Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXIX

Analysis of the Structural and Functional role of Human DEAD-box Helicase 3X responsible for DDX3X Syndrome

PhD Student: Noemi Borgognoni
Molecular Biology and Computational Biophysics Lab, DiSVA
Tutor: Prof. Daniele Di Marino

INTRODUCTION

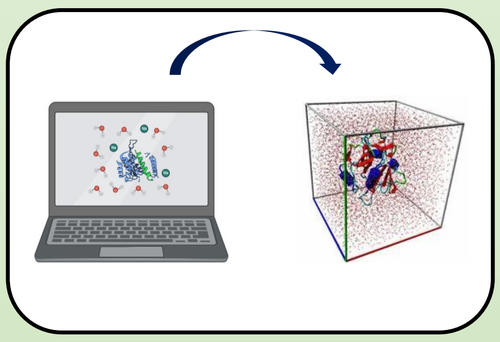
DDX3X is a human RNA-binding DEAD-box helicase, acting as RNA-unwinding enzymes, chaperones for RNA folding, and regulators of protein-RNA complex assembly/disassembly on every stage of RNA metabolism. It comprises conserved domains 1 and 2 with nine signature motifs for DNA and RNA binding, as well as ATP hydrolysis [1]. DDX3X follows a catalytic cycle as a dimer with three stages: an apo state, a pre-unwinding state with RNA binding, and a post-unwinding state where each monomer releases an RNA strand after separation. DDX3X has a crucial role in cell growth control, mRNA transport, and translation. Its influence on RNA metabolism impacts various biological processes, and altered functionality can lead to diseases, including DDX3X syndrome, a rare intellectual disability (ID) linked to mutations in the X-linked DDX3X gene, observed in only 300 individuals to date [2].

AIM

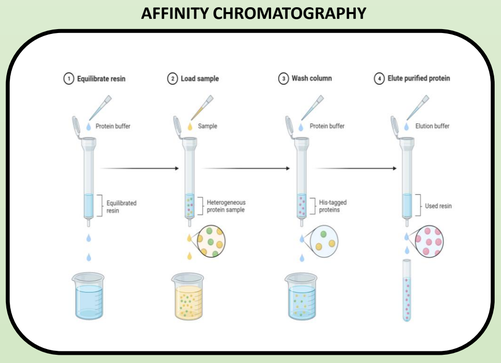
The aim of the project is to investigate, using computational simulations and enzymatic functionality assays based on fluorescence detection, the impact of some of most frequently DDX3X mutations (i.e., R351Q, R362C, R376C, and E348Q), on catalytic cycle of the enzyme. As these mutations are located within the ATPase and RNA binding domain of the protein, they might potentially lead to a diminished ability to bind and subsequently unwind RNAs double strands. This, in turn, raises the prospect of a potential link between these mutants and the development of DDX3X-related syndrome.

METHODS

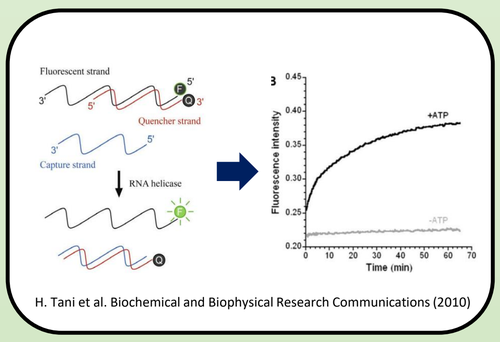
MOLECULAR DYNAMICS SIMULATIONS



AFFINITY CHROMATOGRAPHY

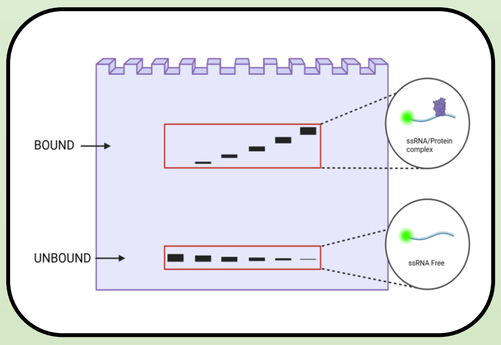


FRET ASSAY



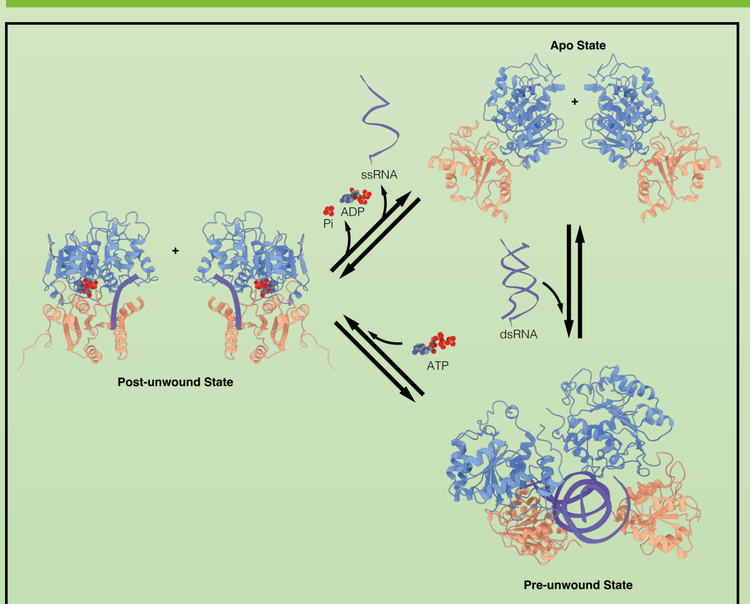
H. Tani et al. Biochemical and Biophysical Research Communications (2010)

EMSA ASSAY



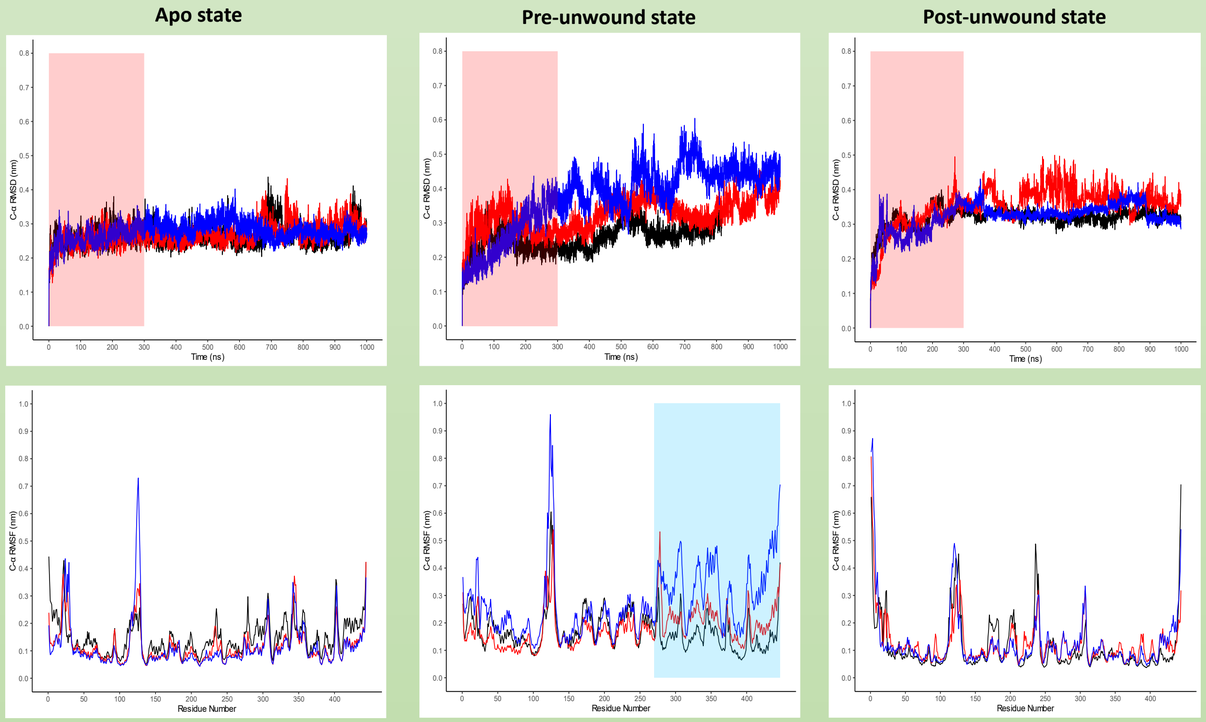
RESULTS

COMPUTATIONAL SOMULATIONS



Ribbon three-dimensional (3D) structural representation of the three catalytic states of WT DDX3X. The Apo, Pre-unwound, and Post-unwound states of WT DDX3X were depicted using Chimera X software [PDB ID: 2I4I; PDB ID: 6O5F].

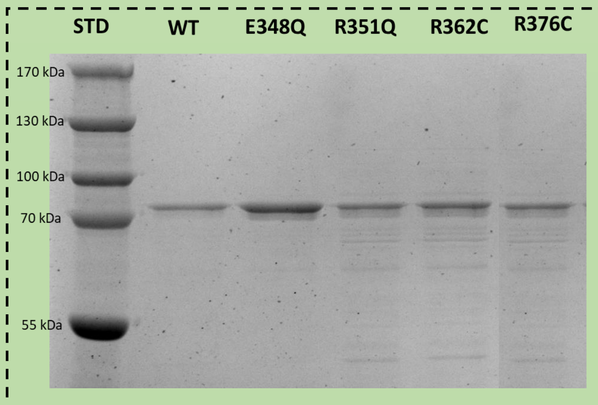
Apo state | **Pre-unwound state** | **Post-unwound state**



WT
R362C
R376C

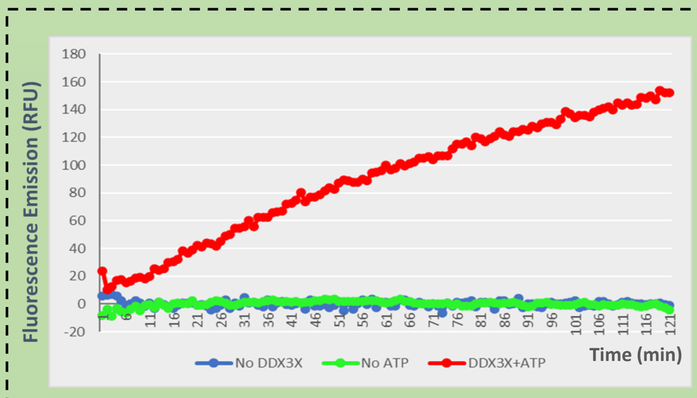
To investigate the roles of the R362C and R376C DDX3X mutants, we conducted a series of molecular dynamics simulations [5] for the enzyme's three catalytic states. Our analysis of Root Mean Square Deviation (RMSD) revealed a pronounced destabilization of the mutants in both pre- and post-unwound states, indicating that the mutations indeed affect the enzyme-RNA complex. Furthermore, the ATP-binding domain of the DDX3X mutants in the pre-unwound state exhibited higher Root Mean Square Fluctuation (RMSF), which may be associated with a reduced RNA unwinding capacity in these mutant enzymes.

PURIFICATION



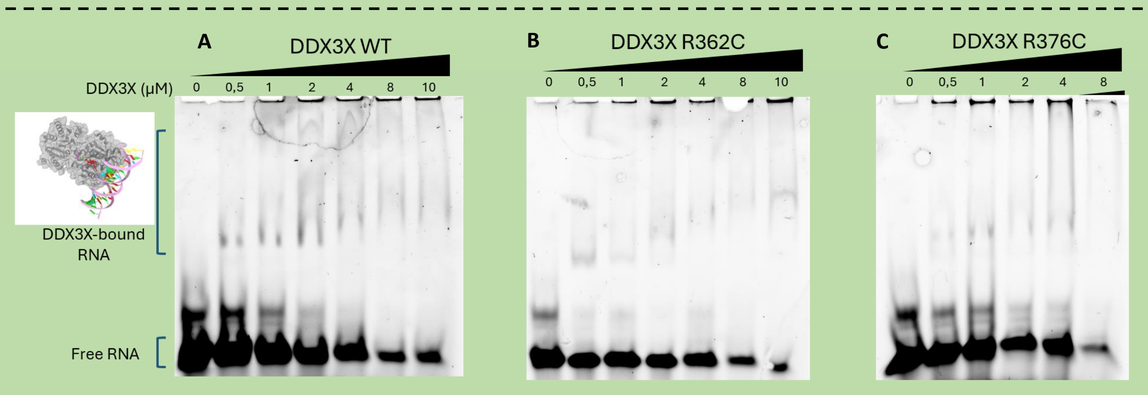
Protein purification of DDX3X and mutants. Proteins expression in E.coli BL21 cells. Bands (79 kDa) of DDX3Xwt, E348Q, R351Q, R362C, R376C.

FRET ASSAY



Förster Resonance Energy Transfer (FRET) assay for DDX3X helicase activity. Three FRET reaction mixes: in the first (blue) we have absence of DDX3X, in the second (green), we have absence of ATP and in the third (red) DDX3X and ATP are present.

EMSA ASSAY



Electrophoretic Mobility Shift Assay (EMSA) binding experiments for DDX3X and mutants. (A) DDX3X WT and **(B)** mutant DDX3X R362C were titrated from 0 to 10 uM in the presence of 50 nM fluorescent ssRNA substrate. **(C)** DDX3X R376C mutant was titrated from 0 to 8 uM in the presence of the same quantity of ssRNA substrate.

CONCLUSIONS

In conclusion, our study demonstrates that mutations impact the enzyme's dynamics when RNA is involved, as evidenced by higher RMSD values in mutants and RMSF analysis. These results potentially indicate reduced RNA unwinding ability. Moreover, we have developed a highly efficient purification protocol for both wild-type and mutant proteins. Furthermore, to validate MD simulations, two fluorescence-based assays have been established for monitoring DDX3X helicase and RNA-binding activity [3][4]. Overall, our analyses indicate that mutations could impact the activity of DDX3X, but it is not yet known at which step of the catalytic cycle.

Future perspectives:

- Perform further molecular dynamic simulations with other mutants
- Study DDX3X variants with FRET assay and optimize EMSA assay for WT protein and mutants to detect catalytic differences

REFERENCES

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[3] Tani H, Fujita O, Furuta A, Matsuda Y, Miyata R, Akimitsu N, Tanaka J, Tsuneda S, Sekiguchi Y, Noda N. Real-time monitoring of RNA helicase activity using fluorescence resonance energy transfer in vitro. Biochem Biophys Res Commun. 2010 Feb 26;393(1):131-6
[4] Epling, L. B., Grace, C. R., Lowe, B. R., Partridge, J. F., & Enemark, E. J. (2015). Cancer-associated mutants of RNA helicase DDX3X are defective in RNA-stimulated ATP hydrolysis. Journal of molecular biology, 427(9), 1779–1796.
[5] Di Marino D., Chillemi G., De Rubeis S., Tramontano A., Achsel T., Bagni C. MD and Docking Studies Reveal That the Functional Switch of CYFIP1 is Mediated by a Butterfly-like Motion. Journal of Chemical Theory and Computation 2015 11 (7), 3401-3410