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INTERACTIONS BETWEEN SIRT1 AND POLY(ADP-RIBOSE) POLYMERASES – NOVEL MODES OF METABOLIC REGULATION?

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SIRT1 is a NAD⁺-dependent protein deacetylase and its NAD⁺-dependence enables SIRT1 to act as a sensor of cellular metabolism. SIRT1 is activated by increases in NAD⁺ levels, or indirectly by different small molecule activators. SIRT1 activation leads to the deacetylation and activation of numerous metabolic transcription factors such as PGC-1 and FOXOs that culminates in the enhancement of mitochondrial biogenesis. Activation of SIRT1 in mice results in higher whole-body energy expenditure (EE) and protection against high-fat diet (HFD)-induced obesity and diabetes. NAD⁺ dependence of SIRT1 activity suggested that artificially silencing other NAD⁺ consumers, such as PARPs, could effectively induce SIRT1 activity. Our results demonstrate that the deletion of either PARP-1, or PARP-2 in mice, equally results in a leaner phenotype, higher EE and SIRT1 activation that was protective against HFD-induced obesity and diabetes. PARP-1 and SIRT1 are interrelated due to the competition for the same limiting NAD⁺ pool. However, the link between PARP-2 and SIRT1 is based on a direct interaction between PARP-2 and the proximal SIRT1 promoter, where PARP-2 acts as a suppressor. We further tested our observations in a model of Doxorubicin (DOX)-induced cardiovascular damage. DOX enhances free radical production and hence induce DNA damage, PARP activation, and cellular and mitochondrial dysfunction. PARP-1 knockout mice are protected against DOX-induced damage, what prompted us to investigate whether the deletion of PARP-2 could be equally protective. Aorta from PARP-2 knockout mice were partially protected against DOX-induced smooth muscle damage. The depletion of PARP-2 does not drastically reduce PARP activity suggesting a different protective mechanism than in PARP-1^{-/-} mice. Therefore we hypothesized that SIRT1-induced mitochondrial stabilization could be the protective factor. We have observed the induction of SIRT1 upon PARP-2 depletion both *in vivo* and *in vitro*. Induction of SIRT1 induced mitochondrial biogenesis and protected mitochondria against DOX-induced damage. The results presented hereby depict a new modality for the activation of mitochondrial biogenesis and presents different means of applicability.

Supported by: Bolyai fellowship to PB, National Innovation Office (FR-26/2009, Baross program Seahorse grant), OTKA CNK80709, K82009, K75864, PD83473, TAMOP-4.2.2/B-10/1-2010-0024 and TAMOP-4.2.2-08/1-2008-0019 and UD MHSCMecenatura (Mec-8/2011).

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DUTPase IS ESSENTIAL FOR GENOME STABILITY AND IMAGINAL TISSUE DEVELOPMENT IN *DROSOPHILA*

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Proper S phase progression during cell cycle requires a balanced and abundant nucleotide pool. Impairment in the nucleotide biosynthesis may lead to DNA replication errors and DNA damage checkpoint activation and also can violate genome integrity. dUTPase has the role to maintain uracil-free genome by eliminating dUTP from the nucleotide pool. However, physiological fate of the unusual uracil-substituted DNA is not described in details. Depletion of dUTPase in some organisms (*E. coli*, *S. cerevisiae*, *A. thaliana*, *C. elegans*) revealed its conservative role to preserve genome integrity and avoid DNA repair responses. Among uracil-DNA glycosylases, UNG is the most important DNA repair enzyme that is responsible for the majority of uracil-DNA processing, and whose depletion can complement dUTPase deficiency at least partially. Since *Drosophila* lacks the UNG enzyme, it provides a hopeful model to study the fate of uracil-substituted DNA.

We showed that dUTPase expression is restricted mainly to undifferentiated proliferating tissues. Furthermore, genomic uracil substitution depends on the dUTPase expression pattern in *Drosophila* larval tissues, since absence of UNG allows the persistence of uracil-DNA in differentiated tissues. Perturbing this pattern by dUTPase silencing increased the rate of genomic uracil substitution in undifferentiated tissues as well. However, lack of UNG does not guarantee complete tolerance to uracil-DNA. We detected an increased rate of DNA strand breaks in imaginal wing precursor tissue and developmental defects in pupal stage. Our suggested candidate for uracil-DNA processing is the mismatch-specific TDG homologue uracil-DNA glycosylase, Thd1, whose silencing complemented pupal lethality of dUTPase silenced animals.