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**Oral Communication Abstract – 6B.01**

# GENETIC AND EPIGENETIC NETWORKS DISRUPTED IN INTELLECTUAL DISABILITY

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*Intellectual disability, genes, epigenetic, network, chromatin*

Intellectual disabilities (ID) comprise a highly diverse group of cognitive disorders. Gene defects account for about half of all patients and mutations causative for impaired cognition have been identified in more than 400 genes. While there are numerous genetic defects underlying ID, a more limited number of pathways is emerging whose disruption appears to be shared by groups of ID genes, for example . One of these common pathways is composed of ID genes that encode regulators of chromatin structure and of chromatin-mediated transcription regulation. Already more than 20 “epigenetic ID genes” have been identified and this number is likely to increase in the coming years when deep sequencing of exomes and genomes will become commonplace. Prominent examples of epigenetic ID proteins include the methyl CpG-binding protein MECP2, involved in Rett syndrome, the CREB binding protein CBP (Rubinstein-Taybi syndrome) and euchromatin histone methyltransferase 1 (EHMT1; Kleefstra syndrome). Interestingly, several epigenetic ID proteins have been found to directly interact with one another or act together in complexes that regulate the local chromatin structure at target genes. Thus, it appears that the functions of individual epigenetic ÌD proteins converge onto similar biological processes that are crucial to neuronal processes. The next challenge will be to gain more insight into patterns of altered DNA methylation and histone modifications that are caused by epigenetic gene mutations and how these will disrupt the brain-specific expression of target genes. To that end, we follow a multi-level strategy that besides neurogenetics includes functional genomics which encompasses the generation and characterization of model organisms (mouse, rat and Drosophila) and molecular & cellular neurobiology to dissect molecular and cellular mechanisms that are key to learning and memory. Such research may reveal that a wide variety of mutations in the genetic code result in a more limited number of disruptions to the epigenetic code. If so, this will provide a rationale for therapeutic strategies.

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**Oral Communication Abstract – 6B.02**

## ROLE OF CELL ADHESION MOLECULES NECTINS 1 AND 4 IN THE PATHOGENESIS OF ECTODERMAL DYSPLASIA SYNDROMES

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*Nectin, ectodermal dysplasia, cell adhesion, morphogenesis*

Ectodermal dysplasias (EDs) are heterogeneous conditions with more than 200 described forms. The presence of hair and teeth anomalies, alopecia, and cutaneous syndactyly defines the ectodermal dysplasia-syndactyly syndrome (EDSS; OMIM 613573). We have recently identified PVRL4 mutations as the cause of EDSS in two families from Algeria and Italy. PVRL4 encodes nectin-4, a cell adhesion molecule with a relevant role in cadherin-based adherens junctions and belongs to a family of four members. Notably, PVRL1 (encoding nectin-1) mutations cause Cleft Lip/Palate-Ectodermal Dysplasia Syndrome (CLPED1; OMIM#225060) also known as Zlotogora-Ogur syndrome, hence the term "nectinopathies". A homozygous pathogenic mutation was detected in the Algerian family. In addition we identified a missense and a truncating mutation in the Italian family, confirming PVRL4 as the disease causative gene of EDSS. Interestingly, in most adult human tissues, nectin-4 expression is almost absent. In turn, high levels of protein are present in the hair follicle and in the interdigital regions of mouse embryos, the mainly affected tissues in EDSS. These data outline a relevant role of nectin-4 in hair cycling and morphogenesis and support the existence of a growing group of EDs secondary to defective cell adhesion. We are currently studying the mechanism by which nectins 1 and 4 cooperates towards the regulation and the maintenance of tissue morphogenesis.

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**Oral Communication Abstract – 6B.03**

# p63 AND DLX RELATIONSHIP: RELEVANCE IN HUMAN HEREDITARY ECTODERMAL DYSPLASIA SYNDROMES

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*p63, hereditary disease, epithelial development and differentiation*

DLX and p63 proteins are families of transcription factors that plays a pivotal role in many developmental processes, ranging from organization of the body plan to differentiation of individual tissues. Mutations in the p63 and DLX genes are responsible for Ectodermal Dysplasia Syndromes (EDs), a group of pathological conditions that share common anomalies in epithelial- and mesenchimal-derived organs such as hair, tooth, nails, and sweat glands and have been associated with abnormalities in other organs (Priolo and Lagana, 2001). DLX3 mutations are responsible for tricho-dento osseous syndrome (TDO) and amelogenesis imperfecta, hypoplastic-hypomaturation, with taurodontism (AIHHT), while mutations in the p63 gene have been associated with ectrodactyly-ectodermal dysplasia cleft lip/palate (EEC), ankyloblepharon-ectodermal dysplasia clefting syndrome (AEC), and split hand/foot malformation (SHFM). We and others have shown that p63 acts upstream of the Dlx genes in a transcriptional regulatory pathway relevant for ectodermal dysplasias. More recently, we have found that DLX3 and p63 proteins can interact and exert a reciprocal regulation on their activities. We propose that DLX genes and p63 are components of multiple regulatory mechanisms and signaling pathways that play a crucial role during development and differentiation.

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**Oral Communication Abstract – 6B.04**

# STUDIES IN YEAST MODEL OF PATHOLOGICAL MUTATIONS OF THE HUMAN GENE *HCCS*

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*Yeast model, mitochondria, cytochrome c, heme lyase, mitochondrial disease*

Microphthalmia with linear skin defects (MLS) is an X-linked dominant male-lethal neuro-developmental disorder associated to mutations in the holocytochrome c-type synthetase (*HCCS*) transcript. Female patients display microphthalmia and linear skin defects, CNS malformation, mental retardation and cardiac defects.

The HCCS gene encodes a mitochondrial protein that catalyzes the attachment of heme to apo-cytochrome c (Cytc) and c1. In yeast the enzyme heme lyase is encoded by nuclear gene CYC3 and then transferred into the mitochondria. Defects in yeast heme lyase (cyc3 null mutant) result in loss development of respiratory growth.

While ectopic expression of human *HCCS* wild-type in a yeast null mutant cyc3 is capable to restore oxidative growth, the expression of *HCCS* mutants associated with MLS disease (E159K; R217C) do not complement the OXPHOS phenotype. Measurement of the mitochondrial cytochrome content were done to evaluate the structural integrity of the respiratory chain complexes. In *cyc3* yeast null strain, transformed with the gene *HCCS* wild type, spectra profile was indistinguishable from the strain carrying the yeast gene *CYC3* wt**.** In contrast, the *HCCS* null strain showed a marked reduction in both the absorption peak of cytochrome c and cytochrome aa3, similar defects were exhibited by the two pathogenic alleles HCCS. In agreement with the reduction in content of cytochrome c we observed a marked reduction in respiratory activity.

Western blotting analysis was performed to check the successful import of cytochrome c into the mitochondria. Pathogenic alleles showed a reduced amount of cytochrome c compared to that of wild-type, indicating the accumulation of apocytochrome c into the mitochondria due to the presence of a heme lyase enzyme, although not catalytically active.

In addition, both strains carrying the *hccs* mutant or null alleles showed a significant decrease in the chronological life span (CLS). Treatment with acetic acid to induce necrosis showed a survival rate of cells of the mutant strains significantly lower than that of wild-type suggesting that mutations in HCCS has led to a decline in life span due to necrotic death. These data confirm the role of *HCCS* in mitochondria and suggest that the MLS should be considered a mitochondrial disease.

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**Oral Communication Abstract – 6B.05**

# GENE-GENE INTERACTION AMONG CYTOKINE POLYMORPHISMS INFLUENCE SUSCEPTIBILITY TO AGGRESSIVE PERIODONTITIS

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*Aggressive periodontitis, cytokine genes, epistasis, genetic susceptibility, gene-gene interaction*

Aggressive periodontitis (AgP) is a complex multifactorial disease: bacterial infection is the primary etiologic agent, but also other host factors are involved, in particular the immune system seems to play an important role in the pathogenesis.

Gene-gene interaction is a general ubiquitous component of the genetic architecture of complex diseases and it is quite reasonable to expect that gene-gene interaction may play a crucial role in periodontitis too. The purpose of this paper was to apply, on a dataset of candidate gene polymorphisms for AgP, different multivariate analysis tools, with the aim to infer biological structures from genetic markers by means of an epistatic analysis.

Our sample is composed by 122 generalized AgP patients and 246 systemically healthy controls, recruited among subjects seeking care for periodontal treatment at three different Italian centers. We focused our attention on 28 polymorphisms, all lying in genes involved in inflammation and/or immunity response. Genotyping was performed using MassArray high-throughput DNA analysis with MALDI-TOF mass spectrometry (Sequenom, Inc., San Diego, CA).

We made a case-control association analysis with PLINK. Subsequently we analyzed combined genotypes using both parametric algorithms, such as General Discriminant Analysis (GDA) and Generalized Linear Model Analysis (GLZ), and the non parametric Multifactor Dimensionality Reduction (MDR) approach.

Our results confirm an important role of IL-6 in susceptibility to AgP: IL-6(-572) variant shows a strong independent effect ($p<0.001$) whereas IL-6(-6106) and IL-6(-1480) contribute to the disease interacting with IL-18 ($p<0.001$), IL-4 ($p<0.001$) and, less significantly, with IL-2 ($p=0.04$). We highlight also a significant contribute to AgP susceptibility of Fc gamma receptor gene variants both independently and as combined genotype: FCGR2A*C-FCGR3B*C shows an increased effect on susceptibility to AgP ($p=0.003$) and this is not attributable to LD between the two polymorphisms, since the two markers are in complete equilibrium.

Two other interesting results emerge: an involvement of Selenoprotein S gene SEPS1 in the determination of AgP, both as independent factor ($p=0.005$), and in association with IL-2 ($p<0.001$); and a relation between Tumor Necrosis Factor Surface Receptor 1 gene and AgP in association with IL-2 ($p<0.001$). At the best of our knowledge, this is the first evidence reported in literature showing a potential association of these latter genes and AgP susceptibility.

As a last consideration, none of the analyses performed revealed an involvement of Interleukin-1 cluster genes, the most studied factor in periodontitis, in determining the pathological phenotype. This is consistent with our previous findings in a similar Caucasian sample.

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**Poster Communication Abstract – 6B.06**

# *CDKN2A*/P16$^{INK4A}$ 5'UTR VARIANTS IN MELANOMA PREDISPOSITION: LOST IN TRANSLATION, SOMEWHERE

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*CDKN2A, melanoma, 5'UTR variants, translation efficiency, polysomal profiling*

The *CDKN2A* gene is the most common high penetrance susceptibility gene identified to date in melanoma families. While functional tests for determining the pathogenicity of missense germline mutations in the *CDKN2A* coding region have been developed, rare polymorphisms or sequence variants at the *CDKN2A/ p16*$^{INK4a}$ 5'UTR, encountered during routine screening, are usually defined as variants with unknown significance after determining their frequency in control population and the cosegregation analysis in the family, when possible. We recently developed reporter assays to study a panel of *p16*$^{INK4a}$ 5'UTR variants identified as heterozygous changes in patients from a hospital-based series of melanoma cases (c.-21C>T; c.-25C>T&c.-180G>A; c.-56G>T; c.-67G>C). Monocistronic as well as bicistronic luciferase-based reporter vectors were developed and used to test wild type and variant p16$^{INK4a}$ 5'UTR activity upon transient transfection in melanoma-derived cells (WM266-4, G361 and SK-Mel-5) and in the breast cancer-derived MCF7 cells. Results revealed that the c.-21C>T variant had a strong negative impact on the reporter activity, similar to that of the known melanoma-predisposing mutation c.-34G>T, included as a control. The variants at -56 and at -25&-180 exhibited a milder impact, while results with c.-67G>C were dependent on the type of reporter vector. Quantification of the luciferase mRNA conducted in parallel with the luciferase assay indicated that the impact of the variants was mainly post-transcriptional. We also applied a polysomal profiling technique to measure allelic imbalance starting from heterozygous patient-derived cell lines and found that the c.-21C>T variant but also c.-56T>G and c.-67G>C exhibited lower association with the polysomes suggestive of reduced mRNA translation efficiency. A panel of eleven additional germline variants in the 5'UTR of *p16*$^{INK4a}$ is being investigated. In particular we are focusing on the functional interactions between wild type and variant *p16*$^{INK4a}$ 5'- and 3'-UTR sequences and on the impact the variants can have on

the targeting of the *p16*$^{INK4a}$ mRNA by microRNAs or RNA binding proteins.

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**Poster Communication Abstract – 6B.07**

# STRIKING CHANGES OF MIRNAS EXPRESSION IN CD4+ T LYMPHOCYTES OCCURRED EVEN IN THE ABSENCE OF AN ESTABLISHED HIV-1 INFECTION

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*miRNAs, HIV infection, immuno-markers, host genetic factors*

miRNAs are known to inhibit HIV-1 expression by modulating host innate immunity or by directly interfering with viral mRNAs. Here, we examined miRNA expression in CD4+ T lymphocytes from HIV-1 élite LTNP (éLTNP), naïve, and multiple exposed uninfected individuals (MEU) by real-time PCR-based arrays. Viro-immunological analysis in CD4+ T cells revealed that éLTNP had a lower amount of activated T lymphocytes, less activated regulatory T cells, more TREC+ cells, and less HIV-DNA than naïve patients. Among the quantified 377 miRNAs, 113 varied (either up or down) of at least 1 $Log_{10}$ between each patients group and healthy controls: 25 miRNAs were up-regulated, while 88 were down-regulated. All the up-regulated miRNAs were undetectable in cells from controls. In all patients' classes, 3 miRNAs (miR-203, miR-449a, miR-502-5p) were up-regulated and 5 (miR-329, miR-337-5p, miR-379, miR-503, miR-518d-3p) were down-regulated, suggesting a hypothetic HIV-1 exposure signature. By hierarchical clustering, éLTNP clustered with naïve whereas all MEU grouped together, supporting that miRNAs may work as HIV-1-related genetic factors. Furthermore, 21 miRNAs significantly differentiated éLTNP from MEU and 23 miRNAs the naïve from MEU (16 miRNAs were in common), only miR-155 characterized éLTNP vs. naïve. Among these miRNAs, only 3 were involved in viral replication (let-7a, miR-34a, miR-485-3p), whereas 5 in immune response (miR-21, miR-23a, miR-125-3p, miR-155, miR-424). On the whole, these findings suggest that miRNA profile observed in all HIV-1+ and MEU subjects could be the result not only of a productive infection, but also of the exposure to viral products (e.g., plasma gp120 that can unspecifically bind CD4+ T cells). Thus, even the exposure to HIV products can leave stable signs in immune cells, whose meaning has to be clarified.

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**Poster Communication Abstract – 6B.08**

# *Fra14A2*, THE MURINE ORTHOLOGUE OF COMMON FRAGILE SITE *FRA3B,* IS UNSTABLE *IN VIVO* IN SOMATIC AND GERM CELLS

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*Common fragile sites, genomic instability, mouse gametogenesis*

Common fragile sites (CFS) are large sequences prone to chromosome breakage, which are hotspots for chromosome rearrangements and colocalise to genomic alterations found in tumours (Durkin & Glover, Annu. Rev. Genet., 41:169-192, 2007). CFS may harbour genes whose mutation is cause of human disease. For example, *PARK2* at *FRA6E* is involved in a form of autosomal recessive juvenile parkinsonism. Breakages at CFS appear *in vitro* after replication stress, and one key question is whether this loci may be considered stable in human subjects under physiological conditions. *FRA3B* (3p14.2) is the most active CFS of the human genome and the tumour-suppressor gene *FHIT* represents its core of instability (Durkin & Glover, Annu. Rev. Genet., 41:169-192, 2007). Sequence conservation between human and murine CFS has been demonstrated (Helmrich et al, Genome Res., 16:1222-1230, 2006); *Fra14A2/Fhit* is the murine orthologue of *FRA3B/FHIT* (Glover et al., Cancer Res., 58:3409-3414, 1998).

Molecular combing is a single molecule approach allowing a fine description of the structural and replication features of chromosomal regions (Palumbo et al., Chromosoma, 119:575-87, 2010). By molecular combing we have found that in normal human cells a small but relevant percentage of sequence abnormalities is present within the core of instability encompassing exon 5 of *FHIT* at *FRA3B*. Interestingly, the region is coincident with that described in cancer (Durkin & Glover, Annu. Rev. Genet., 41:169-192, 2007).

Aim of the study is to evaluate the *in vivo* stability of CFS *Fra14A2,* the murine orthologue of *FRA3B*, in the absence of exogenous replication stress. We are particularly interested in describing the modality of expression of the fragile site in the mouse germ cells, in order to evaluate if these genomic regions maintain the unstable features described in culture, and if they contribute to a genetic risk for the progeny. In mouse embryonic fibroblasts (MEF), used as controls, *Fra14A2* appears to be very active in response to aphidicolin-induced replication stress. Moreover, according to molecular combing results, the untreated MEF cells are characterised by spontaneous genomic instability occurring within *Fhit.* In particular, by using a probe pair identifying the orthologous sequence of the human core of instability, we found a rather heterogeneous pattern of hybridization suggesting the occurrence of deletions and rearrangements within the region under study. This is in agreement with our data on human cells. Molecular combing has been applied also on elongated DNA from epididymal sperm of adult C57Bl/6J mice. Interestingly, sequence instability events, similar to those observed in culture in MEF cells and in human cell lines, were found. Therefore mature gametes may carry sequence abnormalities at *Fra14A2/Fhit*.

We also considered the replication profile of the murine fragile site. Whole genome replication parameters of MEF cells appeared comparable to those observed in human primary

fibroblasts. In particular, fork rates were 1.49±0.06 kb/min, inter-origin distances were 136.7±12.8 kb. The single locus data are in progress. As a further step we aim to evaluate the spontaneous instability of *Fra14A2* in different somatic compartments with diverse proliferation activity, to better understand the relationships between replication activity and instability of CFS.

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**Poster Communication Abstract – 6B.09**

# *SDH6* A NEW GENE OF *SACCHAROMYCES CEREVISIAE* REQUIRED FOR ASSEMBLY OF COMPLEX II

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*Yeast model, mitochondria, succinate dehydrogenase, Saccharomyces cerevisiae, mitochondrial disease*

Yeast is recognised as a model for approaching human diseases-associated gene functions particularly concerning mitochondrial ones due to the yeast ability to survive without a functional mitochondrial respiratory chain, provided that a fermentable carbon source is made available.

Succinate dehydrogenase or complex II is composed of four subunits (*SDHA-D* in humans, *SDH1-4* in yeast), all encoded by nuclear genes. Despite the extensive knowledge on structural and catalytic properties of the complex, only recently two assembly factors specific for the SDH has been found: *SDHAF1* and *SDHAF*2. The *SDHAF1* gene has been identified in humans because linked to infantile leukoencephalopathy. A yeast strain deleted in *SDH6*, the *SDHAF1* ortholog, was OXPHOS incompetent, due to a severe and specific reduction of SDH activity. However, the Km value for succinate was similar in wild-type and in the null mutant, suggesting that defective SDH activity was caused by reduced number of enzyme units rather than by qualitative alterations of complex II. In agreement with the reduction of SDH activity, we found a reduction in the amount of SDH complex on 2D-BNGE. In addition *SDHAF1* is not physically associated and stably bound to complex II. Furthermore, a co-immunoprecipitation study was undertaken in order to determine whether *SDHAF1* interacts with a subunit of SDH. The two pathogenic alleles, R61P and G63R, have been tested for their capability to be translocated into mitochondria.

To gain insight into the molecular basis of the Sdh6-less phenotype, we screened a genomic library for multicopy suppressors of acetate-negative phenotype and characterized the genes identified. Two genes *YAP1* and *YAP2*, encoding transcription factors necessary for the stress response, were found to suppress the OXPHOS growth phenotype of the *sdh6* null mutant, but failed to increase SDH enzymatic activity. These results suggest that Sdh6 might play an additional role besides the SDH assembly.

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**Poster Communication Abstract – 6B.10**

# OVEREXPRESSION OF DNA POLYMERASE ZETA REDUCES THE MITOCHONDRIAL MUTABILITY CAUSED BY PATHOLOGICAL MUTATIONS IN DNA POLYMERASE GAMMA IN YEAST

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*DNA polymerase zeta, Rev1, DNA polymerase gamma pathological mutation, rescue of mtDNA mutability, Rev3 mutagenesis*

DNA polymerase zeta (Pol zeta), which is composed by Rev3 and Rev7 subunits, and Rev1 are polymerases involved in translesion synthesis (TLS). The main role of Pol zeta during TLS is the extension from terminally (mismatched) primers. Rev1 encodes for a deoxycytidyl transferase that preferentially incorporates C opposite to an abasic site. Interaction of Rev1 with Pol zeta trough a Rev3-Rev1 binding stimulates the activity of Pol zeta. Besides their role in the nucleus, yeast Rev3, Rev7 and Rev1 localize also in mitochondria: deletion of any of the genes encoding these enzymes increases the point mutation of mitochondrial DNA (mtDNA) measured as the frequency of mtDNA mutants resistant to erythromycin ($Ery^R$).

It has been previously speculated that, in absence of the TLS by Rev1 and/or Pol zeta, the mitochondrial DNA polymerase gamma (Pol gamma), the mitochondrial replicase, could introduce mutations by replicating the mtDNA lesions. As a consequence, Pol zeta and Rev1, which are responsible in the nucleus for the error-prone bypass of DNA lesions, in the mitochondria could be responsible for an error-free bypass, or maybe less error-prone, compared to Pol gamma. Pol gamma is a protein conserved in fungi and animals. To date, more than 150 pathological mutations in POLG have been identified in severe mitochondrial disorders. In yeast the DNA polymerase gamma is encoded by *MIP1* gene. Thanks to the similarity between human Polg and Mip1 (approximately 43%), yeast was used to validate the role of human putative pathological mutations, to understand the biochemical consequences associated to these mutations and in particular to find mechanisms able to rescue the harmful effects of Mip1 mutations, such as the treatment with antioxidant molecules.

In this perspective, we wondered whether overexpression of Pol zeta and Rev1 could rescue the detrimental effects on mtDNA point and extended mutability caused by mutations mapping in different domains of MIP1. We show that overexpression of Rev3 reduces, in a Rev7-dependent and Rev1-independent manner, the mtDNA extended mutability caused by a subclass of pathological mutations in Mip1, the yeast mitochondrial DNA polymerase orthologous to human Polg, whose detrimental effects on mtDNA stability are not rescued by treatment with antioxidants. This beneficial effect observed is synergistic with the effect achieved by increasing the dNTPs pools, which are the substrate of both Pol zeta and Pol gamma. On the contrary, the overexpression of Rev3 does not rescue the mitochondrial extended mutability caused by Mip1 mutations sensitive to the treatment with antioxidants. Furthermore, overexpression of both Pol zeta and Rev1 reduces mtDNA point mutability in mtDNA mutator *mip1* strains. Since overexpression of Rev3 is detrimental for nuclear DNA mutability, in order to obtain the beneficial effect on mtDNA without

the negative effect on nuclear DNA, we constructed and overexpressed a mutant isoform of Rev3 unable to migrate into the nucleus: it reduced mtDNA mutability without increasing nuclear mutability.