

# MOLECULAR BIOLOGY CENTER

## ***Laboratories:***

- Molecular Glycobiology Laboratory;
- High Performance Liquid Chromatography (Hplc) Laboratory;
- Sequencing and Genetic Analysis Laboratory;
- Drosophila.

Different rooms are allocated to: MARINE BIOLOGY, CHROMATOGRAPHY & SPECTROPHOTOMETRY, GENETIC ANALYSIS, CELL CULTURE, ELECTROPHORESIS & PCR, SAMPLE PREPARATION & OPTICAL MICROSCOPY, STERILIZATION & DECONTAMINATION, BACTERIAL CULTURE, PhD Students and Post-docs. In addition, we recently setup an ANIMAL FACILITY in our Center.

## ***Research topics:***

- Structural and Functional Genomics.
- Molecular Taxonomy and Phylogeny.
- Molecular Biotechnologies.
- Molecular Glycobiology
- Mobile Genetic Elements - Retrotransposons
- Marine Biology
- Cellular and Molecular Immunology

## ***Research team:***

- Octavian Popescu, PhD, professor, [opopescu@biolog.ubbcluj.ro](mailto:opopescu@biolog.ubbcluj.ro)
- Annette DAMERT, PhD, sci. res. gr. III, [adamert@hasdeu.ubbcluj.ro](mailto:adamert@hasdeu.ubbcluj.ro)
- Mircea Teodor CHIRIAC, PhD, sci. res. gr. III, [mtchiriac@yahoo.com](mailto:mtchiriac@yahoo.com)
- Oana HERLEA, PhD, sci. res., [h\\_ariadna77@yahoo.com](mailto:h_ariadna77@yahoo.com)
- Beatrice KELEMEN, PhD, sci. res., [bkelemen@hasdeu.ubbcluj.ro](mailto:bkelemen@hasdeu.ubbcluj.ro)
- Beatrix FERENCZ, PhD, sci. res., [beatrixferencz@yahoo.com](mailto:beatrixferencz@yahoo.com)
- Iulia LUPAN, PhD, asist., [iulia.lupan@gmail.com](mailto:iulia.lupan@gmail.com)
- Băcilă Ioan, PhD Student,
- Basangiu Bianca, PhD Student,
- Bücs Szilárd, PhD Student,

- Chilici Anet, PhD Student,
- Jakab Endre, PhD Student,
- Şuteu Dana, PhD Student,
- Bálint Miklós, PhD Student,
- Nagy Csaba, PhD Student,
- Chira Sergio, PhD Student,
- Csorba Kinga Melinda, PhD Student,
- Marosi Albert Bela, PhD Student,

***Main equipments /Facilities:***

Molecular biology requires technology that is simple to use yet adequately supply for a variety of applications. The most important equipments of the center are represented by:

- ***The ABI Prism 310 Genetic Analyzer***, an automated system for sequencing, sizing and quantifying nucleic acids. This apparatus can be also used for DNA-based human identification (processing of forensic casework, establishing population databases, performing paternity testing, etc).
- ***The Beckman Coulter CEQ 8800***, a flexible enough Genetic Analysis System to adapt to a broad spectrum of applications, yet easily accessible to a wide range of users.
- ***The reversed microscope NIKON Eclipse TS2000*** with LUCIA-measurement software, using high-tech standards for light, phase-contrast and fluorescence microscopy.
- ***The JASCO HPLC system including two PU-2080 pumps***, DG-1580 in-line degasser, HG-1580-30 high pressure mixer, MD-1510 diode array detector, Evaporative Light Scattering detector and PC system (LC-Net II control and JASCO-BORWIN acquisition).



***Current research projects:***

- “Bioinformatics of gene sequences involved in prokaryotic cell division”, (CEEX 52/2005), coordinator;
- “Patogenomic integrate network (platform) for transfer of the research results in biomedical field”, (CEEX 28/2005), partner;

- “Biochemical and genetic characterization of homocysteine metabolism and redox status in autism. Therapeutic implications”, (CEEX 83/2006), partner;
- “Biochemical, neurocognitive and imaging markers in populations with high genetic risk for psychoses and at the first psychotic event”, (CEEX 97/2006);
- “Serological and molecular variability of *Plum Pox* virus isolates from Romania and geographic distribution at the level of main pomicultural regions”, (CEEX 102/2006);
- “Molecular diagnostic of bullous epidermolyses. Modern research techniques for diagnosis, treatment and prevention of bullous epidermolyses. The carrying out of a national genodermatosis data base”, (CEEX 126/2006);
- “Molecular biotechnologies for labeling of L-amino acids and proteins using  $^{15}\text{N}$ ”, (CEEX 129/2006);
- “Inflammation in atherosclerosis: modulation of gene expression for fractalkine, apoE, NADPH oxidoreductase, VEGF by inflammation mediators; the prevention/reversion capacity of certain drugs”, (CEEX 130/2006), partner;
- “Biodiversity of *Pyramidellidae* gastropods from Romanian Black Sea using molecular ecology and taxonomy”, (CNCSIS cod A 1341), coordinator.
- “Structure and dynamics of *Rapana venosa* stock from Romanian Black Sea; characterization of growth, productivity and genetic diversity of this species”, (CNCSIS cod A\_C 67), partner.

***Partners and collaborators:***

- Paul-Ehrlich-Institut, Langen, Germany
- UK-SH Luebeck, Germany
- “I. Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca
- University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca
- The Technical University, Cluj-Napoca
- Institute of Biological Research, Cluj-Napoca
- National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca
- Institute of Cellular Biology and Pathology Nicolae Simionescu, Bucharest
- "Cantacuzino" National Institute of research and development for Microbiology and Immunology, Bucharest
- "Grigore Antipa" National Museum of Natural History, Bucharest

- "Grigore Antipa" National Institute for Marine Research and Development Constanta
- Romanian Marine Station, Agigea

***Publications:***

1. Kirilyuk A, Tolstonog Gv, Damert A, Held U, Hahn S, Loewer R, Buschmann C, Horn Av, Traub P, Schumann Gg,  
*Functional endogenous LINE-1 retrotransposons are expressed and mobilized in rat chloroleukemia cells,*  
Nucleic Acid Res, **36**, 648-665, 2008.
2. Sesarman A, Mihai S, Chiriac Mt, Olaru F, Sitaru Ag, Thurman Jm, Zillikens D, Sitaru C,  
*Binding of avian IgY to type VII collagen does not activate complement and leukocytes and fails to induce subepidermal blistering in mice,*  
Brit J Dermatol, **158**, 463-471, 2008.
3. Mavros M, Chirita V, Popescu O, Ferencz B,  
*The genetic polymorphism of MTHFR gene in schizophrenia,*  
Rev Med Chir Soc Med Nat Iasi, Jan-Mar;112, 76-82, 2008.
4. Chiriac M, Lupan I, Bucurenci N, Popescu O, Palibroda N,  
*Stereoselective synthesis of L-[<sup>15</sup>N] amino acids with glucose dehydrogenase and galactose mutarotase as NADH regenerating system,*  
J. Label Compd Radiopharm, **51**, 171-174, 2008.
5. Balint M, Barnard Pc, Schmitt T, Ujvarosi L, Popescu O,  
*Differentiation and speciation in mountain streams: a case study in the caddisfly Rhyacophila aquitanica (Trichoptera),*  
J Zool Syst Evol Res, **46**, 340-345, 2008.
6. Zagrai, L., Zagrai, I., Ferencz, B., Gaboreanu, I., Kovacs, K., Petricele, I., Popescu, O., Pamfil D., Capote N.,  
*Serological and molecular typing of Plum pox virus isolates in the North of Romania,*  
J Plant Pathol, **90**, 41-46, 2008.
7. Lupan I, Chira S, Chiriac M, Palibroda N, Popescu O,  
*Enzymatic synthesis of 15N-L-aspartic acid using recombinant aspartase from Escherichia coli K12,*  
Rev Chim, **59**, 1216-1217, 2008.
8. Sanziana MICU, Beatrice KELEMEN and Gheorghe MUSTAT

*Current distribution and shell morphotypes of rapana venosa (valenciennes, 1846) in the agigea 4m littoral*  
Analele Științifice ale Universității „Al. I. Cuza” Iași, s. Biologie animală,  
Tom LIV, 2008

### ***Abstracts:***

Kirilyuk A, Tolstonog Gv, Damert A, Held U, Hahn S, Loewer R,  
Buschmann C, Horn Av, Traub P, Schumann Gg,  
*Functional endogenous LINE-1 retrotransposons are expressed and mobilized in rat chloroleukemia cells,*  
Nucleic Acid Res, **36**, 648-665, 2008.

LINE-1 (L1) is a highly successful autonomous non-LTR retrotransposon and a major force shaping mammalian genomes. Although there are about 600 000 L1 copies covering 23% of the rat genome, full-length rat L1s (L1Rn) with intact open reading frames (ORFs) representing functional master copies for retrotransposition have not been identified yet. In conjunction with studies to elucidate the role of L1 retrotransposons in tumorigenesis, we isolated and characterized 10 different cDNAs from transcribed full-length L1Rn elements in rat chloroleukemia (RCL) cells, each encoding intact ORF1 proteins (ORF1p). We identified the first functional L1Rn retrotransposon from this pool of cDNAs, determined its activity in HeLa cells and in the RCL cell line the cDNAs originated from and demonstrate that it is mobilized in the tumor cell line in which it is expressed. Furthermore, we generated monoclonal antibodies directed against L1Rn ORF1 and ORF2-encoded recombinant proteins, analyzed the expression of L1-encoded proteins and found ORF1p predominantly in the nucleus. Our results support the hypothesis that the reported explosive amplification of genomic L1Rn sequences after their transcriptional activation in RCL cells is based on L1 retrotransposition. Therefore, L1 activity might be one cause for genomic instability observed during the progression of leukemia.

Sesarman A, Mihai S, Chiriac Mt, Olaru F, Sitaru Ag, Thurman Jm, Zillikens D,  
Sitaru C,

*Binding of avian IgY to type VII collagen does not activate complement and leukocytes and fails to induce subepidermal blistering in mice,*  
Brit J Dermatol, **158**, 463-471, 2008.

Epidermolysis bullosa acquisita (EBA) is a severe autoimmune skin disease characterized by tissue-bound and circulating autoantibodies to type VII collagen, the major component of anchoring fibrils. When passively transferred into mice, rabbit IgG against type VII collagen induces Fc-dependent activation of complement, the recruitment of leucocytes into the skin, and subepidermal blistering. In addition to these inflammatory mechanisms, clinical and experimental evidence suggests that antibodies against type VII collagen might induce blisters by disrupting the ligand function of type VII collagen by an Fc-independent mechanism. OBJECTIVES: To study noninflammatory mechanisms of blister formation in

experimental EBA. METHODS: We generated chicken IgY antibodies directed to recombinant type VII collagen and examined their pathogenic activity using ex vivo and animal models. RESULTS: Mice injected with these chicken IgY antibodies showed binding of the antibodies to the dermal-epidermal junction of skin sections. However, IgY antibodies did not fix complement C3 in enzyme-linked immunosorbent assay and immunofluorescence complement-binding assays. In addition, IgY antibodies did not induce dermal-epidermal separation ex vivo. Following their passive transfer into Balb/c mice, chicken IgY antibodies against type VII collagen bound at the dermal-epidermal junction, but, in contrast to rabbit IgG, did not fix complement C3, recruit granulocytes, or induce skin blisters. CONCLUSIONS: These findings demonstrate that binding of avian IgY to type VII collagen is not pathogenic in vivo and strongly suggest that in experimental EBA, antibodies to type VII collagen induce blisters not by direct disruption of the ligand function of type VII collagen, but rather by an Fc-dependent engagement of humoral and cellular inflammatory factors.

Mavros M, Chirita V, Popescu O, Ferencz B,  
*The genetic polymorphism of MTHFR gene in schizophrenia,*  
Rev Med Chir Soc Med Nat Iasi, Jan-Mar;112, 76-82, 2008.

The C677T polymorphism of the MTHFR gene, resulting in hyperhomocysteinemia, has been shown to be implicated in the etiology of schizophrenia. Previous studies showed that A1298C polymorphism seems not to be related to schizophrenia. AIM OF THE STUDY: To analyze two genetic polymorphisms of the MTHFR gene, C677T and A1298C in 44 patients with schizophrenia and evaluate its relationship with the risk of schizophrenia and with some clinical aspects. MATERIAL AND METHOD: We determined the presence of the C677T and A1298C mutations of the MTHFR gene in 44 in patients with schizophrenia and in 35 normal controls. The patients were assessed by psychiatric examination and scalar evaluation. RESULTS: 28 (66.7%) of the patient group had the T allele of the C677T genetic polymorphism, compared to 11 (34.3%) subjects of the control group. The intensity of the positive, negative and general symptoms was slightly higher in the patients presenting the T allele. The A1298C missense mutation was more frequent between control subjects (57.5%) compared to the patient group (39%). The intensity of the positive symptoms was slightly increased in the patients with the missense mutation in the position 1298, but the intensity of the negative and general symptoms did not differ. CONCLUSIONS: Our study confirms the role of the C677T genetic polymorphism in the susceptibility for schizophrenia. The relationship between A1298C genetic polymorphism and schizophrenia was not demonstrated in our study.

Chiriac M, Lupan I, Bucurenci N, Popescu O, Palibroda N,  
*Stereoselective synthesis of L-[<sup>15</sup>N] amino acids with glucose dehydrogenase and galactose mutarotase as NADH regenerating system,*  
J. Label Compd Radiopharm, **51**, 171-174, 2008.

We have developed an efficient stereospecific enzymatic synthesis of L-[<sup>15</sup>N]-valine, L-[<sup>15</sup>N]-leucine, L-[<sup>15</sup>N]-norvaline, L-[<sup>15</sup>N]-norleucine and L-[<sup>15</sup>N]-isoleucine from the corresponding  $\alpha$ -keto acids by coupling the reactions catalysed by leucine dehydrogenase and glucose dehydrogenase/galactose mutarotase. Giving high yields of L-amino acids, the

procedure is economical and easy to perform and to monitor at a synthetically useful scale (1–10 g).

Balint M, Barnard Pc, Schmitt T, Ujvarosi L, Popescu O,  
*Differentiation and speciation in mountain streams: a case study in the caddisfly Rhyacophila aquitanica (Trichoptera),*  
J Zool Syst Evol Res, **46**, 340-345, 2008.

Few studies have analysed the biogeography of mountain aquatic organisms, although this habitat provides stable conditions in which many species survived Pleistocene climatic oscillations, usually in the geographical vicinity of their present distribution ranges. The mountain caddisfly *Rhyacophila aquitanica* was selected as a model organism for this habitat type. Morphological measurements of genitalia and external characters of male individuals were obtained from almost the entire range of distribution of the species. Morphometric results were analysed by cluster analysis and multivariate statistics. Important differences were discovered among three population groups of *R. aquitanica* inhabiting different European mountain ranges: (i) mountain ranges north-west of the Alps (Massif Central, Vosges, Schwarzwald, Fribourg), (ii) the southern Alps (Lombardia and Carinthia) and (iii) the western part of the southern Carpathians. This divergence suggests a long-term isolation among these groups, which presumably took place long before the last Pleistocene glaciation, with no secondary contact among these populations. The differentiation centres of the southern Alps and Carpathian groups may have been mostly homotopic to their actual ranges, whereas the western group must have been distributed in the areas west or north-west of the Alps with secondary expansions and disjunctions.

Zagrai, L., Zagrai, I., Ferencz, B., Gaboreanu, I., Kovacs, K., Petricele, I., Popescu, O., Pamfil D., Capote N.,  
*Serological and molecular typing of Plum pox virus isolates in the North of Romania,*  
J Plant Pathol, **90**, 41-46, 2008.

Plum pox virus (PPV) is considered as the most dangerous viral pathogen of stone fruits. Although PPV is widespread in Romania and causes serious yield losses, little is known about the variability of its isolates. To secure this information we investigated 43 PPV isolates collected from five different plum orchards in the North of Romania in the Bistrita plum-growing area. PPV strains were serologically tested by TAS-ELISA using PPV-D and PPV-M specific monoclonal antibodies. Molecular strain typing was done by IC-RT-PCR targeting three genomic regions corresponding to (Cter)CP, (Cter)N1b/(Nter)CP and C1. RFLP analysis was used to distinguish the two major strains, D and M based on a RsaI polymorphism located in (Cter)CP. All PCR products targeting (Cter)CP and 8 PCR products spanning the (Cter)N1b/(Nter)CP cistrons were sequenced. All PPV isolates typed as PPV-M by serological analysis and by molecular differentiation in the genomic region corresponding to (C-ter)CP were confirmed by nucleotide sequencing to be homologous to PPV recombinant (PPV-Rec) previously reported. All these recombinant isolates share the same recombination breakpoint and conserve the DAG motif, which is considered essential for

aphid transmission. This genetic similarity confirms that PPV-Rec may represent an ancestral group with a common evolutionary origin. Overall results provided evidence for endemic distribution of PPV-Rec in plum trees grown in the North of Romania.

Lupan I, Chira S, Chiriac M, Palibroda N, Popescu O,  
*Enzymatic synthesis of 15N-L-aspartic acid using recombinant aspartase from Escherichia coli K12,*  
Rev Chim, **59**, 1216-1217, 2008.

Amino acids are obtained by bacterial fermentation, extraction from natural protein or enzymatic synthesis from specific substrates. With the introduction of recombinant DNA technology, it has become possible to apply more rational approaches to enzymatic synthesis of amino acids. Aspartase (L-aspartate ammoniolyase) catalyzes the reversible deamination of L-aspartic acid to yield fumaric acid and ammonia. It is one of the most important industrial enzymes used to produce L-aspartic acid on a large scale. Here we described a novel method for [<sup>15</sup>N] L-aspartic synthesis from fumarate and ammonia (<sup>15</sup>NH<sub>4</sub>Cl) using a recombinant aspartase.

Sanziana MICU, Beatrice KELEMEN and Gheorghe MUSTAT  
*Current distribution and shell morphotypes of rapana venosa (valenciennes, 1846) in the agigea 4m littoral*  
Analele Științifice ale Universității „Al. I. Cuza” Iași, s. Biologie animală, Tom LIV, 2008

The Asian gastropod species *Rapana venosa*, introduced in the Black Sea waters in 1946, has been spreading quickly enough to affect the natural equilibrium in the ecosystems that it has invaded. Previous studies mentioned a broad dietary preference for bivalves, including soft bottoms infaunal species *Venus gallina*, *Gouldia minima* and *Pitar rudis* (Zolotarev, 1996), and *Cerastoderma glaucum*, *Anadara inaequivalves*. In this study we aim at analysing the length-weight, length-frequency relationships for a group of *Rapana venosa* adult specimens, collected from Agigea 4m littoral, and some aspects about morphological differences between specimens captured in three locations (Agigea, Vama Veche, typical for stony bottom and Eforie Nord, typical for the sandy substratum).

## **Conferences:**

1. Ludwig RJ, Ishii N, Kasperkiewicz M, Chiriac MT, Zillikens D.  
*Intravenous immunoglobulin (IVIG) is effective in experimental epidermolysis bullosa acquisita.*  
5<sup>th</sup> Joint Meeting - International investigative dermatology – Kyoto, Japan – May 14-17. *J Invest Dermatol* 128: S1-S226; doi:10.1038/jid.2008.93.

2. Baican A, Baican C, Chiriac G, Chiriac MT, Dima V, Zillikens D, Sitaru C.

*Autoimmune bullous diseases in a Northwestern region of Romania.*

5<sup>th</sup> Joint Meeting - International investigative dermatology – Kyoto, Japan – May 14-17. *J Invest Dermatol* 128: S1-S226; doi:10.1038/jid.2008.93.

Xray Shimatzu XRD 6000 Diffractometer

3. Chiriac MT, Zillikens D, Sitaru C.

*Myeloperoxidase is required for antibody-induced tissue damage in experimental epidermolysis bullosa acquisita.*

35<sup>th</sup> Annual Meeting of the Arbeitsgemeinschaft Dermatologische Forschung (ADF), Erlangen, Germany. *Exp Dermatol* 17(3), February 28 - March 01, 2008, 241-290.

4. Recke A, Sitaru C, Vidarsson G, Evensen M, Chiriac MT, Zillikens D.

*Autoantibodies of the IgG1 and IgG3 subclasses mediate the main pathogenic effects in epidermolysis bullosa acquisita.*

35<sup>th</sup> Annual Meeting of the Arbeitsgemeinschaft Dermatologische Forschung (ADF), Erlangen, Germany. *Exp Dermatol* 17(3), February 28 - March 01, 2008, 241-290

***PhD thesis:***

**Octavian Ioan HENEGARIU,**

Molecular and functional analysis of chromosome structure.

**Miklos BALINT,**

Pleistocene and Holocene history of *Rhyacophila aquitanica* (Insecta: Trichoptera) in the Carpathian Mountains – potential speciation centers.