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TAC-1, a Regulator of Microtubule Length in the *C. elegans* Embryo

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Summary

Regulation of microtubule growth is critical for many cellular processes, including meiosis, mitosis, and nuclear migration. We carried out a genome-wide RNAi screen in *Caenorhabditis elegans* to identify genes required for pronuclear migration, one of the first events in embryogenesis requiring microtubules. Among these, we identified and characterized *tac-1* a new member of the TACC (Transforming Acidic Coiled-Coil) family [1]. *tac-1(RNAi)* embryos exhibit very short microtubules nucleated from the centrosomes as well as short spindles. TAC-1 is initially enriched at the meiotic spindle poles and is later recruited to the sperm centrosome. TAC-1 localization at the centrosomes is regulated during the cell cycle, with high levels during mitosis and a reduction during interphase, and is dependent on aurora kinase 1 (AIR-1), a protein involved in centrosome maturation [2]. *tac-1(RNAi)* embryos resemble mutants of *zyg-9* [3], which encodes a previously characterized centrosomal protein of the XMAP215 family and was also found in our screen. We show that TAC-1 and ZYG-9 are dependent on one another for their localization at the centrosome, and this dependence suggests that they may function together as a complex. We conclude that TAC-1 is a major regulator of microtubule length in the *C. elegans* embryo.

Results and Discussion

The main microtubule-organizing center (MTOC) in animal cells is the centrosome, which consists of a pair of centrioles and surrounding pericentriolar material (PCM) that contains, among other proteins, the γ-tubulin complexes involved in the nucleation of microtubules (MTs). In *Caenorhabditis elegans*, as in most animals, the sperm contributes the only pair of centrioles, which duplicate to form the new zygotic centrosomes and recruit the PCM components from the cytoplasm, allowing MT nucleation and growth. Prior to mitosis, growth of long astral MTs is required for migration of the maternal toward the paternal pronucleus, for migration of the pronuclear-centrosomal complex to the cell center, and for rotation of the complex onto the anterior-posterior axis [4, 5].

To find new components required for MT-dependent events, we analyzed 491 (50%) RNAi-induced embryonic lethals identified in [6] and [7] by using DIC videomicroscopy (see the Experimental Procedures). For this study, we looked for genes whose inactivation by RNAi led to a specific failure of both pronuclei to migrate, excluding those with additional defects in the pronuclear envelope or where no spindle was made. The genes identified fall into three different categories linked to regulation of microtubule-dependent functions.

The first class comprises Prefoldin subunits 2 and 3 (Table 1). The corresponding proteins in *Saccharomyces cerevisiae*, Pac10 and Gim4p, are components of the Gim protein folding complex, which promotes the formation of functional α- and γ-tubulin in *S. cerevisiae* [8]. Slower or improper folding of the newly synthesized tubulin could impair MT formation and thus pronuclear migration.

The second category contains six components of the *C. elegans* dynein-dynactin complex (Table 1). Components of this complex are required for a range of MT-dependent movements, including migration of the maternal pronucleus toward the sperm-associated centrosomes in various organisms [9–11]. In *C. elegans*, previous work identified five dynein-dynactin subunits as having a role in pronuclear migration (the dynein heavy chain [DHC-1], light chain [DLC-1], light intermediate chain [DLI-1], p150 glued [DNC-1], and p50 dynamitin [DNC-2]) [10, 12, 13]. In addition to three of the above, we identified three additional subunits of this complex as being required for pronuclear migration (see Table 1): the dynein intermediate chain, an Actin-Related Protein 1 (ARP-1) homolog, and a homolog of CAPZ (CAP-2), which is an actin capping protein found at the barbed end of the ARP1 filament (reviewed by Allan [14]).

In the third category, we found the previously characterized gene *zyg-9* and a new gene, *tac-1*. *zyg-9* encodes a centrosomal microtubule-associated protein of the XMAP215 family [3] that is involved in the regulation of MT dynamics (reviewed by Ohkura et al. [15]). ZYG-9 is required for the formation of long MTs, and *zyg-9* mutant embryos fail to undergo pronuclear migration [3]. Below, we show that MT-dependent defects observed in *tac-1(RNAi)* embryos are similar to those of *zyg-9* mutants and that the encoded proteins are likely to function together.

tac-1 encodes a protein of 260 amino acids with a predicted coiled-coil region at its C terminus (see Figure S1 in the Supplemental Data available with this article online). BLAST searches with this domain reveal weak similarity to TACC (Transforming Acid Coiled-Coil) centrosome- and spindle-associated proteins identified in *Drosophila* and mammals that play a role in spindle organization (reviewed by Gergely [16]). TACC proteins share homology only in the C-terminal coiled-coil region, and this region is sufficient for targeting to the centrosome [1, 17]. Although the N-terminal regions of TACC proteins share no obvious homology, they are relatively large compared with that of TAC-1. This suggests the possibility that N-terminal TACC functions might not be

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Table 1. Genes Found in the Screen for which RNAi Prevents Pronuclear Migration

Predicted Gene	Locus	Description	Homolog(s)	BlastP E Value	Reference
H20J04.5	-	Prefoldin subunit 2	*Sc* GIM4	3 e-05	This work
T06G6.9	-	Prefoldin subunit 3 (von-Hippel-Lindau binding protein 1)	*Hs* PFD3 *Sc* PAC10	6.4 e-33 2 e-14	[30]
T21E12.4	*dhc-1*	Dynein heavy chain	*Dm* DYHC-1	0.0	[10, 30]
ZK593.5	*dnc-1*	dynactin-p^{150}glued	*Hs* p150 Glued	2 e-37	[10]
C17H12.1	-	Dynein intermediate chain	*Hs* DNCI 1	4 e-99	This work
Y53F4B.22	-	Actin Related Protein 1 (ARP-1)	*Hs* ARP1	1 e-155	This work
M106.5	*cap-2*	Actin capping protein	*Hs* CAPZB	8 e-89	This work
F22B5.7	*zyg-9*	MAP from XMAP215 family	*Dm* MSPs	1 e-55	[3]
Y54E2A.3	*tac-1*	Transforming Acidic Coiled-Coil protein 1	*Dm* D-TACC	1 e-07	This work

D.I.C. recordings were made after RNAi of 53% of the embryonic lethal genes on chromosomes I [30], II, VI, V, and X; the genes shown are those found in the screen where neither pronuclei migrated after RNA, excluding those displaying an abnormal nuclear envelope or no spindle formation. Chromosome III was excluded from this study, as a similar analysis of this chromosome was performed by Gonczy et al. [12]. The table includes four genes, T06G6.9 [30], *dhc-1* [10, 30], *dnc-1* [10], and *zyg-9* [3], whose pronuclear migration phenotype was identified previously.

conserved in *C. elegans* or could be performed by a different protein. As in *Drosophila*, there is only a single TACC homolog in the *C. elegans* genome. This is in contrast to mammals in which several members can be identified.

In *tac-1(RNAi)* embryos, migration of the maternal pronucleus does not take place (Figure 1F). The sperm centrioles duplicate and migrate around the paternal pronucleus (Figure 1E), but the sperm pronuclear-centrosomal complex does not move toward the center of the embryo or rotate onto the anterior-posterior axis. A transversely aligned spindle is then formed by the centrosomes and the sperm DNA at the posterior of the embryo (Figure 1G). In addition, 30% of early embryos exhibit a meiotic defect (Figure 1I and data not shown).

Shortly before anaphase in *tac-1(RNAi)* embryos, the maternal DNA is captured by only one centrosome (Figure 1H, arrow in the GFP panel); this capture induces the formation of multiple nuclei at the 2-cell stage (data not shown). Interestingly, maternal pronuclear envelope breakdown is delayed compared to the paternal one (see Figures 1C and 1G). Delayed maternal nuclear envelope breakdown in the absence of pronuclear migration has also been observed when dynein heavy chain is inhibited by RNAi [10]. As only the paternal pronucleus is associated with centrosomes, this suggests that activities inducing nuclear envelope breakdown and/or entry into mitosis could be associated with the centrosome. Indeed, work in mammalian cells has shown that nuclear envelope breakdown proceeds via tearing induced by centrosomal microtubules [18], and factors required for mitotic entry, such as cyclin-dependant kinase 1 (cdk1), accumulate at the centrosomes in their active form [19]. Taken together, these defects show that *tac-1* is required for normal MTOC (centrosomal or acentrosomal)-associated functions.

To determine whether these defects are a result of altered MT regulation, we looked at the distribution of MTs in 1-celled *tac-1(RNAi)* embryos. To compare equivalent wild-type and mutant stages, we used the state of DNA condensation to stage the embryos. In wild-type embryos, from rotation of the pronuclear-centrosomal complex to the end of mitosis, MTs nucleated from the centrosome extend all the way to the cortex (Figures 2A and 2G). By contrast, in *tac-1(RNAi)* embryos, these MTs are remarkably short and do not reach the cortex (Figures 2D and 2J). The rest of the cytoplasm is filled with interphase-like MTs (compare Figure 3Aiii to Figures 2D and 2J). This may reflect the fact that in *tac-1(RNAi)* embryos, the tubulin that fails to be recruited to the short astral MTs can still form cortical arrays as in earlier wild-type stages. The presence of these MTs suggests that the absence of TAC-1 specifically affects MTs nucleated from the centrosome.

To determine if TAC-1 regulates mitotic spindle length as well as the length of astral MTs, we measured the distance between the two centrosomes at the metaphase stage of live wild-type or *tac-1(RNAi)* 1-celled embryos (see the Experimental Procedures). We found that spindles in *tac-1(RNAi)* embryos are on average 20% shorter than in wild-type (*tac-1(RNAi)* = 10.4 μm [$\pm$0.5], n = 12; wild-type = 13.1 μm [$\pm$0.3], n = 12). Short spindles are also observed in *Drosophila d-tacc* mutants [17]. However, *tac-1(RNAi)* embryos have normal chromosome alignment on the metaphase plate in contrast to the alignment defect observed in TACC3-depleted human cells [20]. As TACC3 is strongly associated both with the spindle microtubules and with the centrosomes, a localization pattern that is slightly different from the distribution of TAC-1 (see below), TACC3 could regulate spindle MTs differently.

Using antibodies raised against full-length TAC-1, we found that TAC-1 is first associated with the meiotic spindle and is enriched at the spindle poles (Figure 3Aii). As the sperm aster grows, TAC-1 is recruited to the centrosomes (Figure 3Aiv), where it accumulates as they mature, reaching a peak at metaphase (Figure 3Avi). This localization pattern is similar to that of components of the pericentriolar material, such as the nucleating factor γ-tubulin and the microtubule-associated protein ZYG-9 [3, 21]. At telophase, centrosomal TAC-1 association becomes weaker, coinciding with the change of aster shapes observed in the 1-celled *C. elegans* embryo (Figure 3Aviii).

To examine the dynamics of TAC-1 localization, we followed the behavior of a GFP:TAC-1 fusion protein

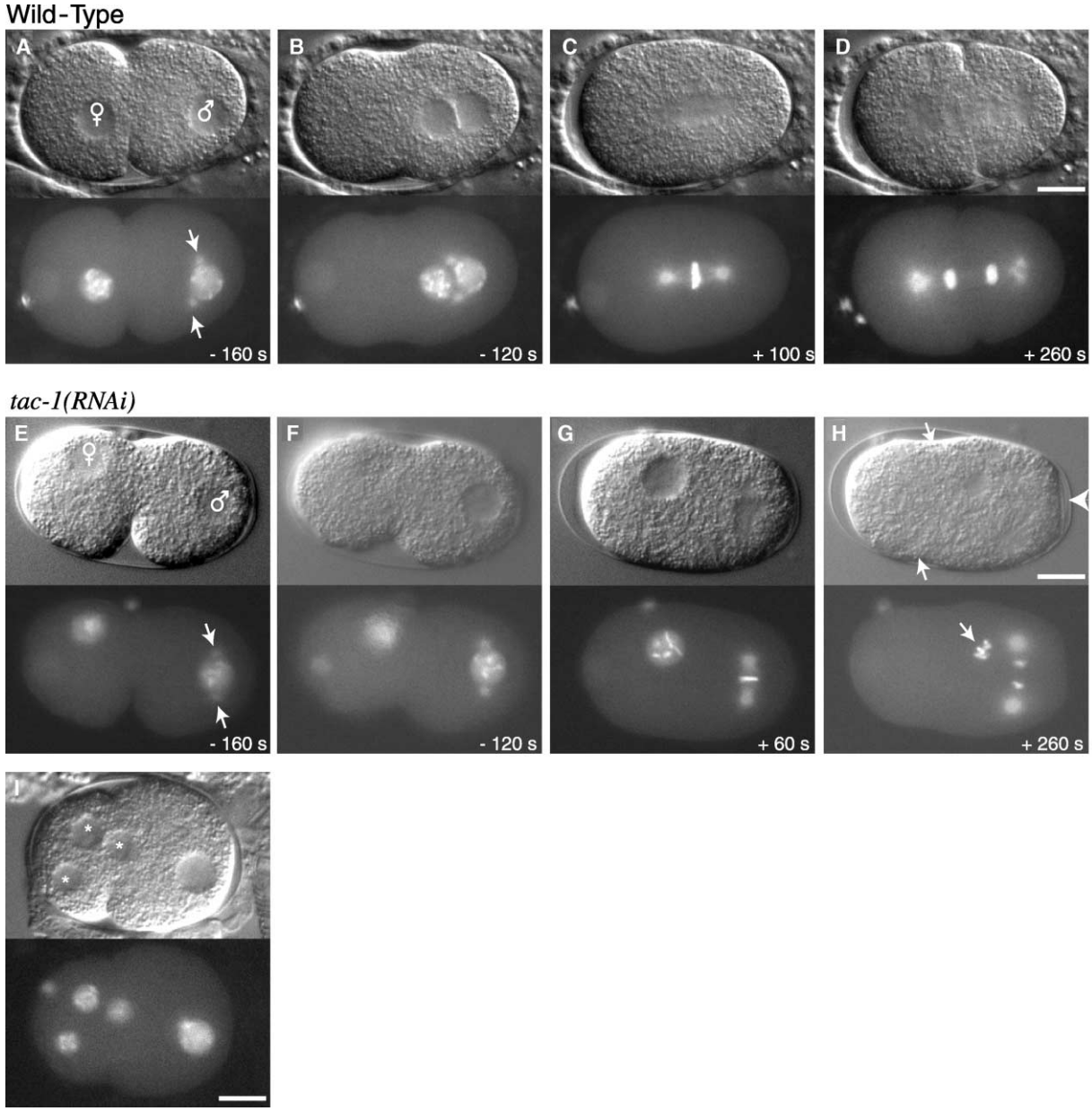

Figure 1. TAC-1 Is Required for MT-Dependant Processes during the First Cell Cycle

(A–H) A time-lapse series analysis of (A–D) wild-type and (E–H) *tac-1(RNAi)* embryos expressing both GFP-α-tubulin and GFP-histone fusion proteins. The top image in each panel shows a DIC image; the bottom image in each panel shows the corresponding fluorescent channel. The anterior portion of the embryos is oriented toward the left. In (A) and (E), the maternal and paternal pronuclei are indicated in the DIC picture, and the centrosomes, highlighted by GFP-α-tubulin, are marked by arrows. The times shown are the seconds before or after nuclear envelope breakdown (NEBD). In (A) wild-type and the (E) *tac-1(RNAi)* embryo at −160 s, the centrosomes have duplicated and have migrated around the male pronucleus. At −120 s in (B) wild-type, the pronuclei have met at the posterior, but in the (F) *tac-1(RNAi)* embryo, pronuclear migration has failed. At +100 s both in (C) wild-type and the (G) *tac-1(RNAi)* embryo, DNA is aligned on the metaphase plate. (G) Note that the female pronucleus has not yet undergone NEBD in the *tac-1(RNAi)* embryo. (C) In wild-type, the spindle is central and is oriented along the anterior-posterior axis following movement to the center and subsequent rotation of the pronuclear-centrosomal complex. (G) In the *tac-1(RNAi)* embryo, the spindle is transverse and is located at the posterior due to failure of these two processes. At +260 s, (D) wild-type and (H) *tac-1(RNAi)* spindles are in telophase. In the *tac-1(RNAi)* embryo, the maternal DNA (arrow in the GFP panel in [H]) has been captured at the metaphase to anaphase transition by one aster, inducing the formation of one cell with multiple nuclei. At the same time, cytokinetic furrows invaginate in three different places (see the DIC panel), one bisecting the spindle (arrowhead) and two perpendicular to the first (arrows). The movies from which these images are taken are available as Supplemental Data.

(I) Meiosis defect in a *tac-1(RNAi)* embryo: three female pronuclei are visible (stars). Meiosis defects were observed in 7/21 *tac-1(RNAi)* embryos: 4/7 had extra female pronuclei, 2/7 had an abnormally large polar body, and 1/7 had no female pronucleus.

The scale bars represent 10 μm.

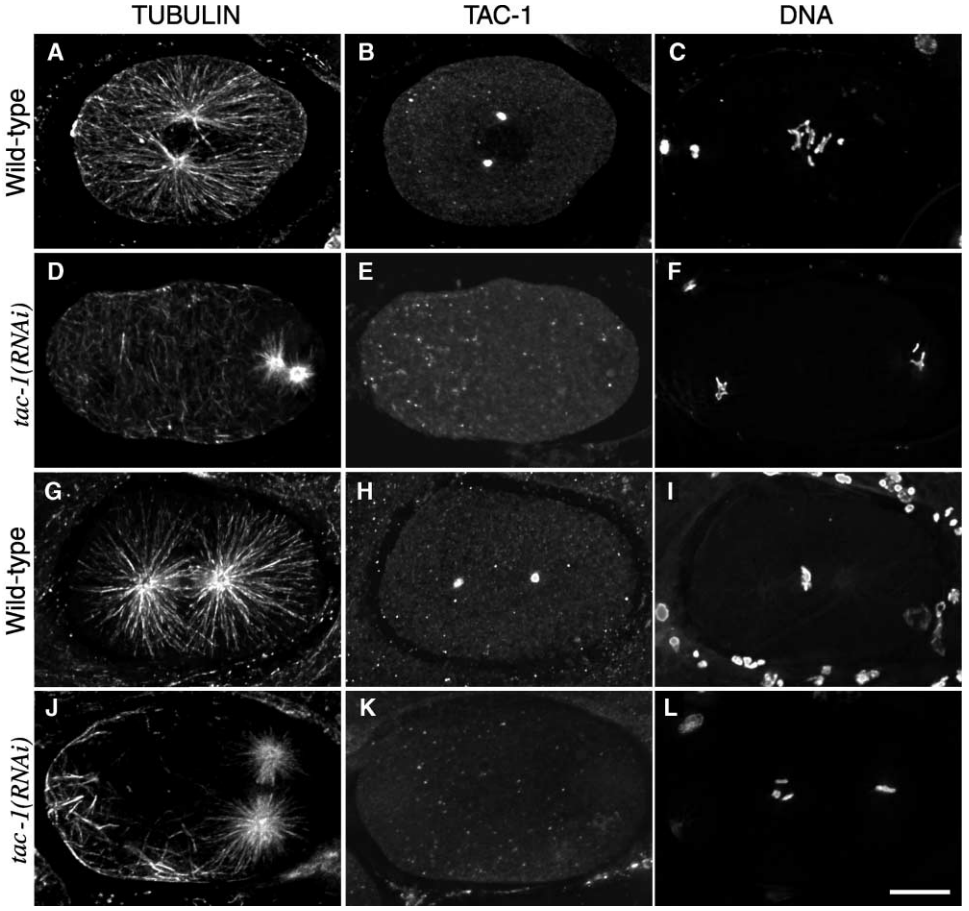

Figure 2. TAC-1 Is Required for the Growth of Long Microtubules

(A–L) Wild-type and *tac-1(RNAi)* embryos stained for MTs (left), TAC-1 (middle), and DNA (right) at (A–F) prophase and (G–L) metaphase. (A–C) and (G–I) In wild-type, MTs nucleated from the centrosome reach the cortex. (D–F) and (J–L) In *tac-1(RNAi)* embryos, MTs nucleated from the centrosome are very short. Note that the cytoplasm contains long interphase-like MTs, even in mitosis, when there are normally no cortical MTs observed. The scale bar represents 10 μm.

(Figure 3B). As seen for the endogenous protein, GFP:TAC-1 is localized to the centrosomes before pronuclear migration and becomes highly concentrated there, reaching a peak at metaphase (Figure 3B, +85 s). During anaphase, GFP:TAC-1 starts to disappear from the center of the aster (see hole in Figure 3B, +130 s and +140 s). By the end of telophase, two foci of GFP:TAC-1, which are probably associated with the newly duplicated centrosome, are observed in the center of the "old" ring of GFP:TAC-1 (Figure 3B, +700 s). During interphase, centrosomal GFP:TAC-1 reaccumulates until the next mitosis (Figure 3B, +805 s). This behavior is very similar to that observed for γ-tubulin GFP [22]. GFP:TAC-1 is also weakly associated with the central spindle around the chromosomes at metaphase (Figure 3C). We conclude that *C. elegans* TAC-1 is present on the meiotic spindle and is dynamically associated with centrosomes.

The *C. elegans* homolog of aurora A, the AIR-1 kinase, is essential for centrosome maturation and for the recruitment of several components of the PCM [2]. Similarly, we found that TAC-1 is absent from the centrosomes in *air-1(RNAi)* embryos (Figure 4M, 7/7 early embryos). In *Drosophila*, aurora A interacts with D-TACC in vivo and is able to phosphorylate the middle part of the protein (absent in TAC-1) very efficiently in vitro [23]. It remains to be determined if AIR-1 can directly phosphorylate TAC-1.

The defects in meiosis, pronuclear migration, and the short microtubules observed in *tac-1(RNAi)* embryos are very similar to the defects of *zyg-9* mutant embryos [3], and we also found the *zyg-9* gene in our RNAi screen. ZYG-9 is a microtubule-associated protein of the XMAP215/MSPs/ChTOG family. Although proteins from this family are associated with the centrosome, where MT minus ends lie, they primarily affect plus end, rather than minus end, dynamics (reviewed by Wittmann et al. [24], Ohkura et al. [15]).

Consistent with the idea that ZYG-9 and TAC-1 might act in a common process, we found that embryos depleted for both proteins have the same phenotypes as those in which only one is inhibited (n = 7; data not shown). To explore further the relationship between TAC-1 and ZYG-9, we asked whether they were dependent on each other for their centrosomal localization. In *tac-1(RNAi)* embryos, centrosomal ZYG-9 is undetectable (Figure 4F). We also found that TAC-1 centrosomal localization depends on the presence of ZYG-9 (Figure

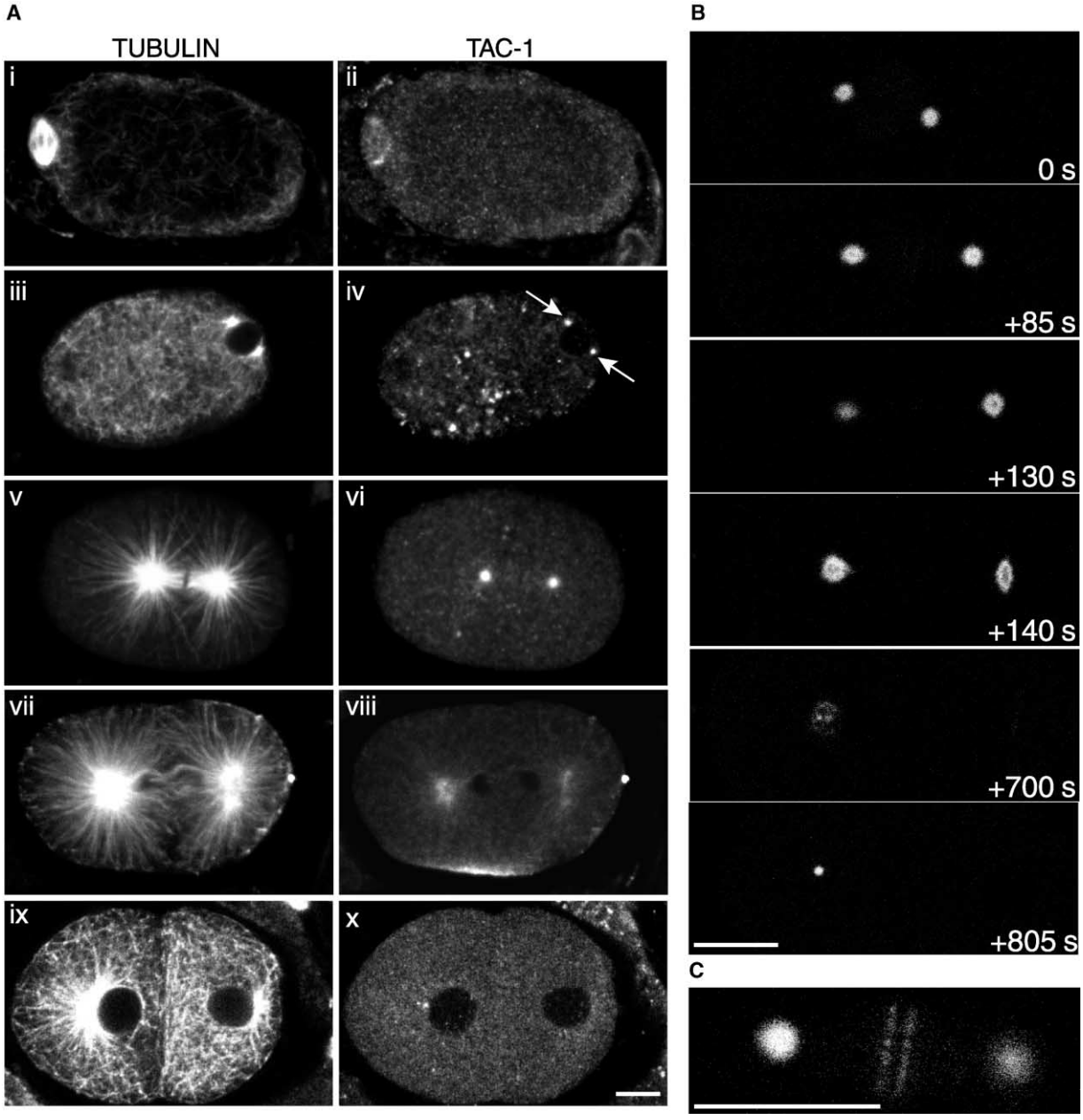

Figure 3. TAC-1 Is Dynamically Associated with the MTOC in *C. elegans* Embryos

(A) Fixed wild-type, 1-celled embryos stained for MTs (left panels; [i], [iii], [v], [vii], and [ix]) and TAC-1 (right panels; [ii], [iv], [vi], [viii], and [x]). (i and ii) TAC-1 is enriched at the poles of the meiotic spindle. (iii and iv) TAC-1 is associated with the sperm centrosomes (arrows in [iv]). (v and vi) High TAC-1 accumulation at the centrosomes in metaphase. (vii and viii) Reduced TAC-1 accumulation in telophase; (ix and x) 2-cell stage. The scale bar represents 10 μm.

(B) Still images from a time-lapse movie of an embryo expressing GFP:TAC-1. The pictures show GFP:TAC-1 behavior from prophase in the 1-celled embryo (just after pronuclear-centrosome rotation) to the 2-cell stage. (0 s) prophase, (+85 s) metaphase, (+130 s) anaphase, (+140 s) telophase, (+700 s) early 2-celled embryo, (+805 s) interphase 2-celled embryo. The scale bar represents 10 μm.

(C) GFP:TAC-1 around the chromosomes on a metaphase plate of a multicellular embryo. The scale bar represents 5 μm.

4H). This codependence is specific and is not a consequence of a general disruption of the centrosome, as γ-tubulin (TBG-1) localization is not disrupted in *tac-1(RNAi)* embryos and TAC-1 is still present at the centrosomes in *tbg-1(RNAi)* embryos (see Figures S2B and S2C in the Supplemental Data).

In a parallel study by Srayko et al. [10], ZYG-9 levels were only partially reduced at the centrosome in *tac-1(RNAi)* embryos. This difference in results might be due to the use of different RNAi conditions and/or different antibodies and staining methods (see the Experimental Procedures). In addition, the authors observed a 50% reduction in ZYG-9 abundance in *tac-1(RNAi)* embryos. Nevertheless, significant codependence was also seen in their study.

Similar to our results, *Drosophila* D-TACC is essential

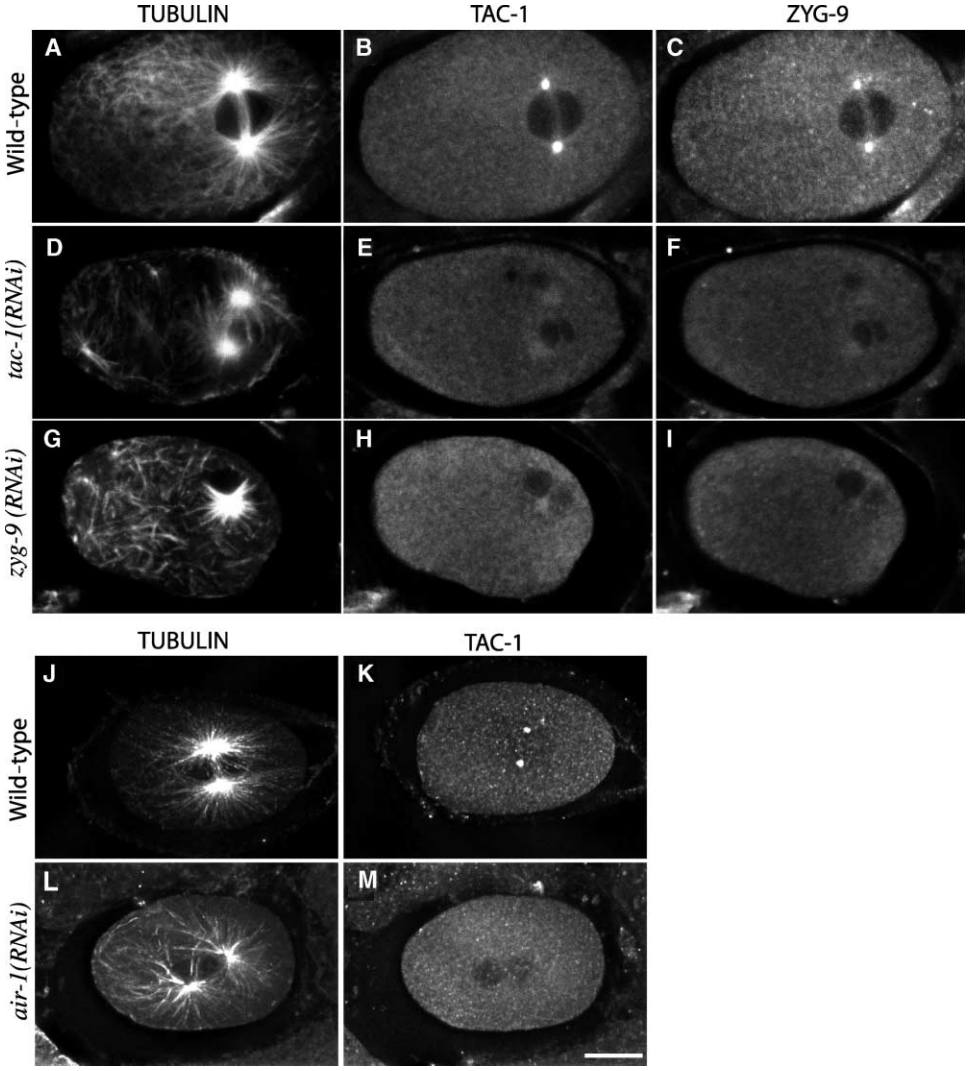

Figure 4. Localization of TAC-1 and ZYG-9 to Centrosomes Is Interdependent, and TAC-1 Localization is Regulated by AIR-1

(A–I) (A–C) Wild-type, (D–F) *tac-1(RNAi)*, and (G–I) *zyg-9(RNAi)* embryos stained for MTs (left), TAC-1 (middle), and ZYG-9 (right). (A–C) A wild-type embryo showing colocalization of TAC-1 and ZYG-9 at the centrosomes. (D–F) *tac-1(RNAi)* 1-celled embryo. In conditions where 80% of *tac-1(RNAi)* embryos show undetectable TAC-1, 89% of embryos showed undetectable ZYG-9 at the centrosome as shown (n = 20). (G–I) *zyg-9(RNAi)* 1-celled embryo. In conditions where 100% of *zyg-9(RNAi)* embryos show undetectable ZYG-9, 80% of *zyg-9(RNAi)* embryos showed undetectable TAC-1 at the centrosome as shown (n = 10).

(J–M) (J and K) Wild-type and (L and M) *air-1(RNAi)* embryos stained for MTs (left) and TAC-1 (right). Centrosomal TAC-1 staining was strongly reduced in 100% of early embryos (n = 7). The scale bar represents 10 μm.

for localization of the ZYG-9 homolog Minispindles (MSPs) to the mitotic [25, 26] and meiotic [27] spindle poles. These proteins were also shown to interact physically [26]. In contrast to our results, the amount of D-TACC associated with the centrosomes is not dramatically affected in a *msps* mutant. Taken together, these results have led to a model whereby D-TACC tethers MSPs to the centrosome. However, all available *msps* alleles produce a significant amount of protein [25], making it difficult to conclusively analyze the requirement of *msps* for D-TACC localization at the centrosome. The codependence we identified between TAC-1 and ZYG-9 suggests that the two proteins may carry out their functions as a complex, rather than one targeting the other to the centrosome. It will be important to further investigate interactions between these proteins in *Drosophila* and other systems to see if they indeed function as a complex.

Although concentrated at MT minus ends, XMAP215 family members primarily influence plus end dynamics [15], and recent work suggests that this is through regulation of the pause state between MT growth and shrinkage [28, 29]. It has been postulated that localization at the centrosome allows efficient recruitment to the plus ends of the newly formed MTs, as movement away from the centrosomes was seen for *Drosophila* D-TACC-GFP [26]. Similarly, we have observed TAC-1 on the metaphase plate, where plus ends lie (Figure 3C). Future imaging improvements and studies in the *C. elegans* embryo may allow better visualization and understand-

ing of the mechanism by which TAC-1 and ZYG-9 affect MT dynamics.

Supplemental Data

Supplemental Data including Experimental Procedures, movies corresponding to Figure 1, and results showing the specificity of the anti-TAC-1 antibodies as well as the dependence between TAC-1 and TBG-1 (γ-tubulin) for their localization at the centrosome are available at http://www.current-biology.com/cgi/content/full/13/17/1499/DC1/.

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