YSF-121
The RNA component of ribonuclease P from *Dictyostelium discoideum*

A. Vourekas[1], V. Stamatopoulou[1], C. Stathopoulos[2] and D. Drainas[1]
[1]*Department of Biochemistry, School of Medicine, University of Patras, Patras, GREECE,* [2]*Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, GREECE*

Introduction: Ribonuclease P is an essential enzyme that endonucleolytically cleaves all precursor tRNA transcripts to produce their mature 5′ ends. The biological importance of RNase P is underscored by its presence in all organisms. All forms contain a similar in size RNA subunit which is absolutely required for catalysis. However, the size and number of protein subunits of the holoenzyme varies significantly, from one small subunit in bacteria to ten subunits in human RNase P. The putative gene of the RNA subunit of *D. discoideum* RNase P was recently identified through phylogenetic comparison (1), but provided no clues for its possible catalytic attributes reported by our lab previously (2).
Methods: *D. discoideum* RNase P RNA sequence identified by Marquez *et al.* (1) was cloned by PCR. The gene sequence was certified by primer extension and 3′ RACE of RNA extracted from active RNase P fractions. Northern blot analysis was used for size estimation of various fragments of RNA subunit present in RNase P preparations. Active RNase P fractions were treated with micrococcal nuclease for the destruction of the native RNA subunit. Various *in vitro* transcripts of the RNA gene were assayed for ribozyme activity and used in reconstitution assays for the rescue of the catalytic activity of the MN treated enzymatic complex.
Results and discussion: *D. discoideum* RNase P has distinctive characteristics compared to other eukaryotic RNase P enzymes. It harbors fewer protein subunits, which are larger than their homologues in other eukaryotes. Our biochemical analysis certified the RNase P RNA gene. Two transcripts of this gene are present in active fractions, the full length and a smaller transcript lacking 70 nts at the 5′ end. Both fragments were cloned and were used in reconstitution assays where they substituted the native RNA subunits. Both fragments were capable of rescuing RNase P enzymatic activity, which was previously eliminated by nuclease treatment. These results provide novel insight on RNase P RNA structure and function.
References:
1. Stathopoulos C, Tekos A, Zarkadis IK and Drainas D. *Eur. J. Biochem.* 2001; **268**: 2134–2140.
2. Marquez SM, Harris JK, Kelley ST, Brown JW, Dawson SC, Roberts EC and Pace NR. *RNA* 2005; **11**: 739–751.

YSF-122
Putative posttranslational modifications of *Paramecium* RAB7 isotypes may result in distinct electrophoretic migration pattern

E. Wypych, M. Osinska, J. Wiejak and E. Wyroba
Nencki Institute of Experimental Biology, Warsaw, POLAND

Introduction Traffic along endocytic pathway is regulated by the family of Rab proteins directing internalized cargo to different destinations. Two paralogous genes encoding Rab7 were cloned by us in evolutionary ancient *Paramecium*. The overall predicted protein sequences of *Paramecium* Rab7 isotypes exhibit higher similarity to human counterpart than to Rab7 deriving from parasitic protozoa. The deduced sequences of 206 amino acids are 97.6% identical. Interestingly, specific antipeptide antibodies detected single cross-reacting polypeptides of ˜21 kDa for Rab7a and ˜23 kDa for Rab7b.
Methods Mass spectrometry and detection of putative phosphorylation/glycosylation modifications were performed to clarify this pattern. Cell homogenate prepared with phosphatase inhibitors was subjected to SDS-PAGE: one half of the gel was silver-stained whereas the identical second half was electrotransferred onto nitrocellulose and immunoanalyzed with specific antibodies.
Results Mass spectral data revealed that silver-stained protein bands corresponding to those observed in Western blot contained the peptides matching both the Rab7 isotypes. Phosphorylation pattern was analyzed with Pro-Q Diamond that detects all phosphorylated residues and with antibodies specific for phospho-serine, -threonine and –tyrosine, respectively. In both cases the subsequent immunodetection of the same blot was performed. No significant difference was observed in phosphorylation pattern for both isotypes. Pro-Q Emerald staining revealed the glycosylated band corresponding to Rab7b isotype.
Conclusions These results indicate that distinct pattern of *Paramecium* Rab7 isotypes migration in SDS-PAGE could be the result of posttranslational glycosylation of Rab7b.
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Cloning and sequence analysis of the human CA9 promoter

H. Yildirim and F. Köçkar
Balikesir University Faculty of Science and Literature, Department of Biology, Balikesir, TURKEY

Carbonic anhydrases (CAs) are a group of zinc-containing metalloenzymes that catalyse the reversible reaction from H_2O and CO_2 to HCO_3^- ions. There are fifteen mammalian CA isozymes with different tissue distributions and locations. Of the fifteen isozymes, CA IX has been linked to the tumors and neoplastic invasion. CA IX is ectopically expressed at relatively high levels and with a high prevalence in some tumor tissues whose normal counterparts do not contain this protein, e.g. carcinomas of the cervix uteri, esophagus, kidney, lung and breast. On the other hand, tumors originating from tissues with high natural CA IX expression, such as the stomach and gallbladder, often lose some or all of their CA IX upon conversion to carcinomas. The aim of the study is to cloning, sequence and functional analysis of human CA9 promoter. Intially, 1284 bp of human CA9 promoter was amplified using PCR-based strategy using the human blood genomic DNA as a template and cloned into the luciferace vector, pGL2 basic. Then, the sequence analysis of human CA9 promoter was performed. Comparison of the sequence of human CA9 promoter to other promoter of mammalian homologues have also been carried out. The putative transcription factor binding sites was found by using TRANSFAC database. For functional analysis of hCA9 promoter, the full length promoter in the pGL2 basic vector was transfected into Hep3B cell lines by Ca phosphate transfection method. The basal promoter activity of human CA9 promoter was determined as time and concentration dependent manner.

YSF-124
Intermediate filament misregulation, GLAST down-regulation and MAPK activation in developing brain of rats with congenital hypothyroidism

A. Zamoner, L. Heimfarth and R. Pessoa-Pureur
Departamento de Bioquimica, Instituto de Ciencias Basicas da Saude, Porto Alegre, BRAZIL

Developmental thyroid hormone (TH) deficiency leads to mental retardation and neurological deficits in humans. In this study congenital hypothyroidism was induced in rats by adding 0.05 % 6-propyl-2-thiouracil in the drinking water during gestation and suckling period. This treatment induced hyperphosphorylation of neuronal intermediate filament (IF) proteins, neurofilaments of high, medium and low molecular weight (NF-H, NF-M and NF-L, respectively) without altering the phosphorylation level of astrocyte IF proteins, glial fibrillary acidic protein (GFAP) and vimentin in cerebral cortex of rats. Furthermore, the immunocontent of GFAP and NF subunits was down-regulated, while vimentin was unaltered in tissue homogenate of hypothyroid animals. Nevertheless, the immunocontent of the Triton-insoluble IF proteins showed that NF subunits and GFAP were also decreased, while vimentin was unaltered, suggesting that hyperphosphorylation partially interfered with NF polymerization/aggregation ability. Moreover, we verified the immunocontent of astrocyte glutamate/aspartate transporter (GLAST), as well as activation of mitogen-activated protein kinases (MAPK) in hypothyroid rats. Results showed that hypothyroidism is associated with decreased GLAST immunocontent. Otherwise, we demonstrated increased extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation without altering Jun N-terminal kinase (JNK) and $p38^{MAPK}$ phosphorylation. However, total JNK levels were down-regulated. Taken together, these results suggest that the thyroid status could modulate the integrity of neuronal cytoskeleton acting on the endogenous NF-associated phosphorylating system and that such effect could be related to glutamate-induced excitotoxicity, as well as ERK1/2 and JNK modulation. These events could be somehow related to the neurological dysfunction described in hypothyroidism.