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Molecular Switch for Signal Transduction: Structural Differences Between Active and Inactive Forms of Protooncogenic *ras* Proteins

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Abstract:

Ras **proteins participate as a molecular switch in the early steps of the signal transduction pathway that is associated with cell growth and differentiation. When the protein is in its GTP complexed form it is active in signal transduction, whereas it is inactive in its GDP complexed form. A comparison of eight three-dimensional structures of *ras* proteins in four different crystal lattices, five with a nonhydrolyzable GTP analog and three with GDP, reveals that the "on" and "off" states of the switch are distinguished by conformational differences that span a length of more than 40 Å, and are induced by the γ-phosphate. The most significant differences are localized in two regions: residues 30 to 38 (the switch I region) in the second loop and residues 60 to 76 (the switch II region) consisting of the fourth loop and the short α-helix that follows the loop. Both regions are highly exposed and form a continuous strip on the molecular surface most likely to be the recognition sites for the effector and receptor molecule(or molecules). The conformational differences also provide a structural basis for understanding the biological and biochemical changes of the proteins due to oncogenic mutations, autophosphorylation, and GTP hydrolysis, and for understanding the interactions with other proteins.**

Structural correlations to biological and biochemical functions. Examination of the crystal structures of the seven normal (Gly^{12}) proteins and one transforming (Val^{12}) catalytic domain supports reasonable correlations between the observed structural features and the known biochemical and biological functions of the *ras* proteins, such as oncogenic mutations, autophosphorylation of viral *ras* protein, GAP interaction, antibody induced neutralization of *ras* function, and GTP hydrolysis. Comparison of all the guanine nucleotide complexes of *ras* protein whose structures have so far been determined reveals that the conformation of the phosphate binding loop, L1, is identical within experimental error. [Our earlier result (*8*) that L1 conformation of normal (Gly^{12}) and transforming (Val^{12}) *ras* protein is different was due to an error in the tracing of the loop (*9*)]. As pointed out earlier, the backbone NH groups of residues 13 or 14 to 17 of this loop and the side chain of Lys^{16} are involved in hydrogen-bonding to the β phosphate. However, a superposition of loop 1 of the GDP-CP complex of normal (Gly^{12}) protein and the GDP complex of oncogenic (Val^{12}) *ras* protein reveals that the hydrophobic side chain of Val^{12} would be in contact with the highly hydrophilic and charged γ phosphate of GDP-CP, creating an energetically unfavorable situation (Fig. 6A). This may result in changes in the catalytically favorable position and orientation of the γ phosphate. Furthermore, the side chain of Val^{12} partially blocks the entrance of the guanine nucleotide pocket, thus possibly preventing the entry of a nucleophilic attacking group or departure of the γ phosphate after hydrolysis (or both). This observation suggests that other substitutions of Gly^{12} would create a similar situation, thus decreasing GTPase activity of the mutants. On the other hand, the observed oncogenic activation by substitution of Gly^{13} by valine or aspartate (*2*) may arise from distortion of the loop 1, which binds β phosphate. This model provides a qualitative structural explanation for the earlier observation that the substitution of Gly^{12} or Gly^{13} by almost any amino acid except proline endows the *ras* protein with transforming activity (*21*). Preliminary model building with proline at these positions show little steric blocking of the entrance or distortion of the loop.

Oncogenic *ras* proteins encoded by retroviral *ras* genes have one additional mutation at residue 59, substituting threonine for alanine, and this threonine is autophosphorylated (*22*). The location of this residue in both GDP complexes and GTP analog complexes is approximately the same, and is near the γ phosphate in the structure of the GDP-CP complex (Fig. 6B). This close proximity immediately suggests that the hydroxyl group of threonine at this position would be a good acceptor of the γ phosphate resulting in a covalently bound phosphate on the threonine. It can even be possible, in case of the virally encoded protein, that the side chain of Thr^{59}, polarized by other side chains or water nearby, may act as an attacking group in the hydrolysis reaction.

GAP binds to GTP complexes of normal and transforming mutants, but catalyzes GTPase activity of normal *ras* proteins only (*6, 23, 24*). Whether GAP is an upstream negative regulator or downstream effector of *ras* proteins is still uncertain. GAP sensitivity of various mutants (*25–28*) suggests that a large portion of switch I and a part of switch II regions are involved with GAP interaction.