

HUMBOLDT-UNIVERSITÄT ZU BERLIN



LEBENSWISSENSCHAFTLICHE FAKULTÄT
INSTITUT FÜR BIOLOGIE

Protokoll

Fachkurs: Biology

Versuch: Testung von Y

Betreuer: Dr. X

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1 Einleitung

Microtubules (MT) mediate various cellular functions such as structural support, chromosome segregation, and intracellular transport. ? They are essential cytoskeletal elements.

MTs consist mainly out of α - and β -tubulin. α - and β -tubulin form the MT as a hollow tube while forming α - and β -tubulin dimers. The end of the MT with α -tubulin is called the minus end and the other end of the MT with the β -tubulin get's called plus end. The minus end is less dynamic than the plus end because the plus end functions as the growth area of the MT by adding tubulin dimers to it.

The properties of the MT are based on the (1.) isoforms and (2.) the covalent posttranslational modifications (PTM) of the different tubulins, which is called the 'tubulin-code'. The existence of 9 α - and 10 β -tubulins isoforms in humans was verified.

Acetylation and detyrosination of tubulins are two of the PTMs of the MT. They are a reversible process and have a cross-talk between each other.

Acetylation of K40 luminal α -tubulin decreases the stiffness of MT and is correlated with a long MT lifetime, because the reduced stiffness makes it more resilient against a mechanical stress force. Detyrosination of MT increases the stability of MT by decreasing the interaction of the MT with depolymerizing proteins.

K40 acetylation is the only PTM within the lumen of MTs.

Most PTMs occur at the C-terminal end of a tubulin amino-acid side chain. The PTMs can produce alterations in cellular function and it's phenotype. ? Detyrosination removes the C-terminal tyrosine of most α -tubulin isoforms.

It has been shown that the VASH1/2:SVBP complex is enzymatic activate during the MT detyrosination.

In this experiment the technique of transfection is used. Transfection is the process of introducing nucleic acids in the form of DNA or RNA into an eukaryotic cells. There exist two different forms of the transfection: either the nucleic acids only get read-out by the cell or they get build into it's genome. The latter has the ability to inherit it to the daughter cells of the transfected cell.

2 Material und Methoden

First order and second order antibodies of different animals types um die Signalstärke zu erhöhen, da Alexa Fluorophor 'anti ANIMAL Type' zum first order antibody ist und somit sonnst an beiden verschieden markierten alpha-Tubulin und detyr-tubulin binden kann.

TrackMate ? ?

'LysoTracker™ Green DND-26 is a green fluorescent dye that stains acidic compartments in live cells with excitation/emission maxima 504/511 nm.' <https://www.thermofisher.com/order/catalog/product/L7526>

'SiR-tubulin is based on the far-red silicon rhodamine (SiR) fluorophore analogue SiR and the microtubule-binding drug Docetaxel. SiR-tubulin allows labeling of endogenous microtubules in live cells with high specificity and low background without the need for genetic manipulation or over-expression. The key features of SiR-tubulin are i) far-red absorption and emission wavelengths, ii) cell permeability, iii) fluorogenic character, and iv) compatibility with super-resolution microscopy (e.g., STED SIM). In addition, SiR-tubulin can be used for wide-field and confocal fluorescent imaging in living cells. The emission in the far-red wavelength minimizes phototoxicity and sample autofluorescence.' <https://www.cytoskeleton.com/tubulins/live-cell-imaging-tubulin/cy-sc014>

'Immunocytochemistry (ICC) is a common laboratory technique that is used to anatomically visualize the localization of a specific protein or antigen in cells by use of a specific primary antibody that binds to it. The primary antibody allows visualization of the protein under a fluorescence microscope when it is bound by a secondary antibody that has a conjugated fluorophore.' <https://en.wikipedia.org/wiki/Immunocytochemistry>

Blocken verhindert unspezifische Bindung und erlaubt somit nur spezifische Bindung der Antikörper

Spinning disk confocal microscopy (SDCM) löst das Problem der geringen Zeitauflösung des Laser Scanning Confocal Microscope (LSCM), indem es eine Nipkow-Scheibe verwendet. Die Scheibe besitzt viele Lochblenden. Diese Scheibe wird bei der Untersuchung rotiert, wodurch die Beobachtungsebene wie homogen beleuchtet ist. With that you can capture a large field of view (FOV) in as little as 5ms, but with a lost of intensity

due to light scattering.

TIRFM stands for 'Total Internal Reflection Fluorescence Microscopy'. Effekt der Totalreflexion wird ausgenutzt, um oberflächennahe Prozess zu studieren. Die evaneszente Welle hat eine Eindringtiefe von ca. 100-200nm und regt nur die Fluorophore in diesen Bereich an. Die Methode wird zur Untersuchung der Unterseite von adhärennten Zellen verwendet.

3 Ergebnisse

4 Diskussion

5 Anhang