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Speckle Microscopy and Microtubules

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Fluorescent Speckle Microscopy is a type of microscopy that uses polymer subunits drawn from a random pool to create a "speckle" appearance in microtubules. Researchers now know that each polymer is composed of a- and b- tubulin heterodimers and how many of the past techniques do not compare to the quality of the fluorescent speckle microscopy procedures. There are many factors that go into the growth of the FSM procedures and the advantages and disadvantages of the fluorescent color quality. Advancements with the cameras used in this procedure and the length the fluorescence lasts is a major factor in the advancement of the procedure and how microtubules can be viewed with the "speckle" colors. Stochastic properties play a large factor into how the tubulin dimers ends generate the fluorescent speckles. With this said, there are many advances that have been made since 1997 with the fluorescent speckle microscopy viewing techniques, but there are also many obstacles that need to be resolved to grow with the microscopy.

Introduction

Cytokinesis is the division of the cytoplasm of a cell after the division of the nucleus during cell division. Before cytokinesis, mitosis occurs which is a multi-step process that produces two identical daughter cells during its phases (prophase, premetaphase, metaphase, anaphase, and telophase). During the last phase of mitosis (telophase), the chromatids arrive at opposite poles of the cell and new membranes form around the nuclei. Completing the mitotic cycle is the process of cytokinesis, which results when a fiber ring composed of actin around the middle of the cell pinches the cell into two daughter cells (each with their own nucleus). The spindle fibers that pull the centromeres to opposite ends are called microtubules. These microtubules are stiff, long, and have a cylindrical structure and are composed of tubulin. Generally, these regulate the shape and movement of a cell. The movements and dynamic assembly of microtubules can be measured using fluorescent speckle microscopy. When fluorescent speckle microscopy was first discovered, it depended on new advances in cameras with cooled charge-couples device detectors and their application to fluorescence microscopy in cell biology in the early mid-1990s (Salmon and Waterman 3940). Today with various researches, it is now known that the speckled pattern in microtubules was a result of unequal distribution of fluorescently labeled tubulin subunits. Fluorescent Speckle Microscopy is now showing that there are various different assemblies that can be detected (besides the ones that are already discovered), but it now possesses the issue of viewing all of the different assembles using the current cameras that are available.

Recent Progress

The various recent discoveries using fluorescent speckle microscopy of microtubules and many of the uses of fluorescent microscopy have been used to further the study of microtubules. Recently, the biological studies world has learned that fluorescent speckle microscopy has been mostly used to study the behavior of actin and tubulin polymers. Speckle Microscopy has helped researchers to discover that each microtubule is a polymer composed of a- and b-tubulin heterodimers organized into thirteen protofilaments that form a hollow cylindrical structure (Barisic 150). With this idea now known, there are many advantages of using FSM such as not having to use photoactivation and photobleaching techniques (which have been used to measure the dynamics of macromolecular structures). With these types of microscopy, the monitoring of the subunits (which are added to the ends of the microtubules from the dimer pool which is randomly determined) are limited to bleached or photoactivated regions. The speckle microscopy method allows the fluorescence to retain for longer periods of

time, giving researchers more time to determine how this speckled pattern occurs. Recently according to Salmon and Waterman-Storer, normal stochastic association of tubulin dimers with growing microtubule ends generates the fluorescent speckles. Apparently, each time a dimer is added to one of the thirteen filaments of the growing ends, the probability that it will be an X-rhodamine-labeled dimer depends on the fraction of labeled dimers in the tubulin pool (Salmon and Waterman-Storer 2059).

There are many ways to image and analyze fluorescent speckles but through years of recent research, there have been many issues that have brought FSM to a halt growth wise. A technical challenge in recent studies is the capacity to perform high-resolution imaging of diffraction-limited regions. Even with the improved technique using spinning-disk confocal microscopes, the microscopy would require a very sensitive imaging system to contrast the speckles more effectively. A second research by Barisic debates that the development of image analysis tools is a great challenge because researchers need them to retrieve maximal quantitative information for the same experimental settings. The microtubule flux can be measured using different tools such as kymograph analysis (a device that graphically records changes in position over time, and is most commonly used to record changes in pressure or motion). The last technical challenge that Barisic discusses is how to reach low expression of fluorescent proteins for fluorescent speckle microscopy. Barisic explains, "In order to reach the goal of low expression (essential for optimal contrast between speckles and neighboring regions), proteins either have to be injected or expressed at very low concentrations (Barisic 151)." Recent advances within Fluorescent Speckle Microscopy include using different bacterium to investigate the techniques such as bacterium with a low number of chromosomes (for example, drosophilia S2). Along with this discovery, researchers can use Drosophilia S2 to investigate spindle dynamics and perform the techniques in various labs, along with distinguishing between kinetochore and nonkinetochore microtubules.

Discussion

There are many questions that still remain unanswered even with these recent results using speckle microscopy. For example, how do the free "minus" ends stabilize during the fluorescent speckle microscopy procedures and assuming that the assembling of microtubules is broken by some type of bond during cytokinesis including hydrogen or electrostatic bonds, how does this breaking affect the speckle microscopy procedure? These questions can be answered with an advance in CCD cameras (need to be more sensitive and have a smaller pixel size which would enhance the detection of very dim signals at higher resolution). Another new development that would change the face of Fluorescent Speckle Microscopy is if something were created that would increase contrast and temporal resolution (by introducing a variable that would promote clustering of fluorescent molecules along microtubule stubs) (Barisic 159). Another advance that would need to be put into place would be software for more precise and simpler analysis so that the evaluation of data could increase. FSM is currently based on nonuniformed distribution of fluorescently labeled subunits, which need to be uniformed because it would show exact assemblies, or at least get closer to the goal. FSM has come a long ways since 1997, but could use many advances to continue to grow the researches.

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