May 2nd, 11:00 AM - 1:00 PM

Interstrand Crosslink Resistance in Escherichia Coli

Jedidiah D. Acott
Portland State University

Jessica M. Cole
Portland State University

Charmain Courcelle
Portland State University

Justin Courcelle
Portland State University

Jedidiah D. Acott, Jessica M. Cole,
Charmain T. Courcelle Justin Courcelle

Interstrand Crosslink Resistance in *Escherichia coli*

Department of Biology
Portland State University
Portland, Oregon

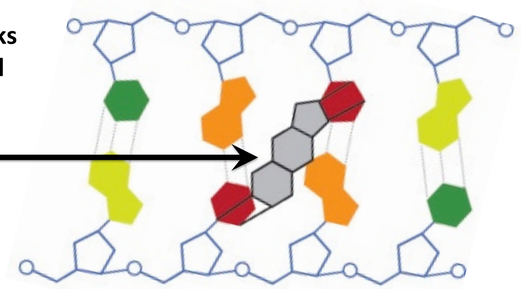
Abstract:

8-methoxypsoralen is a photoactivated DNA-intercalating agent, which after absorbing two high-energy UVA photons, covalently binds pyrimidine bases on both DNA strands, to form an interstrand crosslink (ICL). These lesions completely block replication and transcription, and are widely used in chemotherapies; yet the mechanism by which they are processed remains poorly understood. In 1985, Ahmed and Holland reported an *Escherichia coli* mutant demonstrating hyper-resistance to ICL-inducing agents. The mutation was mapped to 57.2 minutes on the chromosome, and potentially encoded a 55-kDa protein that was induced as part of the SOS response. Although these genes remain unidentified, *hscA* and *hscB* map to this location, have a similar size, and are SOS-inducible. To determine if these genes or others might confer ICL resistance in *E. coli*, we characterized how cells survived PUVA treatment in the absence of HscAB, and when these gene products were overexpressed. In a second approach to screen for ICL resistance genes, we developed a selection system to isolate hyper-resistant strains through the sequential growth and exposure of wild-type cultures to PUVA. We found no effect on cell survival in the *hscAB* mutant compared to its wild-type parent, suggesting that HscAB may not be contributing to ICL resistance as previously hypothesized. However, due to the significant cytotoxicity of plasmids containing *hscAB*, even in the absence of PUVA treatment, we were unable to determine whether over-expression of these gene products might provide a protective effect to cells. Using iteratively PUVA cells, we isolated strains that were >10^4-fold more resistant to this ICL-inducing agent compared to the parent strain. This result suggests that *E. coli* possess mechanisms to repair or tolerate ICL's that contribute to resistance to these agents, similar to what is observed in human cancer cells.

Introduction:

Interstrand crosslinks inhibit replication and transcription in all cells, leading to severe cellular toxicity.

Interstrand crosslinks prevent the physical separation of DNA strands and block replication and transcription



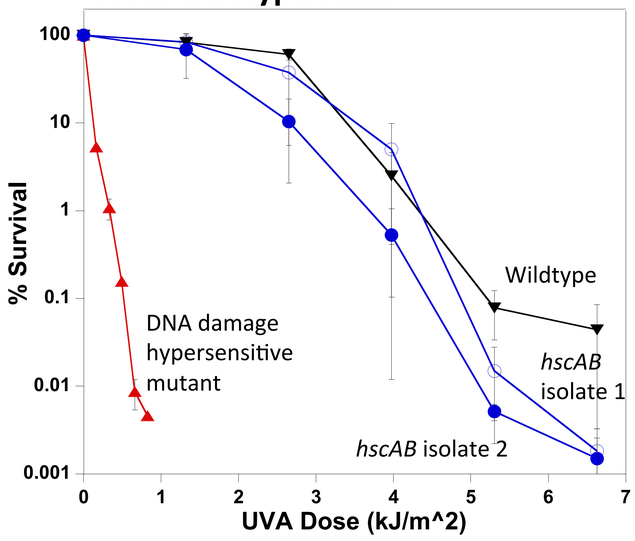
Interstrand crosslinking agents are potent chemotherapeutics, yet cancer cells often develop resistance to these drugs through unknown mechanisms.

The model organism *E. coli* encodes genes for most of the cellular processes thought to be involved in resistance to interstrand crosslinks in human cells, including nucleotide and base excision repair and translesion DNA synthesis.

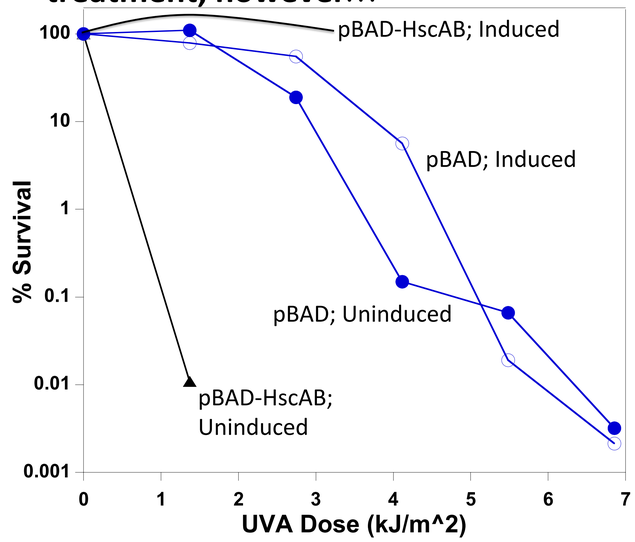
Therefore, we sought to identify genes contributing to ICL-resistance in *E. coli* using targeted mutagenesis and a general selection scheme.

Image from: http://meddev.uio.no/elaring/lcms/ernaeringslaere/nutr-cancer-biology/nutrition-cancerARC04.xml?menuItemIndex=9

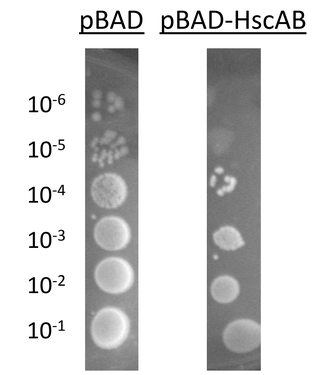
hscAB mutants were as sensitive to PUVA as wildtype cells.



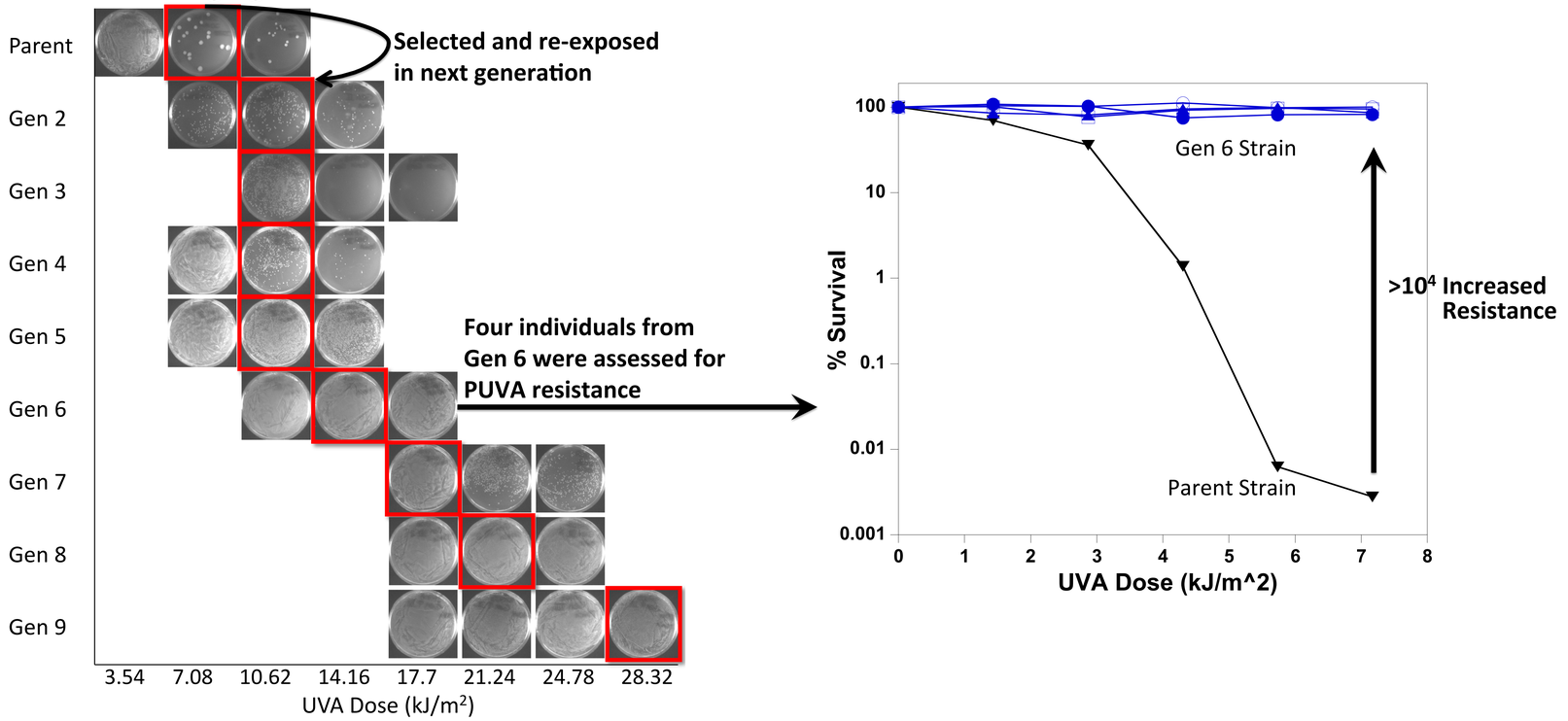
Cells containing HscAB expression plasmids were more sensitive to PUVA treatment, however…



…even in the *absence* of PUVA damage and induction of HscAB overexpression, cells containing plasmids with *hscAB* were less viable.



Sequential selection and re-exposure of cultures to PUVA treatment led to increased resistance to ICL-inducing agents.



Conclusion:

The absence of HscAB was found to have no measurable effect on cell survival following PUVA treatment.

Plasmids containing HscAB were toxic to cells even in the absence of PUVA treatment, such that overexpression of HscAB as a mechanism of ICL resistance could not be tested.

A population of ICL hyper-resistant cells was isolated over successive generations that had >10^4 magnitude of cell survival to PUVA treatment compared to its wild-type parent. Sequencing of this hyper-resistant strain will be performed to identify genes contributing to the altered phenotype.

Acknowledgements: This work was funded by the NIH grant, R15ES025953