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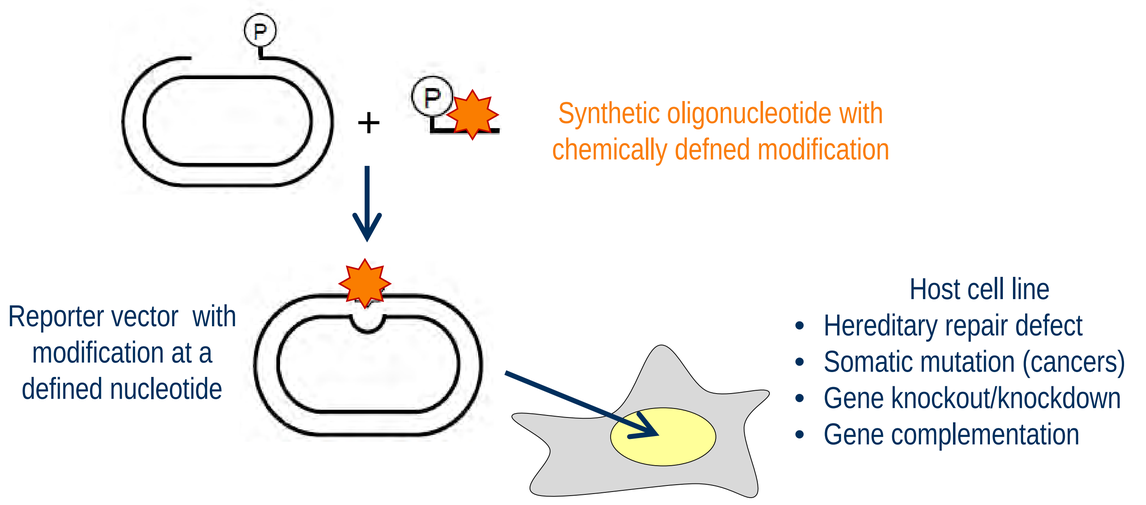
Synthetic DNA modifications reveal the mechanisms of repair and mutagenicity

SUMMARY

To understand the specific genotoxicity pathways of DNA-damaging agents, our lab builds reporter genes that harbour synthetic nucleotide derivatives, which model peculiar types of DNA damage. Here, we present a **comprehensive workflow for characterisation of repair and miscoding properties of structurally defined DNA modifications**:

- transcription blocking capacity of the given DNA modification is first determined in a nucleotide excision repair (NER)-deficient cell line;
- for strongly-blocking DNA lesions, repair capacities of cells of interest can be measured based on the recovery of the reporter gene expression;
- for bypassed DNA modifications, we have developed dedicated gain-of-function reporters to analyse the miscoding properties and measure repair indirectly as suppression of the mutant phenotype;
- genetically manipulated cells can be used to define the repair pathway components specific to the given type of DNA damage;
- moreover, base excision repair (BER) substrates can be protected by additional chemical modification of the sugar-phosphate backbone to unmask the overlapping repair mechanisms.

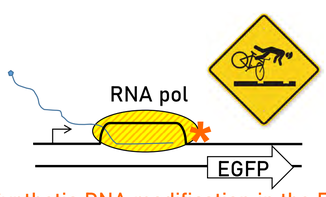


Step 1: Measurement of transcription blockage versus bypass **(1A)**

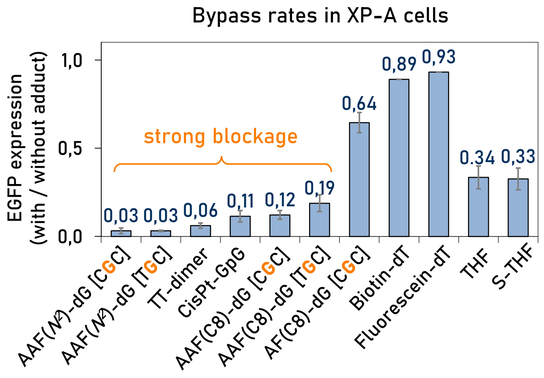
Transcription-blocking damage	Bypassed DNA modifications
Step 2: Measurement of the repair **(1B)**	*Step 2:* Detection of transcriptional miscoding **(4A)**
Step 3: Characterisation of the repair pathway • reverse genetics tools **(2)** • chemical biology tools **(3)**	*Step 3:* Characterisation of the repair, based on suppression of the phenotypic mutations **(4B)**

(1) TRANSCRIPTION BLOCKAGE BY DNA DAMAGE AND THE HOST-CELL REACTIVATION (HCR) PRINCIPLE

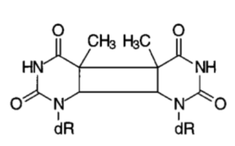
1A) Quantification of transcription blockage in a NER-deficient cell model

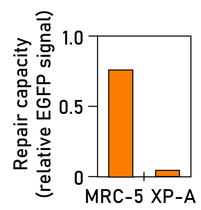


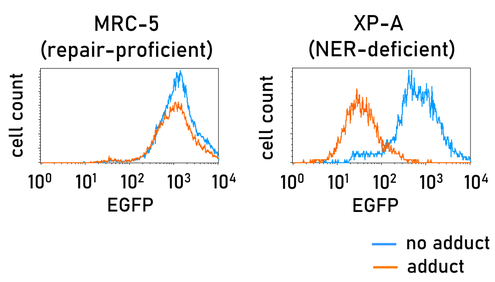
* Synthetic DNA modification in the 5′ UTR



1B) HCR of the reporter gene is a sensitive indicator of DNA repair

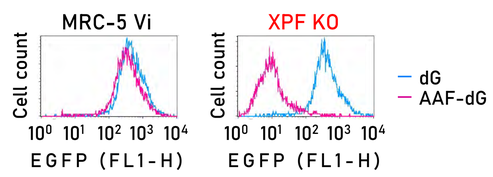




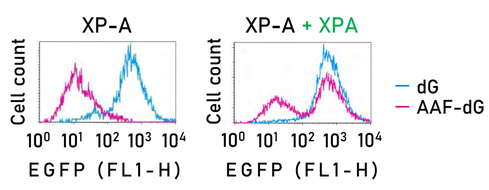


(2) IDENTIFICATION OF THE CRITICAL REPAIR GENES

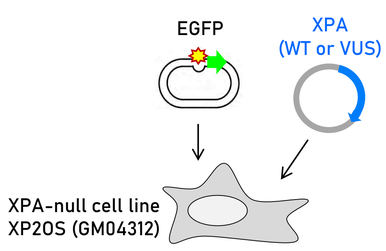
2A) Gene knockout

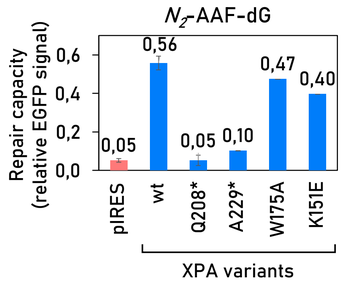


2B) Complementation of the repair defect with a functional gene

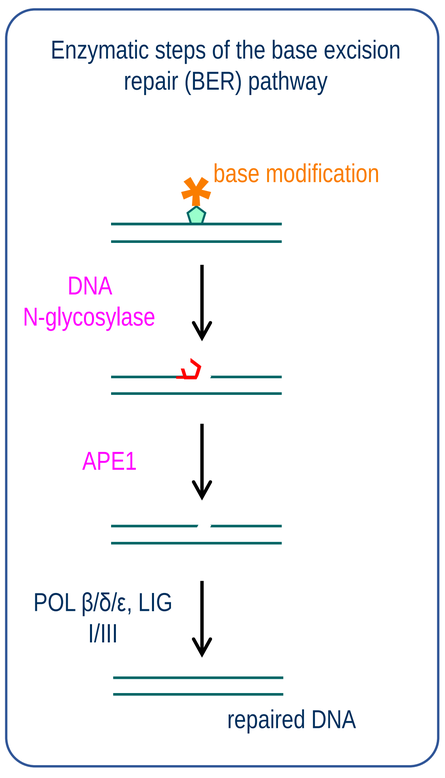


2C) Repair phenotype of VUS („variants of unknown significance“)

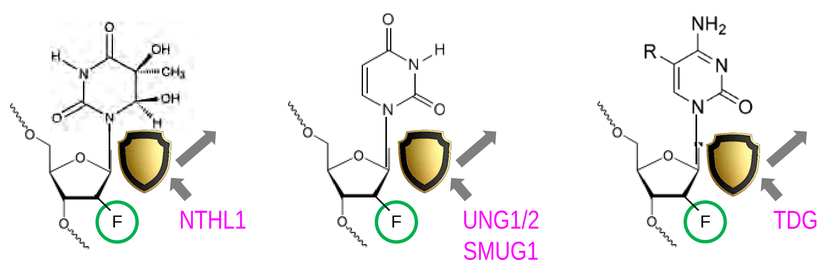




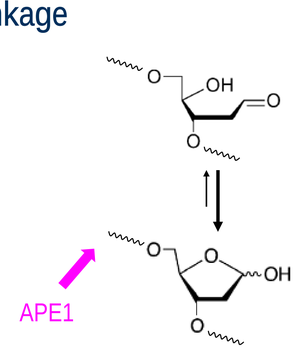
(3) "SUPERLESIONS" PROTECTED FROM BASE EXCISION REPAIR

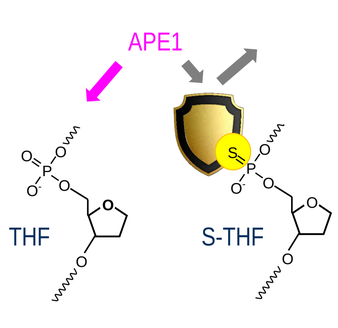


3A) Inhibition of base excision by 2'-fluorination of deoxyribose



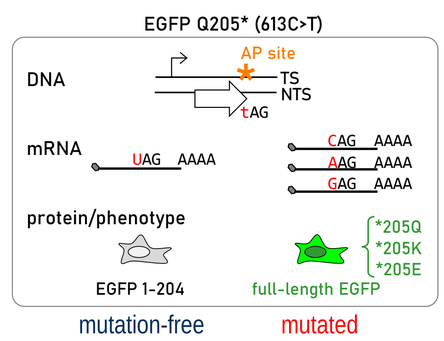
3B) Inhibition of strand scission by sulfurisation of the phosphodiester linkage

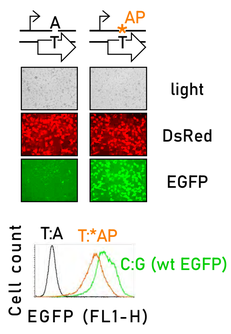




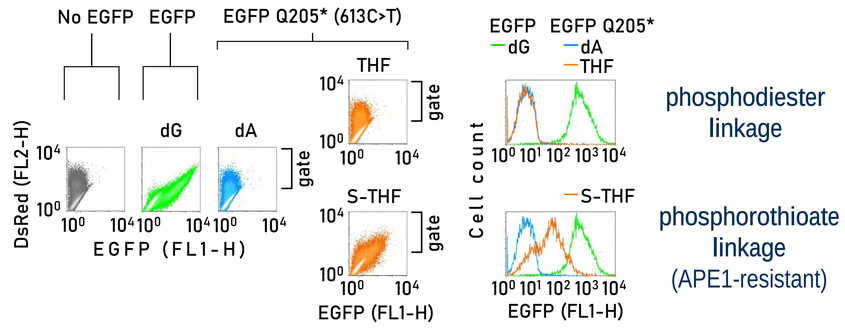
(4) REPAIR OF MISCODING DNA LESIONS

4A) Direct detection of transcriptional miscoding by AP lesion





4B) Suppression of the mutant phenotype by repair in MRC-5 cells



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