Structural and biochemical studies to probe cell cycle regulation by CXC domain proteins in algae

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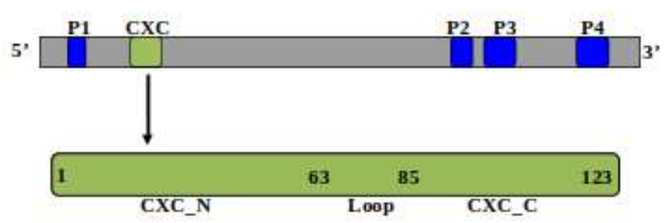
Abstract

CHT7 is a regulator of quiescence repression in *Chlamydomonas reinhardtii*. Initially, CHT7's repression activity was thought to be managed by its DNA-binding CXC domain. But it was later observed that not CXC but CHT7's predicted protein domains were proposed to be involved in its activities. Yet, it remains unclear why and how CHT7 refrains its CXC domain from participating in any transcriptional activities. Through biophysical experiments and molecular dynamics approaches, we studied the DNA recognition behavior of CHT7-CXC. The results indicate that this domain possess sequence selectivity. Further, to understand if CXC loses its DNA binding capabilities in the vicinity of other repressors, we examined CHT7-CXC's DNA binding stability under the spatial constraint conditions created by fusing CHT7-CXC with AsLOV2. The results show limited ability of CHT7-CXC to withstand steric forces and provide insights to why and how algal cells may hold back CHT7-CXC's indulgence in quiescence repression.

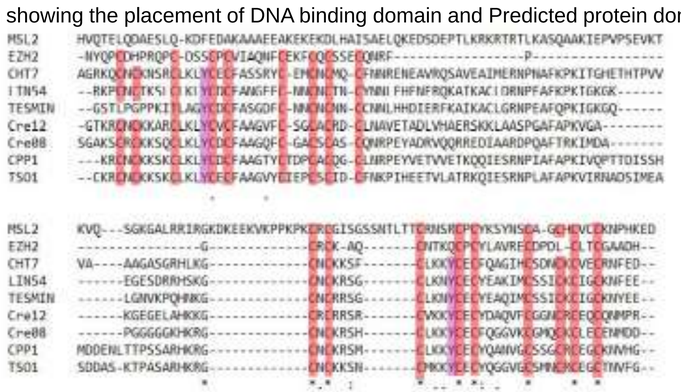
Methodology

PROMOTER PREDICTION	PROTEIN PURIFICATION	BINDING ASSAYS	MD SIMULATION AND MSM	ANALYSIS
Sequence Acquired from Phytozome13 database and NCBI's Genome viewer	Cloning	**EMSA:**	Modelled structure using I-TASSER	Analysis was done using CPPTRAJ and data was plotted using R studios AND MATLAB
Promoter prediction using the PromPredict tool	Expression in Rosetta cells	Electrophoretic mobility shift assay	Docking using HADDOCK	
1000 bps upstream of 5'UTR	GST Pull Down Assay	Titration of constant DNA against increasing concentration of protein.	Molecular dynamic simulation run for 500ns using AMBER 20	
	FPLC PURIFICATION	**Anisotropy Assay:**	MARKOV STATE MODELING	
	MASS SPECTROMETRY	Titration of fluorescently labelled DNA against increasing concentration of protein to calculate dissociation constant.		

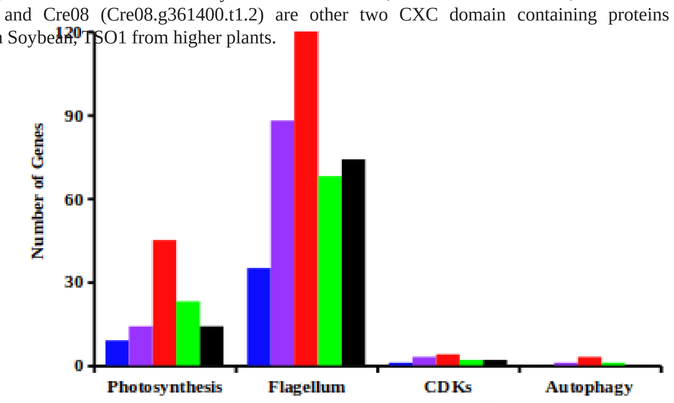
Results



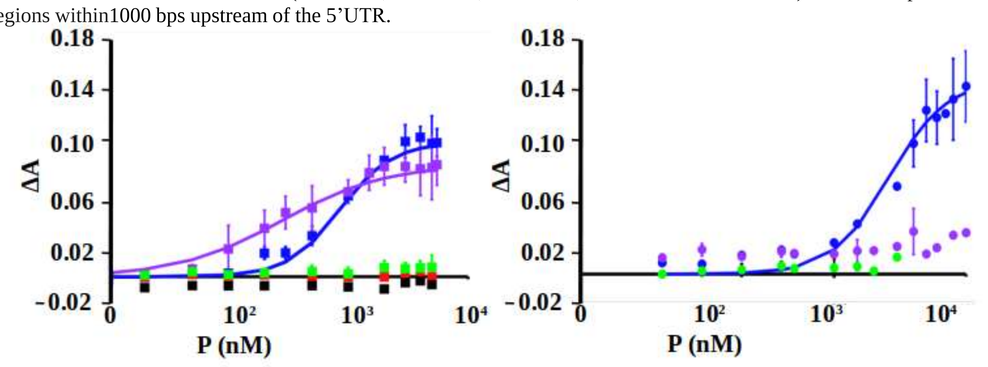
Catoon showing the placement of DNA binding domain and Predicted protein domainss



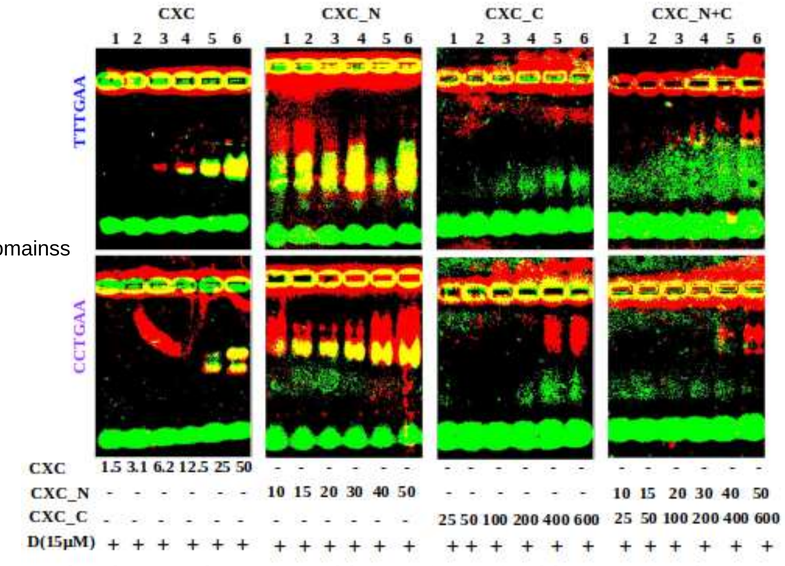
Sequence alignment of CXC domains of different proteins. MSL2 from Drosophila melanogaster, EZH2 from humans, CHT7's (Cre11.g481800.t1.1) CXC domain from Chlmydomonas reinhardtii, LIN54 from humans, TESMIN from Mouse, Cre12 (Cre12.g550250.t1.2) and Cre08 (Cre08.g361400.t1.2) are other two CXC domain containing proteins in Chlmydomonas reinhardtii, CPP1 from Soybean, TSO1 from higher plants.



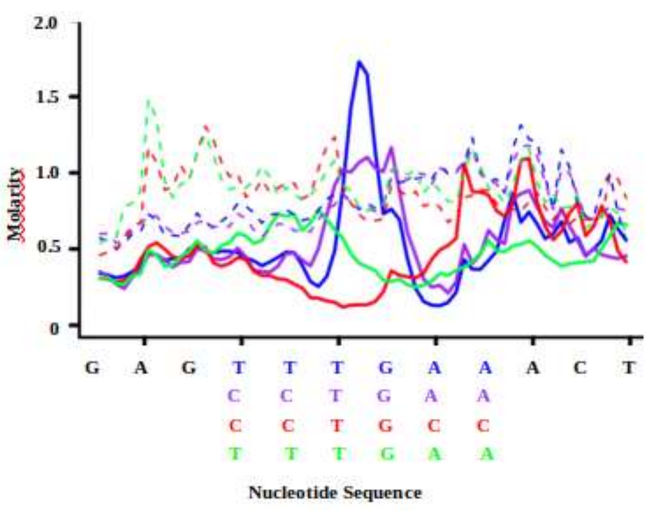
Bar graph shows the number of mis regulated Photosynthetic, Flagellum, CDKs and Autophagy genes in the cht7 mutant that contains six nucleotide (TTYRAA, TTTGAA, CCTGAA, CCTGCC and TTTGCC) sites in the promoter regions within1000 bps upstream of the 5'UTR.



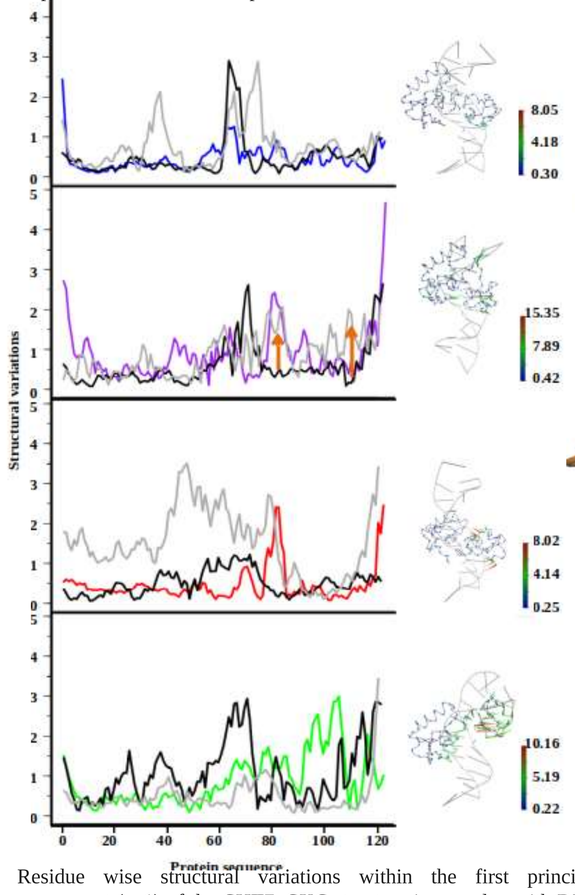
Fluorescence polarization assay (FPA) demonstrating change in fluorescence anisotropy (ΔA) of 5nM 6-FAM labeled DNA with change in concentration



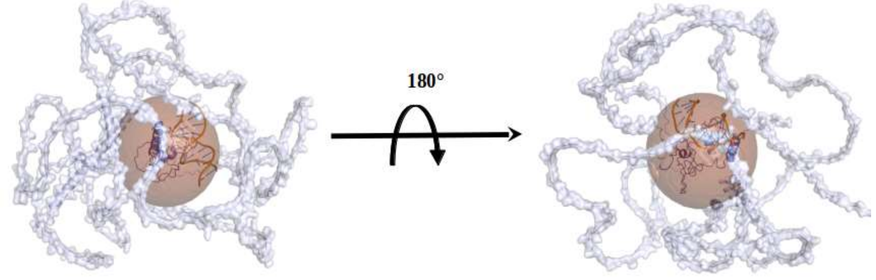
Overlap of EMSA gel images stained for DNA (in green) and for protein (in red). Regions in yellow represent the protein DNA bound complex.



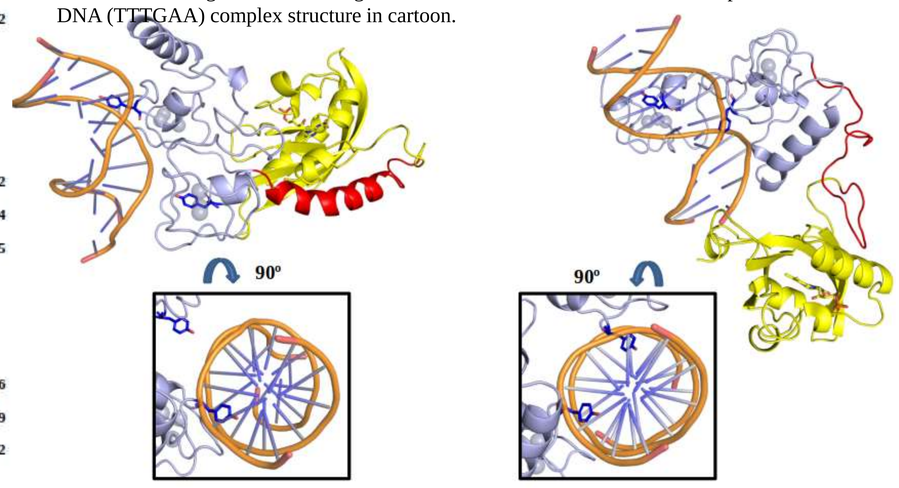
Ionic concentration across the DNA within the CHT7_CXC bound complexes (solid lines) and in isolated DNA (dotted).



Residue wise structural variations within the first principal component (pc1) of the CHT7_CXC structure in complex with DNA containing binding regions TTTGAA, CCTTGAA, CCTGCC and TTTGCC are plotted in blue, purple, red and green, respectively..



CHT7's alpha fold structural model represented in the cartoon is shown from two different angles in grey. Enclosed within it is the brown sphere representing the inner boundary line of the CHT7 residues excluding and surrounding the CXC domain. Enclosed within this sphere is the CXC DNA (TTTGAA) complex structure in cartoon.



Molecular dynamic structures of the molecular fusion of Avena Sativa's LOV2 ($AsLOV_2$) and CHT7_CXC generated under the dark (LOV_D-CXC) and the light (LOV_L-CXC) conditions in complex with 12mer DNA duplex containing TTTGAA as the binding region.

Conclusion

Following are the conclusion drawn from the work:

1. CXC binding is dependent on the DNA length.
2. Possibly, the CXC undergoes a slide and search mechanism to recognize the specific binding stretch of the DNA.
3. Two subdomains possess asymmetric DNA binding abilities.
4. DNA recognition ability of CXC is partly dependent on the loop between the two subdomains.
5. Molecular crowding due to the formation of the CHT7 complex could induce structural changes within the CHT7, which switches CXC to the conformational state incapable of DNA binding.

References

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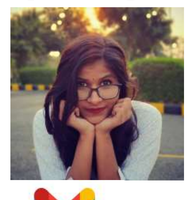

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