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Identification of intrinsic dimer and overexpressed monomeric forms of γ-tubulin in Sf9 cells infected with baculovirus containing the *Chlamydomonas* γ-tubulin sequence

Alexei Vassilev[1], Mary Kimble[1], Carolyn D. Silflow[2,3], Matthew LaVoie[2] and Ryoko Kuriyama[1,*]

[1]Department of Cell Biology and Neuroanatomy, University of Minnesota, 4-135 Jackson Hall, 321 Church St. SE, Minneapolis, Minnesota 55455, USA
[2]Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota, USA
[3]Department of Plant Biology, University of Minnesota, St. Paul, Minnesota, USA

*Author for correspondence

SUMMARY

A new member of the tubulin superfamily, γ-tubulin, is localized at microtubule-organizing centers (MTOCs) in a variety of organisms. *Chlamydomonas* cDNA coding for the full-length sequence of γ-tubulin was expressed in insect ovarian Sf9 cells using the baculovirus expression system. Approximately half of the induced 52 kDa γ-tubulin was recovered in the supernatant after centrifugation of Sf9 cell lysates at 18,000 *g* for 15 minutes. When the cell supernatant was analyzed by FPLC on a Superdex 200 sizing column, *Chlamydomonas* γ-tubulin separated into two major peaks. The lagging peak contained a monomeric form of γ-tubulin with a sedimentation coefficient of 2.5 S, which interacted with the Superdex column in a salt-dependent manner. The leading peak, with an apparent molecular mass of 900 kDa, corresponded to a molecular chaperonin complex, and TCP1 chaperonin released folded γ-tubulin polypeptide from the complex in the presence of MgATP. The released γ-tubulin monomers were capable of binding to microtubules in vitro and biochemical quantities of active monomers were further purified using a combination of size-exclusion and ion-exchange column chromatography. The endogenous Sf9 cell γ-tubulin migrated faster than *Chlamydomonas* γ-tubulin with an apparent molecular mass of 49 kDa on gels. Analyses on gel filtration and sucrose density gradient centrifugation showed that, while overexpressed *Chlamydomonas* γ-tubulin was present in a monomeric form, endogenous γ-tubulin from Sf9 and HeLa cells exists as a dimer. These results may suggest the possibility that γ-tubulin could form a heterodimer with hitherto unknown molecule(s).

Key words: γ-tubulin, baculovirus, Sf9 cell, centrosome, microtubule

INTRODUCTION

Interphase and mitotic microtubules in animal cells originate from microtubule-organizing centers (MTOCs), or centrosomes which are composed of a pair of centrioles plus a surrounding, amorphous cloud of pericentriolar material. The centrosome is involved in determination of the temporal and spatial distribution of microtubule arrays (Porter, 1966). It is therefore essential to identify and characterize molecular components of the centrosome in order to understand the mechanisms of microtubule regulation and function.

Recent progress in immunological approaches has made it possible to identify a number of autoimmune, polyclonal, and monoclonal antibodies specific to hitherto unknown molecular components included in the centrosome (for a review, Kuriyama, 1992). Combined with powerful molecular cloning technologies, more detailed analysis of some of these molecules has been achieved (Joswig et al., 1991; Balczon et al., 1994; Doxsey et al., 1994). Sequence analysis of cloned DNAs has also revealed the presence of several different kinds of kinesin-like motors in the centrosome (Nislow et al., 1992; Dragas-Granoic et al., 1993). Despite a great deal of effort aimed at understanding the role of centrosomal components in the structure and function of the centrosome, the results obtained so far are primarily descriptive in nature.

In 1986, Oakley and his colleagues isolated an extragenic suppressor of a mutation in the β-tubulin gene of the fungus, *Aspergillus nidulans* (Weil et al., 1986). Sequence analysis of the gene showed that, although it was not α- or β-tubulin, the encoded 51 kDa protein product was nearly as similar to the α- and β-tubulins as they are to each other. This protein was proposed to be a new member of the tubulin superfamily called γ-tubulin (Oakley and Oakley, 1989). Immunofluorescence staining of cells with anti-γ-tubulin antibodies showed that the γ-tubulin molecule is a specific constituent of the spindle pole body, a MTOC in *Aspergillus* (Oakley et al., 1990). It was later found that γ-tubulin is a ubiquitous component of MTOCs in a wide range of organisms from fungi to plants to humans