Cellular study of a bacterial DNA-damaging virulence factor, the Cytolethal Distending Toxin

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Abstract

The Cytolethal Distending Toxin (CDT) is a virulence factor produced by several pathogenic bacteria, modulating the immune response, inducing pro-inflammatory signals and disturbing the epithelial barrier. These effects may result in chronic infection and inflammation. Moreover, CDT has been indirectly associated with higher colorectal cancer risk. Indeed, some CDT-producing bacteria have been found around human colorectal tumours and CDT has been shown to induce cancerous markers in mice. Interestingly, at the cell level, CDT induces DNA strand breaks, disturbs the cell cycle, leading to cellular senescence and apoptotic cell death. Thus, CDT is considered as a genotoxin that alter the genetic information by damaging DNA inside living cells, leading to genetic instability and carcinogenesis.

CDT has two distinct biochemical activities, DNase and phosphatase. Their influence on CDT toxicity is not yet fully characterised. Furthermore, cell-cycle phase influences CDT sensitivity. Our preliminary data show that CDT creates DNA damage during mitosis, induces micronucleus formation and an inflammatory response. The objective of this study is to make a link between these different CDT-related cellular defects and to characterize its biochemical activities to better explain CDT physiopathology.

We have developed cellular assays allowing evaluating and comparing the effects of several mutations on CDT activities, aiming to disturb one or the other. We have also designed a live-cell microscopy approach to explore the impact of CDT on cell cycle. Overall, the goal of this project is to better characterise CDT mechanism of action using innovative cellular approaches, in particular its impact on genetic instability.

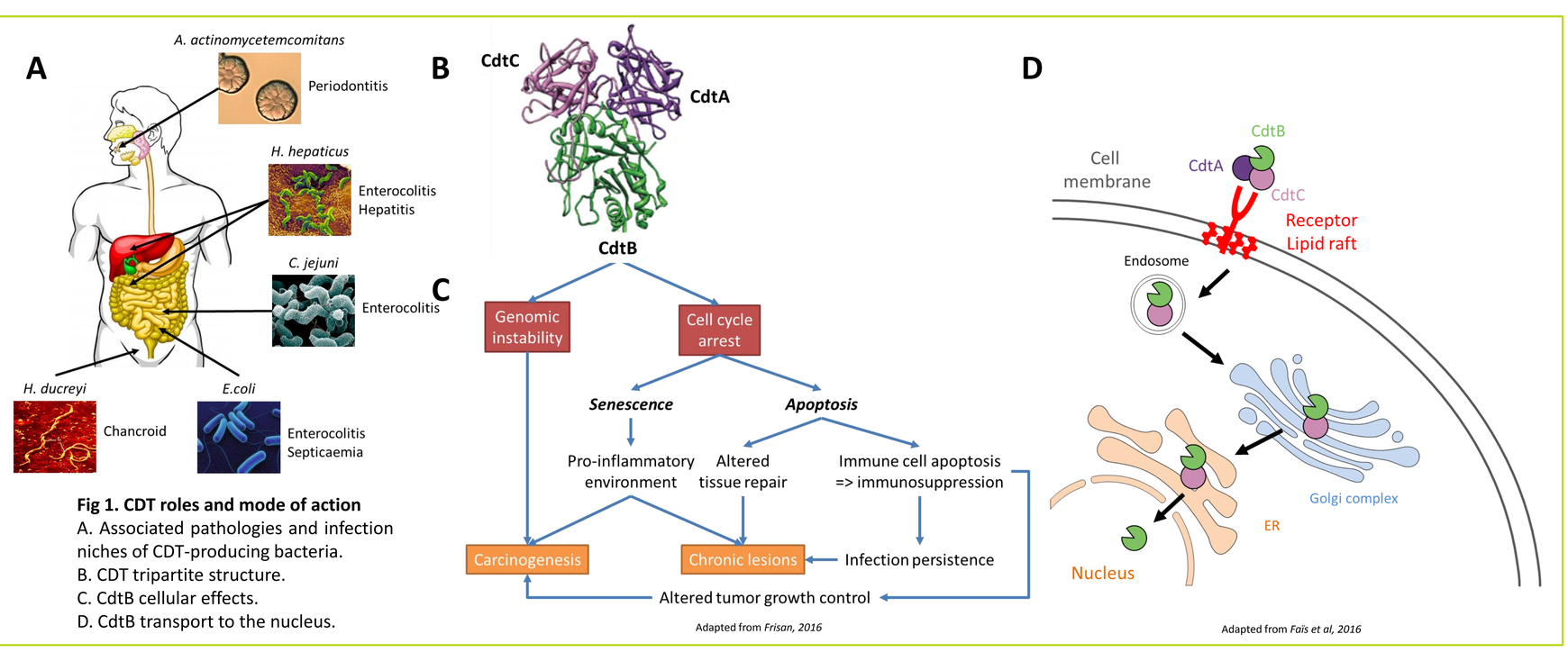

Fig 1. CDT roles and mode of action
A. Associated pathologies and infection niches of CDT-producing bacteria.
B. CDT tripartite structure.
C. CdtB cellular effects.
D. CdtB transport to the nucleus.

CdtB: one active site, two activities ?

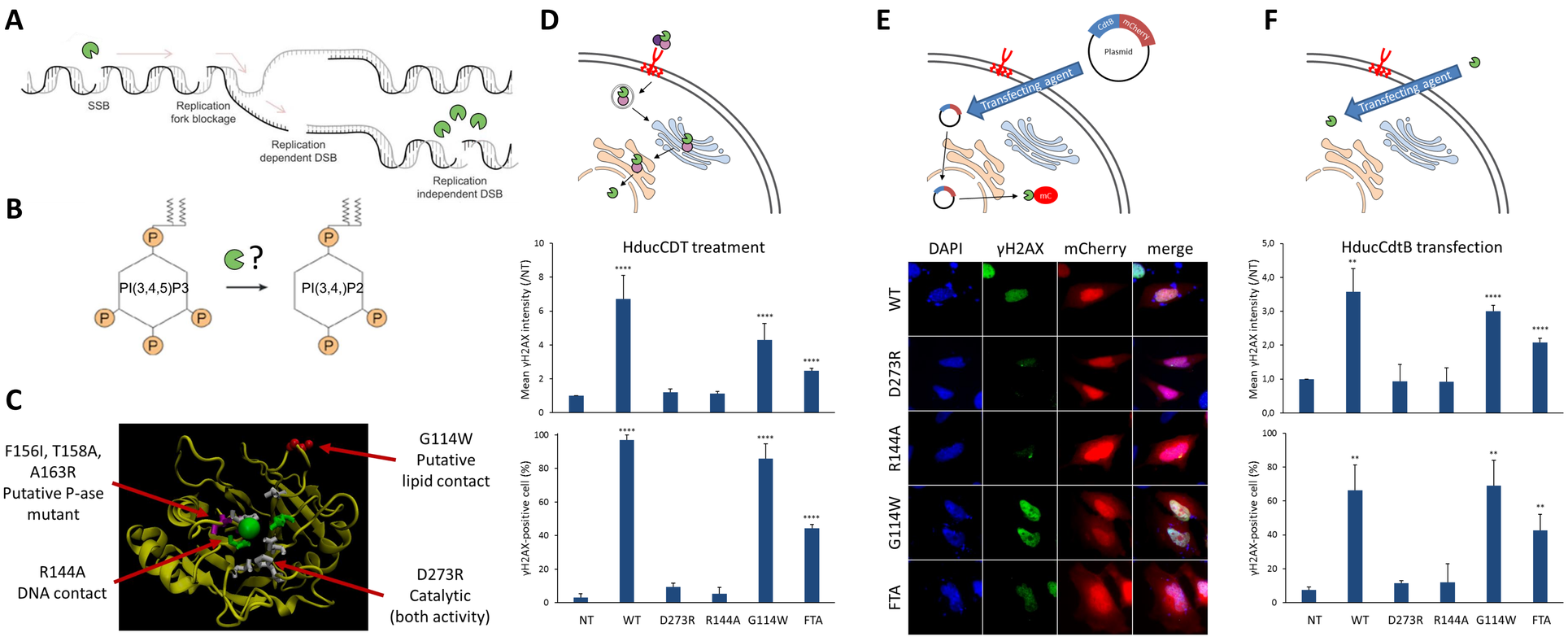

Fig 2. *H. ducreyi* CdtB (HducCdtB) cytotoxicity correlates with its DNase activity
A. CdtB induces SSB at low doses and DSB at high doses.
B. CdtB presents a putative dephosphorylation activity on phosphatydilinositol-3,4,5-triphosphate.
C. HducCdtB structure showing mutations used here.
D. HeLa cells were treated for 24h with HducCdtB mutants before to be scored for γH2AX immunostaining.
E. HeLa cells were transfected for 11h with a mammalian expression plasmids carrying HducCdtB mutants in fusion with mCherry (pmCherry) before to be scored for γH2AX immunostaining.
F. HeLa cells were transfected for 24h with HducCdtB before to be scored for γH2AX immunostaining.
G. HeLa cells were treated for 5 days with HducCdtB mutants before staining with crystal violet.

CDT effects on genetic stability

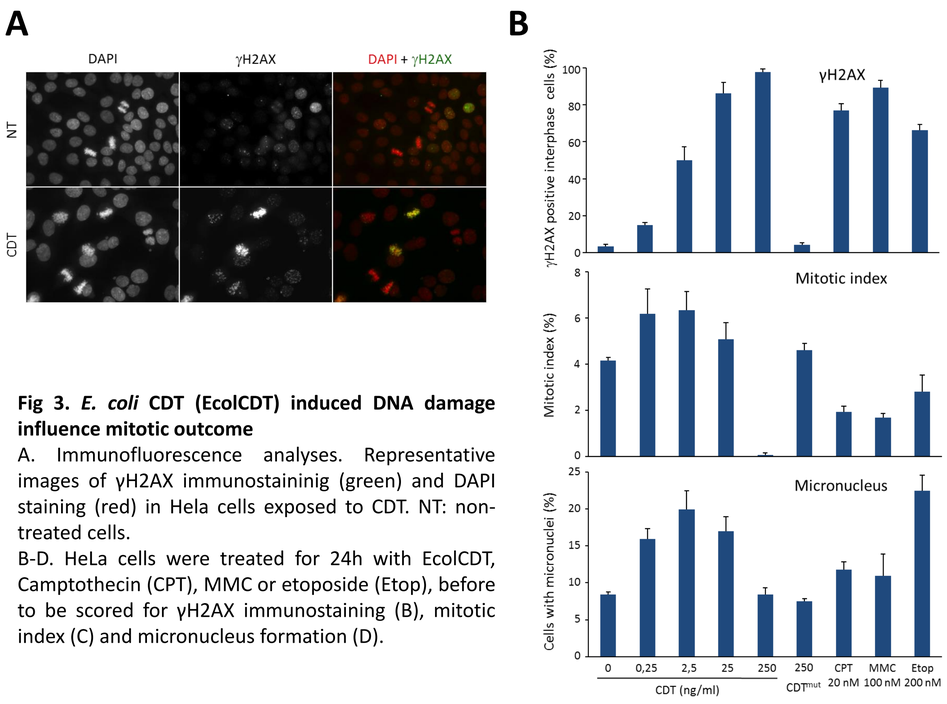

Fig 3. *E. coli* CDT (EcolCDT) induced DNA damage influence mitotic outcome
A. Immunofluorescence analyses. Representative images of γH2AX immunostainig (green) and DAPI staining (red) in Hela cells exposed to CDT. NT: non-treated cells.
B-D. HeLa cells were treated for 24h with EcolCDT, Camptothecin (CPT), MMC or etoposide (Etop), before to be scored for γH2AX immunostaining (B), mitotic index (C) and micronucleus formation (D).

CDT effects on mitosis

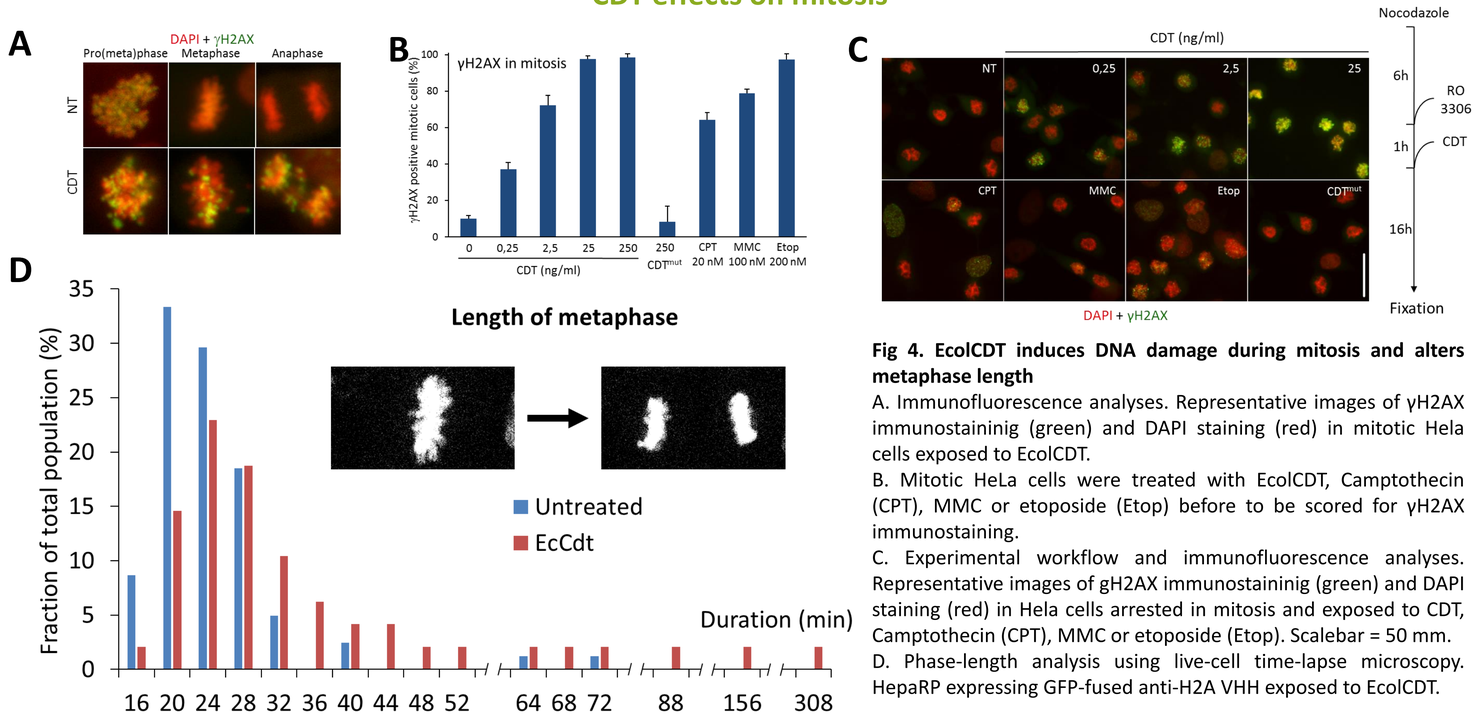

Fig 4. EcolCDT induces DNA damage during mitosis and alters metaphase length
A. Immunofluorescence analyses. Representative images of γH2AX immunostainig (green) and DAPI staining (red) in mitotic Hela cells exposed to EcolCDT.
B. Mitotic HeLa cells were treated with EcolCDT, Camptothecin (CPT), MMC or etoposide (Etop) before to be scored for γH2AX immunostaining.
C. Experimental workflow and immunofluorescence analyses. Representative images of gH2AX immunostainig (green) and DAPI staining (red) in Hela cells arrested in mitosis and exposed to CDT, Camptothecin (CPT), MMC or etoposide (Etop). Scalebar = 50 mm.
D. Phase-length analysis using live-cell time-lapse microscopy. HepaRP expressing GFP-fused anti-H2A VHH exposed to EcolCDT.

Conclusion

- CdtB **nuclease activity correlates with cytotoxicity** while CdtB phosphatase activity has yet to be assessed to decipher their respective roles in CdtB toxicity.
- CDT induces genetic instability as shown by micronuclei formation and DDR activation in mitotic cells. The live-cell microscopy approach is used to further explore CDT impact on cell cycle. This is particularly interesting as cell cycle perturbations and genetic instability are crucial steps in carcinogenesis.
- Our goal is to **understand how CDT can lead to chronic infection and inflammation**. The two approaches allow us to better understand CDT mechanism of action and are therefore a **first step to get more insight on the effects of the toxin on human health.**

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