

**[s8a.m1.o1]** **The Structure of a new intermediate on the Rotary Catalytic Cycle of F<sub>1</sub>-ATPase.** A.G.W. Leslie, R. I. Menz and J. E. Walker. *MRC Laboratory of Molecular Biology, Hills Rd., Cambridge CB2 2QH, UK*  
Keywords: ATPase, aluminium fluoride, rotary catalysis.

ATP synthase is a complex oligomeric assembly which consists of two morphological sectors, an intrinsic membrane domain (F<sub>0</sub>) which contains a proton channel, and a water soluble catalytic domain (F<sub>1</sub>) which contains the nucleotide binding sites. The crystal structure of the F<sub>1</sub> sector from bovine mitochondria revealed a spherical assembly of three  $\alpha$ - and three  $\beta$ -subunits, packed like the segments of an orange. The  $\gamma$ -subunit forms a spindle-like structure which runs through the centre of the ( $\alpha\beta$ )<sub>3</sub> subcomplex. Several features of the structure suggested that the gamma subunit physically rotates relative to the ( $\alpha\beta$ )<sub>3</sub> assembly during catalysis. Subsequently this rotation has been verified by a number of other techniques, including the direct observation of rotation.

Tryptophan fluorescence has been used to monitor nucleotide binding in the *Escherichia coli* F<sub>1</sub>-ATPase and has shown that, on average, all three catalytic sites are occupied by nucleotide during catalysis at physiological concentrations of substrates. In the original crystal structure of the bovine enzyme, only two of the three catalytic  $\beta$ -subunits bound nucleotide. We have recently determined the structure of an aluminium fluoride inhibited form of the enzyme in which all three catalytic sites are occupied by nucleotide. Nucleotide binding to the previously "empty"  $\beta$ -subunit is accompanied by significant conformational changes and a small (20°) rotation of the  $\gamma$ -subunit.

**[s8a.m1.o2]** **The RNA-dependent RNA polymerase of hepatitis C virus: 3D structure and implications for viral replication.** S. Bressanelli, L. Tomei<sup>#</sup>, A. Roussel, I. Incitti<sup>#</sup>, R.-L. Vitale<sup>#</sup>, M. Mathieu, R. De Francesco<sup>#</sup>, F.A. Rey, *Laboratoire de Génétique des Virus, Gif-sur-Yvette, France, # Istituto di Ricerche di Biologia Molecolare, Roma, Italy.*

Keywords: polymerase, RNA, virus replication

Hepatitis C virus (HCV) is an RNA virus that is the major cause of human non-A non-B hepatitis worldwide: an estimated 170 million people are chronically infected with HCV today. Chronic infection leads to liver disease that greatly increases the risk of liver cirrhosis and cancer and often requires liver transplant. As current therapies are effective only in a minority of cases, there is an urgent need for the development of HCV-specific antiviral agents.

The study of the HCV life cycle has been greatly impeded so far by the lack of a reliable cell culture system. Thus, most knowledge about HCV replication has been derived from molecular studies of recombinant viral proteins. By analogy with the Flaviviruses, the closest relatives of HCV, replication of the viral genome is thought to take place *in vivo* at the membranes of the endoplasmic reticulum. The membrane-bound replication complex is likely to involve cellular as well as several viral proteins. The key player in this complex, however, is the virally-encoded RNA-dependent RNA polymerase (RdRp), NS5B. As such, this enzyme is a target of choice for HCV-specific drug design.

We have crystallized a soluble fragment of NS5B deleted of its C-terminal hydrophobic residues. This fragment comprises 536 (out of 591) residues and retains full catalytic RdRp activity. We have solved the structure by the MAD method using a selenomethionine derivative, to a resolution of 2.8 Å.<sup>1</sup>

The structure shows that the enzyme has the characteristic fold of the polynucleotide polymerases, with three subdomains termed "fingers", "palm" and "thumb". However, at variance with all other polymerases described so far, the thumb and fingers subdomains interact directly through an extension of the fingers (the "fingertips"). This interaction greatly reduces the conformational mobility of fingers and thumb relative to each other. Indeed, superposition of the structure of NS5B with that of HIV-1 reverse transcriptase in a ternary complex with DNA and dTTP<sup>2</sup> suggests a possible concerted movement of thumb and fingertips during translocation of the RNA template-primer in successive rounds of polymerisation. This special feature of NS5B will be discussed, as well as its possible implications for the replication of HCV and other RNA viruses.

[1] Bressanelli *et al.*, "Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus", *P.N.A.S.* (1999), 96: 13034-13039

[2] Huang *et al.*, "Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance", *Science* (1998), 282: 1669-1675