Intruder to the Sanctorum: Sub-Nuclear Localization and Interaction of Cytolethal Distending Toxins in Host Cells

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Abstract

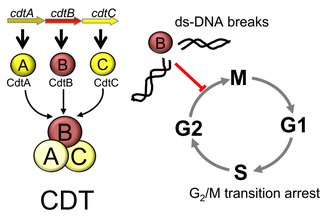
Bacterial toxins constitute a broad class of enzymes with the capability of targeting hosts to its advantage. Exploring the relationship between pathogen and host has led to the understanding of many mechanistic aspects that bacterial effectors exploit to manipulate the host cellular machinery. A unique class of bacterial exotoxins called Cytolethal Distending Toxins (CDT), target the host genome resulting in DNA damage. CDT is secreted by many bacteria such as *Escherichia coli*, *Campylobacter jejuni*, *Salmonella typhi*, and *Haemophilus ducreyi* that persist with high frequency amongst mucocutaneous niches. Uptake of CTDs are dependent on its structural components as a hetero-tripartite toxin with a CdtA and CdtC subunit necessary for binding and delivery of the catalytic CdtB subunit into the host cell. To elicit physiological effects, CDT traffics to the host nucleus upon uptake and causes DNA damage. However, little is known about CDTs nuclear actions that allow it to incur a host response. It is likely that CDT associates with a host factor within the nucleus to induce DNA damage. Our aim is to identify an interaction between CDT and a host factor necessary to invoke DNA damage. Thus far, we have demonstrated that CDT colocalizes to interchromatin granule clusters. To further analyze this interaction, we will conduct immunoblot analysis and subnuclear fractionation techniques. Understanding the localization and mechanism of CDT necessary for inducing a host cellular response will not only help us to better understand genotoxins, but also allow us to explore the interactions of host factors within minimally characterized nuclear compartments.

Bacterial Genotoxins

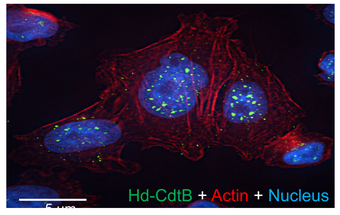
- Secreted by gram negative mucosal pathogens
- Intracellular acting heterotrimeric AB_2 toxin
- CdtB - catalytic subunit – DNAse activity
- Causes DNA breaks, DNA damage & G_2/M cell cycle arrest
- Chronic infection – continued DNA assault – risk for cancer

Cytolethal Distending Toxins (CDT)

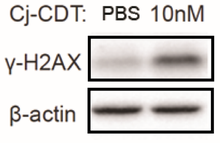
Heterotrimeric AB_2 toxin



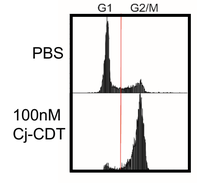
CdtB localizes to the nucleus



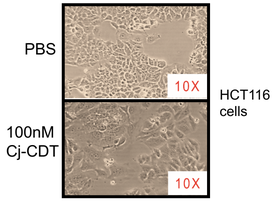
CDT mediated DNA damage response



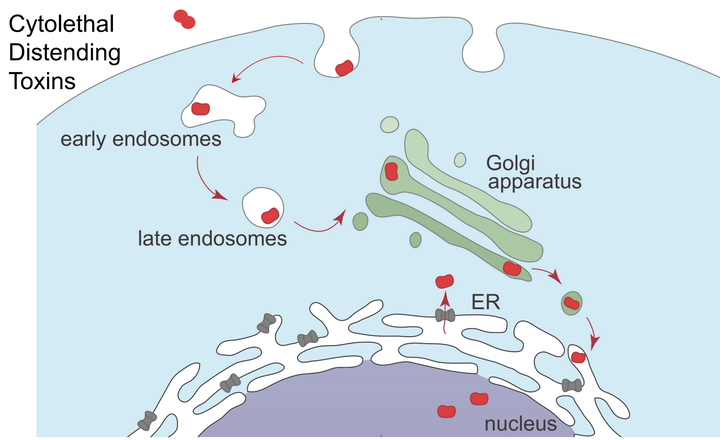
CDT mediated cell cycle arrest



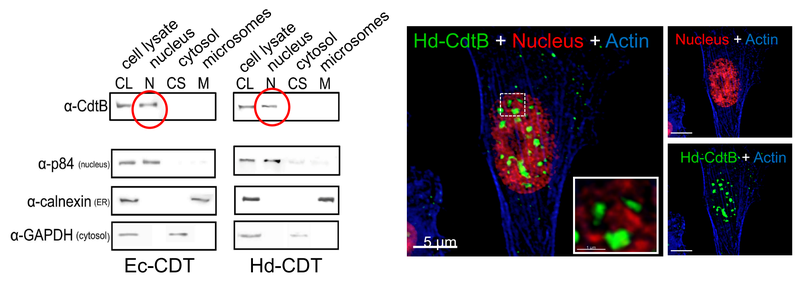
CDT mediated cellular distension



Retrograde trafficking of CDT to nucleus

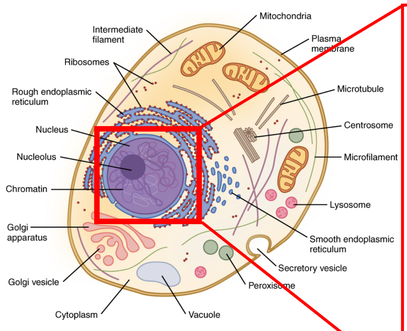


Nuclear localization of CdtB

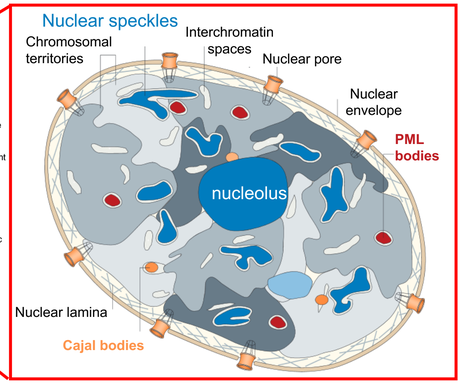


Nuclear architecture

Subcellular Compartments

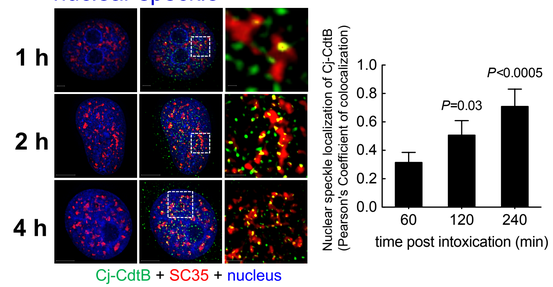


Subnuclear Compartments

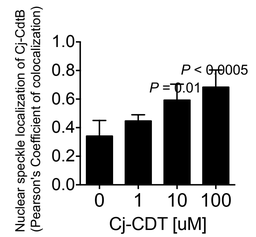


Cj-CdtB localizes to nuclear speckles

Time dependent Cj-CdtB localization to nuclear speckle

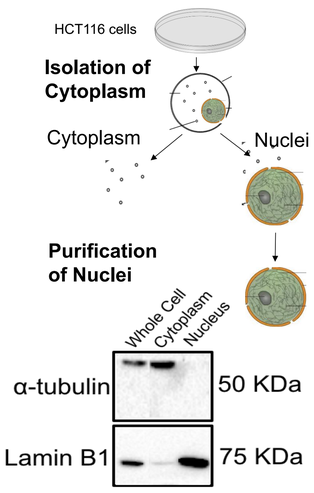


Dose dependent Cj-CdtB localization to speckle

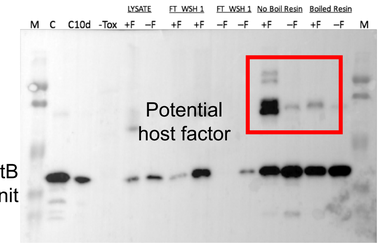


Identification of interacting host factor with the toxin

Subnuclear fractionation to identify host factor interaction with the toxin



Identification of host factor interaction with the toxin using formaldehyde cross linking



1% Formaldehyde cross linking & Cobalt resin pulldown

Conclusion

- CdtB subunit localizes with nuclear speckles.
- Nuclear speckles are associated with gene transcription and accessibility to nascent DNA.
- Nuclear compartments can be isolated to identify toxin localization.
- A host factor must exist to assist in a CDT mediated host cellular response.
- Future directions include optimizing our method for isolating CDT with a localized compartment and identification of a potential host factor

Acknowledgements

Steven R. Blanke lab members & NIH – AI059095, GM098756