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Alfred Wittinghofer's Lecture Part 1:
GTP binding proteins as molecular switches

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1. Keywords and Terms

GTPase, GTP binding, Molecular Switch, GAP, GTPAse Activating Protein, GEF, Guanine Nucleotide Exchange Factor, Effector

2. Lecture Notes

GTP-binding proteins

GTP-binding proteins, also more commonly called GTPases or G proteins, are molecular switches which switch between an inactive, GDP-bound, OFF state and an active, GTP-bound, ON-state.

GEFs

GTP-binding proteins have a high affinity for nucleotides, in the subnanomolar to picomolar range. Thus, the dissociation of GDP (or any other nucleotide) is very slow. Another protein called a Guanine Nucleotide Exchange Factor, or GEF, increases the nucleotide dissociation rate by many orders of magnitude.

GAPs

The GTPase reaction that hydrolyses GTP to GDP and converts G proteins from the active to the inactive state, is very slow. GTPase Activating Proteins, or GAPs, accelerate the reaction by many orders of magnitude. Usually, the molecular machinery of the GTPase is incomplete and is complemented by residues from the GAP.

Effectors

Effectors (also called downstream effectors) are proteins that interact with the GTP-bound, active form of the GTP-binding proteins, but not the GDP-bound, inactive form. They are the mediators of the biological reaction.

Structure and Function

The G domain is a protein fold common to all of the several hundred or so GTP-binding proteins. In its simplest form, it consists of six -strands alternating with five helices and is thus a classical protein, like many other nucleotide binding proteins.

All G domain proteins have five more or less conserved sequence motifs, which are the fingerprint to classify them. The first of these motifs is the P-loop (Phosphate binding), a motif that is also found in many ATP-binding proteins such as motors, ABC transporters, and AAA proteins (but not in protein kinases).

Ras

The Ras superfamily is a particular family of GTP-binding proteins consisting of just the G domain plus or minus various additions and deletions. The Ras proteins have a molecular mass of around 20-25 kDa. The superfamily can be divided into different subfamilies based on sequence homology. The different subfamilies also specify different functions such as signalling, vesicular transport, actin remodelling etc. The

different functions are determined by the surface of the G domains, which are completely different in different proteins.

The structural basis of the molecular switch can be described by a loaded spring model where the elements of the structure called switch I and II are connected to the -phosphate by two totally conserved hydrogen bonds. After GTP hydrolysis, the -phosphate is released and the switches relax in to a different conformation. The switch mechanism is canonical but there are many subtle or dramatic variations on the common theme.

Switch I and II are the business part of GTP-binding proteins and change conformation based on the presence or absence of the -phosphate. Switch I contains a conserved threonine (in the G2 motif) and switch II a conserved glycine (part of the DxxG G3 motif) that are bound to the -P by main chain hydrogen bonds.

A basic conserved biochemical feature of Ras-like G proteins is a high affinity for nucleotide and, consequently, a very slow dissociation of nucleotide. The specificity for guanine nucleotide is very high. For Ras, the difference in affinity between guanine and adenosine is more than 106 fold. The binding of nucleotide is usually dependent on Mg2+ and therefore, nucleotide dissociation can be accelerated dramatically by EDTA. Nucleotide exchange is also accelerated by GEFs (see above). The GTPase activity of G proteins is also usually very slow. It too requires the presence of particular bivalent ions such as Mg2+, although, it will also work with Mn2+ but not Ca2+. GTPase activity is stimulated by GAPs (see above).

While G proteins of the Ras superfamily consist of just the G domain, there are many other GTP-binding proteins that contain several domains, such as ribosomal biosynthesis factors EF1 or EF2, proteins of the dynamin superfamily, or the G subunit of heterotrimeric G proteins.

3. Recommended Reading

1. R. Vetter and A. Wittinghofer. The guanine nucleotide-binding switch in three dimensions. Science 294, 1299-1304 (2001)
2. J.L. Bos, H. Rehmann, and A. Wittinghofer. GEFs and GAPs: Critical Elements in the Control of Small G Proteins. Cell 129, 865-877 (2007)

3. J. Cherfils and M Zeghouf. Regulation of small GTPases by GEFs, GAPs and GDIs. Physiol Rev. 93, 269-309 (2013)

4. Review Questions

1. What is the basic function of GTP-binding proteins?
2. How do other proteins modify the basic cycle of GTP-binding proteins?
3. What are effectors?
4. What is the G domain?
5. What is the structure of the G domain?
6. What is the Ras superfamily?
7. What is the structural basis of the molecular switch?
8. What are the switch regions?

5. Answers to Review Questions

1. To switch between an inactive GDP-bound OFF state and an active GTP-bound ON state.
2. The slow dissociation of GDP is accelerated by GEFs, the slow GTPase reaction by GAPs.
3. Effectors are proteins that recognize and bind (with reasonable affinity) to GTP-bound but not GDP-bound G proteins.
4. It is the simplest structural and functional unit of GTP-binding proteins.
5. It is a typical -protein and consists of six -strands and five -helices
6. It consists of many 20-25 kDa GTP-binding proteins that are classified into different subfamilies such as Ras, Rho, Rab, Ran, and Arf.

7. In the GTP-bound state, the switch regions are connected to the -phosphate by hydrogen bonds that are released during/after GTP hydrolysis and loss of the -P.
8. Most G proteins contain two regions called switch I (G2) and switch II (G3) which change structure between the GDP-bound and GTP-bound state.

6. Discussion Questions

1. How is it that the G domain is conserved between Ras-like proteins and yet each protein has its own set of interacting proteins such as GEFs, GAPs, and effectors?
2. How do you envision the activation or deactivation of GTP-binding proteins in a signal transduction chain being regulated?
3. Why is the presence or absence of a phosphate group a convenient mode of regulation?

7. Answers to Discussion Questions

1. Proteins with very low sequence identity (as little as 15-20%) can still have the same protein fold. The conserved G domain contains the active site (ie the GTPase reaction center) of the Ras family proteins. The non-conserved amino acid residues, however, are most likely on the surface of the protein and thus mediate different interactions. A few residues on the surface of the protein can make the difference; a good example is the interaction of Raf kinase
2. The amount of active G protein is directly proportional to the GTP binding reaction and inversely proportional to the GTPase reaction. Thus, a particular G protein can be activated by activating or recruiting its GEF, or by inhibiting or removing its GAP. And conversely, a particular G protein can be deactivated by inhibiting or removing its GEF and activating or recruiting its GAP.
3. Phosphoesters or phosphoanhydrides (like ATP and GTP) have the interesting property that they are kinetically stable, which means hydrolysis is slow, yet the reaction is energetically favorable (thermodynamically unstable). Thus, enzymes are required for both phosphorylation of a protein by the transfer of the -phosphate from

ATP or GTP, and the dephosphorylation of a protein by hydrolysis. This allows for tight control of this, in principle, easily reversible protein modification.

8. Research the Literature on Your Own

1. Where else is reversible phosphorylation used in biology? (Hint: see Susan Taylor and Bob Lefkowitz's iBioSeminars)
2. What other reversible modifications do you know, and how do they compare with phosphorylation? (Hint: see Carolyn Bertozzi's iBioSeminar)