s8a.m10.p3 **Functional expression and preliminary X-ray crystallographic study of human Survivin**. S. Shin, B.-H. Oh, *Department of Life Science, and Division of Molecular and Life Science, Pohang University of Science and Technology, Pohang, Kyungbuk, 790-784, Korea.*
Keywords: protein protein interactions.

Survivin, an apoptosis inhibitor/cell-cycle regulator, is critically required for suppression of apoptosis and ensuring normal cell division in the G2/M phase of the cell cycle. It is highly expressed in a cell cycle-regulated manner and localizes together with caspase-3 on microtubules within centrosomes. Whether survivin is a physiologically relevant caspase inhibitor has been unclear due to the difficulties with obtaining correctly folded survivin. In this study, recombinant, active human survivin was expressed in *Escherichia coli*, purified to homogeneity and characterized physico-chemically, revealing that survivin exists as a homodimer in solution and binds caspase-3 and -7 tightly with a dissociation constant of 20.9 nM and 11.5 nM, respectively. Consistently, survivin potently inhibits the cleavages of a physiological substrate poly(ADP-ribose) polymerase and an artificial tetrapeptide by caspase-3 and -7 in vitro with apparent inhibition constants of 36.0 nM and 16.5 nM, respectively. The data suggest that the suppression of default apoptosis checkpoint by survivin in the G2/M phase is likely through sequestering caspase-3 and -7 in the inhibited state on microtubules. The localization of survivin, which is essential for its function, should increase the protective activity beyond a threshold level at the action site. To further clarify survivin's protective mechanism, the structure needs to be delineated at atomic level.

Single crystals were obtained using a precipitant solution containing 30% ethylene glycol and 100 mM Hepes (pH 7.5) at 4°C. Initial X-ray diffraction data shows relatively weak intensities to only 5.0 Å resolution and permit the assignment of the crystallographic unit cell to a primitive hexagonal space group with the cell dimensions, a=147.492, b=147.492, and c=143.001. Refinement of the crystalization conditions for structure determination is under way.

[1] Li, F., "Control of apoptosis and mitotic spindle checkpoint by survivin", Nature, (1998), 396, 580-584.
[2] Ambrosini, G., "A novel anti-apoptosis gene, *survivin*, expressed in cancer and lymphoma", Nature Medicine, (1997), 3, 917-921.
[3] Tamm, I., "IAP-family protein Survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs", Cancer Research, (1998), 58, 5315-5320.

s8a.m10.p4 **Structural basis for the activation of the nuclear GTPase Ran by its β-propeller exchange factor RCC1**. L. Renault , J. Kuhlmann, and A. Wittinghofer, *Max-Planck Institut für molekulare Physiologie, Postfach 50 02 47, 44202 Dortmund, Germany.*
Keywords: regulatory GTP-binding protein, Ran, guanine nucleotide exchange factor (GEF).

Guanine nucleotide exchange factors (GEF) activate GTP-binding proteins (GTPase) by stimulating the exchange of GDP for GTP in a multistep reaction mechanism which involves binary and ternary complexes between GTPase, guanine nucleotide, and GEF. Activation of the small nuclear GTP-binding protein Ran by its chromatin-associated GEF RCC1 is a key step for its regulatory role both in nucleo-cytoplasmic transport[1] and cell cycle progression[2]. How GEFs accelerate the dissociation of GDP to promote the loading of GTP (in excess in cellular medium) is only partially understood at the molecular level.

GEFs of different GTP-binding protein family do not share any notable analogy to each other in their primary and tertiary structure. The structure of RCC1 forms a seven-bladed β-propeller, constituted by internal repeats of 51-68 residues per blade[3]. These repeats are found in more than 20 different proteins, not related to RCC1. Based on biochemical and structural docking experiments, we could identified the RCC1 interacting face with the GTPase Ran on one face of the β-propeller[3,4].

From the different intermediate and transition steps of the exchange reaction involving modulatory interactions of GTPase and GEF, only the binary nucleotide-free GTPase/GEF complex has been structurally investigated in three other GTP-binding protein families to suggest the molecular basis for the overall mechanism. Here we are reporting the 1.8 Å X-ray crystallographic structure of a complex formed between Ran and its β-propeller GEF, RCC1. The nucleotide-binding site of Ran is filled by a divalent anion mimicking the binding of the β-phosphate of the nucleotide. A preliminary analysis suggests that the GEF RCC1 is competing with the nucleotide for stabilizing the nucleotide free form of Ran by interacting with the nucleotide phosphate-binding sites and by controlling the nucleotide base-binding sites in an allosteric manner[5]. The structure of the β-propeller RCC1/Ran may represent a basis for further mutational analysis of paralogous proteins containing RCC1-repeats.

[1]. Gorlich, D., et al., "Transport between the cell nucleus and the cytoplasm. " [Review], Annu. Rev. Cell Dev. Biol., (1999), 15, 607-660.
[2] Desai, A.et al., "Microtubule cytoskeleton: No longer an also Ran. " [Review], Curr. Biol., (1999), 9, R704-7.
[3] Renault, L., et al. "The 1.7 A crystal structure of the regulator of chromosome condensation (RCC1) reveals a seven-bladed propeller.", Nature, (1998), 392, 97-101.
[4] Azuma, Y., et al., "Model of the ran-RCC1 interaction using biochemical and docking experiments. ", J. Mol. Biol., (1999), 289, 1119-30.
[5] Renault, L., et al., "Structural basis for the activation of the nuclear GTPase Ran by its β-propeller exchange factor RCC1 .", *in preparation.*