Small GTPases

ISSN: 2154-1248 (Print) 2154-1256 (Online) Journal homepage: https://www.tandfonline.com/loi/ksgt20

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To cite this article: Scott M. Coyle (2016) Reverse engineering GTPase programming languages with reconstituted signaling networks, Small GTPases, 7:3, 168-172, DOI: 10.1080/21541248.2016.1178367

To link to this article: https://doi.org/10.1080/21541248.2016.1178367

Accepted author version posted online: 29 Apr 2016.
Published online: 09 May 2016.

SMALL GTPASES
2016, VOL. 7, NO. 3, 168–172
http://dx.doi.org/10.1080/21541248.2016.1178367

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COMMENTARY

Reverse engineering GTPase programming languages with reconstituted signaling networks

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ABSTRACT

The Ras superfamily GTPases represent one of the most prolific signaling currencies used in Eukaryotes. With these remarkable molecules, evolution has built GTPase networks that control diverse cellular processes such as growth, morphology, motility and trafficking.[1-4] Our knowledge of the individual players that underlie the function of these networks is deep; decades of biochemical and structural data has provided a mechanistic understanding of the molecules that turn GTPases ON and OFF, as well as how those GTPase states signal by controlling the assembly of downstream effectors. However, we know less about how these different activities work together as a system to specify complex dynamic signaling outcomes. Decoding this molecular "programming language" would help us understand how different species and cell types have used the same GTPase machinery in different ways to accomplish different tasks, and would also provide new insights as to how mutations to these networks can cause disease. We recently developed a bead-based microscopy assay to watch reconstituted H-Ras signaling systems at work under arbitrary configurations of regulators and effectors.[5] Here we highlight key observations and insights from this study and propose extensions to our method to further study this and other GTPase signaling systems.

ARTICLE HISTORY
Received 28 March 2016
Revised 8 April 2016
Accepted 11 April 2016

KEYWORDS
biochemistry; dynamics; in vitro reconstitution; signaling; Ras

The majority of biochemical work on Ras systems to date has typically focused on individual steps in the GTPase reaction cycle: GEF assays monitor the first exchange of fluorescently labeled nucleotide for unlabeled nucleotide; GAP assays monitor the hydrolysis of GTP under conditions in which exchange is typically not possible[6]; effector binding is investigated when GTPases are loaded with non-hydrolyzable GTP analogs.[7-9] These approaches have allowed us to develop an understanding of how GEF, GAP, effectors, and GTPase operate in isolation. However, in the cell all of these activities can be present at once, such that the GTPase is actively cycling as it propagates signals downstream to multiple competing effectors. While one can query Ras under such conditions with cell-based experiments, it is challenging to map cellular signaling outcomes to specific configurations of the underlying network. How can we determine how the configuration of the underlying GTPase network defines how signals are transmitted and the ways in which cells can "program" the system to produce specific dynamic responses to external inputs?

To address this problem, we sought a new type of *systems level reconstitution* of GTPase signal processing that would allow for multiple cycles of activation and deactivation of the GTPase and in which outputs would be measured by effector binding to the GTPase—precisely the same way in which cells couple GTPase activation to outputs. Thus, we developed a bead-based microscopy assay to literally watch H-Ras signal processing, in which microspheres coated with GTPase serve as a platform for input-dependent assembly and disassembly of fluorescently-labeled effector molecules from a solution of network components (Fig. 1).[5]

When an input is applied to an H-Ras signaling network, what dynamic outputs are produced as a result? Our assay enabled us to explore this question because we could prepare signaling networks with different concentrations and identities of GEFs, GAPs, and effectors and then monitor the dynamics of the system response over time to a perturbation. In doing so, we found that the nature of the underlying configuration of the GTPase network had dramatic effects on the type of output dynamics that we observed (Fig. 2).

Increasing amounts of step-input derived from a constitutive GEF activity led to increased amplitudes of system output (i.e. effector binding) and faster turn-on response. Surprisingly, these same inputs in the presence

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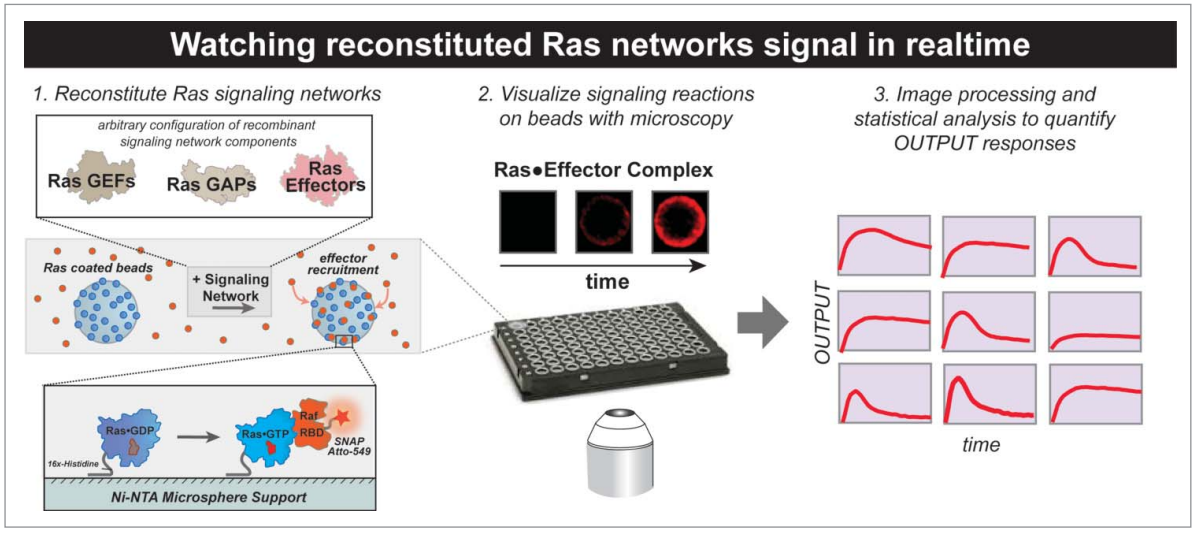

Figure 1. A bead-based microscopy assay for watching Ras GTPase networks signal under arbitrary system configurations. Depiction of the assay developed in ref. 5. Beads loaded with the H-Ras GTPase are placed in a solution containing fluorescent effector molecules and user-defined concentrations of network components like GAP and GEF. When an INPUT that changes the activity of one of these components is applied to the system (such as a change in [GEF]), activation of the GTPase will lead to recruitment of fluorescent effectors to the bead surface, defining the OUTPUT of the system. This recruitment can be monitored in real time by microscopy for hundreds of unique beads in a multiplexed fashion to see how different network configurations result in different dynamic signaling OUTPUTs.

of increasing amounts of GAP activity did not simply lower the amplitude of the response, but substantially altered the dynamics as well. High levels of GAP activity led to overshoot of the final steady state and a transient, pulsatile output response, in which shorter pulse-widths were associated with higher concentrations of GAP in the system. This implies that both sustained and transient outputs can be naturally encoded by these GTPase systems.

In addition, we found that the density of GTPase present on the bead surface played an important role in the apparent output dynamics we observed. In response to the exact same input, low GTPase density systems exhibited more transient character in their outputs than high density systems, likely representing a shift in network configuration from one in which the GTPase was limiting to one in which the GTPase was in excess.

Another surprising observation was that effectors played a much more active role in signal processing than we had anticipated. We had initially thought that higher concentrations of effector would simply scale the amplitude of the output response. However, increasing the levels of effector in the system led not only to more amplitude in the system response, but also wildly different dynamics. This is because higher concentrations of effector enable faster equilibration against the time-varying concentration of activated GTPase, thus allowing higher effector concentrations to capture more transient features of the upstream GTPase signal in the output.

The active roles that effectors play in shaping signaling dynamics were even more striking in networks that contained more than one effector. These bear a stronger resemblance to the actual networks inside cells, in which

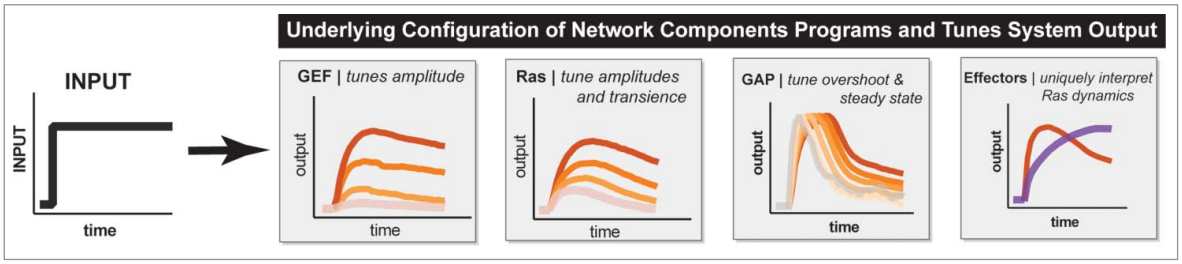

Figure 2. Each Ras network component programs Ras signaling output dynamics in a unique way. When an input is applied to a Ras signaling system, the underlying network configuration will shape the resulting output dynamics. We found that each signaling component in the network impacted the timing, duration, and amplitude of signaling output in a unique way. The way each network component—GEF, GAP, Ras, or effector—programs the system output is summarized in this figure.

H-Ras can communicate signals to many different competing downstream targets such as Raf, PI3 Kinase, RalGDS, as well as many others.[10,11] Remarkably, we found that in multi-effector networks, different effectors could interpret an input to the signaling network with completely different output dynamics. For example, one effector might produce a sustained response, while a second would respond with a transient pulse. These complex dynamics appear to be the result of competition between effectors that possess different affinities and assembly/disassembly kinetics toward the supply of activated GTPase.

An interesting consequence of this is that effector usage naturally partitions into a particular order as dictated by the abundance and biophysical properties of the effectors in the network, such that a particular sequence of effector use occurs in response to an input. This may be a simple method by which cells can use Ras GTPase signaling networks to build temporal programs in which different activities peak and decline at different times during the signaling response.

By simultaneously varying GEF, GAP, effector, and GTPase levels in this way, we were able to build a design manual describing the dynamic signaling programs that can be generated from the simplest-case GTPase signaling system, and observed a rich space of complex signaling outputs that could arise in response to a simple a step-input. The fact that so many different dynamic outputs and response shapes were possible suggests that the Ras machinery is highly versatile and readily reconfigured across different cell types or species to build complex temporal signaling programs and fulfill particular signaling needs.

However, this versatility is not without trade-offs. Mutations in Ras family signaling networks are associated with cancer as well as many other so-called RASopathies such as Noonan syndrome.[12] Perhaps the most notorious mutations occur in the Ras GTPase itself, where substitutions at the G12, G13, and Q61 positions are frequently associated with cancer.[13] In these cases, the mutations are thought to disrupt signaling through 3 mechanisms: decreasing the intrinsic GTPase hydrolysis rate, blocking GAP-catalyzed hydrolysis, and altering affinity and preference for downstream effectors.[10,13,14] More recently, the Ras G12C was shown to actively cycle in a "hyper-excitable" state in cells, illustrating that some undesirable features of oncogenic alleles may only be manifest in a cycling network context.[15] In agreement with this, we found that the network context was critical for revealing differences between oncogenic and wildtype alleles of Ras (Fig. 3). In networks that lacked GAP activity, step-inputs resulted in system outputs in which oncogenic and wildtype Ras were nearly indistinguishable from one another. In contrast, the same step-input in a high GAP context led to massive differences in signaling output amplitudes and dynamics: wildtype Ras produced a low-amplitude transient pulse of signaling output, whereas oncogenic Ras produced a high-amplitude sustained signal.

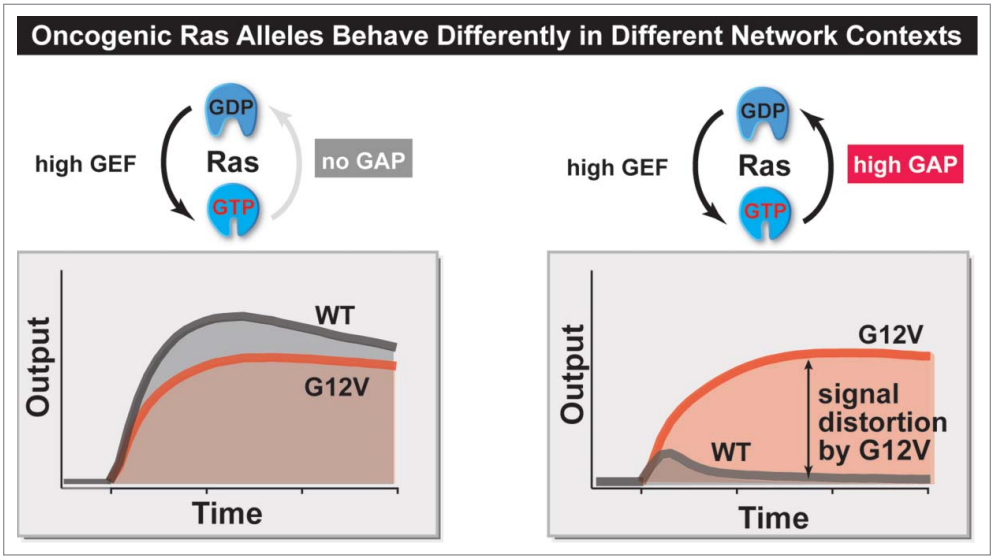

Figure 3. The extent to which oncogenic alleles distort signal processing depends on the underlying network configuration. The difference between signaling outputs of networks harboring either wildtype Ras or oncogenic Ras networks was compared using our assay. When an input was applied to a low-GAP network context, the outputs from wildtype and oncogenic Ras networks were largely indistinguishable. In contrast, when that same input was applied to a high-GAP network context, the outputs were completely different: wildtype Ras produced a low-amplitude transient pulse of output, while oncogenic Ras produced a high-amplitude sustained signal.

By comparing the differences between wildtype and oncogenic Ras across many different network configurations, we were able to determine regimes in which oncogenic alleles distorted wildtype signals most strongly. We found that oncogenic alleles of Ras in a low GEF input / high GAP background could distort integrated signals by over 1000 fold compared to wildtype Ras. Intuitively, these correspond to weak or noisy inputs to the system that would normally be filtered out by the high basal GAP activity, but that in the context of the oncogenic allele are now sustained to high levels. This is consistent with models of oncogenic Ras signaling in which the system becomes hyper-activatable and erroneously responds to noisy inputs, as opposed to simply being 'constitutively active'. This is also in agreement with the recent observation that Ras G12C can actively cycle in a hyper-excitable state in cells. It further suggests that one reason why Ras mutations are oncogenic in one cell or tissue type but not another may reflect underlying differences in the network context in which those mutations are manifest.

The fact that the most striking differences between wildtype and oncogenic alleles of H-Ras occur under network configurations in which wildtype Ras is normally fast-cycling suggests that these differences in the lifetimes of certain GTPase states could be used as a selectivity-handle to target only the mutated form of Ras. For example, if a drug has slow assembly kinetics on the GTPase, it may be unable to target a fast-cycling wildtype Ras but would be able to assemble on a slow-cycling mutant. The fact that we have a simple high-throughput fluorescence-based assay in which the behavior of wildtype and oncogenic Ras can be compared side-by-side could prove useful for screening for small molecules that can exploit these *dynamical* differences in the GTPase.

In the future, we hope to extend the approach we have taken with H-Ras to explore other aspects of both Ras signaling as well as the signaling properties of other small GTPase systems, such as the Rho and Rab GTPases. How do the dynamic properties of the system change if fluid lipid supports are used? Are the ways in which the GEF/GAP/Effector shape the amplitude and duration of H-Ras outputs similar in other small GTPase systems, or do idiosyncratic features of the molecules create a different "programming language" for other systems? What happens when signaling states are coupled to mechanical systems, such as actin polymerization? What happens when multiple GTPases are connected together, as in the Ras → Ral → Rho cascade?[16] By building up networks of these signaling molecules *in vitro* and watching how they operate with new methods like the one we developed, we can begin to shed light on these questions about these fascinating signaling molecules.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

[1] Chang F, Steelman LS, Shelton JG, Lee JT, Navolanic PM, Blalock WL, Franklin R, McCubrey JA. Regulation of cell cycle progression and apoptosis by the Ras/Raf/MEK/ERK pathway (Review). Int J Oncol 2003; 22:469-80; PMID:12579299.

[2] Sjölander A, Yamamoto K, Huber BE, Lapetina EG. Association of p21ras with phosphatidylinositol 3-kinase. Proc Natl Acad Sci U S A 1991; 88:7908-12; PMID:1716764; http://dx.doi.org/10.1073/pnas.88.18.7908

[3] Hofer F, Fields S, Schneider C, Martin GS. Activated Ras interacts with the Ral guanine nucleotide dissociation stimulator. Proc Natl Acad Sci 1994; 91:11089-93; PMID:7972015; http://dx.doi.org/10.1073/pnas.91.23.11089

[4] Bourne HR, Sanders DA, McCormick F. The GTPase superfamily: a conserved switch for diverse cell functions. Nature 1990; 348:125-32; PMID:2122258; http://dx.doi.org/10.1038/348125a0

[5] Coyle SM, Lim WA. Mapping the functional versatility and fragility of Ras GTPase signaling circuits through in vitro network reconstitution. eLife 2016; 5:e12435; PMID:26765565.

[6] Eberth A, Ahmadian MR. In Vitro GEF and GAP Assays [Internet]. In: Current Protocols in Cell Biology John Wiley & Sons, Inc; 2001 [cited 2015 Jul 27]. Available from: http://onlinelibrary.wiley.com/doi/10.1002/0471143030.cb1409s43/abstract

[7] Geyer M, Schweins T, Herrmann C, Prisner T, Wittinghofer A, Kalbitzer HR. Conformational transitions in p21ras and in its complexes with the effector protein Raf-RBD and the GTPase activating protein GAP. Biochemistry (Mosc) 1996; 35:10308-20; PMID:8756686.

[8] Sydor JR, Engelhard M, Wittinghofer A, Goody RS, Herrmann C. Transient kinetic studies on the interaction of ras and the Ras-Binding domain of c-Raf-1 reveal rapid equilibration of the complex. Biochemistry (Mosc) 1998; 37:14292-9; PMID:9760267.

[9] Herrmann C, Horn G, Spaargaren M, Wittinghofer A. Differential interaction of the ras family GTP-binding proteins H-Ras, Rap1A, and R-Ras with the putative effector molecules Raf kinase and Ral-guanine nucleotide exchange Factor. J Biol Chem 1996; 271:6794-800; PMID:8636102; http://dx.doi.org/10.1074/jbc.271.36.21848

[10] Smith MJ, Ikura M. Integrated RAS signaling defined by parallel NMR detection of effectors and regulators. Nat Chem Biol 2014; 10:223-30; PMID:24441586; http://dx.doi.org/10.1038/nchembio.1435

[11] Herrmann C. Ras–effector interactions: after one decade. Curr Opin Struct Biol 2003; 13:122-9; PMID:12581669; http://dx.doi.org/10.1016/S0959-440X(02)00007-6

[12] Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nat Rev Cancer 2007; 7:295-308; PMID:17384584; http://dx.doi.org/10.1038/nrc2109

[13] Barbacid M. ras Genes. Annu Rev Biochem 1987; 56:779-827; PMID:3304147.

[14] Trahey M, McCormick F. A cytoplasmic protein stimulates normal N-ras p21 GTPase, but does not affect oncogenic mutants. Science 1987; 238:542-5; PMID:2821624; http://dx.doi.org/10.1126/science.2821624

[15] Patricelli MP, Janes MR, Li L-S, Hansen R, Peters U, Kessler LV, Chen Y, Kucharski JM, Feng J, Ely T, et al. Selective Inhibition of Oncogenic KRAS Output with Small Molecules Targeting the Inactive State. Cancer Discov 2016; 6:316-29; PMID:26739882; http://dx.doi.org/10.1158/2159-8290.CD-15-1105

[16] Neel NF, Martin TD, Stratford JK, Zand TP, Reiner DJ, Der CJ. The RalGEF-Ral Effector Signaling Network. Genes Cancer 2011; 2:275-87; PMID:21779498; http://dx.doi.org/10.1177/1947601911407329