and the serine protease domain positioned on top. The structure of the individual apple domains is represented by a novel topological motif. The FXI structure combined with our previous structural analysis of the Glycoprotein Ib receptor domain[1] allows us to construct a model of the activating ternary complex formed with thrombin.

[1] Uff et al., *J. Biol. Chem*.,2002, **277**, 35657 - 35663.

Keywords: receptor binding, coagulation, protease

MS43.27.6

Acta Cryst. (2005). A**61**, C59

Structural Studies of the CLD from Aggrecan

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Aggrecan is an important protein in the extracellular matrix (ECM) in the cartilage and its function is to organize the forming of the hyaluronan-lectican complexes in the ECM. Aggrecan consists of three globular domains and a central region of an elongated glycosaminoglycans-carrying region. The N-terminal domain (G1) binds to hyaluronan and the C-terminal domain (G3) has been shown to be involved in the binding to two types of ligands: sulphated glycolipids on the cell surface and dimeric/multimeric ECM proteins, e.g. fibulin-2 and tenascin-R. The CLD within the G3 domain of aggrecan has been observed to make a tight protein-protein interaction (K_D 12nM) with fibronectin type III repeats (FnIII) 3-5 from tenascin-R (TN3-5). Interestingly it has been shown that, though the complex is totally dependent on Ca^{2+}, as would be expected of a CLD, the interaction does not depend on carbohydrate. This is one of only a few direct protein-protein interactions of CLDs involving Ca^{2+}.

The structural studies will give us a first insight how lecticans use the CLD domain to interact with different ECM proteins. This is the first structure showing a calcium-dependent protein-protein interaction involving a C-type lectin domain that is not mediated by a carbohydrate. We have also solved the CLD of aggrecan in a unbound state, giving clues of the importance of the calcium ions.

Keywords: protein-protein interaction, calcium-binding protein, complex structure

MS44 NUCLEIC ACIDS - TRANSCRIPTION, TRANSLATION AND REPAIR

Chairpersons: Stephen Neidle, Eric Westhof

MS44.27.1

Acta Cryst. (2005). A**61**, C59

Eukaryotic Translesion Synthesis DNA Polymerases: Structure and Function

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Cellular DNA is continually damaged by external and internal agents, and both eukaryotes and prokaryotes possess DNA polymerases that can replicate through DNA lesions. Humans have four such (Y-family) polymerases – Polκ, Polι, Polη, and Rev1 – each with a unique DNA damage bypass and fidelity profile. Polη, for example, is unique in its ability to replicate through UV-induced cyclobutane pyrimidine dimers (CPDs), while Polκ is inefficient at replicating through a T-T dimer but can readily extend from mispaired termini. Polι is perhaps the most unusual with varied efficiencies and fidelities opposite different template bases. I will present our structural work on these eukaryotic DNA repair polymerases, with an emphasis on the basis of their specialization in lesion bypass.

Keywords: DNA-polymerase, DNA-repair, replication

MS44.27.2

Acta Cryst. (2005). A**61**, C59

Mechanism for *de novo* RNA Synthesis by T7 RNA Polymerase

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DNA-directed RNA polymerases (RNAP) are distinguished by their ability to initiate *de novo* synthesis of polymer, RNA, from a promoter without the requirement for a 3'OH primer-terminus. The RNAP from bacteriophage T7 requires no accessory factors for RNA synthesis during the initiation phase of transcription. T7 RNAP is unique, however, in its requirement for *de novo* synthesis to start with the incorporation of two GTPs at the beginning of transcription.

We examined the structural and kinetic basis for *de novo* synthesis of RNA by using a series of novel DNA constructs which varied the template DNA initiation sequence and the incoming NTP analogues. Nine structures of T7 RNAP with promoter DNA and/or an incoming pair of NTPs were determined to 2.2 to 3.2 Å resolution and are *the first structural examples of de novo RNA synthesis by an RNA polymerase*. Different promoter-template DNA constructs are bound to the enzyme in virtually identical conformations. The two initiating NTPs are accommodated in the enzyme by changes to both the geometry of the active site and a novel bend in template DNA. The incoming NTPs are recognized by novel enzyme and template DNA interactions. Active site residues make a large contribution to the recognition of the initiating NTPs in addition to the specific DNA template base-pair interactions. Pre-steady state kinetic measurements support the idea that discrimination of initiating nucleotide by the enzyme plays a greater role than template specification of nucleotide selection for *de novo* synthesis of RNA by T7 RNA polymerase.

Keywords: transcription initiation, polymerase, nucleotide selection

MS44.27.3

Acta Cryst. (2005). A**61**, C59

Structural Basis for RNA-regulated Gene Expression

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RNA molecules have been discovered at the heart of several central aspects of gene expression, from protein biosynthesis by the ribosome to the targeting of new proteins to the correct intracellular locale to RNA interference (RNAi). Understanding how these RNA-mediated processes work will illuminate central aspects of modern cell biology and also provide important clues to the possibly fundamental role of RNA in the evolution of life. I will describe our efforts to understand the structural basis for RNA function, highlighting recent discoveries about the recognition and cleavage of double-stranded RNA in the early steps of the RNAi pathway.

Keywords: RNA structure, RNA, gene expression

MS44.27.4

Acta Cryst. (2005). A**61**, C59-C60

Structural Basis for Specific Recognition of the UsnRNP m_3G-cap by Snurportin1

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The small nuclear ribonucleoprotein particles (snRNPs) are the major components of the splicing machinery that removes introns from pre-mRNA. In metazoans, the snRNP biogenesis is an ordered process requiring both nuclear and cytoplasmic phases. After transcription, the snRNAs U1, U2, U4, and U5 are exported into the cytoplasm, where the assembly with seven Sm proteins occurs and the snRNA 5′-cap nucleotide is modified from a 7-methyl-guanosine (m^7G-) to a 2,2,7-trimethyl-guanosine (m_3G-) cap. The hyper-methylated m_3G-cap represents one of the two nuclear localisation