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Oral Communication Abstract – 5A.01

YEAST AS A MODEL SYSTEM TO SHED LIGHT ON THE ROLE OF THE HUMAN DISEASE PROTEIN MPV17

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Yeast model, mitochondria, mtDNA, MPV17/SYM1, mtDNA instability

An intriguing gene necessary for the maintenance of mtDNA is human MPV17, mutation of which leads to a peculiar form of hepatocerebral mtDNA depletion syndrome (MDS). Even though Mpv17 mutations are one of the causes of MDS in humans and the discovery of this protein has been reported more than 20 years ago, its function is not yet understood. Originally considered as a peroxisomal membrane protein, it was later demonstrated that Mpv17 is localized to the inner mitochondrial membrane, as also previously demonstrated for the yeast orthologue Sym1, identified as a heat shock protein with a role in metabolism and/or tolerance to ethanol. With the aim of clarifying the role of MPV17 pathological alleles in MDS, we took advantage of *S. cerevisiae* as a model system. These studies in yeast have shed some light on the function of Sym1. The *sym1* mutant mitochondria are morphologically abnormal, with flattened mitochondrial cristae and accumulation of electron-dense particles, suggesting a role for Sym1 in the structural preservation of the inner mitochondrial membrane. This defect is not a consequence of the mtDNA instability because it has been observed under cultural conditions where no defect of mtDNA was observed, indicating that the morphogenetic effects of Sym1 are likely to precede and possibly determine its effects on mtDNA stability. The phenotypes of double mutants (*cit1 sym1*, *cit2 sym1*) and the nature of multicopy suppressors (*ODC1, YMC1*) suggest for *sym1* null mutant a defect in Krebs cycle confirmed by an enzymatic analysis that clearly indicates a heavy reduction of succinate dehydrogenase activity. Accordingly, *sym1Δ* displays a significant reduction in the amount of glycogen that is dependent on gluconeogenesis, which is in turn regulated by the anaplerotic flux of tricarboxylic acid intermediates from mitochondria to the cytosol. Interestingly, patients with Mpv17 mutations suffer from drastic, often fatal, hypoglycaemic crises, which are likely due to glycogen shortage in liver. Moreover blue-native gel electrophoresis immunovisualization clearly demonstrated that Sym1 is part of a high-molecular weight complex. While further work is necessary to identify the primary role of Sym1, including the molecular dissection and characterization of the Sym1-containing protein complex, these results indicate that Sym1 is involved in the structural and functional stability of the inner mitochondrial membrane, thus

controlling crucial mechanisms related to this compartment, including respiratory chain complexes activity, mitochondria morphology and mtDNA maintenance.

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Oral Communication Abstract – 5A.02

HORSE MITOCHONDRIAL GENOMES: AT LEAST 17 MATRILINEAL LINEAGES UNDERWENT DOMESTICATION

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Horse mitochondrial genome, domestication, mtDNA haplogroups, origin of Equus caballus, Przewalskii horse

Archaeological and genetic evidences concerning the time and mode of wild horse (*Equus ferus*) domestication are still debated. High levels of genetic diversity in the horse mitochondrial DNA (mtDNA) have been detected when analyzing the control region, whose recurrent mutations, however, tend to blur the structure of the phylogenetic tree. To overcome the likely limitations of control-region data and to improve the resolution of the horse mtDNA phylogeny, we analyzed a total of 83 mitochondrial genomes (81 new and two from the literature) from modern horses (*Equus caballus*) across Asia, Europe, the Middle East and the Americas. Our data indicate a major founder event around 120,000 years ago and reveal 18 distinct haplogroups (A-R) whose radiation times are essentially confined to the Neolithic. All haplogroups were detected in Asia, but one (L) is most common and equally diverse in Europe, and another one (F) is only found in *Equus przewalskii*, the only remaining wild horse. Therefore, at least 17 matrilineal lineages from the extinct *Equus ferus* underwent domestication, probably in the Eurasian steppes but possibly even in Western Europe, and were transmitted to modern breeds. The identification of these haplogroups is a prerequisite for ancient DNA studies and for evaluating the role of mitochondrial DNA backgrounds in racecourse performance.

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Oral Communication Abstract – 5A.03

THE NUCLEOPORIN mRNA EXPORT PROTEIN, RAE1, EXERTS PLEIOTROPIC EFFECTS ON MITOTIC AND MEIOTIC CELL CYCLE, AND VIABILITY IN *DROSOPHILA MELANOGASTER*

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Meiosis, spermatogenesis, fertility, cell cycle

Drosophila is a particularly suitable organism to study the complex events of male germ cell differentiation and meiosis that culminate in mature sperm formation. Here, we report the identification of the gene responsible for the fully viable, male sterile phenotype of an EMS-induced mutant strain. Immunofluorescence assays revealed early defects of nuclear envelope morphology and chromosome condensation in primary spermatocytes. Upon meiosis, these mutants display a unique behavior since they execute a strongly impaired first meiotic division and skip the second one, resulting into onion stage spermatids that, nonetheless, attempt a highly defective spermiogenesis. By combining traditional gene mapping techniques and DNA sequencing with RNA interference experiments, we identified the locus affected by EMS treatment in *rae1* (ribonucleic acid export) gene. Rae1 is a nucleoporin that has been shown to be required for nuclear mRNA export and mitotic spindle assembly from yeast to mammals. Driven knockdown of Rae1 by RNAi in *Drosophila* neuroblasts and imaginal discs surprisingly altered the normal progression of mitotic cell cycle eventually resulting into lethality. The missense mutation we identified in *Z2-5584* line brings about a substitution of an evolutionarily highly conserved amino acid on the third putative WD40-repeat domain, that accounts for a very severe phenotype in testis whereas it has no effect on development and viability of *Drosophila*. As a whole, these results lead us to speculate that the domain affected by the mutation in RAE1 protein is specifically required for meiotic cell cycle, thus providing the first evidence of the RAE1 involvement in *Drosophila* male germ line differentiation, where its function is strictly necessary to ensure male fertility.

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Oral Communication Abstract – 5A.04

A ROLE FOR THE *DROSOPHILA* HISTONE VARIANT H2AV IN MITOTIC CHROMOSOME SEGREGATION

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Drosophila, histone variants, chromosome segregation, cell division, chromatin

We found that mutations in the H2Av gene, which encodes the Drosophila H2A variant, impair chromatin compaction of pericentric chromosome regions and lead to irregular chromosome segregation during mitotic divisions of larval neuroblasts. Immunostaining with tubulin, DNA and centrosomal proteins revealed that the most straightforward phenotype elicited by H2Av-depleted mutant cells is the presence of apparent anaphase-looking bipolar spindles (20%; n= 125), with chromosomes not connected to the spindle poles by bundles of kinetochore microtubules (MTs). In addition, mutant chromosomes were not able to congress to the equator of the cell spindle, failed to separate in sister chromatids and appeared to segregate randomly to the poles. This was also confirmed by immunolocalization experiments using kinetochore markers such as Cenp-C, Cid and Hec1/Ndc80, which appeared localized on mutant chromosomes always as two twin spots. We have also found that most of the irregular mitotic figures (85%, n= 45) exhibited high levels of Cyclin B with respect to anaphase control cells, in which Cyclin B was almost absent (3%, n= 55). This indicates that all anaphase-looking bipolar spindles with scattered chromosomes are indeed in a metaphase-like status (ana/metaphase-like figures). Consistent with these findings, we observed that checkpoint proteins ZW10 and BubR1 remained strongly localized at centromeres of mutant chromosomes, whereas in wild-type ana/telophase figures both proteins are mostly absent from segregating chromosomes. Moreover, in the mutant ana/metaphase-like figures ZW10 failed to stream towards the cell poles, as occurs in normal metaphases, suggesting that depletion of H2Av causes defective microtubule attachment to the kinetochore. Finally, MT regrowth experiments after cold exposure revealed that mutations in *H2aV* inhibit kinetochore-driven MT growth. As a consequence Dgt6, an Augmin component that plays a pivotal role in K-fibers formation, is strongly reduced in mutant neuroblasts.

Taken together our results suggest that H2Av might be required for the regulation of mitotic chromosome segregation in *D. melanogaster*. Furthermore, our data highlight an unanticipated role of this specific histone variant in controlling the interactions between kinetochore and k-fibers.

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Oral Communication Abstract – 5A.05

VITAMIN B6 IS REQUIRED FOR *DROSOPHILA* CHROMOSOME INTEGRITY

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Vitamin B6, chromosome aberrations, glucose, AGEs, Drosophila

Chromosome aberrations (CAs) are one of the major contributing factors to carcinogenesis. Recent work has shown that micronutrients such as folic acid and vitamins B6 and B12 play important roles in the maintenance genome integrity and cancer prevention. We isolated mutations in the *Drosophila dPdxk* gene that encodes pyridoxal kinase, a highly conserved enzyme required for vitamin B6 (PLP) biosynthesis. *dPdxk* mutants exhibit elevated CA frequencies (~ 6% *vs* 0.5 % in controls) in *Drosophila* larval brain cells. Cytological analysis of brain preparations from *dPdxk* mutant larvae grown in food supplemented with PLP showed complete rescue of the CA phenotype. In addition, wild type larvae treated with vitamin B6 antagonists (4-deoxypyridoxine hydrochloride, penicillamine, cycloserine or isonazid) displayed high CA frequencies (ranging from 3 to 19%), confirming that PLP plays an essential role in the maintenance of genome integrity. Surprisingly, *dPdxk* mutant larvae and isolated brains grown in the presence of D-glucose (1%) showed a strong increase in the frequency of CAs (from 6% to 30%); D-glucose treatment of wild type larvae and brains did not result in detectable effects on chromosome integrity. These results indicate that in absence of PLP, D-glucose is genotoxic. Larval brains and hemolymph of *dPdxk* mutants showed a higher glucose concentration than their wild type counterparts. We also observed that mitotic spindles of *dPdxk* mutants are resistant to colchicine-induced microtubule depolymerization, an effect due to tubuline glycosilation. Together, our results show that an elevated intracellular concentration of glucose has clastogenic effects that are likely to result from AGEs (Advanced Glycation End-products) accumulation. Consistent with this hypothesis, *dPdxk* mutant brains treated with both glucose and a-lipoic acid (a well-known AGE inhibitor) showed fewer CAs than brains treated only with glucose. The clastogenic effect of glucose in the absence of vitamin B6 is evolutionarily conserved. Inactivation of *PDXK* in human fibroblasts and HeLA cells by either RNAi or chemical inhibitors resulted in chromosome breakage, which was potentiated by glucose addition. These results suggest that patients with vitamin B6 deficiency or treated with vitamin B6 inhibitors may suffer chromosomal damage when their blood sugar is too high.