Xrs2 phosphorylation keeps DNA tidy despite the MMS

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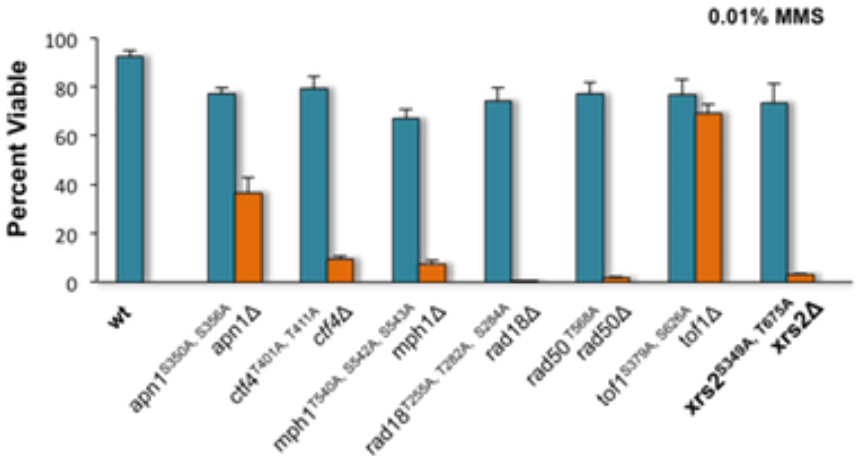

Viability of yeast strains in the presence of DNA alkylating agent, MMS. Novel phosphorylation sites on seven genes were identified by mass spectrometry and mutated to prevent phosphorylation (cyan) or genes were deleted (orange). Deletion strains are highly sensitive to MMS, and non-phosphorylatable mutants have intermediate survival.

Figure provided by Dongqing Huang

The blueprints for organismal development are contained within DNA. For this reason the fidelity of DNA is maintained through many mechanisms: polymerases that copy DNA are equipped with 'proof-reading' activities, mismatched DNA base pairs are excised for repair, and extensive damage actually arrests cell division. The diverse types of DNA damage each activate unique combinations of repair enzymes; however, it is clear that phosphorylation is an extremely common signal used to activate or silence these pathways. In fact, previous work has documented 100s of protein phosphorylation events involved in DNA damage repair. While previous studies characterized protein phosphorylation changes in response to severe DNA damage that halts cellular growth and division, researchers in the Paulovich Lab (Clinical Research Division) used mass spectrometry to explore how phosphorylation events change under mild DNA damaging conditions that cause DNA replication stress. This work was recently published in *Genetics* and identified many unknown phosphorylation events induced by DNA damage, including sites on Xrs2 that contribute to repairing DNA breaks and maintaining telomeric DNA.

This work was performed on Saccharomyces cerevisiae, a yeast that provides unparalleled genetic tools for understanding essential eukaryotic processes, including DNA damage repair. To induce DNA damage, yeast cells were grown in 0.01% MMS, a DNA alkylating agent, for 3 hours. These cells were also grown in media containing isotopically labeled amino acids (^{13}C Arg and ^{15}N Lys). The DNA damaged cells and untreated cells are then mixed and analyzed by mass spectrometry. The "heavy" ^{13}C and ^{15}N labeled amino acids from MMS-treated yeast are isotopically distinct giving those proteins slightly different masses in the mass spectrometric analysis compared to unlabeled or "light" ^{12}C and ^{14}N-containing wild type proteins. This method is termed SILAC and allows researchers to make quantitative comparisons from mass spec results. Using this technique, researchers identified 5,524 phosphorylation sites of which 4,449 had been previously identified, leaving 1,075 unique modifications. From this list, 264 proteins were prioritized because MMS treatment induced their phosphorylation by two-fold or greater.

Identification of these novel phosphorylation sites does not necessarily mean they contribute to DNA damage repair. Molecular manipulation of the protein targets in yeast allowed researchers to understand the function of these modifications. Seven genes containing 15 novel MMS- inducible phosphorylation sites were selected for further characterization. When these genes were fully deleted, yeast growth in MMS was severely limited. The phosphorylation sites appear to be important for growth in MMS because expressing non-phosphorylatable versions of these genes conferred intermediate sensitivity to the alkylating agent. The non-phosphorylatable mutations were combined with other genetic deletions to uncover how these phosphorylation sites contribute to different DNA repair pathways.

Researchers focused on the gene XRS2 for detailed studies because it is implicated in many DNA-related activities. The non-phosphorylatable mutant was paired with single gene deletions of proteins involved in many DNA-specific activities including: homologous recombination, DNA replication, non-homologous end joining, telomere maintenance, and sister chromatid cohesion. When the genes EXO1 and YKU80 were deleted in the XRS2 mutant cells growth defects in MMS were exacerbated, suggesting these genes are involved in activities that are also regulated by XRS2 phosphorylation. Further experiments revealed that Yku80 and Xrs2 phosphorylation protects the ends of linear chromosomes, called telomeres. This was observed by decreased telomere length and faster onset of cellular senescence in the XRS2 mutant strain. The connection between Xrs2 and Exo1 seems to be in repairing DNA double strand breaks. A key step in this process is the resection of DNA to create a single stranded DNA tail. Xrs2 forms a protein complex at double strand breaks and stimulates the nuclease activity of Mre11 to generate single stranded DNA, and then is evicted from

the site. Exo1 deletion further sensitized cells to MMS because it can substitute for Mre11 in generating single stranded DNA at sites of damage.

This study used a proteome-wide method to expand the scientific understanding of DNA damage signaling. Moreover, they verified that some of these novel phosphorylation events contribute to recovery from MMS-induced DNA damage. Such work is important for understanding human biology, and is particularly relevant in cancer patients where DNA repair enzymes are commonly mutated and DNA alkylating agents are common chemotherapies.

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Huang D, Piening BD, Kennedy JJ, Lin C, Jones-Weinert CW, Yan P, Paulovich AG. 2016. DNA Replication Stress Phosphoproteome Profiles Reveals Novel Functional Phosphorylation Sites on Xrs2 in Saccharomyces cerevisiae. *Genetics*. 203(1):353-68. doi: 10.1534/genetics.115.185231.