

Mechanisms of Allostery and Membrane Attachment in Ras GTPases: Implications for Anti-Cancer Drug Discovery

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Abstract: Ras GTPases are membrane-anchored molecular switches that mediate signaling pathways controlling a variety of cellular processes, including cell division and development. Despite their prominent role in many forms of cancer, little is known about the structure of the membrane bound protein or the mechanism and thermodynamics of membrane insertion. The modulation of membrane binding by the catalytic domain is another area of on-going scrutiny. Recent computational and experimental efforts that have begun to shed some light on these issues are the subject of this review. The bulk of the available structural and thermodynamic information on membrane-bound Ras has been obtained by studying peptides derived from the membrane-anchoring regions of N-ras and H-ras proteins. However, those results have been complemented by data, though limited, on the membrane binding of the full-length Ras as well as by predictions about putative communication routes between the GTP-hydrolyzing catalytic domain and the membrane-interacting C-terminus. A tentative mechanistic picture of Ras signaling that is emerging from these studies will be discussed in connection with allostery and implication for the design of selective anti-cancer drugs.

Keywords: Membrane orientation, molecular dynamics, allostery, Ras signaling, lipid-anchor, thermodynamics, dynamics.

INTRODUCTION

Ras proteins are monomeric GTP-hydrolyzing enzymes (GTPases or G-proteins). They are crucial molecular switches in major signaling pathways, such as the mitogen-activated protein kinase (MAPK) cascade that controls cell proliferation and differentiation [1,2]. Numerous biological, biochemical and biophysical studies over the last four decades have documented the involvement of Ras in tumorigenesis and developmental disorders [1,3-5]. Indeed, malfunction in the switching function of Ras is associated with about a third of all human tumors.

Humans have three RAS genes that encode for three ubiquitously expressed and highly homologous proteins: N-ras, H-ras and K-ras4B (hereafter K-ras) [1,5-7]. These isoforms interact with overlapping downstream targets yet produce specific signal outputs [8]. They are also associated with different diseases [9,10]: disease-causing mutations in K-ras are associated with Noonan syndrome or pancreatic, lung, and colorectal cancers; mutations in H-ras are associated with Costello syndrome and bladder cancer whereas those of N-ras are implicated in a variety of leukemia. Therefore, understanding the molecular basis of functional specificity in Ras signaling is a topic of much current research. A longstanding challenge in this endeavor is the absence of structural and thermodynamic data that would provide a mechanistic picture of the signaling process; atomic-resolution structure determination of fully functional, membrane-bound, Ras proteins remains elusive.

Recent studies using computer simulations and biophysical experiments are beginning to play key roles in providing the initial clues into the structural and thermodynamic factors underlying specificity in Ras signaling. Both the simula-

tion and experimental studies thus far focused on model peptides representing the membrane-interacting regions of Ras [11-19]. However, full length N- and H-ras have also been investigated, albeit to a limited extent [18-21]. Several recent reviews extensively discussed results from these studies and their implications for basic and applied research in Ras signaling [20,22,23]. The current review covers results from the most recent computational and experimental efforts that more directly shed light on the issue of Ras isoform-specific signal transduction. A major focus is on the structural and thermodynamic aspects of Ras-membrane interactions and their interpretation in terms of allostery. Allostery was originally defined as the regulation of function by a ligand-induced change in protein quaternary structure [24], and an allosteric effect [25-28] as a concerted action between two coexisting discrete states (Monod-Wyman-Changeux model [29]) or a sequential conformational change (Koshland-Nemethy-Filmer model [30]). This definition does not account for communication within monomeric proteins, such as the coupling between the catalytic and membrane binding regions of Ras to be discussed here. However, the concept of allostery was recently expanded to "a change in the tertiary and quaternary structure, or both, induced by a small molecule or another protein" [31] and "a re-distribution of protein conformational ensembles" [25,32]. Despite their seemingly contradictory premises, i.e., "induced" versus "re-distribution" of conformations, these definitions imply that allostery "could be a change in the flexibility of the protein rather than simply a change in the structure" [31]. It follows that allosteric transition can occur in single domain monomeric proteins. This is supported by the observed allostery in the monomeric nitrogen regulatory protein C (NtrC) [33] and human p38 MAP kinase [34], and by the introduction of allostery into nonallosteric proteins (reviewed in [32]). This latest definition of allostery will be used throughout this review to explain both computational and experimental observations.

This review is organized as follows. First, the biological and biochemical knowledge pertinent to the issue of func-

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tional specificity is briefly discussed. Then, aspects of Ras-membrane interaction that provide pointers to the determinants of specificity, and open new possibilities for the design of isoform-specific therapeutic agents, are discussed in some detail. This is followed by a discussion on the possible link between membrane orientation of H-ras and conformational changes at the nucleotide binding site. Finally, a brief perspective into the future of Ras research is presented as a concluding remark.

LIPID MODIFICATION AND MEMBRANE BINDING

Membrane binding is essential for the biological function of Ras [35]. Membrane-targeting is achieved through lipid-anchors, i.e., protein motifs formed by posttranslational lipid-modification of C-terminal Cys residues. Lipid-modification involves a series of enzymatic steps (Fig. 1). The first is farnesyltransferase (FTase)-catalyzed attachment of a farnesyl group to the Cys side chain of a CAAX signal (*A* usually represents aliphatic and *X* any amino acid) [36]. The second step is proteolysis of the AAX sequence by the Ras-converting enzyme (RCE) [37]. The now terminal Cys is carboxymethylated by isoprenylcysteine carboxymethyltransferase (ICMT) [38]. This step completes the processing of K-ras because K-ras contains an additional membrane-interacting motif consisting of a stretch of positively charged residues preceding the farnesylated Cys. At this stage, K-ras is therefore transported to the plasma membrane *via* an unknown mechanism. N- and H-ras undergo further modification involving reversible palmitoylation of one (N-Ras) or two (H-ras) Cys residue(s) by the enzyme palmitoyltransferase [39]. The fully processed and mature N- and H-ras are

then transported to the plasma membrane *via* the exocytic pathway [40,41]. It is important to note, however, that unlike K-ras, N- and H-ras also signal from endomembranes and cycle between the plasma membrane and Golgi depending on whether or not they are palmitoylated [42]. Furthermore, plasma membrane-bound Ras proteins are in dynamic equilibrium between cholesterol-enriched and cholesterol-deficient membrane subdomains [43-45]. These subdomains, termed nanoclusters, are small with typical radii of 6-20 nm containing ~7 Ras proteins per cluster [22,44,46-49]. Intriguingly, nanoclustering was found to be activation state dependent so that GTP-H-Ras is populated in cholesterol-insensitive nanodomains while GDP-H-ras segregates to cholesterol-sensitive subdomains. Recent experimental and computational studies demonstrated that nanoclusters play a crucial role in cellular function: they maintain signaling fidelity by converting graded input signals to digital signals [46-48]. This control mechanism of signal transmission, namely, the analog-to-digital conversion of signal by nanoclusters, has been the subject of an excellent recent review [48].

RAS ISOFORMS ARE IDENTICAL IN THEIR BIOCHEMICAL FUNCTION BUT DIVERGENT IN THEIR BIOLOGICAL FUNCTION

The biological function of Ras GTPases as binary switches in signaling transduction involves cycling between a guanine diphosphate (GDP)- and guanine triphosphate (GTP)-bound off and on states. In response to stimulation by external signals, such as activation of a receptor tyrosine kinase (RTK, Fig. 1) by a growth factor, a guanine nucleo-

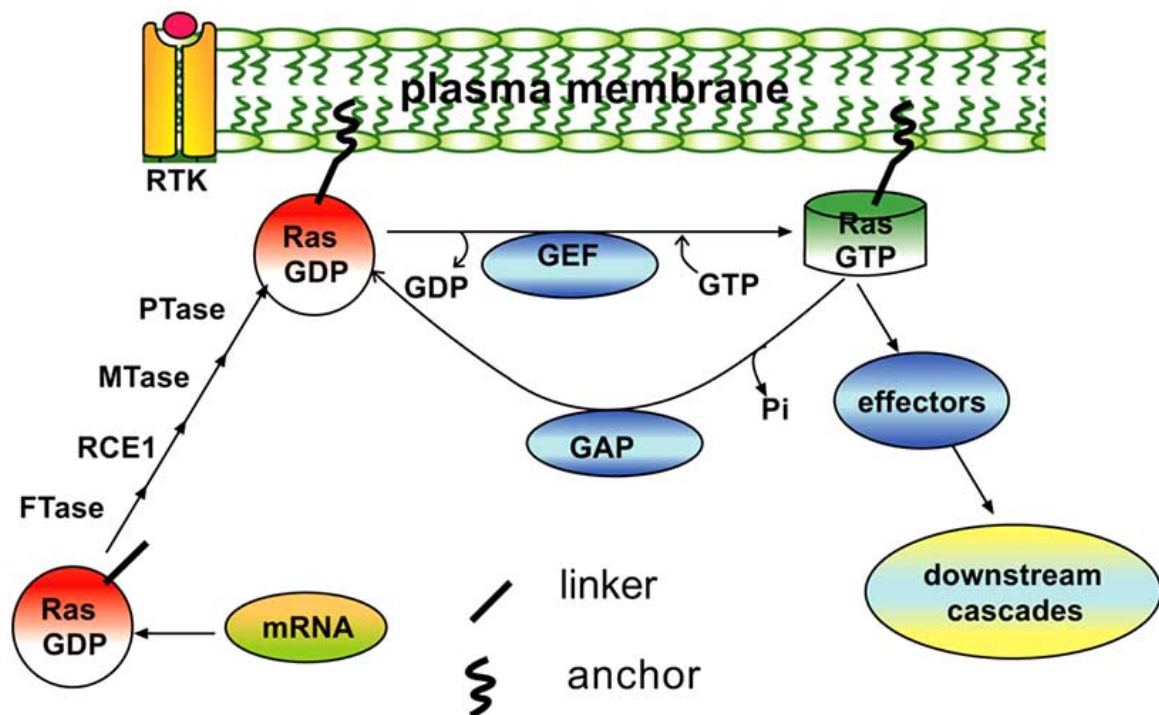


Fig. (1). A schematic showing the post-translational enzymatic processing and membrane targeting of Ras proteins, together with their biochemical and cellular functions. FTase represents farnesyl transferase; RCE1, Ras converting enzyme; MTase, methyltransferase (also called isoprenylcysteine carboxymethyltransferase (ICMT)); PTase, palmitoyltransferase; GEF, guanine nucleotide exchange factor; GAP, guanine nucleotide activating protein; RTK, receptor tyrosine kinase; and Pi, phosphate. [Inspiration for the figure is from ref. [77].

tide exchange factor (GEF) interacts with inactive Ras to catalyze the exchange of GDP for the more abundant GTP. GTP loading induces a conformational change that leads to transmission of signal to the nucleus *via* interaction with a variety of effector kinases (Fig. 1). Signal flow is terminated when negative regulators, called GTPase activating proteins (GAP), bind to active Ras and accelerate the slow intrinsic hydrolysis of GTP. A molecular defect in Ras that impairs the ability of the Ras-GAP complex to hydrolyze GTP leads to uncontrolled cell growth or cancer. All Ras isoforms undertake these reactions both *in vivo* and *in vitro*. However, unlike the case *in vivo*, the catalytic domain (or G-domain, residues 1-166) is sufficient for *in vitro* function. Furthermore, the isoforms exhibit identical reactivity *in vitro* but not *in vivo*.

The catalytic domain is highly conserved among the Ras isoforms, with the first 80 amino acids being identical and the next 85 differing only by 5% (Fig. 2A). Such a very high degree of sequence similarity suggests structural similarity as well. Indeed, comparison of N- and K-ras structural models with crystallographic structures of H-ras did not find significant differences [50]. Furthermore, inspection of the recently determined x-ray structures of N- and K-ras (Protein Data Bank (PDB) [51,52] codes 3CON and 3GFT, respectively) also suggests that the overall structure of these proteins is similar to the over 50 crystallographic structures of H-ras.

The sequence and structural homologies, together with the fact that the catalytic domain is sufficient for hydrolyzing GTP *in vitro*, were taken to imply that functional specificity is encoded in regions outside the catalytic domain. An obvious candidate is the hypervariable region (HVR) comprising the C-terminal 23/24 residues where Ras isoforms share only 15% sequence similarity (Fig. 2B). It has therefore been a focus of much scrutiny [7,8,53,54]. Indeed, both the unstructured linker region (residues 166-179 in N- and H-ras and residues 166-174 in K-ras) and the membrane interacting lipid-anchor (residues 180-189 in N- and H-ras and residues 175-188 in K-ras, underlined in Fig. 2B) were shown to be essential for biological activity [53,54]. An example is the study that combined Fluorescence Recovery after Photo-bleaching (FRAP) to measure membrane affinity on live cells and Electron Microscopy (EM) of intact plasma membrane sheets to spatially map microdomains of wild-type and mutant H-ras [53]. Using numerous mutations on the HVR, this work showed that the lipid anchor and the adjacent linker provide high-affinity interactions with lipid raft and nonraft plasma membrane microdomains, respectively [53]. The critical elements required for membrane binding by the lipid anchor include the farnesyl moiety at position 186 in all three isoforms plus a palmitoyl group at position 181 of N-ras, two palmitoyls at positions 181 and 184 of H-ras, and a polylysine domain in K-ras (Figs. 1 and 2B) [35-39,55,56]. These differences in lipid modification have direct relevance for the membrane binding behaviors of the isoforms. For example, a study using FRAP and EM techniques found that removal of the H-ras palmitoyl at position 184 results in a variant that emulates the GTP-regulated microdomain interactions of N-ras [57]. Understanding the basis of functional specificity in Ras signaling thus requires a detailed characterization of not only the catalytic domain in

solution, but also of the full-length protein in a membrane environment.

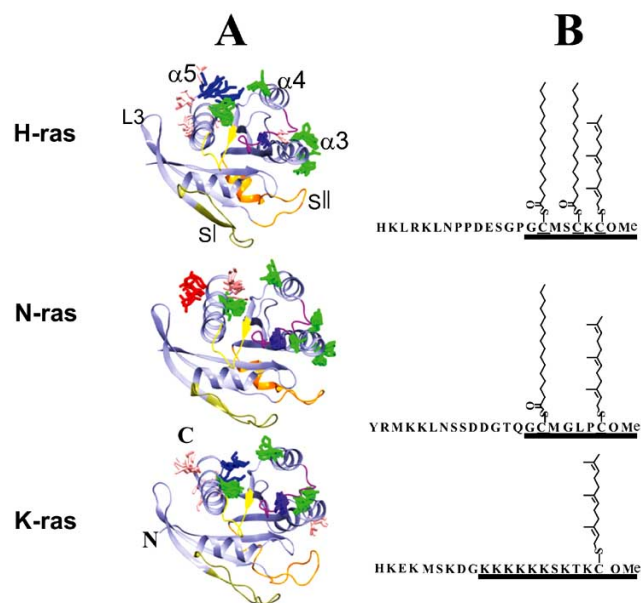


Fig. (2). Comparison of N-, H- and K-ras proteins. The structures for N- and K-ras were modeled based on that of H-ras. (A), Non-conservative amino acid substitutions in the catalytic domain (residues 1-166) [50] are mapped onto the three-dimensional structures. Multiple side chain-orientations indicate the mobility of these residues during a 10ns molecular dynamics simulation. Notice the absence of substitution in the N-terminal lobe (towards bottom) comprising the first 80 amino acids. The ~5% amino acid variations are only at the C-terminal lobe in the upper portion of the structures, especially on the three surface helices $\alpha 3$, $\alpha 4$ and $\alpha 5$. Structural regions involved in function and nucleotide binding are colored in light green (sI), orange (sII), pink ($\beta 5$ - $\alpha 4$, which contains the conserved NKXD motif), and yellow ($\beta 6$ - $\alpha 5$, which contains the conserved Ala146). The two switch regions and the $\beta 2$ - $\beta 3$ turn (or loop 3, L3) are labeled. (B), Sequences and lipid-modifications of the hypervariable region (HVR) with the amino acids comprising the lipid-anchors underlined. [The images in A are adopted from Fig. (5B) of ref. [50].

STRUCTURAL AND DYNAMIC ASPECTS OF THE CATALYTIC DOMAIN

The hallmark of all G-proteins is the γ -phosphate-dependent conformational change at their catalytic domain, as documented by three decades of structural and spectroscopic studies [58]. For H-ras alone, over 50 high-resolution structures have been deposited in the PDB. These structures typically comprise the first 166 residues (i.e., the catalytic domain) of the 189 amino acid protein and were solved for both the GDP- and GTP (or analogue)-bound forms and in complex with GEF, GAP or effector proteins [58,59]. Furthermore, efforts by the Structural Genomic Consortium enabled determination of the GDP-N-ras and GTP-K-ras catalytic domain structures. In all cases, the catalytic domain is comprised of a 6-stranded β -sheet surrounded by five α -helices (Fig. 2). Some of the functionally critical regions

include a conserved phosphate-binding loop (P-loop, residues 10–17) and two nucleotide-binding site switch regions (sI, residues 25–40 and sII, residues 57–75). Oncogenic mutations frequently occur in these three regions. Switches sI and sII are highly dynamic and undergo major conformational changes upon GDP/GTP exchange, as demonstrated by many crystallographic and spectroscopic studies on the wild-type and mutant H-ras [60,61].

Bioinformatics-based principal component (PC) analysis of the available H-ras crystal structures found two major clusters of conformers representing the GDP- and GTP-bound conformational states [50]. A number of distinct clusters that are intermediate between the major GDP and GTP clusters were also discovered. These intermediate clusters were populated by structures with point mutations at sI, sII and the P-loop, the most interesting examples being Y32C at sI and G12V at the P-loop. The structure of the oncogenic G12V variant in its GDP-bound form (pdb code 2Q21) has several unique features that resemble the corresponding features in the GTP clusters, implying this mutation may lower the energetic barrier between inactive and active conformational states. This unique feature of the G12V mutant was exploited in an MD study aimed at exploring the reaction path between the nucleotide states [50,62]. The simulations, which were carried out in two different force fields and programs, found that when a γ -phosphate is introduced into the GDP-G12V x-ray structure, a spontaneous transition towards the main GTP cluster occurs. The transition involves a multiphase process in which initial sII reorganization is followed by that of sI. Furthermore, the relative arrangement of the two switches was found to determine the nature of the nucleotide states [50].

An initial MD-based analysis of N- and K-ras, based on structural models built using H-ras as a template (Fig. 2A), found very little difference in the overall structure of the isoforms [50]. Furthermore, apart from the somewhat enhanced flexibility of K-ras at sI, and loops β 4- β 5 and α 4- β 5, the overall dynamics of the isoforms also appeared to be similar. On the other hand, a closer analysis of the non-conservative amino acid substitutions by mapping them on the 3D structures identified an invariant N-terminal lobe that contains the two canonical switches and the P-loop, and a C-terminal lobe that contains all of the major sequence variations within the catalytic domain. These mutations are distributed on structural elements (such as α 3, α 4, and α 5) that have no known (direct) involvement in function. However, when long-range side-chain contacts were analyzed in terms of their role in inter-lobe engagement, two-to-three unique putative routes of communications were found to link sites directly in contact with the nucleotide with those distal from it [50]. If verified by further studies using the now available N- and K-ras x-ray structures, this information will provide useful insights into how subtle variations in inter-lobe communication may lead to functional diversity. Future studies along these lines should help create opportunities for selective intervention of Ras-related carcinogenesis.

However, the abundant information on the catalytic domain of Ras proteins does not fully explain their functional diversity. A comprehensive understanding of the molecular basis of functional diversity requires detailed structural and

thermodynamic information on the membrane binding properties of each protein. As discussed below, recent computational studies and innovative experiments have started to provide interesting clues that helped generate plausible hypotheses that explain, at least in part, the origins of Ras diversity in cellular function [15-23,63].

STRUCTURE AND DYNAMICS OF MEMBRANE-BOUND RAS

The similarity in the catalytic domains of the isoforms led to the notion that their functional diversity is coded in the HVR [7,8]. This notion found support from experiments that demonstrated the functional role of HVR, as discussed above. It is therefore vital that the structure and dynamics of Ras is characterized in its membrane-bound form. Because structure determination of membrane-bound full-length Ras is still not possible, biophysical experiments and computer simulations have been used as the principal techniques for predicting Ras-membrane complex structures [11-14,17,21,23,63,64]. One of the important findings of such studies is that the membrane-anchor alone stably binds to the plasma membrane and therefore can serve as a suitable model system to study Ras-membrane interactions. Spectroscopic investigations of the N-ras anchor in lipid bilayers enabled characterization of many features of Ras-membrane binding, including the role of flexibility, localization and distribution of the anchor backbone and side-chains in membranes (reviewed in ref. [23]). These studies were extended to the full-length N-ras protein, enabling deduction of the overall structural features of the monolayer-bound protein [19,65]: the anchor adopts a “horse-shoe” like shape [17] and the catalytic domain undergoes some reorientation upon binding to a lipid monolayer [19].

Much of the currently available atomistic information on the structural and dynamical features of Ras-membrane interactions has been derived from MD-based techniques and using heptapeptides representing the Ras lipid-anchors [11,14,16,21]. In the case of H-ras, membrane binding properties of the HVR and the full-length protein were also investigated [13,21]. In all simulations, once a barrier is crossed through the formation of 5-7 carbon-carbon contacts between Ras and DMPC lipid tails, the anchor quickly inserts and stabilizes in the bilayer. Typical final configurations look like the one displayed in Fig. (3), which is a snapshot from a simulation of the farnesylated and dually palmitoylated H-ras in a DMPC bilayer. The distribution of the lipid-anchor atoms is such that the hydrophobic side chains (which include the lipid-modified moieties plus Leu in N-ras and Met in H-ras) insert deep into the hydrophobic core of the bilayer and the polar side chains point towards solvent and interact with the head group. The backbone localizes either in the hydrophobic-hydrophilic interface near the glycerol oxygens or at the lipid-water interface near the phosphate oxygens. Note that all the previous simulations discussed in this review used a hexadecyl group to model farnesyl. Ongoing research with farnesyl suggests that the hexadecyl group has no major effect on the overall structure of Ras-membrane complexes, although variations are found in the details of the atomic interactions (Gorfe AA, unpublished data).

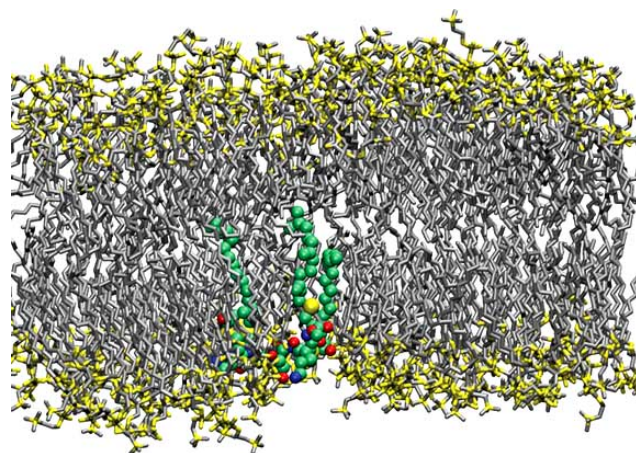


Fig. (3). A snapshot from an MD simulation of an H-ras lipid-anchor in a DMPC bilayer. The DMPC lipids are shown in silver stick models with the non-carbon heavy atoms at the glycerol and head group regions colored in yellow. The H-ras anchor is shown in a space-filling model with carbon in light green, oxygen in red, nitrogen in blue and sulfur in orange. Lipid molecules that were on top of the anchor and prevented visualization were removed for clarity, as were water molecules and hydrogen atoms.

Simulations of full-length H-ras and of the HVR found that the insertion depth of the anchor lipids and the localization of the backbone are modulated by the linker and the catalytic domain [13,21]. Most intriguing, however, was that the GTP-loaded H-ras underwent a major conformational change upon membrane binding so that the catalytic domain makes direct contact with the bilayer. This result, therefore, predicted that H-ras has two modes of membrane binding [21]. In one of these, a pair of positively charged residues at helix $\alpha 4$ of the catalytic domain (Arg128 and Arg135) interacts with the bilayer head group to stabilize a GTP-H-ras conformation. In the other mode, another pair of positively charged residues from the HVR (Arg169 and Lys170) stabilizes a GDP-bound conformation (Fig. 4). To test the validity of these predictions, a series of experiments were carried out. The experiments were designed to probe H-ras membrane orientation, effector/modulator interaction, and signaling. The major techniques that were used include medium-throughput Fluorescence Resonance Energy Transfer (FRET) and Fluorescence Lifetime Imaging (FLIM), coupled with functional assays on live cells (ERK activation in BHK cells and PC12 cell differentiation) [21,63]. FRET occurs when a donor and an acceptor are close to each other (within 10nm). Maximal FRET-efficiencies at high acceptor concentrations are characteristic for different H-ras mutant/ nanodomain marker FRET pairs [20, 63]. A set of FRET values, termed FRET vectors [66,63], were used to describe the lateral segregation and orientation of wild-type and mutant H-ras with respect to a large set of nanodomain markers. These experimental results fully validated the predictions. For instance, mutation of the basic residues Arg128/Arg135 in helix $\alpha 4$ or Arg169/Lys170 of the HVR to alanine selectively altered the FRET behavior of GTP- or GDP-loaded H-ras, confirming a critical role of these residues in stabilizing GTP- or GDP-H-ras interactions with the plasma membrane. The corollary to this result is that the plasma membrane serves a regulatory function by helping align active Ras into

a signaling competent orientation. FLIM-FRET was used to study the interaction of the Ras-binding domain (RBD) of effector C-Raf (residues 51–131) with GTP-H-ras in which the basic residues in $\alpha 4$ (Arg128/Arg135) and those in the HVR (Arg169/Lys169) were replaced by alanine [63]. The mutations in the HVR significantly increased the interaction of Ras with C-Raf RBD compared with the control G12V-GTP-H-ras; the mutations in $\alpha 4$ weakened the RBD interaction. As a result, ERK activation was reduced in BHK cells expressing the $\alpha 4$ mutant while the ppERK levels were more than two-fold greater in BHK cells expressing the HVR mutant than in cells expressing G12V-GTP-H-ras [63]. The results from the ERK activation assays were corroborated by previous PC12 differentiation assays [21]. Interestingly, mutation of all four basic residues restored the RBD interaction and ERK phosphorylation levels to the G12V-GTP-H-ras levels, suggesting that the balance of the GTP-H-ras orientation [63] is similarly restored.

In an attempt to explore how the re-orientation occurs, salt bridge interactions involving Asp47/Glu48 of the $\beta 2/\beta 3$ loop and Arg161/Arg164 of helix $\alpha 5$ (both at the catalytic domain) were eliminated through alanine substitution [20,63]. This perturbation (i.e., of the $\beta 2/\beta 3$ - $\alpha 5$ interaction) affected the FRET-vector in a manner indicative of the residues' involvement in the membrane re-orientation of H-ras [20,63]. The perturbation also affected effector (C-Raf RBD) binding and ERK activation in BHK cells [63]. For instance, alanine-substitution of Asp47/Glu49 resulted in a variant that more effectively binds to C-Raf RBD and is hyper-active in phosphorylating ERK [63]. Further analysis of the FRET data suggested that these structural elements together serve as a novel conformational switch (sIII) that operates through helix $\alpha 4$ and the HVR to reorient the H-ras catalytic domain with respect to the membrane plane [63]. By combining these experimental data with results from the MD simulations, a "balance" model was proposed to describe the regulation of Ras function through modulation of its membrane orientation. Using the $\beta 2/\beta 3$ - $\alpha 5$ salt bridge as a fulcrum, the tilt of the balance can be shifted between the signaling competent H-ras conformation (where $\alpha 4$ contacts membrane) and the signaling incompetent conformation (where the HVR interacts with membrane) [63]. The implication of this model to a wide range of issues in Ras signaling has been discussed elsewhere [20].

GTP-dependent conformational change at membrane interacting regions has been observed in other GTPases as well. In Arf, GTP binding involves detachment and membrane insertion of an N-terminal helix [67,68]. In Ran, a large conformational change in sI is accompanied by a major reorganization of a C-terminal region [69-71]. Conversely, in Rap1 phosphorylation of a C-terminal Ser affects the structure of the canonical switches [72]. Coupling of conformational change and membrane binding is also observed in non-G-proteins. An interesting example is the matrix domain of the HIV-1 Gag precursor protein. In this protein, trimerization flips out a hidden myristic acid for membrane insertion, and clusters basic residues for a high specificity binding of PI(4,5)P2 [73].

MD simulations also indicated localized structure perturbations of lipid bilayers upon insertion of Ras [13,22]. These

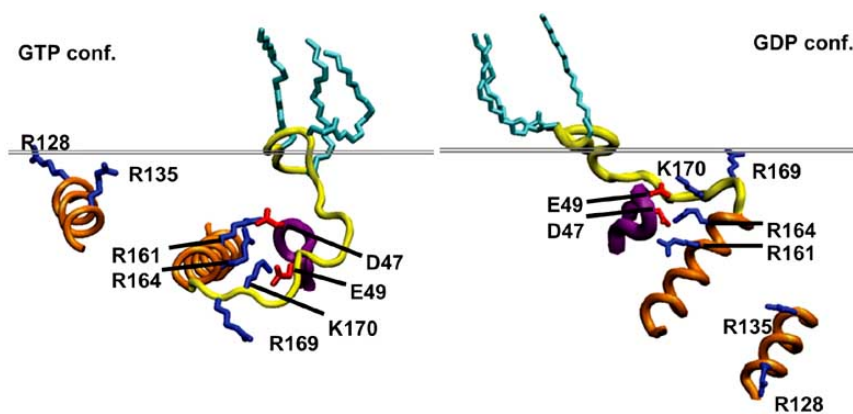


Fig. (4). Nucleotide-dependent membrane orientation of H-ras derived from molecular dynamics simulations [21] and validated by experiments [20,63]. The approximate relative positions of the DMPC head group at the lower leaflet of the model bilayer are indicated by gray lines. The Ras lipids are shown in cyan-colored stick model. The model at the left is highly populated in H-ras MD simulations with bound GTP while the one to the right is more populated in simulations with GDP. In contrast to the dramatic difference in membrane orientation, the two models are characterized by a similar overall structure with small variations at the HVR and its interaction with the $\beta 2/\beta 3$ turn [21,22]. Note the proximity to the bilayer of a pair of positively charged residues (blue sticks) at helix $\alpha 4$ (Arg128 and Arg135) and the HVR (Arg169 and Lys170); these residues interact with the lipid head group and stabilize the GTP-H-ras and GDP-H-ras conformations, respectively. The salt bridge interactions involving Asp47/Glu49 (red) of the $\beta 2$ - $\beta 3$ turn (purple) and Arg161/Arg164 in $\alpha 5$ are also highlighted.

perturbations were shown to be modulated by the membrane-insertion depth of the Ras lipids and interfacial localization of the backbone either near the hydrophobic-hydrophilic or bilayer-water interfaces. Moreover, membrane-insertion depth and backbone localization were modulated by the linker and the catalytic domain, providing clues that help rationalize the biochemical and biophysical observations on the role of the catalytic domain and the HVR on membrane binding and lateral segregation [13].

THERMODYNAMICS OF RAS-MEMBRANE INTERACTION

Insight into the thermodynamic basis of Ras-membrane association has been obtained from free energy profile (or potential of mean force, PMF) calculations for the insertion of H-ras anchor into a DMPC bilayer [12,64]. In the absence of lipid-modification, the PMF sharply rises when the peptide backbone contacts the DMPC head group [64]. This shows, as expected, that lipid-modification is absolutely required for membrane binding. For the triply lipid-modified peptide, however, insertion of a few of the terminal methyl and/or ethyl carbons of Ras lipids into the upper chain region of DMPC triggers a rapid decline of the PMF. A deep minimum is reached when the distribution of side chain and backbone atoms of the anchor is optimal; optimal membrane binding is achieved when the backbone is roughly 12-16 Å away from the bilayer center (see Fig. 3). One can conclude from the slope and depth of the profile, or the large free energy gain of ~ -30 kcal/mol, that spontaneous dissociation is unlikely [12]. The driving force for the insertion was investigated by parsing the free energy of insertion into enthalpic/entropic and solute/solvent contributions [14]. Despite a significant entropic barrier arising from conformational reorganization of the anchor, membrane insertion was found to be predominately enthalpic in nature [14].

The contribution of individual lipid modifications to membrane affinity was investigated by eliminating the lipid

modifications one after another and computing the PMFs for the membrane insertion of the mutant peptides [64]. The estimated free energy changes for dually lipidated H-ras were large (ranging between ~ -20 and -30 kcal/mol) and close to that of the triply lipidated wild-type anchor. Indeed, the palmitoyl at Cys181 and the farnesyl at Cys186 were found to be sufficient for tight membrane binding, with the palmitoyl at Cys184 providing little extra affinity. Thus, the contributions of the individual lipid-modifications to membrane affinity are location dependent and non-additive. Furthermore, the location of the free energy minimum varies depending on whether the palmitoylation is at Cys181 or Cys184. Because the computed PMFs are likely to be modulated by the linker and the activation state of the catalytic domain [12], one can imagine a corresponding effect on lateral segregation due to a shift in the location of the free energy minimum or the insertion depth of the anchor. Based on the predicted differences in the level of membrane penetration by GTP- and GDP-H-ras [21], one may speculate that the palmitoyl moiety at position 184 modulates insertion in an activation state dependent manner, which is consistent with a previous finding that the palmitate at Cys184 supports correct GTP-regulated lateral segregation [57].

COMMUNICATION BETWEEN THE CATALYTIC AND MEMBRANE BINDING REGIONS

A number of cell biological and biophysical experiments documented the modulation of Ras membrane binding and lateral segregation by the bound nucleotide [53,74]. Furthermore, residues at disparate locations (at the HVR, sIII and $\alpha 4$) are found to be involved in (re)orienting H-ras with respect to the membrane plane [20,63]. All of these suggest some kind of dynamic communication between the canonical switches sI and sII, and the HVR. They also suggest that this communication involves sIII and $\alpha 4$. The question is how do these regions communicate?

The putative inter-lobe communication pathways described in an earlier section may provide initial clues into

how the nucleotide binding region and the C-terminal part of the catalytic domain communicate [50]. Additional clues came from MD simulations of the oncogenic G12V H-ras, where it was noted that the $\beta 2/\beta 3$ turn and the end of $\alpha 5$ become flexible during the nucleotide-dependent conformational change [62]. A better picture of the structural changes occurring at these sites emerged from accelerated MD (aMD) simulations of wild type H-ras [62]. aMD is a technique in which simulations are performed in a smoother energy surface so that crossing of barriers is possible, thus allowing sampling of conformational space inaccessible to conventional simulations [75,76]. The aMD simulations, which enabled an almost complete inter-conversion between the GDP and GTP states of wild-type H-ras, revealed that the $\beta 2/\beta 3$ turn and the C-terminus of $\alpha 5$ move in concert and adopt a unique conformation during the transition (i.e., when sI and sII undergo maximal conformational change, see Fig. (2) of ref. [62]). Such a dynamic linkage between the switching apparatus and the membrane interacting C-terminus may be the key to nucleotide-dependent modulation of membrane binding. Interfering with this allosteric communication, as well as with the inter-lobe communication discussed earlier, may have useful implications for the design of isoform-selective anti-cancer drugs.

PERSPECTIVE AND CONCLUDING REMARKS

In order to visualize the signaling function of Ras in a living cell, a simple analogy may be helpful. Perhaps the most obvious is the electrical wiring in a typical building with the accompanying switches and fuses. The isolated catalytic domain can be viewed as a switch on a shelf, waiting to be connected. The HVR links-up the switch to the wiring, which in the cell is a cascade of interacting proteins. The proteins need to be properly organized in a (deformable) wall, or the plasma membrane. With this analogy, one can imagine that the catalytic domain remains switchable irrespective of its location (i.e., on a shelf or a wall) as long as sufficient energy is supplied to turn it on and off. This energy comes from GTP hydrolysis (which derives the conformational change of sI and sII). In the case of the electrical switch, the energy to break or make contact comes from the force expended in pressing a button. A switch (catalytic domain) does not transmit or break a signal until wired into the electrical system; in our example the wiring runs through the wall or the plasma membrane. For a biological switch to be operational, therefore, it should be integrated into the plasma membrane and precisely connected to the signaling cascade it regulates.

There can be multiple switches within a single system of wiring, controlling the same or different outlets, such as, for instance, a lamp in a hallway. A defective switch will not work, nor will a normal switch that is not properly connected. Analogously, if we wish to design a drug that selectively targets a defective switch, we first need to identify the defective switch from among many practically identical switches. The data discussed in this review suggest that the three Ras switches are connected to the wiring slightly differently. However, we still have to find the "blue-print" of the connectivity and the means by which to test defective connections; knowledge of how the switch breaks, i.e., how

the catalytic domain changes conformation is necessary but not sufficient. The studies discussed in the current and several other reviews laid down some of the initial stepping stones towards the blue-print of Ras wiring.

It should be emphasized that the analogy described above is useful but simplistic because the plasma membrane is far from a passive wall. It plays an active role in modulating Ras signaling through, for example, its role in nanoclustering. Little is known about exactly how membrane structure and dynamics affects and is modulated by Ras-membrane interaction. Future studies aimed at a comprehensive understanding of Ras signaling from a structural perspective should adopt an approach that would allow study of Ras in the context of membrane and its interaction partners. Rational design of effective and selective anti-cancer drugs further requires that special emphasis be placed on the role of allostery and the communication between the canonical switch regions and the membrane-interacting C-terminus.

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REFERENCES

- [1] Karnoub, A.E.; Weinberg, R.A. Ras oncogenes: Split personalities. *Nat. Rev. Mol. Cell. Biol.*, **2008**, *9*, 517-531.
- [2] Barbacid, M. Ras genes. *Annu. Rev. Biochem.*, **1987**, *56*, 779-827.
- [3] Waldmann, V.; Rabes, H.M. What's new in ras genes? Physiological role of ras genes in signal transduction and significance of ras gene activation in tumorigenesis. *Pathol. Res. Pract.*, **1996**, *192*, 883-891.
- [4] Bar-Sagi, D. Ras proteins: Biological effects and biochemical targets (review). *Anticancer Res.*, **1989**, *9*, 1427-1437.
- [5] Bos, J.L. Ras oncogenes in human cancer: A review. *Cancer Res.*, **1989**, *49*, 4682-4689.
- [6] Plowman, S.J.; Hancock, J.F. Ras signaling from plasma membrane and endomembrane microdomains. *Biochim. Biophys. Acta*, **2005**, *1746*, 274-283.
- [7] Wolfman, A. Ras isoform-specific signaling: Location, location, location. *Sci STKE*, **2001**, *2001*, PE2.
- [8] Hancock, J.F. Ras proteins: Different signals from different locations. *Nat. Rev. Mol. Cell Biol.*, **2003**, *4*, 373-384.
- [9] Kratz, C.P.; Niemeyer, C.M.; Zenker, M. An unexpected new role of mutant ras: Perturbation of human embryonic development. *J. Mol. Med.*, **2007**, *85*, 227-235.
- [10] Schubbert, S.; Bollag, G.; Shannon, K. Deregulated ras signaling in developmental disorders: New tricks for an old dog. *Curr. Opin. Genet. Dev.*, **2007**, *17*, 15-22.
- [11] Gorfe, A.A.; Pellarin, R.; Caflich, A. Membrane localization and flexibility of a lipidated ras peptide studied by molecular dynamics simulations. *J. Am. Chem. Soc.*, **2004**, *126*, 15277-15286.
- [12] Gorfe, A.A.; Babakhani, A.; McCammon, J.A. Free energy profile of H-ras membrane anchor upon membrane insertion. *Angew. Chem. Int. Ed. Engl.*, **2007**, *46*, 8234-8237.
- [13] Gorfe, A.A.; Babakhani, A.; McCammon, J.A. H-ras protein in a bilayer: Interaction and structure perturbation. *J. Am. Chem. Soc.*, **2007**, *129*, 12280-12286.
- [14] Gorfe, A.A.; Baron, R.; McCammon, J.A. Water-membrane partition thermodynamics of an amphiphilic lipopeptide: An enthalpy-driven hydrophobic effect. *Biophys. J.*, **2008**, *95*, 3269-3277.
- [15] Huster, D.; Vogel, A.; Katzka, C.; Scheidt, H.A.; Binder, H.; Dante, S.; Gutberlet, T.; Zschornig, O.; Waldmann, H.; Arnold, K. Membrane insertion of a lipidated ras peptide studied by FTIR,

- solid-state NMR, and neutron diffraction spectroscopy. *J. Am. Chem. Soc.*, **2003**, *125*, 4070-4079.
- [16] Vogel, A.; Katzka, C.P.; Waldmann, H.; Arnold, K.; Brown, M.F.; Huster, D. Lipid modifications of a ras peptide exhibit altered packing and mobility versus host membrane as detected by 2H solid-state NMR. *J. Am. Chem. Soc.*, **2005**, *127*, 12263-12272.
- [17] Reuther, G.; Tan, K.T.; Kohler, J.; Nowak, C.; Pampel, A.; Arnold, K.; Kuhlmann, J.; Waldmann, H.; Huster, D. Structural model of the membrane-bound C-terminus of lipid-modified human N-ras protein. *Angew. Chem. Int. Ed. Engl.*, **2006**, *45*, 5387-5390.
- [18] Reuther, G.; Tan, K.T.; Vogel, A.; Nowak, C.; Arnold, K.; Kuhlmann, J.; Waldmann, H.; Huster, D. The lipidated membrane anchor of full length N-ras protein shows an extensive dynamics as revealed by solid-state nmr spectroscopy. *J. Am. Chem. Soc.*, **2006**, *128*, 13840-13846.
- [19] Bringezu, F.; Majerowicz, M.; Wen, S.; Reuther, G.; Tan, K.T.; Kuhlmann, J.; Waldmann, H.; Huster, D. Membrane binding of a lipidated N-ras protein studied in lipid monolayers. *Eur. Biophys. J.*, **2006**, 491-498.
- [20] Abankwa, D.; Gorfe, A.A.; Hancock, J.F. Mechanisms of ras membrane organization and signalling: Ras on a rocker. *Cell Cycle*, **2008**, *7*, 2667-2673.
- [21] Gorfe, A.A.; Hanzal-Bayer, M.; Abankwa, D.; Hancock, J.F.; McCammon, J.A. Structure and dynamics of the full-length lipid-modified H-ras protein in a 1,2-dimyristoylglycero-3-phosphocholine bilayer. *J. Med. Chem.*, **2007**, *50*, 674-684.
- [22] Abankwa, D.; Gorfe, A.A.; Hancock, J.F. Ras nanoclusters: Molecular structure and assembly. *Semin. Cell Dev. Biol.*, **2007**, *18*, 599-607.
- [23] Brunsveld, L.; Waldmann, H.; Huster, D. Membrane binding of lipidated ras peptides and proteins--the structural point of view. *Biochim. Biophys. Acta*, **2009**, *1788*, 273-288.
- [24] Monod, J.; Changeux, J.P.; Jacob, F. Allosteric proteins and cellular control systems. *J. Mol. Biol.*, **1963**, *6*, 306-329.
- [25] Tsai, C.J.; del Sol, A.; Nussinov, R. Allostery: Absence of a change in shape does not imply that allostery is not at play. *J. Mol. Biol.*, **2008**, *378*, 1-11.
- [26] Goodey, N.M.; Benkovic, S.J. Allosteric regulation and catalysis emerge via a common route. *Nat. Chem. Biol.*, **2008**, *4*, 474-482.
- [27] Swain, J.F.; Gierasch, L.M. The changing landscape of protein allostery. *Curr. Opin. Struct. Biol.*, **2006**, *16*, 102-108.
- [28] Cui, Q.; Karplus, M. Allostery and cooperativity revisited. *Protein Sci.*, **2008**, *17*, 1295-1307.
- [29] Monod, J.; Wyman, J.; Changeux, J.P. On the nature of allosteric transitions: A plausible model. *J. Mol. Biol.*, **1965**, *12*, 88-118.
- [30] Koshland, D.E., Jr.; Nemethy, G.; Filmer, D. Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry*, **1966**, *5*, 365-385.
- [31] Kuriyan, J.; Eisenberg, D. The origin of protein interactions and allostery in colocalization. *Nature*, **2007**, *450*, 983-990.
- [32] Gunasekaran, K.; Ma, B.; Nussinov, R. Is allostery an intrinsic property of all dynamic proteins? *Proteins*, **2004**, *57*, 433-443.
- [33] Volkman, B.F.; Lipson, D.; Wemmer, D.E.; Kern, D. Two-state allosteric behavior in a single-domain signaling protein. *Science*, **2001**, *291*, 2429-2433.
- [34] Pargellis, C.; Tong, L.; Churchill, L.; Cirillo, P.F.; Gilmore, T.; Graham, A.G.; Grob, P.M.; Hickey, E.R.; Moss, N.; Pav, S.; Regan, J. Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site. *Nat. Struct. Biol.*, **2002**, *9*, 268-272.
- [35] Hancock, J.F.; Magee, A.I.; Childs, J.E.; Marshall, C.J. All ras proteins are polyisoprenylated but only some are palmitoylated. *Cell*, **1989**, *57*, 1167-1177.
- [36] Lane, K.T.; Beese, L.S. Thematic review series: Lipid posttranslational modifications. Structural biology of protein farnesyltransferase and geranylgeranyltransferase type I. *J. Lipid Res.*, **2006**, *47*, 681-699.
- [37] Ashby, M.N. CAAX converting enzymes. *Curr. Opin. Lipidol.*, **1998**, *9*, 99-102.
- [38] Winter-Vann, A.M.; Casey, P.J. Post-prenylation-processing enzymes as new targets in oncogenesis. *Nat. Rev. Cancer*, **2005**, *5*, 405-412.
- [39] Linder, M.E.; Deschenes, R.J. New insights into the mechanisms of protein palmitoylation. *Biochemistry*, **2003**, *42*, 4311-4320.
- [40] Apolloni, A.; Prior, I.A.; Lindsay, M.; Parton, R.G.; Hancock, J.F. H-ras but not K-ras traffics to the plasma membrane through the exocytic pathway. *Mol. Cell. Biol.*, **2000**, *20*, 2475-2487.
- [41] Prior, I.A.; Hancock, J.F. Compartmentalization of ras proteins. *J. Cell. Sci.*, **2001**, *114*, 1603-1608.
- [42] Rocks, O.; Peyker, A.; Kahms, M.; Verveer, P.J.; Koerner, C.; Lumbierres, M.; Kuhlmann, J.; Waldmann, H.; Wittinghofer, A.; Bastiaens, P.I. An acylation cycle regulates localization and activity of palmitoylated ras isoforms. *Science*, **2005**, *307*, 1746-1752.
- [43] Parton, R.G.; Hancock, J.F. Lipid rafts and plasma membrane microorganization: Insights from ras. *Trends Cell Biol.*, **2004**, *14*, 141-147.
- [44] Plowman, S.J.; Muncke, C.; Parton, R.G.; Hancock, J.F. H-ras, K-ras, and inner plasma membrane raft proteins operate in nanoclusters with differential dependence on the actin cytoskeleton. *Proc. Natl. Acad. Sci. U. S. A.*, **2005**, *102*, 15500-15505.
- [45] Hancock, J.F. Lipid rafts: Contentious only from simplistic standpoints. *Nat. Rev. Mol. Cell Biol.*, **2006**, *7*, 456-462.
- [46] Harding, A.; Hancock, J.F. Ras nanoclusters: Combining digital and analog signaling. *Cell Cycle*, **2008**, *7*, 127-134.
- [47] Tian, T.; Harding, A.; Inder, K.; Plowman, S.; Parton, R.G.; Hancock, J.F. Plasma membrane nanoswitches generate high-fidelity ras signal transduction. *Nat. Cell Biol.*, **2007**, *9*, 905-914.
- [48] Harding, A.S.; Hancock, J.F. Using plasma membrane nanoclusters to build better signaling circuits. *Trends Cell Biol.*, **2008**, *18*, 364-371.
- [49] Inder, K.; Harding, A.; Plowman, S.J.; Philips, M.R.; Parton, R.G.; Hancock, J.F. Activation of the MAPK module from different spatial locations generates distinct system outputs. *Mol. Biol. Cell*, **2008**, *19*, 4776-4784.
- [50] Gorfe, A.A.; Grant, B.J.; McCammon, J.A. Mapping the nucleotide and isoform-dependent structural and dynamical features of ras proteins. *Structure*, **2008**, *16*, 885-896.
- [51] Kouranov, A.; Xie, L.; de la Cruz, J.; Chen, L.; Westbrook, J.; Bourne, P.E.; Berman, H.M. The RCSB pdb information portal for structural genomics. *Nucleic Acids Res.*, **2006**, *34*, D302-305.
- [52] Deshpande, N.; Adress, K.J.; Bluhm, W.F.; Merino-Ott, J.C.; Townsend-Merino, W.; Zhang, Q.; Knezevich, C.; Xie, L.; Chen, L.; Feng, Z.; Green, R.K.; Flippen-Anderson, J.L.; Westbrook, J.; Berman, H.M.; Bourne, P.E. The RCSB protein data bank: A redesigned query system and relational database based on the mmcif schema. *Nucleic Acids Res.*, **2005**, *33*, D233-237.
- [53] Rotblat, B.; Prior, I.A.; Muncke, C.; Parton, R.G.; Kloog, Y.; Henis, Y.I.; Hancock, J.F. Three separable domains regulate gtp-dependent association of H-ras with the plasma membrane. *Mol. Cell Biol.*, **2004**, *24*, 6799-6810.
- [54] Jaumot, M.; Yan, J.; Clyde-Smith, J.; Sluimer, J.; Hancock, J.F. The linker domain of the Ha-ras hypervariable region regulates interactions with exchange factors, RAF-1 and phosphoinositide 3-kinase. *J. Biol. Chem.*, **2002**, *277*, 272-278.
- [55] Silvius, J.R. Mechanisms of ras protein targeting in mammalian cells. *J. Membr. Biol.*, **2002**, *190*, 83-92.
- [56] Magee, T.; Hanley, M. Protein modification. Sticky fingers and CAAX boxes. *Nature*, **1988**, *335*, 114-115.
- [57] Roy, S.; Plowman, S.; Rotblat, B.; Prior, I.A.; Muncke, C.; Grainger, S.; Parton, R.G.; Henis, Y.I.; Kloog, Y.; Hancock, J.F. Individual palmitoyl residues serve distinct roles in H-ras trafficking, microlocalization, and signaling. *Mol. Cell Biol.*, **2005**, *25*, 6722-6733.
- [58] Vetter, I.R.; Wittinghofer, A. The guanine nucleotide-binding switch in three dimensions. *Science*, **2001**, *294*, 1299-1304.
- [59] Herrmann, C. Ras-effector interactions: After one decade. *Curr. Opin. Struct. Biol.*, **2003**, *13*, 122-129.
- [60] Ford, B.; Hornak, V.; Kleinman, H.; Nassar, N. Structure of a transient intermediate for GTP hydrolysis by ras. *Structure*, **2006**, *14*, 427-436.
- [61] Spoerner, M.; Nuehs, A.; Ganser, P.; Herrmann, C.; Wittinghofer, A.; Kalbitzer, H.R. Conformational states of ras complexed with the GVTP analogue GPPNHp or GPPCh2P: Implications for the interaction with effector proteins. *Biochemistry*, **2005**, *44*, 2225-2236.
- [62] Grant, B.J.; Gorfe, A.A.; McCammon, J.A. Ras conformational switching: Simulating nucleotide-dependent conformational transi-

- tions with accelerated molecular dynamics. *PLoS Comput. Biol.*, **2009**, *5*, e1000325.
- [63] Abankwa, D.; Hanzal-Bayer, M.; Ariotti, N.; Plowman, S.J.; Gorfe, A.A.; Parton, R.G.; McCammon, J.A.; Hancock, J.F. A novel switch region regulates H-ras membrane orientation and signal output. *EMBO J.*, **2008**, *27*, 727-735.
- [64] Gorfe, A.A.; McCammon, J.A. Similar membrane affinity of mono- and di-s-acylated ras membrane anchors: A new twist in the role of protein lipidation. *J. Am. Chem. Soc.*, **2008**, *130*, 12624-12625.
- [65] Guldenhaupt, J.; Adiguzel, Y.; Kuhlmann, J.; Waldmann, H.; Kottling, C.; Gerwert, K. Secondary structure of lipidated ras bound to a lipid bilayer. *FEBS J.*, **2008**, *275*, 5910-5918.
- [66] Abankwa, D.; Vogel, H. A FRET map of membrane anchors suggests distinct microdomains of heterotrimeric G proteins. *J. Cell Sci.*, **2007**, *120*, 2953-2962.
- [67] Goldberg, J. Structural basis for activation of ARF GTPase: Mechanisms of guanine nucleotide exchange and GTP-myristoyl switching. *Cell*, **1998**, *95*, 237-248.
- [68] Antonny, B.; Beraud-Dufour, S.; Chardin, P.; Chabre, M. N-terminal hydrophobic residues of the G-protein ADP-ribosylation factor-1 insert into membrane phospholipids upon GDP to GTP exchange. *Biochemistry*, **1997**, *36*, 4675-4684.
- [69] Vetter, I.R.; Arndt, A.; Kutay, U.; Gorlich, D.; Wittinghofer, A. Structural view of the ran-importin beta interaction at 2.3 a resolution. *Cell*, **1999**, *97*, 635-646.
- [70] Vetter, I.R.; Nowak, C.; Nishimoto, T.; Kuhlmann, J.; Wittinghofer, A. Structure of a ran-binding domain complexed with ran bound to a GTP analogue: Implications for nuclear transport. *Nature*, **1999**, *398*, 39-46.
- [71] Chook, Y.M.; Blobel, G. Structure of the nuclear transport complex karyopherin-beta2-ran x GppNHp. *Nature*, **1999**, *399*, 230-237.
- [72] Edreira, M.M.; Li, S.; Hochbaum, D.; Wong, S.; Gorfe, A.A.; Ribeiro-Neto, F.; Woods, V.L.; Altschuler, D.L. Phosphorylation-induced conformational changes in rap1b: Allosteric effects on switch domains and effector loop. *J. Biol. Chem.*, **2009**, *284*, 27480-27486.
- [73] Saad, J.S.; Miller, J.; Tai, J.; Kim, A.; Ghanam, R.H.; Summers, M.F. Structural basis for targeting HIV-1 gag proteins to the plasma membrane for virus assembly. *Proc. Natl. Acad. Sci. U. S. A.*, **2006**, *103*, 11364-11369.
- [74] Prior, I.A.; Harding, A.; Yan, J.; Sluimer, J.; Parton, R.G.; Hancock, J.F. GTP-dependent segregation of H-ras from lipid rafts is required for biological activity. *Nat. Cell Biol.*, **2001**, *3*, 368-375.
- [75] Hamelberg, D.; Mongan, J.; McCammon, J.A. Accelerated molecular dynamics: A promising and efficient simulation method for biomolecules. *J. Chem. Phys.*, **2004**, *120*, 11919-11929.
- [76] Hamelberg, D.; de Oliveira, C.A.; McCammon, J.A. Sampling of slow diffusive conformational transitions with accelerated molecular dynamics. *J. Chem. Phys.*, **2007**, *127*, 155102.
- [77] Ahmadian, M.R. Prospects for anti-ras drugs. *Br. J. Haematol.*, **2002**, *116*, 511-518.