s1.m7.p11 **Structural studies of the Talin VBS3 with Vinculin Head.** Alexandre R. Gingras, Evangelos Papagrigoriou, Igor Barsukov, David R. Critchley and Jonas Emsley, *Department of Biochemistry, University of Leicester, University Road, Leicester, LE1 7RH, UK. E-mail: arg9@le.ac.uk*

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Integrin mediated cell adhesion and signal transduction is implicated in key physiological processes such as cell growth, regulation of apoptosis, hemostasis and pathological processes such as thrombosis and tumour metastasis. The interaction between integrin cytoplasmic domains and the cytoskeletal protein talin plays a dynamic role in the assembly of structures called focal adhesions which regulate the above cellular processes. Talin couples integrins to F-actin, and contains three binding sites (VBS1-VBS3) for vinculin, a protein implicated in the negative regulation of cell motility and whose activity is modulated by an intramolecular interaction between the Vinculin head (Vh) and Vinculin tail (Vt) domains [1]. In the present study, we have determined the crystal structure of the Vh (1-258) in complex with a synthetic VBS3 peptide (1944-1969) to 2.6Å resolution using one multiple anomalous diffraction dataset from selenomethionine labelled crystals. These crystals belong to space group $P2_12_12$ with cell dimensions of a=52Å b=72Å c=96Å α=90° β=90° γ=90°. The asymmetric unit contained one Vh-VBS3 heterodimer and had a solvent content of 53%. The Vh-VBS3 complex form an elongated shape structure of 101Åx40Åx27Å. In the Vh-VBS3 crystal structure one molecule of Vh binds to one molecule of VBS3. The interaction is mainly hydrophobic between the VBS3 amphipathic α-helix and the Vh helices α1-4, forming a new 5 helix bundle. The structure of the VBS3 helix within the talin rod domain is unknown. We expressed a recombinant 20 kDa talin rod fragment containing the VBS3 helix and characterised it using NMR. NMR spectra of the 20 kDa talin polypeptide shows evidence that the protein adopts a stable folded state in solution. The addition of unlabelled Vh to the talin polypeptide leads to large chemical shift changes in most cross-peaks indicative of large talin rod structural rearrangement upon Vh binding. The VBS released form of talin could be a key trigger underlying integrin mediated signal transduction and assembly of the actin cytoskeleton. The mechanism of activation is still unclear, but we have strong evidence that the talin rod makes large structural rearrangements in order to bind vinculin head. Moreover, talin binding to the vinculin head displaces the Vinculin tail, allowing it to bind F-actin and other cytoskeletal proteins.

[1] Bass, M.D., B. Patel, I.G. Barsukov, I.J. Fillingham, R. Mason, B.J. Smith, C.R. Bagshaw, and D.R. Critchley, Further characterization of the interaction between the cytoskeletal proteins talin and vinculin. *Biochem J*, 2002. **362**(Pt 3): p. 761-8.

s1.m7.p12 **Mitochondrial KAS.** Anette Henriksen, Penny von Wettstein-Knowles, *Carlsberg Laboratory, Department of Chemistry, Gamle Carlsberg Vej 10, Valby, Denmark. E-mail: anette@crc.dk*

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The synthesis of fatty acids for cell membrane generation and maintenance is catalysed by fatty acid synthase (FAS). FAS consists of a series of enzymatic activities, either gathered on very large multifunctional polypeptides (type I) or organized from discrete enzymes (type II). Type I systems are found in animal and fungi cytosol, while the presence of Type II systems characterize bacteria and plant plastids. However, it has recently been shown that FAS II proteins are also encoded by nuclear DNA in animals and fungi. In these organisms, the FAS II proteins contain mitochondrial targeting sequences [1]. FAS enzymes are essential for lipid and fatty acid synthesis and are attractive targets for therapeutics. β-Ketoacyl-acyl carrier protein synthase (KAS) is the FAS enzyme catalysing the biosynthetic Claisen condensation reaction. The substrate and product profiles for mitochondrial KAS (mtKAS) from *Arabidopsis thaliana* are different from those characterizing the bacterial enzymes [2].

The crystal structure of the *Arabidopsis thaliana* mtKAS has been determined to 2.0 Å resolution. It is the first structure of a mtKAS. The structure reveals a dimeric enzyme with an αβαβα-fold and a somewhat extended cap region. In contrast to other KAS enzymes, mtKAS can utilize the decarboxylation product of malonyl-ACP in a productive way in the absence of acyl-ACP substrates and can catalyse the condensation of the complete range of saturated acyl-ACP substrates (C_3-C_{16}) with malonyl-ACP as the only substrate. The role of the active site architecture and possible mtKAS:ACP interactions are discussed and correlated with proposed catalytic mechanisms.

[1] Zhang *et al*. (2003). *J. Biol. Chem*. **278**, 40067-40074.
[2] Yasuno *et al.* (2003). *J. Biol. Chem*. **279**, 8242-8251.