MS78.P10

Acta Cryst. (2011) A**67,** C691

Structural biology of transcription factor ci by crystallography and small angle scattering

Kim Krighaar Rasmussen,[a,b] Kristian Frandsen,[a] Margit Pedersen,[c] Lise Arleth,[b] Leila Lo Leggio,[a] [a]*Biophysical Group, Departament of Chemistry, Faculty of Science, University of Copenhagen.* [b]*Biophysics, Department of Basic Sciences and Enviroment, Faculty of Life Sciences, University of Copenhagen.* [c]*Center for Models of Life, Niels Bohr Institute, Faculty of Science, University of Copenhagen.* E-mail: KKR@chem.ku.dk

The molecular mechanisms that determine the expression of one or another set of genes resulting in different development pathways have been studied intensively in bacteriophages. Temperate phages, as opposed to virulent virus, may choose to enter either a lytic or a lysogenic lifestyle following infection of a sensitive host. In the lytic infection cycle, new phages are produced, followed by lysis of the host cell and liberation of phage progeny into the surroundings. Whereas in the lysogenic infection cycle, the phages genome typically integrates into the bacterial chromosomal DNA, resulting in a dormant prophage, mainly expressing genes required for maintenance of the lysogenic state. The decision to enter either the lytic or the lysogenic life cycle is controlled by a bistable genetic switch consisting of phage promoters responsible for transcription of genes required for either the lytic or lysogenic growth, including phage-encoded protein regulating these promoters. In the temperate lactococcal phage TP901-1 one of the main players involved in the regulation of the lytic and lysogenic promoters is a phage-encoded repressor protein, CI, which represses transcription from the lytic promoter P_L by binding to multiple operator sites on the DNA [1], [2]. In this project we aim to characterize CI, various truncated versions of CI, and CI-DNA complexs using crystallography and small-angle scattering techniques, to obtain further understanding of the mechanism of repression.

Various truncations of CI were cloned, expressed in *E.coli* and purified. Subsequently, crystals were obtained for two of the constructs namely the C-Terminal Domain of CI (CTD-CI; residues 91-180) and the N-Terminal Domain (NTD-CI: residues 1-73). The crystals grown from CTD-CI belonged to space group $P3_121$ with unit cell parameters a=30.2 Å, b=30.2 Å and c=177.7 Å. CTD-CI is responsible for the oligomerization of the presumed CI hexamer [3]. Because of low sequence homology with known structures SAD/MAD techniques have been applied to overcome the phase-problem. The crystals were initially soaked in NaBr and CsI solutions and data were recorded. However, because of the unspecific binding of the HA we were not able to solve the phase problem, and we ended up producing a selenomethonine derivative.

We have furthermore succeeded in crystallizing the NTD-CI, which contains the DNA-binding domain of CI. The NTD-CI fold up in a putative Helix Turn Helix (HTH) domain. The crystal diffracted to 1.6 Å and belongs to the space group $P2_12_12_1$ with cell parameters a= 23.68 Å b=43.84 Å and c=72.58 Å. Also here only low sequence identity is found with known HTH structures which complicates structure determination by molecular replacement technique, thus experimental phases will also be sought for this domain.

Δ58CI, a truncated variant of the CI wild type protein, [3] was also expressed and purified. The Δ58CI in contrast to the CI protein only forms dimers and therefore only binds to only one operator site consisting of inverted repeated sequences. Parallel with crystallization studies Δ58CI was used to perform small angle scattering studies with or without DNA. DLS and SAXS results showed a change in particle size when decreasing salt concentration in a solution of DNA and delta58. This preliminary indicates DNA-protein complex formation, and will be investigated further by SANS (small angle neutron scattering).

[1] A.H. Johansen, L. Brøndsted, K. Hammer, *Virology* **2003**, *311,* 144-156. [2] M. Pedersen, K. Hamme, *J. Mol. Biol.* **2008**, *384,* 577-589. [3] M. Pedersen, L. Lo Leggio, J. G. Grossmann, S. Larsen, K. Hammer, *J. Mol. Biol.* **2008**, *376,* 983-996.

Keywords: SAXS, Transcription factor, DNA

MS78.P11

Acta Cryst. (2011) A**67,** C691

C. albicans SerRSs: understanding the evolution of an ambiguous genetic code

Sandra Macedo-Ribeiro,[a] Rita Rocha,[a] Manuel S. Santos,[b] Pedro J.B. Pereira,[a] [a]*IBMC- Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, (Portugal).* [b]*RNA Biology Laboratory, Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro (Portugal).* E-mail: sribeiro@ibmc.up.pt

The discovery of genetic code alterations, of non-canonical amino acid biosynthesis and of natural and artificial incorporation of non-standard amino acids into the proteome of various organisms demonstrated its intrinsic flexibility and non-universality. In the pathogenic yeast *Candida albicans* the universal leucine CUG codon has been reassigned to serine [1], [2]. A unique $tRNA_{CAG}^{Ser}$ mediates this codon reassignment and its ability to be recognized by both leucyl- and seryl-tRNA synthetases (LeuRS and SerRS), introduces a residual level of CUG-decoding ambiguity (2-5% leucine misincorporation). It has been previously demonstrated that leucine misincorporation can be increased up to 28% in *C. albicans* without visible effects on growth rate but with a remarkable impact in cell morphology [1].

In an approach to elucidate the molecular mechanisms underlying the tolerance of several fungal species to genetic code ambiguity, we carried out a large-scale comparative structural analysis of CUG-containing proteins in *C. albicans* and determined the structure and function of the two natural isoforms of the *C. albicans* CUG-containing seryl-tRNA synthetase (SerRS), a key regulator of CUG-decoding ambiguity.

The CUG-encoded residues are predominantly found in non-conserved and surface accessible sites of *C. albicans* proteins, where both serine and leucine can be accommodated without major structural impact. For some CUG-containing proteins, serine or leucine incorporation is likely to result in subtle functional changes, indicating that CUG-codons were not randomly relocated but have been selected to confer advantage to this pathogen under Ser/Leu ambiguity. In agreement, the high-resolution three-dimensional structures of the two *C. albicans* SerRS isoforms show that the presence of a serine or a leucine at the CUG-encoded position at the dimer interface does not induce gross structural changes. However, the two enzymes have different $tRNA_{CAG}^{Ser}$ serylation activities *in vivo*, suggesting that incorporation of Leu/Ser in SerRS might contribute to CUG ambiguity regulation. The data provides experimental evidence for a critical role of codon-decoding ambiguity in protein evolution, showing for the first time how mistranslation can be integrated in a living system upon relocation of ambiguous codons in quasi-neutral positions to minimize protein misfolding. We propose that Ser/Leu incorporation at these positions further provides effective mechanisms to create structural and functional plasticity as a survival strategy with potential implications in pathogenesis.

[1] A.C. Gomes *et al. Genome Biol,* **2007**, *8*, 206. [2] T. Suzuki, T. Ueda, K. Watanabe. *EMBO J,* **1997**, *16*, 1122-1134.

Keywords: tRNA, genetic code, morphology