Nucleotide excision repair of oxidatively induced DNA damage

Leen Sarmini, Mohammed Meabed, and Andriy Khobta
Institute of Nutritional Sciences, Friedrich Schiller University Jena

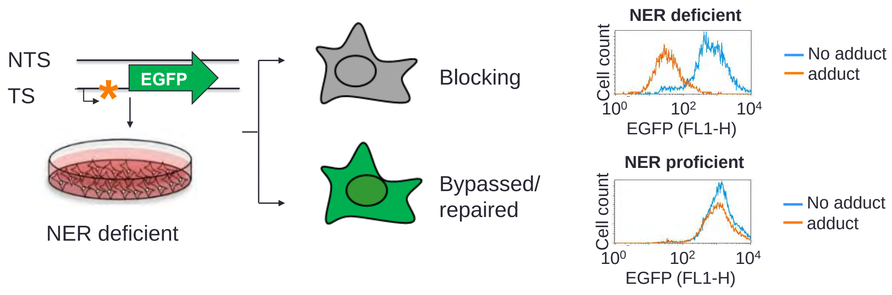
Background and aims

Individuals with hereditary defects of nucleotide excision repair (NER), for instance Cockayne syndrome (CS) and some xeroderma pigmentosum (XP) patients, manifest features of segmental ageing and neurodegenerative disease. Studies suggested a role of damage induced by reactive oxygen species (ROS) as a causal factor. On the other hand, the great part of oxidatively induced DNA damage is efficiently removed by the base excision repair (BER) mechanism, independently from NER. This raises the question whether NER is required for the removal of a sub-class of oxidatively induced DNA modification.

The aim of present work was to assess the involvement of NER in the repair of cyclopurine nucleotides and thymine glycol, whose repair mechanisms were incompletely characterised previously.

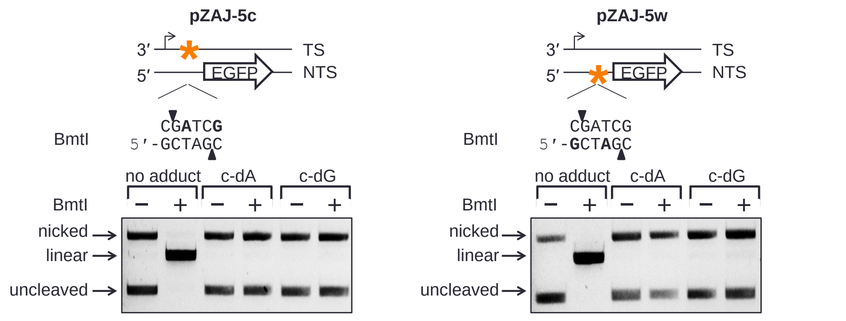
Experimental approach

Synthetic DNA modifications were incorporated into an EGFP reporter gene. EGFP expression was measured in cells with various NER statuses as an indicator of transcription blockage or recovery as a result of repair activity.

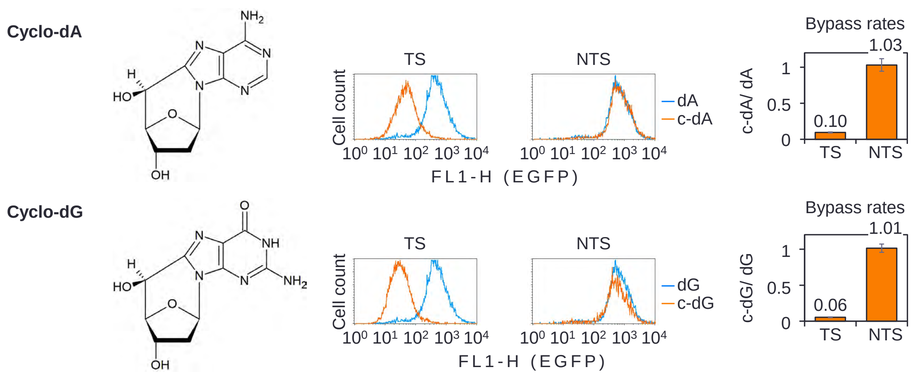


1 Cyclo-dA and cyclo-dG incorporated into the transcribed DNA strand eliminate transcription

(A) Characterization of reporter constructs containing no adduct or cyclopurine adducts at a defined position in the transcribed (TS) and non-transcribed (NTS) DNA strands.

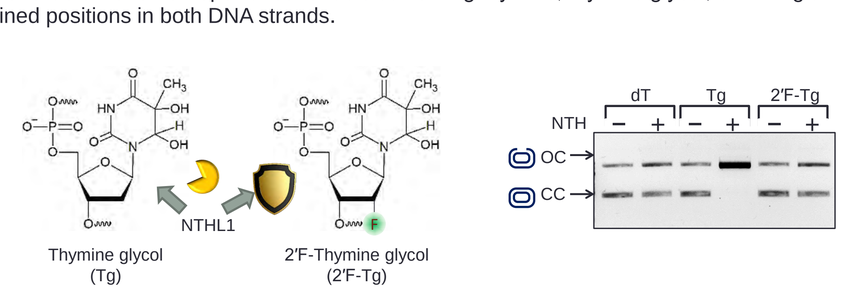


(B) Expression analysis of constructs harboring Cyclo-dA and cyclo-dG in the 5′ untranslated region, analyzed in XP-A cells (GM 04312).

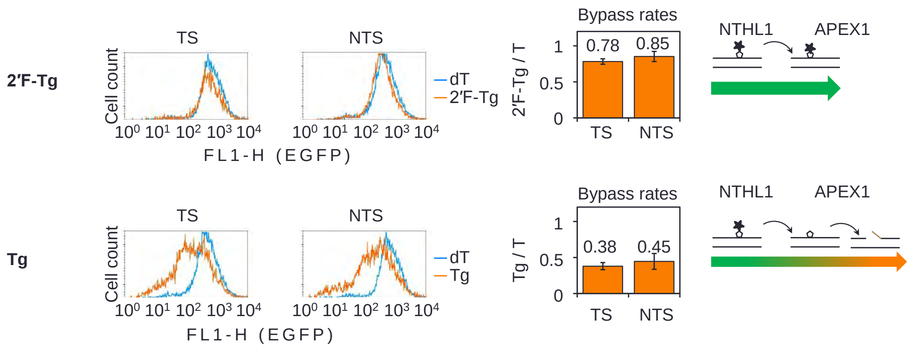


3 Thymine glycol is bypassed during transcription

(A) Characterization of reporter constructs containing thymine, thymine glycol, or 2′F-Tg at defined positions in both DNA strands.



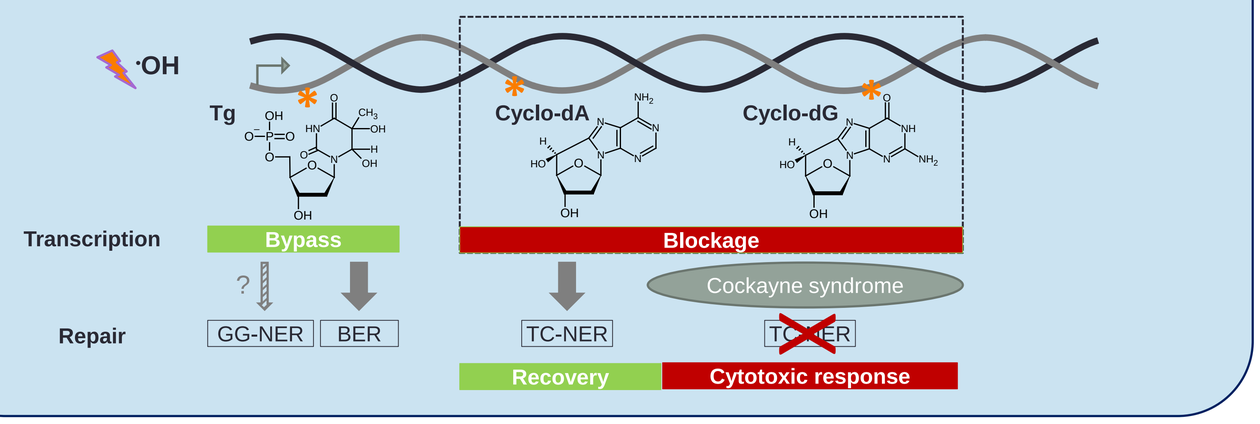
(B) Expression analysis of constructs harboring Tg or 2′F-Tg in the 5′ untranslated region, analyzed in XP-A cells (GM 04312).



Key findings

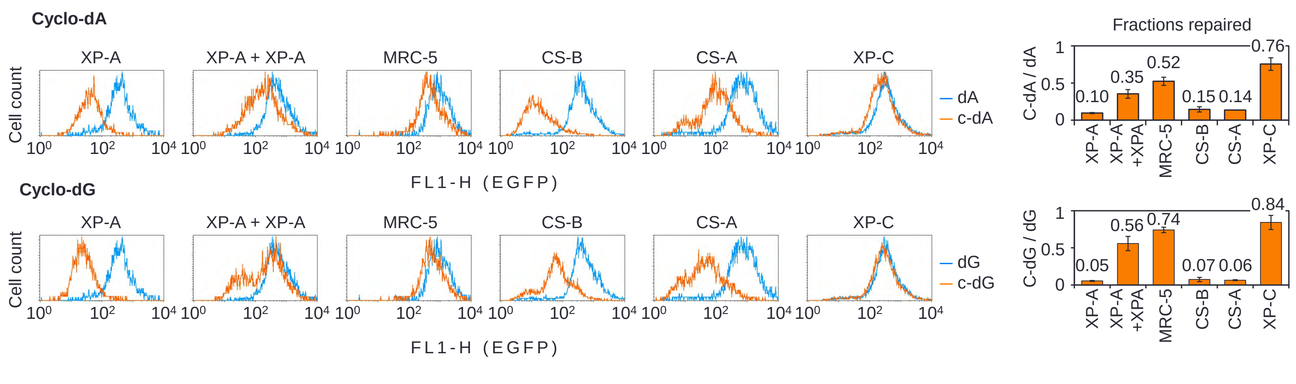
- Cyclo-dA and cyclo-dG cause transcription blockage *(Figure 1)*
- Cyclo-dA and cyclo-dG can only be repaired by TC-NER *(Figure 2)*
- In contrast, thymine glycol is bypassed during transcription and is repaired primarily by BER, initiated by NTHL1 *(Figures 3 and 4)*

A model: Based on the finding of a strict requirement of TC-NER for repair of cyclopurines, we propose that these physiologically relevant DNA lesions may contribute to pathogenesis of CS and XP neurological disease.

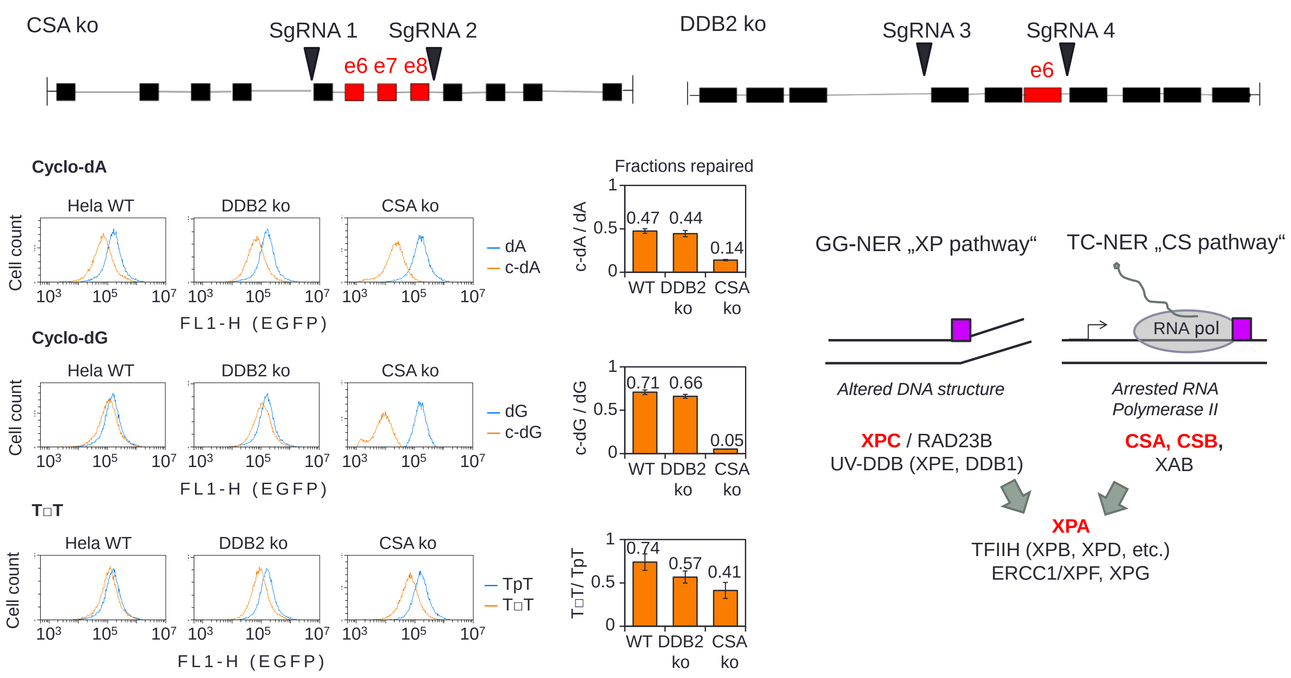


2 Cyclo-dA and cyclo-dG require TC-NER

(A) Host cell reactivation in human NER deficient cell line XP-A (GM04312) and the complemented XP-A/+XP-A (GM15876), repair proficient MRC-5 (AG10076), TC-NER deficient CS-B (GM16095) , CS-A (GM16094), and GG-NER deficient XP-C (GM15983).



(B) Expression analysis of reporters with cyclo-dA, cyclo-dG and thymine dimer in isogenic cell lines generated by CRISPR-Cas9 knock out (ko) of *DDB2* (GG-NER) and *CSA* (TC-NER) genes.



4 Thymine glycol is repaired predominantly by NTHL1 glycosylase

Generation of NTHL1 knock out (ko) by CRISPR-Cas9 and expression analyses of constructs harbouring Tg and 2′F-Tg in both DNA strands, transfected to Hela WT and NTHL1 ko