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CANCER RESEARCH UK

Cell Cycle Genetics Research Group

UNIVERSITY OF CAMBRIDGE

Department of Genetics

For the Attention of the Foundation for Polish Science,

The Cell Cycle Genetics Group, located at the Department of Genetics of the University of Cambridge and led by Professor David Glover, has been collaborating with the Laboratory of Mass Spectrometry of IBB PAS for the past three years.
Our group in Cambridge is interested in understanding the role of key regulators of the cell cycle as well as in dissecting the molecular structure and function of multiprotein complexes involved in the process of cell division principally at the centrosome and kinetochore. To carry out such studies we need to identify networks of protein-protein interactions and determined how they are affected by post-translational modifications. Mass spectrometry (MS) can deliver crucial information about these topics and for mainstream molecular cell biology it has become a method of choice due to its sensitivity, accuracy, flexibility and the large amount of data coming out of single experiments. Recognising the strength of this approach we have teamed up with the group in Warsaw, because of the variety of instrumentation in this laboratory and the deep knowledge in the field, which the group of Michal Dadlez has to offer.

We have every intent of continuing with this collaboration and in expanding it into new areas. So far we have successfully cooperated in identifying proteins present in complexes affinity-purified as interactors with known tagged targets (examples in Dzhindzhev et al. Nature (2010) 467, 714; Przewloka et al. (2011) Curr Biol 21, 399). Now we wish to know precise details of posttranslational modifications (PTMs) and how these influence the binding of the partner proteins in the complexes and thereby their function. We will initiate this study by targeting the centromeric component CENP-C, a key regulator of kinetochore assembly. To this end we will isolate this protein from Drosophila tissue culture cells expressing tagged CENP-C using different cell growth and purification conditions and identify PTMs, which will be later analyzed in vitro. Further going into biology of those, we need to know enzymes mediate the crucial modification events and understand biological relevance of their activities. We anticipate that many of these will be phosphorylations brought about by the mitotic protein kinases that we study in the Cambridge lab although additional modifications can also be anticipated. We can address the specificity of the modification in vitro, for example by phosphorylation assays, and in vivo by treating cells with specific (kinase) inhibitors. These approaches are established in the lab.

We would also like to explore the structural characterization of the protein complexes we work on. The approach here would be to co-express proteins of interest from a complex in bacterial or insect cells. Complexes would be purified, crystallized and subjected to the classical X-ray diffraction analysis. However, several of these complexes are multisubunit (3, 4 and 5 subunit-large) and may not crystallize readily. It is therefore possible that the

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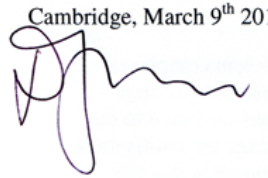
structures of single subunits or domains might be obtained more easily. We would like to try to transfer fragmentary structural knowledge to the level of the multi-subunit complexes following the strategy already employed in Professor Dadlez's laboratory for a multidomain protein (Kupniewska-Kozak et al. (2010) J Mol Biol 403, 52). This novel method is based on measuring the rates of hydrogen-deuterium exchange of amide hydrogens by mass spectrometry. Although not as accurate as crystallography, this technique allows identification of fast- and slow-exchanging regions of the protein and can thus map regions of different dynamics, thus providing experimental constraints for structural modelling. As an example of the complex, which we would like to analyze using this approach, is the structure composed of the fragment of the centromeric protein CENP-C bound to subunits of the Mis12 complex and the kinetochore protein Spc105 from Drosophila melanogaster. Knowing the structural basis for the interaction between Mis12 complex and CENP-C is a very important step towards the understanding of cell cycle-based regulation of kinetochore assembly, since we know that the core kinetochore proteins are recruited by CENP-C (Przewloka et al (2011) Curr Biol 21, 399). The combination of structural and functional approaches would give us an important multi-angle insight into this field of study.

Overall, the proteomics facility at the Institute of Biochemistry and Biophysics can convey to us essential information about a protein's presence, modification status and structure. This will be complemented by our functional studies of proteins and protein complexes at the cellular and organismal levels using cell biology and genetic tools available at our lab in Cambridge. Together these approaches should give us a comprehensive view of regulatory function of our targets both in vitro and in vivo.

A prospective new Ph.D. student participating in those studies would be directly involved in such experiments at all levels. During his/her stay at the proteomics facility in Warsaw that person would be trained in using MS, specifically focusing on the identification and analysis of posttranslational modifications as well as in using MS-based structural methods. S/he would be visiting the Cambridge laboratory on regular basis in order to carry out molecular cell biology. Additionally, after being trained in molecular biology techniques for studying protein-protein interactions, the Ph.D. student would bring this knowledge to Warsaw and with the help of local scientists (IBB PAS offers expert help and support in many areas of molecular biology) would be able to set up by him/her-self many experiments on that site.

These interactions will benefit both of our labs and give the opportunity for a prospective Ph.D. student to be trained in the highly skilled and research-oriented environments of both the Warsaw and Cambridge institutions.

David M. Glover Marcin Przewloka
Cambridge, March 9th 2011



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