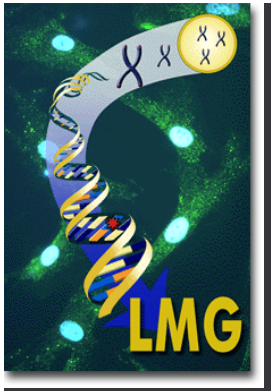

A Novel Cofactor and Diverse Nucleic Acid Interactions of CSB, a Human Swi/Snf2 Protein

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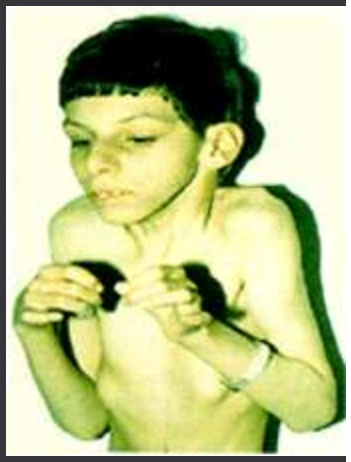
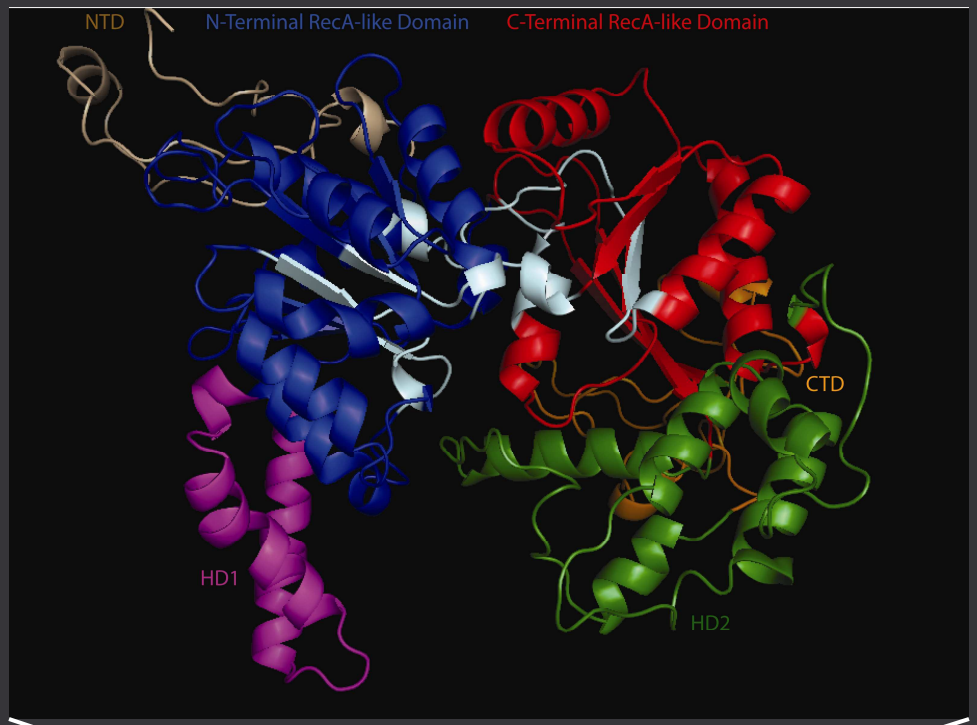
Cockayne Syndrome

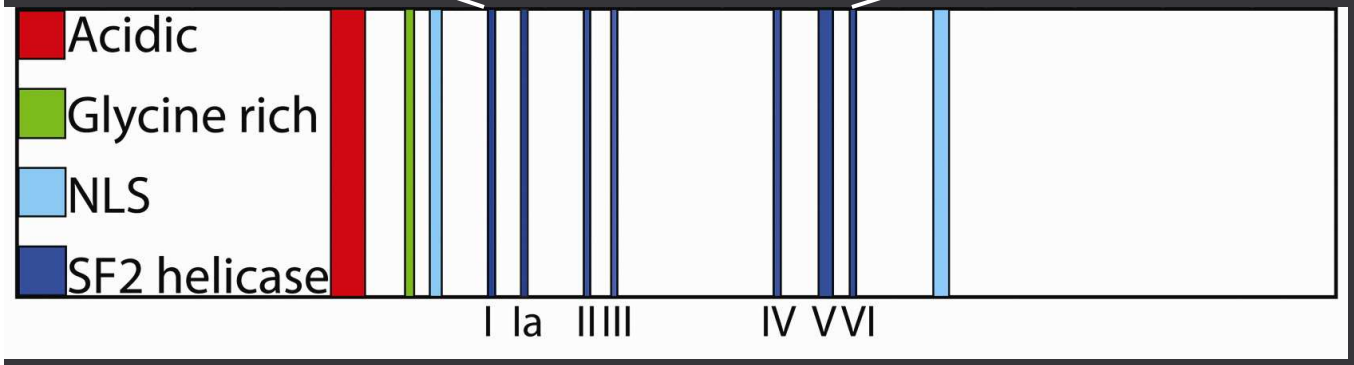
- Cockayne syndrome (CS) is a rare autosomal recessive segmental premature aging disorder.
  - cachectic dwarfism
  - cutaneous photosensitivity
    - cells failure to recover RNA synthesis after UV
  - neuropathologies
  - no increased cancer incidence
- CS has two strict complementation groups.
  - CSA (*ERCC8*) and CSB (*ERCC6*)
- Mutations in the CSB gene account for ~80% of CS cases.



CSB Protein



- SWI/SNF2 family member
- DNA dependent ATPase.
- Essential for transcription coupled nucleotide excision repair.
- Plays a role in general transcription.
- An auxiliary factor in base excision repair.



ATP Dependent and Independent Functions?

- 50% of non-synonymous amino acid mutations in CSB from CS patients reside within the ATPase core.
- Complete complementation of UV sensitivity in CS cells requires an intact ATPase core.
- With or without ATP, CSB causes a change in DNA conformation.
- Incision activity for 8-oxoG is restored by CSB_{E646Q} ATPase mutant protein.
- CSB_{E646Q} partially complements MMS sensitivity.
- CSB stimulates Ape1 and Neil1 incision activity and RNA polymerase I elongation independent of ATP.

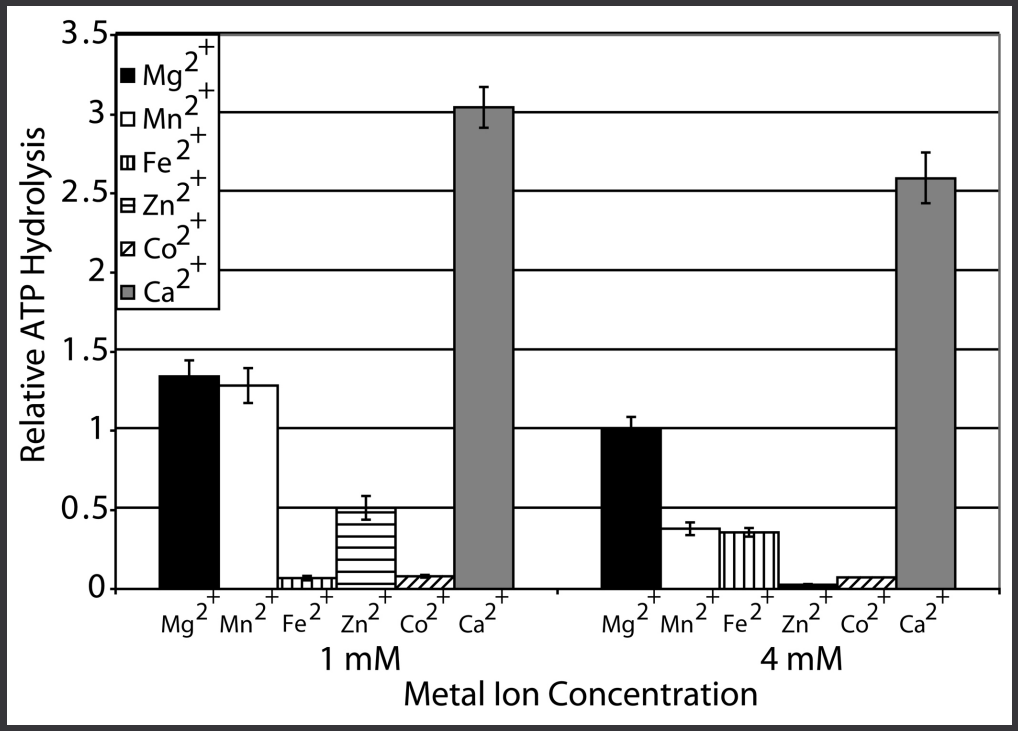
Rationale

- Hypothesis: CSB can act in two distinct modes: one that is dependent on ATP and one that is independent of ATP.

- Aim
  - To characterize additional ATP dependent and independent biochemical properties of human CSB.

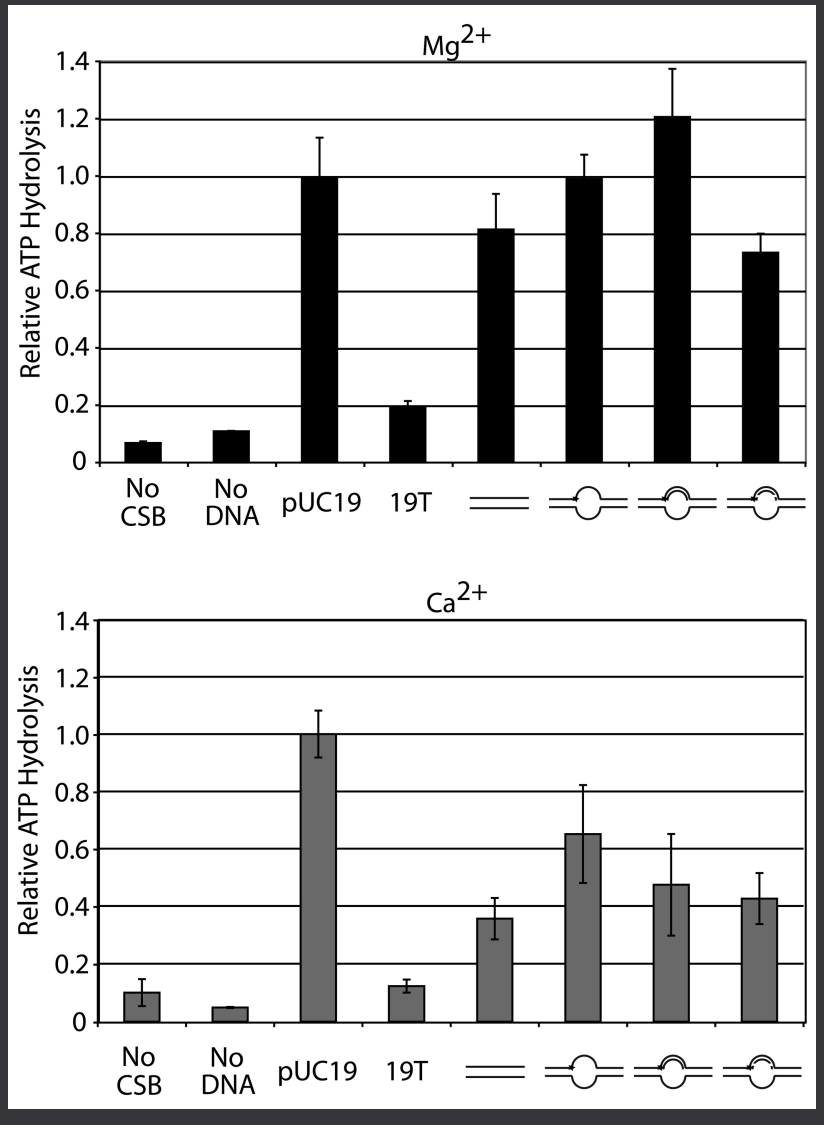
What do these activities tell us about lack of CSB in CS and *in vivo* biological roles of CSB?

Mg^{2+} and Ca^{2+} serve as co-factors for CSB DNA dependent ATP hydrolysis

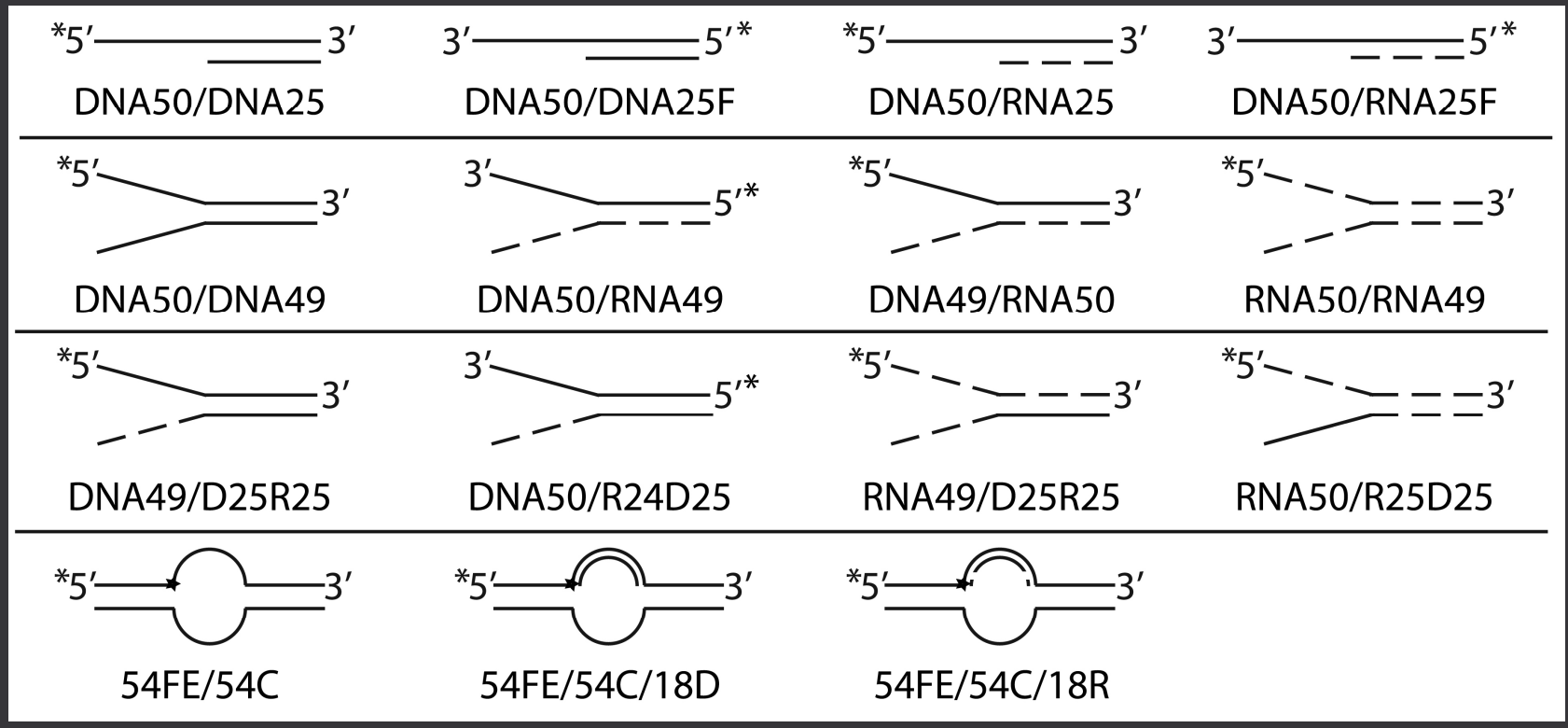


Ca^{2+} is the preferred metal cofactor for DNA dependent ATP hydrolysis by CSB

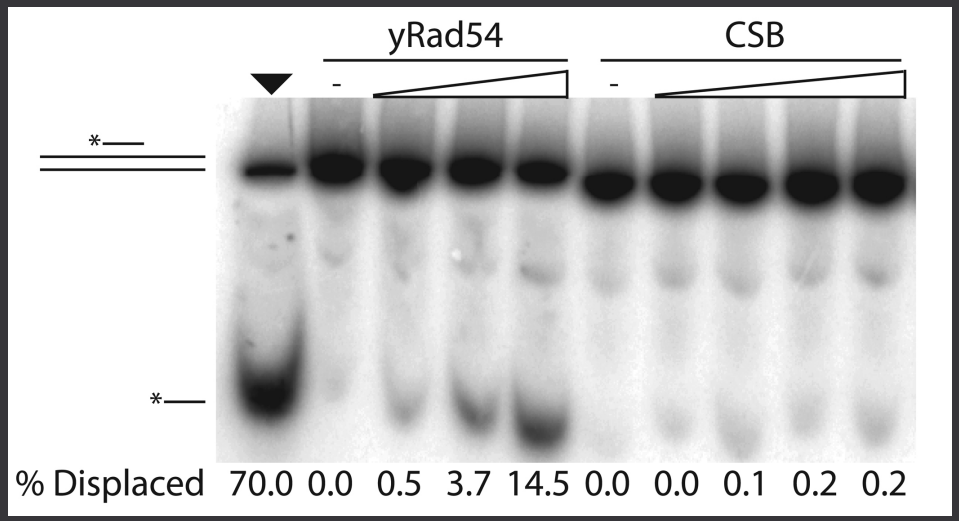
Alternate model nucleic acid substrates serve as cofactors for CSB dependent ATP hydrolysis

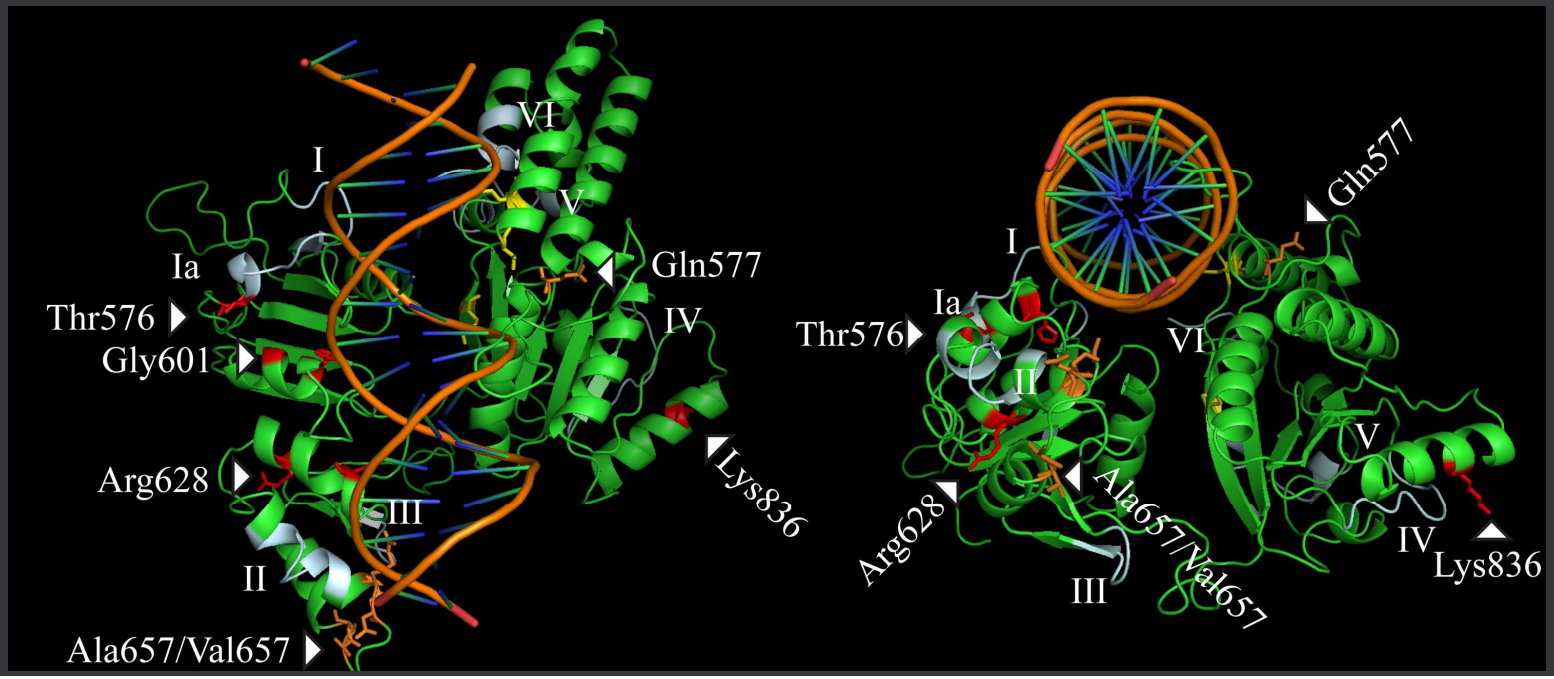


CSB does not possess ATP dependent unwinding activity

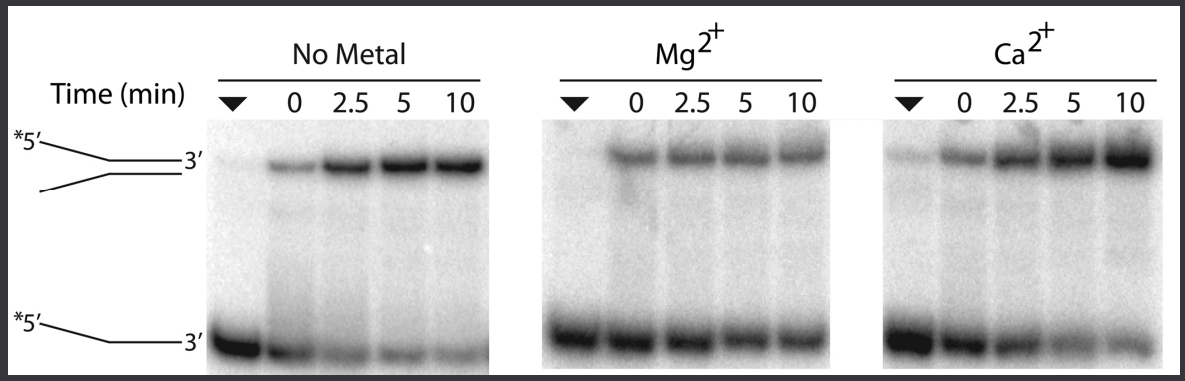


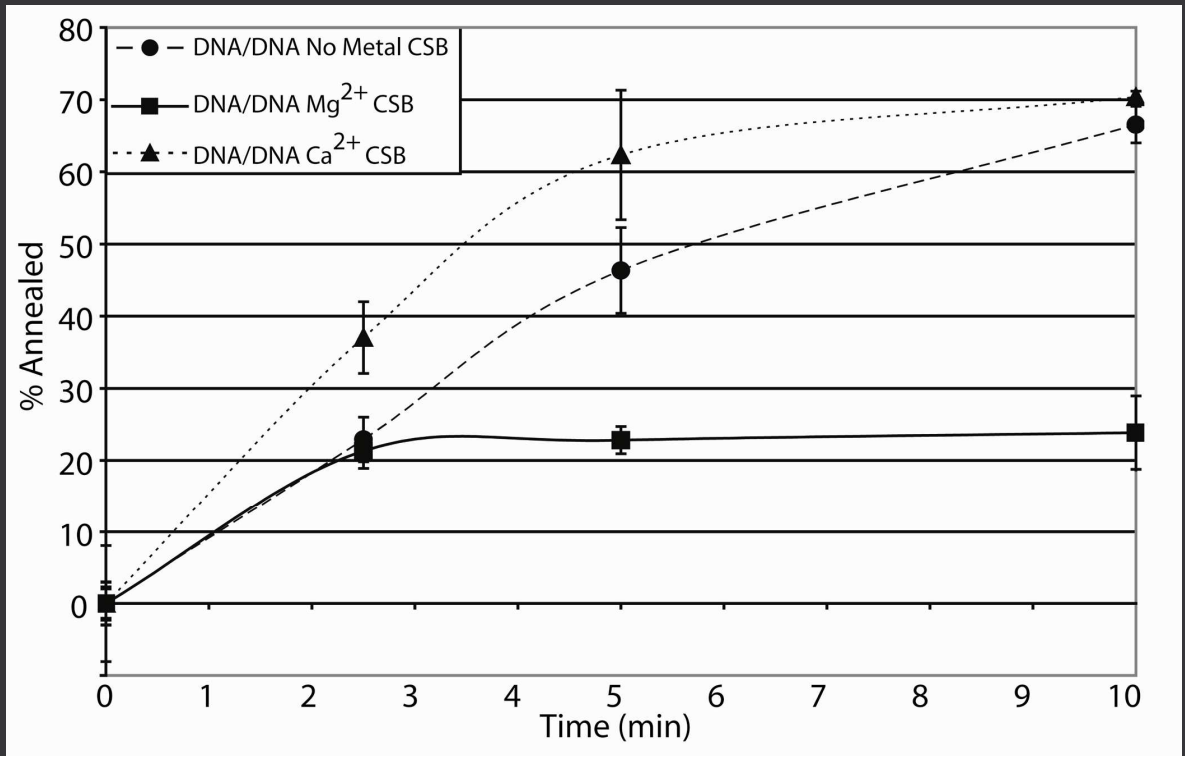
CSB is unable to translocate along dsDNA



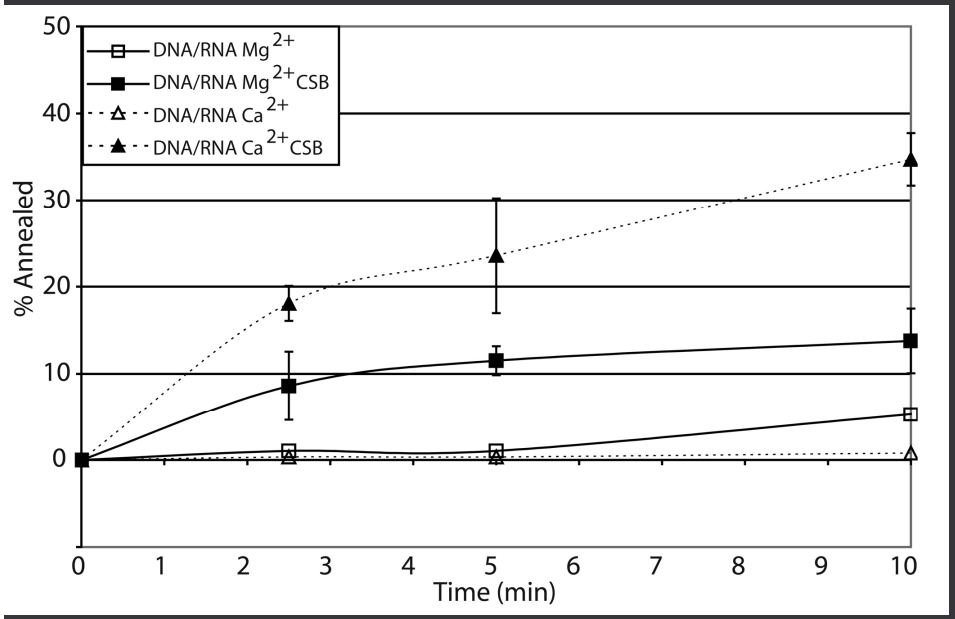


Ca^{2+} is the preferred metal cofactor for CSB DNA/DNA complementary strand pairing

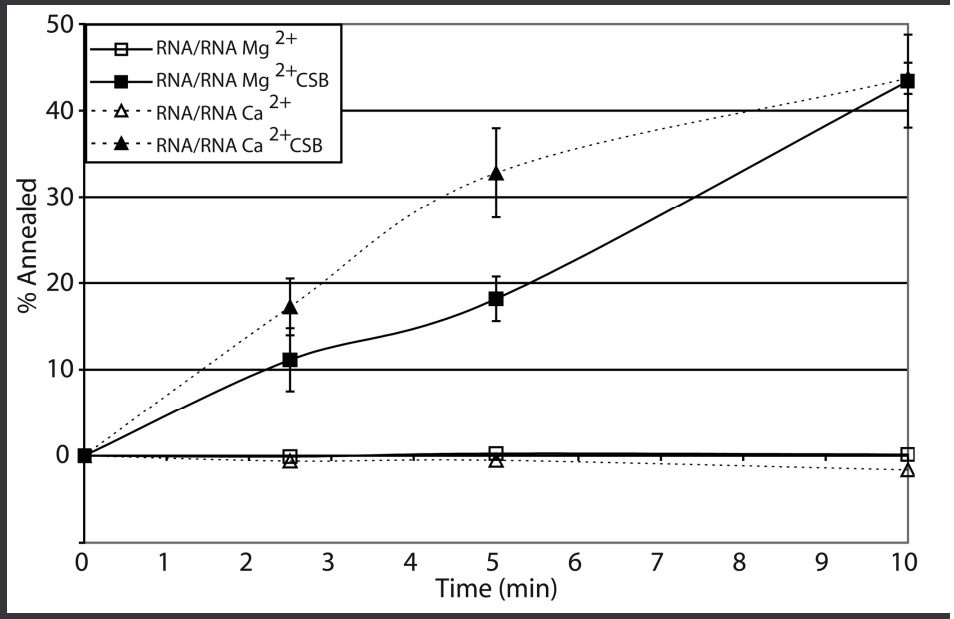




CSB complementary strand pairing extends to DNA/RNA and RNA/RNA

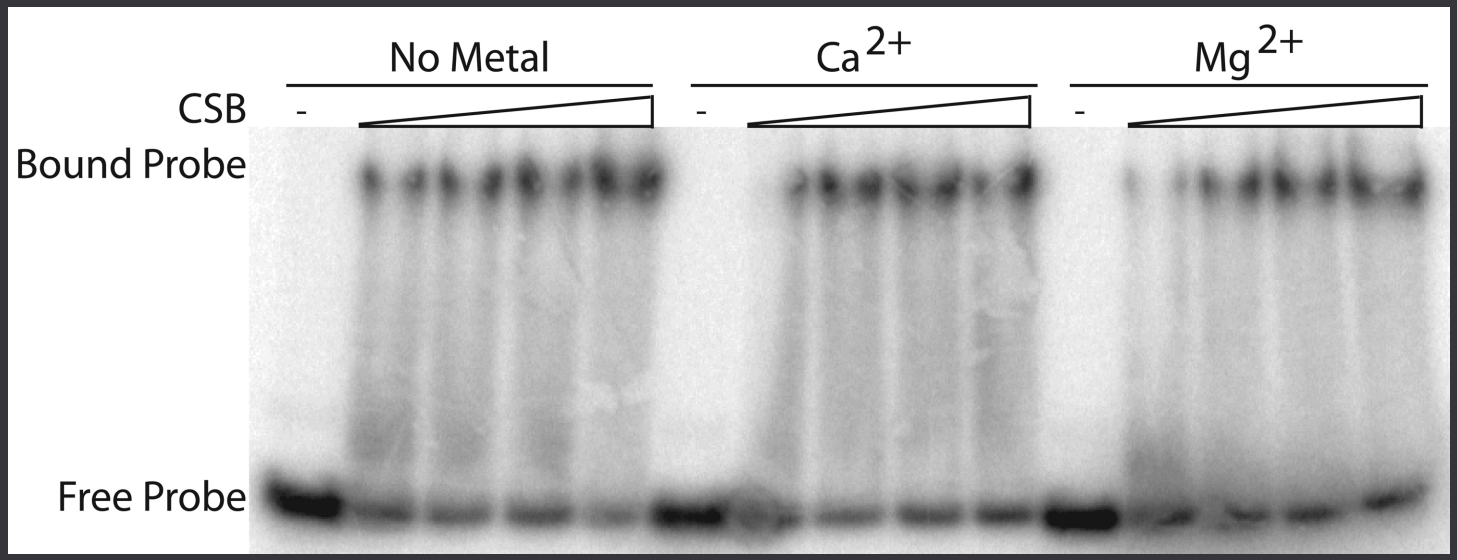


DNA/RNA Annealing



RNA/RNA Annealing

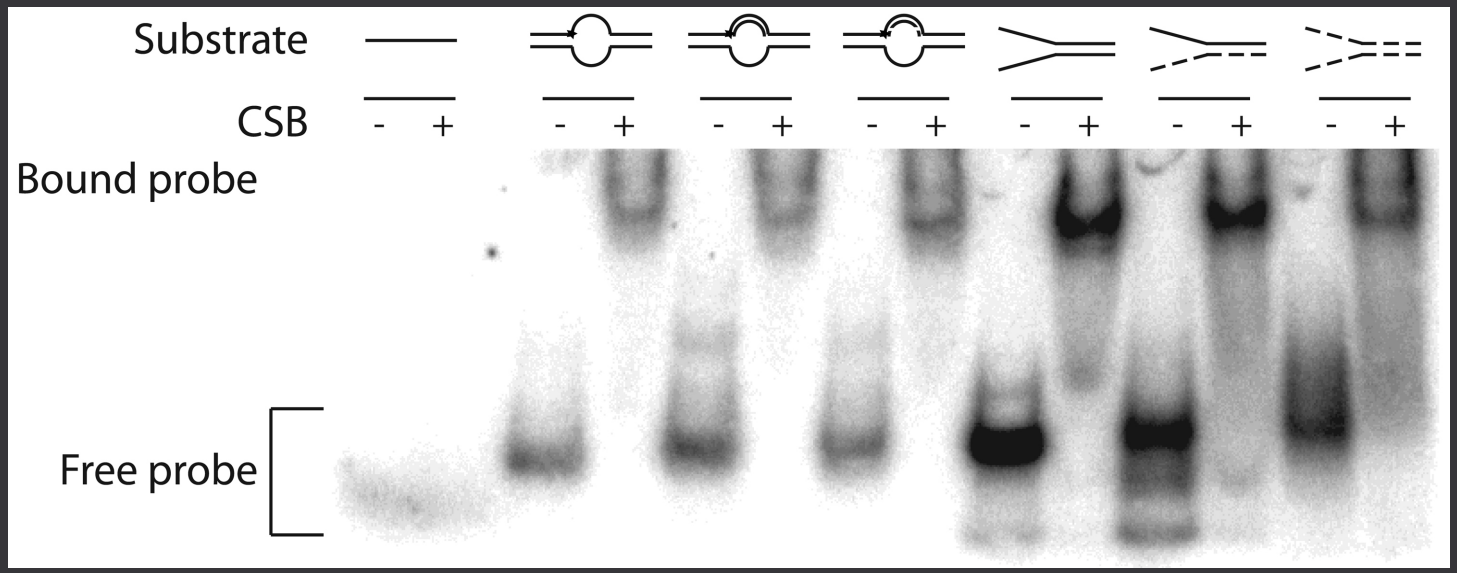
dsDNA binding by CSB



K_D (nM)	7.7	9.3	11.1

Binding is independent of divalent metal and ATP

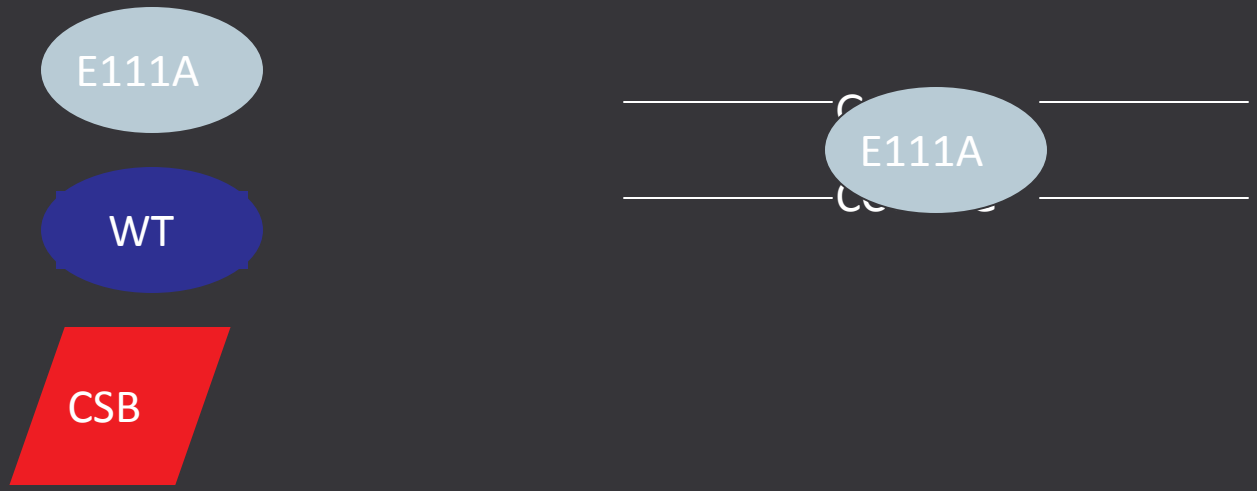
Nucleic acid binding by CSB

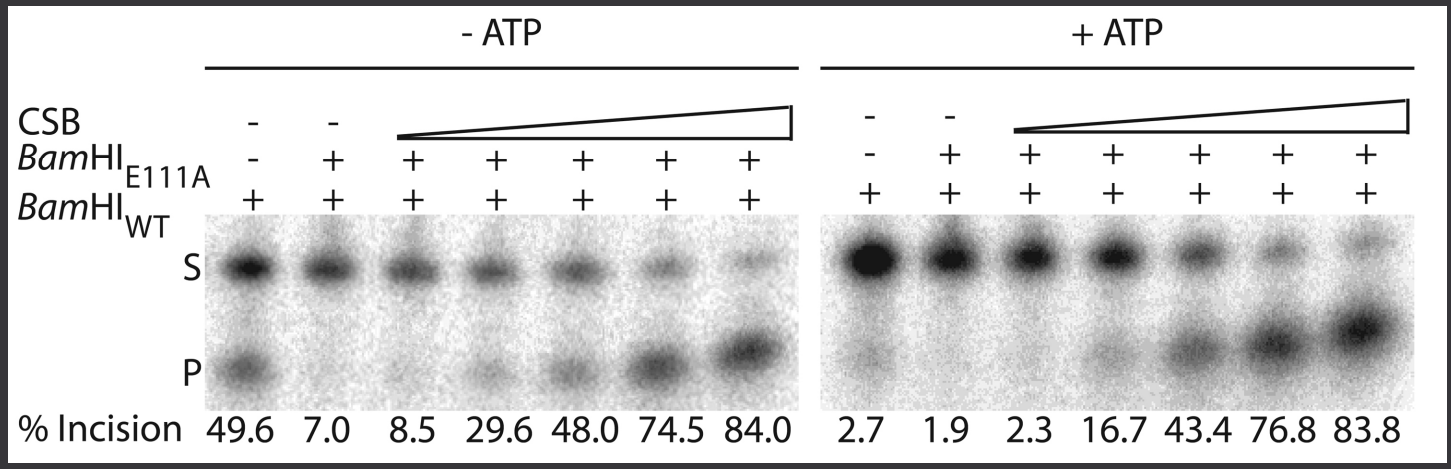


K_D (nM)	N.D.	2.3	1.4	3.1	4.2	2.3	4.7

CSB binds diverse substrates

CSB is able to displace protein from dsDNA in an ATP independent manner





Conclusions

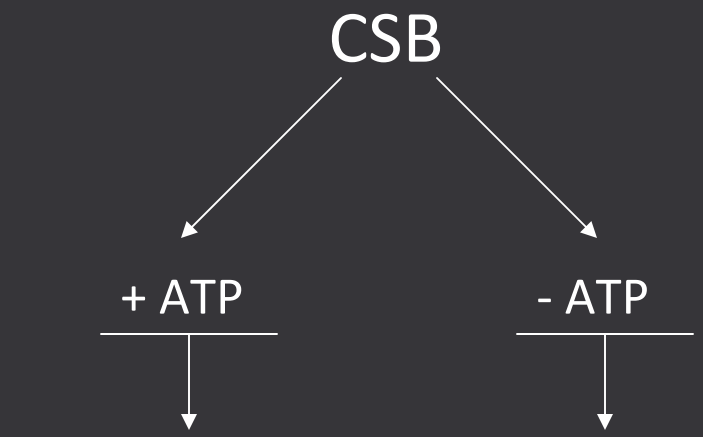
- Ca^{2+} is a novel metal cofactor for ATP hydrolysis and complementary strand pairing.
- Unable to unwind nucleic acid strands and unable to translocate along nucleic acid.
- Complementary strand pairing extends to DNA/RNA and RNA/RNA.
- DNA binding is independent of metal or nucleotide (ATP, ADP, or AMP-PNP).
- Binds to diverse substrates.
- Can remove or re-arrange a pre-bound protein.

What is it using energy for?

Implications and future directions

- Ca^{2+} use may relate to neuroabnormalities found in CS patients.
- Ability to pair DNA and RNA suggests multiple roles.
- Is there a unique, unidentified substrate for CSB?
- Are there protein factors required for revealing an intrinsic motor activity of CSB?
- DNA binding and protein displacement/re-arrangement reveal important ATP independent activities.
- Dissect the ATP dependent and independent roles of CSB and how these activities relate to pathology of CS and lack of increased cancer incidence in CS patients.

Working Model



- Repair of UV Damage
- Nucleosome Remodeling?

- Repair of Endogenous DNA Damage
- Physical and Functional interaction with BER enzymes (Neil1, Ape1, & PARP1)

Acknowledgements

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