Exploration of the Activation Mechanism of Small GTPase RhoA

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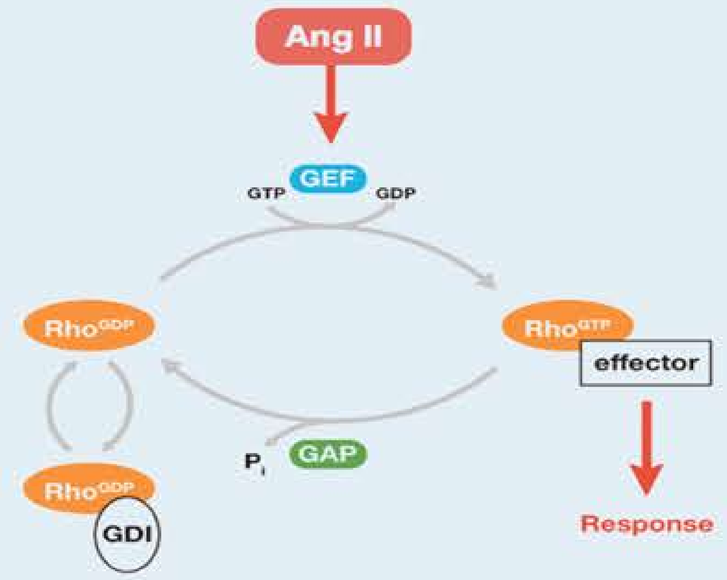
Background:

The Ras superfamily is one of the largest protein families involved in cell signal transduction, with 166 members.
These proteins play a role in the vascular smooth muscles cells in response to angiotensin-II stimuli. This superfamily shares a common activation mechanism where a GTP-bound is the activated state and a GDP-bound ras member is found in the unactivated form. Once the GTP is dephosphorylated in GDP, ras members have to be reactivated through a nucleotide exchange mechanism catalyzed by a ras-specific Guanine Exchange Factor (GEF).

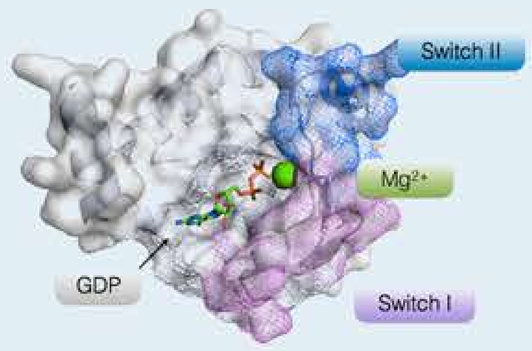


Abstract:

The activation cycle of one member of the Ras family, rhoA, and its specific partner p115-GEF (also known as Arhgef-1)is the subject of our study.
The protein-protein interaction involves two region switches on rhoA, the nucleotide binding pocket and a magnesium.

Goals:

In order to understand the activation mechanism at an atomic level, an in silico approach using molecular dynamics (MD) on all-atom simulations is used to investigate the dynamic of the interaction between Rhoa and Arhgef1 and when does the nucleotide exchange take part.



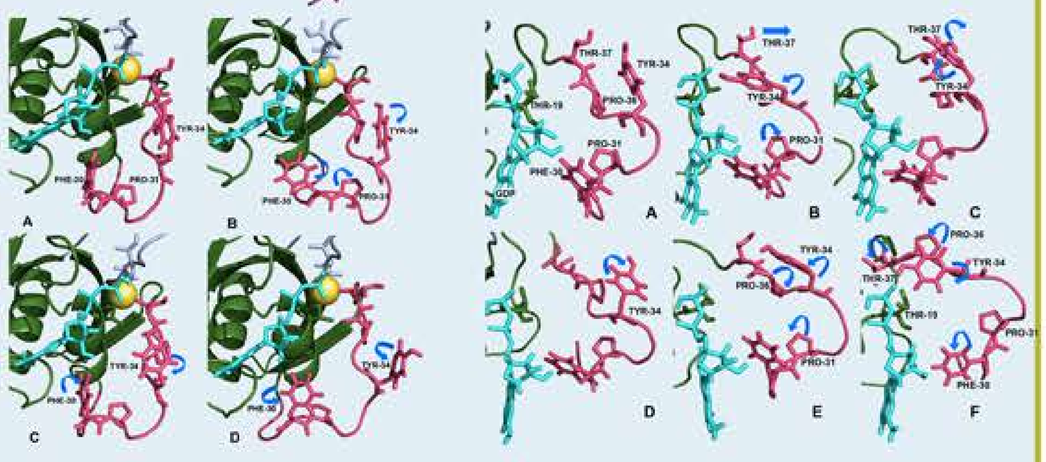
Hypothesis:

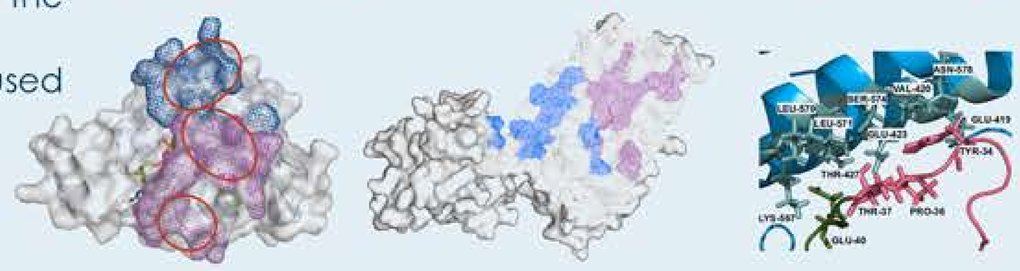
The magnesim is always present and seems to have a key role in the binding of the GDP and the comformation of RhoA.
Arhgef1 must play a role in the destabilisation of the GDP binding for it to get expulsed to let the place for a GTP.
The Exchange seems to occurs when complexed together, element of the interface may influ the expulse of the GDP.

Methods:

- Using molecular dynamic simulations with the Ambers forcefield AMBER99, parametrisation of the GDP using Ambertools and antechambers from files of the AMBER parameter database.
- MD simulations were conducted using GROMACS 5.1.2, in all atoms.

The Interface have been analysed with Alanin scanning and docking study (both protein-protein and small molecule-protein).





Observations:

- The Magnesium is moving towards the Phosphates of the GDP, and stay there as it seems to be its more stable state (red sphere).
- During simulations of RhoA+GDP+Mg^{2+} , we noticed that the most important amino acids were having significant conformational shifting. As showed in the figures, the simulations without the Mg^{2+} display more significant shifting.
- The interface analysis showed some pockets on RhoA where are located some key amino acids, thoses amino acids have conformational changes due to the absence of Mg^{2+}, and this shifting is toward the "outside" of the protein, pointing in the direction of the interface, toward Arhgef1.
- The docking study confirmed that thoses key amino acid where also used as hotspot for the interaction with Arhgef1

Conclusion:

- The Position of Mg^{2+} is one of the key factor in the GDP/GTP exchange, a displacement of the ion showed drastic conformational changes. And is involded in the binding interaction with the GDP and GTP.
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References:

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3 - Oleksy A, Opaliński Ł., Derewenda U., Derewenda Z. S., Otlewski J. (2006) The molecular basis of RhoA specificity in the guanine nucleotide exchange factor PDZ-RhoGEF. J. Biol. Chem. 281, 32891–32897