Coding properties of abasic sites during DNA synthesis in human cells

Leen Sarmini, Jacqueline Behrendt, Marta Rodríguez-Alvarez, Andriy Khobta
Institute of Nutritional Sciences, Friedrich Schiller University Jena, Germany

FRIEDRICH-SCHILLER-UNIVERSITÄT JENA
Fakultät für Biowissenschaften

Background: Apurinic/apyrimidinic (AP) sites belong to the most frequent pre-mutagenic DNA lesions. Because of their chemical reactivity, most of the existing knowledge about miscoding properties of AP sites derives from studies of a structurally analogous synthetic tetrahydrofuran (THF) lesion, which codes preferentially for dA during DNA synthesis under cell-free conditions. However, the nucleotide incorporation profiles of THF and, especially of the natural, deoxyribose (dR), AP lesions were not satisfactorily analysed in the cellular context.

Aim: We aimed at comparison of the mutagenic properties of the THF and dR lesions. The loss-of-function *EGFP* mutants were modified in the coding strand by incorporation of synthetic THF or dR opposite to a gap to detect reverse mutations arising during the gap filling DNA synthesis.

Main Findings

- **Synthetic AP lesions were successfully integrated into the mutant EGFP reporter at defined positions in the coding strand (Fig. 1.1)**
- **Translesion synthesis (TLS) over THF led to a substantial non-dA incorporation (Fig. 2.2)**
- **The natural AP lesion was even more prone to erroneous bypass, compared to THF, strongly favouring the incorporation of non-A nucleotides (Fig. 2.2)**
- **In-depth RNA-sequencing confirmed differences in the mutation spectra of dR and THF AP sites: for dR AP sites it is A > G > C > T, whereas for THF it is A >> C > T ≥ G (Fig. 3)**
- **In summary, we conclude that natural abasic sites are a more appropriate model to study mutagenicity compared to the synthethic THF analogue**

(1) Incorporation of different types of AP lesions into the EGFP reporter gene

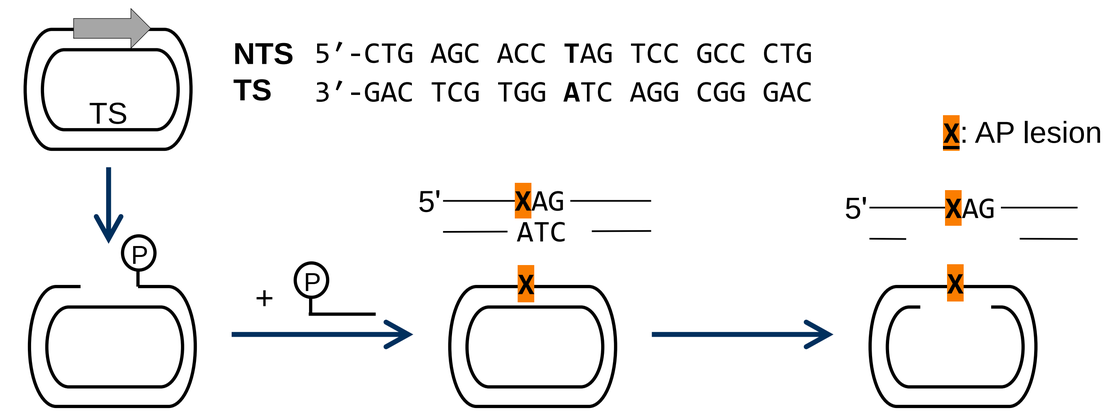

Figure 1.1 | Design of a TLS reporter based on an EGFP mutant (c.613G>T shown as an example). AP lesions were incorporated into the coding DNA strand precisely at the mutation site, opposite to a gap. During the gap filling DNA synthesis any miscoding event is detectable by reversal to the fluorescent EGFP phenotype.

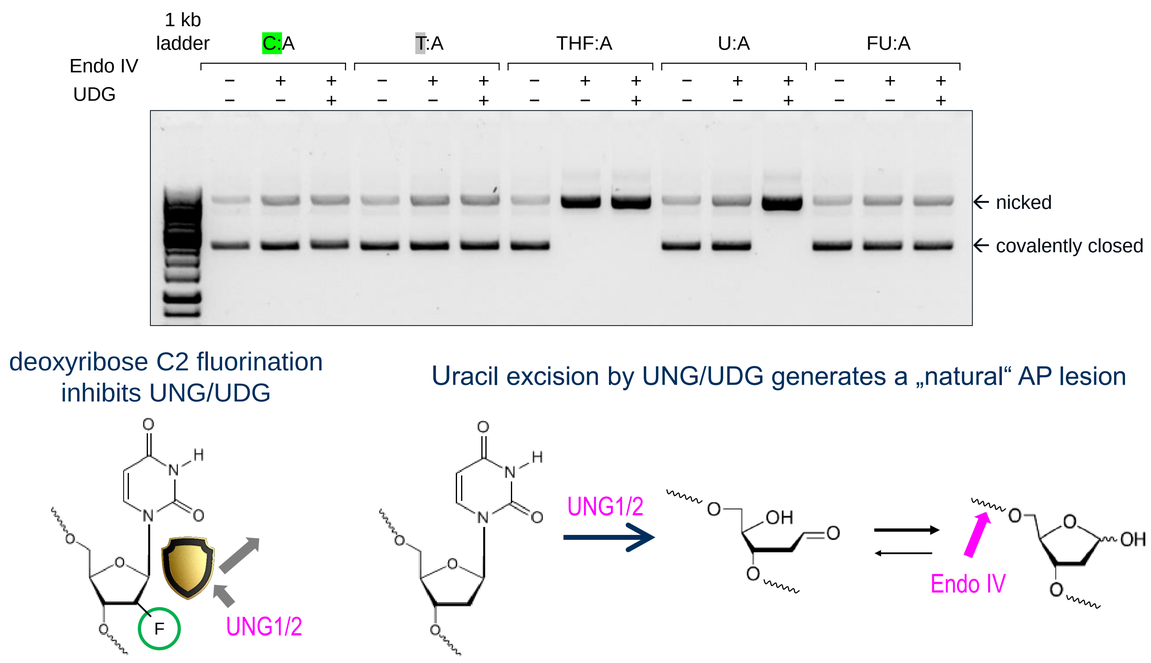

Figure 1.2 | Analysis of the incorporated modifications. Prior to the gap generation in the transcribed strand, plasmid DNA was incubated with *E. coli* UDG and/or endonuclease IV (Endo IV). Chemical structures illustrate the modifications and their sensitivity to the base excision repair enzymes.

(2) Gap filling DNA synthesis over abasic sites leads to high non-A incorporation rates

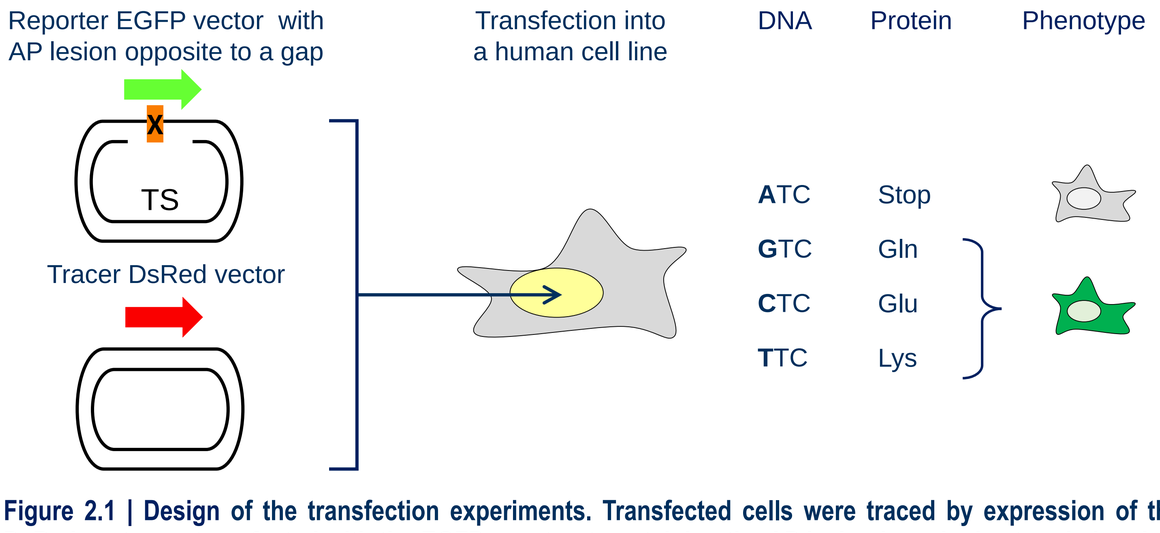

Figure 2.1 | Design of the transfection experiments. Transfected cells were traced by expression of the DsRed vector co-transfected with the EGFP construct containing the AP lesion in a single stranded region. Incorporation of G, C, or T during the gap filling DNA synthesis leads to recovery of the EGFP fluorescence.

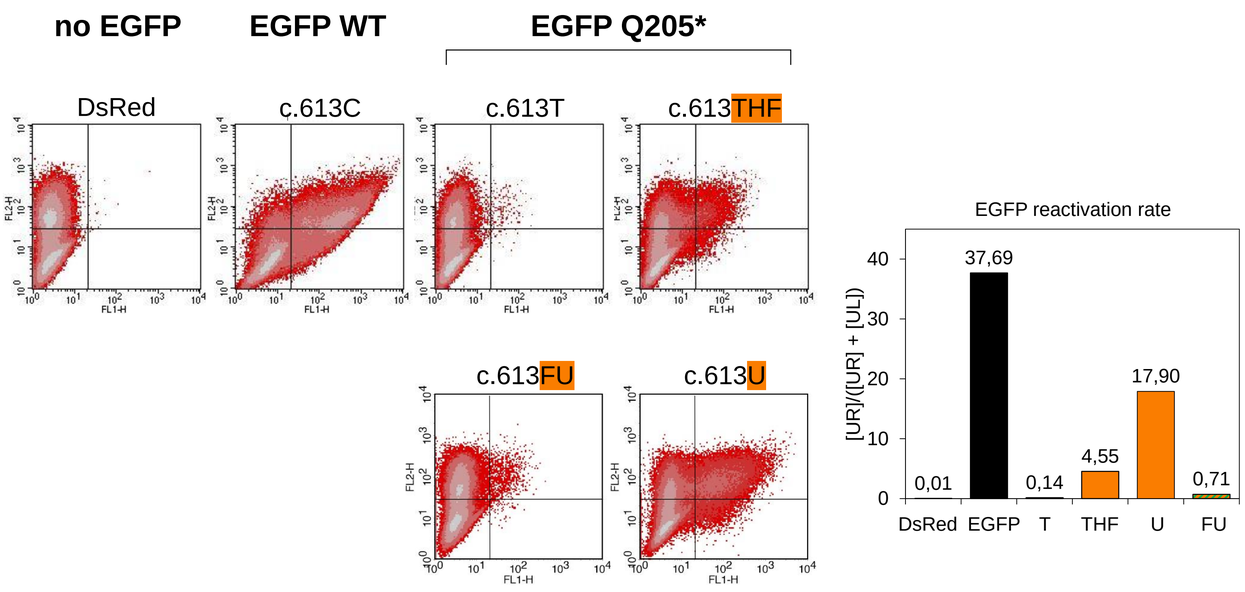

Figure 2.2 | Flow cytometry data of HeLa (DsRed, c.613C, T, THF) or HeLa pEps (c.613U, FU) 24 hours after transfection with the reporter constructs harboring the modifications at c.613. The absence of the EGFP signal in the case of the modification-free c.613T construct indicates that newly-synthesized DNA strand essentially contains only A at the analysed position. Right shift in the fluorescence distribution plots indicates non-A incorporation in cells transfected with constructs containing either THF (c.613THF) or the natural AP site (c.613U).

(3) THF and dR AP lesions have different mutation profiles

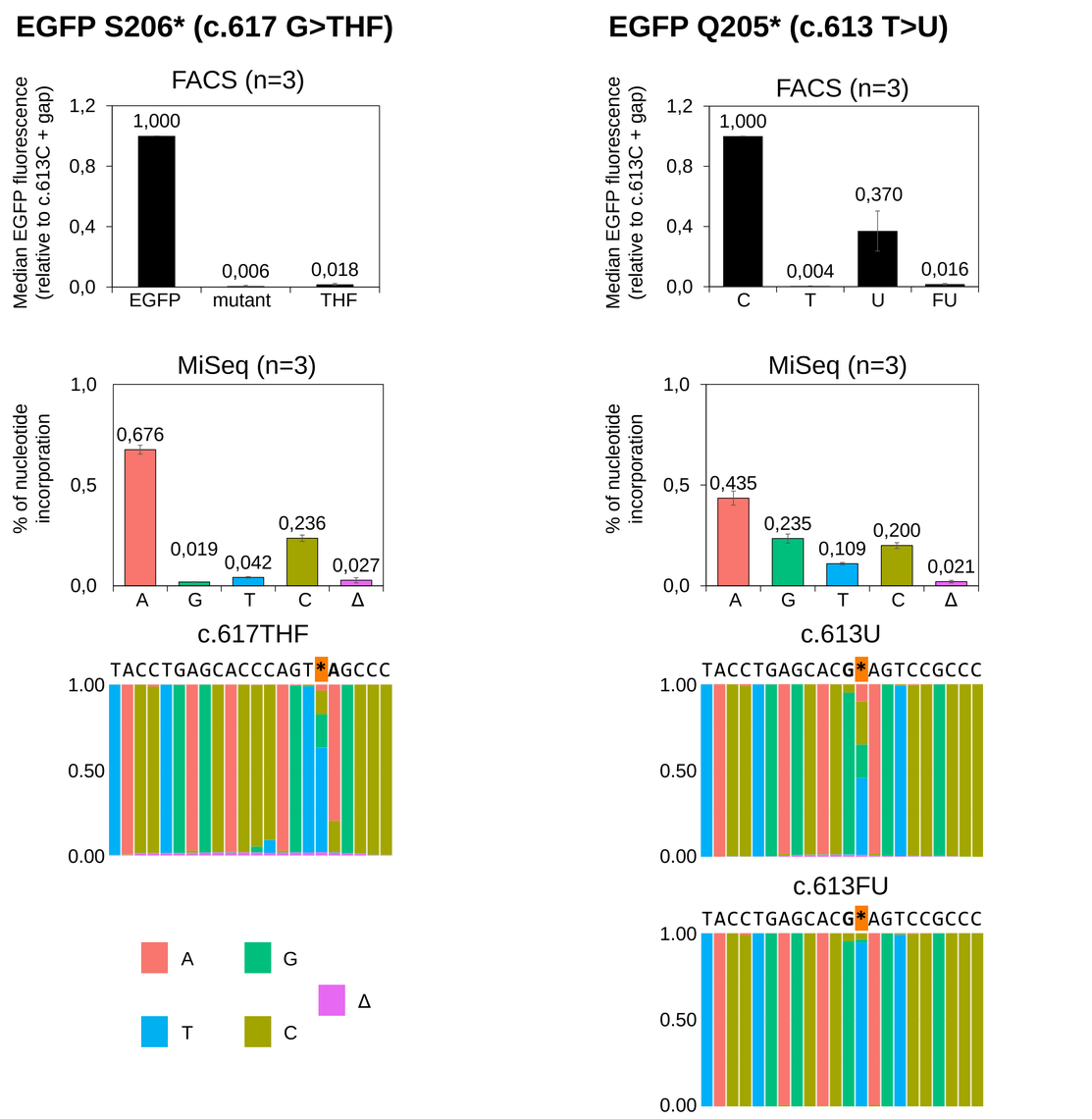

Figure 3 | Representative flow cytometry (FACS) and the respective RNA sequencing results of the THF (c.617THF), dR (c.613U), and c.613FU reporter constructs recovered from transfected HAP1 c.631 cells. Besides the indicated modifications at positions c.617 and c.613, both constructs contain a second silent mutation next to the modification (c.618C>A and c.612C>G) to monitor carry over of unmodified plasmid DNA.

Acknowledgements

Maria Mendez-Lago (Genomics core facility)
Sergi Sayols Puig (Bioinformatics core facility)

Funding

International PhD Programme Mainz
imb Institute of Molecular Biology
DFG
SFB 1361

We Are Recruiting

https://www.etox.uni-jena.de