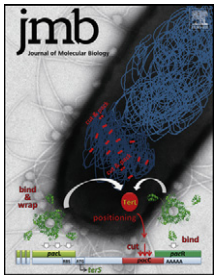

Structural Insights of WHAMM's Interaction with Microtubules by Cryo-EM

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http://dx.doi.org/10.1016/j.jmb.2017.03.022
Edited by E. Nogales

Abstract

WASP homolog associated with actin, membranes, and microtubules (WHAMM) is a vertebrate protein functioning in membrane tubulation for intracellular membrane trafficking and specific organelle formation. Composed of multiple domains, WHAMM can bind to membrane and microtubule (MT) and promote actin polymerization nucleation. Previous work revealed that WHAMM's activity to promote actin nucleation is repressed upon binding to MTs. Here, we discovered that WHAMM interacts with αβ-tubulin through a small peptide motif within its MT-binding domain. We reconstructed a high-resolution structure of WHAMM's MT-binding motif (MBM) assembling around MTs using cryo-electron microscopy and verified it with chemical cross-linking and mass spectrometry analysis. We also detected a conformational switch of this motif between the non-MT-bound state and the MT-bound state. These discoveries provide new insights into the mechanism by which WHAMM coordinates actin and MT networks, the two major cytoskeletal systems involved in membrane trafficking and membrane remodeling.

Introduction

WASP homolog associated with actin, membranes, and microtubules (WHAMM) is a mammalian protein belonging to the WASP family of actin polymerization nucleation-promoting factors (NPFs). It is composed of multiple domains: an N-terminal WMD interacting with membrane, a middle predicted coiled-coil (CC) region (termed CC domain) for microtubule (MT) binding, and a conserved C-terminal proline-rich region and WASP homology 2 peptides plus connector and acidic segments (PWCA) domain promoting Arp2/3-mediated actin-polymerization [1]. In a cell, WHAMM mostly localizes near ER and Golgi apparatus, and it is critical to maintain the shape of Golgi and mediate intracellular vesicle transportation from ER to Golgi [1]. WHAMM was also reported to be involved in autophagosome biogenesis through an actin comet tail mechanism, suggesting a WHAMM-mediated link between Arp2/3-driven actin polymerization and autophagy [2]. Moreover, a recent genetic study identified that WHAMM gene has risk haplotype associated with systemic lupus erythematosus, underlining WHAMM's pathological significance [3].

WHAMM was originally discovered as a homolog of NPFs that all have a conserved C-terminal PWCA domain but various N-terminal sequences [1,4,5]. The common PWCA domain is responsible for promoting Arp2/3-related actin branching polymerization. The actin nucleation-promoting activities of NPFs are highly regulated via various means in a cell. Different from the classical NPFs such as N-WASP, WHAMM's NPF activity is not auto-inhibited [1]. Instead, its NPF activity can be largely repressed by MTs [6]. Therefore, the MT-binding property and actin nucleation-promoting activity of WHAMM are mutually exclusive, suggesting the presence of at least two populations of WHAMM molecules with distinct functions for a cell's intracellular vesicle trafficking. As a matter of fact, it has been found that WHAMM's NFP activity is not

absolutely required for regulating the intracellular VSV-G anterograde transport and maintaining Golgi's positioning and morphology, whereas a full-length WHAMM is necessary to promote WHAMM-mediated membrane tubulation [1]. So, investigating the interaction between WHAMM and MTs will help us understand WHAMM's specific roles for different aspects of vesicle trafficking and remodeling in a cell.

WHAMM's CC domain was identified to be responsible for its interaction with MTs [1,6]. The CC domain shares no sequence homology to any known MT-binding proteins including the kinesin or dynein motors or other classical MT-associated proteins (MAPs). Therefore, WHAMM stands as a unique type of MT-binding protein. Using cryo-electron microscopy (cryo-EM), we had previously revealed that WHAMM can decorate MTs following the heterodimeric tubulin lattice by its CC domain and directly tether lipid vesicles to MTs [6]. The low-resolution nature of the EM reconstruction at ~20-Å resolution, however, hindered us from understanding the detailed mechanism of WHAMM's MT-binding property and the regulation of its NPF activity by MTs.

In this work, we dissected WHAMM's interaction with MTs and identified a novel MT-binding motif (MBM) of WHAMM. Using high-resolution cryo-EM reconstruction and chemical cross-linking of proteins coupled with mass spectrometry (CXMS) analysis, we determined the structure of WHAMM–MT interaction moiety at high resolution and detected a conformational switch of WHAMM's MBM upon binding to MTs. These discoveries provide new mechanistic insights into WHAMM function in anchoring lipid vesicles onto MTs and regulation of its NPF activity.

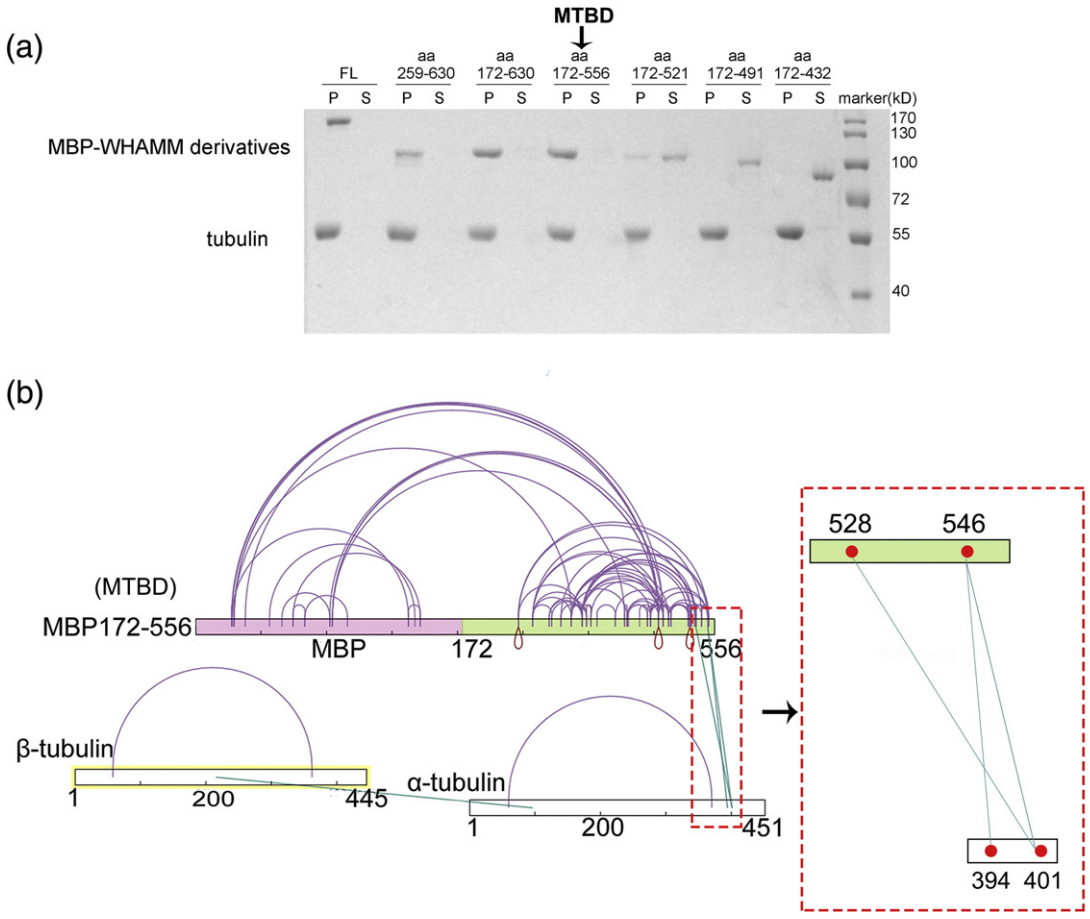

Fig. 1. Biochemical identification of the MT-binding region of WHAMM. (a) MT co-sedimentation assay of different MBP-WHAMM derivatives. "P" represents the pellet fraction and "S" represents the supernatant fraction. The arrow points the assay result of MTBD (aa 172–556). (b) The cross-linked lysine pairs identified from the MT–MTBD complex, depicted using xiNET [30]. The intermolecular cross-links between α-tubulin (K394 and K401) and MTBD (K528 and K546) are emphasized in red box.