of group A and B kinases. It was supposed that these residues might be responsible for the difference in ROP GTPase-binding. Although these amino acid motifs are dispersed along the kinase sequences, on 3D kinase models most of them form a common surface. We replaced these amino acids one by one in the ROP-activated RLCK VI_A2 kinase with typical amino acids of B-type kinases, using site-specific mutagenesis. Changing the motifs prevented ROP GTPase binding in yeast two hybrid system. The mutant kinases were produced in and purified from a bacterial protein expression system. The in vitro auto-phosphorylation activity of the kinase was completely eliminated in the case of certain mutations, while in other instances the activity become independent of the presence of the ROP GTPase. These results were confirmed with the RLCK VI_A3 kinase. As this kinase (in contrast to VI_A2) well phosphorylates the myelin basic protein substrate, we could demonstrate that the mutations affect substrate phosphorylation and auto-phosphorylation in the same way.

We were interested in how ROP-activated kinases are widespread in the plant kingdom. Therefore the evolutionary conservation of the position of ROP-binding amino acids was investigated in kinase sequences of different plant taxa with available whole genome sequence. We could conclude that RLCK VI A-type kinases exist in multicellular plants but not in unicellular algae. Consequently, their function is probably not essential for basic cell functions, but is rather required for the development of a multicellular organisms. This is supported by our experiments using transgenic and mutant plants with altered kinase expression. These studies show that the kinases regulate plant growth and morphology. That the in planta functions of these kinases are dependent on ROP GTPases, still need to be justified. The ROP-binding mutant kinase forms we produced may serve as a tool to answer this question.

Supervisor: Attila Fehér
E-mail: lajko.dezi@brc.mta.hu

Cell response to UV irradiation: Bomapin is a new member in the damage response

Hajnalka Majoros

Department of Biochemistry and Molecular Biology, University of Szeged, Szeged, Hungary

Ultraviolet light-induced DNA damage response is an extensively studied process because improper or delayed repair of the errors could lead to cancerous malformations. Although several proteins involved in DNA damage repair have been identified, the entire repair cascade and the protein-protein interactions remain to be explored. Based on the wavelengths UV light is classified as UV A, B and C. UV C irradiation is absorbed mostly by the ozone layer, therefore under normal circumstances, UV A and UV B are the major causes of DNA damage in epidermal cells. In order to study the process of DNA damage response upon UV B irradiation, we used keratinocyte cells to test their DNA repair abilities and analyze the possible mechanism which could lead to skin cancer progression.

In a primary screen we identified Bomapin as one of the most dramatically up-regulated gene in keratinocytes following UV B irradiation. Bomapin (SPB10) is a serine protease inhibitor which promotes cell proliferation under normal circumstances in hematopoietic and myeloid leukemia cells. In the absence of growth factors Bomapin can induce apoptosis. However, only a few pieces of data have been reported about the function of Bomapin. Based on these findings, it seemed to be interesting to determine whether this protein has a gain-of-function effect in cancer evolution. To learn about the possible biological function of *Bomapin* induction upon UV B treatment we used immortalized keratinocyte (Hker E6SFM, HaCat) and melanoma (A375) cell lines. As a first step we analyzed the kinetics of UVB induction by determining the mRNA level in different time intervals following irradiation (2h, 8h, and 24h). We found that *Bomapin* mRNA level was increased 2 and especially 8 hours after UVB irradiation (80 J/m^2), while 24h after the treatment the mRNA level was decreased in each the examined cell lines. The mRNA level of *Bomapin* was also increased upon a low intensity (20 J/m^2) UV B irradiation in primer keratinocyte cells. Accordingly, the transient change of mRNA level suggests that Bomapin might be involved in the UV B induced stress response. In accord with the elevated Bomapin mRNA level, higher amount of Bomapin protein could be detected after DNA damage induced by ultraviolet irradiation (2 h, 8 h, and 24 h) in Hker cells, suggesting Bomapin involvement in UV B damage response. Since the protein seems to be a possible player in UV B repair in the following step we investigated the subcellular localization of Bomapin protein. We found that under normal circumstances, Bomapin protein was mostly present in the cytoplasm and co-localized with tubulin. Few hours after DNA damage Bomapin is transported to the nucleus from the cytoplasm and it is detectable in chromatin bound foci suggesting its possible importance in the repair process of damaged DNA.

In summary, our results suggest that Bomapin protein could be an essential player in the UV B induced stress response. Further experiments are needed to clarify its regulatory function in UV B response.

This work was supported by grants from *TÁMOP-4.2.2-08/1-2008-0001*, *TÁMOP-4.2.2.A-11/1/KONV-2012-0035* and *TÁMOP-4.1.1.C-12/1/KONV-2012-0014*.

Supervisor: Imre Miklós Boros
E-mail: borosi@bio.u-szeged.hu