expression of Bar is repressed by the cooperative mechanism of Al and CII in the distal-most region. These two homeodomain proteins bind cooperatively to the Bar enhancer element and repress its gene expression.

To elucidate the structural basis of the cooperative DNA binding mechanism of Al and CII, we determined the minimal region of Al and CII that are indispensable for the cooperative DNA binding mechanism of these proteins at first. Then, we tried to determine the homeodomain structures of Al and CII, a binary complex structure of Al-DNA, and a ternary complex structure of Al-CII-DNA to analyse the structural basis of the cooperativity of these proteins. In this study, we determined four structures by X-ray crystallography: the structure of Al homeodomain, the structure of Hox11L1 homeodomain (human homolog of CII), the binary complex structure of Al-DNA, and the ternary complex structure of Al-CII-DNA. Our results show a novel ternary complex structure formation of homeodomain proteins. In the ternary complex structure, the extended conserved region of CII homeodomain plays a critical role for the cooperative DNA binding mechanism. The extended conserved residues located in the N-terminal to the CII homeodomain are well ordered in the ternary complex structure and inserted into a minor groove of DNA. We show that three residues (His-10, Tyr-8, and Arg-5 of CII homeodomain) are indispensable for both sequence recognition and cooperative DNA binding mechanism of Al and CII. On the other hand, the extended conserved residues located in the C-terminal to the CII homeodomain form intermolecular interactions with Al to increase binding affinity of CII homeodomain. The structural analysis of Al-DNA and Al-CII-DNA complexes shows that the cooperative DNA binding of Al and CII is caused by a structural modification of DNA by the binding of Al homeodomain to DNA. Our result provides a novel possibility of cooperative DNA binding of homeodomain proteins for accurate gene regulations.

Keywords: biology, complex, transcription

MS22.P18

Purification and crystallization of a Protein-DNAComplex
Anna Rubio-Cosials, Jasmin Sydow, Nereida Jiménez-Menéndez, Pablo Fernández-Millán, Miquel Coll, Maria Solà. "Institut de Biologia Molecular de Barcelona (CSIC) and Institut de Recerca Biomédica, Parc Científic de Barcelona, Baldiri Reixac 10, 08028 Barcelona, (Spain). E-mail: msverri@ibmb.csic.es

Our project deals with a transcription factor (25 kDa) in complex with a DNA that harbours the cognate binding sequence. The protein was fused to a C-terminal His, tag by genetic engineering and overexpressed under standard conditions (37ºC, 225rpm, LB medium, IPTG induction) in the BL21(DE3)pLysS E.coli strain. The protein purification was done in two steps, which consisted in an affinity column (His-Trap Chelating, GE Healthcare) followed by a gel filtration (Superdex 75, GE Healthcare).

For the DNA-protein complex different oligonucleotides were designed based on the specific DNA-binding sequence and taking into account a more general binding sequence described for the protein superfamily. The oligos, of blunt or cohesive ends, included sequences of different number of base pairs.

In order to understand the behaviour of the complex and to know the correct ratio between DNA and protein, electrophoretic mobility shift assays (EMSA) were performed at different protein and DNA stoichiometries. Two retardation bands systematically appeared at higher protein ratios, suggesting the formation of a dimer onto the DNA. This oligomerisation state was confirmed by ultracentrifugation assays.

Crystalization was tried by the vapour diffusion method at 20ºC and 4ºC, dispensing both hanging and sitting drops with the protein alone or the DNA-protein complex. The initial crystalization conditions were subsequently optimised. The best crystals obtained belonged to the orthorhombic space group P222 (cell parameters a=56.12; b=118.41; c=120.05; α=β=γ=90º) and diffracted to 4 Å resolution.

In order to improve the crystal quality and the resolution several methods were tried, like micro and macroseeding [1] or dehydration methods [2]. Additional methods will be tried in combination with different types of oligos.


Keyword: protein-DNA complex, purification, crystallization

MS22.P19

An unfolding model for the GDP/GTP conformational switch of the GTPase Arf6

Institut de Chimie des Substances Naturelles, CNRS, Gif-sur-Yvette.

Synchrontron SOLEIL, Gif-sur-Yvette (France). E-mail: aizel@lebs.cnrs-gif.fr

Small G proteins of Arf family (ADP ribosylation factor) are ubiquitously implicated in membrane trafficking. Arf1 and Arf6, two members of the family, are highly similar: they have over 60% sequence identity, and structural studies have shown that the surfaces they use to interact with regulators and effectors are essentially identical in sequence and structure [1]. Yet, they have different functions in cells. Arf1 is a major regulator of most aspects of vesicular traffic. Indeed, Arf1 regulates the recruitment of coated vesicles of the Golgi apparatus, while Arf6 is restricted to the plasma membrane where it acts at the crossroads of trafficking and cytoskeleton functions [2]. Consistent with their cellular specificities, Arf1 and Arf6 also have distinctive biochemical properties in vitro, for which no straightforward structural explanation has been put forward.

Arf proteins alternate between a GDP-bound inactive form in the cytosol and a GTP-bound active form, which is bound to membranes and able to interact with effectors to induce a cellular response. We show that a truncated Arf6 mutant, which mimics membrane-bound Arf6-GDP is partially unfolded in the crystal compared to cytosolic, full-length Arf6. This unusual conformation is the major Arf6-GDP species in solution, as shown by synchrotron SAXS analysis [3]. In contrast, the equivalent Arf1 mutant is essentially identical to full-length, cytosolic Arf1-GDP, as shown by NMR analysis [4]. Taken together, these experiments suggest that the structural routes for the activation of Arf1 and Arf6 diverge at the step where GDP-bound Arf is recruited to membranes prior to nucleotide exchange. These differences may account for the biochemical differences between Arf1 and Arf6, and yield their functional specificities.


Keywords: GTPases, crystallography, SAXS