ACETYLATION IN STABLE MICROTUBULES

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Introduction

Microtubules are an essential part of the cytoskeleton and assist in multiple cellular processes including intracellular transport and cell division. They also play an important role in neuronal function.

Acetylation in microtubules is a prominent post-translational modification in cells with stable microtubule networks such as neurons[1]. Microtubule acetylation in neurons may play an important role in neurodegenerative diseases like Alzheimer's[2]. However, it is not known why stable microtubules are more acetylated than dynamic microtubules. There are two models to explain this phenomenon.

The first is that stable microtubules are preferentially acetylated, and the second is that both are acetylated equally, but dynamic microtubules are destabilized by acetylation.

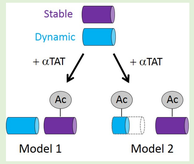
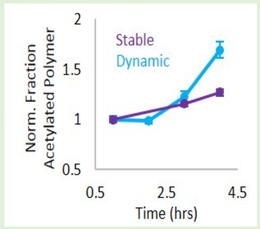


Figure 1: Two models for acetylation in microtubules.



Preliminary research has shown that stable microtubules are not preferentially acetylated, in fact dynamic microtubules may have higher rate of acetylation.

Figure 2: Acetylation rate in stable and dynamic microtubules.

Experimental methods

1. Effect of potassium chloride on microtubule acetylation

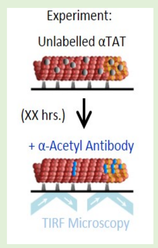


Figure 3: In-vitro acetylation of microtubules observed using TIRF microscopy.

- Microtubules were stuck down on the coverslip.
- Acetylation reaction mixtures were flowed into the chamber to allow acetylation.
- 0mM, 150mM and 400mM KCl acetylation mixtures were used.
- TIRF microscopy used to record data.

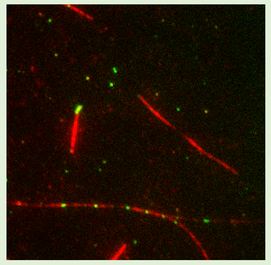


Figure 4: Stable microtubules (red) with acetylation (green) in 400mM KCl solution.

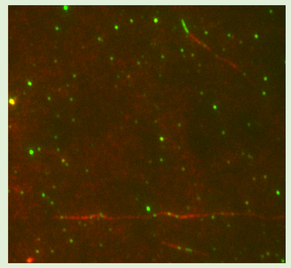


Figure 5: Dynamic microtubules (red) with acetylation (green) in 400mM KCl solution.

- Percent acetylation measured using MATLAB codes
- Relation between microtubule length and percent acetylation studied

2. Length measurement of acetylated stable microtubules

- Microtubules were stuck down on coverslip.
- Labelled tubulin added to chamber to allow fresh stable microtubules to grow
- Acetylation mix flowed in to chamber
- Data recorded on TIRF microscope for 30 minutes
- Depolymerization rates measured and compared with control group

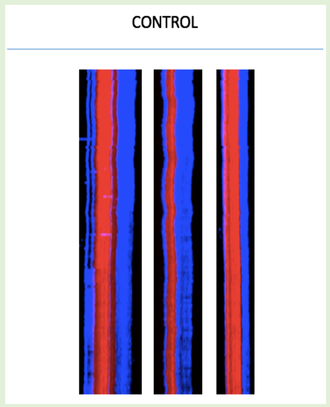


Figure 6: Depolymerization of control stable microtubules (blue) over time.

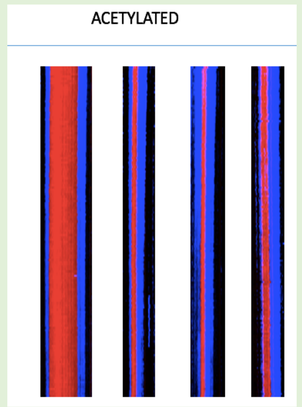


Figure 7: Depolymerization of acetylated stable microtubules (blue) over time.

Results and Conclusions

- Stable microtubules are more acetylated than dynamic microtubules, but acetylation rate is similar

1. Effect of potassium chloride on microtubule acetylation

- At high potassium chloride concentrations, highly acetylated dynamic microtubules are shorter in length.

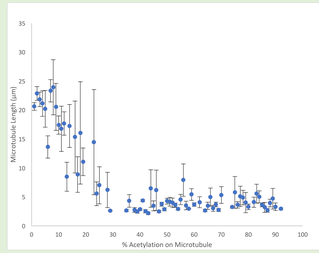


Figure 8: When exposed to 150mM KCl, the most acetylated dynamic microtubules were shorter in length.

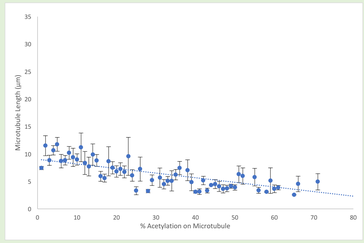


Figure 9: When exposed to 150mM KCl, there was no strong correlation between per cent acetylation and stable microtubule length.

- Less of a correlation is seen between acetylation and length in stable MTs

2. Length measurement of acetylated stable microtubules

- Acetylated dynamic microtubules have shorter catastrophe time and lengths
- Acetylated stable microtubules have depolymerization rate similar to control microtubules

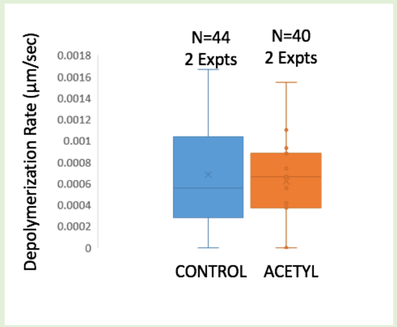


Figure 10: Acetylated stable microtubules depolymerize at a rate similar to stable microtubules which are not acetylated.

Conclusion:
Model 2 is supported: rate of acetylation is similar for dynamic and stable microtubules, but dynamic microtubules are destabilized by acetylation.

References:

1. Song, Y & Brady, ST. (2015). Post-translational modifications of tubulin: pathways to functional diversity of microtubules. *Trends Cell Biol* 25: 125-36.
2. Brion BH and J-P (1996) Reduction of Acetylated a-Tubulin Immunoreactivity in Neurofibrillary Tangle-bearing Neurons in Alzheimer's Disease. *J Neuropathol Exp Neurol* 55(9):964–972.

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