

GAPs

Terminator versus effector functions and the role(s) of ArfGAP1 in vesicle biogenesis

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Whether your passion is to understand and reverse disease processes or “simply” a better understanding of how cells work, anyone wishing to understand cell regulation today must have a detailed and accurate understanding of regulatory GTPase mechanisms and their application to specific pathways. This is becoming increasingly difficult as the details of signaling by members of different families of GTPases and their regulators expand. But this is all the more reason to continually ask, which aspects of GTPase signaling are distinct to a GTPase or its sub-family and which are conserved throughout the superfamily? We each have slightly different views of the key aspects of GTPase signaling that are derived from the main GTPases studied in our own labs; e.g., translocation onto a membrane is an essential and integral aspect of Arf activation but not of other GTPases. However, one aspect of GTPase signaling that I had come to believe to be widespread and of general importance is not universally accepted. In fact, through my conversations at the recent FASEB summer research conference on “Arf Family GTPases” and reading of the literature in a graduate tutorial class, I realized that it is not known or accepted by the majority of researchers. The question is the role of GTPase activating proteins (GAPs) in signaling. Are they “pure” terminators of signaling or do they serve effector functions?

It is universally agreed that in cells GTPases are activated by guanine nucleotide exchange factors (GEFs), which promote the exchange of GTP for GDP, and inactivated by GTPase activating proteins (GAPs, or RGS proteins for G proteins), which promote the hydrolysis of bound GTP. This cycle of GTP binding and hydrolysis is accompanied by changes in conformation of the GTPase, which can (rarely) be coupled to changes in spatial location but are always coupled to changes in affinity for different binding partners. During the time interval that the GTPase is in its active conformation it displays higher affinity for the “effectors” that are the mediators of the changes in cellular activities and biology.[†] There can be other protein partners of GTPases that modulate this cycle [e.g., guanine nucleotide dissociation inhibitors (GDIs) for some members of the Ras superfamily and

$\beta\gamma$ dimers for G proteins] but these are not universal. And regulatory roles for specific lipids have been clearly established in several cases but this topic is beyond the scope of this article. Thus, we strive to learn the identities and actions of the GEFs, GAPs and effectors to understand the biology, signaling pathway, and regulation of each GTPase.

GAPs were identified through a variety of techniques, including genetic screens in *S. cerevisiae*^{1,2} and *C. elegans*³ as negative regulators of GTPase signaling, in early studies of p21 ras signaling as an activity that is inactive against oncogenic Ras mutations⁴ and, importantly, as activities associated with known GTPase effectors, including phospholipase C⁵ and cGMP phosphodiesterase.⁶ Despite the fact that GAP and RGS proteins in several cases emerged as negative modulators of GTPase signaling, there were clear indications very early that positive signaling was also possible, if not documented. The idea that a protein with GAP activity may serve a positive role in signaling was the subject of a review as early as 1989.⁷ Since then the numbers of examples have only grown so there should be no need for discussion as to whether GAPs can serve effector functions. So it is surprising to me to discover the high percentage of researchers who still describe GAPs only as terminators of GTPase signaling or who express confusion when a mutation that attenuates GAP activity retains or displays enhanced biological responses.

I must confess that my own research history in Arf biology has contributed greatly to my complete acceptance that most, if not all, Arf GAPs have effector functions. The fact that Arf proteins lack intrinsic GTPase activity and thus require a GAP to promote GTP hydrolysis initially suggested a greater need for Arf GAPs as terminators of Arf signaling. But in 1998 a very gifted researcher in my group at that time, Chun-jiang Zhang, identified a number of genes in yeast as suppressors of the loss of Arf1 signaling in the yeast *S. cerevisiae* and showed them to encode proteins with Arf GAP activity and distinct cellular effects.^{8,9} The fact that there exists a family of proteins with Arf GAP domains in every eukaryotic cell, with more than 30 members

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[†]Though not the purpose of this article, I would like to point out that referring to the GTP-bound state as active and GDP-bound state as inactive can be misleading (or flat-out wrong) with at least some GTPase; e.g., Ran moves in and out of the nucleus in a nucleotide-dependent fashion with equal signaling outcomes from both. Better may be to simply state that the two nucleotide-dependent conformers have different affinities for different sets of proteins.

in humans that resolve into ten clades in phylogenetic analyses¹⁰ I also interpret as evidence for distinct actions and likely biological outcomes beyond termination of activated Arf. The fact that most Arf GAPs are large proteins with multiple domains that bind directly to many other partners¹¹ reveals a high level of regulation of localization, Arf GAP activity, and other effects. Whether such proteins are viewed as protein scaffolds that nucleate assembly of multi-protein complexes with effector functions or as highly evolved and regulated Arf GAP complexes will depend upon the readout used in the assay and perhaps the preconception of the researchers involved. I believe that these types of observations have been made in most every other family of regulatory GTPases and the conclusions likely extend to them as well. Perhaps one or more experts in Ras, Rho, Ran, Rab or other families would offer their views?

Why would GAPs have evolved to possess both terminator and effector functions? Here again our views of GTPase signaling are challenged. Most models of GTPase signaling show activation by the GEF on a bilayer, with an effector to be engaged floating nearby. This implies/requires a freely diffusible, activated GTPase existing on the membrane whose biological activity will depend upon the first partner encountered, be it a GAP, effector A, effector B, etc. I must confess that I had this view until quite recently and thought that it was the relative concentrations of those proteins that dictated the outcome. But recent experiments in my lab by an outstanding graduate student, Amanda Caster, revealed data that are inconsistent with the formation of a diffusible pool of activated Arfs at the Golgi (Caster, Shrivastava-Ranjan and Kahn, manuscript submitted). Probably the most developed model for the positive role for GAP activity by an effector is found in work from Ken Harden's lab on phospholipase C- β 3.¹² They argue convincingly that the GAP activity is important to noise suppression in signaling as well as signal amplification. The importance of a solid conceptual understanding of kinetics as a feature of GTPase signaling and GAP actions was brought home to me by a beautifully written review by Eliot Ross¹³ that I recommend highly to everyone. It touches on kinetic aspects of GTPase signaling and helps the reader better understand the links between signal amplitude modulation and signal termination as well as "kinetic scaffolding." Although written from the perspective of G proteins and RGS's, it is equally relevant and important for all GTPases and GAPs. There are likely correlates in other GTPase families that I hope readers may share with the rest of us by blogging examples.

There are three aspects of Arf GAP biology that I find the most underdeveloped or misunderstood and I suspect this is true of GAP biology in other GTPase families. (1) How are Arf GAP activities regulated? (2) What is the specificity of each Arf GAP for GTPases in the family? (3) How does the rate of GTP hydrolysis contribute to the kinetics of signaling by the substrate GTPases? Confusion on each of these issues is exacerbated by the high percentage of papers that use the isolated GAP domains from much larger proteins, which is very likely to result in the loss of regulation, altered specificity and kinetics. Each family of

GTPases (Ras, Rho, Arf, Rab, etc.) use distinct GAPs, with distinctive GAP domains, which have largely indeterminate specificity within the family. If two GTPases with distinct activators and downstream effectors share a common GAP, there is obvious potential for cross-communication and regulation of those two pathways. I believe this to be a fundamental aspect of GTPase biology that is slowly emerging. While regulation of any one pathway can be critical to cell biology, I believe it is the integration of different pathways that will ultimately prove to be the most profound function of GTPases.

While no one today can seriously dispute the potential for some GAPs to have effector function(s) there is widespread disagreement on the frequency with which this occurs. I come dangerously close to believing that ALL Arf GAPs have effector functions, though this is obviously a difficult position to argue. Thus, I have been following with great interest the controversy over the past several years as to whether the function of ArfGAP1 in vesicle biogenesis is solely to promote GTP hydrolysis and release of Arf and COPI or is an obligate component in the budding vesicle and thus an Arf effector. ArfGAP1 is the founding member of the Arf GAP family¹⁴ and smallest of the members as it contains no other domains. Thus, while it would clearly be a mistake to extrapolate any conclusions from one Arf GAP to all Arf GAPs, if ArfGAP1 is proven to serve effector functions it lowers the bar for all the others.

I have enjoyed the controversy that in recent years appears to have focused in the laboratories of Felix Wieland^{15,16,17} at Heidelberg University, and Victor Hsu^{18,19,20} at Harvard University, because in my view each group is doing very clever, appropriate, and similar experiments to test their hypotheses and yet continue to reach opposing conclusions. There has never been any hint of impropriety or subterfuge yet the data lead the authors to different conclusions. This is a wonderful "teachable moment" for students of all ages and an ideal topic for the CellLogBlog. I co-authored a recent review on Arf GAPs called "Models for the functions of Arf GAPs" in *Seminars in Cell & Developmental Biology*²¹ in which I discussed the controversy over the functions of ArfGAP1, so I won't re-state my arguments here. To properly assess the controversy one must understand the details of the assays used as I assume the resolution will be found in those details. Rather than have me describe those, I have asked these two leaders in this field to state their case and argue for their models to allow us all to better judge and form our own opinions. (www.landesbioscience.com/journals/cellularlogistics/blog/4) (www.landesbioscience.com/journals/cellularlogistics/blog/5) The outcome will not resolve the larger question of whether all GAPs are effectors, but it is certain to move us significantly forward in our understanding of the regulation of vesicle biogenesis and provide more detailed and accurate molecular models of Arf, COPI, and Arf GAP actions.

We welcome your opinions and ideas about this discussion at the CellLogBlog. Contribute your thoughts immediately online at www.landesbioscience.com/journals/cellularlogistics/blog/3 Select discussion entries will be published in the next issue.

Note

I believe that controversies of this kind are important in all fields as they push us all to design better, more critical experiments that inevitably teach us more than we would have learned without them. It is important that these issues receive a thoughtful, critical, and respectful airing to help direct all of us toward better experimental design. I can't think of a better use of the CellLogBlog (www.landesbioscience.com/journals/cellularlogistics/blog) to highlight our intentions in setting up and promoting constructive discourse in important topics to cell biology and biochemistry of the cell. If you have a controversy in your field that you would like to be the subject of a review or blog post, please contact one of the blog organizers:

Rick Kahn (rkahn@emory.edu), Alberto Luini (luini@tigem.it) or Nava Segev (nava@uic.edu) with your suggestion.

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