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Expression Levels of DNA Damage Response Genes in Zebrafish (Danio rerio) following Treatment Methylnitrosourea, Doxorubicin, or Ultraviolet light (abstract)

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Expression Levels of DNA Damage Response Genes in Zebrafish (Danio rerio) following Treatment Methylnitrosourea, Doxorubicin, or Ultraviolet light.

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While much of the signaling of DNA damage is coordinated through post-translational modification, the expression levels of five genes, ATM, p53, p21, chk1, and chk2 have been examined in zebrafish at different stages of life ranging from two hours to adults (males and females) three years of age. Zebrafish are vertebrate, freshwater fish that grow 3-4 cm in size and are relatively easy to maintain at low costs. Each pair of fish can produce hundreds of eggs biweekly with a generation time of 3 months. The embryos develop ex vivo and are completely transparent during the first week of life, allowing for the observation of internal structures. This study utilized RNA collected from both untreated zebrafish embryos, and embryos treated with Ultraviolet light, N-nitroso-N-methylurea (MNU), or Doxorubicin. The level of mRNA expression from these genes at each stage was determined using quantitative reverse transcriptase polymerase chain reaction (q-RT PCR) and SYBER Green in relation to actin. Both ATM and p21 exhibited high levels of gene expression within untreated samples isolated from older zebrafish such as 3 years of age. Every gene treated with MNU and Doxorubicin increased in expression as the concentration was raised, however, ATM was the only gene that showed an increase in its expression following exposure to ultraviolted radiation. The expression of DNA damage response genes examined in zebrafish will further establish this vertebrate as a model organism to study DNA repair mechanisms.

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Evolution of novel response element specificity in the glucocorticoid receptor.

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Specific recognition of DNA response elements by transcription factors is a key step in the regulation of gene expression. Some transcription factors recognize multiple, distinct response elements to mediate transrepression of transactivation of target genes. However, the mechanism by which a single transcription factor can evolve to recognize multiple response elements is unclear. Here, we study the evolution of the glucocorticoid receptor, which recognizes distinct response elements to mediate activation or repression its target genes. The glucocorticoid receptor binds to activating glucocorticoid response elements, or (+)GREs, as a dimer to activate transcription. Alternatively, GR binds to negative glucocorticoid response elements (nGREs) in a monomeric fashion to repress transcription. We demonstrate that, of the extant 3-keto steroid receptors, only the glucocorticoid receptor can bind to and repress transcription in a nGRE-dependent manner, despite the ability of all 3-keto steroid receptors to bind to and activate gene transcription from (+)GREs. Surprisingly, using ancestral gene reconstruction, we find that nGRE binding and gene repression was a feature of the ancestor of all