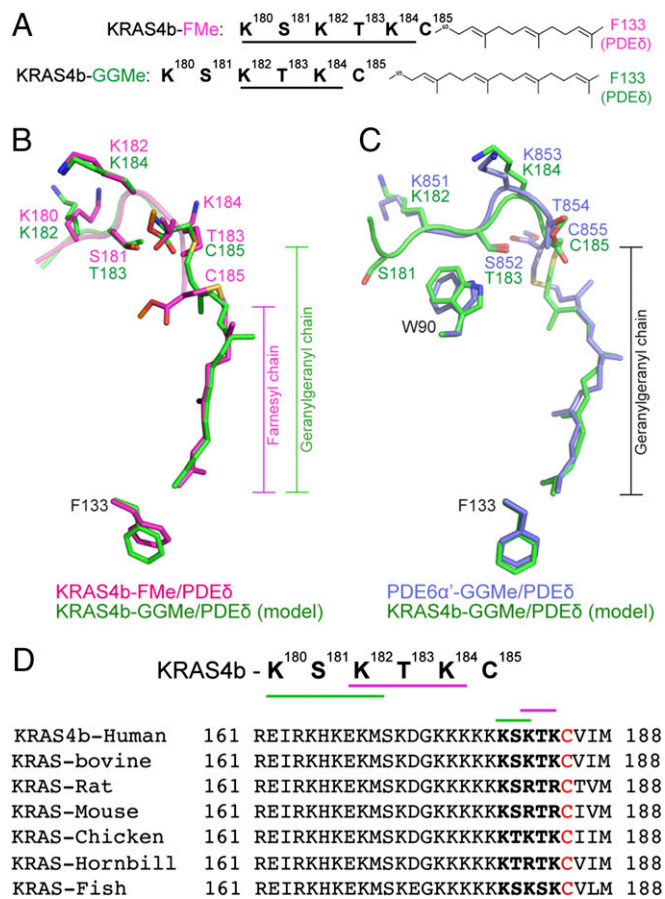


Fig. 6. KRAS4b-PDE δ structure in crystal form II mimics the binding of KRAS4b-GGMe to PDE δ and identification of a new sequence motif in the HVR of KRAS4b. (A) Schematic diagram showing the additional five carbon atoms present in the geranylgeranyl chain compared with the farnesyl chain. (B) Structural superposition of prenylated C185 and the HVR residues from KRAS4b-FMe-PDE δ structure in crystal form I (colored magenta) and modeled structure of KRAS4b-GGMe-PDE δ (colored green) using KRAS4b-FMe-PDE δ structure in crystal form II. Positions of farnesyl and geranylgeranyl chains are highlighted. (C) Structural superposition of geranylgeranylated-methylated pentapeptide of PDE6 α' in complex with PDE δ (colored light blue; PDB ID code 5ETF) and modeled structure of KRAS4b-GGMe in complex with PDE δ (colored green). Positions of geranylgeranyl chains in these two structures are similar. PDE6 α' -GGMe, geranylgeranylated-methylated PDE6 α' . (D) Sequence alignment of amino acid sequence of the HVR of KRAS4b from various organisms shows the presence of a unique 5-aa-long sequence motif that allows the binding of prenylated KRAS4b to PDE δ as seen in this study. The HVR residues proposed to maintain similar protein-protein interaction in the KRAS4b-FMe-PDE δ and the KRAS4b-GGMe-PDE δ complexes are highlighted by magenta and green-colored lines above the sequence alignment.

interaction interface but also, reveal how PDE δ can bind to different forms of prenylated KRAS4b and why only some prenylated proteins bind to PDE δ . Our binding studies show that the carboxymethyl group present on farnesylated C185 plays a key role in the KRAS4b-PDE δ interaction (Fig. 1 B-E and Fig. S1). The weak binding observed in the absence of the methyl group is likely caused by the charge repulsion effect of the carboxylic acid group and the decreased hydrophobicity of farnesylated C185. Our results are in accordance with the previous findings showing that carboxymethylation of prenylated Rho peptide and catalytic subunits of PDE6 significantly increases their affinity for RhoGDI and PDE δ , respectively (33, 36). Carboxymethylation is the only step in the posttranslational modification of the CaaX motif that is potentially reversible (37). The importance of the methyl group in KRAS4b

binding to PDE δ highlights the potential regulatory significance of this modification in trafficking KRAS4b to cellular membranes. Thus, our results suggest that KRAS4b molecules that are not fully processed (not farnesylated or methylated) will not bind to PDE δ and will not be delivered to the plasma membrane.

Although a large number of RAS structures have been solved during the preceding 25+ years of structural studies on RAS proteins, these studies did not provide any structural information on residues present in the HVR. The structure of the KRAS4b-PDE δ complex (crystal form II) described here provides atomic details of the entire HVR (165-185) (Fig. 2B and Fig. S2B) and likely represents a conformation of HVR residues in the cytoplasm when KRAS4b is bound to PDE δ . Additional studies are required to determine if some of the HVR residues could also form a similar α -helical conformation when KRAS4b is anchored to the membrane. Previous studies suggest that, in membrane-bound KRAS4b, the farnesyl and methyl groups are inserted inside the membrane bilayer, and the polybasic region present in the HVR interacts with acidic phospholipids, such as phosphatidylserine or phosphatidylinositol, that are preferentially located in the inner leaflet of the plasma membrane (38). Our mutational analysis of KRAS4b HVR residues showed that, other than the prenylated Cys (C185), the composition of the residues located upstream of C185 also plays a role in KRAS4b-PDE δ interactions. Although mutation to alanine of four residues (S181, K182, T183, and K184) preceding the prenylated C185 was tolerated, mutations of K184 and S181 to an acidic and larger residue like glutamate resulted in a significant loss of binding (Fig. 3C and Fig. S3C). Residue S181 is a part of the protein-protein interaction interface only in the crystal form I. The larger protein-protein interaction interface and role of S181 of KRAS4b in KRAS4b-FMe-PDE δ interaction

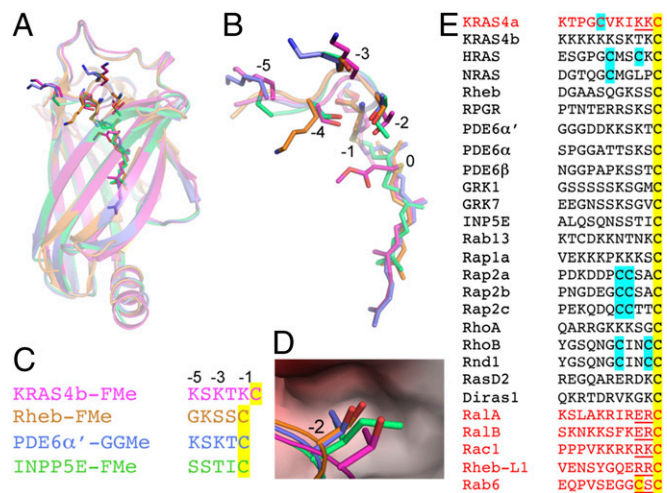


Fig. 7. Structural and sequence analysis of prenylated proteins that have been tested previously for binding to PDE δ . (A) Structural superposition of PDE δ in complex with KRAS4b-FMe (crystal form I), farnesylated-methylated Rheb (Rheb-FMe; PDB ID code 3T5G), geranylgeranylated-methylated PDE6 α' (PDE6 α' -GGMe; PDB ID code 5ETF), and farnesylated-methylated INPP5e (INPP5E-FMe; PDB ID code 5F2U). (B) Enlarged view of prenylated protein/peptide shown in A. The residues located upstream of prenylated C185 are numbered from -1 (K184) to -5 (K180). (C) Structure-based sequence alignment of prenylated protein/peptide shown in A and B. (D) Enlarged view of the binding pocket formed by PDE δ residues, where Thr183 in KRAS4b and corresponding Ser, Thr, and Ile residues in Rheb, PDE6 α' , and INPP5E localize at protein-protein interface. (E) Sequence alignment of various proteins that have been tested previously for binding to PDE δ . Prenylated and palmitoylated Cys are highlighted in yellow and cyan, respectively. Prenylated proteins that contain either large polar amino acids at -1 and -2 positions or a second prenylated Cys site (shown in red) would cause steric clash with PDE δ residues and are unlikely to form a protein-protein complex.

indicate that the structure observed in crystal form I represents the biological interaction of KRAS4b-FMe with PDE δ .

Chandra et al. (20) showed that eight lysine residues present in the polybasic region of the KRAS4b are not necessary for binding to PDE δ and that the interaction becomes stronger when these lysine residues are mutated to glutamate. They also showed that diminishing the charge on the polybasic region by stimulating phosphorylation of S181 with bryostatin-1 enhances the ability of PDE δ to solubilize KRAS4b. Recent studies have also shown that KRAS suppresses Wnt/Ca²⁺ signaling pathway by direct binding with calmodulin and that the KRAS4b–calmodulin interaction is attenuated by phosphorylation of S181 by PKC (39, 40). Our structural data show that six lysine residues present between 175 and 180 do not interact with PDE δ and thus, are not likely to play any role in KRAS4b–PDE δ interaction. In contrast to the above-mentioned study, we saw that mutation of K184 (in the KRAS4b double-mutant T183A-K184E) to glutamate showed significant loss of binding affinity between KRAS4b and PDE δ . Also, the S181E mutant showed significant loss in the binding affinity compared with S181A. Analysis of the KRAS4b–PDE δ interaction interface suggests that phosphorylation of S181 is likely to decrease the binding affinity between KRAS4b and PDE δ because of steric hindrance and charge repulsion, because the side chain atoms of Glu88 of PDE δ form hydrogen bonds with the side chain atoms of KRAS4b S181.

Structure and sequence alignment of prenylated proteins that have been shown to bind to PDE δ shows that the residues involved at the protein–protein interface are not conserved and that prenylated cysteine can occupy multiple positions inside the central hydrophobic pocket of PDE δ . These observations are in accordance with the known broad specificity of PDE δ for prenylated proteins. The hydrophobic pocket in PDE δ is too small to accommodate another lipid chain. Thus, the prenylated proteins that undergo palmitoylation upstream of the prenylated Cys can bind to PDE δ only in the depalmitoylated state. For example, although Rab6 contains smaller amino acids upstream of the prenylated Cys, it cannot bind to PDE δ , because it becomes geranylgeranylated at the –3 position as well. Unlike palmitoylation, prenylation is not a reversible modification. Based on our structural and sequence analysis, we propose that prenylated proteins that contain a residue with a small side chain at –1 and –2 positions upstream of the prenylated Cys are likely to bind to PDE δ . This hypothesis explains why other prenylated members of the RAS superfamily, such as KRAS4a, RalA, RalB, and Rac1, do not bind to PDE δ . With this reasoning, one could predict if a given prenylated protein is likely to bind to PDE δ or not.

Unlike KRAS4b, the HVRs of KRAS4a, HRAS, and NRAS are mono- or dipalmitoylated at Cys residues located upstream of the C-terminal Cys. Palmitoylation of RAS proteins is readily reversible *in vivo* and plays a key role in their subcellular localization. Palmitoylation of HRAS/NRAS at the Golgi stabilizes their association with membranes. Depalmitoylation releases HRAS/NRAS into the cytoplasm, allowing their return to the Golgi for another round of palmitoylation. Sequence and structure analysis of KRAS4b and HRAS/NRAS indicates that PDE δ should be able to bind to depalmitoylated HRAS/NRAS, because it contains small side chain-containing residues upstream of prenylated Cys. As shown previously (20), it is possible that, in a steady state, the fraction of depalmitoylated HRAS is much less than that of depalmitoylated NRAS, because HRAS becomes palmitoylated at two positions in the HVR as opposed to just one position in NRAS. In accordance with the previous findings that, unlike KRAS4b, KRAS4a does not bind to PDE δ (31), our sequence and structural analysis suggests that the steric hindrance caused by two lysine residues located upstream of prenylated Cys is likely to interfere with interaction between depalmitoylated KRAS4a and PDE δ . NRAS has been shown to bind to PDE δ only in the farnesylated form and not in the geranylgeranylated

form (20). Although NRAS contains small side chain-containing amino acids (Met-Gly-Leu-Pro) upstream of prenylated Cys, it is likely that the presence of a proline residue at –1 position results in limited flexibility, because proline and its preceding residue will have restricted ϕ – ψ torsion angles in the protein backbone. Limited flexibility of residues present at –1 and –2 positions could possibly affect the binding of geranylgeranylated NRAS to PDE δ .

Structural superposition of PDE δ in complex with prenylated proteins/peptides shows that the prenylated Cys of KRAS4b binds deeper inside the hydrophobic pocket of PDE δ , and thus, four additional KRAS4b residues (181–184), rather than three, are located inside the binding pocket of PDE δ . Comparison of the KRAS4b–PDE δ structure in crystal form I with the previously solved Rheb–PDE δ structure (26) shows that W90 and F133 residues in PDE δ undergo conformational changes to allow more extensive interaction between KRAS4b and PDE δ compared with that between Rheb and PDE δ (Fig. S5 A and B). This observation indicates that PDE δ interaction with KRAS4b could be more specific than other prenylated proteins and thus, despite PDE δ having broad specificity for prenylated proteins, it could serve as a potential target for KRAS-driven cancers. GTP-bound Arl2/Arl3 has been shown to disrupt RAS–PDE δ interaction at the membrane (26). Superposition of the KRAS4b–PDE δ and GTP-bound Arl2–PDE δ structures shows the possible formation of a ternary complex. However, based on an Rheb–PDE δ structure, it was suggested that binding of GTP-bound Arl2 to PDE δ forces the latter into a closed conformation by altering the conformation of two β -strands in PDE δ with the result that the RAS protein can no longer bind to PDE δ –Arl2 complex (26).

Recently, it was shown that PDE δ binds to geranylgeranylated–methylated PDE6 α' pentapeptide with an affinity ($K_d = 2.3 \pm 0.9$ nM) \sim 1,000 times higher than that of the KRAS4b-FMe (30). Sequence and structural similarity between our modeled structure of KRAS4b-GGMe–PDE δ and geranylgeranylated–methylated PDE6 α' pentapeptide in complex with PDE δ supports the potential binding of KRAS4b-GGMe by PDE δ (Fig. 6C). It is likely that the interaction formed by the additional five carbon atoms present in the geranylgeranyl chain within the hydrophobic pocket will result in a higher affinity between these two proteins.

Analysis of the amino acid sequence upstream of C185 showed the presence of a 5-aa sequence motif (K-S-K-T-K) that allows the formation of similar protein–protein interactions in both crystal forms of KRAS4b–PDE δ complex. The crucial role played by this motif in allowing prenylated KRAS4b binding to PDE δ is supported by the fact that this motif is highly conserved in KRAS4b across various organisms (Fig. 6D). Identification of this sequence motif strengthens our hypothesis that both the KRAS4b-FMe and KRAS4b-GGMe could bind to PDE δ by shifting the HVR residues by two amino acids toward the N terminus at KRAS4b–PDE δ interface, allowing PDE δ to target both forms of prenylated KRAS4b to cellular membranes. Based on our sequence and structural analyses, we suggest that crystal form I shows how KRAS4b-FMe binds to PDE δ , whereas crystal form II indicates how KRAS4b-GGMe could bind to PDE δ . It is important to note here that, until now, the only evidence that KRAS4b can be geranylgeranylated is in the presence of an FTI. No physiological condition has yet been found under which the KRAS4b modification is shifted to geranylgeranylation.

Despite the significant progress in characterizing the role of PDE δ in targeting RAS to the membrane and identifying nanomolar inhibitors that can break KRAS4b–PDE δ interactions, we need to overcome additional challenges before we can successfully target PDE δ for KRAS4b-driven cancers. Previous studies have shown that KRAS KO in mice is lethal (41, 42) but that PDE δ KO is not (43). This difference may be because PDE δ helps in the localization of KRAS4b, not of KRAS4a, and therefore, only affects one isoform of KRAS. Future studies need to focus on determining

exactly how dependent RAS proteins are on PDE δ for proper localization to the membrane (20) and on PDE δ inhibitors that could target specific interactions between KRAS4b–HVR and PDE δ . The biggest challenge would be to design a specific inhibitor that can avoid any toxic or off-target effects of inhibiting PDE δ . One might also search for molecules that lock KRAS4b and PDE δ together so that they become resistant to dissociation in the presence of GTP-bound Arl2/Arl3 and thus, decrease population of active KRAS4b at the plasma membrane. A therapeutic strategy to alleviate, instead of eliminate, specific RAS proteins inside the cell might prove effective in targeting cancer, making PDE δ a target highly worthy of attention.

Materials and Methods

Cloning, Expression, and Purification. KRAS4b-FMe (human) was cloned, expressed, and purified using the procedures described previously (44). Human PDE δ was cloned in pDest-566 vector with N-terminal His₆-maltose binding protein tag and expressed in BL21 STAR (rne131) *Escherichia coli* strain. Highly purified untagged proteins were purified using a multistep purification process. *SI Materials and Methods* has a detailed methodology on cloning, expression, and purification.

Crystallization and Data Collection. Before crystallization setup, purified KRAS4b-FMe and PDE δ proteins were mixed in a molar ratio of 1:1 and incubated on ice for 1 h. The complex crystals were grown with the sitting drop vapor diffusion method by mixing the protein–protein complex with an equal volume of reservoir solution containing 0.1 M citrate (pH 5.0) and 20% (wt/vol) PEG 6000 (crystal form I) and 0.1 M Hepes:NaOH (pH 7.5) and 2 M ammonium sulfate (crystal form II). Small crystals appeared in 2–3 d and grew to full size in 10 d. For data collection, crystals were flash frozen (100 K) in the above reservoir solutions supplemented with 30% (vol/vol) ethylene glycol as a cryoprotectant. Crystallographic datasets were integrated and scaled using the XDS (45). The high-resolution cutoffs of 2.0 and 1.9 Å were selected for crystal forms I and II, respectively, because reflections present in the high-resolution shell have average $I/\sigma(I)$ above three, multiplicity close to seven, and completeness close to 100%, despite having relatively high R_{merge} values. Crystal form I belongs to the space group P2₁ and contains one copy of KRAS4b–PDE δ complex in the asymmetric unit. Crystal form II belonged to the space group P2₁2₁2₁ and contains two copies of KRAS4b–PDE δ complex in the asymmetric unit. The crystal parameters and the data collection statistics are summarized in Table S1.

Structure Determination and Analysis. Crystal structures of KRAS4b and PDE δ complex were solved by molecular replacement as implemented in the program PHASER (46) using the apo-structure of truncated KRAS4b [1–169; Protein Data Bank (PDB) ID code 4OBE] and protein atoms corresponding to PDE δ in the farnesylated–methylated Rheb peptide–PDE δ complex structure (PDB ID code 3T5G). Initial map showed interpretable electron density, in which both the proteins could be built manually. Additional model improvement was carried out with alternate rounds of model building via COOT (47) and refinement using Phenix.refine (48, 49) and Refmac (50), with rigid body, simulated annealing, positional, and translation–libration–screw (TLS) refinement at different stages. After a few rounds of refinement, inspection of the difference ($F_o - F_c$) map showed positive density for the farnesylated and methylated C185 and GDP. Placement of ligands were followed by identification of potential

sites of solvent molecules by automatic water-picking algorithm in COOT (47). The positions of these automatically picked waters were checked manually during model building. The majority of the model has a clear and well-interpretable electron density map with the exception of switch 1 residues and three to five residues in HVR in crystal form I, which were omitted in the final model. Refinement statistics of both crystal forms are given in Table S1. Structural comparisons were performed using the SSM algorithm (51) implemented in COOT. Figures were prepared using the program PyMOL (52). All crystallographic and structural analysis software used in this study was compiled and maintained by the SBGrid consortium (53).

Binding Affinity Measurements by ITC. ITC experiments were performed in a MicroCal PEAQ-ITC (Malvern). Both KRAS4b and PDE δ proteins were extensively dialyzed in 20 mM Hepes (pH 7.3), 300 mM NaCl, 5 mM MgCl₂, and 1 mM TCEP [tris(2-carboxyethyl)phosphine]. Before titration, both proteins were centrifuged at 14,000 \times g for 5 min to remove any debris and air bubbles. Protein concentration was measured using absorbance at 280 nm. Dialyzed KRAS4b and PDE δ proteins were loaded into the calorimeter cell and titration syringe, respectively. The calorimetric titrations were carried out at 25 °C using 15–18 injections of 2.4–2.6 μ L each injected at 150- to 180-s intervals. A control experiment was performed by titrating PDE δ into a sample cell that contained buffer alone, with no significant heat release observed. Data analysis was performed based on a binding model containing “one set of sites” using a nonlinear least squares algorithm incorporated in the MicroCal PEAQ-ITC analysis software (Malvern).

SV-AUC. To study the interaction of PDE δ with the different forms of KRAS4b, ~1:1 stoichiometric mixtures of PDE δ :KRAS4b-FMe and PDE δ :KRAS4b-Far were studied at different concentrations ranging from ~2–80 μ M using SV-AUC technique. *SI Materials and Methods* has detailed information on sedimentation velocity experiments.

PDB ID Codes. The atomic coordinates and structure factors for KRAS4b-FMe–PDE δ complex in crystal forms I and II have been submitted to the PDB and assigned ID codes 5TB5 and 5STAR, respectively.

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