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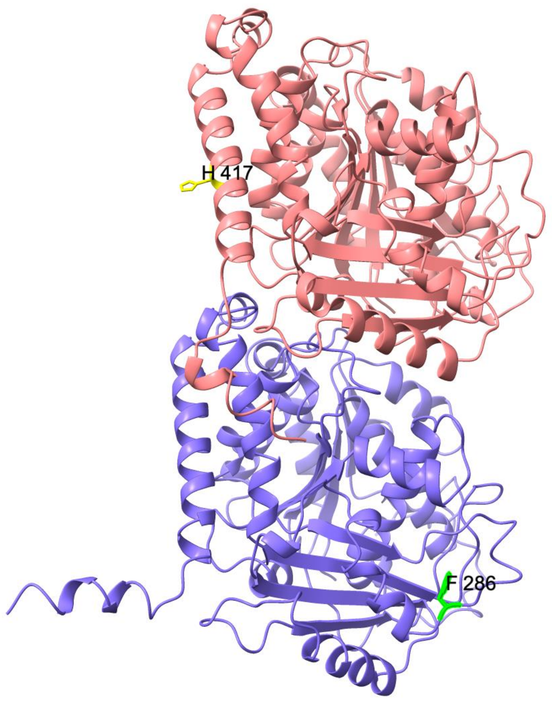
Effect of Disease-Related Mutations of Tubulins on Microtubule Properties and on Neuronal Development and Differentiation

Introduction

Microtubules are non-covalent polymers formed from heterodimers of α- and β- tubulin molecules and is important in cell functions such as chromosome segregation, cell cytoskeleton and organelles intracellular transport. Numerous disease-associated point mutations have been found present on different tubulin genes causing a of neurodevelopmental disorder diseases, such as lissencephaly (smooth brain), polymicrogyria and cortical malformations, collectively known as tubulinopathies (Cushion et al., 2023).

L286F on TUBA1A is identified in patients diagnosed with severe lissencephaly with previous study suggesting that it may disrupt cortical formation due to failure to interact or bind with microtubule associated proteins or motors (Poirier et al., 2007; Tian et al., 2010). While D417H on TUBB3 is one of 8 mutations which is found to cause ocular motility disorder CFEOM3, with all subjects showing signs of maldevelopment of spinal motor neurons (Tischfield, 2010). Previous studies showed that D417H alters microtubule conformation, dynamics at both ends and affects binding of kinesins (Ti et al., 2016).

However, due to challenges in generating recombinant mutant tubulins for characterization in vitro and in vivo via animal models, the effects of these disease-related mutant tubulins are not well studied. Here, we will generate and purify these mutant tubulins for characterization.



Objectives

To dissect the effect of neurological disease-related mutations on microtubule properties with an initial focus on the D417H mutation on β-tubulin L286F mutation in α-tubulin and evaluate the possibility of using recombinant *C. elegans* tubulins Mec-12 and Mec-7 to study the effects of disease-related mutations on tubulins.

Method

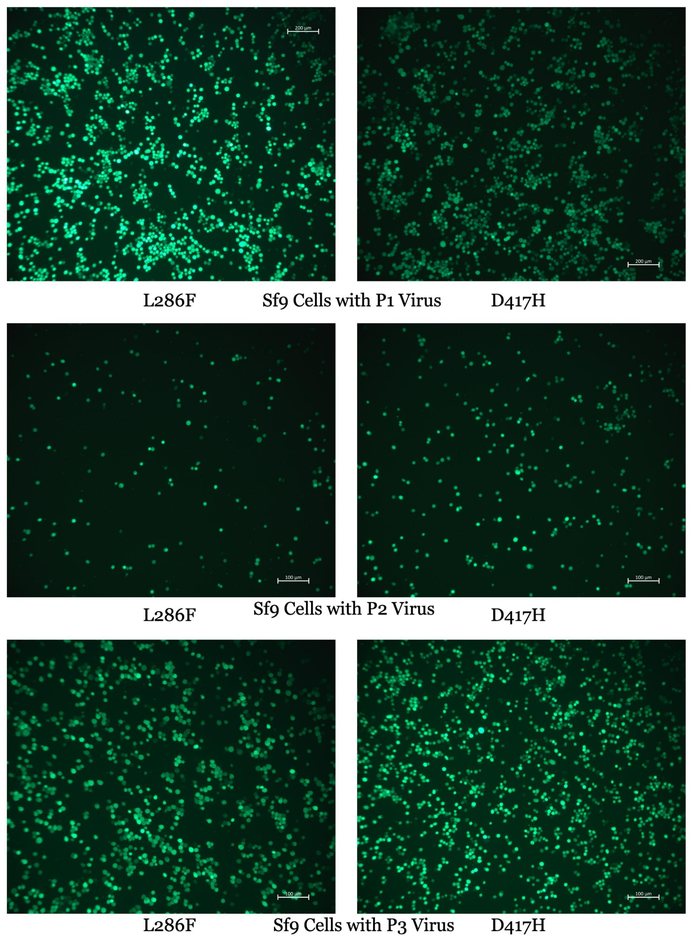
Using *C. elegans* as a model organism, we generate mutated sequences of Mec-12^{L286F} and Mec-7^{D417H} in pFastBac Dual Vector with His and Strep tag using the IVA cloning method.

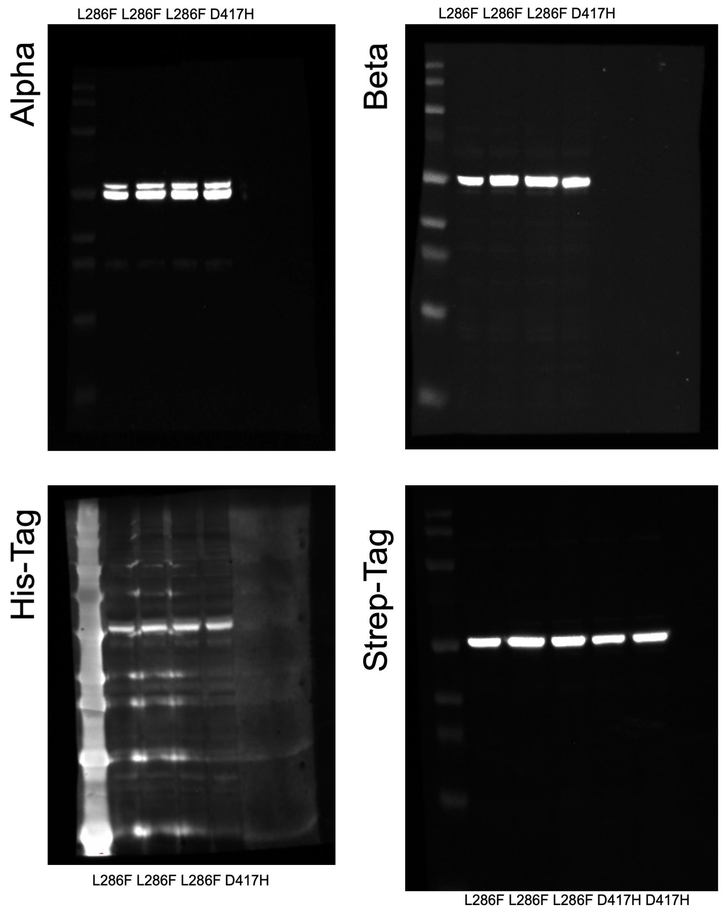
Plasmids are then transformed in DH10EMBacY (E. coli) competent cells to produce bacmid, which is then used to generate recombinant baculovirus.

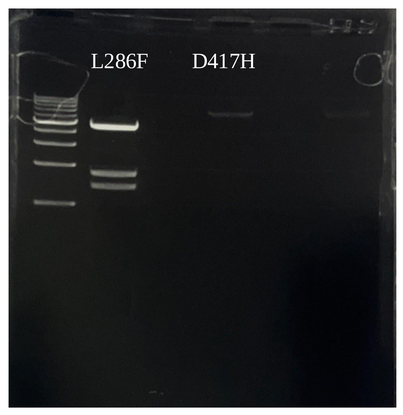
Virus are then used to infect insect cells to produce recombinant *C. elegans* tubulins Mec-12^{L286F} and Mec-7^{D417H} which are α- and β- tubulins needed for the polymerization of mechanosensory neurons in *C. elegans* (Fukushige et al., 1999; Savage et al., 1989).

Pure tag-free recombinant tubulins can then be purified from lysed insect cells and used for in vitro studies, using TIRF microscopy to observe microtubule dynamics, and possible in vivo studies in *C. elegans* (Ti et al., 2020).

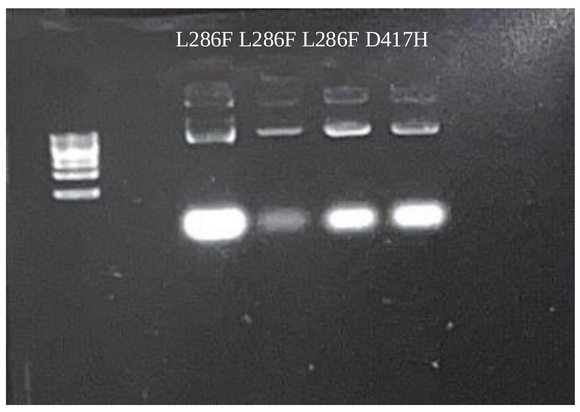
Results







PCR product check using gel electrophoresis



Bacmid expression check

Mutant tubulins are generated and confirmed with fluorescence in Sf9 cells and Western Blot.

Discussion

Western blot results showed that affinity tag-free recombinant C. elegans tubulins is successfully generated and purified from insect cells. The next steps of this project is to run in vitro assays such as TIRF microscopy to observe the dynamics of microtubules polymerized from these mutated tubulins compared to wild type. In vivo characterization of mutated Mec-12 and Mec-7 such as in touch neuron receptors of *C. elegans* is also possible in the future.

Further studies on the interaction between kinesins or other microtubule associated proteins and these mutant microtubules are still needed to know the exact mechanism of how neuronal disorders are developed from a single point mutation in one isotype of microtubules.

Acknowledgements

I would like to thank Dr. Ti for his extremely helpful teachings and guidance for this research project, and Ti Lab members for their help and support throughout the journey. I would also like to express gratitude to Lord Laidlaw, The Laidlaw Foundation and HKU Horizons office for the funding, research opportunity and leadership trainings provided.

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