Structure-based hypothesis on active role of RasGEF αG-helix

Valeria Mapelli, Elena Accardo, Sonia Fantinato, Elena Sacco, Luca De Gioia and Marco Vanoni

Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca, Piazza della Scienza 2, I 20126, Milan, Italy

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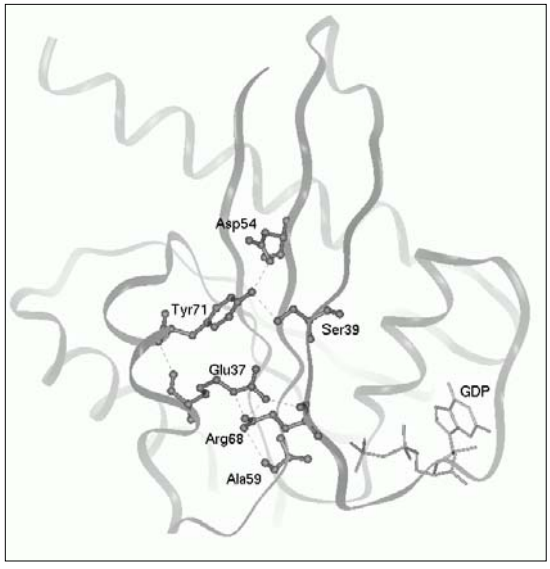
Introduction

Ras proteins are small GTPases ivolved in signaling pathways controlling cell growth and differentiation. They act as molecular switches by cycling between an active GTP- and an inactive GDP-bound state. Following the activation of specific cell-surface receptors, Ras proteins switch from inactive to active state through the catalytic action of specific Guanine nucleotide Exchange Factors (GEFs), that promote the dissociation of GDP from Ras, allowing GTP entrance into the Ras nucleotide poket. The *Saccharomyces cerevisiae* Ras-GEF Cdc25 ($Cdc25^{Sc}$) was the first Ras-exchanger to be identified [1]. In higher eukaryotes there are two different classes of Ras-specific $Cdc25^{Sc}$ homologs, Sos proteins [2] and $Cdc25^{Mm}$ [3], also referred to as Ras GRF. Ras-specific GEFs are made of several functional and structural domains, Ras GEF activity is contained within a domain showing very high similarity to the $Cdc25^{Sc}$ catalytic domain and called, for this reason, Cdc25 homology domain. Structural studies on Ras crystallized in complex with nucleotide [4] (GDP or GTP-analogs) and human exchange factor Sos [5] respectively have allowed both to identify conformational differences between active and inactive state of Ras, and to make hypothesis on molecular determinants of interaction and catalytic activity of human Sos.
Mutational and structural studies on Ras GEFs catalytic domain have pointed to a major role for the helical-hairpin formed by αH and αI helixes (catalytical hairpin) in the catalytic mechanism [5,6,7,8] of Ras-specific GEFs. In the present work we investigate the Ras GEF αG-helix role in Ras-GDP to GTP exchange.

Results and Discussion

Ras-nucleotide and Ras-Sos complexes: Structural Analysis

By comparing Ras structures in complex with nucleotide and human exchange factor Sos respectively it's possible to note that it undergoes substantial structural modifications after interaction with GEF. Major conformational changes are at the level of the so called Ras *switch* regions (*switch1* aa 25-40, *switch2* aa 57-75): *switch1* region is displaced from the typical position assumed in the Ras-GDP/GTP complexes. This displacement leads to the opening of Ras nucleotide pocket and to the consequent nucleotide release. In the Ras-nucleotide crystal structure an hydrogen bond network involving E37, S39, D54, A59, R68, Y71 residues seems to be crucial to keep the two Ras switch regions closed. These intramolecular interactions are peculiar to the Ras-nucleotide binary complex. Analysis of the Ras-Sos complex suggests that αG-helix of Sos may play a relevant role in exchange catalytic activity, possibly destabilizig the interaction network typical of Ras-GDP and so aiding in displacing the nucleotide from Ras.



Ras-GDP complex crystal structure [4].
Aminoacids side-chains forming Hydrogen bond network are shown.

Methods and Results

The role of the αG-helix is likely to be conserved in different Ras-specific GEF as suggested by the analysis of the model of $Cdc25^{Mm}$ catalytic domain in complex with Ras, obtained by homology modelling (using the on-line Swiss Model server), exploiting the Ras-Sos solved structure as template. This model was refined by Molecular Mechanics (Discover module of *InsightII*). The role of αG-helix residues in GDP/GTP exchange activity was tested by virtual and experimental mutagenesis of three specific $Cdc25^{Mm}$ residues to alanine. The structural model of the mutant Ras-GEF complex was refined by Molecular Mechanics and all intra- and intermolecular interactions was computed. In silico, the triple mutant GEF presents only local rearrangements at the level of αG-helix, loosing all the interactions thought important for destabilizing the Ras-nucleotide interaction network, but it conserves those interactions stated determinant for Ras-GEF interaction [9]. Thus it can be predicted that this mutant should interact with nucleotide-bound Ras, but it should have an extremely residual activity, if any.
The function of mutant GEF was then studied by a combination of *in vitro* and *in vivo* assays. In keeping with our structural-based hypothesis, the triple-mutant retains its ability to bind to Ras as detected by BIAcore technology,but it is completely unable to catalytically promote guanine nucleotide exchange on Ras. Its activity was tested using purified Ras in complex with a fluorescent analog Guanine nucleotide (mant GTP/GDP). This assays allowed us to monitor both the release and the exchange of fluorescent guanine nucleotides. Finally, when expressed in *Saccharomyces cerevisiae* strains carrying *cdc25* mutant gene (lethal at 37°C), the mutant GEF doesn't restore $cdc25^{Ts}$ yeast strains vitality, in keeping with *in vitro* results.

Conclusions and Perspective

This work confirms and extend to other Ras GEF previous results [9] on Sos GEF, indicating a role for αG-helix in promoting nucleotide exchange on nucleotide-bound Ras. Iterative computational and molecular experiments are under way in order to fuller carachterize the role of αG-helix in GEF function.

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