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GEF-effector interactions

Catherine L Jackson*

Institut Jacques Monod, CNRS; Université Paris Diderot; Sorbonne Paris Cité; Paris, France

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Members of the Arf family of small GTP-binding proteins, or GTPases, are activated by guanine nucleotide exchange factors (GEFs) that catalyze GDP release from their substrate Arf, allowing GTP to bind. In the secretory pathway, Arf1 is first activated by GBF1 at the *cis*-Golgi, then by BIG1 and BIG2 at the *trans*-Golgi and *trans*-Golgi network (TGN). Upon activation, Arf1-GTP interacts with effectors such as coat complexes, and is able to recruit different coat complexes to different membrane sites in cells. The COPI coat is primarily recruited to *cis*-Golgi membranes, whereas other coats, such as AP-1/clathrin, and GGA/clathrin, are recruited to the *trans*-Golgi and the TGN. Although Arf1-GTP is required for stable association of these various coats to membranes, and is sufficient *in vitro*, other molecules, such as vesicle cargo and coat receptors on the membrane, contribute to specificity of coat recruitment in cells. Another mechanism to achieve specificity is interaction of effectors such as coats with the GEF itself, which would increase the concentration of a given coat in proximity to the site where Arf is activated, thus favoring its recruitment. This interaction between a GEF and an effector could also provide a mechanism for spatial organization of vesicle budding sites, similar to that described for Cdc42-mediated establishment of polarity sites such as the emerging bud in yeast. Another factor affecting the amount of freely diffusible Arf1-GTP in membranes is the GEF(s) themselves acting as effectors. Sec7p, the yeast homolog of mammalian BIG1 and BIG2, and Arno/cytohesin 2, a PM-localized Arf1 GEF, both bind to Arf1-GTP. This binding to the products of the exchange reaction establishes a positive feedback loop for activation.

Many factors could affect the level of freely diffusible Arf1-GTP in cellular membranes. In reconstituted in vitro systems, Arf1-GTP diffuses very rapidly within membranes, with $D = 4.7\ \mu m^2/s$ as measured in GUVs by FRAP analysis.[1] Although fully quantifying pools of Arf1-GTP in the endo-membranes of cells, such as that of the Golgi, is not feasible with current technologies, interactions of Arf1 regulators and effectors contribute important information to this question. The active, GTP-bound, form of GTPases binds specifically to proteins called effectors, and hence the availability of effectors in proximity to the site of GTPase activation could have a major effect on the level of free Arf1-GTP available for diffusion. Interactions between the activating GEF and effectors have been described for several members of the Arf and Rab GTPase families. The early Golgi-localized Arf1 GEF, GBF1 in mammalian cells and its homologues in yeast, Gea1 and Gea2, interact with the Arf1 effector COPI.[2] This interaction is specific, in that the related GEFs BIG1 and BIG2 do not interact with COPI.[2] Gea1 and Gea2 also interact with the tethering complex TRAPPII, which itself interacts with COPI, leading to a larger GEF-effector interaction loop.[3] In the case of COPI, AP-1/clathrin, GGA/clathrin and AP-3 coats, inhibition of Arf1 activation prevents coat localization to membranes in cells, indicating that Arf1-GTP is required for stable association of these coats with membranes. In vitro, a GEF is not required for binding of coats such as COPI to liposomes, but only depends on the presence of Arf1-GTP. It is likely that the interaction between GEFs and effectors serves to increase the concentration of an effector in proximity to where a GTPase is activated, but is not sufficient to mediate stable binding of the effector to membranes. This theme of GEF-effector interactions is also evident in other GTPase pathways.

In early endosomal trafficking, the Rab5 GEF Rabex5 interacts with the Rab5 effector Rabaptin5,[4] the Ypt7 GEF Vam6/Vps39 is part of the Ypt7 effector HOPS complex, which mediates vacuolar fusion,[5,6] and the Sec4p GEF Sec2p interacts with the Sec4p effector Sec15p, which is a component of the exocyst complex.[7] In these systems, the GEF-effector interaction creates a positive feedback loop for activation of the small G protein, which in the case of Sec4p, is important for polarized delivery of secretory vesicles to their destination at the PM.[7,8] In mammalian cells, the Cdc42/Rac GEFs, termed PIX, interact with the p21-activated kinases (PAKs), which are Cdc42 effectors.[9,10] In yeast, a similar complex between the Cdc42 GEF Cdc24 and effector PAK is formed through the intermediary of the Bem1 protein, and this GEF-Bem1-PAK complex is an essential component of

*Correspondence to: Catherine L Jackson; Email: jackson@ijm.univ-paris-diderot.fr

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a Turing-type mechanism for establishment of a single site per cell at which Cdc42 is enriched.

Turing developed a theoretical model that showed how patterns of components in a reaction-diffusion system can emerge from an initial homogeneous distribution through amplification of initial random fluctuations.[11] In the case of GEF-GTPase-effector assemblies, this type of model is sufficient to generate a small number of active GTPase clusters through the operation of an autocatalytic amplification mechanism (positive feedback), starting from multiple, random GTPase activation events.[12]

Cdc42 marks the site of the emerging daughter cell (bud) in yeast, and a Cdc42 GEF-effector interaction is essential for bud site selection under conditions where extrinsic spatial cues are absent.[12,13] In this situation, establishment of a unique bud site is mediated by an initial stochastic Cdc42 activation event, which is amplified via the positive feedback loop created by the Cdc42p GEF—effector complex, leading to formation of a single cluster of activated Cdc42p at the cell cortex, which marks the site for new bud growth. The parameters required for this type of Turing mechanism are the switch-like properties of the GTPase, a GEF (Cdc24 in the yeast polarity system) an effector (Bem1-PAK), and interaction between the GEF and effector.[12] In addition to mathematical modeling, and testing of the resulting predictions by experimental approaches, the robustness of this model has been demonstrated by reprogramming bud site selection through manipulation of parameters predicted by the mathematical model.[14]

Could a similar Turing-type mechanism be involved in establishment of COPI budding sites? One major difference compared to Cdc42-mediated polarity establishment is the fact that there are multiple COPI budding sites in a cell, not just one as in the case of a bud site in yeast or the leading edge of a migrating cell. However, adjustment of the parameters in the system, as well as additional feedback loops, including inhibitory loops, determines the total number of sites established at steady state.[14,15] Hence the system for Arf1-mediated vesicle budding could be set to allow a specific spatial organization of budding sites, including multiple sites, within the Golgi. In addition to an active GTPase and effectors, cargo proteins are also implicated in the establishment of vesicle budding site, as described in the first article in this series by Rick Kahn.[16] One way that GEF-effector positive feedback loops could be coordinated with cargo incorporation is through coat-cargo interactions. COPI binding to membranes is enhanced in the presence of the cytoplasmically exposed tail of the cargo proteins such as p23.[17,18] Coat-cargo interactions also regulate COPII[19,20] and AP-1 [19,21] vesicle budding. It is likely that GEF-effector associations are dynamic and not high-affinity, stable interactions, as they are generally not sufficient to maintain a significant pool of the effector on the membrane. In the case of the Sec4p GEF Sec2p, enhancing its binding to the exocyst is detrimental to the dynamic cycle of vesicle formation and targeting.[7] For AP-1/clathrin, Arf1-GTP interacts at three distinct sites on the AP-1 coat adaptor complex, and induces a large conformational change that opens up an additional cargo binding site,[22] further stabilizing the coat on the membrane.

An additional mechanism that could contribute to the levels of freely diffusible Arf1-GTP in cellular membranes is the fact that Arf GEFs themselves can be effectors. This involves the binding of Arf to two distinct sites on the GEF, one the catalytic site for nucleotide exchange and the other a regulatory site. In mammalian cells, Arf1-GTP activates its GEF Arno/cytohesin 2, binding to the PH domain just downstream of the catalytic domain.[23-26] In yeast, Arf1-GTP activates the Golgi-localized GEF Sec7 (orthologue of mammalian BIG1 and BIG2), through interaction with the membrane-binding HDS1 domain also located just downstream of the catalytic domain.[27] In both cases, a positive feedback loop is created which results in a high rate of nucleotide exchange on the Arf.[26,28] In the case of Sec7, interaction of the HDS1 domain with Arf1-GTP is required for its localization to the late Golgi,[27,28] and Arno/cytohesin 2 requires interaction of its PH domain with a PM-localized Arf protein (Arf6 or Arl4), for its PM localization.[23,24] Interestingly, the PH domain of Arno/cytohesin has a lower affinity for Arf1-GTP than does a Golgi effector, the GRAB domain of the golgin GMAP-210.[26] Along similar lines, PM-localized Arf6-GTP is more effective at activating cytohesins than Arf1-GTP.[23,24,26] It is reasonable that the GEF itself would bind to the product of its own activity less well than other effectors do, to ensure that the latter could compete with the activating GEF for the Arf-GTP that the GEF produces. It would also be expected from this result that the levels of Arf-GTP can exceed the number of classic effectors available, in order to provide a pool for interaction with the GEF itself.[26]

In conclusion, when considering the amount of freely diffusible active GTPase produced by a GEF, the availability of effectors associated with the GEF, and capacity of the GEF itself to bind the active GTPase should be taken into consideration. The importance of the resulting feedback loops has been shown for Cdc42 in polarity establishment, and similar mechanisms could be at work in other GTPase-regulated systems.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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