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Title: Purification of Tubulin with Controlled Posttranslational Modifications and Isotypes from Limited Sources by Polymerization-Depolymerization Cycles

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **N**

3. **Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **48**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Magda Maria Magiera**: This method allows the generation of pure, assembly-competent tubulin with a defined isotype composition or with post translational modifications in quantities sufficient to perform repeat in vitro experiments **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera NOTE: last one

REQUIRED:

1.2. Satish Bodakuntla ~~**Magda Maria Magiera**~~: This protocol, based on cycles of tubulin polymerization, is easy to perform in any cell biology lab and facilitates the generation of large amounts of tubulin with little hands-on time **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera NOTE: last one

Introduction of Demonstrator on Camera

~~1.3. **Magda Maria Magiera**: Demonstrating the procedure will be Satish Bodakuntla, a post-doc, and Jijumon, a Ph.D. student, from my laboratory **[1][2]**.~~

~~1.3.1. INTERVIEW: Author saying the above~~
~~1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera~~

Protocol

2. Amplification and Lysis of Cells in Suspension Cultures

2.1. To purify tubulin from suspension cultures, nine days before the tubulin prep, revive and amplify the desired cell type to obtain six confluent fifteen-centimeter dishes **[1]**.

2.1.1. WIDE: Talent adding cells to dish(es)

2.2. Add 1 liter of pre-warmed medium to every spinner bottle per dish under a cell culture cabinet **[1]** and place the spinners on a stirring table inside the cell culture incubator **[2]**. Leave the lateral spinner caps slightly open to allow the medium to equilibrate to the atmosphere of the incubator **[3]**.

2.2.1. Talent adding medium to spinner bottle, with medium container visible in frame NOTE: MED + CU
2.2.2. Talent placing spinners on stirring table in incubator
2.2.3. Lateral cap(s) being slightly opened

2.3. The next day, use the cells from the confluent dishes to inoculate the spinner bottles **[1]**. Return the spinner bottles to the incubator for one week with the lateral valves slightly open **[2]**.

2.3.1. Talent adding cell suspension to spinner bottle
2.3.2. Talent placing spinner bottles into incubator, with open lateral cap visible in frame as possible

2.4. To harvest the cells, transfer the cultures from the spinner bottles into 1-liter centrifuge bottles **[1]** and collect cells by centrifugation **[2-TXT]**.

2.4.1. Talent adding cells to bottle(s)
2.4.2. Talent placing bottle(s) in centrifuge **TEXT: 15 min, 250 x g, RT** NOTE: take 2

2.5. Resuspend each pellet in 10 milliliters of ice-cold PBS **[1]** and pool the resuspended cells in a 50-millliter tube for centrifugation at 4 degrees Celsius **[2]**. After discarding the supernatant, determine the cell pellet volume **[3]**.

2.5.1. Shot of pellet, then PBS being added to bottle, with PBS container visible in frame NOTE: take 1 : MED, take 2 : CU
2.5.2. Talent adding cells to 50-mL tube
2.5.3. Supernatant being discarded, then shot of pellet volume

2.6. Add 10 milliliters of lysis buffer to the cell pellet **[1]** and invert the tube several times to resuspend the cells **[2]**.

2.6.1. Buffer being added to the tube, with buffer container visible in frame *Videographer: Important step*
2.6.2. Talent inverting the tube to resuspend cells

3. Amplification and Lysis of Adherent Cells and Mouse Brain Tissue Lysis

3.1. To purify tubulin from adherent cell cultures, revive and amplify the desired cell type to obtain ten confluent fifteen-centimeter dishes on the day of the tubulin prep **[1]**.

3.1.1. WIDE: Talent adding cells to dish(es) NOTE: use 2.1.1

3.2. To harvest the adherent cells, treating three dishes at a time, tilt the culture dishes to remove the medium **[1]** while a second person gently washes the cells with 7 milliliters of PBS-EDTA **[2]**.

3.2.1. Talent tilting plate/discarding supernatant *Videographer: Important/difficult step*
3.2.2. Second Talent washing plate with PBS-EDTA, with PBS-EDTA container visible in frame *Videographer: Important/difficult step* NOTE: take 1 : MED, take 3 : CU

3.3. After the washing, the second person should treat the cells with 5 milliliters of PBS-EDTA per dish **[1]** before having a third person use a cell lifter to gently push the cells to one edge of the dish **[2]**.

3.3.1. Second Talent adding PBS-EDTA to dish, with PBS-EDTA container visible in frame *Videographer: Important/difficult step* NOTE: use 3.2.2
3.3.2. Third Talent shoveling cells to side of dish *Videographer: Important/difficult step* NOTE: take 2 + MED at the end

3.4. When the cells have been detached, pool the cells from all three dishes into a 50-milliter tube on ice **[1]** and rinse each plate with an additional 2 milliliters of PBS-EDTA to collect any remaining cells **[2]**.

3.4.1. Talent adding cells to a tube

3.4.2. Talent rinsing a plate, with PBS-EDTA container visible in frame

3.5. After collecting the cells by centrifugation, discard the supernatant to determine the volume of the cell pellet **[1-TXT]**. Add the lysis buffer, resuspend the cells, and transfer them into a 14-milliliter round-bottom tube **[2]**.

3.5.1. Supernatant being discarded/volume being visualized **TEXT: 10 min, 250 × *g*, 4 °C**
3.5.2. Talent adding buffer to cells and transferring them to a tube

3.6. Sonicate the cells for approximately 45 pulses **[1-TXT]**.

3.6.1. Tube being sonicated **TEXT: Confirm lysis by light microscopy** NOTE: CU at the end

3.7. For mouse brain lysis, add 500 microliters of lysis buffer to a single brain harvested from an adult mouse **[1]** and use a tissue blender to lyse the tissue **[2]**.

3.7.1. Talent adding buffer to brain, with buffer container visible in frame
3.7.2. Talent using blender on the tissue

4. Sample preparation for analysis

4.1. After obtaining the lysate, add a 1/100th of its volume to an equal volume of 2x Laemmli buffer **[1]** and boil the solution for 5 minutes **[2]**. Store it at minus 20 degrees Celsius until quantification **[3]**.

4.1.1. WIDE: Talent adding a sample aliquot to the Laemmli buffer
4.1.2. Talent placing sample on a hot plate for boiling NOTE: 2nd part
4.1.3. Talent placing sample at -20 °C TEXT: Prepare samples at each step of the protocol

5. Clarification, Polymerization, and Depolymerization of the Lysate

5.1. To clarify the lysate **[1]**, centrifuge the sample **[2-TXT]** and use a long needle to carefully transfer the clear supernatant to a new tube, taking care to avoid the upper floating layer **[3-TXT]**.

5.1.1. LAB MEDIA: Scheme for video. *Video Editor: please emphasize Lysate clarification text and arrows*
5.1.2. Talent placing a tube in a centrifuge **TEXT: 30 min, 150,000 x g, 4 °C**
5.1.3. Shot of supernatant and floating layer, then supernatant being collected *Videographer: Important step* **TEXT: Supernatant 1**

5.2. To polymerize tubulin from the clarified lysate **[1]**, mix the entire volume of supernatant 1 with a 1/200th-volume of 0.2-molar GTP (G-T-P) and half a volume of warm glycerol in an appropriately sized tube **[2-TXT]**.

5.2.1. LAB MEDIA: Scheme for video. *Video Editor: please emphasize 1st polymerization text and arrows*
5.2.2. Talent adding GTP and/or glycerol to the supernatant, with GTP and glycerol containers and empty ultracentrifuge tube visible in frame *Videographer: Important step* **TEXT: GTP: guanosine triphosphate**

~~5.3.~~ Gently pipet the mixture, take care to avoid introducing air bubbles **[1]**.

5.3.1. Talent pipetting mixture NOTE: with 5.2.2
~~5.3.2. Talent adding solution to tube~~

5.4. Seal the tubes with parafilm **[1]** and place them in a 30-degree Celsius water bath for 20 minutes **[2]**.

5.4.1. Talent covering tube with parafilm
5.4.2. Talent placing tubes in the water bath

5.5. After centrifugation **[1-TXT]**, transfer the supernatant to a new tube **[2-TXT]**.

5.5.1. Talent placing tube(s) into centrifuge **TEXT: 30 min, 150,000 x g, 30 °C** NOTE: twice same slate, check the file name
5.5.2. Talent aspirating supernatant, with new tube visible in frame **TEXT: Supernatant 2**

5.6. To depolymerize the microtubules present in the pellet 2 **[1]**, resuspend the pellet in ice-cold BRB80 (B-R-B-eighty) for 5 minutes on ice **[2]**.

5.6.1. LAB MEDIA: Scheme for video. *Video Editor: please emphasize 1st depolymerization text and arrows*
5.6.2. Talent adding BRB80 to tube, with BRB80 container visible in frame

5.7. Pipet the sample first with a P1000 pipette **[1]** then with a P200 pipette every 5 minutes for 20 minutes on ice **[2-3]**.

5.7.1. Talent pipetting sample with a P1000 pipette *Videographer: Important step*
5.7.2. Talent pipetting sample with a P200 pipette *Videographer: Important step*
5.7.3. Added shot: final result , homogeneous mix

5.8. Once the sample is homogeneous, centrifuge it **[1-TXT]** and transfer the obtained supernatant to a new tube **[2-TXT]**.

5.8.1. ~~Talent transferring supernatant to a tube~~ **TEXT: 20 min, 150,000 x g, 4 °C** NOTE: use 5.7.3

5.8.2. Shot of the pellet in focus **TEXT: Supernatant 3**

5.9. To remove microtubule associated proteins from the tubulin **[1]**, mix the entire volume of supernatant 3 with an equal volume of pre-heated 1-molar PIPE-S, the same volume of pre-heated glycerol, and a 1/100th-volume of 0.2-molar GTP **[2]**.

5.9.1. LAB MEDIA: Scheme for video. *Video Editor: please emphasize 2nd Polymerization text and arrows*

5.9.2. Talent adding PIPES to tube containing supernatant 3, with PIPES, GTP, and glycerol containers in the frame

5.10. Incubate the mix for 20 minutes at 30 degrees Celsius before centrifuging **[1-TXT]**. Transfer the microtubule-associated protein-containing supernatant 4 into a new tube **[2-TXT]**. The pellet contains polymerized microtubules **[3-TXT]**.

5.10.1. Talent placing the tubes containing mix in water bath set at 30 °C **TEXT: 30 min, 150,000 x g 30 °C**

5.10.2. Talent transferring supernatant to tube **TEXT: Supernatant 4** NOTE: take 2

5.10.3. Shot of the pellet **TEXT: Pellet 4**

5.11. After performing a third polymerization and depolymerization **[1]**, the amount of harvested tubulin can be quantified by SDS-PAGE (S-D-S-page) analysis **[2-TXT]**.

5.11.1. Video editor will focus on the 3rd polymerization and depolymerization steps on the scheme

5.11.2. LAB MEDIA: Figure 2A **TEXT: See text for full sample clarification and purification details**

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
2.6., 3.2., 3.3., 5.1., 5.2., 5.7.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
3.2., 3.3. Harvesting cells growing as adherent cultures. We work in a trio, each person performing one step of the harvesting: wash – detach – collect.
5.1. removing the supernatant during cell lysate clarification. We remove the clear supernatant with a long needle, thus avoiding to contaminate it with the material floating above.

Results

6. Evaluation, Quantification, and Characterization of Purified Tubulin

6.1. The success of the purification process can be confirmed on a Coomassie-stained SDS-PAGE **[1-TXT]**.

6.1.1. LAB MEDIA: Figure 2A *Video Editor: please emphasize bands in P4 lane* **TEXT: A Coomassie-stained SDS-PAGE**

6.2. A lower-than-expected yield of the purified tubulin can be due an inefficient tubulin polymerization **[1]**, as evidenced by a lower amount of tubulin in the second, fourth, and sixth pellet fractions **[2]** and higher amount in the second, fourth, and sixth supernatant fractions **[3]**.

6.2.1. LAB MEDIA: Figure 2B
6.2.2. LAB MEDIA: Figure 2B *Video Editor: please emphasize bands in lanes P2, and P4*
6.2.3. LAB MEDIA: Figure 2B *Video Editor: please emphasize bands in lanes SN2, and SN4*

6.3. To quantify the purified tubulin **[1]**, the samples can be run next to known quantities of bovine serum albumin **[2]**. Quantitative densitometry of the protein bands then allows to calculate the quantity of purified tubulin **[3]**.

6.3.1. LAB MEDIA: Figure 3A *Video Editor: please emphasize Tubulin lanes*
6.3.2. LAB MEDIA: Figure 3A *Video Editor: please emphasize BSA lanes*
6.3.3. LAB MEDIA: Figure 3B *Video Editor: please emphasize correlation line*

6.4. The modification status of the tubulin can be confirmed by immunoblot analysis of the samples **[1]**.

6.4.1. LAB MEDIA: Figure 4 *Video Editor: please emphasize glutamylation bands in Ttll5, Ttll7, and ttll1-/- lanes*

Conclusion

7. Conclusion Interview Statements

7.1. **Jijumon**: It is essential to avoid contaminating the supernatant with the floating material layer after cell lysate clarification, as this material will negatively affect the tubulin purification **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (4.1.) NOTE: last one

7.2. **Carsten Janke**: Our protocol facilitates the isolation of tubulin with controlled modifications, allowing the impact of tubulin modifications on microtubule properties and their molecular interactions by in vitro reconstitution experiments to be directly addressed **[1]**.

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera NOTE: last one