Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1764 (2006) 920–928
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Characterization of the microtubule proteome during post-diapause development of *Artemia franciscana*

Paul A. O'Connell [a], Devanand M. Pinto [b], Ken A. Chisholm [b], Thomas H. MacRae [a,*]

[a] *Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1*
[b] *Institute for Marine Biosciences, National Research Council of Canada, Halifax, Nova Scotia, Canada B3H 3Z1*

Received 10 December 2005; received in revised form 23 February 2006; accepted 13 March 2006
Available online 5 April 2006

Abstract

The microtubule proteome encompasses tubulin and a diverse group of proteins which associate with tubulin upon microtubule formation. These proteins either determine microtubule organization and function or their activity is influenced by microtubule association. To characterize the microtubule proteome in *Artemia franciscana*, tubulin assembly was induced with taxol in vitro after 0 and 12 h of post-diapause development. Proteins obtained by extraction of microtubules with 0.5 M NaCl were electrophoresed in two-dimensional gels and analyzed by mass spectrometry. Fifty-five proteins were identified with 10 of these occurring at both developmental stages, and multiple isoforms were observed for some proteins of the *Artemia* proteome. Their functions include roles in membrane transport, metabolism, chaperoning and protein synthesis, thus reflecting physiological properties of encysted *Artemia* such as stress resistance and the ability to rapidly initiate post-diapause development. For example, chaperones may protect tubulin during encystment and facilitate folding in metabolically active embryos. Additionally, the interaction of metabolic enzymes with microtubules funnels reaction intermediates, potentially enhancing efficiency within biochemical processes. This study represents the first systematic characterization of a crustacean microtubule proteome. Although it is difficult to be certain that all protein associations documented herein occur in vivo, the results suggest how protein–protein interactions contribute to cytoplasmic organization while implying how *Artemia* embryos resist stress and remain capable of development once diapause terminates.

Keywords: Microtubule proteome; Two-dimensional gel electrophoresis; Mass spectrometry; Development; Post-diapause cyst; *Artemia franciscana*

1. Introduction

Microtubules are dynamic polymers that influence eukaryotic cell shape and polarity, mediate chromosome movement and transport membrane bound organelles. The main structural protein of microtubules is tubulin, a heterodimer composed of α- and β-tubulin, each usually encoded by a family of genes [1–3]. Other tubulin isotypes include γ, δ, ε, ζ and η, but with exception of γ-tubulin which localizes at the MTOC and nucleates microtubules [4], distribution is restricted primarily to centrioles and basal bodies [5]. Microtubules are polar with polymerization and depolymerization occurring predominantly at the plus end by dynamic instability [6,7]. Disassembly of microtubules may exert sufficient force to power movement of intracellular components [8].

Microtubule function is mediated by a diverse group of proteins traditionally called microtubule-associated proteins (MAPs) [3]. These proteins co-purify with tubulin during cycles of assembly and disassembly and they have been divided into two groups termed structural and dynamic. This division has become less distinct with the discovery of proteins that share characteristics of both classes and those that fall into neither [9,10], leading to the more general concept of the microtubule proteome [11–13]. Members of the microtubule proteome such as MAP1A and B, MAP2, MAP4 and tau, the classical structural MAPs, modulate dynamic instability and influence polymer organization by promoting tubulin polymerization and microtubule stabilization [3,14,15]. In contrast, the kinesins and dyneins are dynamic MAPs known as molecular motors; they promote microtubule-dependent movement during spindle elongation, chromosome segregation, and trafficking of membranous organelles [16–19]. The kinesin variant Kim1 mediates ATP-dependent microtubule severing and reorganization,

* Corresponding author. Tel.: +1 902 494 6525; fax: +1 902 494 3736.
E-mail address: tmacrae@dal.ca (T.H. MacRae).

1570-9639/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbapap.2006.03.003

functions shared by spastin, katanin and stathmin (Op18) [3,10,20]. For proteins of the microtubule proteome that do not mediate cytoskeleton organization and function, microtubules serve as anchoring platforms. Examples are metabolic enzymes, signal transduction proteins and protein synthesis factors, some of which also cross-link microtubules and modulate cytoskeleton organization [11,21–25].

Embryos of the brine shrimp, *Artemia franciscana*, a Branchiopod crustacean, develop either oviparously or ovoviviparously, the former leading to release of encysted gastrulae (cysts) and the latter to swimming larvae [26,27]. Cysts enter diapause, a dormant state characterized by resistance to extreme physiological stress [27–30]. Once diapause is broken encysted gastrulae resume development when hydration, aeration and temperature are appropriate, and larvae are released from cysts. This complex differentiation process occurs in the virtual absence of cell division [31]. Under these conditions, changes to the microtubule proteome during development and differentiation can be studied without the complication of modifications related to mitosis and cytokinesis. Moreover, because many different cells exist in post-diapause developing *Artemia*, potential exists to find a wide range of microtubule interacting proteins. In this context, previous work on *Artemia* identified a 49-kDa microtubule cross-linking protein as coenzyme A-transferase [32,33] and p26, a small heat shock protein (sHSP) with molecular chaperone activity was shown to bind microtubules and suppress tubulin denaturation [34].

Proteins interacting with microtubules from *Artemia* at two development stages are documented in this work, the first systematic characterization of a crustacean microtubule proteome. The 0 h post-diapause cysts exhibit low metabolic activity and limited differentiation, whereas the 12 h post-diapause *Artemia*, which under the incubation conditions in this study are beginning to emerge, are metabolically active and have undergone extensive cell differentiation. Tubulin assembly was induced by taxol addition to *Artemia* cell free extracts and proteins stripped from the stabilized microtubules were resolved in two-dimension gels and identified by mass spectrometry. Of 133 *Artemia* proteins selected for analysis, fifty-five were identified, including those involved in metabolism, signaling, chaperoning and protein synthesis. The results, although generated in vitro and thus requiring additional physiological verification, contribute to our understanding of protein–protein interactions in the cytoskeleton and to the role they play in cell activities. Moreover, the approach is applicable to characterization of the microtubule proteome in any organism with tubulin amenable to taxol-induced assembly.

2. Materials and methods

2.1. Preparation of Artemia cell free extracts

Sixty grams (dry weight) of *A. franciscana* cysts (INVE Aquaculture, Inc., Ogden, UT, USA) were hydrated overnight in distilled water at 4 °C, collected with suction on a Buchner funnel, washed with cold distilled water and either homogenized after washing with Pipes buffer [100 mM 1,4-piperazine-*N,N′*-bis (2-ethanesulfonic acid) as free acid, 1 mM EGTA, 1 mM $MgCl_2$, pH 6.5] (0 h development), or incubated at 27 °C in Hatch Medium [35] with shaking at 220 RPM for 12 h. After 12 h at 27 °C approximately 20% of the cysts had emerged but hatching had not occurred and no swimming larvae were observed. Incubated cysts were collected on a Buchner funnel under light vacuum and washed with cold distilled water and Pipes buffer. Three milliliters of Pipes buffer was added to 60 g (wet weight) of sample which was then homogenized in a Retsch motorized mortar and pestle (Brinkman Instruments Canada, Rexdale, ON, Canada) for 5 min in the presence of protease inhibitors [33]. The homogenate was centrifuged at 16,000 ×*g* for 10 min at 4 °C, filtered through two layers of Miracloth (Calbiochem, San Diego, CA, USA), and centrifuged at 40,000 ×*g* for 30 min at 4 °C. The upper three-quarters of the supernatant was placed in a fresh tube and centrifuged at 40,000 ×*g* for 20 min at 4 °C.

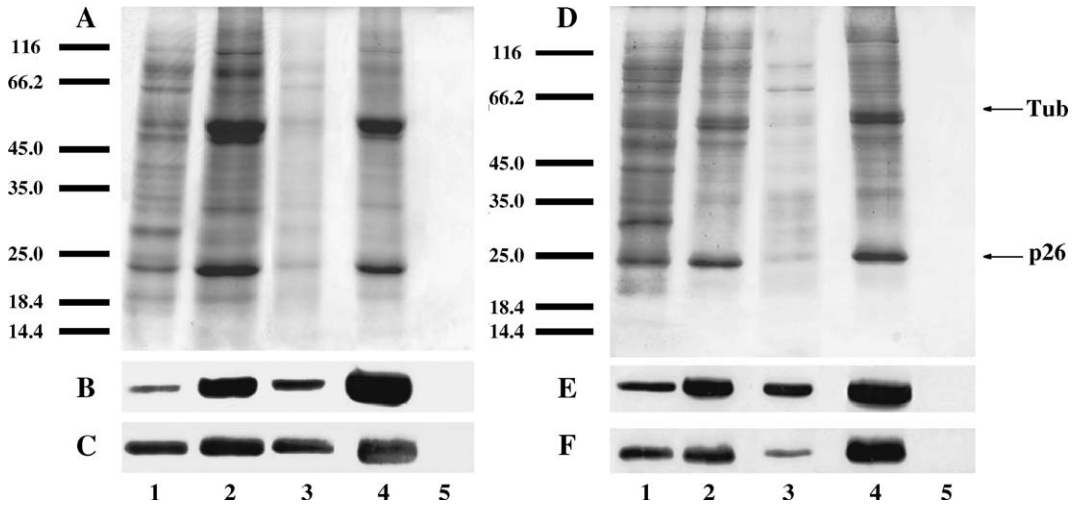

Fig. 1. Preparation of microtubule proteomes from *Artemia*. Protein samples from 0 h (A, B, C) and 12 h (D, E, F) post-diapause *Artemia* were electrophoresed in 12.5% SDS-polyacrylamide gels and either stained with Coomassie Blue (A, D) or blotted to nitrocellulose for staining with DM1A, a monoclonal antibody to α-tubulin (B, E), or a polyclonal antibody to the sHSP p26 (C, F). Lane 1, 5 μg of *Artemia* protein extract; lane 2, 5 μg of protein from the microtubule pellet obtained upon incubation of *Artemia* extract with taxol, centrifugation through sucrose and subsequent resuspension in buffer; lane 3, 5 μg of protein from the *Artemia* microtubule proteome obtained by salt extraction of taxol stabilized microtubules; lane 4, 5 μg of protein consisting of taxol stabilized microtubules that were extracted with 0.5 M NaCl, centrifuged through sucrose cushions and suspended in buffer; lane 5, control showing that no proteins were obtained when cyst protein extract was incubated in the absence of taxol. Labeled arrows indicate tubulin and p26.