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Anthony Hyman's Lecture Part 2

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1. Keywords and Terms

polymers, microtubules, microtubule dynamics, *Xenopus* cytoplasm extracts, total internal reflection microscopy (TIRF), catalyst, polymerase, secondary screen

2. Lecture Notes

Lecture 1 described a genome-wide RNAi screen for genes involved in cell division, and this lecture discusses a secondary screen and detailed follow-up on genes important for microtubule growth. Students will learn about the organization and dynamics of microtubules and about the different techniques and systems with which they are studied.

3. Review Questions

1. Microtubules have a "plus" end and a "minus" end. What gives them this polarity?

2. How do microtubules grow? Mark ALL correct answers.

    a. Tubulin dimers are added to the plus end of the microtubule
    b. Tubulin dimers are added to the minus end of the microtubule
    c. Tubulin dimers are added to the middle of the microtubule
    d. Tubulin monomers are added to the plus and minus ends of the microtubule

3. What properties of microtubule growth differ between microtubules in vivo (in a cell) and in vitro (in a test tube)? Mark ALL correct answers.

    a. Microtubules grow faster in a cell.
    b. Microtubules grow faster in a test tube.
    c. Microtubules exhibit more dynamic instability (growing/shrinking cycles) in a cell.
    d. Microtubules exhibit more dynamic instability (growing/shrinking cycles) in a test tube.

4. What is Xenopus cytoplasmic extract?

5. What happens to microtubule growth when XMAP215 is removed from cytoplasmic extract? How does this relate to what was seen in *C. elegans*?

6. Stabilized microtubule seeds are created using a non-hydrolysable analogue of GTP. This means that these microtubules (mark ALL correct answers):

    a. have no dynamic instability
    b. can no longer grow
    c. can no longer shrink
    d. grow at the same rate as microtubules with GTP
    e. grow slower than microtubules with GTP

7. Where does XMAP215 localize on microtubules?

8. True or false? Total Internal Reflection Microscopy (TIRF) allows you to look at single molecules of GFP that are very close to the surface of the coverslip.

9. What is the function of the TOG domains of XMAP215?

10. XMAP215 is:

    a. A polymerase
    b. An enzyme that stabilizes an intermediate
    c. A catalyst

d. All of the above

4. Answers to Review Questions

1. Tubulin dimers are heterodimers made up of two different subunits: alpha and beta. These dimers assemble head-to-tail in long rows to form protofilaments, such that one tip of a protofilament ends in an alpha subunit, while the opposite tip ends in a beta subunit. The protofilaments associate side-to-side to form a tube, the microtubule. The beta subunit marks the plus end of the microtubule, and the alpha subunit marks the minus end.
2. (a) and (b) are correct
3. (a) and (c) are correct
4. *Xenopus* cytoplasmic extract is the cytoplasm and cytoplasmic components from frog eggs, in the absence of cell membranes.
5. There is hardly any microtubule growth when XMAP215 is removed from *Xenopus* cytoplasmic extract. When Zyg-9, a member of the XMAP family, is removed from *C. elegans* embryos using RNAi, microtubule growth is also impaired.
6. (a), (c) and (d) are correct
7. XMAP localizes to the ends of microtubules
8. True
9. TOG domains bind tubulin.
10. (d) All of the above

5. Discussion Questions

1. Why is a microtubule a polymer? What does it mean to be a polymer?
2. Genes required for microtubule growth were found using a secondary genetic screen. Describe what this means, in general, and with regards to the screens discussed in the lecture.
3. Antibodies are very useful tools in molecular biology. In Lecture 1, antibodies were used in the immunoprecipitation technique. How were antibodies used in this lecture

when studying XMAP215 in *Xenopus* extract? Compare and contrast this technique with immunoprecipitation.

4. Why do MTs shrink when XMAP215 is added in the absence of tubulin?

6. Answers to Discussion Questions

1. A polymer is formed when many monomers or small subunits are linked together to create a larger molecule. Microtubules are made up of many, repeating tubulin heterodimer subunits.

2. A secondary genetic screen refines the phenotypes of genes found in a broader, primary genetic screen. Lecture 1 described a genome-wide RNAi screen for genes important for cell division, which uncovered 800 genes. Because microtubule growth is necessary for cell division, any gene that controls microtubule growth should be among the 800 genes found using the RNAi screen. To find genes necessary for microtubule growth, another genome-wide screen is not necessary. Rather, a secondary screen can be performed on the 800 genes (or an even further refined subset) to look specifically for phenotypic defects in microtubule growth.

3. An antibody that binds specifically to XMAP215 was used to deplete it from Xenopus extract in order to determine what happens to the growth rate of microtubules in the absence of XMAP215. In immunoprecipitation, an antibody that binds specifically to one protein can be used to pull it out of a solution in order to study it further and determine with what other proteins it forms a complex. In both techniques, an antibody is used to isolate a specific protein from a mix of proteins or cytoplasmic components. However, the purpose of antibody depletion is similar to

   RNAi: to infer the function of a protein by taking it away and observing the consequences.

4. XMAP 215 acts as a catalyst by stabilizing an intermediate in the reaction that turns tubulin into microtubules, and vice versa. The reaction could be drawn like this: tubulin ßà intermediate state ßà microtubules. XMAP215 helps the reaction proceed faster, in both directions, by making it easier to reach the intermediate state. When tubulin is abundant, it drives the reaction forward towards microtubule growth. The removal of tubulin, the substrate of the reaction, drives the reaction in the opposite direction, towards microtubule shrinkage. Since XMAP215 helps the reaction

proceed faster in both directions, adding XMAP215 to microtubules in the absence of tubulin substrate will thus drive the reaction backwards.

7. Explain or Teach These Concepts to a Friend

1. Explain how microtubules grow and shrink. What are the building blocks of microtubules; where and how are they added and removed? What is the role of GTP in this process?
2. Explain why microtubules are important for cell division. Why is it important for microtubules to grow and shrink during this process? (This will require some background reading beyond the lecture.)

8. Research the Literature on Your Own

1. What is a microtubule-destabilizing factor and how does it work?
2. Research another discovery that has been made using *Xenopus* cytoplasmic extracts.
3. XMAP215 is a microtubule polymerase. What is a polymerase? What are some other important polymerases in the cell and what do they do?

9. Papers for Journal Club

1. Tournebize R, Popov A, Kinoshita K, Ashford AJ, Rybina S, Pozniakovsky A, Mayer TU, Walczak CE, Karsenti E, Hyman AA. Control of microtubule dynamics by the antagonistic activities of XMAP215 and XKCM1 in Xenopus egg extracts. Nat Cell Biol. 2000 Jan;2(1):13-9. [PMID: 10620801]

   This paper provides more detail on the investigation of the function of XMAP215 using *Xenopus* egg extracts. Students will also learn about the microtubule destabilizing factor XKCM1, which is not described in the lecture.

2. Srayko M, Kaya A, Stamford J, Hyman AA. Identification and characterization of factors required for microtubule growth and nucleation in the early *C. elegans* embryo. Dev Cell. 2005 Aug;9(2):223-36. [PMID: 16054029]

This paper provides more detail on the secondary screen, searching for genes required for microtubule dynamics, described in the lecture

3. Joe Howard & Anthony A. Hyman. Dynamics and mechanics of the microtubule plus end. Nature. 2003 Apr 17;422(6933):753-8. Review. [PMID:12700769]

   This review describes how the dynamics and properties of microtubules translate into the ability to move cellular structures around inside cells.