Hydrophobic network between AB and HI hairpins suggests a new role for AB hairpin in GEF action mechanism

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Keywords: Homology Modelling**,** Ras, Guanine nucleotide Exchange Factors.

Introduction

The analysis of protein 3D structure is an important step to understand their mechanism of action, regulation, function and family's belonging. Experimental methods for proteins structure determination don't keep up with the increasing number of genomic sequence available: this led to an increase of computational methods that predict three-dimensional model for a protein of unknown structure (target) on the basis of sequence similarity to proteins of known structure (templates). There are different kinds of Homology Modelling methods, but all of them can't recover from an incorrect target-template alignment: a good alignment is the first think to be considered when we're talking about model's confidence. SWISS MODEL, an automated comparative protein modelling server [5] starts with the analysis of the structural conserved regions in the target-templates alignment.
Ras protein are highly conserved GTPase playing a pivotal role in different important cellular events: cell proliferation, differentiation, cellular traffic and cytoskeleton organization. Within cells, Ras proteins exist both in a GTP-bound form ("on" state) or a GDP-bound ("off" state). The level of the GTP-bound state derives from the balance of the activity of the GTPase Activating Proteins (GAPs) and Guanine nucleotide Exchange Factors (GEFs). Common feature of all Ras GEFs is the presence of a domain, the RasGEF domain, carrying all the main structural features needed to interact with Ras and to exchange the nucleotide. A notable feature of this catalytic domain is the protrusion of a hairpin, formed by helices αH and αI, out of the core of the domain. It has been proposed helix αH plays an important role in the nucleotide-exchange mechanism opening up the nucleotide-binding site [3].

Results and discussion

In our laboratory, a GEF mutant with dominant-negative properties (GEF-DN) has been isolated. The mutant, $Cdc25^{MmW1056E}$, carrying a substitution of Trp1056 to glutamate, is catalytically inactive and able to efficiently displace wild-type GEF from Ras and to originate a stable Ras·GEF binary complex [1,2]. Surprisingly, the corresponding mutation in $Cdc25^{Sc}$, the first GEF to be identified, doesn't originate a GEF-DN protein: the $Cdc25^{W1334E}$ mutant displays a full catalytic activity and doesn't show a dominant negative behaviour.
Using the online server SWISS MODEL [5], we obtained the RasGEF domain structure of *M.musculus* and *S.cerevisiae* GEF ($Cdc25^{Mm}$ and $Cdc25^{Sc}$), using the crystal structure of hSos1 as a template [4]. The model's confidence is supported by the high degree of similarity between the sequences, which gives an accurate target-template alignment, and by a model's check with WHAT IF, a useful model evaluation program. The $Cdc25^{Mm}$ and $Cdc25^{Sc}$ Ras-GEF domain, as well as the hSos1 one, consists of a series of helical hairpins that pack against each other where we can find the protrusion of HI hairpin.
Based on the structural analysis of $Cdc25^{Mm}$ and $Cdc25^{Sc}$ RasGEF domain, Trp1056 (mouse) and Trp1334 (yeast) are located within another helical hairpin, conserved among all Ras GEFs and named AB (according to the Boriack-Sjodin nomenclature [4]), which is located at the interface with Ras in proximity of its nucleotide-binding site, as occurs for the HI hairpin. While HI hairpin (also referred to the catalytic hairpin) is directly involved in the nucleotide exchange mechanism, no functional role for the AB hairpin has yet been reported. Moreover, in the mammalian GEF, the Trp1056 coordinates an hydrophobic network between the AB hairpin and the catalytic one (Fig.1). This hydrophobic pocket disappears in $Cdc25^{Sc}$, where only some one-to-one interactions remain. In this contest is clear how the introduction of the carboxilate charged residue in the $Cdc25^{MmW1056E}$ mutant could alter the conformational properties of the two hairpins required for an optimal interaction with Ras and useful for the nucleotide exchange mechanism of the GEF.

Conclusions and perspectives

While HI hairpin is directly involved in the nucleotide exchange mechanism, no functional role for the AB hairpin has yet been reported. These data suggest that AB hairpin seems to play an active role in GEF action mechanism on the basis of its structural properties and by functional data regarding the dominant negative properties of $Cdc25^{MmW1056E}$ mutant. These structural and functional considerations, together with the normal phenotype of the $Cdc25^{ScW1334E}$ mutant, indicate that the destruction of the hydrophobic network between the AB and HI hairpin could be, to a great extent, important for the GEF-DN properties.

Thus, the “one finger hypothesis” proposed to explain the catalytic functioning of Ras-specific GEF needs to be extended: other regions within the catalytic domain, beside the HI helical hairpin, could play an active role in GEF action mechanism and are required to originate a catalytically active GEF.

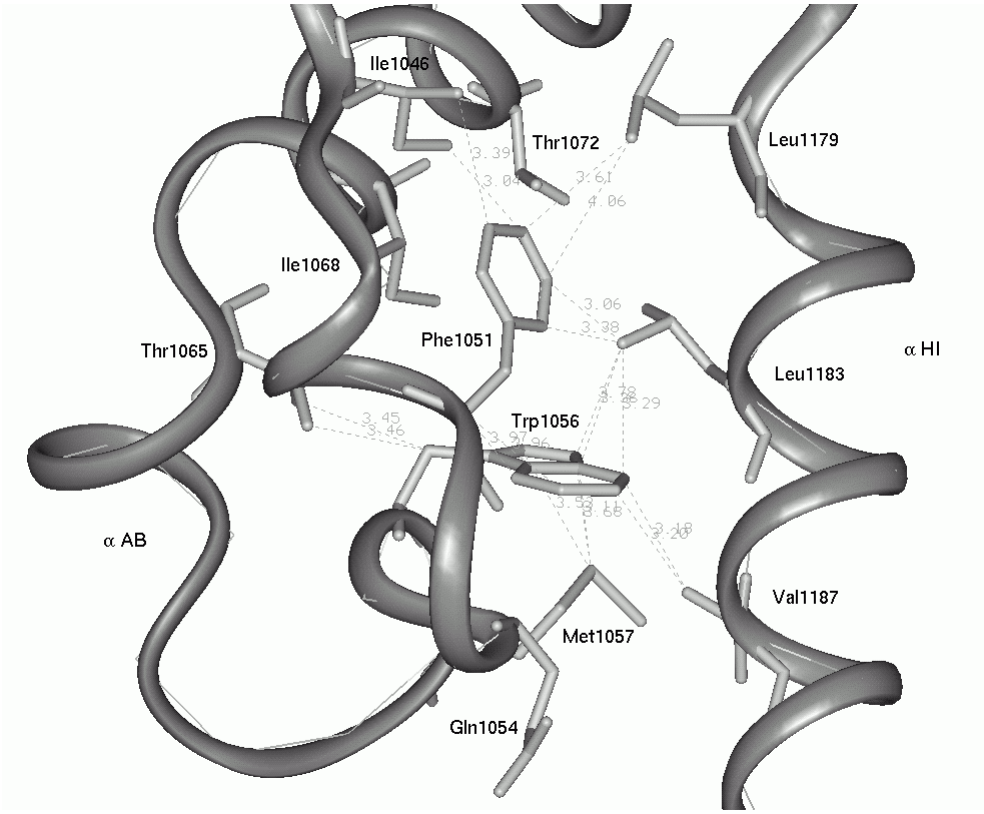

Fig.1: Hydrophobic network between the AB hairpin and the catalytic one in $Cdc25^{Mm}$

Acknowledgements

This work has been partially supported by grants from MIUR.

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