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Project title: Functional analysis of CCT-PDCL3 complex using a reconstituted human in vitro translation system

CCT is a 16-subunit, protein folding machine which folds the cytoskeletal proteins, actin and tubulin. We have previously built a functional CCT system in vitro from the yeast Saccharomyces cerevisiae proteins and we want to achieve this for the human CCT machine so we can investigate and chemically perturb its sequential allosteric cycle. A critical component in the yeast system is the co-factor PLP2 which stabilises CCT-actin-foldingintermediate complexes and increase the yields of native actin. The human orthologue of yeast PLP2 is PDCL3 and its activity is poorly understood because it inhibits actin folding on CCT. Dr Machida and Prof Willison performed experiments to investigate the effect of phosphorylating the CCT co-factor protein PDCL3 on folding of actin in an *in vitro* human protein translation system which has been built in the laboratory of Prof Imataka. Human CCT was produced in BHK-21 cells using the vaccinia virus T7 polymerase system developed in the Imataka lab (Machida, K et al; Reconstitution of the human chaperonin CCT by co-expression of the eight distinct subunits in mammalian cells. Protein Expression and Purification 82, 61-69, 2012). Recombinant PDCL3 was produced in E.coli. and phosphorylated in vitro using casein kinase II. Phosphorylation activity was monitored using a native gel shift assay. The controls and the experiments worked but the phosphorylation of PDCL3 did not enhance the cell free translation system to increase the yield of native actin. Further experiments are required to discover the function of PDCL3 in the human CCT system. We successfully transferred technology and reagents from London to Hyogo and we will continue the collaboration.

Personal experiences:

Prof Willison has lived and worked in 3 Japanese cities; Tokyo – 1 month stay in 1984, Osaka – 1 year stay in 1990 and Himeji – 2 months stay in 2013. During this most recent visit he met several foreign, early career researchers working in Japanese laboratories and he found them enjoying the scientific environment and their everyday living experiences. Since there are so many opportunities for scientists of all nationalities to win fellowships to work in Japan these days many laboratories are becoming much more international than when he first visited Japan 30 years ago. The internet has made living in Japan much easier for foreigners who can speak Japanese but still cannot read enough kanji! Hotels are bookable online and JR train timetables in English can be found too and this makes tourism so much easier than in the past. Prof Willison is happy for new JSPS fellows to get in touch by email if they would like some advice about working in Japanese laboratories and/or living in Japan.

HYOGO - 07/11/2013 IMATAKA-ken - Students and JSPS host - Dr Kodai Machida (in front of door)

