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# Catalytic antibodies in autoimmunity. Development of catalytic vaccines

**A. Gabibov**

M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences
16/10, Miklukho-Maklaya Str., Moscow, Russian Federation, 117984

gabibov@ibch.ru

Discovery of catalytic antibodies (abzymes) was a revolutionary event that created new junctions between chemistry, biochemistry, immunology and pathology. Creation of abzymes is based on the intrinsic properties of immunoglobulin superfamily to produce complementary «molecular imprints». These «catalytic imprints» could be made from the stable chemical analogs of transition state (TSA) of the enzyme reaction. This approach was successfully developed by Richard Lerner group. The alternative way to create abzymes was proposed by French colleagues (Daniel Thomas and Alain Friboulet, University of Compiegne) and the author. This allowed us to generate abzymes with acetylcholinesterase and protease activities. We used also autoimmune mice models to create epitop-specific abzymes.This may give rise to biocatalysts with new functions, previously unknown for common enzymes, which may be very profitable for fine organic synthesis. This method stimulated our attempts to make antibody-like acceptors for phosphorus-based poisons. Recombinant antibodies with such functions were obtained in this lab using chemical selection of «naïve» phage-display library. The X-ray of this antibody was made and 3D structure was solved. Other advantage of abzyme field is the opportunity to make «catalytic vaccines». Numerous attempts to combat HIV infection using drug therapy as well as classical vaccination turned out to be ineffective. One of the targets for the novel therapeutic approach may be the main surface antigen, viral envelope protein gp120. The specific cleavage of this protein can lead to the dramatic changes in the immune response toward virus and decrease binding of HIV to CD4 receptor.

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# Use of molecular mimicry for the generation of new bio-active molecules

**E. Stephan-Queffeulou, S. Bun, B. Avalle, A. Friboulet**

UMR 6022 CNRS «Enzyme and Cell Engineering» Universite de Technologie de Compiegne
BP 20529–60206, Compiegne, France

alain.friboulet@utc.fr

The research developed in the «Enzyme and Cell Engineering» laboratory is devoted to the study of biocatalysis, mainly by creating new functions not existing in nature to explain actual life properties and to produce new tools for biotechnology and medicine. Catalytic antibodies (abzymes) were first developed as tools in chemistry, or as laboratory curiosities. Our group has shown that the structural and functional mimicry of the idiotypic network of immune system can be exploited to elicit catalytic anti-idiotypic antibodies. Monoclonal antibodies with esterase, amidase and protease activity were thus obtained. The data obtained indicate that the idiotypic network is capable, to a significant extent, to replicate sophisticated catalytic apparatus of serine proteases, and further validates the use of mimicry of enzyme active centers by the immune system for induction of catalytic antibodies. On the other hand, other groups have clearly demonstrated the presence of antigen-specific hydrolytic antibodies in a number of inflammatory, autoimmune and neoplastic disorders, but also in the serum of healthy donors; their pathogenic effects have been demonstrated occasionally. The origin of disease-associated catalytic antibodies may have been «induced» by the antigen implicated in the disease, may result from the loss of repressive control, or may be an expression of the idiotypic network with exacerbated self-recognition in autoimmune diseases. To try to understand the mechanisms resulting in the expression of catalytic antibodies in some diseases, the physiopathological role of catalytic antibodies has been investigated in various diseases. Finally, the field of catalytic antibodies has brought lessons to understand the molecular architecture necessary for regulating catalytic activity. On the one hand the selection of peptides from the diversity of libraries expressed on the surface of bacteriophages allow the characterization of efficient peptides. On the other hand, the preparation of molecularly imprinted polymers (MIP) is another way using molecular mimicry to obtain synthetic compounds able to act as artificial antibodies, or as specific inhibitors of enzyme activities.

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# Enchanced release ofMyelin Basic Protein encephalitogenic peptide by brain-derived proteasome in autoimmune demyelination.

**A. A. Belogurov, Jr., A. G. Gabibov**

M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences
16/10, Miklukho-Maklaya Str., Moscow, Russian Federation, 117984

gabibov@ibch.ru

One of the major functions of the proteasome is to generate peptides that are further presented on the MHC I molecules. This particular process is extremely important for immune system and specifically for cytotoxic lymphocytes to distinguish friend from foe. IFN- -inducible immunoproteasome reveals different protein degradation pattern and is thought to be key player in antigen presentation during inflammation. This investigation is devoted to the elucidation of Myelin Basic Protein (MBP) degradation via proteasome pathway. This autoantigen is highly involved in neurodegeneration and neuroinflamation processes and has intimate links with the development of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE). Quantitative LC-ECI-MS comparative analysis of MBP degradation by proteasomes from mice brain and set of MS-associated proteases displayed unique property of proteosome machinery during autoimmune demyelination to efficiently generate MBP immunodominant fragments – especially encephalitogenic peptide ENPVVHFF. Consequent presentation of processed myelin antigens to autoreactive T-cells may induce their activation and cytotoxicity thus enhancing inflammation and demyelination and even serve as a trigger of the disease. To understand the nature of this process we analyzed the influence of proteasome catalytic subunits composition on MBP degradation pattern. We showed that the level of immunoproteasome is dramatically elevated in brain of EAE SJL mice in comparison with naïve SJL and BALB/c mice. Resident neuronal cells, oligodendrocytes and astrocytes were shown to carry mainly immunoproteasome with catalytic subunit LMP2 while another one LMP7 was colocalized with infiltrating lymphocytes. Specific LMP2 inhibitor was shown to be efficient blockader of brain-derived immunoproteasome activity. This may be countered by giving immunoproteasome-targeted inhibitors for therapy of ongoing autoimmune neurological diseases.

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# Strong association locus with juvenile idiopathic arthritis on Chromosome 14: a possible tool for autoimmune disease prognosis

**I. Trapina[1, 2], O. Sugoka[1], I. Rumba-Rozenfelde[2], R. Lunin[1, 2], N. Sjakste[2], T. Sjakste[1]**

[1]Institute of Biology, University of Latvia
3, Miera, Salaspils, Latvia, LV2169

[2]University of Latvia
1a, Sarlotes Str., Riga, Latvia, LV1001

Nikolajs.Sjakste@lu.lv

**Aim**. Pediatric autoimmune diseases including juvenile idiopathic arthritis (JIA), type 1 diabetes, bronchial asthma, form an important cause for physical handicap and disablement in early age. These are multifactorial pathologies developing on hereditary basis as result of interaction between environmental factors and the patient's organism [1]. Despite numerous studies on molecular genetics of autoimmune diseases prominent associations were not identified up till now. The odds ratio (OR) values are usually low and change depending on populations. It seems that the identified risk genes are responsible for a small part of the genetic contribution, but predisposition is determined by other, yet unrevealed loci [2, 3]. Creation of genetic markers strongly associated with human autoimmune diseases could form a basis for elaboration of a method for early diagnosis of autoimmune diseases. Chromosome 14q polymorphisms appear to be prospective markers of autoimmune diseases, as it encodes several proteasomal genes. Proteasomes are involved in pathogenesis of autoimmune diseases. **Methods**. The polymorphisms were genotyped in a case/control study composed of 170 JIA patients and 233 subjects without JIA. Conventional genotyping techniques (fragment analysis, primer-specific PCR, restriction site polymorphism and sequencing) were applied. **Results**. Our findings have proven the initial hypothesis. Alleles of two microsatellites (short reiterated sequences) localized in the region appeared to be associated with juvenile idiopathic arthritis (JIA) with a low P values and high OR values. The (AC)5AT(AC)15 allele of the HSMS701 marker is strongly associated with the risk of JIA development ($P = 4.9\ \ 10^{-5}$, OR = 15.43). The (AC)5AT(AC)18 allele of the HSMS701 marker and HSMS702 (TG)10 allele also turned out to be JIA risk factors ($P = 1.09\ \ 10^{-3}$, OR = 3.16 un $P = 2.00\ \ 10^{-3}$, OR = 7.39 correspondingly) [4]. Several single nucleotide polymorphisms, especially replacement of cytosine codon with adenine codon in –110 position of the PSMA6 gene also can be considered to be a JIA risk factor [5]. Proteasomal ATPase gene PSMC 6 (14q21.1) SNP c. 86–104 T > A appears to be associated with JIA oligoarthritis form ($P < 0.05$). These results indicate impact of alleles on the disease development risk. Thus the existing preliminary data indicate strong association of this locus with JIA. Determination of the locus allele polymorphisms could be used for prognosis of autoimmune disease development during the first year of the life.

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# Antibodies–Antidotes toward chemical weapons: promiscuity of catalytic sites

**I. V. Smirnov, I. N. Kurkova, E. Carletti, P. Masson, A. A. Jr. Belogurov, N. A. Ponomarenko, A. Tramontano, A. Friboulet, A. G. Gabibov**

M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences
16/10, Miklukho-Maklaya Str., Moscow V-437, Russian Federation, 117997

Centre de Recherches du Service de Sante des Armees (CRSSA)
24, avenue des Maquis du Gresivaudan, La Tronche, France, 38700

Institut de Biologie Structurale (CEA-CNRS-UJF)
41, rue Jules Horowitz, Grenoble, France, 38027

University of California-Davis
Davis, California, 95616

smirnov@mx.ibch.ru

Immunoglobulins were shown to serve as an excellent template to generate *de novo* catalytic active centers. Both combinatorial and rational design approaches were used to improve their catalytic efficiency for numerous reactions. Organophosphorous poisons (OP, nerve gases) may be regarded as one of the intriguing targets to develop antibody-based capturing machinery. Previously we obtained rationally designed antiidiotypic antibodies 9A8 against acetylcholinesterase which was shown to have pronounced specificity of its active center toward OP analogs. Its 3D model showed Ser-His dyad, responsible for catalysis. Alternatively using combinatorial selection of human phage display library by non-toxic phosphorus compounds we selected A17 ScFv antibody able to capture OP analogs. This antibody was expressed as full length antibody in CHO cells, Fab-fragment of it was crystallized and two 3D structures of non-modified and modified by phosphonate residues were solved with resolution 1.5 and 1.36 Å respectively. The 3D structures of 9A8 and both A17 antibodies were compared and mechanism of OP capturing was described. We failed to show any similarities on the level of primary and 3D structures with corresponding enzymes. The microcalorimetric studies allowed us to make quantitative conclusions concerning active sites of both antibodies. Rationally designed 9A8 has more dance active site, alternatively combinatorial selected A17 poses «primitive» rigid active center. 9A8 antibodies was interact with fluorescent analog of soman. A17 antibody hydrolyze paraoxon with low rate constant. These antibodies displayed certain specificity toward DNA. Rationally designed 9A8 was characterized as specific dsDNA binder and A17 was shown to catalyze dsDNA cleaving reaction. Thus both Abs has pronounced promiscuity of their catalytic centers.

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# Epstein-Barr virus contributes to Burkitt's lymphoma by rearranging the nuclei of B lymphocytes

**M. Klibi, A. Pichugin, V. Ribrag[1], O. V. Iarovaia[2], M. Lipinski, S. V. Razin[1], Y. S. Vassetzky**

UMR8126, CNRS, Institut de Cancerologie Gustave Roussy; Universite Paris XI Sud
Villejuif, France

[1]Institut de Cancerologie Gustave Roussy
Villejuif, France

[2]Institute of Gene Biology, Russian Academy of Sciences
34/5, Vavilov Str., Moscow, Russian Federation, 119334

vassetzky@igr.fr

Burkitt's lymphoma (BL) is a relatively rare non-Hodgkin lymphoma. Most BLs carry a translocation of the c-myc oncogene from chromosome 8 to either the immunoglobulin (Ig) heavy-chain region on chromosome 14 [t(8;14)] or one of the light-chain loci on chromosome 2 (kappa light chain) [t(8;2)] or chromosome 22 (lambda light chain) [t(8;22)]. The major translocation juxtaposes Ig heavy chain gene (/IGH/, 14q32) sequences with the c-myc locus, leading to an overexpression of a number of genes, including the c-myc gene. In many cases, particularly in African children and AIDS patients, BLs are associated with Epstein-Barr virus (EBV). Several hypotheses have been put forward to explain this association, but the issue is not completely resolved. We have studied the intranuclear localization of c-myc and IgH loci in normal B lymphocytes and found that they are co-localized in the nuclear space only in 3 % of studied nuclei, with the IgH locus being localized in the center of the nucleus, and c-myc in the nuclear periphery. C-myc moves to the center of the nucleus upon EBV infection and IgH-c-myc co-localization is increased fivefold, to 15 %. This co-localization is a necessary pre-requisite of translocation that leads to BL. We are currently studying the role of specific EBV proteins in induction of this rearrangement.

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# Novel regulatory elements of human RUNX1 gene

**E. N. Markova, O. L. Kantidze, S. V. Razin**

Institute of Gene Biology, Russian Academy of Sciences
34/5, Vavilov Str., Moscow, Russian Federation, 119334

lidlein@mail.ru

**Aim**. Mammalian proteins RUNX1, RUNX2 and RUNX3 belong to the family of transcription factors CBF. As these factors appeared early in the evolution they participate in the regulation of the pivotal processes in the organism. So, the human RUNX1 protein coordinates hematopoiesis, nerve system development and angiogenesis. In the past 10 years the structure of the protein and it's downstream targets as well as the intron-exon structure of the gene RUNX1 were studied thoroughly. The point mutations, deletions and duplications of the locus, containing the RUNX1 gene, were elucidated shortly after the structure became clear. But the most attention was drawn to the chromosomal translocations of *RUNX1* gene as they are the cause of acute myeloid leukemia. Therefore, the RUNX1 gene itself has been a good model for studying the mechanisms of chromosomal translocations. However, though so much is known about the RUNX1 and it`s product it remains quite unclear how the tissue-specific regulation of the gene is established. There are two promoters that guide the expression of RUNX1, the distal one placed before the first exon and the proximal one inside the second intron of the gene. But it was proved that the promoters do not have any tissue specificity. Thus, there should be some extra regulatory elements that control the promoter activity. So the aim of the present study was to identify such elements. **Methods**. Genetic constructs on the basis of *pGL3* vector containing promoters and potential regulatory elements from RUNX1 gene were prepared. Lymphoid and epithelial cells were transfected with these vectors. After 24 hours incubation the luciferase luminescence was measured in the cell extracts using standard Double Luciferase assay kit. The *in vitro* interaction of tissue-specific transcription factors with these regulatory elements was checked with the help of electrophoretic mobility shift assay (EMSA). The *in vivo* interactions of the promoters with their regulatory elements was established through 3C (Chromosome Conformation Capture) analysis. **Results**. In the transient transfection experiments we managed to identify two potential enchancers regulating the corresponding promoters. In electrophoretic mobility shift experiment we observed the formation of DNA-protein complexes with the enchancer elements only in the extracts prepared from lymphoid cells. Using 3C assay we managed to characterize the spatial organization of the RUNX1 gene and to verify *in vivo* promoter-enchancer interactions. **Conclusions**. In this work we identified two novel *cis*-regulatory elements that guide tissue-specific expression of human RUNX1 gene. Considering the genomic positions of these newly characterized elements our study might be helpful for further investigation of molecular mechanisms leading to leukemogenic chromosomal translocations.

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# IGF-system and oncogenic redundancy in brain tumor development

**V. Kavsan, S. Avdieiev, P. Areshkov, O. Balinska, V. Rymar, V. Dmitrenko, A. Iershov, Yu. Zozulya[1], T. Malisheva[1], V. Rozumenko[1], M. Sanson[2]**

Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine
150, Akademika Zabolotnogo Str., Kyiv, Ukraine, 03680

[1]A. P. Romodanov Institute of Neurosurgery of Academy of Medical Sciencies of Ukraine
32, Manuilskoho Str., Kyiv, Ukraine, 04050

[2]Service de Neurologie Mazarin et INSERM, Hospital de la Salpetriere
Paris, France

kavsan@imbg.org.ua

The role that the IGF system plays in tumor progression makes it a target for cancer therapy. At the same time, oncogenic redundancy is a significant obstacle to successful treatment. Although IGF-I was proposed as one of the targets for tumor therapy, enhanced IGF-I expression was not found in astrocytomas. This may explain why the anti-IGF-I treatment does not give positive results, supposing that the development of glial tumors is activated by some other way. IGF-I participation in cellular signaling pathways of glioblastoma may be substituted by highly expressed IGF-II as well as high expression of IGF-IIR. Overexpressed IGFBPS activates IGF even without increasing ligand expression that also leads to anti-apoptotic consequences. Other proteins may also stimulate both main signaling pathways in glial tumors. In fact, CHI3L1 works in a synergistic fashion with IGF-I: both initiate MAPK and PI3K signaling cascades (Recklies et al., 2002) and activate mitogenesis. As we have shown recently, CHI3L1 with the pronounced increased expression in tumor stimulates proliferation and possesses the oncogenic properties. CHI3L2 also has a significantly increased expression in glioblastoma and activates ERK1/2 but decreases mitogenesis and inhibits cell proliferation. While stimulation of ERK1/2 phosphorylation by CHI3L1 gave short activation of ERK1/2 leading to a proliferative signal, the treatment with CHI3L2 gave the sustained activation. The arguments put forward in the previous and present works propose that when cells make decision about proliferation versus differentiation, they do it by differences in the duration of ERK activation and by translocation of ERK1/2 to the cell nucleus. However, a serious limitation of this model is that it was based wholly on experiments with PC12 cells, which may represent an abnormal system. Here, other cell type, namely HEK293 cells, had also sustained ERK1/2 activation after treatment by CHI3L2 that makes differences in the duration of ERK activation as a common signaling effect leading to proliferation or differentiation. What is a difference between CHI3L1 and CHI3L2? They are quite high homologous, have the same NH2-residues; their 3D-structures are also very close. The main difference between them is that YKL-40 is N-glycosylated at Asn60. IGF-system targeting for anticancer therapy is rapidly becoming a clinical reality, so an understanding of the complexity of this system is very timely.

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# Dopamine synthesis in non-dopaminergic neurons: physiological and morphological evidence

**M. V. Ugrumov, A. Calas[1]**

Koltzov Institute of Developmental Biology, Russian Academy of Sciences
26, Vavilov Str., Moscow, Russian Federation, 119991

[1]INSERM U 862, Universite de Bordeaux
Bordeaux, France

michael.ugrumov@mail.ru

**Aim**. In addition to dopaminergic (DA-ergic) neurons expressing all the enzymes of DA synthesis, tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AACD), the neurons expressing one of the enzymes, TH or AADC, were discovered. These neurons are distributed all through the brain though they appear to be particularly numerous in the hypothalamic tubero-infundibular system (TIS). This study was aimed to test our hypothesis that the neurons expressing individual complementary enzymes of DA synthesis produce this neurotransmitter in cooperation. It means that L-tyrosine is transformed to L-DOPA in TH-containing neurons that is followed by L-DOPA transfer to AADC-containing neurons for its conversion to DA. **Methods**. DA synthesis was estimated under static and perifusion incubation of tissue slices of TIS and substantia nigra (control) of adult rats in the presence or in the absence of L-leucine or L-methionine. These amino acids were used as competitive inhibitors of L-DOPA uptake to AADC-containing neurons. The total amount of DA in tissue extracts and in the incubation medium measured with the high performance liquid chromatography with electrochemical detection after 1.5 hour incubation was considered as an index of DA synthesis. Moreover, we have used the double-immunolabeling of AADC and TH on vibratome sections of TIS with peroxidase and gold particles intensified with silver, respectively. This has been done for evaluation of topographic relations between the monoenzymatic neurons. **Results**. An incubation of the TIS slices in the presence of either L-leucine or L-methionine resulted in the decrease of the DA contents both in slices and the incubation medium. The same procedures with the slices of the substantia nigra did not modify the DA contents in the tissue and incubation medium. The above data show that L-leucine and L-methionine inhibit DA synthesis in the TIS enriched in monoenzymatic neurons whereas they do not influence on this synthesis in the substantia nigra consisting of bienzymatic DA-ergic neurons. These data are considered as the direct proof of DA synthesis by monoenzymatic non-dopaminergic neurons. According to our double-immunolabeling study, the monoenzymatic axons, TH- and AADC-immunopositive axons, in the TIS are in apposition with each other as well as with bienzymatic axons. Close topographic relations between the monoenzymatic axons suggest their functional interaction serving to transfer L-DOPA from TH-containing axons to AADC-containing axons. **Conclusions**. The TIS monoenzymatic non-dopaminergic neurons expressing complementary enzymes of DA synthesis produce this neurotransmitter in cooperation that is facilitated by close topographic relations in-between.

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# Effect of high frequency stimulation of subthalamic nucleus on adult neurogenesis in an animal model of Parkinson's disease

**V. Khaindrava[1, 2], L. Kerkerian-Le-Goff[1], A. Daszuta[1]**

[1]IC2N, IBDML, CNRS
Marseille, France

[2]Institute of Normal Physiology, Russian Academy of Medical Sciences
6, korp. 4, Bolshaya Nikitskaya, Moscow, Russian Federation, 103009

daszuta@lncf.cnrs-mrs.fr

**Aim**. Two early symptoms in Parkinson's disease (PD) are alteration in olfaction and depression. Intriguingly, these symptoms may target neuronal changes in the two restricted regions showing adult neurogenesis, the subventricular zone (SVZ) – rostral migratory stream (RMS)-olfactory bulb (OB) continuum and the dentate gyrus (DG) of the hippocampus. In both regions, production of new interneurons is involved in memorization processes, as odor recognition in OB and hippocampal dependent spatial memory in DG. Furthermore, adult neurogenesis in DG has also been associated to stress and antidepressant effects in animal models of depression. Finally, loss of dopamine, which is the pathological hallmark of PD, also affects adult neurogenesis in rodent and human. The aim of the present study was to examine the effect of a recent efficient treatment of PD: the high frequency stimulation of subthalamic nucleus (STN HFS), on adult neurogenesis in rats with unilateral lesion of DA cells in the substantia nigra pars compacta. **Method**. Male wistar rats were divided in three experimental groups: sham of lesion and STN electrode implantation ($n$ = 7), lesioned rats stereotaxically injected with 6-hydroxydopamine (6-OHDA; 12 µg/6 µl saline plus ascorbic acid 0.1 %) in the left substantia nigra and implanted with an electrode in STN ($n$ = 8), and 6-OHDA lesioned rats with STN electrode implantation and stimulation ($n$ = 8). Electrode implantation was performed 2 weeks after lesion; stimulation started 1 week later and lasted for 8 days. STN HFS was applied continuously on freely moving rats with the following parameters: frequency 130 Hz, pulse width 80 µs, intensity 80 µA over the stimulated period. To study new cell survival, bromodeoxyuridine (BrdU), a mitotic marker that remains in the DNA even after cell division, was injected the first day of stimulation (5 75 mg/kg i. p. every 2 hours). Rats were perfused at the end of the stimulation period. Cell proliferation was studied using immunostaining for Ki67. Confocal microscope analyses allowed phenotype determination using double immunostaining for BrdU and DCX (a marker of neuroblasts). To determine the extent of DA depletion, tyrosine hydroxylase immunostaining (optical density) was quantified in the striatum and accumbens. **Results**. Unilateral DA depletion was almost complete in the striatum (3 % of sham) and partial in the nucleus accumbens (35 % of sham); STN HFS increases TH level in both regions (14 % in striatum and 73 % in the accumbens). In the SVZ, cell proliferation was slightly (17 %) but significantly decreased in the lesioned side, an effect correlated to TH level, while cell survival was decreased in both sides and not correlated to TH level. STN HFS did not affect cell proliferation, but increased cell survival. Such a stimulatory effect of STN HFS on cell survival was also observed in the RMS and OB. In the DG no changes in cell proliferation were observed after lesion with or without STN HFS, while significant decreases in cell survival were observed on both sides of lesioned animals, and reversed by STN HFS. Lesion or stimulation did not affect the phenotype of newly formed cells. These results indicate a potent restorative effect of STN HFS on adult neurogenesis in every regions examined that may have positive consequences on OB and DG functions.

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# Kruppel-like factor KLF15: a key player in facioscapulohumeral dystrophy

**P. Dmitriev, A. Petrov, E. Ansseau[2], S. Charron[2], F. Coppee[2], A. Belayew[2], G. Carnac[1], A. Turki[1], D. Laoudj[1], M. Lipinski, Ye. S. Vassetzky**

UMR 8126, Universite Paris-Sud 11, CNRS, Institut de Cancerologie Gustave-Roussy
Villejuif, France, F-94804

[1]INSERM EA 4202 ERI25
371, Avenue du Doyen Gaston Giraud, Montpellier, France, F-34295

[2]Service de Biologie Moleculaire, Universite de Mons-Hainaut
6, avenue du champ de Mars, Mons, Belgium, 7000

dmitriev@igr.fr

**Introduction**. Facioscapulohumeral muscular dystrophy (FSHD), a dominant hereditary disease with a prevalence of 1 in 20,000 individuals, is caused by a partial deletion in the subtelomeric D4Z4 repeat array on chromosome 4q. **Results**. Here we show that a strong transcriptional enhancer within the D4Z4 repeat unit interacts with the Kruppel-like factor KLF15 and that the expression level of KLF15 regulates the activity of the D4Z4 enhancer which, in turns, induces expression of DUX4c in a KLF15-dependent manner. During muscle differentiation, increased expression of KLF15 activates the D4Z4 enhancer leading to activation of the promoter of the DUX4c gene. Furthermore, we show that KLF15 is expressed at higher levels in muscle biopsies from FSHD patients compared to controls. Higher expression of KLF15 in muscles of FHSD patients could induce untimely overexpression of DUX4c with deleterious effects on muscle cell differentiation. **Conclusions**. We suggest that the KLF15-controlled D4Z4 enhancer may play a role in normal and pathological development of muscular cells.

# Poly(ADP-ribose) polymerase 1 is a key regulator of damage processing in base excision repair

**O. I. Lavrik, S. N. Khodyreva, E. S. Ilina, M. M. Kutuzov**

Institute of Chemical Biology and Fundamental Medicine, SB Russian Academy of Sciences
8, Lavrentiev Str., Novosibirsk, Russian Federation, 630090

lavrik@niboch.nsc.ru

**Aim**. Poly(ADP-ribose) polymerase 1 (PARP1), functioning as DNA nick-sensor, interacts with base excision repair (BER) DNA intermediates containing single-strand breaks. Bound to DNA breaks, PARP1 catalyzes synthesis of poly(ADP-ribose) covalently attached to some nuclear proteins and itself. Autopoly(ADP-ribosyl)ation of PARP1 facilitates its dissociation from DNA breaks and is considered as a factor regulating DNA repair. The aim is to study the role of PARP1 and its autopoly(ADP-ribosyl)ation in regulation of damage processing by the BER enzymes. **Methods**. Affinity modification, MALDI-TOF-MS, enzyme functional tests. **Results**. We identified PARP1 among the BER proteins cross-linked to the photoreactive branch-point BER DNA intermediate. Apurinic/apyrimidinic endonuclease 1 (APE1), DNA polymerase (Pol ), flap endonuclease 1 (FEN1) were found to crosslink to the same BER intermediate. By functional assays in reconstituted systems, as well as in cell extracts, we demonstrated that PARP1 and its poly(ADP-ribosyl)ation was involved in regulation of activity of the base excision repair enzymes – APE1 (3'-5' exonuclease activity), Pol , FEN1. PARP1 was shown to more efficiently influence DNA synthesis in long patch BER and its poly(ADP-ribosyl)ation pathways. PARP1's ability to interact with intact AP sites and AP sites processed by APE1 via covalent via Schiff base intermediate was demonstrated in cell extracts and with pure PARP1 protein. The identity of PARP1 as the target for cross-linking to AP sites in cell extracts was proved by peptide mapping on the data of MALDI-TOF-MS. PARP1 is unable to cleave AP sites, but instead forms with AP sites a stable intermediate. PARP1 is unable to be autopoly(ADP-ribosyl)ated upon binding with AP sites until processing of AP site by APE1. **Conclusions**. Thus, in addition to well-known role of PARP1 as nick-sensor, we demonstrated potential role of this protein in regulation of earlier stage of the BER process preceding incision of sugarphosphate backbone of DNA. Interaction of PARP1 with AP sites along with the previously detected interaction with DNA breaks demonstrates that PARP1 is a key sensor of lesions appeared in BER. PARP1 can regulate initial stage of BER persisting on AP site until APE1 could come and initiate AP site cleavage. The PARP1 activation and resultant automodification appears to facilitate the BER factor recruitment to stimulate the repair process. In the absence or deficiency of APE1, PARP1 can provide temporal protection of AP sites. PARP1 is able to interact also with AP sites cleaved by APE1. However, in the absence of Pol , when the removal of 5' deoxyribose is failed, interaction of PARP1 with this intermediate stimulates synthesis of poly(ADP-ribose) polymer, which is known as a death signal. **Acknowledgements**. This work was supported by RFBR, projects N 10-04-01083, 09-04-93106, 08-04-00704 Program of RAS «Molecular and Cellular Biology» and State contract N 02.740.11.0079.

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# Thyroid-transcription factor-1: a new gene targeted by the Wnt/ -catenin pathway

**M. Gilbert-Sirieix, L. Massade**

UMR CNRS 8203 Vectorologie et Therapeutiques anticancereuses Institut Gustave Roussy
Villejuif, France

liliane.massade@igr.fr

**Introduction**. The Wnt signaling pathway has recently emerged as a critical pathway in carcinogenesis. Furthermore, many -catenin targets have been shown to play important roles in cancer including c-myc, cyclin D1, matrix metalloproteases, CD44 or homeodomain-containing genes. In the Papillary Thyroid Carcinoma (PTC), the RET/PTC rearrangement is known to induce the disease but the mechanisms involved in the promotion of the pathology are still unknown. On the other hand, thyroid-transcription factors like thyroid transcription factor-1 (TTF-1), thyroid transcription factor-2 (TTF-2) or paired box gene 8 (Pax8) seemed also to be involved in thyroid cancer genesis and progression. **Aim**. The purpose was to investigate whether Wnt/ -catenin pathway would regulate TTF-1 expression and to assess the role of molecular canonical Wnt pathway components in the TTF-1 regulation. **Results**. We first demonstrated the activation of the pathway in a thyroid carcinoma cell line which express the RET/PTC1 oncogene (*TPC-1*): we showed the presence of the different components of the pathway by immunocytochemistry and PCR and use RNA interference to target some of them. We observed a down-regulation of the *TTF-1* gene and protein expression. We next proceeded to the reverse study where the Wnt pathway is over activated in the cell line by using LiCl and SB216763 and we showed a TTF-1 gene and protein induction confirmed by the presence of an active TCF/LEF response element in the TTF-1 promoter detected with different promoter deletions and ChIP analysis. **Conclusions**. This study underlines the regulation of TTF-1 by the Wnt/ -catenin pathway at transcriptional and transductional levels and provides evidence that TTF-1 would be a new target gene of the Wnt/ -catenin pathway and could be investigated in the treatment of aggressive or persistent PTC.

# Latent membrane protein 2A (LMP2A) of Epstein-Barr virus interacts with endocytic adaptors: intersectin 1 and amphiphysin 1

**O. Dergai, M. Dergai, I. Skrypkina, L. Matskova[1], L. Tsyba, G. Winberg[1], A. Rynditch**

Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine
150, Academika Zabolotnogo Str., Kyiv, Ukraine, 03680

[1]Karolinska Institute
Stockholm, Sweden

o.dergai@gmail.com

**Aim**. Epstein-Barr virus is a member of the herpes virus family and one of the most common human viruses. EBV is associated with a number of human malignancies, such as Burkitt's lymphoma, Hodgkin's lymphoma and the epithelial cell malignancy nasopharyngeal carcinoma (NPC). Only restricted set of viral genes is expressed within latent phase: LMP1, LMP2A, LMP2B, EBNAs and EBERs. Latent membrane proteins are key player of infected cells transformation. But little is known about mechanism governing internalization and trafficking through cells compartment The aim of current work is to identify protein-protein interaction that allows latent membrane proteins to get access to the host endocytic machinery. **Methods**. Using molecular cloning techniques and site-directed mutagenesis set of plasmids harboring CDS and its variants of LMP2A, LMP2B, LMP1 and endocytic proteins have been obtained. Standard GST-pull down and immunoprecipitation assays were used to assess interactions, conditions of complexes formation between host targets and viral proteins. Immunofluorescence analysis with set primary monoclonal antibodies followed by appropriated Texas-Red or Alexa405-conjugated antibodies. **Results and conclusions**. Here we report about interaction between viral protein LMP2A and endocytic adaptor intersectin 1. Our immunoprecipitation data evidence about complex formation between LMP2A and ITSN1 *in vivo* in HEK 293, MCF-7 cells and in B-lymphocyte cell line CBMI. SH3 domains of ITSN1 are sufficient to precipitate LMP2A *in vitro*, thus it was supposed that ITSN1 binds -PXXP- motives of LMP2A. Moreover, another endocytic adaptor – amphiphysin 1 has been found to bind -PXXP- of LMP2A through its SH3 domain. Mutational analysis of LMP2A sequence indicates at least two binding sites for ITSN1 and 3 for Amphiphysin. According to our data intersectin1 interacts with both isoforms: LMP2A and 2B, while amphiphysin binds only LMP2A. Amphiphysin 1 associates with two sites at the N-terminus of LMP2A which were known to mediate interaction of this viral protein with ubiquitin-ligases. Thus, it is tempting to speculate that amphiphysin 1 compete with mentioned ubiquitin-ligases inhibiting down regulation of LMP2A associated proteins like Syk and Lyn. Analysis of subcellular distribution revealed co-localization of LMP2A and ITSN1 in clathrin-coated pits. Summarizing these data we can propose a model of clathrin-mediated internalization of LMP2A which could be ITSN1-dependent.

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# Responses of base excision repair proteins to oxidative stress

**A. Bravard, A. Campalans, R. Amouroux, J. Hall[1], B. Epe[2], J. P. Radicella**

Institut de Radiobiologie Moleculaire et Cellulaire
CEA, Fontenay aux Roses, France

[1]Institut Curie
Orsay, France

[2]Institute of Pharmacy, University of Mainz
Mainz, Germany

jpradicella@cea.fr

Oxidative stress is the consequence of reactive oxygen species (ROS) accumulation that disrupts the intracellular redox balance. All cellular macromolecules are potential targets for ROS, among them nucleic acids. Because of its weak oxidative potential, the guanine base is the most susceptible to be damaged by ROS. The 8-oxoguanine (8-oxoG), resulting from guanine oxidation, is highly mutagenic and can lead to G:C to T:A transversions after two replication cycles. Mammalian cells possess a specific DNA glycosylase, OGG1, which recognizes and excises specifically this oxidized base; these first steps initiate the base excision repair (BER) pathway. Here we will present two different aspects of the response to oxidative stress of the proteins involved in the initial steps of BER. Recent work from our laboratory concluded that after a strong oxidative stress OGG1 could by inactivated by thiol oxidation. Here we show that the replacement of serine 326 for a cysteine, a polymorphic variant associated with cancer predisposition, leads to a lower and more vulnerable cellular 8-oxoG repair capacity. Lymphocyte cell lines homozygote for either Cys or Ser alleles were analyzed for their *in vitro* and *in vivo* repair capacities, showing that cells carrying the Cys variant systematically displayed a reduced *in vivo* capacity of 8-oxoG removal from their genome and increased genetic instability. Consistently, extracts from these cell lines had 50% of the DNA glycosylase activity found in cells carrying the Ser allele. Surprisingly, the activity of the Cys extracts could be taken to the levels of those from the Ser variant by addition of a reducing agent, while the Ser one was not affected. Conversely, the Cys variant was more readily inactivated by exogenous oxidative stress. Biochemical assays confirm that the oxidation of the form carrying the extra cysteine residue is at the basis of the impaired DNA repair capacity of OGG1 S326C and therefore could explain its association with cancer predisposition. Secondly, we will show some recent cellular and biochemical results describing the relocalization to specific regions of the nucleus of OGG1, APE1 and XRCC1, proteins involved in the initial steps of the 8-oxoG BER. After exposure to an oxidative stress generating large amounts of 8-oxoG in DNA, OGG1, together with the other two BER proteins is recruited to chromatin. By both, biochemical and microscopy approaches we show that the DNA glycosylase specifically relocates to the euchromatin fraction. Compaction of chromatin using hypertonic treatment of the cells strongly inhibits *in vivo* repair. Furthermore, kinetic experiments using OGG1 mutants indicate that completion of repair is required for release of the DNA glycosylase. Taken together our results suggest a mechanism by which, in response to oxidative stress, the cell triggers a preferential repair of 8-oxoG in active chromatin regions.

# Poly(ADP-ribose) polymerases in the DNA damage response: new incomers

**V. Schreiber, J.-C. Ame, A. Hakme, M. Kutuzov[1], H.-K. Wong, E. Fouquerel, T. Kalisch, A. Bresson, C. Boehler, S. Khodyreva[1], O. Lavrik[1], F. Dantzer**

FRE3211, IREBS, CNRS, Universite de Strasbourg, ESBS
Bd S. Brant, BP 10413, 67412 Illkirch, France

[1]Institute of Chemical Biology and Fundamental Medicine, SB Russian Academy of Sciences
8, pr. Lavrentieva, Novosibirsk, Russian Federation, 630090

valerie.schreiber@unistra.fr

Poly(ADP-ribosyl)ation is a post-translational modification of proteins mediated by Poly(ADP-ribose) polymerases (PARPs). It occurrs in various cellular processes such as DNA repair, replication, transcription, telomere homeostasis, mitotic segregation or cell death. PARP-1 is a molecular sensor of DNA breaks that signals their presence by synthesizing poly(ADP-ribose) (PAR), which modifies chromatin architecture and recruits DNA repair factors at the damaged site. Whereas activation of PARP-1 by mild genotoxic stimuli facilitates DNA repair and cell survival, intense PAR production upon massive DNA attack triggers cell death. PARP-1 inhibitors have entered clinical trials to potentialize the cytotoxicity of clastogens in radio- and chimiotherapy, but also to sensitize cells with genetic defects in double-strand break repair such as BRCA-deficient cells. Our laboratory has established *in silico* the sequence of novel PARPs, bringing to 17 the number of PARP family members. The diversity of functional domains associated to the PARP domain, the va- rious subcellular localizations and the novel functions discovered considerably extended the field of poly(ADP-ribosyl)ation reactions. We have cloned and characterized PARP-2, demonstrating that it is also a DNA damage dependent PARP, required for efficient DNA strand breaks repair. Cellular and animal models deficient in PARP-1 or PARP-2 developed in the laboratory revealed redundant and complementary functions of these two proteins in the surveillance and maintenance of genome integrity. These models pointed also to specific functions in differentiation processes. Biochemical analyses are now being conducted to determine the mechanism of action of PARP-2 in the DNA damage response. Results on the identification of PARP-2 DNA targets and activators will be presented. PARP-9 is a protein overexpressed in some high-risk B lymphomas. It contains two macro domains, the non-histone domain present in the histone variant macroH2A involved in transcriptional silencing and chromosome X inactivation. PARP-9 macro domains were reported to efficiently bind PAR, questioning whether PARP-9 could be recruited at sites of intense poly(ADP-ribose) synthesis such as DNA strand breaks. To gain insights into the function of PARP-9, we have generated a human cell line that efficiently down regulates the expression of PARP-9. The results will be presented on the consequences of PARP-9 deficiency on the cell response to DNA damage.

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# The siRNA targeted to mdr1b and mdr1a mRNAs *in vivo* sensitizes murine lymphosarcoma to chemotherapy

**M. A. Zenkova, O. A. Patutina, N. L. Mironova, V. V. Vlassov**

Institute of Chemical Biology and Fundamental Medicine, SB Russian Academy of Sciences
8, pr. Lavrentieva, Novosibirsk, Russian Federation, 630090

marzen@niboch.nsc.ru

**Background**. A substantial progress in the study of cancer development was achieved during last decades. However, mortality from these diseases still remains high despite the appearance of new highly efficient therapeutics and new polychemotherapy programs. One of the main obstacles for successful polychemotherapy of cancer diseases is the development of multiple drug resistance (MDR) phenotype, the resistance to a wide range of chemotherapeutic agents, acquired by cancer cells. Currently, RNA in- terference represents a perspective strategy to overcome MDR via silencing the genes involved in de- velopment of this deleterious phenotype (genes of ABC transporters, antiapoptotic genes, etc.). **Aim**. In this study, we used the siRNAs targeted to mdr1, mdr1a, and bcl-2 mRNAs to reverse the MDR of tu- mors and increase tumor sensitivity to chemotherapeutics. **Methods**. In our previous work (Mironova et al., Ann. NY Acad. Sci. 2006, 1091: 490–500) RLS40 cell line, exhibiting MDR. The MDR of RLS40 cells was provided by a high expression of mdr1b and mdr1a genes and a moderate expression of bcl-2 and appeared as a 20-fold decrease in the cell sensitivity to a wide range of cytostatics (cyclophospha- mide, cysplatin, vinblastine, and rubomycin). The obtained RLS40 cell line generates tumors in mice and mimics the tumor status observed in patients after several chemotherapy courses (Zenkov et al., Nucleosides, Nucleotides, Nucleic Acids 2004, 23: 843–847). Thus, we had at our disposal two lymphosarcoma models displaying MDR: the MDR of RLS40 is mdr1b/1a-associated and the MDR of RLS tumor is predominantly bcl-2-associated. The antitumor therapy consisting in *ex vivo* or *in vivo* application of siRNA targeted to mdr1a, mdr1b and bcl-2 mRNAs followed by cyclophosphamide administration was applied to the mice bearing RLS40 or RLS lymphosarcoma. The dynamics of tumor growth as well as levels of genes expression and sensitivity of siRNAs-treated tumor cells to vinblastine was examined. **Results**. Our data show that mdr1b/1a and bcl-2 siRNAs caused five- and two-fold de- crease in the corresponding mRNA levels, respectively, and mdr1b/1a siRNA exerted an increase in cell sensitivity to cytostatics. The application of siRNA followed by cyclophosphamide administration was studied *ex vivo* and *in vivo* using RLS40 and the related RLS lymphosarcomas developed in mice. Single application of mdr1b/1a siRNA and cyclophosphamide resulted in a decrease in the MDR and a three- fold decrease in the tumor size as compared with the control animals treated only with cyclophos- phamide, while bcl-2 siRNA was ineffective *in vivo*. **Conclusions**. In perspective, mdr1b/1a siRNA may become a well-reasoned adjuvant tool in the therapy of MDR malignancies.

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# Cellular localization of YB-1 under varying anti-cancer drug exposure and stress

**V. Joshi, L. Hamon, L. P. Ovchinnikov[1], D. Pastre, P. A. Curmi**

Laboratoire Structure-Activite des Biomolecules Normales et Pathologiques, INSERM/UEVE U829
EA3637, Evry, France, 91025

[1]Institute of Protein Research, Russian Academy of Sciences
Pushchino, Moscow Region, Russian Federation, 142290

pcurmi@univ-evry.fr

The ubiquitous Y box-binding protein (YB-1) binds to nucleic acids and participates in the regulation of gene expression and translation. In addition, this protein may protect cells from the toxic effects of agents that lead to DNA cross-linking, suggesting a role in multidrug resistance. We generate novel YB-1 antibodies against synthetic peptides corresponding to a wild type of P-YB-1 to probe it intracellular localization in control cells or after their exposure to different agents such as cisplatin, mitomycin C, vinblastine, doxorubicin. The results show variation of the YB-1 protein localization under different conditions as probed by western blotting and indirect immunofluorescence microscopy.

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# YB-1–PABP interplay in specific regulation of YB-1 mRNA translation

**D. N. Lyabin, I. A. Eliseeva, O. V. Skabkina, L. P. Ovchinnikov**

Institute of Protein Research, Russian Academy of Sciences
4, Institutskaya Str., Pushchino, Moscow Region, Russian Federation, 142290

lyabin@vega.protres.ru

**Aim**. The Y-box binding protein 1 (YB-1) is a member of the protein family with the cold shock domain. It is involved in virtually all DNA- and mRNA-dependent events. The amount of YB-1 in the cell strongly influences the transcriptome and proteome profiles of the cell, affects its phenotype, prevents or promotes cell oncotransformation, and contributes to tumor aggressiveness. Therefore, knowledge of the regulatory mechanisms of YB-1 synthesis may be useful for development of novel approaches to cancer therapy. Previously, we found that the YB-1 mRNA contains a regulatory element in its 3' untranslated region. This element specifically binds two major mRNP proteins, YB-1 and poly(A) binding protein (PABP). Their binding sites on YB-1 mRNA overlap, and the two proteins compete for binding to the regulatory element. In doing so, YB-1 selectively inhibits (autoregulates) YB-1 mRNA translation, while PABP, in contrast, stimulates it in rabbit reticulocyte lysates. In studying the mechanism of YB-1-PABP interplay in regulation of YB-1 mRNA translation, a question arises as to whether these proteins act independently or the role of one of them is reduced solely to displacement of the other from the regulatory element. The specific task of our study was to answer this question. **Methods**. We obtained YB-1 mRNA forms without the regulatory element or with a mutated element lacking specific affinity for either YB-1 or PABP. The effect of YB-1 and PABP on translation of these mRNAs was studied in the rabbit reticulocyte cell-free translation system. **Results**. The deletion of the entire regulatory element from YB-1 mRNA results in elimination of both the negative effect of YB-1 and positive effect of PABP on YB-1 mRNA translation. The mutation in the YB-1 binding site strongly decreases the inhibitory effect of YB-1 and leaves the stimulating effect of PABP almost unchanged. With partially deleted PABP binding site, YB-1 mRNA translation keeps being inhibited by YB-1, and PABP becomes incapable of preventing this inhibition. Nevertheless, PABP preserves its ability to activate translation of this mRNA form in the cell-free system. **Conclusions**. Thus, translation of YB-1 mRNA is affected directly and independently by the both proteins: negatively by YB-1 and positively by PABP. This results from their specific binding to the regulatory element of this mRNA. The opposite effects of these proteins on YB-1 mRNA translation can be enhanced through their competition for binding to the regulatory element. Besides, PABP appears to be able to stimulate translation of YB-1 mRNA even without specific affinity for the regulatory element. This ability remains to be explained.

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# Chitinase 3-like 2 gene as a potential marker of human glial tumors

**S. Avdieiev, P. Areshkov, O. Boyko, V. Filonenko, V. Kavsan**

Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine
150, Academika Zabolotnogo Str., Kyiv, Ukraine, 03680

stasavdieiev@gmail.com

Identification and characterization of differentially expressed genes in astrocytic gliomas, most common glial tumors, can be expected to provide relevant and important insights into the molecular determinants of tumor initiation, progression and diagnostics. Serial Analysis of Gene Expression found CHI3L1 (YKL-40) and CHI3L2 (YKL-39) genes among the most abundant transcripts in glioblastoma. Overexpression of CHI3L1 at the mRNA and protein levels in glioblastoma was shown in our previous study CHI3L1 and CHI3L2 belong to the glycohydrolase family 18. Kawamura et al. (1999) showed that a family of growth factors for imaginal disc cells of the fruit fly belongs to the chitinase-3 like protein family. Recklies et al. (2002) found that CHI3L1 stimulated DNA synthesis and proliferation in a synergistic fashion with IGF-I by activation of both extracellular signal-regulated kinase (ERK) – and protein kinase B (AKT)-mediated signaling cascades. Taking into account the lack of similar data for CHI3L2, very closely related in size, amino acid and nucleotide sequences (52 and 56 % homology) to CHI3L1, the present study was initiated to analyze the expression and functional role of CHI3L2 in glial tumors. Northern hybridization showed the expression of CHI3L2 in the majority of glioblastomas. Anaplastic astrocytomas were possible to divide on two groups: in one of them the CHI3L2 expression was completely undetectable, but in another it was possible to detect quite high contents of CHI3L2 mRNA. CHI3L2 mRNA has not been detected in eight samples of diffuse astrocytoma under investigation and was determined only in 1 from 13 samples of normal brain. Western blot analysis of brain tumors revealed the 39 kDa product in five of eleven analyzed glioblastoma samples and in five of thirteen anaplastic astrocytomas, but none from five samples of normal brain. CHI3L2 activates signal-regulated kinases ERK1/ERK2 in human embryonic kidney (HEK293) and human glioblastoma (U87 MG) cells. Effect of CHI3L2 on PI3K/AKT signaling pathway is currently under investigation. Unexpectedly, dose dependent decreases in total DNA content and [$^3$H]thymidine incorporation were observed in HEK293 cells treated with CHI3L2 at concentrations 50 and 100 ng/ml. CHI3L2 induced sustained phosphorylation of ERK1/ERK2 in HEK293 cells in a very similar way to that was shown for PC12 cells treated with nerve growth factor (NGF). These data suggest that CHI3L2 may cause differentiation phenotype, but not proliferation. Thus, increased expression of CHI3L2 at the mRNA and protein levels in glioblastoma and its involvement in activation of MAPK signaling cascade may suggest its potential role in carcinogenesis.

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# Assembly of the tRNA3Lys packaging complex in HIV-1

**L. Kobbi[1], G. Octobre[1], J. Dias[1], M. Comisso[1], M. Kaminska[1], V. Shalak[1, 2], M. Mirande[1]**

[1]Laboratoire d'Enzymologie et Biochimie Structurales, CNRS
1, Avenue de la Terrasse, Gif-sur-Yvette, France, 91190

[2]Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine
150, Academika Zabolotnogo Str., Kyiv, Ukraine, 03680

mirande@lebs.cnrs-git.fr

The human immunodeficiency virus, HIV-1, infects cells of the immune system, and is responsible for the acquired immunodeficiency syndrome (AIDS). This retrovirus contains a dimer of a single-stranded RNA genome that must be converted into double-stranded DNA during viral replication. The reverse transcriptase involved in this process is encoded by the viral genome. The $tRNA_3^{Lys}$ species from the host cell is used by the reverse transcriptase of HIV-1 as a primer for the synthesis of the first, viral minus-strand DNA. During the budding process, all the viral components assemble at the cell membrane in order to form new HIV-1 particles, which are then released outside of the cell. During this step, the three $tRNA^{Lys}$ species of the host cell are specifically packaged into the viral particle1. One of the proteins responsible for the specific packaging of tRNALys has been identified as lysyl-tRNA synthetase (LysRS)2. In humans, there are two isoforms of LysRS, the cytoplasmic (cLysRS) and the mitochondrial (mLysRS) species. These two forms are encoded by the same gene by means of alternative splicing. The two LysRS species share a large part of their sequence, but it has been shown that the only source of LysRS detected in the virions is the mitochondrial form3, while the cytoplasmic form is exclusively found at the level of the Multi-Aminoacyl-tRNA Synthetase Complex (MARS), a stable multiprotein assembly containing eight other aminoacyl-tRNA synthetases. We used the yeast two-hybrid system to screen viral proteins that may form a specific complex with LysRS. The Pol region of the polyprotein GagPol is able to interact with LysRS. This interaction involves the p6* and IN domains of Pol. On the other hand, this interaction involves the catalytic domain of LysRS, a domain shared by mLyRS and cLysRS. LysRS is also a component of the cytosolic MARS complex. LysRS is associated within this complex via its interaction with the scaffold protein p38. We showed that this interaction is also mediated by the catalytic domain of LysRS. The two LysRS species, cLysRS and mLysRS, are equally able to bind p38 in a two-hybrid assay, but only cLysRS is recovered within MARS. Thus, cLysRS and mLysRS have the capacity to bind p38 or Pol *in vitro*, but only cLysRS is targeted to MARS, and mLysRS to HIV-1 particles, *in vivo*. This implies that the two LysRS species follow distinct targeting pathways, to MARS or to the mitochondria, *in vivo*.

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# Human cartilage glycoprotein gp-39 (YKL-40) has oncogenic properties

**O. Balynska, V. Baklaushev[1], V. Kavsan**

Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine
150, Academika Zabolotnogo Str., Kyiv, Ukraine, 03680

[1]V. P. Serbsky National Research Centre for Social and Forensic Psychiatry, RMH

l.v.balynskay@gmail.com

**Aim**. Gliomas are the most frequent malignant primary brain tumors. They are highly aggressive, invasive, neurologically destructive and characterized by very high biological heterogeneity. It was found earlier that CHI3L1 (human cartilage 39-kDa glycoprotein) gene is expressed at markedly higher level in glioblastomas as compared to human normal brain. The previously published results indicate that YKL-40, encoded by CHI3L1 gene, may be used as a potential molecular marker for brain glial tumors. The aim of present study was to investigate oncogenic properties of YKL-40. **Methods**. We established cell line stably expressed YKL-40. Presence of YKL-40 in cells was tested by Western-blot and immunocytochemistry. To determine whether YKL-40 possesses oncogenic properties, we check ability of stably expressed YKL-40 cell line form colonies in soft agar. To determine whether YKL-40 can influence tumor initiation and development *in vivo*, HEK293 cells stably expressing YKL-40, and HEK293 cells transfected with the empty vector, were used for stereotaxic intracerebral implantation to female Wistar rats. Tumor growth was monitored weekly for up to 6 weeks. **Results**. Western-blot and immunocytochemistry analysis confirmed a presence of YKL-40 in cells stably transfected by recombinant construction. The YKL-40-expressing clones grew as colonies in soft agar. This property was not observed in the parental HEK293 cells or in HEK293 cells transfected with the empty vector. After 21$^{th}$ day after implantation, tumors appeared in three rats with implanted HEK293 cells which stably expressed YKL-40, two rats had smaller tumor after one month. The volume of tumor was determined by approximating it with an ellipsoid. The volume of the ellipsoid was equal to 443,9 mm$^3$. No tumor growth was observed for at least six weeks in five rats which were injected by empty vector-transfected HEK293 cells as a negative control. Histochemical analysis of intracerebral tumor HEK293_YKL40 showed arrangement of YKL-40 on the periphery of the tumor. **Conclusions**. Expression of YKL-40 in HEK293 cells was sufficient to induce *in vivo* tumorigenicity.

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# Functional aspects of adaptor proteins of intersectin family

**L. Tsiba, O. Novokhatska, I. Skrypkina, M. Dergai, O. Nikolaenko, O. Dergai, S. Kropyvko, T. Gryaznova, D. Morderer, J. Moreau[1], L. Drobot[2], A. Rynditch**

Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine
150, Academika Zabolotnogo Str., Kyiv, Ukraine, 03680

[1]Institut Jacques Monod
Batiment Buffon, 15, rue Helene Brion, Paris 13, France, 75252

[2]Palladin Institute of Biochemistry of National Academy of Sciences of Ukraine
9, Leontovicha Str., Kyiv, Ukraine, 01601

a.v.rynditch@imbg.org.ua

**Aim**. The rich binding capability of the multidomain, adaptor and scaffolding proteins of intersectin (ITSN) family has linked them to multiple functions such as clathrin-mediated endocytosis, mitogenic signaling and actin cytoskeleton rearrangements. Abnormalities of ITSN1 expression were associated with the endocytic anomalies reported in Down syndrome brains, early stages of Alzheimer's disease as well as neurodegeneration in Huntingon's disease. Overexpression of INSN1 was sufficient to induce oncogenic transformation. Meantime, high level of ITSN2 expression in breast cancer was associated with good prognosis suggesting that minor differences in the amino acid residues between these proteins could affect the binding properties. The other sources of functional diversity can include alternative splicing and usage of alternative promoters. Recently we identified 17 alternative splicing events for ITSN1 pre-mRNA. However, the function of ITSN1 isoforms and their expression are not well defined. Moreover, little is known about ITSN2. **Methods**. Immunoprecipitations, GST pull-down experiments, determination of sub-cellular localization by direct and indirect immunofluorescence were used to study protein interactions. To analyze the effect of ITSN2 domains overexpression on embryonic development, microinjection of mRNA into the animal hemisphere of two-cell stage embryos were performed. **Results**. We have compared interaction interfaces of ITSN1 and ITSN2 for their binding partners and found four novel ITSN2 interacting proteins involved in endocytosis, RTK signaling and rearrangements of actin cytoskeleton. Both intersectins interact with RLIP-associated Eps domain-containing proteins, Reps1 and Reps2/POB1.The latter regulates the transport function of RLIP76, an energy dependent transporter for glutathione-conjugates and chemotherapeutic agents, associated with multi-drug resistance. Phenotypic changes seen after manipulation of ITSN function *in vivo* revealed the effect of ITSN2 on early embryonic development. Microinjections of mRNA encoding different domains of ITSN2 into Xenopus embryos showed the strong hyperpigmentation, loss of cell-cell contacts and gastrulation failure what was observed in case of overexpression of C-terminal part of ITSN2 long isoforms (DH, PH and C2 domains). This effect was correlated with alteration in the cortical actin cytoskeleton. Further experiments demonstrated that mechanism of DH/PH domain function involved activation of small GTPase Cdc42. Dominant negative Cdc42 rescued loss of cell-cell contacts in case of DH/PH overexpression, increased embryos survival rate and reduced severe hyperpigmentation effect. Moreover, wild type Cdc42 co-expressed with DH/PH increased its deleterious effect supporting the idea of the role of DH domain GEF activity towards Cdc42 in observed phenotype. Obtained results suggest a possible role of ITSN2 in the coordinated changes of actin cytoskeleton during early embryonic development. **Conclusions**. INSN1 and ITSN2 long isoforms are implicated in actin regulation and recruitment of endocytic machinery. Defining the functional activities of intersectin alternative spliced isoforms is particularly relevant given that the intersectins are involved either in neurodegenerative diseases or in cancer.

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# The Transcription Factor Cycle (TFC) hypothesis and the genon-concept

**K. Scherrer**

Institut Jacques Monod
CNRS, Paris, France

scherrer.klaus@ijm.univ-paris-diderot.fr

The Gene and Genon (GENe-operON) concept has emphasised the necessity to conceptually separate genomic product information (the coding triplet sequence) from regulative information. Regulative cis-programs are based on the sequential alignment of sites in DNA and RNA binding regulative protein or RNA (within RNAi) factors. On an individual mRNA, added to and superposed onto the coding sequence is the cis-program representing, thus, it's Genon. The cis-genon picks up an ensemble of factors from trans (it's transgenon) by the sequential oligomotifs recognising protein or si/miRNA. The pre-mRNA carries the pre-genon program (controlling e. g. differential splicing) and the DNA of a genomic domain its proto-genon controlling chromatin modulation as well as transcription start and extent. The notion of such programs at DNA and (pre-)mRNA level became most important recently, to interpret post-genomic data bearing on the unexpected extent of the transcribed genome and the size of individual primary transcripts. Thus, all CRMs (cis-regulatory modules) of promoter, enhancer and other types are transcribed. The sequence of CRMs way upstream, downstream and in intronic or intergenic positions represents thus a pre-genon; clear-cut phenotypes relate to mutation of such CRMs without affecting the coding sequence. This may allow interpreting old data in a new manner showing that factors that are assembled on DNA may be carried away by the nascent transcript, to exert a function at RNA level. Certain factors binding DNA with high affinity and site specificity are found predominantly on pre-mRNA. Some TFs and promoter-binding factors also bind RNA. Hence, the possibility arises that TFs and other DNA-binding factors might be transferred from chromatin to pre-mRNA or full domain transcripts (FDTs); they may cycle back to the DNA in a balance controlled by relative affinity/specificity. DNA might serve as «entry site» binding, and possibly organising into ensembles factors to be carried away by the nascent RNA. FDTs have two functions: (i) control mRNA synthesis in cis and (ii) bind from trans and store RNA-binding factors controlling gene expression. (1) In interphase cells FDTs produce mRNA under the control of the pre-genon programs. (2) Producing some mRNA during mitosis and meiosis (e. g. histone mRNA) they might maintain the organisation of factors fixed on pre-mRNA to be reinserted site-specific on «virgin» DNA. In mitosis, these factors might be stored on not processed FDTs to be transferred back to the DNA in interphase. This might perpetuate and control euchromatin open for transcription. In meiosis, when the genome is fully transcribed and oocytes are full of high Mr RNA, the FDTs with their attached factors might be disposed in sectors of the ooplasm. During cleavage stage they may end up in specific cells, feeding specific factors to chromatin of individual cells. This process would correspond to a first stage of determination of later differentiation in space and time. The TFC concept also implies that mutations of TF sites will affect not gene products per se, but interfere in the systems of regulative networks, which control gene expression, leading to specific phenotypes.

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# Ku antigen interacts with DNA containing abasic sites

**E. S. Ilina, A. E. Berezhnoy[1], S. S. Larin[1], O. I. Lavrik, S. N. Khodyreva**

Institute of Chemical Biology and Fundamental Medicine, SB Russian Academy of Sciences
8, pr. Lavrentieva, Novosibirsk, Russian Federation, 630090

[1]Institute of Gene Biology of the Russian Academy of Sciences
34/5, Vavilova Str., Moscow, Russian Federation, 119334

katya.plekhanova@gmail.com

**Aim**. Prominent lesions in DNA are abasic (AP) sites arising spontaneously or as intermediates during base excision repair. An AP site can form a Schiff base intermediate with primary amino groups of proteins. This intermediate can be stabilized by $NaBH_4$ treatment and, therefore, AP DNA can be used as a tool in detecting proteins that interact with AP sites. This approach named borohydride trapping (BHT). **Methods**. BHT and immunoprecipitation or MALDI-TOF-MS analysis were used for identification of cross-linked protein to AP DNA. BHT and dot-ELISA were used to monitor the amount of Ku antigen in different cell extracts. **Results**. In HeLa cell extract, a predominant product with an apparent molecular mass of 95 kDa was observed. Analogous covalent adducts of proteins with AP DNA were revealed in the extracts derived from human fibroblast, HL-60, K562, and several melanoma cell lines unlike bovine testis nuclear extract. The cross-linked protein was identified as the p80 subunit (Ku80) of Ku antigen (Ku) by immunoprecipitation and MALDI-TOF-MS analysis. Ku is an abundant DNA end binding protein in human cells. Human Ku is composed of two tightly associated polypeptide chains with molecular masses of 69.8 and 82.7 kDa (Ku70 and Ku80, respectively) and is the DNA binding component of DNA-dependent protein kinase (DNA-PK). AP DNAs of different structure were used to study peculiarities of Ku interaction with DNA. Considering the extreme selectivity of AP site-containing DNA probes, we decided to examine the use of this approach for measuring levels of Ku in different cell extracts. The level of Ku in cancer cells is considered to be a relevant factor for the prediction of tumour radiosensitivity and may play a role in treatment outcome. Ku expression was shown to correlate with tumour and metastatic progression. Ku may act as either a tumor suppressor or an oncoprotein, depending on its expression level. We found that quantitation by dot-ELISA and AP site cross-linking were comparable. Cross-linking detected allows revealing truncated variants of Ku80 polypeptide (Ku80v) that is truncated at the C terminus, which are detected in some cells. The ability of Ku80v complexes to interact with DNA-PK catalytic subunit (DNA-PKcs) is greatly reduced and resulted in increased sensitivity to some DNA damage agents as a consequence of reduced DNA repair. **Conclusions**. BHT unlike western blot or estimation of the Ku content, based on mRNA levels, reveals Ku forms that are active in DNA binding, including those, which have aberrations in Ku80, but retain ability to heterodimerize with Ku70 and bind DNA. In addition, in proposed assays no special precautions for equal loading of samples on gels are required since the amount of Ku80 is estimated as the ratio of AP DNA cross-linked to Ku80 to total AP DNA in the sample. Moreover, this approach can be used to monitor the levels and forms of Ku80 separately in cytoplasmic and nuclear fractions of cells. As a routine test, this method is also less time and reagent consuming than blotting and EMSA. This work was supported in part by RFBR (09-04-93106 and 10-04-01083), Program of RAS «Molecular and Cellular Biology», Helmholtz-Russia joint research groups 2008 (HRJRG-102).

# Epigenetic changes of macrosatellite 3.3-kb repeats in human diseases

**N. Kisseljova, A. Katargin, D. Ezerina, E. Kim[1], Y. S. Vassetzky[1]**

Institute of Carcinogenesis, N. N. Blokhin Cancer Research Center
24, Kashirskoe shosse, Moscow, Russian Federation, 115478

[1]UMR8126, CNRS, Institut de Cancerologie Gustave Roussy; Universite Paris 11 Sud
Villejuif, France

natalia-kis@yandex.ru

**Aim**. The members of 3.3-kb repeat family reside in the subtelomeric regions of chromosomes 4 and 10 as tandem repeats containing 11–100 monomers and on the short arms of all acrocentric chromo- somes where repeats interspersed with 68 bp satellite DNA (van Geel et al., 2002; Lyle et al., 1995). Partial deletions of repeat array on chromosome 4 named D4Z4 are associated with facioscapulohu- meral muscular dystrophy (FSHD), an autosomal dominant disorder. In many normal somatic tis- sues, 3.3-kb repeats are mostly but not completely methylated and are hypomethylated in glioblasto- mas, leukemia cell lines, and tissues of patients with FSHD (Cadeux et al., 2006; Tsumagari et al., 2008; de Greef et al., 2010). Previously, we identified macrosatellite 3.3-kb repeats as hypermethy- lated in cervical cancer in genome-wide screening. Thus, hypo- and hypermethylation of 3.3-kb re- peats are associated with several human diseases. The aim of present study is: 1) to confirm that hy- permethylation of 3.3-kb repeats is tumor-specific event; 2) to investigate consequences of hypo- and hypermethylation of 3.3-kb repeats for disease cells. **Results**. For the first time, we have de- monstrated by bisulfite genomic sequencing that more than 50 % of cervical cancers display hyper- methylation of 3.3-kb repeats compared to normal cervical tissues. Different regions within the 3.3- kb monomer exhibit inter-individual variation in the methylation patterns in normal tissues and dif- ferent susceptibility to hypermethylation in tumors. Mechanisms of these changes remain unknown. Recently, it has been shown that a nuclear matrix attachment ragion (S/MAR) is located in the vi- cinity of the clusters of 3.3-kb repeats on 4 and 10 chromosomes. The S/MAR adjacent to the D4Z4 array was found to be prominent in normal human myoblasts and non-muscular human cells, and was much weaker in muscle cells derived from FSHD patients, suggesting that the D4Z4 repeat array and upstream genes are located in two distinct chromatin loops in non-muscular cells and in normal human myoblasts vs a single loop in FSHD myoblasts (Petrov et al., 2006). We have shown that methylation status of S/MAR is changed in cervical carcinoma cell lines compared to normal cervical tissues and correlates with changes of 3.3-kb repeats methylation status. **Conclusions**. It is known that some proteins that mediate specific association of DNA with nuclear matrix interact with the methylated DNA only (Horike et al., 2005; Stratling and Yu, 1999). We propose that chan- ges in methylation of the subtelomeric regions of chromosomes 4 and 10 are linked with changes in domain structure of chromatin in cells of cancer and FHSD patients. This hypothesis is currently under study.

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