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DECIPHERING THE AHR:ARNT DIMERIZATION PROCESS: HOW TO ASSEMBLY THE FUNCTIONAL PUZZLE OF INTERACTING INTERFACES.

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Introduction

The Aryl hydrocarbon Receptor (AhR) is a basic Helix-Loop-Helix PER-ARNT-SIM (bHLH-PAS)-containing transcription factor activated by binding to a wide range of xenobiotics, including polycyclic- and halogenated-aromatic hydrocarbons. Upon ligand binding, the AhR translocates in to the nucleus and dimerizes with the AhR Nuclear Translocator (ARNT) protein, the complex binds to DNA and promotes the expression of genes, including many responsible of metabolic detoxification pathways[1,2]. Elucidating the dimerization process is crucial for understanding transformation of the AhR into its transcriptionally active form. The process requires the N-terminal region of both proteins, defined by a bHLH motif followed by a tandem repeat of two PAS domains.

No experimentally determined structure is available for the AhR:ARNT dimer, but several X-ray structures of homologous complexes have been deposited in the PDB[3–6]. Accordingly, only theoretical models based on a comparative approach can provide insight into the AhR:ARNT dimerization mode and the complex network of Protein-Protein Interactions (PPIs).

Our initial studies focused on the homology modeling of individual dimers of the PAS-A and PAS-B domains[7]. These models allowed us to outline the essential interacting interfaces, but they were not sufficient to completely define the determinants of AhR:ARNT dimerization that involve both PAS-A/PAS-B and PAS-A/bHLH crosstalk. The very recent availability of a novel full-length X-ray structure of homologous bHLH-PAS complex (HIF2α:ARNT)[6] opens new avenues to develop a more complete structural model of the AhR:ARNT dimer.

Materials and Methods

Homology modelling. The 3D models of the murine AhR:ARNT dimer were generated using MODELLER[8]. Homologous bHLH-PAS dimers were chosen as structural templates (PDB ID: 3F1P; 4F3L; 4M4X; 4ZP4). Due to the lack of structural data, some loop regions were reconstructed using an ab initio strategy[9].

Analysis of ΔSASA. The dimerization interfaces were defined by selecting residues that show variations in Solvent Accessibile Surface Area (ΔSASA), with the web service POPSCOMP[10].

Evaluation of the $\Delta G_{binding}$ and hot spots identification. The binding free energy (ΔGbinding) was calculated according to the Molecular Mechanics Generalized Born Surface Area (MM-GBSA) method[11]. The groups of interacting residues providing significant contributions to the overall $\Delta G_{binding}$ were defined as hot spots[12,13].

Results and Discussion

The bHLH-PAS CLOCK:BMAL1 complex[3], the HIF2α:ARNT PAS-B heterodimer[4], and the AhR:AhR PAS-A homodimer[5] were initially chosen as structural templates to build individual models of PAS-A and PAS-B domain dimers. The shape and electrostatic properties of the modeled dimerization interfaces comply with the geometrical and physico-chemical properties of the AhR and ARNT proteins. Since the alternative models developed for each domain dimer suggested distinct putative hot spots (ΔGbinding signatures), a set of mutagenesis experiments was designed for validation and assessment of the dimerization modes. Ligand-dependent DNA binding of the AhR:ARNT heterodimer mutants was used as a measure of functional AhR:ARNT dimerization[7].

Currently, starting from the X-ray structures of the N-terminal regions of both the CLOCK:BMAL1 dimer[3] and the recently available HIF2α:ARNT complex[6], as well as from our models of the PAS-A and PAS-B AhR:ARNT dimers, we have developed structural models of the AhR:ARNT complex covering the entire bHLH-PASA-PASB region. Modeling of the inter-domain linkers and of the PAS-A loops, which are absent in the reported template structures, has required the use of ab-initio loop modeling

techniques. Apparently, the two models show remarkable differences in the quaternary architectures, with distinct interaction networks among the six domains, associated to different arrangements of the flexible inter-domain linkers. Actually, analysis of the dimerization interfaces indicates that the most important PPIs in the models greatly overlap. Indeed, both the models exhibit similar ΔGbinding values. Calculation of ΔSASA reveals a core interface in which the PPIs of the bHLH motifs and the PAS-A domains are deeply intertwined. The spatial distribution of the hot spots emphasizes how the major contributions to the dimerization are in such region. This observation enforces the hypothesis that the dimerization event is strongly coupled with the transcriptional activation, since the bHLH motif is also responsible for DNA binding[14]. Besides, the interaction between PAS-B domains appears isolated from the rest of the dimerization interface, with a little contribution to the total ΔGbinding. This findings support the hypothesis that PAS-B interaction plays a regulatory role mediated by ligand binding[15].

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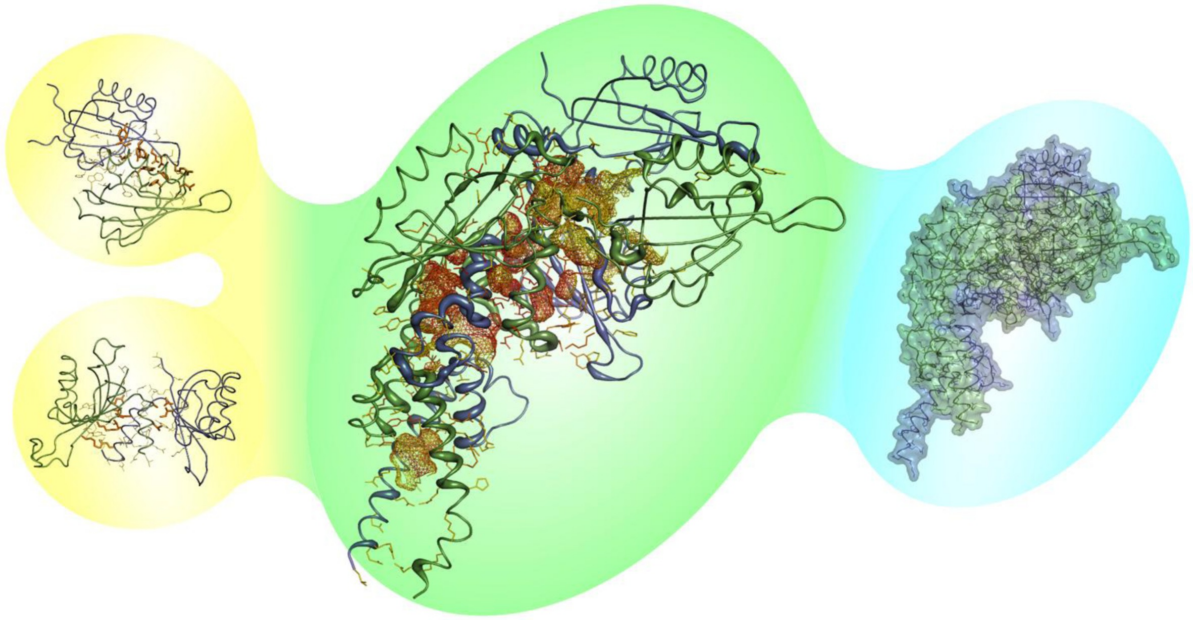

Building the homology models. *On the left, the two individual dimer models for the PAS-A and PAS-B domains, hot spots are shown in orange sticks. On the right, the structure of the bHLH-PAS CLOCK:BMAL1 complex, one of the two reference templates chosen. In the middle, the homology model of the bHLH-PAS AhR:ARNT complex. The thickness of the tube representation highlights the regions more involved in the dimerization interface, the wireframe volumes depict PPIs that mostly contribute to the* $\Delta G_{binding}$.