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DNA checkpoint override and redox signaling in *Schizosaccharomyces pombe*

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Abstract

This thesis covers intracellular stress signaling through genotoxic stress, overriding of checkpoint control, as well as cellular redox status in hypoxic and oxidative stress

Papers I and II: Caffeine has been shown to override cell cycle checkpoints in humans as well as in the fission yeast *Schizosaccharomyces pombe*. Understanding of the mechanism may aid in the development of compounds with similar overriding mechanisms for sensitization in cancer therapy. We show that caffeine induces accumulation of the mitotic inducer protein Cdc25, which removes inhibitory phosphorylation from the CDK Cdc2. Deletion of genes encoding the fission yeast checkpoint proteins Rad3 or Cds1 resulted in a higher constitutive level of Cdc25, suggesting a constitutive role in regulation of the Cdc25 level. Importantly, however, caffeine-induced Cdc25 accumulation is Rad3 independent. Mechanistically our results indicate that caffeine stabilizes and induces nuclear accumulation of Cdc25 as well as preventing Wee1, the kinase phosphorylating the same residue that Cdc25 dephosphorylates, from increasing in response to DNA damage, thereby enforcing progression into mitosis. Our results are in agreement with the known caffeine inhibition of TORC1 contributing to checkpoint override.

Paper III: FHIT, a human tumor suppressor, modulates DNA damage sensing, checkpoint control, proliferation and apoptosis. We investigated Aph1, the fission yeast homolog of FHIT, and found that deletion of the *aph1*$^{+}$ gene led to enhanced proliferation in sublethal concentrations of genotoxins. This phenotype was accompanied by elevated chromosome fragmentation and/or missegregation. Moreover, we show that an *aph1* deletion leads to knock-down of the checkpoint protein Rad1 in the 9-1-1 complex, and that Aph1 as well as all 9-1-1 proteins are downregulated in hypoxia.

Paper IV: H_2O_2 induces oxidative stress, but is also a signaling molecule that exerts its function through reaction with selected thiols of protein cysteines. MAP kinase (MAPK) pathways are induced by H_2O_2 in both human and fission yeast. We observed that an active site cysteine, shown to be involved in negative regulation of a human MAP kinase kinase (MAPKK), is evolutionarily conserved in all MAPKKs of budding yeast, fission yeast and humans, indicating that regulation of kinase activity through this cysteine may be a conserved feature of MAPK signaling in these organisms. The active site cysteine C458 in fission yeast MAPKK has no plausible cysteine partner for intramolecular disulfide bond formation. However, Wis1 kinase activity was still inactivated by reversible thiol oxidation in a C458 dependent way. The synthetic allosteric MAPKK modulator molecule INR119, predicted to bind in a site next to C458, protected against negative oxidative regulation *in vitro* targeting C458, resulting in enhanced MAPK signaling *in vivo.*

Keywords: Caffeine, Cell cycle, Checkpoint, Cdc25, Wee1, TORC1, FHIT, Proliferation, Aph1, Redox, Hypoxia, H_2O_2, Cysteine, Thiol, Allosteric, MAPK, Sty1, MAPKK, Wis1