

Bik1, the Yeast Ortholog of the Human Microtubule Binding Protein CLIP-170
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Microtubules are long, tubular protein complexes that give structure to the cell. They form a sub-cellular organization called the cytoskeleton which plays a major role both in cell-division and in transporting molecules throughout the cell. Microtubules are able to grow and shrink through a process called dynamic instability [1]. It is written in the literature that a class of molecules called microtubule associated proteins is responsible for regulating microtubule growth and shrinkage rates by affecting the dynamic instability [2].

Bik1 is a yeast microtubule associated protein and is related to, is a homolog of, the mammalian protein CLIP-170. Both of these proteins preferentially associate with the growing end of microtubules, and as such they fall into the class of molecules called plus-end tracking proteins [2]. While the end result is the same in both cases, that Bik1 and CLIP-170 track the growth of the microtubule plus-end, the literature says that the mechanisms by which each of these proteins operate are different [3]. It was the goal of this project to gather data to either verify this hypothesis or to contradict it. This information would then be useful in other areas of research, especially within the lab, such as in computational models of how microtubule associated proteins affect dynamic instability. Additionally, the data collected would help deepen and broaden the general understanding of the mechanisms involved in plus-end tracking by microtubule associated proteins.

In order to accomplish the goal of testing the hypothesis that Bik1 and CLIP-170 track the plus-end by different mechanisms I ran a set of experiments to quantitatively determine the strength of the binding reaction between Bik1 and microtubules. These experiments, called cosedimentation assays, had already been done for CLIP-170, and they showed that a direct interaction does exist between that protein and microtubules, and that the bind between them is relatively strong. Thus, if Bik1 behaved similarly in these experiments it would suggest that it may have a similar mechanism, and if not, then it most likely would have a different *modus operandi* altogether.

In the experiment I combined a set amount, expressed as a micromolar concentration, of Bik1 with increasing amounts of microtubules to see whether binding would occur. If Bik1 were to bind the microtubules it would become a part of a heavy complex, and thus it would sediment into the pellet, rather than remaining in the supernatant, after centrifugation of the mixture. This sedimentation could then be quantified by looking at a protein separation gel run of the experiment. Protein gels are made by adding the experimental solution to a polyacrylamide gel electrophoresis (PAGE) setup and sending electrical energy across the gel, causing the charged proteins to move at rates dependent upon their size. In this way the constituent proteins can be separated and identified. By running two sets of gels, one for the supernatant and one for the pellet, the location of the various proteins after centrifugation can also be determined, and thus it is possible to judge whether one has bound to another, for in this case they would both be in the pellet due to the greater size of the complex. It is important to note that of the three proteins discussed here – microtubules, Bik1, and later Bim1 – only microtubules sediment into the pellet alone, whereas the other two remain in the

supernatant under normal conditions. Thus, any non-microtubule bands would show that a complex has formed. Upon interpreting the results of my Bik1-microtubule cosedimentation assays I determined that Bik1, unlike its mammalian relative, has little or no ability to bind directly to microtubules under the conditions of the experiment (Fig. 1).

There were various reasons why Bik1 might not bind microtubules, the two main possibilities were that either the protein could have “turned itself off” when one end of it bound to its other end and not to the microtubule, or that there really is no direct interaction between Bik1 and microtubules. To determine which possibility was truly the case I decided to substitute shortened fragments of Bik1 for the full-length protein. In these fragments the area which has been determined to be the site of interaction between the protein and microtubules was conserved, while the opposite end which may have interfered with the reaction was cut off. I ran the same cosedimentation experiments, this time with two lengths of Bik1 fragments, and I obtained the same data. This suggested strongly that there really is no direct interaction between Bik1 and microtubules (Fig. 2).

As was stated earlier, Bik1 and CLIP-170 fall into a class of proteins which track the plus-end of microtubules. There is a good deal of literature describing the interactions between CLIP-170, microtubules, and another mammalian protein called EB1 [4, 5]. EB1 is also a plus-end tracker, but its affinity for, or strength of direct interaction with, microtubules is much weaker than that of its counterpart CLIP-170. Furthermore, research has shown that the presence of CLIP-170 in the reaction causes EB1 to bind to the microtubule to a much greater extent.

Like Bik1 and CLIP-170, there is a protein in yeast called Bim1 which is the homolog of EB1. Since Bim1 gets its name from the fact that it has been shown that it binds to microtubules, I decided to see whether I could get this to occur using the same type of experiment as I did with Bik1 and its fragments. It ended up working out that I was able to see a direct binding interaction between Bim1 and microtubules through a cosedimentation assay, and I determined its strength of interaction to be comparable to that of CLIP-170 and microtubules (Fig. 3).

Given the research that shows that the human CLIP-170 is needed for EB1 to bind to microtubules I decided to see whether Bik1 might need Bim1 in order to bind. To test this I performed a cosedimentation assay, but I kept the amounts of microtubules and Bim1 constant, and added increasing amounts of Bik1 to the different reactions. Due to difficulties with the apparatus and procedure involved I was unable to get quantifiable results on whether Bim1 influences the binding of Bik1 to microtubules, but I was able to see qualitatively that Bim1 has an increased affinity for microtubules in the presence of Bik1, and that the same may be true for Bik1 (Fig. 4).

This research allows me to conclude that Bik1 and Bim1 have different behaviors and mechanisms of plus-end tracking compared to their respective homologs CLIP-170 and EB1. Furthermore, the two yeast proteins appear to have reversed their roles in mammals. Whereas Bim1 seems necessary for Bik1 to bind to microtubules, CLIP-170 is necessary for strong binding in EB1.

The addition of these data into a computational model of microtubule dynamic instability in the presence of various microtubule associated proteins should make for a robust model. This research shows that numerous factors must be involved in determining what is required for a protein to bind to the microtubule. It is not just

sequence homology, or how closely two proteins are related, that determines this, for if it were then Bik1 would necessarily behave more like CLIP-170 than EB1.

The future of the project can take a number of different paths. While doing these experiments I designed a version of Bik1 that is “tagged” with a protein that glows green under the microscope so that it can be inserted into a cell and followed with a camera. This would be of use because Bik1 is a yeast protein, and this tagged version was made for use in mammalian cells. One of the other possible reasons why Bik1 was unable to bind to the microtubules in my experiments was that they were purified from a cow, and there are some differences between mammalian and yeast microtubules. This tagged Bik1 should show whether Bik1 can bind mammalian microtubules *in vivo*, something it could not do *in vitro*. Another important set of experiments would extend those I did with Bim1 and Bik1 together. More cosedimentation assays would be run to get a truly quantitative measure of how each protein affects the interaction of its counterpart with microtubules to determine whether there is a real cooperation between them or not.

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