Atlas of Science
another view on science
http://atlasofscience.org

Substrate phosphoswitching: a novel way to regulate enzymatic activity by phosphorylation of its substrate

Cells sense a vast variety of signals by receptors that are distributed on their surface. This perception transduces internal physiological responses using signal pathways, a cascade of protein interacting to bring a change in the cell behavior. Among the various types of receptors, G protein-coupled receptors (GPCRs) are the most studied not only because there are so many but also because they are important in human health and disease. The GPCR-mediated signaling pathway contains in its cascade of interacting proteins a guanine nucleotide (G)-binding protein complex that acts like a molecular switch. The G protein complex contains Gα, Gβ, and Gγ subunits. When the Gα subunit binds GDP, the complex is off. Upon stimulation by an extracellular signal, the GPCR catalyzes exchange of GDP for GTP thus switching the complex on, consequently Gα and Gβγ dimer propagate signal transduction in this cascade. Signaling turns off again when the Gα hydrolyzes its bound GTP back to GDP. This process is termed the GTPase cycle.

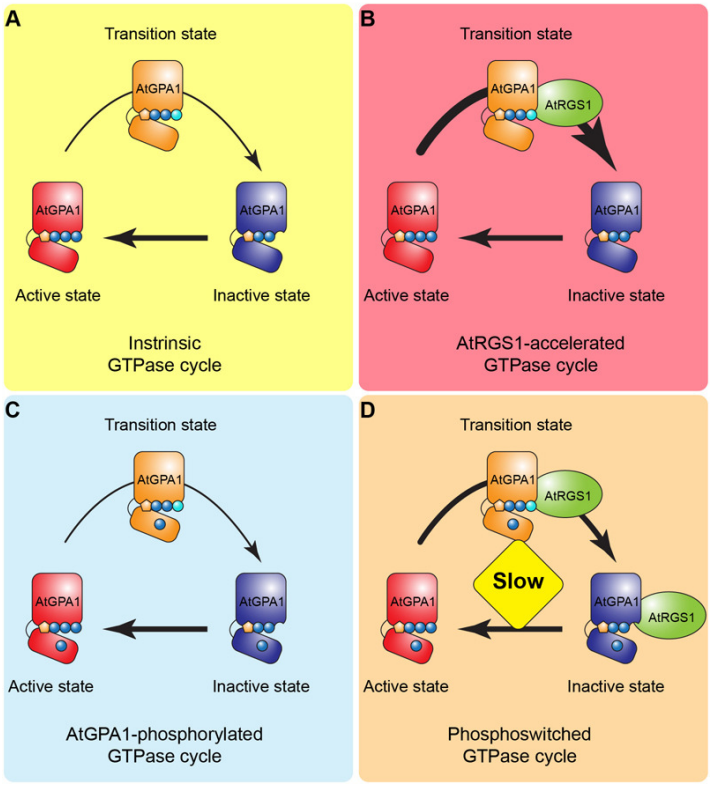

Fig. 1. Schematic diagrams for the GTPase cycle in the absence or presence of the modulation by AtRGS1 (green) or AtGPA1 (red, gold, blue) phosphorylation. A, the intrinsic GTPase cycle; B, AtRGS1 accelerated GTPase cycle; C, phosphorylation on Tyr-166 does not influence the modulated GTPase cycle. The size of the arrow represents the relative speed of each step in the cycle. Guanine nucleotide is represented by the yellow pentagon (base) with blue circles (phosphates). In the transition state the nucleotide is in a higher energy state, GDP +Pi (cyan circle). When AtGPA1 is not phosphorylated at Y166 as shown in panels A and B, AtRGS1 has GAP activity. When AtGPA1 is phosphorylated at Y166 as shown in panels C and D, AtRGS1 has quasi-GDI activity.

Despite conservation of G protein complexes throughout eukaryotes, the GTPase cycles are regulated differently in animals vs. much of the rest of eukaryota. In contrast with the activation mechanism described above for animals, plant and protist G proteins do not require a GPCR because these Gα subunits spontaneously bind GTP to self-activate (Fig. 1A). To terminate activation, a repressor is required. In the genetic model *Arabidopsis thaliana*, a receptor-like protein called Regulator of G-protein Signaling 1 (AtRGS1) modulates the GTPase cycle by accelerating the intrinsic GTP hydrolysis rate of the Gα (called AtGPA1), consequently keeping it off. De-repression occurs when AtRGS1 physically dissociates from Gα (Fig. 1B). However, this dissociation/activation model is insufficient to explain the entire mechanism because AtGPA1 is at least transiently activated prior to the AtRGS1 dissociation. This paradox indicates that there is another mechanism for G protein inactivation. What is it?

We hypothesized that specific and reversible phosphorylation of AtGPA1 might be the missing mechanism to regulate or at least modulate activation. By structural analyses, we identified an evolutionary conserved residue on AtGPA1, Tyr-166, locating in the intramolecular domain interface where nucleotide binding and hydrolysis occurs. A mutation mimicking constitutive phosphorylation selectively impaired AtRGS1-accelerated GTPase cycle without influencing the intrinsic activity of AtGPA1 (Fig. 1C and D). Interestingly, this mutation changes the binding affinity between AtRGS1 and AtGPA1. AtRGS1 selectively binds Gα subunit in its transition state (GDP + Pi) which is intermediate between active and inactive states. However, AtRGS1 strongly bound both the transition and inactive state of the phosphomimetic AtGPA1 mutant (Fig. 1D).

Proteins like AtRGS1 that bind and stabilize this transition state are called GTPase Accelerating Proteins (GAPs) whereas proteins that bind and stabilize the GDP-bound form are called GDP Dissociation Inhibitors (GDIs). Phosphorylation of AtGPA1 changes AtRGS1 from a GAP to a quasi-GDI. While reversible phosphorylation is well known to change the activity of an enzyme (in fact a Nobel Prize was awarded for this discovery), never before was there an example where phosphorylation of the substrate changes the functionality of its cognate enzyme. Being unprecedented, it was necessary that we coin this novel substrate-enzyme relationship “substrate phosphoswitching”.

So, what is the kinase that phosphorylates AtGPA1 at this tyrosine necessary for substrate phosphoswitching? We identified BAK1, a Leucine-Rich-Repeat Receptor-Like Kinase, as a kinase of AtGPA1. We showed that BAK1 phosphorylates AtGPA1 on Tyr-166. *In vitro* results suggested that phosphomimetic AtGPA1 influenced its interaction with AtRGS1 and the response to a signal shed by bacteria (flg22). Considering Tyr-166 phosphorylation is induced by several plant hormones as well including abscisic acid, indole-3-acetic acid, gibberellic acid, jasmonate and kinetin, substrate phosphoswitching may be a mechanism in a variety of plant physiological processes.

Bo Li, Alan M. Jones
University of North Carolina, Department of Biology, Chapel Hill, NC, USA

Publication

Tyrosine phosphorylation switching of a G protein.
Li B, Tunc-Ozdemir M, Urano D, Jia H, Werth EG, Mowrey DD, Hicks LM, Dokholyan NV, Torres MP, Jones AM
J Biol Chem. 2018 Mar 30