THE ROLE OF INOSITOL METABOLITES IN THE MODULATION OF THE DNA DAMAGE RESPONSE

RAFAELLA JEKABSON[1], JOSÉ RENATO ROSA CUSSIOL[2]

Department of Biochemistry –
Instituto de Farmacologia e Biologia Molecular
Universidade Federal de São Paulo
e-mail: rafaella.jekabson@unifesp.br ; cussiol@unifesp.br

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Introduction - The integrity of the DNA molecule is crucial for the preservation of life as the accumulation of lesions may lead to consequences such as genomic instability and loss of genetic information. Under such circumstances, a comprehensive DNA Damage Response (DDR) was selected, enabling the recognition and repair of such lesions along with effector roles in diverse cell pathways such as DNTP pool sensing, cell cycle checkpoints and telomere maintenance. The budding yeast protein kinases Mec1/Tel1 (ortholog of mammalian ATR/ATM) constitute an important element in the DDR acting as sensors of damaged DNA, but also catalyzing the phosphorylation of proteins. More recently there have been reports correlating enriched Mec1 phosphorylation with the inositol metabolism pathway under conditions of genotoxic stress caused by the alkylating agent methyl methanesulfonate (MMS) or the ribonucleotide reductase inhibitor hydroxyurea (HU). Inositol is present in all cells and can be synthetized from glucose, originating diverse metabolites, amongst which the inositol polyphosphate signaling family. It has been previously demonstrated that knockouts in proteins of such family lead to sensitivity to genotoxic stress. We investigated in *Saccharomyces cerevisae* the specific Mec1/Tel1 targets Opi1 and Kcs1, related to the regulation of inositol synthesis and pyrophosphate synthesis (PP-IPs) respectively. Importantly, we elucidated further the relevance of the inositol pyrophosphates in the context of replication stress induced either by MMS or HU.

Materials and methods - We generated yeast cell lines following the epitope tagging and gene disruption protocols. Genes initially selected for analysis are Opi1 and Kcs1, a repressor of transcription of inositol pathway genes and an inositol polyphosphate kinase respectively. To access the contribution of these genes for the DDR we employed cell viability assays, cell cycle progression analysis and *western blots*

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Preliminary results - We have initially established that there is an accumulation of protein levels of Kcs1 in response to exposure to MMS in WT cells. The knockout has also demonstrated increased sensitivity to MMS and HU, as well as hyperactivation of the protein kinase Rad53, an important effector of the DNA damage signaling. We hypothesize the hyperactivation of Rad53 could lead to defective DNA repair through homologous recombination based on previous reports. Moreover, we have demonstrated that deletion of the transcriptional repressor Opi1, rescue the MMS/HU sensitivity of cells lacking Kcs1.

Conclusions - Kcs1 could promote genomic stability through the synthesis of inositol pyrophosphates. However, it is still unclear how these molecules affect the DDR. We hypothesize IPs can regulate direct or indirectly Rad53 kinase activity through non-enzymatic phosphorylation or allosteric regulation. Furthermore, Opi1 might play an important role in this process probably by regulating Kcs1 expression levels.

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