

MINI REVIEW

Non-canonical functions of the mitotic kinesin Eg5

Min Liu , Jie Ran & Jun Zhou

College of Life Sciences, Collaborative Innovation Center of Cell Biology in Universities of Shandong, Institute of Biomedical Sciences, Shandong Provincial Key Laboratory of Animal Resistance Biology, Shandong Normal University, Jinan, China

Keywords

Eg5; kinesin; localization; microtubule; mitotic spindle.

Correspondence

Min Liu, College of Life Sciences, Shandong Normal University, 88 East Wenhua Road, Jinan, Shandong 250014, China.
Tel: +86 531 8618 2518
Fax: +86 531 8618 2518
Email: minliu@sdsnu.edu.cn

Received: 2 May 2018;
Accepted: 24 May 2018.

doi: 10.1111/1759-7714.12792

Thoracic Cancer **9** (2018) 904–910

Abstract

Kinesins are widely expressed, microtubule-dependent motors that play vital roles in microtubule-associated cellular activities, such as cell division and intracellular transport. Eg5, also known as kinesin-5 or kinesin spindle protein, is a member of the kinesin family that contributes to the formation and maintenance of the bipolar mitotic spindle during cell division. Small-molecule compounds that inhibit Eg5 activity have been shown to impair spindle assembly, block mitotic progression, and possess anti-cancer activity. Recent studies focusing on the localization and functions of Eg5 in plants have demonstrated that in addition to spindle organization, this motor protein has non-canonical functions, such as chromosome segregation and cytokinesis, that have not been observed in animals. In this review, we discuss the structure, function, and localization of Eg5 in various organisms, highlighting the specific role of this protein in plants. We also propose directions for the future studies of novel Eg5 functions based on the lessons learned from plants.

Introduction

Mitosis is an essential cellular process in eukaryotic organisms. To ensure that mitosis is carried out with accuracy and at the appropriate frequency, eukaryotic cells have evolved complex and finely tuned mechanisms. At the core of these regulatory mechanisms is the mitotic spindle, a complex structure comprising microtubules; microtubule-dependent motor proteins, such as dynein and kinesin family proteins; and non-motor microtubule-binding proteins.^{1,2} Microtubules, as the key structural component of the bipolar mitotic spindle, are essential for its assembly and maintenance.^{3,4} Microtubule-binding proteins, especially motor proteins, are also essential for spindle integrity.^{5–7}

A subset of kinesins have been shown to play vital roles in the process of cell division, including the establishment of spindle bipolarity, chromosome alignment, and cytokinesis; these processes all rely on the synergistic effects of kinesins.⁸ Based on sequence similarity, kinesin proteins have been divided into more than a dozen subfamilies.^{9,10} Eg5, also known as kinesin-5 or kinesin spindle protein, is a member of the kinesin family and has been shown to play a critical role in the establishment of spindle bipolarity.¹¹

Recent studies in plants have found that Eg5 is also involved in spindle organization, chromosome segregation, and cytokinesis. Additionally, Eg5 possesses a post-anaphase function in plants that has not been observed in animals.^{12–16} In this review, we discuss the structure, localization, and function of Eg5, with a focus on its unique localization and function in plants.

Eg5 structure and function

The amino acid sequences and structures of Eg5 are conserved across multiple species (Fig 1).^{10,11} Most Eg5 proteins contain a motor domain, an internal stalk domain, and a tail domain. Four Eg5 proteins form a bipolar homotetrameric complex via interactions between the stalk domains, resulting in the positioning of two motor domains at each end of the tetramer (Fig 2a).¹¹ The tetramers can simultaneously move toward the plus ends of two anti-parallel microtubules, a movement that pushes anti-parallel microtubules in opposite directions (Fig 2b).^{17,18} Both ATPase activity and the microtubule-binding property are carried out by the motor domain of Eg5.¹⁹ However, the non-motor stalk and tail domains are also required for the protein to crosslink microtubules and slide apart anti-parallel

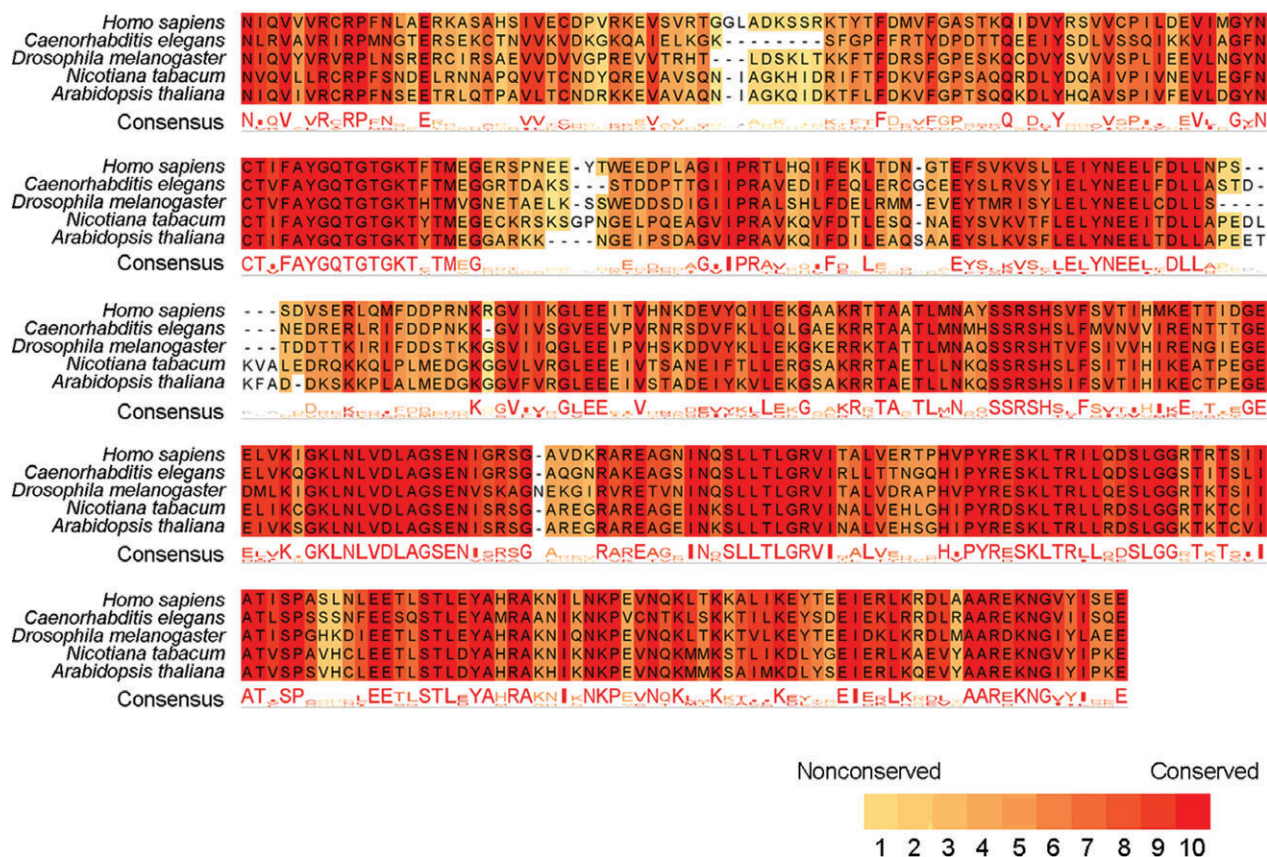


Figure 1 Multiple sequence alignment of conserved regions in the motor domain of Eg5 from five different species. The consistency of amino acids is classified from 0 (nonconserved) to 10 (conserved).

microtubules.^{20,21} In addition, the tail domain of Eg5 contributes to its localization during mitosis and enhances its binding to microtubules.²²

In multiple eukaryotic organisms, the core functions of Eg5 are similar. In animals, fungi, and plants, Eg5 monomers form tetramers that play an essential role in the establishment of spindle bipolarity. In addition, stable Eg5 dimers have been reported to promote microtubule polymerization in vitro.²³ A requirement for Eg5 during mitosis has been demonstrated experimentally in cells from multiple species. Deletion of Eg5, reduction of its expression, or inhibition of its activity results in the formation of a monopolar spindle (Fig 3), leading to the activation of the spindle checkpoint and a subsequent block in cell division.^{11,24–26} The formation of a monopolar spindle upon the impairment of Eg5 supports the general consensus that the core function of Eg5 is to slide apart anti-parallel microtubules during mitosis.

In mammalian cells, overexpression of Eg5 leads to abnormal cell division and genomic instability, anomalies associated with tumorigenesis.²⁷ For example, overexpression of Eg5 in pancreatic cancer cells causes the formation of multipolar spindles.²⁸ Given that Eg5 plays an essential role during

cell division and that it is highly expressed in many human cancer types,^{28–34} this protein is considered a promising target for cancer therapy. Numerous small-molecule compounds that inhibit Eg5 activity have been shown to inhibit cancer cell division, induce apoptosis, and effectively block tumor growth in mice; in addition, several Eg5 inhibitors are already in clinical trials as potential anti-cancer agents.^{25,35}

Subcellular localization of Eg5

The subcellular localization of Eg5 varies between species. For example, in *Drosophila melanogaster* cells, Eg5 localizes uniformly along spindle microtubules. In *Xenopus laevis* cells, Eg5 is enriched near the spindle poles, a localization pattern that results from dynein-dependent transport.^{36,37} In *Caenorhabditis elegans*, Eg5 is mainly expressed in the hermaphrodite germline of fertilized embryos; after fertilization, the subcellular localization changes during the cell cycle.³⁸ In prophase, Eg5 begins to interact with microtubules, and in metaphase, Eg5 is enriched on the spindle poles and kinetochore microtubules. During anaphase, Eg5 is positioned at the central spindle, while in telophase, Eg5 localizes to the midbody.

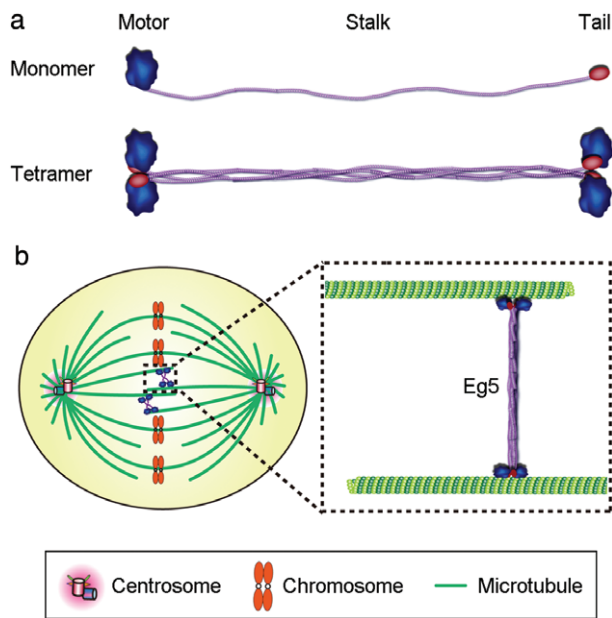


Figure 2 Eg5 structure and function. (a) An Eg5 monomer contains a motor domain, a stalk domain, and a tail domain (top). Four Eg5 monomers form a homotetramer via interactions between the stalk domains (bottom). (b) Schematic model showing that the Eg5 tetramer cross-links and slides apart anti-parallel microtubules, contributing to bipolar spindle formation and maintenance.

The localization of Eg5 in prophase has recently been shown to depend on TPX2, a microtubule nuclear factor.^{39,40}

In plants, Eg5 localization shows substantial divergence from the localization patterns observed in animals and fungi. In *Arabidopsis thaliana*, Eg5 is distributed along microtubules throughout the cell cycle, irrespective of whether cells are in interphase or mitosis.^{14,41–43} In *Nicotiana tabacum*, the distribution of microtubules changes with the progression of the cell cycle, as does the localization of Eg5.^{13,44} During the S phase of the cell cycle, Eg5 distributes along cortical microtubules. In pre-mitotic cells, it localizes along microtubules in the pre-prophase band and along perinuclear microtubules. During mitosis, Eg5 is distributed along spindle microtubules and on the equatorial plate, while in cytokinesis, Eg5 localizes to phragmoplast microtubules.¹³ In *Physcomitrella patens*, Eg5 localizes to cytoplasmic microtubules in prophase and to phragmoplast microtubules in prometaphase.¹⁵ Surprisingly, however, there is little localization of Eg5 to the equatorial plate, an area where anti-parallel microtubules are enriched.

Non-canonical functions of Eg5 in plants

The unique localization of Eg5 in plants suggests that this protein may possess novel functions in plants. These non-

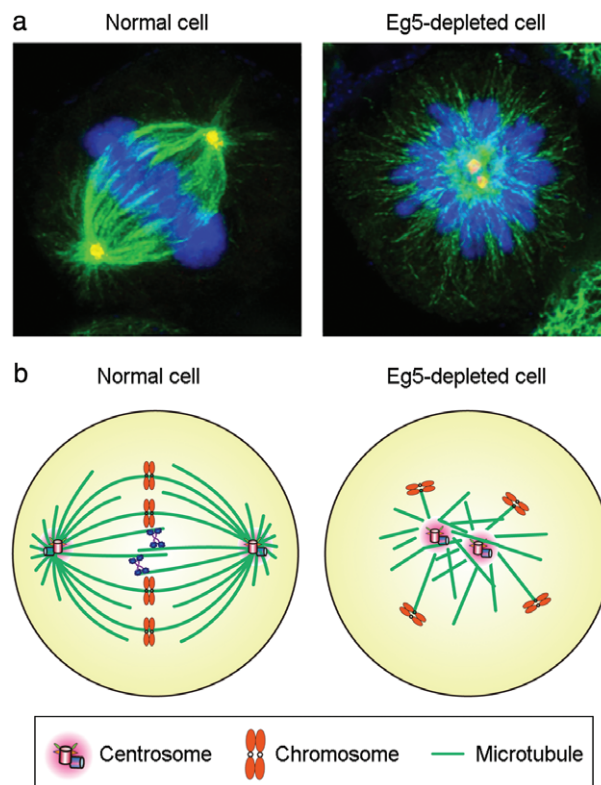


Figure 3 Depletion of Eg5 impairs mitotic spindle formation. (a) Immunofluorescence microscopy of microtubules (green), γ -tubulin (red), and chromosomes (blue) in normal metaphase or Eg5-depleted HeLa cells. (b) Schematic model illustrating a bipolar spindle in the normal metaphase cell and a monopolar spindle in the Eg5-depleted cell.

canonical activities of Eg5 also suggest the possibility that additional roles for Eg5 and other kinesins may exist in animal cells. In *Nicotiana tabacum*, Eg5 is reportedly involved in separating anti-parallel microtubules in the phragmoplast.^{13,45,46} Inhibition of Eg5 using a peptide that targets its motor domain blocks the translocation of phragmoplast microtubules, suggesting that the motor activity of Eg5 is required for the organization of phragmoplast microtubules. Additionally, this phenotype indicates a vital role for Eg5 in microtubule translocation, an event that is critical for the formation and maintenance of the bipolar structure of the phragmoplast.

In *Arabidopsis thaliana*, disruption of Eg5 activity leads to disorganized intracellular microtubules during interphase and disrupted spindle microtubules.^{14,47} These changes affect the formation of the bipolar spindle, suggesting that the function of Eg5 in *Arabidopsis thaliana* may be similar to that in animals. However, in mammalian epithelial cells depleted of Eg5, expression of *Arabidopsis thaliana* Eg5 does not rescue the formation of bipolar spindles, despite the localization of exogenous Eg5 to spindle microtubules.^{14,48} In *Physcomitrella patens*, Eg5 plays a

significant role in spindle organization and chromosome segregation.¹⁵ Depletion of Eg5 induces the formation of multinucleated cells, a phenotype resulting from aberrant chromosome segregation. Additionally, spindle microtubules are disrupted in Eg5-depleted cells, with metaphase spindles appearing longer and slackened and phragmoplast microtubules forming later and failing to properly align.¹⁵ However, depletion of Eg5 in *Physcomitrella patens* does not affect spindle bipolarity, an observation inconsistent with the classical function of Eg5 in the formation and maintenance of bipolar spindles.

Conclusions and perspectives

Given the vital role Eg5 plays during cell division, characterization of the regulation of Eg5 structure and function may promote a deeper understanding of the molecular mechanisms that underlie cell division. In animals, exploring the regulation of Eg5 may provide new knowledge regarding the pathogenesis of cancer and other diseases, leading to improved diagnosis and treatments.^{49–56} In plants, exploring the function, localization, and motor activity of Eg5 may provide important insights into its roles in plant growth and development; furthermore, these studies will provide new knowledge for Eg5 research in animals.^{7,57–66} It is worth noting that the localization of kinesins during mitosis has been systematically analyzed in *Physcomitrella patens*.¹⁵ Forty-two kinesins are found in microtubule-based structures, such as the kinetochores, spindles, and phragmoplasts. Only one kinesin shows the same localization pattern as its animal homologs, and many kinesins are enriched at unexpected locations.¹⁵ Therefore, studying the localization and function of Eg5 and other kinesins in plant cells could contribute to the discovery of novel Eg5 functions that may be difficult to uncover in animals.

Accumulating evidence indicates that Eg5 localization, motor activity, and function are modulated by post-translational modifications (Fig 4).^{67–75} For example, the threonine at position 926 in the tail domain of human Eg5 can be phosphorylated by Cdk1; this modification is important for the interaction of Eg5 with microtubules and its localization to the spindle.⁷⁶ In addition, the serine at position 1033, also in the tail domain of human Eg5, is phosphorylated by Nek6/7, a modification that contributes to its localization to the spindle pole.⁷⁷ In contrast to the Eg5 tail

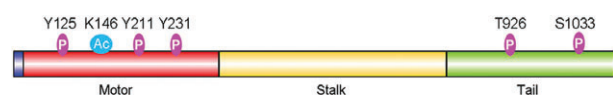


Figure 4 Schematic illustration of phosphorylated (P) and acetylated (Ac) amino acid residues in Eg5.

domain, it is unclear whether the motor domain of Eg5 is phosphorylated, and whether such phosphorylation is involved in the modulation of Eg5 localization, motor activity, or function in spindle assembly and maintenance. Recent studies have suggested acetylation of Eg5 at lysine 146, which is located in the $\alpha 2$ helix of the motor domain, enhances its mechanochemical coupling, and alters its mitotic function.⁷⁵ In addition, Src is reported to phosphorylate three tyrosines in the motor domain of human Eg5⁷⁸ however, the molecular mechanisms regulating this modification and its functional significance remain unknown. It also remains to be determined whether the phosphorylation of Eg5 affects its role in plant growth and development. Additional studies identifying other forms of post-translational modifications, and determining how these modifications affect Eg5 localization and function may lead to the discovery of novel non-canonical activities for Eg5.

Acknowledgment

This study was supported by grants from the National Natural Science Foundation of China (31671403 and 31741039).

Disclosure

No authors report any conflict of interest.

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