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DISCUSSION

Session Chairperson: Margaret A. Titus
Scribe: Alexander L. Friedman

JOHNSON: We have other direct evidence for showing that kind of processivity that we're not quite ready to share. But the fundamental question is the underlying basis for that processivity. And I've always felt that this observation of one head binding and the other hanging off in solution is contrary to what you might expect for a processivity where you would require if anything you should have two heads attached and rarely only one attached. It seems like a bit of a contortion to get it to fit into a model of hand-over-hand movement. The other question is, you've done some experiments on the mant-ADP measurements of release, and the question is whether you've extended those measurements into a time resolution sufficient to be able to ask whether you're getting half of the heads coming off after the ADP is coming off fast and half coming off slow.

HACKNEY: The short answer to that is "no we have not" tried to do those experiments yet, but we plan to.

JOHNSON: The other question is, it seems that the processivity in fact is very ionic strength-dependent as a function of the rebinding rate. Some of the differences that we see, I think, are a function of the differences in the rate of kinesin binding as a function of ionic strength. In particular I want to remind you, in the early studies using ATP-induced association as an affinity purification tool, that's always with ATP and salt being added. That shifts the equilibrium toward dissociation.

HACKNEY: That's also the fact that it folds up, and we think that's part of why it doesn't work.

JOHNSON: Yeah.

HACKNEY: If you stop and think about it, that's the wrong way for a motor to work. It shouldn't fall off when you add ATP. It falls off because it's in this folded form, which is designed not to work. So in fact if there were really active motors lurking around you wouldn't find them that way because they may stay bound after you add the ATP.

JOHNSON: Yeah. The final question is, it seems that processivity is apparent at lower salt concentrations and the real question is, to what extent processivity of a small molecule bound to a microtubule by itself continues into the physiological ionic strength conditions of 150 mM salt.

HACKNEY: I think it's going to be very much less. These experiments are done roughly at, I believe, 48 mM as the ionic strength. So it's not physiological, but it's not one of the really low ionic strength buffers. And if I can comment on his first comment, I would say in fact that I find these types of models to be just what one would like. I think they're nice because they solve two thing[s] a motor has to do. If the heads are both bound and one has to move, it is in many cases going to be trying to pull the attached head. That represents a load on the head that's going to move. The other thing is there's likely to be some sort of refractory phase after a head is released before it can bind back to the microtubule. And this model leaves this head there long enough for it to go through this refractory phase and to be primed and ready to add back when it's allowed to add back by the ATP binding to the attached head.

JOHNSON: I hope we're not going to resurrect the Eisenberg-Taylor controversy on refractory states.

WONG: I have a question about your calculation of the maximum collision rate between a microtubule and kinesin in your abstract. If I understood this correctly, the equation you used was based on assuming the microtubule was a perfect absorber for kinesin.

HACKNEY: Yes.

WONG: OK. If that's true, that means that every collision that kinesin makes with a microtubule would cause the disappearance of kinesin. That would create a depletion zone next to the microtubule, and that would definitely make your calculation of the maximum collision rate lower than what really happens if it were a diffusion-limited reaction.

HACKNEY: There will be a zone where there will be a lower concentration but, presumably, it comes off at some rate too. The calculation was just to give the absolute maximal rate that it might bind. The real rate is thought to be lower than that. Also, these rates are highly dependent on the salt concentration.

WONG: Definitely. I did a rough calculation assuming it's not a perfect absorber, just to calculate the collision rate. Of course, it's very rough. I think it's right on the order of magnitude. The number comes out 600 times higher than what you estimated.

HACKNEY: I would have to look at those calculations before I could comment on them. One thing you have to realize: the rate that the head binds to this large microtubule is going

to be very, very high. But there are many of tubulin subunits per microtubule, and you have to divide by the number of subunits. That's what brings the net rate down. That rate is on a per subunit rate, which is what you actually measure experimentally.

WONG: As I discussed with George Oster earlier, it's probably possible to model the whole thing with a 3D to 1D diffusion kinetic problem and figure how much is the residual time on the microtubule and find out how many ATPs are hydrolyzed per step with a more thorough boundary condition problem.

HACKNEY: That could well be possible to model that. I haven't tried it.