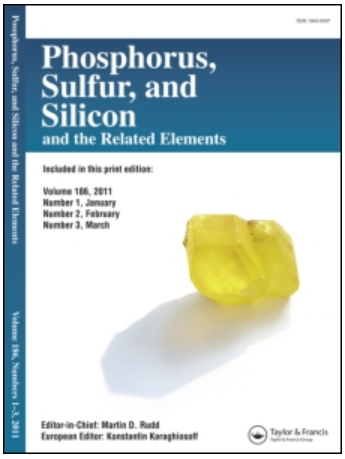

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The Ras-RasGAP Complex: How to Complement an Inefficient Active Site

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GTP-hydrolysis as carried out by GTP-binding proteins[1] is intrinsically very slow but can be accelerated by orders of magnitude upon interaction with GTPase Activating Proteins, GAPs, which are specific for the respective GTP-binding proteins. Focusing on p21ras (Ras), a key element in growth control and differentiation, we have used biochemical and structural methods to elucidate the mechanism of GTPase activation. An arginine side chain is supplied into the active site of Ras to contact the nucleotide and stabilize the transition state of the reaction, consistent with mutational analyses. The switch II region of Ras is stabilized by GAP-334 to allow Gln61, the mutation of which activates the oncogenic potential of Ras, to participate in catalysis. The structure provides an explanation how Gly12 and Gln61 mutations might escape regulation by GAPs.

Introduction: The small GTP binding protein p21ras (Ras) plays a crucial role in cellular growth control[2]. It functions like a molecular switch cycling between GTP-bound 'ON' and GDP-bound 'OFF'. In the active form Ras interacts specifically with effector molecules. The transmitted signal is switched off by GTP hydrolysis mediated by Ras itself, a process that is intrinsically slow but can be accelerated dramatically upon interaction with GTPase activating proteins (GAPs)[3]. Oncogenic Ras mutants, as found in a large number of tumors, are unable to hydrolyze GTP at a rate sufficient to turn off the signal and are not sensitive to GAPs[2]. This makes the mechanism of Ras mediated GTP hydrolysis and how it is accelerated by GAPs an important target for the development of anticancer drugs. Starting with p120GAP[4], a number of RasGAPs are know to date [3]. They are modular proteins of various sizes and domain composition sharing a region of sequence homology (spanning approximately 350 amino acids) that is responsible for the GAP activity of these proteins.

The GTPase activating domain (residues 714-1047) of the human p120GAP (GAP-334) is a helical protein consisting of 2 domains: a small extra domain (GAP_{ex}; residues 718-764; 982-1037) and a central domain (GAP_c; residues 765-981) that contains all residues conserved among RasGAPs[5] (Fig 1). Conserved residues are clustered around a surface groove which was identified to be the Ras binding site. To find out how GAPs interact with Ras, how they accelerate GTP hydrolysis, and why oncogenic Ras mutants are not sensitive to GTPase

activation we have determined the structure of a complex between Ras•GDP and the catalytic domain of human p120GAP (GAP-334) crystallized in the presence of aluminum fluoride.

Experiments with aluminum fluoride: It was found that RasGAPs and Ras•GDP form a ternary complex with aluminum fluoride [6], a putative transition state analogue mimicking the phosphoryl group transferred during GTP hydrolysis. Since Ras•GDP alone does not bind AlF_x and given the requirement of stoichiometric amounts of GAP for complex formation with Ras, these experiments favored a model, according to which GAP participates actively in catalysis.

The Ras-RasGAP complex: In the structure of a Ras-RasGAP complex crystallized from H-Ras•GDP and GAP-334 in the presence of aluminum fluoride, Ras binds to the surface groove with its switch regions, P-loop, and a part of α3[7](Fig. 1). On the GAP side the interaction is mediated mainly by residues belonging to loop $L1_c$, (finger loop), helices $\alpha6_c$ and $\alpha7_c$ together with $L6_c$ (variable loop). The interface is stabilized by hydrophobic and hydrophilic contacts involving several water molecules.
The effector region participates in a number of polar interactions that particularly involve the $L6_c$ region of GAP and several water molecules. Lys949 and Glu950, belonging to loop $L6_c$ are tightly involved in these interaction, and in a revised sequence comparison that suggest $L6_c$ to be of variable length (hence 'variable loop') in RasGAPs, define a previously unrecognized 'KE'-motif. Of five acidic residues in the effector region only Asp33 and Asp38 are in orientations to interact closely with GAP, contacting directly or indirectly Lys949 and Glu950. The N-terminal part of helix α3 (residues 87-102) of Ras contains another region to contact GAP, with Asp92 being a residue crucial for the interaction[8].

The active site and mechanism of GTPase activation: In the Ras-RasGAP complex the nucleotide GDP is bound in a similar way as observed in isolated Ras[9, 10]. Aluminum fluoride is bound in the γ-phosphate position but in contrast to α-subunits of heterotrimeric G-proteins[11, 12] and to the structure of the catalytic domain of p50rhoGAP complexed with RhoA•GDP and aluminum fluoride [13] only three fluoride ligands appear to be present. Although AlF_3 has also been found in other phosphotransfer enzymes the difference in the number of fluorides coordinating aluminum is presently not explained [14].
In the complex, the nucleotide binding pocket is bordered by $L1_c$ of GAP-334. This causes placement of the guanidinium group of Arg789 in proximity of the phosphate moieties and of AlF_3 to stabilize the transition state of the GTPase reaction (Fig. 2). In addition, the presence of $L1_c$ results in stabilization of switch II and especially of Gln61, which is mobile in isolated Ras[9, 10]. Mutational analyses of conserved arginines confirm the critical role of Arg789 for GAP catalysis[15]. Oncogenic mutations in position 12[16] or 61[17] would not be compatible with the transition state geometry explaining why they are not sensitive to GTPase activation.

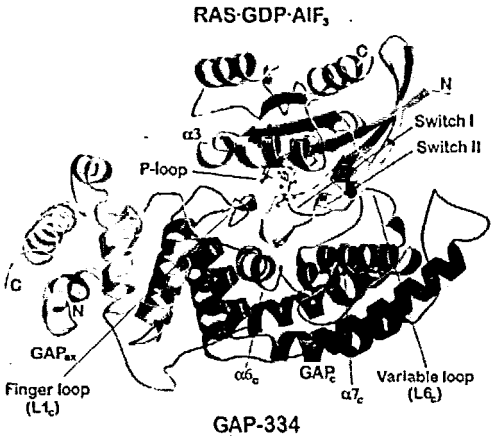

Fig. 1: GAP-334 and its interactions with Ras. Ribbon representation of the complex showing the architecture of GAP-334 and its binding to Ras, with important elements indicated (see text), GDP·AlF3 is in ball and stick.

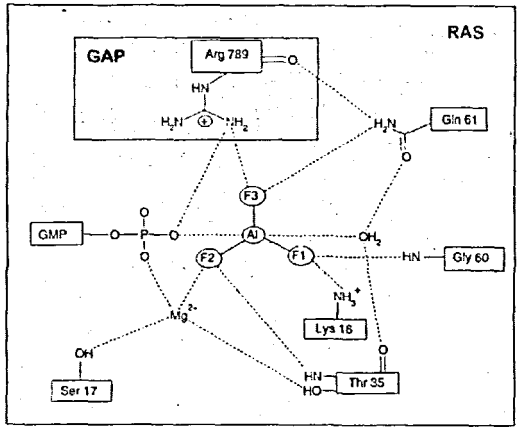

Fig.2: Schematic diagram of the active site, with polar interactions symbolized as dashed lines. The active site is constituted by Ras and GAP-334, forming a heterodimer to accelerate the rate of GTP hydrolysis.

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References

[1] H.R. Bourne, D.A. Sanders, F. McCormick, *Nature* **348,** 125–132, (1990).
[2] D.R. Lowy, B.M. Willumsen, *Annu Rev Biochem* **62,** 851–891 (1993).
[3] A. Wittinghofer, K. Scheffzek, M.R. Ahmadian, *FEBS Letters* **410,** 63–67 (1997).
[4] M. Trahey, F. McCormick, *Science* **238,** 542–545 (1987).
[5] K. Scheffzek, A. Lautwein, W. Kabsch, M.R. Ahmadian, A. Wittinghofer, *Nature* **384,** 591–596 (1996).
[6] R. Mittal, M.R. Ahmadian, R.S. Goody, A. Wittinghofer, *Science* **273,** 115–117 (1996).
[7] K. Scheffzek, M.R. Ahmadian, W. Kabsch, L. Wiesmüller, A. Lautwein, F. Schmitz, A. Wittinghofer, *Science* **277,** 333–338 (1997).
[8] D.R. Wood, P. Poullet, B.A. Wilson, M. Khalil, K. Tanaka, J.F. Cannon, F. Tamanoi, *JBiol Chem* **269,** 5322–5327 (1994).
[9] E.F. Pai, U. Krengel, G.A. Petsko, R.S. Goody, W. Kabsch, A. Wittinghofer, *EMBO J9,* 2351–2359 (1990).
[10] M.V. Milburn, L. Tong L, A.M. DeVos, A. Bruenger, Z. Yamaizumi, S. Nishimura, S.H. Kim, *Science* **247,** 939–945 (1990).
[11] D.E. Coleman, A.M. Berghuis, E. Lee, M.E. Linder, A.G. Gilman, S.R. Sprang, *Science* **265,** 1405–1412 (1994).
[12] J. Sondek, D.G. Lambright, J.P. Noel, H.E. Hamm, P.B. Sigler, *Nature* **372,** 276–279 (1994).
[13] K. Rittinger, P.A. Walker, J.F. Eccleston, S.J. Smerdon, S.J. Gamblin, *Nature* **389,** 758–762 (1997).
[14] A. Wirtinghofer, *Current Biology* **7,** 682–685 (1997).
[15] M.R Ahmadian, P. Stege, K. Scheffzek, A. Wittinghofer, *Nat. Struct. Biol.* **4,** 686–689, (1997).
[16] P.H. Seeburg, W.W. Colby, D.J. Capon, D.V. Goedel, A.D. Levinson, *Nature* **312,** 71–75 (1986).
[17] C.J. Iter, T. Finkel, G.M. Cooper, *Cell* **44,** 167–176 (1986).