Ras isoform-specific expression, chromatin accessibility, and signaling

Ruth Nussinov \(^1,2\) · Mingzhen Zhang \(^1\) · Ryan Maloney \(^1\) · Hyunbum Jang \(^1\)

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Abstract

The anchorage of Ras isoforms in the membrane and their nanocluster formations have been studied extensively, including their detailed interactions, sizes, preferred membrane environments, chemistry, and geometry. However, the staggering challenge of their epigenetics and chromatin accessibility in distinct cell states and types, which we propose is a major factor determining their specific expression, still awaits unraveling. Ras isoforms are distinguished by their C-terminal hypervariable region (HVR) which acts in intracellular transport, regulation, and membrane anchorage. Here, we review some isoform-specific activities at the plasma membrane from a structural dynamic standpoint. Inspired by physics and chemistry, we recognize that understanding functional specificity requires insight into how biomolecules can organize themselves in different cellular environments. Within this framework, we suggest that isoform-specific expression may largely be controlled by the chromatin density and physical compaction, which allow (or curb) access to “chromatinized DNA.” Genes are preferentially expressed in tissues: proteins expressed in pancreatic cells may not be equally expressed in lung cells. It is the rule—non an exception, and it can be at least partly understood in terms of chromatin organization and accessibility state. Genes are expressed when they can be sufficiently exposed to the transcription machinery, and they are less so when they are persistently buried in dense chromatin. Notably, chromatin accessibility can similarly determine expression of drug resistance genes.

Keywords KRAS · HRAS · NRAS · K-RAS4A · K-RAS4B · Gene accessibility · Signaling · Inhibitor

Introduction

Quantifying Ras isoform-specific expression in distinct tissues has been challenging. Here, we suggest that a key factor determining their cell-specific levels is their epigenetics and chromatin accessibility status. Chromatin accessibility relates to the local density level of proteins including histones, transcription factors, chromatin-modifying enzymes, and chromatin-remodeling complexes on the DNA. Their depletion at cis-regulatory elements commonly points to candidate genomic regions which may be available for transcription (Minnoye et al. 2021). Post-translational modifications (PTMs) of chromatin, such as DNA methylation, and histone methylation and acetylation, are dynamic, varying across cell states and types and correlating with chromatin accessibility and gene expression. The dynamic density levels make profiling of accessibility an extremely difficult task (Ashwin et al. 2019; Barth et al. 2020; Wachsmuth et al. 2016). An added complexity is the interpretation of the data as it relates to enhancer–promoter proximity and functional transcription factor binding (Minnoye et al. 2021). As we discuss below, these complexities combine with additional ones underscoring the challenge of quantitative studies of Ras isoform-specific expression.

Here, we describe isoform-specific activities and review experimental observations from a structural dynamic standpoint. This conformational perspective of isoform-specific activities mimics nature: biomolecules are not static sculptures. Molecular behavior, which dominates the structure–function paradigm, is shaped by biomolecules which are always switching between a variety of structures with varying
energies, with the most populated being those which are energetically most favored. This dynamic (Kumar et al. 2000; Tsai et al. 1999) behavior can be described by the free energy landscape (Frauenfelder et al. 1991). The populations of the conformational species are influenced by multiple factors, including sequence alterations, which dictate specific interactions. Thus, with different sequences and chemistries (charge, hydrophobicity) and distinct combinations of hydrophobic PTMs in the hypervariable C-terminal tails (Fig. 1), the isoforms present different favored interactions with membrane lipids and protein partners.

Our views are influenced by concepts from physics and chemistry. We believe that molecular fluctuations are harnessed for life (Nussinov 2016; Wei et al. 2016) and that biomolecules must be described statistically, not statically. Understanding functional specificity requires insight into how biomolecules can organize themselves and their assemblies in different cellular environments, including molecular concentrations (e.g., of scaffolding proteins, as was shown for galectin-1 (Blazevits et al. 2016)). It is also manifested by the distinct segregations into nanoclusters, and the isoform dynamics between the substructures where they congregate and their movements in the plasma membrane into—and out of—the membrane domains (Nussinov et al. 2019b).

**Background information on Ras**

**Ras isoforms, structure, and biology**

Ras proteins control cell proliferation pathways, cell growth and division (Lavoie et al. 2020; Nussinov et al. 2020b; Smith et al. 2020). Three RAS genes lead to four proteins, HRas, NRas, and splice variants KRas4A and KRas4B. The incidence of mutated RAS genes differs among human cancers: KRAS is the most highly mutated isoform (85%), followed by...
NRAS (11%), and HRAS (4%). They predominate in different cancer types and the distributions of the mutations differ as well (Cox et al. 2014; Hobbs et al. 2016; Li et al. 2018a; Prior et al. 2012). Sequence comparisons indicate that the catalytic domains of the isoforms (residues 1–166) are almost identical. Binding to exchange factors exchanges GDP by GTP activating the Ras isoforms; binding to GTPase-activating proteins (GAPs) deactivates them (Tsai et al. 2015; Vigil et al. 2010). When activated, Ras proteins bind and activate their effectors, initiating their respective signaling pathways, primarily mitogen-activated protein kinase (MAPK) via Raf/MEK/ERK (MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal regulated kinase) and PI3K/AKT/mTOR (PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; mTOR, mechanistic target of rapamycin) phosphorylation cascades. Both signaling pathways feed into the cell cycle, together leading to cell proliferation (Nussinov et al. 2017).

Over 150 experimentally determined structures of the catalytic domain have been deposited in the structural database. In contrast, the 22–23 residue C-terminal tail which constitutes the hypervariable region (HVR) is disordered, precluding crystallization. However, its populated conformations have been sampled by NMR and molecular dynamics (MD) simulations (Chavan et al. 2015a). Membrane anchorage, executed by the tails, is required for Ras activation, with the tails’ hydrophobic PTMs docking at favored membrane environments (Fig. 1). The HVRs are farnesylated, proteolyzed, and carboxyl methylated. In addition, NRas, HRas, and KRas4A cysteines are palmitoylated. These PTMs stabilize the anchor- age. Lacking a palmitoyl, KRas4B attachment to negatively charged membranes is assisted by a lysine polybasic stretch (175KKKKKK180). The covalent palmitoylation thioester modification linkages are irreversible; the farnesyl thioether linkages are not. The HVR of KRas4B can also be phosphorylated. The introduction of the large, negatively charged phospholipid group dissociates KRas4B from the negatively charged plasma membrane (Bivona et al. 2006; Jang et al. 2015). The HVR of KRas4A resembles that of KRas4B: it is also highly positively charged, albeit not to the extent of KRas4B (Nussinov et al. 2016). Whereas the lysines are almost continuous in KRas4B, they are not in KRas4A and there are fewer of them (Tsai et al. 2015). KRas4A’s pattern of PTMs varies as well and lies in-between NRas and KRas4B. Like NRas, KRas4A can have farnesy1 and palmitoyl; however, with the palmitoyl thioester linkage being irreversible, it may be hydrolyzed, resulting in KRas4A retaining only its farnesy1. This can explain KRas4A’s acting as NRas (when the tail is farnesylated and palmitoylated) and as KRas4B (when the palmitoyl is hydrolyzed), thus NRas- and KRas4B-like cell transformation patterns (Tsai et al. 2015). Like the C181S NRas, a C180S mutation in KRas4A does not stop it from shuttling to the plasma membrane. However, in the absence of palmitoylation and diminished polybasic region as compared to KRas4B, KRas4A retains lower affinity for the plasma membrane (Chakrabarti et al. 2016; Muratcioglu et al. 2017; Nussinov et al. 2016). The HVR sequence and palmitoylation status also govern the isoforms’ preferred plasma membranes (Eisenberg et al. 2011) (Fig. 2). The positively charged KRas HVR, but not HRas or NRas, strongly favors acidic disordered membranes (Fig. 1), making membrane composition an important consideration in isoform-specific signaling (Chavan et al. 2015b; Nussinov et al. 2018a). Isoform-specific HVR activities also include binding to membrane shuttling factors as in the case of phosphodiesterase-δ (PDEδ) which shuttles KRas4B (Dharmaiah et al. 2016; Klein et al. 2019; Kuchler et al. 2018; Muratcioglu et al. 2017). NMR data and MD simulations of GDP-bound KRas4B suggest that the HVR obstructs access to the active site (Banerjee et al. 2016; Jang et al. 2016a), which apparently is not exhibited by other isoforms likely due to the absence of a sufficiently strong positive charge. Ras isoforms are also differentially ubiquitylated, which may affect their membrane attachment status (Ahearn et al. 2018; Dohlman and Campbell 2019; Hobbs et al. 2016; Sasaki et al. 2011). Activation-related effects include monoubiquitylation at Lys147, which increases the levels of KRas4B-GTP due to impaired GAP binding (Baker et al. 2013a; Sasaki et al. 2011), and HRas ubiquitylation at Lys117, which promotes GDP-GTP exchange (Abe et al. 2020; Baker et al. 2013b).

Earlier discussions on Ras isoforms

Several comprehensive reviews of Ras isoforms have been published in the last few years. These broadly discussed their different biochemical and biological (many cancer-related) roles, localization, sublocalization, and more. To avoid reviewing the topics which were covered, we refer the reader to these recent excellent publications. These include subcellular localization and considering exploiting the membrane in therapeutics (Kattan and Hancock 2020; Zhou et al. 2018), mutational analysis and isoform signaling (Li et al. 2018a; Munoz-Maldonado et al. 2019; Prior et al. 2020; Randic et al. 2021), subcellular localization and tumor growth (Garcia-Ibanez et al. 2020), Ras–ERK signaling (Zaballos et al. 2019) and MAPK inhibition (Heppner and Eck 2021; Ullah et al. 2021), isoform-specific differences in the effector binding regions (Nakhaeizadeh et al. 2016), and the recent contributions from the Mark Philips lab on KRas4A reversible palmitoylation and colocalization (Amendola et al. 2019) and on membrane association/colocalization (Zhou et al. 2020). Isoform signaling specificity at the membrane (Nussinov et al. 2018a), KRas mobility in the membrane (Nussinov et al. 2019b) and nanoclustering (Nussinov et al. 2019a) were also reviewed as well as genetic aspects of KRas signaling networks (Jinesh et al. 2018). Thus, below, we only briefly...
touch on some of these aspects. Instead, we provide new views which we hope will help guide future research.

**Ras isoforms segregate into nanoclusters which favor distinct membrane composition: family members with similar tail chemistry can join**

GTP-bound Ras forms nanoclusters in the membrane. Nanoclustering is required for Ras signaling via the MAPK, but not PI3Kα/AKT/mTOR pathways (Bandaru et al. 2019; Boriack-Sjodin et al. 1998; Cherfils and Zeghouf 2013; Hancock et al. 1989; Jang et al. 2016a; Jang et al. 2016b; Muratcioglu et al. 2017; Nussinov et al. 2018a; Nussinov et al. 2019a; Schmick et al. 2016). The reason for this distinction is that for MAPK signaling, Raf kinase domains need to be activated. This requires their side-to-side (homo- or hetero-) dimerization (Freeman et al. 2013a; Freeman et al. 2013b; Jambrina et al. 2016; Lavoie et al. 2018; Rezaei Adariani et al. 2018; Tsai and Nussinov 2018; Udell et al. 2011; Varga et al. 2017; Verkhivker 2016; Yuan et al. 2018), which is not the case for PI3Kα (Nussinov et al. 2018a; Nussinov et al. 2019a). The two kinase domains are contributed by two Raf molecules, with the Ras binding domain (RBD) of each Raf binding to the catalytic domain of an active Ras molecule (Fig. 3). This requires that Ras molecules either be in direct contact (Ras dimers) (Jang et al. 2020; Muratcioglu et al. 2020; Nan et al. 2015) or in spatial vicinity which can be achieved in sufficiently populated nanoclusters. Membrane anchored nanoclusters increase the probability of such favorable proximity (Nussinov et al. 2019a), and galectin dimers were proposed to scaffold Raf-effectors to further promote Ras nanoclustering (Blazevits et al. 2016). In this model, at high concentrations, galectin dimerizes in the cytoplasm and binds to two Raf’s RBDs which are Ras-bound. The galectin–Raf complexes cooperatively nucleate Ras nanoclustering, thereby promoting dimerization of Raf’s kinase domains. Computational modeling supported by experimental data suggested that cooperativity between KRas4B dimerization and Raf-1 (C-Raf) RBD–Ras binding may also emerge though the engagement of Raf-1’s cysteine-rich domain (CRD) at the membrane (Jang et al. 2020). In this model, Raf-1’s RBD-CRD can cooperatively support stable KRas4B

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**Fig. 2** Subcellular localization of Ras proteins. Ras isoforms, HRas, NRas, and two splice variants KRas4A and KRas4B terminate in a CAAX motif are cleaved, carboxymethylated, and farnesylated in the endoplasmic reticulum (ER). HRas, NRas, and KRas4A are further palmitoylated in the Golgi apparatus, while KRas4B traffics to the plasma membrane shuttled by phosphodiesterase-δ (PDEδ). KRas4A in state 1 (only farnesylated) can also traffic to the plasma membrane via a similar mechanism as in KRas4B. After palmitoylation, HRas, NRas, and KRas4A in state 2 (with both farnesyl and palmitoyl modifications) are translocated to the plasma membrane via vesicular transport. HRas favors localization in the ordered caveolae and lipid rafts, and fluidic disordered (non-raft) regions of the plasma membrane. NRas can localize to cholesterol-rich lipid raft and non-raft regions. Depalmitoylation removes HRas and NRas from the plasma membrane. KRas4A in state 2 can localize to the non-raft region, while both KRas4A in state 1 and KRas4B highly localize to the acidic lipid enriched membrane microdomains. Phosphorylation of Ser181 or calmodulin extracts KRas4B from the plasma membrane.
The varied chemistry of the HVRs, thus membrane preferences, favors distinct isoform nanoclusters. However, recently KRas4B nanoclusters were shown to be co-inhibited also by other isoforms and notably, by a subset of prenylated small GTPase family members, confirming the importance of dimer/co-cluster formation on cell membranes (Li et al. 2020b), in line with an earlier hypothesis making such prediction (Nussinov et al. 2020a).

A few years ago, wild-type HRas was observed to suppress HRas driven cancer (Spandidos et al. 1990). This was followed by the observation that wild-type KRas can inhibit lung cancer (Diaz et al. 2002) and that wild-type NRas can also suppress its mutant as potently as can KRas (Zhang et al. 2001). However, the data (To et al. 2013; Xu et al. 2013; Zhou et al. 2016) as to how would wild-type Ras suppress the oncogenicity of its mutant was not understood (Kong et al. 2016; Qiu et al. 2011; Staffas et al. 2015) despite its significant implications to Ras drug discovery (Zhou et al. 2016). Nanoclustering can resolve these apparent perplexing observations. In the absence of an external cue, wild-type Ras spends most of its life in the inactive state. "Diluting" the population of active, oncogenic Ras by the inactive wild type would lower the probability of spatially nearby active Ras molecules (Fig. 3), thus Raf dimerization, activation, and MAPK signaling. This effect is particularly large in cells with high KRas populations. Segregated nanoclustering also resolves the next question of why the wild-type state of one isoform type cannot suppress the oncogenic mutant of another (Matallanas et al. 2011; Xu et al. 2013). The apparent observed suppression of MAPK by Rap1A small GTPase, whose HVR resembles that of KRas4B, might also be explained along similar lines (Nussinov et al. 2020a).

**Ras isoforms display distinct favored interactions with Raf isoforms**

Ras/Raf/MEK/ERK is a major Ras signaling pathway involving protein kinases phosphorylation cascade (Lavoie and Therrien 2015). The sequences, structures and functions of Raf isoforms, A-Raf, B-Raf, and Raf-1 are mostly similar, and they share conserved regions (CRs); CR1, CR2, and CR3 (Shaw et al. 2014; Yaeger and Corcoran 2019). CR1 at the N-terminal, contains the RBD and the CRD. In active Raf, RBD interacts with Ras, and CRD binds to the membrane (Garcia-Gomez et al. 2018; Ghosh et al. 1994; Li et al. 2018b; Terrell and Morrison 2019; Travers et al. 2018) (Fig. 3). Inhibition of Raf can allosterically promote...
its paradoxical activation (Hatzivassiliou et al. 2010; Heidorn et al. 2010; Poulakakos et al. 2010) via heterodimeric interactions between the kinase domains of Raf isoforms (Jin et al. 2017; Pfister et al. 2008; Ritt et al. 2010). Dimerization of the kinase domains is required for full activation (Durrant and Morrison 2018; Hu et al. 2013; Lavie et al. 2013; Rajakulendran et al. 2009). Under normal circumstances, B-Raf/Raf-1 heterodimers predominate (Freeman et al. 2013a).

Wild-type Raf is mostly in the “closed” autoinhibited state, where the CRD and RBD shield the kinase domain dimerization surface (Nussinov et al. 2020c), with the 14-3-3 proteins stabilizing this organization (Kondo et al. 2019; Park et al. 2019). Dephosphorylation of pSer365 in B-Raf (pSer259 in Raf-1) and release of RBD-CRD following a growth activation signal at the membrane relieve the autoinhibition. The high affinity binding of Raf’s RBD to active Ras, coupled with pSer365 dephosphorylation which destabilizes the 14-3-3 interaction, shifts the Raf ensemble toward the open state (Zhang et al. 2021). This exposes the kinase domain dimerization surface enabling full activation (Fetics et al. 2015; Hatzivassiliou et al. 2010; Lu et al. 2016; Nussinov et al. 2015; Nussinov et al. 2019a). Cryo-EM structures (Kondo et al. 2019; Park et al. 2019) of the Raf kinase domain, RBD and CRD complexed with dimeric 14-3-3 validate the organization of the assembly and the paradoxical activation of Raf (Kondo et al. 2019).

Recent experiments probed the preferred interactions of Ras isoforms with Raf isoforms, by shuffling their N’ termini, which precede the RBD-CRD. The N’ termini vary significantly among the Raf isoforms (Fig. 4), whereas it is long (154 residues) and negatively charged in B-Raf, it is more neutral in Raf-1 (55 residues) and A-Raf (18 residues). The experiments indicated that the N’ termini of Raf isoforms selectively bind the HVRs of Ras isoforms (Terrell et al. 2019). Replacing the Raf-1 terminus with B-Raf’s reduced the HRas–Raf-1 binding but had no significant effect on KRas–Raf-1 interaction. As to Ras HVRs, Raf-1 has high affinity to all Ras isoforms. However, the lysine-rich polybasic region of KRas4B is important to B-Raf selectivity due to its acidic N’ terminal region, resulting in the high-affinity KRas4B–B-Raf RBD interaction. Taken together, Raf-1 binds all Ras proteins; however, B-Raf favors KRas4B, with the sequences responsible for these preferences residing in the N-terminal (for B-Raf) and the HVR at the C-terminal for KRas4B. Additionally, Raf-1 is important for the HRas interaction, with a fairly neutral HVR.

Even though these observations provide critical information, understanding them is marred by lack of structural data. This reflects the disordered nature of the N’ termini of Raf, especially the long B-Raf sequence. It is also unclear how the HVR concomitantly interacts with the membrane and Raf’s termini. With structural data only for a fraction of the sequence, it is challenging to reliably model it.

Isoform-specific localization

Two-decades ago, Alan Wolfman raised the apt question of “How is it that similar proteins carry out different jobs in the cell?” (Wolfman 2001); his answer was “Location, Location, Location.” He proceeded to survey literature reports and concluded that different functions may stem from the distinct subcellular localizations to which the isoforms are directed by their HVRs and patterns of prenylation. Nonetheless, even if Ras isoforms are localized to unique sets of cellular structures, the number of Ras isoform-specific interaction sites is limited, which underscores the importance of understanding why they apparently signal through distinct pathways. This may imply that a Ras isoform-dependent oncogenic phenotype necessitates (i) cooperation of additional cellular Ras proteins, (ii) a steady-state pool of their complexes, and (iii) availability of other proteins in the pathway in the cancer cell. Notably, Ras sublocalization at the plasma membrane is highly dynamic and can be altered depending on the GDP/GTP load or the palmitoylation status (Agudolbinez et al. 2015). Electron microscopy indicated that HRas is mainly in the endoplasmic reticulum and Golgi of acinar cells; KRas in the membrane of ductal cells. Overall, Ras isoforms were observed to have distinct and separate cellular and subcellular distributions that likely persist even in transformed cells (Kocher et al. 2005).

Differential Ras isoform expression

RAS isoforms are preferentially expressed in different cancer types

The question of why the oncogenic RAS isoforms are preferentially expressed in different cancer types has been baffling (Der 2014; Hobbs et al. 2016), and several hypotheses have been proposed to address it (e.g., Der 2014; Haigis et al. 2008; Lampson et al. 2013; Rauen 2013; Schuhmacher et al. 2008; more below). Among these, here we reason that the patterns of isoform expression in different cells can relate to the accessibility of the gene’s regulatory regions. The density of chromatin in regulatory regions of highly expressed isoforms could be lower than those with lower expression. Temporal expression profiles of different isoforms across developmental stages identified “isoform switching” of the predominant isoform (>60% of all isoforms of the given gene at the given stage) (Li et al. 2020a). The chromatin density of specific genes differs between differentiated cells as compared to cells during embryogenesis. The local chromatin density can also vary among the differentiated cells, making the regulatory regions of some genes more accessible to the transcription machinery than others. This, along with the epigenetic features, control gene expression (Klemm et al. 2019), including in our case here
wild-type and mutant Ras isoforms (Nussinov et al. 2021), thus signaling.

This picture becomes even more complex when Raf’s expression and mutational patterns are considered (Desideri et al. 2015). Mutations in B-Raf, but not in A-Raf and Raf-1, are common in human cancers, with Raf-1 mutations at under 1% (Imielinski et al. 2014). Mutations were observed in pancreatic and lung adenocarcinoma and colorectal cancer, where KRas4B is commonly involved. It is overexpressed in bladder cancer, hepatocellular carcinoma, squamous cell carcinoma, and lung adenocarcinoma (Maurer et al. 2011) and is MEK independent (Blasco et al. 2011; Karreth et al. 2011).

Quantitative data on isoform-specific expression are limited

Isoform-specific expression has been probed, as well as its relation to signaling (Newlaczyl 2016) and prognosis (Yang and Kim 2018). KRas4B was confirmed as the most highly expressed isoform and KRas4A as the most dynamically regulated. Quantification of Ras isoform expression during development by real-time polymerase chain reaction (PCR) in mouse tissues indicated a relative contribution of KRas4B > NRas ≥ KRas4A > HRas to total Ras expression (Newlaczyl et al. 2017), where KRas4B is about 60–99% of all Ras transcripts. Recent data have also suggested that spliced variants are translationally significant (Raso 2020). This may reflect the dependence of multiple factors, including cell type, tissue heterogeneity, timing, sparsity of data, defining flexible statistical frameworks for complex differential patterns in gene expression, assigning a reference, errors and missing data, and from our standpoint as we discuss here, key factors are measuring chromatin accessibility (Buenrostro et al. 2015; Cusanovich et al. 2015) and epigenetics, such as DNA methylation (Karemaker and Vermeulen 2018) and more (Lahnemann et al. 2020).
HRas, KRas, and NRas have specific context-dependent functions (Hobbs et al. 2016; Nussinov et al. 2018a; Nussinov et al. 2020b), and mutations (Li et al. 2018a; Munoz-Maldonado et al. 2019). They also display cancer type specific incidence: KRas in pancreatic, lung, and colorectal carcinomas. NRas in cutaneous melanoma (Cox et al. 2014), and HRas in head and neck and bladder squamous cell carcinomas. Several theories have been put forward to explain the cell (tissue)-specificity. Among them (Der 2014), (i) isoform specificity reflects the level of expression. Yet, the significant-ly higher incidence of KRAS mutations as compared to NRAS (Haigis et al. 2008) and HRAS (Schuhmacher et al. 2008) in colorectal carcinoma was offered as questioning this explanation. Another explanation (ii) relates to possible differential potencies in promoting cancers across tissues (Haigis et al. 2008; Russo et al. 2014). (iii) An alternative explanation offered differential DNA repair as a consequence of Ras isoform-specific activating mutations (Ise et al. 2000). This suggested that KRAS regulatory elements are responsible for tissue specificity, rather than the Ras protein (Chin et al. 1997; To et al. 2008). Still other explanations suggested (iv) that isoform translational efficiency encoded by codon usage could be the origin of the isoform specificity (Lampson et al. 2013) and finally, as analysis of The Cancer Genome Atlas indicated (v), tumor RAS gene expression levels are influ-enced by the mutational status of RAS genes and of upstream and downstream Ras pathway genes (Stephens et al. 2017).

Furthering the differential potencies of mutants in promoting cancers across tissues (Haigis et al. 2008; Russo et al. 2014), we propose a role for chromatin accessibility (Fig. 5). The genome of all cells is identical. However, not all genes are equally expressed during the developmental lineage and across tissue microenvironments. One major reason is the status of the chromatin density in gene regulatory regions. Regulatory regions can be buried in compact dense chromatin or be in low-density re-gions. In low density regions, the local chromatin conformation is controlled by nucleosome dynamics. These regions can be-come available to the transcription machinery upon a relatively minor conformational change (Nussinov et al. 2020b). There is a continuum of accessibility across the genome (Klemm et al. 2019), stretching from genes expressed only in embryonic cells, to those in differentiated cells, as in the case of Ras isoforms. Accessibility reflects the cell’s epigenetic landscape (Haigis et al. 2019; Sack et al. 2018) and relates to the density of proteins interacting directly (mostly histones) or indirectly with the DNA and their fractional residence times. Accessibility is a maj-ior factor determining gene expression, thus protein availability and consequently, pathways that consist of interactions of these proteins. Protein availability differs among tissues: protein levels in pancreatic cells where KRas expression is abundant may differ from those in skin cells, where NRas is (Nussinov et al. 2020b). Taken together, KRas and NRas can be differentially expressed in specific cell types because their chromatin accessibility status differ (Brubaker et al. 2019). This could clarify why the height-ened abundance of the same active mutant, e.g., KRas^{G12D}, would differ in pancreatic cancer and melanoma skin cancer. On the other hand, the differential outcome of NRas^{Q61R} versus NRas^{G12D} in melanoma may stem from the differential mutation strengths, which depends on the activation mechanisms (Burd et al. 2014). Exploring the respective mechanisms would be of interest and could aid in development of isoform- and mutant-specific inhibitors.

**Chromatin accessibility and genome organization are cell-type and cell-state specific**

To fit into the limited nuclei space, act in regulation and guard genome integrity, chromatin is compacted. Compactness has been assessed by several methods, including a quantitative fluctuation-based assay (Hinde et al. 2012) and FRET; however, the low resolution does not permit correlation of the in vivo signals with specific higher order chromatin organization (Lleres et al. 2009). Quantitative super-resolution microscopy assay (Dultz et al. 2018) and algorithms for the quanti-fication of chromatin condensation from microscopic data have been developed, but to date their applications have been limited (Sosnik et al. 2017). Chromatin accessibility reflects changes in the local density, or compaction (Magana-Acosta and Valadez-Graham 2020). Dynamic changes in chromatin landscapes are associated with cell differentiation during em-bryogenesis and dedifferentiation in pluripotent stem cell (iPSC) in cancers. Among the factors defining chromatin states are the composition and post-translational modifications (PTMs) of the nucleosomes, concentration and interaction of transcription factors, and chromatin remodelers (Klemm et al. 2019). The mechanisms controlling accessible chromatin regions include competition between transcription factors and histones, chromatin remodeling in cis through proximal linker histone displacement, and in trans through accessible, distal regulatory elements, binding of the pioneer transcription fac-tors to nucleosomal DNA and more (Klemm et al. 2019). Landscapes vary in different tissues and cell types. Most chromatin conformation capture experiments focused on intrachromosomal interactions. Recent observations reveal that the patterns of interchromosomal interactions are tissue-specific, differing between the heart and liver (Chapski et al. 2018). The experiments (Nothjunge et al. 2017; Rosa-Garrido et al. 2017) show preferential localization of genes in 3D in the nuclei of the organs in which the genes are transcribed. Comparisons of liver and cardiac chromatin structures identify widespread differences in compartmentalization, with these not fully correlating with the organ transcriptional states. Localization of genes within organ-specific chromatin scaffolds relate to cell type but can reflect stress conditions as well. Genome structures indicate that the promoter to transposable element loops differ between the organs, pointing to cell type
specific organization of the epigenome. Interchromosomal interactions were enriched in genes associated with the function of that cell type, localizing nearby (Cremer and Cremer 2001) in “transcription factories” (Papantonis and Cook 2013). In the heart, 66.7% of cardiac-specific genes are in the center of one compartment (marked compartment A), while 66.1% of liver-specific genes are toward the periphery in compartment B. The locations of the Ras isoforms on the human chromosomes also differ (Pellicer 2011; Rajasekharan and Raman 2013): HRAS gene is localized to the short arm (p) of chromosome 11 at position 5, the KRAS gene is located on the p arm of chromosome 12 at position 12.1, and the NRAS gene is on chromosome 1 at position 13.2. Even though these are positions along the linear chromosome sequence organization, and to date data about their 3D locations are unavailable, the distinct locations of isoforms suggest distinct organization and expression patterns.

Advancements in super-resolution imaging (e.g., Nir et al. 2018) coupled with measurements of mRNA expression in distinct cell types and states can test whether the patterns of Ras isoform expression are associated with chromatin accessibility (Nussinov et al. 2021; Nussinov et al. 2020b).

Ras isoforms, their functions, and cell signaling

Differential isoform signaling

A key question is how a specific pathway can be selected when the affinities of the effectors for each Ras isoform do not show appreciable differences (Sieburth et al. 1998; Wolfman 2001). That is, how can highly similar proteins carry out different actions in the cell? Possible explanations to this conundrum include (i) cell type-specific expression levels of the Ras isoforms, and of all nodes (proteins) in the respective pathway. For the signal to propagate, the levels of expression of these nodes need to be sufficiently high. (ii) Observations for over two decades (reviewed in, e.g., Castellano and Santos 2011; Garcia-Ibanez et al. 2020; Hobbs et al. 2016; Kattn and Hancock 2020; Mo et al. 2018) suggest that isoform functions may emerge from the subcellular localization favored by their HVRs (Wolfman 2001) (Fig. 1). Isoform-specific functions of Ras can be at least partly explained by localization (Fig. 2). For example, NRas and HRas, but not KRas, are expressed on the Golgi as well as the plasma membranes and it was recently
reported that this localization inhibits malignant transformation (Casar et al. 2018). KRas4A but not KRas4B directly regulates hexokinase I by virtue of its unique localization on the outer mitochondrial membrane driven by depalmitoylation (Amendola et al. 2019). Compartmentalized signaling based on HVR-driven subcellular localization has been established in model organisms (Onken et al. 2006) and finally, a stark example of differential signaling from distinct subcellular locations is in T cell thymic selection (Daniels et al. 2006). (iii) Mutational potency is often isoform tissue specific (Munoz-Maldonado et al. 2019). The strong KRas4B G12D driver interferes with GTP hydrolysis. It occurs broadly, but especially in pancreatic cancers. In contrast, the less frequent, weaker KRas4B A146T drives cancer by promoting GDP by GTP exchange. It has been observed in colorectal and hematopoietic cancers, but not in pancreatic adenocarcinomas where it is not sufficiently powerful for cell transformation (Bera et al. 2019; Poulin et al. 2019).

Major considerations in signaling outputs in distinct tumors

Thus, taken together, signaling outputs in distinct tumors reflect several major components. These include first, the expression levels which depend on chromatin accessibility in the respective cell type at that time window (Fig. 5). The local density of chromatin at the regulatory region of the gene and nearby in cis has to be low to permit binding of the transcription machinery and high expression rates. Indeed, even very high expression level on its own can promote cancer. Second, expression of other proteins in the respective pathway should not be low for the signal to propagate. Third, the mutations should be potent. When considering different mutations in the same cell/tumor, heightened abundance of activated Ras species depends on the expression level of that gene and the potency of the mutation. The NRas Q61R mutations versus KRas G12D in melanoma cell line provide an example (Burd et al. 2014). As clinical data have shown, the number of activated KRas G12D molecules in pancreatic cancer is extremely high.

Calmodulin interacts selectively with oncogenic KRas4B

With a negatively charged linker and hydrophobic pockets, calmodulin (CaM) interacts with KRas4B (Abraham et al. 2009; Chavan et al. 2013; Jang et al. 2017; Jang et al. 2019; Villalonga et al. 2001; Wu et al. 2011), and likely also with KRas4A, but not with the HRas and NRas isoforms (Nussinov et al. 2016). The high affinity charge-charge interaction coupled with the farnesyl nestling in CaM’s hydrophobic pocket, shifts the ensembles toward this energetically favored state, extracting KRas molecules from the membrane (Fivaz and Meyer 2005; Sidhu et al. 2003; Sperlich et al. 2016). While CaM’s interaction appears KRas4B GTP-dependent (Abraham et al. 2009; Chavan et al. 2013; Villalonga et al. 2001; Wu et al. 2011), it can also involve the GDP-bound state (Agamasu et al. 2019; Fivaz and Meyer 2005; Sidhu et al. 2003; Sperlich et al. 2016). This can be understood in terms of the availability of the HVR for the interaction (Jang et al. 2019). In solution, the HVR in the GDP-bound state interacts with the catalytic domain, populating an autoinhibited state (Chavan et al. 2015a; Lu et al. 2015). However, the interaction is unstable which is why it has not been captured in the crystal, suggesting a minor GDP-bound KRas4B population with the HVR available for CaM interaction (Jang et al. 2016a; Nussinov et al. 2018b). At the membrane, likely being sandwiched between the catalytic domain and the bilayer surface the autoinhibited state can persist (Jang et al. 2016a).

CaM’s binding to mutant KRas4B is vastly important to understand. Two possible reasons have been advanced to explain its role: (i) CaM–KRas4B–specific binding reduces the number of available free CaM molecules for Ca2+-dependent protein kinase II activation (Wang et al. 2015); (ii) phosphorylated CaM and mutant KRas4B bind PI3Kα and activate it (Joyal et al. 1997; Wang et al. 2018; Zhang et al. 2017; Zhang et al. 2018). Together, mutant KRas4B and CaM can stimulate the PI3Kα/AKT pathway even in its absence of an incoming receptor tyrosine kinase growth signal. CaM’s fundamental significance in KRas-driven adenocarcinoma made it a prime drug discovery target.

Conclusions

Even though there are some sequence differences in the catalytic domains, the distinction among Ras isoforms rests mainly in their HVR membrane-binding segments (Fig. 1). This distinction underscores the significance of the attachment to membrane domains in determining isoform functions, cellular sublocalization and shuttling vehicles to get them there (Fig. 2). The chemical uniqueness of the HVRs stemming from the variable amino acid sequences and the combination of prenyl modifications, with the consequent separation into mostly homogeneous nanoclusters, emphasizes their specific roles under normal conditions and the resulting mutational distributions observed in cancer. As recent work elegantly demonstrated (Terrell et al. 2019), Ras isoforms interact differentially with Raf isoforms, with Raf-1 binding all mutant Ras proteins with high affinity, whereas B-Raf exhibiting a strong preference for mutant KRas. It is thus quite likely that Ras isoforms also differentially interact with other Ras effectors, such as PI3Kα (Thevathasan et al. 2013). Even though differential KRas and HRas regulation by galectin isoforms was also observed (Elad-Sfadia et al. 2004; Shalom-Feuerstein et al. 2005), more recently the interaction was proposed to be mediated via Raf’s RBD (Blazevits et al. 2016), thereby...
cooperatively scaffold Ras nanoclusters, which would increase dimerization of Raf’s kinase domains and activation.

All proteins are encoded and can be expressed by all cells (Kosti et al. 2016). However, genes are preferentially expressed in tissues (Farahbod and Pavlidis 2019; Honore 2020); proteins expressed in pancreatic cells may not be equally expressed in lung cells. This holds for isoforms of Ras and other proteins, including receptor tyrosine kinases and lipid kinases. It is the rule—not an exception, and it can be at least partly understood in terms of chromatin organization and incoming signaling cues (Fig. 5) and mRNA levels (Lorch and Komberg 2017; Rolicka et al. 2020). Genes are expressed when they can be sufficiently exposed for the transcription machinery to trigger remodeling of the chromatin conformation, and they are less so when they are persistently buried in dense chromatin (Magaña-Acosta and Valadez-Graham 2020). Chromatin remodeling takes place in cancer (Arildsen et al. 2017; Lafon-Hughes et al. 2008; Morgan and Shilatifard 2015). Cell type and cell state epigenetic organizations are key factors determining gene expression status, and the expressed proteins are wired in the cellular protein-protein interaction network. Super high-resolution electron microscopy and computational prediction methodologies are rapidly advancing, and they are being applied to cell-specific cancer genomes. This raises hope that RAS isoform-specific gene scale topologically associating domains (TAD) will be identified not only in specific tissues (Szabo et al. 2019), but also at different cell-transformation states. Such detailed maps could help forecast gene expression and alternative signaling pathways in drug resistance (Nussinov et al. 2021; Nussinov et al. 2020b). The anchorage of Ras isoforms in the membrane and their nanoclustering have been studied extensively, including their detailed interactions, sizes, and preferred membrane environments, chemistry and geometry (Lee et al. 2019). However, the challenge of their epigenetics and its linkage to rewired networks in distinct cell states and types is still waiting to be unraveled. The landscape of accessibility changes dynamically in response to external and developmental cues (Klemm et al. 2019). But its tissue-specific footprints may help in deciphering the impending pathways in drug resistance.

To date, pharmacology has successfully targeted KRas4B<sup>G12C</sup> (Zeng et al. 2020; Zhang et al. 2020). Ras isoform-specific pharmacology at the membrane has been deliberated. However, considering membrane fluidity, the common presence of phosphatidylserine, and the non-uniqueness of the PTMs, toxicity is a challenge. Whereas pharmacological innovations are compelling, reliably forecasting future developments is formidable, making the harnessing of the signaling pathways appear more tractable venues.

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References

Abe T, Umeki I, Kanno SI, Inoue SI, Niihori T, Aoki Y (2020) LZTR1 facilitates polyubiquitination and degradation of RAS-GTPases. Cell Death Differ 27:1023–1035. https://doi.org/10.1038/s41418-019-0395-5

Abraham SJ, Nolet RP, Calvert RJ, Anderson LM, Gaponkeno V (2009) KRAS4A directly regulates hexokinase 1. The landscape of accessibility changes dynamically in response to external and developmental cues (Klemm et al. 2019). But its tissue-specific footprints may help in deciphering the impending pathways in drug resistance.

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References

Abe T, Umeki I, Kanno SI, Inoue SI, Niihori T, Aoki Y (2020) LZTR1 facilitates polyubiquitination and degradation of RAS-GTPases. Cell Death Differ 27:1023–1035. https://doi.org/10.1038/s41418-019-0395-5

Abraham SJ, Nolet RP, Calvert RJ, Anderson LM, Gaponkeno V (2009) KRAS4A directly regulates hexokinase 1. The hypervariable region of K-Ras4B is responsible for its specific interactions with calmodulin. Biochemistry 48:7575–7583. https://doi.org/10.1021/bi900760j

Agamasu C, Ghirlando R, Taylor T, Messing S, Tran TH, Bindu L, Tonelli M, Nissley DV, McCormick F, Stephen AG (2019) KRAS perylation is required for bivalent binding with calmodulin in a nucleotide-independent manner. Biophys J 116:1049–1063. https://doi.org/10.1016/j.bpj.2019.02.004

Agudo-Ibanez L, Herrero A, Barbacid M, Crespo P (2015) H-ras distribution and signaling in plasma membrane microdomains are regulated by acylation and deacylation events. Mol Cell Biol 35:1898–1914. https://doi.org/10.1128/MCB.01398-14

Ahearn I, Zhou M, Philips MR (2018) Posttranslational modifications of RAS proteins. Cold Spring Harb Perspect Med 8:a031484. https://doi.org/10.1101/cshperspect.a031484

Amendola CR, Mahaffey JP, Parker SJ, Ahearn IM, Chen WC, Zhou M, Court H, Shi J, Mendoza SL, Morten MJ, Rothenberg E, Gottlieb E, Wadhgiri YZ, Possemato R, Hubbard SR, Baumann A, Kimmelman AC, Philips MR (2019) KRAS4A directly regulates hexokinase 1. Nature 576:482–486. https://doi.org/10.1038/s41586-019-1832-9

Arildsen NS, Jonsson JM, Bartuma K, Ebbesson A, Westbom-Fremer S, Masback A, Malander S, Nilbert M, Hedenfalk IA (2017)
malignant phenotype in the presence or absence of its oncogene. Cancer Res 62:4514–4518

Dohlman HG, Campbell SL (2019) Regulation of large and small G proteins by ubiquitination. J Biol Chem 294:18613–18623. https://doi.org/10.1074/jbc.R119.011068

Dultz E, Mancini R, Polles G, Vallotton P, Alber F, Weis K (2018) Quantitative imaging of chromatin decompaction in living cells. Mol Biol Cell 29:1763–1777. https://doi.org/10.1091/mbc.E17-11-0648

Durrant DE, Morrison DK (2018) Targeting the Raf kinases in human cancer: the Raf dimer dilemma. Br J Cancer 118:3–8. https://doi.org/10.1038/bjc.2017.399

Eisenberg S, Beckett AJ, Prior IA, Dekker FJ, Hedberg C, Waldmann H, Elad-Sfadia G, Haklai R, Balan E, Kloog Y (2004) Galectin-3 augments metastasis in breast cancer cell lines. J Cell Sci 117:743–750. https://doi.org/10.1242/jcs.20040915

Eisenberg S, Beckett AJ, Prior IA, Dekker FJ, Hedberg C, Waldmann H, Elad-Sfadia G, Haklai R, Balan E, Kloog Y (2004) Galectin-3 augments metastasis in breast cancer cell lines. J Cell Sci 117:743–750. https://doi.org/10.1242/jcs.20040915

Freeman AK, Ritt DA, Morrison DK (2013b) The importance of Raf-RBD. Structure 23:505–510. https://doi.org/10.1016/j.s收费标准.2014.12.017

Fivaz M, Meyer T (2005) Reversible intracellular translocation of KRas but not HRas in hippocampal neurons regulated by Ca2+calmodulin. J Cell Biol 170:429–441. https://doi.org/10.1083/jcb.200409157

Frauenfelder H, Sligar SG, Wolynes PG (1991) The energy landscapes and motions of proteins. Science 254:1598–1603. https://doi.org/10.1126/science.1799933

Freeman AK, Ritt DA, Morrison DK (2013a) Effects of Raf dimerization and its inhibition on normal and disease-associated Raf signaling. Mol Cell 49:751–758. https://doi.org/10.1016/j.molcel.2012.12.018

Freeman AK, Ritt DA, Morrison DK (2013b) The importance of Raf dimerization in cell signaling. Small GTPases 4:180–185. https://doi.org/10.4161/sptg.26117

Garcia-Gomez R, Bustelo XR, Crespo P (2018) Protein-protein interactions: emerging oncotargets in the RAS-ERK pathway. Trends Cancer 4:616–633. https://doi.org/10.1016/j.trecan.2018.07.002

Garcia-Ibanez Y, Riesco-Eizaguirre G, Santisteban P, Casar B, Crespo P (2017) The cysteine-rich region of raf-1 kinase contains zinc, translocates to the plasma membrane and activation pattern. Mol Cell Biol 37:1774–1777. https://doi.org/10.1091/mbc.E17-11-221.

Ghosh S, Xie WQ, Quest AF, Mabrouk GM, Strum JC, Bell RM (1994) Phosphorylation of RAF kinase dimers drives conformational changes that facilitate transactivation. Angew Chem Int Ed Eng 33:209–218. https://doi.org/10.1002/anie.199401858

Glattorick K, Cichowski K, Elledge SJ (2019) Tissue-specificity in cancer: the rule, not the exception. Science 363:1150–1151. https://doi.org/10.1126/science.aaw3472

Hancock JF, Magee AI, Childs JE, Marshall CJ (1989) All ras proteins are polyisoprenylated but only some are palmitoylated. Cell 57:1167–1177. https://doi.org/10.1016/0092-8674(89)90054-8

Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R, Ludlam MJ, Stokoe D, Gloor SL, Viggers G, Morales T, Aliagas I, Liu B, Sideris S, Hoeflich KP, Jaiswal BS, Seshagiri S, Koepfen H, Belvin M, Friedman LS, Malek S (2010) RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature 464:431–435. https://doi.org/10.1038/nature08833

Heidorn SJ, Milagre C, Whittaker S, Nourry A, Niculescu-Duvaz I, Dhomen N, Hussain J, Reis-Filho JS, Springer CJ, Pritchard C, Marais R (2010) Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell 140:209–221. https://doi.org/10.1016/j.cell.2009.12.040

Hepper DE, Eck MJ (2021) A structural perspective on targeting the RTK/Ras/MAP kinase pathway in cancer. Protein Sci. 30:1535–1553. https://doi.org/10.1002/pro.4125

Hinde E, Cardarelli F, Digman MA, Gratton E (2012) Changes in chromatin compaction during the cell cycle revealed by micrometer-scale measurement of molecular flow in the nucleus. Biophys J 102:691–697. https://doi.org/10.1016/j.bpj.2011.11.0426

Hobbs GA, Der CJ, Rossman KL (2016) RAS isoforms and mutations in cancer at a glance. J Cell Sci 129:1287–1292. https://doi.org/10.1242/jcs.182873

Honore B (2020) Proteomic protocols for differential protein expression analyses. Methods Mol Biol 2110:47–58. https://doi.org/10.1007/978-1-0716-0255-3_3

Hu J, Stites EC, Yu H, Germaino EA, Meharena HS, Stork PJS, Kornep AP, Taylor SS, Shaw AS (2013) Allosteric activation of functionally asymmetric RAS kinase dimers. Cell 154:1036–1046. https://doi.org/10.1016/j.cell.2013.07.046

Imelinski M, Greulich H, Kaplan B, Araujo L, Amann J, Hom L, Schiller J, Villalona-Calero MA, Meyerson M, Carbone DP (2014) Oncogenic and sorafenib-sensitive ARAF mutations in lung adenocarcinoma. J Clin Invest 124:1582–1586. https://doi.org/10.1172/JCI72763

Ise K, Nakamura K, Nakao K, Shimizu S, Harada H, Ichise T, Miyoshi J, Gondo Y, Ishikawa T, Aiba A, Katsuki M (2000) Targeted deletion of the H-ras gene decreases tumor formation in mouse skin carcinogenesis. Oncogene 19:2951–2956. https://doi.org/10.1038/sj.onc.1203600

Jambirina PG, Rauch N, Pilkington R, Rybakova K, Nguyen LK, Kholodenko BN, Buchete NV, Kolch W, Rosta E (2016) Phosphorylation of RAF kinase dimers drives conformational changes that facilitate transactivation. Angew Chem Int Ed Engl 55:983–986. https://doi.org/10.1002/anie.201509272

Jang H, Abraham SJ, Chavan TS, Hitchinson B, Khavrutskii L, Tarasova NI, Nussinov R, Gaponenko V (2015) Mechanisms of membrane binding of small GTPase K-Ras4B farnesylated hypervariable region. J Biol Chem 290:9465–9477. https://doi.org/10.1074/jbc.M114.620724

Jang H, Banerjee A, Chavan TS, Lu S, Zhang J, Gaponenko V, Nussinov R (2016a) The higher level of complexity of K-Ras4B activation at the membrane. FASEB J 30:1643–1655. https://doi.org/10.1096/fj.15-279091

Jang H, Muratcioglu S, Gursoy A, Keskin O, Nussinov R (2016b) Membrane-associated Ras dimers are isospecific: K-Ras dimers differ from H-Ras dimers. Biochem J 473:1719–1723. https://doi.org/10.1042/BCJ20160331

Jang H, Banerjee A, Chavan T, Gaponenko V, Nussinov R (2017) Flexible-body motions of calmodulin and the farnesylated hypervariable region yield a high-affinity interaction enabling K-Ras4B membrane extraction. J Biol Chem 292:12544–12559. https://doi.org/10.1074/jbc.M114.620724

Jang H, Banerjee A, Marcus K, Makowski L, Matsud C, Gaponenko V, Nussinov R (2019) The structural basis of the farnesylated and methylated K-Ras4B interaction with calmodulin. Structure 27(1647–1659):e1644. https://doi.org/10.1016/j.str.2019.08.009
Jang H, Zhang M, Nussinov R (2020) The quaternary assembly of KRas4B with Raf-1 at the membrane. Comput Struct Biotechnol J 18:737–748. https://doi.org/10.1016/j.csbj.2020.03.018

Jin T, Lavoie H, Sahmi M, David M, Hilt C, Hammell A, Therrien M (2017) RAF inhibitors promote GAS-RAF interaction by allosterically disrupting RAF autoinhibition. Nat Commun 8:1211. https://doi.org/10.1038/s41467-017-01274-0

Jinesh GG, Sambandam V, Vijayaraghavan S, Balaji K, Mukherjee S (2018) Molecular genetics and cellular events of K-Ras-driven tumorigenesis. Oncogene 37:839–846. https://doi.org/10.1038/onc.2017.377

Joyal JL, Burks DJ, Pons S, Matter WF, Vlahos CJ, White MF, Sacks DB, Kattan WE, Hancock JF (2020) RAS Function in cancer cells: translating of gene and protein expression in normal and cancer tissues. Sci Rep 10:10044

Kattan WE, Hancock JP (2020) RAF Function in cancer cells: translating of gene and protein expression in normal and cancer tissues. Sci Rep 10:10044

Klein CH, Truxius DC, Vogel HA, Harizanova J, Senkus R, Moorhead J, Al-Nawab M, Patel AG, Benjamin Klemm SL, Shipony Z, Greenleaf WJ (2019) Chromatin accessibility and the regulatory epigenome. Nat Rev Genet 20:207–220. https://doi.org/10.1038/s41576-018-0089-8

Kocher HM, Senkus R, Moorhead J, Al-Nawab M, Patel AG, Benjamin IS, Hendry BM (2005) Expression of Ras GTPase isoforms in normal and diseased pancreas. Pancreatology 5:205–214. https://doi.org/10.1159/000085273

Kondo Y, Ognjenovic J, Banerjee S, Kanarud D, Merk A, Kulhanek K, Kong W, Roose JP, Subramaniam S, Kuriyan J (2019) Cryo-EM structure of a dimeric B-Raf:14-3-3 complex reveals asymmetry in the active sites of B-Raf kinases. Science 366:109–115. https://doi.org/10.1126/science.aay5043

Kong G, Chang YI, Dannennawad A, You X, Du J, Ranheim EA, Lee W, Ryu MJ, Zhou Y, Xing Y, Chang Q, Burd CE, Zhang J, Ryu MJ, Zhou Y, Xing Y, Chang Q, Burd CE, Zhang J (2016) Loss of wild-type Kras promotes activation of all Ras isoforms in the active sites of B-Raf kinases. Science 366:109–115. https://doi.org/10.1126/science.aay5043

Kosti I, Jain N, Aran D, Butte AJ, Sirota M (2016) Cross-tissue analysis of RAS protein interactions in living cells reveals a mechanism that dictates KRas(G12D) diffusion and trafficking. Elife 8:e46393. https://doi.org/10.7554/eLife.46393

Lee S, Balmain A, Counter CM (2018a) A model for RAS mutation patterns in cancers: finding the sweet spot. Nat Rev Cancer 18:767–777. https://doi.org/10.1038/s41568-018-0076-6

Li S, Jang H, Zhang J, Nussinov R (2018b) Raf-1 cysteine-rich domain is required for the initiation of lung cancer by K-Ras(G12D). Cancer Discov 1:128–136. https://doi.org/10.1158/2159-8290.CD-17-10044

Lavoie H, Therrien M (2020) RAF inhibitors promote RAS-RAF interaction by allosterically disrupting RAF autoinhibition. Nat Commun 8:1211. https://doi.org/10.1038/s41467-017-01274-0

Lampson BL, Pershing NL, Prinz JA, Lacsina JR, Marzluff WF, MacAlpine DM, Counter CM (2013) Rare codons regulate Kras oncogenesis. Curr Biol 23:70–75. https://doi.org/10.1016/j.cub.2012.11.031

Lavoie H, Therrien M (2015) Regulation of RAP protein kinases in ERK signalling. Nat Rev Mol Cell Biol 16:281–298. https://doi.org/10.1038/nrm3979

Lavoie H, Thevakumaran N, Gavory G, Li JJ, Padeganeh A, Guirol S, Duchaine J, Mao DY, Bouvier M, Sicheri F, Therrien M (2013) Inhibitors that stabilize a closed RAF kinase domain conformation induce dimerization. Nat Chem Biol 9:428–436. https://doi.org/10.1038/nchembio.1257

Lavoie H, Sahmi M, Maignonneuve P, Marullo SA, Thevakumaran N, Jin T, Kurinov I, Sicheri F, Therrien M (2018) MEK drives BRAF activation through allosteric control of KSR proteins. Nature 554:549–553. https://doi.org/10.1038/nature25478

Lavoie H, Gagnon J, Therrien M (2020) ERK signalling: a master regulator of cell behaviour, life and fate. Nat Rev Mol Cell Biol 21:607–632. https://doi.org/10.1038/s41580-020-0255-7

Lee Y, Phelps C, Huang T, Mostofian B, Wu L, Zhang Y, Tao K, Chang YH, Stork PJ, Gray JW, Zuckerman DM, Nan X (2019) High-throughput, single-particle tracking reveals nested membrane domains that dictate KRas(G12D) diffusion and trafficking. Elife 8:e46393. https://doi.org/10.7554/eLife.46393

Li Y, Liu Y, Yang H, Zhang T, Naruse K, Tu Q (2020a) Dynamic transcriptional and chromatin accessibility landscape of medaka embryogenesis. Genome Res 30:924–937. https://doi.org/10.1101/gr.258871.119

Li YC, Lytle NK, Gammon ST, Wang L, Hayes TK, Sutton MN, Bast RC Jr, Der CJ, Piniwnica-Worms D, McCormick F, Wahl GM (2020b) Analysis of RAS protein interactions in living cells reveals a mechanism for pan-RAS depletion by membrane-targeted RAS binders. Proc Natl Acad Sci U S A 117:12121–12130. https://doi.org/10.1073/pnas.200848117

Lleres D, James J, Swift S, Norman DG, Lamond AI (2009) Quantitative analysis of chromatin compaction in living cells using FLM-FRET. J Cell Biol 187:481–496. https://doi.org/10.1083/jcb.200907029

Lorch Y, Komberg RD (2017) Chromatin-remodeling for transcription. Q Rev Biophys 50:e5. https://doi.org/10.1017/QRB.2017.08.033

Luk S, Banerjee A, Zhang J, Gaponenko V, Nussinov R (2015) GTP Binding and oncogenic mutations may attenuate hypervariable region (HVR)-catalytic domain interactions in small GTPase KRas4B, exposing the effector binding site. J Biol Chem 290:28887–28900. https://doi.org/10.1074/jbc.M115.664755

Lu S, Banerjee A, Zhang J, Gaponenko V, Nussinov R (2015) GTP Binding and oncogenic mutations may attenuate hypervariable region (HVR)-catalytic domain interactions in small GTPase K-Ras4B, exposing the effector binding site. J Biol Chem 290:28887–28900. https://doi.org/10.1074/jbc.M115.664755

Luo S, Jiang H, Muratcioglu S, Gursoy A, Keskin O, Nussinov R, Zhang J (2016) Ras conformational ensembles, allostery, and signaling. Chem Rev 116:6607–6665. https://doi.org/10.1021/acs.chemrev.5b00542

Magana-Acosta M, Valadez-Graham V (2020) Chromatin remodelers in the 3D nuclear compartment. Front Genet 11:600615. https://doi.org/10.3389/fgene.2020.600615

Matallanas D, Romanon D, Al-Mulla F, O’Neill E, Al-Ali W, Crespo P, Doyle B, Nixon C, Sansom O, Drostenn M, Barbadic M, Koleh W
Prior IA, Hood FE, Hartley JL (2020) The frequency of Ras mutations in cancer. Cancer Res 80:2969–2974. https://doi.org/10.1158/0008-5472.CAN-19-3682

Qi W, Sahin F, Iacobuzio-Donahue CA, Garcia-Carracedo D, Wang WM, Kuo CY, Chen D, Arking DE, Lowy AM, Hruban RH, Remotti HE, Su GH (2011) Disruption of p16 and activation of Kras in pancreas increase ductal adenocarcinoma formation and metastasis in vivo. Oncotarget 2:862–873. https://doi.org/10.18632/oncotarget.357

Rajakulendran T, Sahmi M, Lefrancois M, Sicheri F, Therrien M (2009) Dimerization-dependent mechanism drives RAF catalytic activation. Nature 461:542–545. https://doi.org/10.1038/nature08314

Rajasekharan SK, Raman T (2013) Ras and Ras mutations in cancer. Central European Journal of Biology 8:609–624. https://doi.org/10.2478/s11535-013-0158-5

Randic T, Kozar I, Marguc C, Utkal J, Kreis S (2021) NRAS mutant melanoma: towards better therapies. Cancer Treat Rev 99:102238. https://doi.org/10.1016/j.ctrv.2021.102238

Raso E (2020) Splice variants of RAS-translational significance. Cancer Metastasis Rev 39:1039–1049. https://doi.org/10.1007/s10555-020-09920-8

Rauen KA (2013) The RASopathies. Annu Rev Genomics Hum Genet 14:355–369. https://doi.org/10.1146/annurev-genom-091212-153523

Rezaei Adariani S, Buchholzer M, Akbarzadeh M, Nakhaei-Rad S, Dvorsky R, Ahmadian MR (2018) Structural snapshots of RAF kinase interactions. Biochem Soc Trans 46:1393–1406. https://doi.org/10.1042/BST20170528

Ritt DA, Monson DM, Specht SL, Morrison DK (2010) Impact of feedback phosphorylation and Raf heterodimerization on normal and mutant B-Raf signaling. Mol Cell Biol 30:806–819. https://doi.org/10.1128/MCB.00569-09

Rolia A, Guo Y, Ganeez Abat, Tariq K, Quin J, Vintermist A, Rolicka A, Guo Y, Ganez Zapater A, Tariq K, Quin J, Vintermist A, Rosa-Garrido M, Chapski DJ, Schmitt AD, Kimball TH, Karbassi E, Rezaei Adariani S, Buchholzer M, Akbarzadeh M, Nakhaei-Rad S, Sasaki AT, Carracedo A, Locasale JW, Anastasiou D, Takeuchi K, Kras in pancreas increase ductal adenocarcinoma formation and metastasis in vivo. Oncotarget 2:862–873. https://doi.org/10.18632/oncotarget.357

Shalom-Feuerstein R, Cooks T, Raz A, Kloog Y (2005) Gaelectin-3 regulates a molecular switch from N-Ras to K-Ras usage in human breast carcinoma cells. Cancer Res 65:7292–7300. https://doi.org/10.1158/0008-5472.CAN-05-0775

Shaw AS, Kornev AP, Hu J, Ahuja LG, Taylor SS (2014) Kinases and pseudokinases: lessons from RAF. Mol Cell Biol 34:1538–1546. https://doi.org/10.1128/MCB.00557-14

Sidhu RS, Clough RR, Bhullar RP (2003) Ca2+/calmodulin binds and regulates K-Ras from membrane. Biochem Biophys Res Commun 304:655–660. https://doi.org/10.1006.bbrc.2003.0635-1

Sieburth DS, Sun Q, Han M (1998) SUR-8, a conserved Ras-binding protein with leucine-rich repeats, positively regulates Ras-mediated signaling in C. elegans. Cell 94:119–130. https://doi.org/10.1016/s0092-8674(00)81227-1

Smith SF, Collins SE, Charest PG (2020) Ras, PI3K and mTORC2-three’s a crowd? J Cell Sci 133:jcs234930. https://doi.org/10.1242/jcs.234930

Smith KA, Webster WA, Siegried KR, McCusker CD (2017) A new and improved algorithm for the quantification of chromatin condensation from microscopic data shows decreased chromatin condensation in regenerating axolotl limb cells. PLoS One 12: e0185292. https://doi.org/10.1371/journal.pone.0185292

Spanididos DA, Frame M, Wilkie NM (1990) Expression of the normal H-ras1 gene can suppress the transformed and tumorigenic phenotypes induced by mutant ras genes. Anticancer Res 10:1543–1554

Sperlich B, Kapoor S, Waldmann H, Winter R, Weise K (2016) Regulation of K-Ras4B membrane binding by calmodulin. Biophys J 111:113–122. https://doi.org/10.1016/j.bpj.2016.05.042

Staflas A, Karlsson C, Persson M, Palmeqvist L, Bergo MO (2015) Wild-type KRAS inhibits oncogenic KRAS-induced T-ALL in mice. Leukemia 29:1032–1040. https://doi.org/10.1038/leu.2014.315

Stephens RM, Yi M, Kessing B, Nissley DV, McCormick F (2017) Tumor KRAS gene expression levels are influenced by the mutational status of KRAS genes and both upstream and downstream KRAS pathway genes. Cancer Informat 16:1176935117711944. https://doi.org/10.1177/1176935117711944

Szabo Q, Bantignies F, Cavalli G (2019) Principles of genome folding into topologically associating domains. Sci Adv 5:eaaaw1668. https://doi.org/10.1126/sciadv.aaw1668

Terrell EM, Morrison DK (2019) Ras-mediated activation of the Raf family kinases. Cold Spring Harb Perspect Med 9:a033746. https://doi.org/10.1101/cshperspect.a033746

Terrell EM, Durrane RE, Ditt R, Sealover NE, Sheffels E, Spencer-Smith R, Esposito D, Zhou Y, Hancock JF, Kortum RL, Morrison DK (2019) Distinct binding preferences between Ras and Raf family members and the impact on oncogenic Ras signaling. Mol Cell 76(872-884):e875. https://doi.org/10.1016/j.molcel.2019.09.004

Thayathasan JV, Tan E, Zheng H, Lin YC, Li Y, Inoue T, Fivaz M (2013) The small GTPase HRas shapes local PI3K signals through positive feedback and regulates persistent membrane extension in migrating fibroblasts. Mol Biol Cell 24:2228–2237. https://doi.org/10.1091/mbc.E12-12-0905

To MD, Wang CE, Karnezis AN, Del Rosario R, Di Lauro R, Balmain A (2019) Kras regulatory elements and exon 4A determine mutation specificity in lung cancer. Nat Genet 40:1240–1244. https://doi.org/10.1038/ng.211

To MD, Rosario RD, Westcott PM, Banta KL, Balmain A (2013) Interactions between wild-type and mutant Ras genes in lung and skin carcinogenesis. Oncogene 32:4028–4033. https://doi.org/10.1038/onc.2012.404
Travers T, Lopez CA, Van QN, Neale C, Tonelli M, Stephen AG, Gnanakaran S (2018) Molecular recognition of RAS/RAF complex at the membrane: role of RAF cysteine-rich domain. Sci Rep 8: 8461. https://doi.org/10.1038/s41598-018-26832-4

Tsai CJ, Nussinov R (2018) Allosteric activation of RAF in the MAPK signaling pathway. Curr Opin Struct Biol 53:100–106. https://doi.org/10.1016/j.sbi.2018.07.007

Tsai CJ, Kumar S, Ma B, Nussinov R (1999) Folding funnels, binding funnels, and protein function. Protein Sci 8:1181–1190. https://doi.org/10.1110.ps.8.6.1181

Tsai FD, Lopes MS, Zhou M, Court H, Ponce O, Fiordalisi JJ, Gierut JJ, Cox AD, Haigis KM, Philips MR (2015) K-Ras4A splice variant is widely expressed in cancer and uses a hybrid membrane-targeting motif. Proc Natl Acad Sci U S A 112:779–784. https://doi.org/10.1073/pnas.1412811112

Udell CM, Rajakulendran T, Sicheri F, Therrien M (2011) Mechanistic principles of RAF kinase signaling. Cell Mol Life Sci 68:553–565. https://doi.org/10.1007/s00018-010-0520-6

Ullah R, Yin Q, Snell AH, Wan L (2021) RAF-MEK-ERK pathway in cancer evolution and treatment. Semin Cancer Biol. https://doi.org/10.1016/j.semcancer.2021.05.010

Varga A, Ehrenreiter K, Aschenbrenner B, Kocieniewski P, Kocianczyk V, Varga A, Ehrenreiter K, Aschenbrenner B, Kocieniewski P, Kochanetz M, Lipniacki T, Baccarini M (2017) RAF1/BRAF dimerization integrates the signal from RAS to ERK and ROKalpha. Sci Signal 10: eaai8482. https://doi.org/10.1126/scisignal.aai8482

Verkhivker GM (2016) Molecular dynamics simulations and modelling of the residue interaction networks in the BRAF kinase complexes with small molecule inhibitors: probing the allosteric effects of ligand-induced kinase dimerization and paradoxic activation. Mol BioSyst 12:3146–3165. https://doi.org/10.1039/c6mb00298f

Vigli D, Cherfils J, Rosman KL, Der CJ (2010) Ras superfamily GEFs and GAs: validated and tractable targets for cancer therapy? Nat Rev Cancer 10:842–857. https://doi.org/10.1038/nrc2960

Villalonga P, Lopez-Alcala C, Bosch M, Chilochea A, Rocamora N, Gil J, Marins R, Marshall CJ, Bachs O, Agell N (2001) C31mulin binds to K-Ras, but not to H- or N-Ras, and modulates its downstream signalling. Mol Cell Biol 21:7345–7354. https://doi.org/10.1128/MCB.21.21.7345-7354.2001

Wachsmuth M, Knoch TA, Rippe K (2016) Dynamic properties of independent chromatin domains measured by correlation spectroscopy in living cells. Epigenetics Chromatin 9:57. https://doi.org/10.1186/s13072-016-0093-1

Wang MT, Holderfield M, Galeas J, Delrosario R, To MD, Balmain A, McCormick F (2015) Wildtype Kras2 can inhibit lung carcinogenesis in mice. Nat Genet 29:768–774. https://doi.org/10.1038/ng.2960

Zhang M, Zhang Z, Vikis HG, Johnson L, Liu G, Li J, Anderson MW, Sills RC, Hong HL, Devereux TR, Jacks T, Guan KL, You M (2001) Wildtype Kras2 can inhibit lung carcinogenesis in mice. Nat Genet 29:25–33. https://doi.org/10.1038/ng.721

Zhang M, Jang H, Gaponenko V, Nussinov R (2017) Phosphorylated calmodulin promotes PI3K activation by binding to the SH2 domains. Biochim Biophys Acta 1853:841–857. https://doi.org/10.1016/j.bbalip.2017.09.008

Zhang M, Li Z, Wang G, Jang H, Gaponenko V, Nussinov R (2018) Calmodulin (CaM) activates PI3Kalpha by targeting the “soft” CaM-binding motifs in both the nSH2 and cSH2 domains of p85alpha. J Phys Chem B 122:11137–11146. https://doi.org/10.1021/acs.jpcb.8b05982

Zhang M, Jang H, Nussinov R (2020) PI3K inhibitors: review and new strategies. Chem Sci 11:5855–5865. https://doi.org/10.1039/d0sc01676d

Zhang M, Jang H, Li Z, Sacks DB, Zhang J, Gaponenko V, Nussinov R (2018) Calmodulin (CaM) activates PI3Kalpha by targeting the “soft” CaM-binding motifs in both the nSH2 and cSH2 domains of p85alpha. J Phys Chem B 122:11137–11146. https://doi.org/10.1021/acs.jpcb.8b05982

Zhang M, Jang H, Nussinov R (2020) PI3K inhibitors: review and new strategies. Chem Sci 11:5855–5865. https://doi.org/10.1039/d0sc01676d

Zhang M, Jang H, Li Z, Sacks DB, Nussinov R (2021) B-Raf autoinhibition in the presence and absence of 14-3-3. Structure. 29:768–777. https://doi.org/10.1016/j.str.2021.02.005

Zhou Y, Hancock JF (2015) Ras nanoclusters: Versatile lipid-based signaling platforms. Biochim Biophys Acta 1853:841–849. https://doi.org/10.1016/j.bbamac.2014.09.008

Zhou B, Der CJ, Cox AD (2016) The role of wild type RAS isoforms in cancer. Semin Cell Dev Biol 58:58–69. https://doi.org/10.1016/j.semcdb.2016.07.012

Zhou Y, Prakash P, Gorfe AA, Hancock JF (2018) Ras and the plasma membrane: a complicated relationship. Cold Spring Harb Perspect Med 9:a031831. https://doi.org/10.1101/cshperspect.a031831

Zhou M, Kuruvilla L, Shi X, Viviano S, Ahearn IM, Amendola CR, Su W, Badi S, Mahafey J, Feherbacher N, Skok J, Schlessinger J, Kurve BK, Calchowder DA, Phillips MR (2020) Scaffold association factor B (SAFB) is required for expression of prenyltransferases and RAS membrane association. Proc Natl Acad Sci U S A 117:31914–31922. https://doi.org/10.1073/pnas.2005712117

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