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Generation of oxidative products by phagocytic cells is known to be one of the important host defence mechanisms directed towards the killing of invading microorganisms (Gallin at al. 1993). Catalases may provide protection against reactive oxygen species produced by neutrophile granulocytes of the human immune system (Chang et al. 1998). Neutropenia is a considerable risk factor of zygomyçoses. In this study, catalase encoding genes of *R. oryzae* have been isolated, and their functional analysis has been started.

Four possible catalase genes were found in the *R. oryzae* genome database (*Rhizopus oryzae* Sequencing Project) by similarity searches with known fungal catalases. These genes and their adjacent regions were amplified by PCR from the genomic DNA of *R. oryzae* and cloned into the vector pBluescriptII SK+ (Stratagene). To reveal their function and to investigate their possible role in the pathogenicity, deletion mutants were created in the case of each isolated genes. Four vectors suitable to create deletions in the different genes were constructed; in each vector, the *pyrG* gene of *R. oryzae* encoding orotidine-5'-monophosphate decarboxylase was placed between the 5' and 3' flanking regions of the appropriate catalase genes. To ensure double crossover gene replacement, linear fragments were cut from the plasmids and used to transform protoplasts of a uracil auxotrophic *R. oryzae* strain using the polyethylene glycol-mediated method.

Integration of the transferred DNAs into the host genome and deletion of the appropriate catalase genes was proven by PCR and Southern blot analysis. Catalase activity of the recipient strain and the four mutants constructed were *in vitro* tested. Effect of hydrogen peroxide on the fungal growth was examined on agar plates and in a microtiter plate assay. All four catalase genes proved to be functional. In all types of mutants, deletion of a catalase gene increased markedly the sensitivity of the transformants to hydrogen peroxide. The strain deficient in the gene designated as 16995 was the less susceptible to hydrogen peroxide whereas the strains deficient in the other genes proved to be more sensitive. Further gene expression studies with isolated genes are in progress and we also plan to use the constructed deletion mutants in pathogenicity tests.

Chang YC, Segal BH, Holland SM, Miller GF, ÉS Kwon-Chunn KJ (1998) Virulence of Catalase-deficient *Aspergillus nidulans* inp47phox-/-,Mice. J Clin Invest 101:1843-1850.
Gallin JI, Buescher ES, Seligmann BE, Nath J, Gaither T, Katz P (1983) NIH conference. Recent advances in chronic granulomatous disease. *Ann. Intern. Med.* 99:657–674.
Papp T, Nyilasi I, Csernetics Á, Galgóczy L, Vágvölgyi Cs (2008) Molecular studies on Zygomycetes fungi causing opportunistic infections. *Rev Med Microbiol* 19:47-55.
*Rhizopus oryzae* Sequencing Project, Broad Institute of Harvard and MIT 2004, htp://www.broad.mit.edu
Ribes JA, Vanover-Sams CL, Baker DJ (2000) Zygomycetes in Human Disease. Clin Microbiol Rev 13:236-301.

Supervisor: Tamás Papp
E-mail: bealinka@gmail.com

## Comprehensive genetic and biochemical examination of the polyubiquitin receptors in *Drosophila melanogaster*

Zoltán Lipinszki

Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

The ubiquitin-proteasome system is responsible for the polyubiquitilation and selective degradation of damaged, misfolded and short-lived regulatory proteins to ensure the proper homeostasis of the eukaryotic cell. Recognition of polyubiquitinated substrates by the proteasome is a highly regulated process that requires polyubiquitin receptors (p54/Rpn10: proteasome receptor subunit; Dsk2 and Rad23: non-proteasomal receptors). The mechanism of substrate recognition and delivery to the proteasome is well known in single cell eukaryotes (e.g. yeast), but unresolved in Metazoans.

We found that the subunit composition of the regulatory particle (RC) of the *Drosophila* 26S proteasome changes in a developmentally regulated fashion (Lipinszki et. al. submitted manuscript). The concentration of the p54/Rpn10 subunit falls suddenly at the end of embryogenesis, remains low throughout the larval stages, starts to increase again in the late third instar larvae and remains high in pupae and adults. A similar developmentally regulated fluctuation could be observed in the concentrations of the Rad23 and Dsk2 extraproteasomal polyubiquitin receptors. Our *in vitro* experiments revealed that protein extracts of first or second instar larvae can selectively degrade the embryonic p54/Rpn10 subunit of the 26S proteasome and the Dsk2 and Rad23 polyubiquitin receptors; whereas all the other tested proteins remained intact. The above observations and the fact that the gene expressions of the receptors remain constant during the development suggest that a selective protease is activated during the early larval stages. We successfully purified and identified this protease. Moreover, all the three receptors carry an extended intrinsically unstructured segment within the molecule, which can be the hot spot for the regulator protease.

To follow the *in vivo* fate of subunit p54/Rpn10, transgenic *Drosophila melanogaster* lines encoding the N-terminal half (NTH), the C-terminal half (CTH) or the full-length p54/Rpn10 subunit have been established in the inducible Gal4-UAS system. The daughterless-Gal4 driven whole-body expression of the full-length subunit or its NTH did not produce any detectable phenotypic changes and the transgenic products were incorporated into the 26S proteasome. The transgene-encoded CTH was not incorporated into the 26S proteasome, caused third instar larval lethality and it was found to be multiubiquitinated. This modification, however, did not appear to be a degradation signal, the half-life of the CTH is over 48 hours. The accumulation of the CTH disturbed the developmentally regulated changes of the subunit composition of the RP and, interestingly, the emergence of the selective proteolytic activity responsible for the depletion of the polyubiquitin

receptors. The accumulation of CTH also suspended the MG132 insensitive (proteasome inhibitor), but PMSF (serine-type endopeptidase inhibitor) sensitive proteolytic degradation of the p54/Rpn10, Dsk2 and Rad23 during the early larval stages.

Interestingly, CTH carrying three active UIM sequences extra-proteasomally traps the Dsk2 protein, hindering its interaction with the 26S proteasome. Our *in vitro* and *in vivo* studies revealed that in *Drosophila* UIM motifs of p54 can selectively bound the N-terminal UBL (ubiquitin like) domain of Dsk2. We suppose that contrary to the yeast model in which Rpn1 and Rpn2 scaffold subunits of the RC anchor Dsk2, Rad23 and Ddi1, in *Drosophila* the major polyubiquitin receptor Dsk2 (Lipinszki et. al. manuscript in preparation) docs to the C-terminally localized UIMs of the p54. Nevertheless, is has been demonstrated that p54 is a shuttling subunit of the proteasome (Kiss et. al., Szabó et. al.). It is conceivable that under regulation (e.g. ubiquitilation) p54 dissociates from the proteasome, and forms a heteromer with the Dsk2/substrate dimer, which is followed by the reassociation of the whole complex to the proteasome for degradation of the substrate protein.

Kiss P, Szabó A, Hunyadi-Gulyás E, Medzihradszky KF, Lipinszki Z, Pál M, Udvardy A (2005) Zn2+-induced reversible dissociation of subunit Rpn10/p54 of the Drosophila 26 S proteasome. Biochemical J (2005) 391, 301-310.

Lipinszki et al. (Submitted manuscript; JCS)

Szabó Á, Pál M, Deák P, Kiss P, Újfaludi Zs, Pankotai T, Lipinszki Z, Udvardy A (2007) Molecular characterization of the Rpt1/p48B ATPase subunit of the *Drosophila melanogaster* 26S proteasome. Mol Genet Genomics 278(1):17-29.

Supervisor: Andor Udvardy
e-mail: lzoltan@brc.hu

## Neuroprotection in ischemic adult rat brain

Máté Marosi

Department of Physiology, Anatomy and Neuroscience, University of Szeged, Szeged, Hungary

Transient global ischemia elicits selective, delayed neuronal death. If the ischemia is short, neuronal damage occurs only in vulnerable areas (Pulsinelli et al. 1985). The pyramidal neurons in the hippocampal CA1 region are very vulnerable. Global ischemia impairs memory and learning functions. It is widely accepted that activation of the excitatory amino acid receptors plays an important role in neuronal death in stroke (Choi 1988). It has recently been reported that glutamate-induced excitotoxicity and a cellular calcium overload are among the key factors of cell death in brain ischemia, especially in the gray matter. By definition, excitotoxicity is a result of overexcitation of the glutamate receptors. In turn, neuroprotective strategies have utilized antagonists of the glutamate receptors to prevent excitotoxic neuronal loss.

The neuroprotective effect of L-kynurenine sulfate (KYN) was studied. KYN pretreatment decreased the number of injured pyramidal cells in the CA1 region of the hippocampus in the four-vessel occlusion (4VO)-induced ischemic adult rat brain. KYN post-treatment proved to be much less effective. In parallel with the histology, a protective effect of KYN on the functioning of the CA1 region was observed: long-term potentiation (LTP) was abolished in the 4VO animals, but its level and duration were restored by pretreatment with KYN. It is concluded that the administration of KYN elevates the KYNA concentration in the brain to neuroprotective levels (Sas et al. 2008).

The excess Glu which causes neuronal death via excitotoxicity, is normally controlled by members of a family of $Na^+$-dependent Glu transporters. By pumping Glu, they guarantee the presence of Glu in brain fluids at levels at which its exerts neither excitotoxic nor unsolicited excitatory effects. Glu transporters located on the brain vasculature may also play an important role in controlling extracellular Glu levels via a brain-to-blood Glu efflux. The scavenging of blood Glu increases the driving force for the brain-to-blood Glu efflux and causes a decrease of the excess Glu present in the brain. (Teichberg et al. 2008)

In the second series of experiments we evaluated the effects of the blood glutamate scavenger oxaloacetate on the impaired LTP observed in the rat 2-vessel occlusion ischemia model. Transient incomplete forebrain ischaemia was produced 3 days before LTP induction. Although the short transient brain ischaemia did not induce histologically identifiable injuries, it resulted in an impaired LTP function in the hippocampal CA1 region without damaging the basal synaptic transmission between the Schaffer collaterals and the pyramidal neurons. This impairment could be fended off in a dose-dependent manner by the i.v. administration of oxaloacetate immediately after the transient hypoperfusion. These results suggest that oxaloacetate-mediated blood and brain glutamate scavenging contributes to the restoration of the LTP after its impairment by brain ischaemia. (Marosi et al. 2009)

Our results suggest that both agents have potential clinical usefulness for the prevention of neuronal loss in stroke conditions.

Choi DW (1988) Glutamate neurotoxicity and diseases of the nervous system. Neuron 1:623-634.

Marosi M; Fuzik J; Nagy D; Rákos G; Kis Z; Toldi J; Teichberg VI; Ruban-Matuzani A; Farkas T (2009) Oxaloacetate restores the long-term potentiation impaired in rat hippocampus CA1 region by 2-vessel occlusion. Eur. J. Pharm. 604:51-57.

Pulsinelli WA (1985) Selective neuronal vulnerability: morphological and molecular characteristics. Prog. Brain Res. 63:29–37.

Sas K, Robotka H, Rozsa E, Agoston M, Szenasi G, Gigler G, Marosi M, Kis Z, Farkas T, Vecsei L, Toldi J, (2008) Kynurenine diminishes the ischemia-induced histological and electrophysiological deficits in the rat hippocampus. Neurobiol Dis. 32:302–308.

Teichberg VI, Cohen-Kashi-Malina K, Cooper I, Zlotnik A, (2009). Homeostasis of glutamate in brain fluids: an accelerated brain-to-blood efflux of excess glutamate is produced by blood glutamate scavenging and offers protection from neuropathologies. Neuroscience 158(1):301-308. Epub 2008 Mar 18.

Supervisor: Tamás Farkas
E-mail: marosi.mate@gmail.com