s8a.m10.o1 **Novel types of interactions of the small GTPase Ran with its effectors**. I.R. Vetter[‡], C. Nowak[‡], T. Nishimoto*, J. Kuhlmann[‡], A. Arndt[‡], U. Kutay[§], D. Görlich[§], A. Wittinghofer[‡], [‡]*Max-Planck-Institut für molekulare Physiologie, Otto-Hahn-Str. 11, D-44227 Dortmund, Germany,* [§]*Zentrum für Molekulare Biologie der Universität Heidelberg, D-69120 Heidelberg, Germany, *Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-82, Japan*.
Keywords: nuclear transport, RanBp2, importin β.

Transport receptors of the Importin β family shuttle between nucleus and cytoplasm and mediate transport of macromolecules through nuclear pore complexes. They interact specifically with the GTP-binding protein Ran which in turn regulates their interaction with cargo. The three-dimensional structure of a complex between Ran•GppNHp and a 462 residue fragment from Importin β[1] shows that the interface with Ran is at the concave side of the irregular crescent formed by the α-helical repeats of importin β.

Another class of Ran effectors is formed by the Ran binding domains (RanBDs) which are defined by a conserved sequence motif found in several proteins involved in nucleocytoplasmic transport. RanBP2 contains four such domains and constitutes a major part of the cytoplasmic fibrils of the nuclear pore complex. The crystal structure of Ran•GppNHp in complex with the first Ran binding domain (RanBD1) of human RanBP2[2] revealed that RanBD1 has a pleckstrin-homology domain fold. Ran•GppNHp shows extensive conformational changes compared to Ran•GDP: The switch I region resembles the canonical Ras•GppNHp structure, whereas the C-terminus of Ran wraps around RanBD1 and contacts a basic patch on RanBD1 with its acidic end. This "molecular embrace" suggests that the primary role of RanBDs is the sequestering of the Ran C-terminus. This in turn helps to trigger the dissociation of RanGTP from importin β-related transport factors and facilitates GTP-hydrolysis by RanGAP[3].

Another binding partner of Ran is NTF2. The structure of the complex[4] shows a binding site which again differs from the binding modes described above.

These structures reveal a remarkable flexibility of the small GTPase Ran to interact with at least five different types of effector and regulator molecules (RanGAP, RCC1, RanBPs, Importins/Exportins, NTF2).

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[3] Bischoff, F.R., Görlich, D. "RanBP1 is essential for the release of RanGTP from Importin β-related nuclear transport factors.", FEBS Lett., (1997), 419: 249-254.
[4] Stewart, M., Kent, H.M., Mccoy, A.J. "Structural basis for molecular recognition between nuclear transport factor 2 (NTF2) and the GDP-bound form of the Ras family GTPase Ran.", J. Mol. Biol., (1998), 277:535-646.

s8a.m10.o2 **Socialise your proteins and make them happy! - Coexpression as a tool to improve and study protein behaviour.** C. Kambach, *Paul Scherrer Institute, Life Sciences/OSRA 007, CH5232 Villigen PSI, Switzerland*
Keywords: overexpression, solubility, protein complexes

In overexpressing recombinant proteins in heterologous systems, it is often observed that proteins which are soluble and well-behaved in their natural cellular environment are either not overexpressed at all, or produced as insoluble aggregates. Post-translational modifications of a eukaryotic protein are generally not reproduced in commonly used bacterial overexpression hosts, which can lead to the production of non-functional protein.

Both kinds of problems can sometimes be overcome by coexpressing natural binding partners or effectors of the target protein, preferably from the same vector. This approach has successfully been used in pro- and eukaryotic expression systems. Apart from improving the overexpression behaviour of a given target, coexpression provides a handle for the study of multi-component protein complexes that are otherwise inaccessible.

Choice of coexpressed species and expression system depend on the biochemistry of the target and its intended use. Since the establishment of parameters such as composition of the coexpressed multi-component system and order of cDNAs on the expression vector is subject to trial and error, construction of such vectors should allow for a modular, highly versatile scheme based on interchangeable expression cassettes.

The seminar will cover some basic considerations on choice of coexpression system, expression cassette and coexpression vector construction, and coexpression protocols. Some applications will be described, and practical examples from the *E.coli* expression system will be given.