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The Formation of Basal Body: Which Comes First Egg or Chicken?

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Basal bodies are the microtubule organizing center for flagellar axonemal microtubules and, as a centriole, are an essential component of the centrosome of an animal cell. Even though the mechanism that regulates basal body (or centriole) assembly is not understood accumulated studies showed two different (templated or *de novo*) pathways for the formation a new basal body (or centriole). In cells that have basal body (or centriole), the new ones are always formed adjacent to the preexisting ones. Based on these observations, it has been suggested that the preexisting organelle plays essential roles for the formation of new ones, the templated pathway. Since most cells have the preexisting organelles, the *de novo* formation of basal body (or centriole) has been regarded as a unique event that occurred in a limited numbers of cell types including *N. gruberi*. However, recent reports that showed the *de novo*formation of centrosomes in CHO cells following their destruction and *de novo* formation of basal bodies in *Chlamydomonas* suggest that many cells are capable of forming this microtubule organizing center *de novo* if necessary. To explore the mechanism of basal body formation, we have been using the differentiation of *N. gruberi* amoebae into swimming flagellates as a model system. *N. gruberi* amoebae have no microtubules except for the intranuclear mitotic spindles that form briefly during cell division. Basal bodies are formed *de novo* about 55 min after the initiation of differentiation, and then the flagella are formed. At 70 min after initiation, cells are round and 50% of the cells have visible flagella. As the flagella elongate, cytoskeletal microtubules begin to emanate from the base of the flagella (80 min) and the cells begin to assume an elongated shape. At the end of differentiation (120 min after initiation), more than 95% of cells have two flagella and cytoskeletal microtubules that run, in parallel, beneath the cell membrane from the base of the flagella toward the opposite end of the cell. The *de novo*formation of basal bodies in *N. gruebri* accompanied with the transient concentration of γ-tubulin and pericentrin (hereafter refer as γ-tubulin concentration) during the *de novo* formation of basal bodies in *Naegleria*. The concentration of γ-tubulin was observed in 10 ~ 20% of cells at the beginning of the differentiation. The percentage of cells with a concentrated region of γ-tubulin was maximal (~ 70%) at 40 min when α - and β-tubulin synthesis for flagella and cytoskeletal microtubules had just commenced, but no assembled microtubules were visible. At 60 min, newly synthesized α-tubulin was concentrated at the γ -tubulin spot from which flagella began to elongate. Once cells contained elongated flagella (80 min), concentrated γ-tubulin moved to the opposite end of the cell without any attached microtubules and disappeared. The transient concentration of γ-tubulin at the site of assembly of the basal bodies in *N. gruberi* was accompanied by reorganization of actin filaments and occurred without the formation of microtubules. We noticed that the initial events that occur during *de novo* basal body formation in *N. gruberi* are very similar to the events during *de novo* formation of centrosomes in CHO cells. First, γ -tubulin and pericentrin are concentrated at a discrete "spot" before the formation of centrioles (or basal bodies) in both systems. Second, the concentration of γ-tubulin is accomplished without the formation of microtubules and third, centrioles (or basal bodies) are formed at the site of the γ-tubulin concentration. These similarities suggested to us that the formation of a protein complex containing γ-tubulin and pericentrin might be a common event in the *de novo* formation of centrioles and basal bodies. Based on these ideas, we developed biochemical procedures for the purification of γ-tubulin containing complexes from *N. gruberi* and examined the possible role of these complexes in the formation of basal bodies. In this seminar, we present evidence that: 1) a protein complex containing γ-tubulin, pericentrin, and myosin II (GPM-complex) is transiently formed during the differentiation; 2) *in vitro* nucleation of MT by this complex is dependent on γ-tubulin; 3) the MT-nucleating activity of GPM-complexes is tightly regulated during basal body formation by phosphorylation; 4) inhibition of the regulation resulted in the formation of multiple flagella; and 5) γ-tubulin is one of the targets for the phosphorylation.