MS04 O1

How covalent and noncovalent interaction with SUMO can regulate the Ubl conjugation process Puck Knipscheer[a] , Andrea Pichler[b], Willem J. van Dijk[a], Jesper V. Olsen[c], Matthias Mann[c], Frauke Melchior[d], Titia K. Sixma[a] [a]*Netherlands Cancer Institute, Amsterdam,* [b]*Max F- Perutz Laboratories, Medical University Vienna,* [c]*Max Planck Institute for Biochemistry, Martinried,* [d]*Göttingen University , Italy*. E-mail: t.sixma@nki.nl

Keywords: SUMO, Ubc9, Ubiquitin conjugation

Covalent modification with SUMO can affect enzymatic function of the target protein. In this manner SUMO can regulate both ubiquitin and SUMO modification pathways. We studied the structure of two E2 conjugating enzymes with covalently modified with SUMO: Modification of the ubiquitin E2-25K inhibits its interaction with the ubiquitin E1 enzyme and therefore strongly inhibits ubiquitin chain formation. In contrast, SUMO modification of the SUMO E2, Ubc9 has a different effect, regulating target choice. Dependent on the target it can cause an lower, equal or much higher sumoylation dependent on the target. Structural analysis explains the different function of SUMO on the two E2 enzymes.
Not only covalent bound SUMO can affect the Ubc9 activity, but SUMO can also bind to a different site on Ubc9 in a non-covalent manner. New mutants based on the crystal structure interfere with this function specifically. These mutants show that non-covalent interaction is important for SUMO chain formation.

MS04 O2

Ras/Nore1 interaction: Mechanism of apoptotic signaling Christian Herrmann [a], Benjamin Stieglitz [a], [a]*Physical Chemistry 1, Ruhr-University Bochum, 44780 Bochum, Germany* E-mail: chr.herrmann@rub.de

Keywords: protein complex structure, protein interactions, protein kinetics

The molecular switch Ras activates different signalling pathways upon binding to its respective downstream effector via a Ras binding domain (RBD). Ras association domain family (RASSF) represents a class of putative Ras effectors which play an important role in growth inhibition and tumor suppression [1]. Promoter hypermethylation of RASSF1A and RASSF5A also known as NORE1A is one of the most common events in human cancer [2]. Tumor suppressive function of these proteins is related to their ability to bind and stabilize microtubules [3]. The mechanism by which Ras might control this process is unknown. We have solved the crystal structure of NORE1A-RBD in complex with Ras and show that NORE1A-RBD binds to microtubules in a Ras dependent manner. The Ras binding domain of NORE1A is enlarged compared to other known RBD structures and shows an N-terminal helix-loop extension. This forms distinct hydrophobic contacts with switch II of Ras and is responsible for the life time of the complex much longer than observed for other Ras effectors. The primary sequence which comprises the RBD matches the minimal fragment necessary for microtubule association *in vivo*. As probed by light scattering activated Ras suppresses
NORE1A induced microtubule bundling, indicating an inhibitory binding model. Our results demonstrate competitive binding of Ras·GTP and tubulin to NORE1A-RBD. These novel findings provide a direct molecular connection between reshaping the microtubule cytoskeleton and Ras mediated signal transduction. The structural basis of effector recognition of RASSF5 via Ras and its implications for tubulin binding might be a good starting point for a more detailed understanding of the biological role of this important class of tumor suppressors.

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MS04 O3

Structural basis for messenger RNA movement on the ribosome Jenner L., Yusupova, G., Rees, B., Yusupov, M. *University of Strasbourg, 1, rue Laurent Fries, 67404, Illkirch, France* E-mail : lasse@igbmc.u-strasbg.fr

Keywords: Riboso e, Bio Synthesis, RNA

Translation initiation is a major determinant of the overall expression level of a gene. The translation of functionally active protein requires the messenger RNA (mRNA) to be positioned on the ribosome in such a way that the start/initiation codon will be read first and in the correct frame. Very little is known about the molecular basis for interaction of mRNA with the ribosome at different states of translation. Recent crystal structures of the ribosomal subunits, empty 70S ribosome and 70S ribosome containing functional ligands provided information about the general organization of the ribosome and its functional centres. Here we compare X-ray structures of eight ribosome complexes modelling translation initiation, post-initiation and elongation states. In the initiation and post-initiation complexes, the presence of the Shine-Dalgarno (SD) duplex causes strong anchoring of the 5'-end of mRNA on the platform of the 30S subunit, where numerous interactions between mRNA and the ribosome take place. Conversely, the 5'-end of the "elongator" mRNA lacking SD interactions is flexible suggesting a different exit path for mRNA during elongation. The post-initiation ribosome complex reveals that after initiation of translation, while SD interaction is still present, mRNA moves in the 3'-5' direction with simultaneous clockwise rotation and lengthening of the SD duplex bringing it in contact with ribosomal protein S2.

MS04 O4

Crystal structure of a phage T4 endonuclease VII - Holliday junction complex Dietrich Suck[a], Christian Biertuempfel[b], Wei Yang[b], Structural *and Computational Biology Unit, EMBL, Heidelberg, Germany.* [b]*Laboratory of Molecular Biology,NIDDK,NIH, Bethesda, USA.*
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Keywords: Holliday junction resolvase, Endonuclease VII, crystal structure

Holliday junctions (HJ) are universal intermediates in repair and reorganization of DNA by homologous recombination. They represent mobile links between two homologous DNA duplexes and generate new segments of