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A highly conserved centrosomal kinase, AIR-1, is required for accurate cell cycle progression and segregation of developmental factors in *Caenorhabditis elegans* embryos

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SUMMARY

***S. cerevisiae* Ipl1, *Drosophila* Aurora, and the mammalian centrosomal protein IAK-1 define a new subfamily of serine/threonine kinases that regulate chromosome segregation and mitotic spindle dynamics. Mutations in *ipl1* and *aurora* result in the generation of severely aneuploid cells and, in the case of *aurora*, monopolar spindles arising from a failure in centrosome separation. Here we show that a related, essential protein from *C. elegans*, AIR-1 (Aurora/Ipl1 related), is localized to mitotic centrosomes. Disruption of AIR-1 protein expression in *C. elegans* embryos results in severe aneuploidy and embryonic lethality. Unlike *aurora* mutants, this aneuploidy does not arise from a failure in centrosome separation. Bipolar spindles are formed in the absence of AIR-1, but they appear to be disorganized and are nucleated by abnormal-looking centrosomes. In addition to its requirement during mitosis, AIR-1 may regulate microtubule-based developmental processes as well. Our data suggests AIR-1 plays a role in P-granule segregation and the association of the germline factor PIE-1 with centrosomes.**

Key words: Aurora, *Caenorhabditis elegans*, Microtubule, Centrosome, PIE-1, P-granule

INTRODUCTION

Several microtubule-based events must occur for a cell to complete mitosis and faithfully segregate its chromosomes to each daughter cell (Sorger et al., 1997). The interphase array of microtubules must be broken down, and the centrosome must be duplicated to produce a pair of centrosomes that separate and migrate to opposite sides of the nucleus. The microtubule-nucleating capacity of the centrosomes must be greatly increased and the microtubules emanating from the centrosomes must contact the chromosomes via an association with the kinetochores. The forces generated by a number of motor proteins present on the spindle microtubules, kinetochores and chromosomes must also cooperate to form a functional bipolar spindle (Barton and Goldstein, 1996; Nigg et al., 1996). These processes are tightly regulated by $p34^{cdc2}$ and a number of other protein kinases (Vandre and Borisy, 1989; Nicklas et al., 1993; Verde et al., 1990; Buendia et al., 1992; Nasmyth, 1996; Nigg et al., 1996; Blangy et al., 1995). However, many of the proteins involved in the phosphorylation cascades controlling these events have not yet been identified (Nasmyth, 1996).

An increasing body of evidence suggests that the Ipl1 and Aurora family of protein kinases plays a key role in regulating mitotic events (Chan and Botstein, 1993; Francisco et al., 1994; Glover et al., 1995; Gopalan et al., 1997; Kimura et al., 1997). Ipl1 (increase-in-ploidy) was identified in a screen for mitotic mutants that failed to undergo normal chromosome segregation in *S. cerevisiae* (Chan and Botstein, 1993). Temperature-sensitive (ts) *ipl1* mutants suffer from severe chromosome nondisjunction that eventually results in the accumulation of unbudded, polyploid cells at the restrictive temperature (Chan and Botstein, 1993). Further cytological and immunochemical analysis of *ipl1* mutant cells has revealed no obvious defects in spindle pole body duplication, separation or bipolar spindle formation (Francisco et al., 1994). *Ipl1* encodes a putative serine/threonine protein kinase (Francisco et al., 1994).

Genes highly related to *ipl1* have recently been identified in *Drosophila*, mouse and humans. Mutations at the *Drosophila aurora* locus result in pupal lethality and mitotic arrest that is characterized by the presence of monopolar spindles (Glover et al., 1995). Less severe mutations result in female sterility where embryos from *aurora* mutant mothers also show defects in spindle morphogenesis that apparently arise from a failure in centrosome separation (Glover et al., 1995). Two recently published reports describing a mammalian relative of Ipl1 and Aurora, called IAK-1 or AIK-1, have both shown that IAK-1/AIK-1 is a mitotically active protein kinase that is localized to the centrosomes and microtubules of the mitotic spindle (Gopalan et al., 1997; Kimura et al., 1997). Further

experiments show that, although mammalian IAK-1 cannot substitute for Ipl1 function in yeast cells, it does appear to participate in the same pathway since its expression enhances the defects seen in an *ipl1* ts mutant (Gopalan et al., 1997). Together, these results suggest that the Ipl1 and Aurora family of protein kinases is highly conserved and that its members regulate mitotic spindle function and chromosome segregation.

Microtubule dynamics are central not only to the events of mitosis, but also to the developmental processes that generate cellular asymmetry and embryonic polarity (Nelson and Grindstaff, 1997; Guo and Kemphues, 1996a; Gonczy and Hyman, 1997; Schierenberg and Strome, 1992). In the soil nematode, *C. elegans*, microtubules and centrosomes are required for the proper orientation of cleavage axes, and are thought to be partially responsible for the accurate localization of P-granules and PIE-1 to germline blastomeres (Hyman and White, 1987; Hyman, 1989; Hird et al., 1996; Mello et al., 1996). To further define the role of the Ipl1 and Aurora family of protein kinases in mitosis and to address the possible role of this kinase family in microtubule dynamics during development, we have identified and characterized two members of this family found in *C. elegans*, AIR-1 and AIR-2 (Aurora/Ipl1 related). Here we show that AIR-1 is required for embryogenesis and that it is associated with mitotic centrosomes. In addition to participating in mitotic spindle formation and chromosome segregation, AIR-1 also appears to be required for microtubule-based developmental processes. We show that the proper segregation of P-granules and the association of PIE-1 with centrosomes can be disrupted in the absence of AIR-1. The localization of PIE-1 to germline blastomeres has been suggested to be dependent on its association with centrosomes (Mello et al., 1996). Although AIR-1 appears to be necessary for the association of PIE-1 with centrosomes, our results suggest that this association is not necessary for the localization of PIE-1 to specific blastomeres. Rather, PIE-1 may be properly segregated via its association with P-granules.

MATERIALS AND METHODS

air-1 and *air-2* cDNA synthesis

Total RNA was prepared from N2 gravid hermaphrodites by standard methods. First strand cDNA was prepared with a GeneAmp RT-PCR kit (Perkin-Elmer, Branchburg, NJ) as described by the manufacturer. *air-1* and *air-2*-specific cDNAs were PCR amplified as described in the text. PCR products were gel-purified using Wizard columns (Promega, Madison, WI) and cloned into a pBluescript vector (Stratagene, La Jolla, CA). Automated DNA sequencing of each cDNA was performed using standard methods. The cDNA sequences confirmed the predictions of Genefinder, a program used by the *C. elegans* Sequencing Consortium, except as described in the text. Amino acid sequence alignments were performed using the NCBI Blast Program, and the ClustalW1.7 Multiple Sequence Alignment Program (Higgins et al., 1996), accessed through the Baylor College of Medicine Search Launcher, Houston, TX.

AIR-1 antibody production

A peptide corresponding to the carboxyl 12 amino acids of the predicted AIR-1 protein was coupled to KLH by standard methods and injected into rabbits. Rabbits were boosted for a period of 6 months and final bleeds were collected. For affinity-purification, the AIR-1 antigenic peptide was coupled to BSA and immobilized using an AminoLink Kit (Pierce Biochemical, Rockford, IL) as described by the manufacturer. Using this column, AIR-1-specific antibodies were purified essentially as described by Walczak et al. (1996).

Immunocytochemistry

Immunocytochemistry experiments and gonad dissections were performed on N2 gravid hermaphrodites and *air-1* antisense RNA-injected hermaphrodites essentially as described by Seydoux and Dunn (1997). Anti-α-tubulin antibodies were purchased from Sigma (St. Louis, MO) and the P-granule-specific antibody OIC1D4 (Strome and Wood, 1983) was obtained from the Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA). Monoclonal PIE-1 and rabbit polyclonal P-granule-specific antibodies were generous gifts of C. Schubert (FHCRC, Seattle, WA) and S. Strome (Univ. of Indiana, Bloomington, IN), respectively. Immunofluorescence microscopy was performed with a Nikon Microphot FXA Microscope and a Zeiss Confocal Microscope. Images were processed using Adobe Photoshop (Adobe, Mountain View, CA).

Antisense RNA injections

Antisense RNA corresponding to the entire coding region of the *air-1* cDNA was synthesized using a T7 or T3 In Vitro Transcription Kit (Ambion, Austin, TX) as described by the manufacturer. L4 and adult N2 hermaphrodites were injected by standard methods (Mello et al., 1991) and were allowed to recover for 12-24 hours before fixation for immunocytochemistry.

RESULTS

Cloning *air-1* and *air-2* cDNAs from *C. elegans*

We have identified two new members of the Aurora/Ipl1 protein kinase family in the *C. elegans* genome database. The *air-1* genomic sequence was found on cosmid K07C11 from chromosome V, and the *air-2* genomic sequence was found on cosmid B0207 from chromosome I. An oligonucleotide primer corresponding to the SL1 trans-spliced leader RNA (found on the majority of *C. elegans* mRNAs) (Blumenthal, 1995), and primers specific for the region surrounding the predicted translation stop codon of the *air-1* and *air-2* sequences were used to RT-PCR amplify both cDNAs from total RNA isolated from gravid adult *C. elegans* hermaphrodites. Sequencing of both cDNAs revealed that they each lacked the first exon predicted by the *C. elegans* Sequencing Consortium, but otherwise reflected the predicted exon structures. An alignment of the predicted protein sequences for AIR-1, AIR-2, IAK-1, Aurora and Ipl1 showed that all five coding regions share a high degree of homology throughout the predicted kinase domain and only diverge significantly at their amino termini (Fig. 1). Further analysis of AIR-2 will be presented elsewhere (J. M. S., unpublished data).

Immunolocalization of AIR-1 in *C. elegans* embryos

To determine the subcellular location of the AIR-1 protein, a rabbit polyclonal antiserum was raised against a synthetic peptide corresponding to the carboxyl-terminal 12 amino acids of AIR-1. Immunocytochemistry using affinity-purified antibodies on fixed *C. elegans* embryos of varying ages revealed that AIR-1 is present on mitotic centrosomes (Fig. 2). Counter-staining with DAPI and anti-α-tubulin antibodies confirmed these conclusions (Fig. 2C-E). Immunostaining was not seen with preimmune sera and was entirely competed by

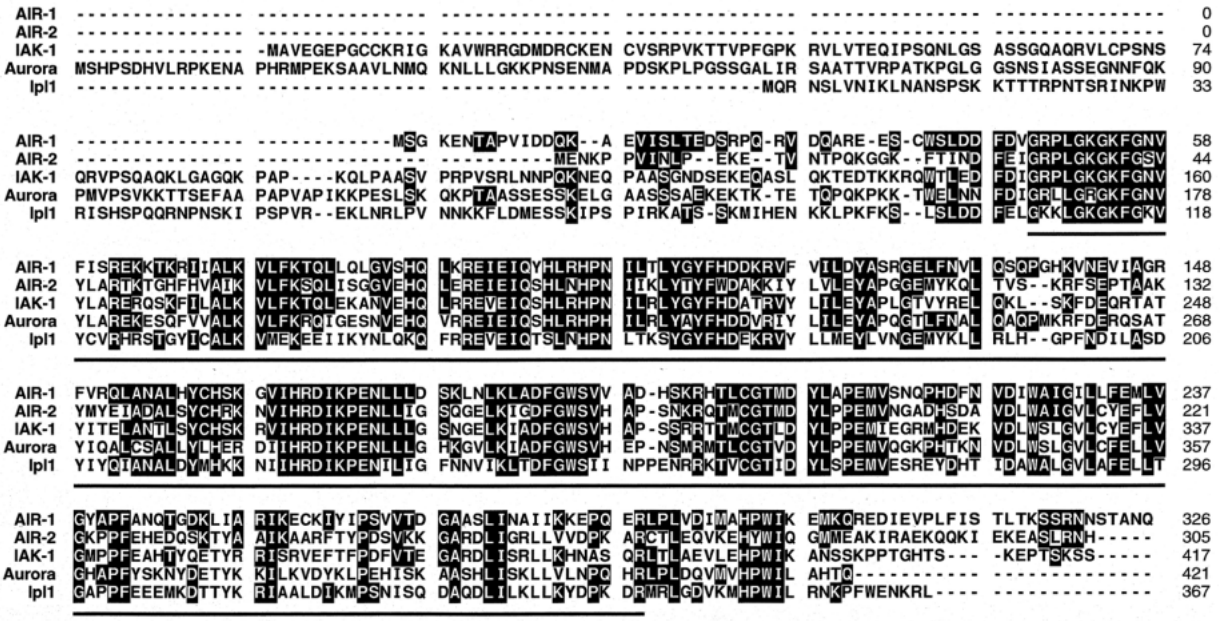

Fig. 1. An alignment of the predicted protein products of the AIR-1 (Genbank accession number AF071206), AIR-2 (AF071207), IAK-1 (AF007817), Aurora(PIR locus number A56220), and Ipl1(Genbank accession number U07163) proteins is shown. Black shading indicates residues that are identical to AIR-1. The predicted kinase domain is underlined. The AIR-1 kinase domain has the following amino acid identities and similarities with the kinase domains of 1) Aurora: 51% identical, 73% similar; 2) IAK-1: 55% identical, 71% similar; 3) AIR-2: 51% identical, 69% similar; 4) Ipl1: 43% identical, 67% similar.

preincubation of the AIR-1 antibody with the antigenic peptide (data not shown). Diffuse cytoplasmic staining was found during interphase but, as cells entered mitosis, AIR-1 became localized to the centrosomes and remained associated with them throughout the mitotic phase of the cell cycle. Developmentally, AIR-1 was diffuse throughout oocytes and was not detected on the centrosomes of the sperm pronucleus (data not shown). AIR-1 staining of centrosomes was initially detected in the first mitotic division following pronuclear fusion, and was present in all mitotic cells up to the limits of detection in the hundreds of cells comprising late-stage embryos (data not shown). In the adult, AIR-1 staining was also found on the centrosomes of mitotic germ cells in the distal gonad (data not shown). These expression patterns suggest that AIR-1 is likely to be both a maternally supplied and a zygotically expressed gene product.

Disruption of AIR-1 expression by RNA-mediated interference

We disrupted the function of AIR-1 during embryogenesis by injecting *air-1* antisense RNA, corresponding to the entire coding sequence of the *air-1* cDNA, into the syncytial gonads of adult *C. elegans* hermaphrodites. Injection of antisense, sense or double-stranded RNA into the gonads of wild-type hermaphrodites has been shown to result in gene-specific loss-of-function phenotypes in the embryos of injected mothers (Guo and Kemphues, 1996b; Powell-Coffman et al., 1996, Fire et al., 1998). This method of disrupting protein expression is commonly referred to as RNA-mediated interference (RNAi) (Rocheleau et al., 1997). Although the mechanism is not understood, it presumably acts by depleting maternally provided mRNA and may also prevent the expression of zygotic transcripts (Fire et al., 1998). Injection of *air-1* antisense RNA completely abolished AIR-1 staining in the embryos of injected animals (Fig. 3), but did not affect the staining pattern of the highly related AIR-2 kinase (data not shown). Loss of AIR-1 protein expression resulted in embryonic lethality in 100% of the embryos laid 12 or more hours postinjection (>200 embryos/injected animal). By Normarski analysis, the embryos appeared to arrest as disorganized masses of more than 100 cells. DAPI staining revealed that these embryos were severely aneuploid, having numerous cells with very large polyploid nuclei, as well as cells that appeared to be completely anucleate (Fig. 3A,B). As controls, we injected RNAs corresponding to several unrelated cDNAs. None mimicked the effect of *air-1* antisense injection nor resulted in the loss of AIR-1 protein expression (data not shown). Disruption of AIR-2 protein expression via the same method resulted in a very different lethal phenotype (J. M. S., unpublished data). For simplicity, we will refer to AIR-1-deficient embryos as *air-1*(*RNAi*) embryos in the remainder of the text.

Immunofluorescent analysis of early *air-1*(*RNAi*) embryos

To determine how the *air-1(RNAi)* terminal phenotype arose, we dissected younger embryos from the gonads of injected mothers and examined them by DAPI, anti-AIR-1 and anti-α-tubulin staining (Fig. 3C-K). This analysis revealed that the AIR-1 protein was no longer detectable at any stage of the cell cycle (Fig. 3C-K). Furthermore, affected embryos had a variety of chromatin and microtubule abnormalities. To quantify these defects, embryos up to the 2-cell stage were classified as follows: (1) meiotic: newly fertilized embryos with maternal

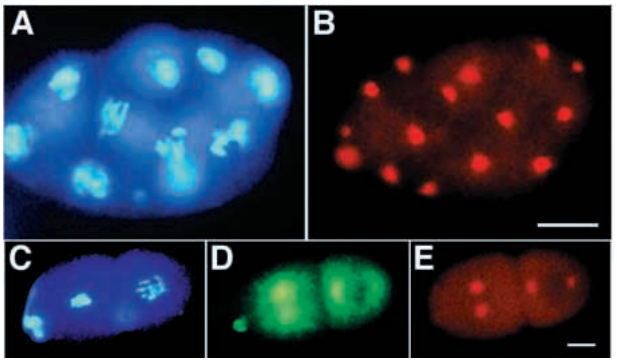

Fig. 2. (A,B) A wild-type *C. elegans* embryo was stained with DAPI (blue) and anti-AIR-1 antiserum (red). AIR-1 is associated with mitotic centrosomes. (C-E) A wild-type 2-cell embryo stained with (C) DAPI (blue), (D) anti-α-tubulin (green) and (E) anti-AIR-1 antiserum (red). Anterior is to the left in C-E. The two daughters of the first embryonic division in *C. elegans* are referred to as AB and P_1. The AB cell (at left) is in metaphase and the P_1 cell (at right) is in prophase. The polar bodies are apparent at the anterior of the embryo (at left, C). Bars, approx. 10 μm.

chromatin undergoing meiotic divisions I and II, (2) pronuclear: both the maternal and paternal pronuclei had decondensed but had not yet fused in the posterior half of the embryo, (3) 1-cell mitotic: embryos undergoing the first mitotic division after pronuclear fusion, and (4) 2-cell: embryos consisting of two cells (regardless of the amount of chromatin in each cell). *air-1*(*RNAi*) embryos of each classification were then scored for chromatin and microtubule defects (as compared to wild-type controls) (Tables 1, 2; Figs 4, 5).

air-1(*RNAi*) embryos have multiple chromatin defects

Meiotic stage *air-1*(*RNAi*) embryos did not differ significantly from wild-type controls. In pronuclear stage embryos, chromatin defects not often found in wild-type controls (Fig. 4A) were apparent in a significant fraction of the *air-1*(*RNAi*) embryos (Table 1). Defects included pronuclei that appeared to be polyploid or had string-like or hypercondensed chromatin and/or misplaced nuclei. However, by the first mitotic division, the percentage of *air-1(RNAi)* embryos displaying chromatin abnormalities increased dramatically. The various defects seen in these embryos are catalogued in Table 1 and examples of *air-1(RNAi)* embryos at various stages of the first cell cycle are shown in Fig. 4. Some of these embryos appeared to enter into the first mitotic division despite incomplete fusion of the maternal and paternal pronuclei (Table 1; Fig. 4E-L). Such events possibly contribute to the other chromatin organization problems that we have observed in these embryos. Wild-type metaphase and anaphase mitotic figures were not found in any of the 1-cell mitotic *air-1*(*RNAi*) embryos examined (n>84) (Table 1; Fig. 4M-T). Metaphase chromatin was not well condensed and did not congress in a tight band at the metaphase plate as in wild-type cells (Fig. 4M-P). In anaphase cells, chromatin bridges were often found between separating chromatin masses that were not well condensed (Fig. 4Q-T).

Of the 2-cell *air-1*(*RNAi*) embryos examined, 80% had clear chromatin defects as compared to wild-type controls (Table 1;

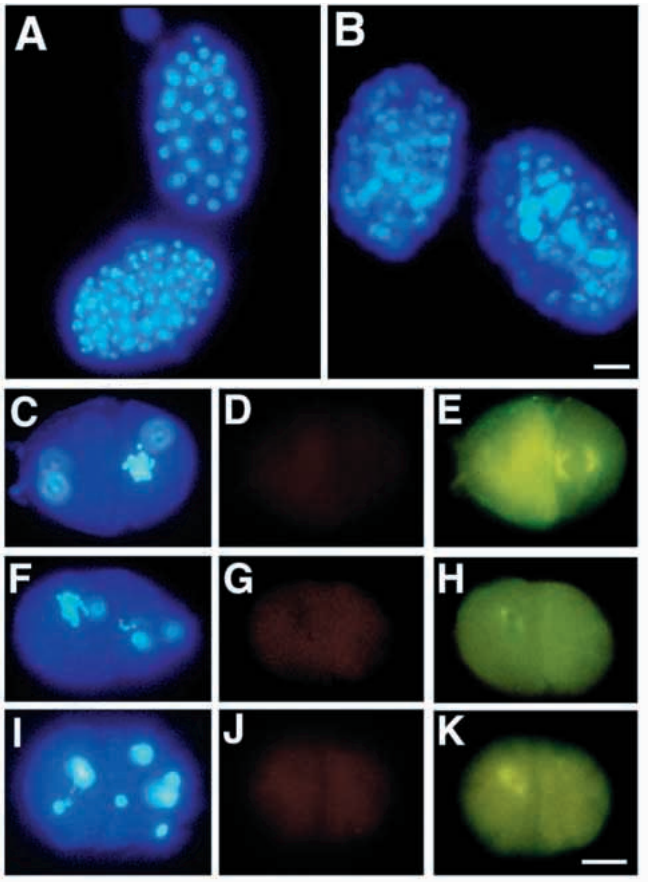

Fig. 3. (A) Wild-type *C. elegans* embryos were stained with DAPI. (B) Two *air-1*(*RNAi*) DAPI-stained embryos typical of the aneuploid 'terminal' phenotype are shown. Bar, approx. 5 μm. (C-K) Young embryos dissected from *air-1* antisense-injected mothers were fixed and stained with DAPI (left panel), anti-AIR-1 (middle panel) and anti-α-tubulin antiserum (right panel). The AIR-1 protein is not detectable over background staining in any of *air-1(RNAi)* embryos examined. *air-1(RNAi)* embryos have severe chromatin and microtubule defects. Bar, approx. 10 μm.

Fig. 5). These included similar defects to those seen in 1-cell mitotic *air-1(RNAi)* embryos. Additionally, AB and P_1 sister cells often did not have equal amounts of chromatin. In the most extreme cases, all chromosomes were found in a single cell (Table 1; Fig. 5E-H). Interestingly, a significant fraction of the embryos also had AB and/or P_1 cells that contained two or more nuclei that had progressed into the next cell cycle (Table 1). These results suggest that, in addition to chromosome segregation defects, *air-1*(*RNAi*) embryos may also be defective in cell cycle progression and/or cytokinesis.

air-1(RNAi) embryos have abnormal mitotic spindles

To address the effect of the loss of AIR-1 protein expression on microtubule dynamics during mitosis, the DAPI and anti-α-tubulin-stained *air-1*(*RNAi*) embryos described above were rescored for the presence and appearance of mitotic centrosomes and spindles (Table 2; Figs 4, 5). Tubulin staining of meiotic and pronuclear stage *air-1(RNAi)* embryos was indistinguishable from wild-type controls (Table 2; Fig. 4A-D, and data not shown). However, nearly all of the *air-1(RNAi)*

embryos undergoing the first mitotic division had discernible defects in the formation and appearance of mitotic spindles. Tubulin staining of embryos that appeared to have a failure in pronuclear fusion revealed disorganized arrays of microtubules (Fig. 4G,H). Further defects at distinguishable stages of mitosis included the presence of interphase-like arrays of microtubules in cells with condensed chromatin (Table 2; Fig. 4I-P) and spindles that consisted of ill-defined, small centrosomes which nucleated fewer microtubules than wild-type centrosomes (Fig. 4I-P). Although spindles in *air-1*(*RNAi*) embryos were bipolar, they were not well organized and were distinctly different from similar-staged mitotic spindles found in wild-type cells (Fig. 4M-T). Similar microtubule defects to those seen in 1-cell mitotic embryos were also noted in almost all of the 2-cell *air-1*(*RNAi*) embryos examined (Table 2; Fig. 5). These results suggest that *air-1*(*RNAi*) embryos may be defective in the breakdown of interphase microtubule arrays and the subsequent nucleation of spindle microtubules from mitotic centrosomes.

A small fraction of 1-cell mitotic and 2-cell *air-1*(*RNAi*) embryos had cells with multiple centrosomes (these cells were invariably polyploid by DAPI staining; Table 2 and data not shown). Thus, as above, some *air-1*(*RNAi*) embryos appear to undergo multiple cell cycles in the absence of cytokinesis. Altogether, our data suggests that, in the absence of AIR-1, cell cycle progression is dramatically affected at the transition from interphase to mitosis and can also be defective in the coupling of the completion of mitosis with entry into the next cell cycle.

Defects in the orientation and timing of mitotic divisions in *air-1*(*RNAi*) embryos

In wild-type embryos, the first cleavage axis lies parallel to the long axis of the embryo (Fig. 4I,J,M,N,Q,R). In the 2-cell embryo, AB invariably divides before its sister P_1, and the cleavage axes are perpendicular to one another (Fig. 5A,B) (Guo and Kemphues, 1996a). The spindle in P_1 rotates to lie parallel to the long axis of the embryo (Hyman and White, 1987; Hyman, 1989). Examination of 1-cell mitotic and 2-cell *air-1*(*RNAi*) embryos by DAPI staining revealed that a small fraction of affected embryos had DNA and/or spindles that were dividing in the wrong orientation (Table 1). However, the majority of divisions in each cell type did occur in the proper orientation. Thus, although AIR-1 does appear to have a small affect on spindle orientation, it does not appear to be absolutely required for spindle rotation. An additional defect noted in 2-cell *air-1*(*RNAi*) embryos included a small fraction of embryos with synchronous divisions of AB and P_1 (Table 1).

Distribution of germline factors in *air-1*(*RNAi*) embryos

Since *air-1*(*RNAi*) embryos showed chromosome segregation and microtubule defects as early as the first mitotic division, we wanted to determine whether cells resulting from such

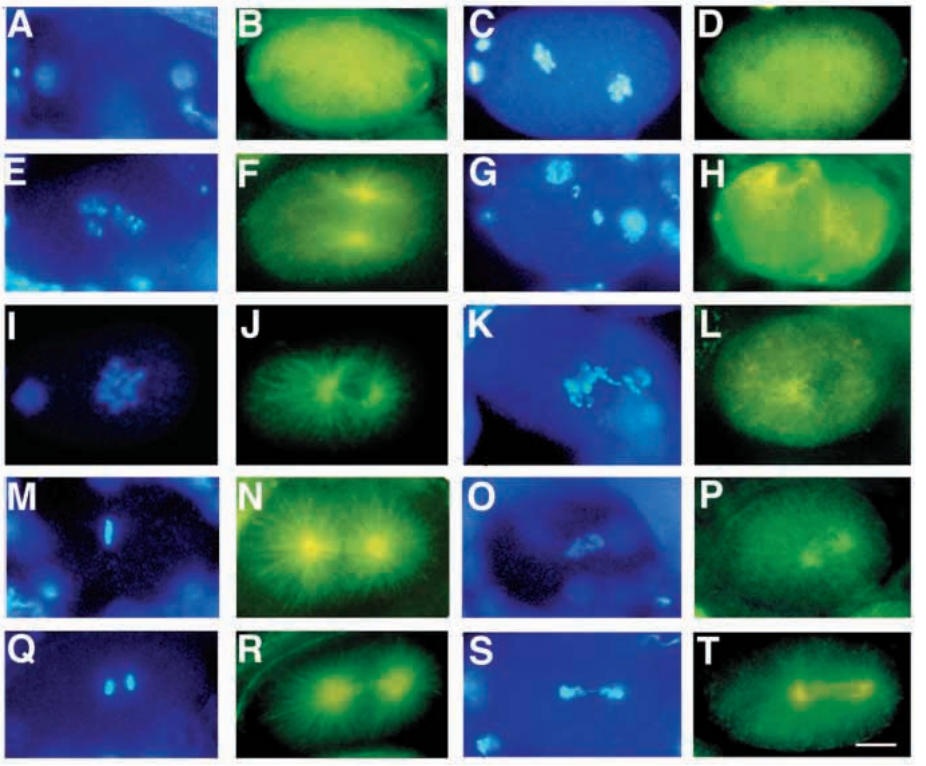

Fig. 4. Early stage wild-type and *air-1*(*RNAi*) embryos were dissected from adult hermaphrodites, fixed and stained with DAPI (A,C,E,G,I,K,M,O,Q,S) and an α-tubulin-specific antibody (B,D,F,H,J,L,N,P,R,T). Anterior is to the left, posterior to the right in each panel. (A,B) Wild-type pronuclear stage embryo, one polar body is apparent at left. (C,D) An *air-1*(*RNAi*) pronuclear stage embryo, polar bodies are apparent at left (this embryo is at a slightly later stage in pronuclear migration than the wild-type pronuclear stage embryo in A,B). (E,F) A wild-type 1-cell embryo just after pronuclear fusion. The spindle in F has not yet rotated, but the centrosomes are readily apparent. (G,H) An *air-1*(*RNAi*) embryo in which the pronuclei have not fused. Free chromosomes are present. Microtubules in H are disorganized. (I,J) A wild-type 1-cell embryo in prometaphase. (J) The spindle has rotated to lie parallel to the long axis of the embryo. (K,L) An *air-1(RNAi)* embryo in prometaphase. Note the incomplete fusion of the pronuclei. (L) A spindle is barely visible against an interphase-like microtubule array. (M,N) A wild-type 1-cell embryo in metaphase. (N) The centrosomes are large and nucleate many microtubules that reach the cell cortex. (O,P) An *air-1(RNAi)* 1-cell embryo in metaphase or early anaphase. (O) The chromatin is not well condensed as in normal metaphase and anaphase figures. (P) A bipolar spindle consisting of smaller centrosomes and many fewer and shorter microtubules than wild type is apparent against a background of interphase-like microtubules. Note the very small size of the centrosomes as compared to those in N. (Q,R) A wild-type 1-cell embryo in anaphase. (Q) The wild-type chromatin is condensed and well separated. (R) The centrosomes are large and the spindle is well-organized. (S,T) An *air-1(RNAi)* 1-cell embryo undergoing an aberrant anaphase. (S) The chromatin is not well organized and a chromatin bridge is apparent. (T) A disorganized *air-1(RNAi)* bipolar spindle is clearly abnormal as compared to wild type (R). Bar, approx. 10 μm.

Table 1. Chromatin defects in meiotic, pronuclear, 1-cell mitotic and 2-cell *air-1(RNAi)* embryos

	Meiotic		Pronuclear		1-cell mitotic		2-cell			
DNA	Total	%	Total	%	Total	%	Total		%	
Normal	69/69	100	30/44	68	20/84	24	14/70		20	
Abnormal	0/69	0	14/44	32	64/84	76	56/70		80	
							AB		P_1	
							Total	%	Total	%
-polyploid			4/14[1]	29	11/64[2]	17	19/56[3]	34	22/56	39
-string-like chromatin[4]			5/14	36	22/64	34	5/56	9	8/56	14
-hyper-condensed chromatin			6/14	43	3/64	5	8/56	14	5/56	9
-misplaced nucleus[5]			3/14	21	5/64	8	5/56	9	1/56	2
-free chromosome(s)[6]					13/64	20	16/56	29	13/56	23
-chromatin bridges					20/64	31	12/56	21	11/56	20
-wrong orientation[7]					9/64	14	8/56	14	7/56	13
-failed pronuclear fusion					12/64	19				
-all chromosomes in one cell							0/56	0	5/56	9
-two or more nuclei/cell[8]							7/56	13	11/56	20
							Total		%	
Cell-cycle timing										
-synchronous							2/56		4	

Fixed *air-1(RNAi)* embryos were stained with DAPI and α-tubulin antibodies. All embryos were staged as meiotic, pronuclear, 1-cell mitotic or 2-cell embryos as described in the text. Embryos were then scored for the commonly found chromatin defects listed at left. Most embryos had multiple defects, therefore the percentages in each column do not add up to 100%.

[1]Pronuclear stage embryos were designated as polyploid if DAPI staining of the maternal and paternal pronuclei were not of equal intensity. DAPI-staining intensity was not quantitatively measured.

[2]1-cell mitotic embryos were designated as polyploid if DAPI staining intensity (as judged by eye) was significantly increased over wild-type controls of the same cell cycle stage, or if free chromosomes in addition to the normal chromosome complement were seen.

[3]A cell of a 2-cell-stage embryo was designated as polyploid if DAPI staining intensity (as judged by eye) relative to its sister cell was increased (see Fig. 5).

[4]String-like chromatin refers to abnormal chromatin that appears to be 'unraveling' or fragmenting and is less condensed than DNA found in wild-type cells at similar stages of the cell cycle.

[5]Nuclei were judged to be misplaced if their position deviated significantly from wild-type controls (as judged by eye). For instance, many of the misplaced nuclei present in AB cells were adjacent to the cell membrane between the AB and P_1 cells, a position that is not found in wild-type controls.

[6]A cell was designated as having a free chromosome or chromosomes if at least one chromosome was found to be well separated from the main chromatin mass of the cell.

[7]Wrong orientation refers to metaphase or anaphase cells that had chromosomes that were aligned in an abnormal orientation as compared to wild-type cells. For instance, some abnormal 1-cell mitotic embryos had anaphase figures that were perpendicular to the long axis of the embryo rather than parallel to the long axis as in wild-type cells of the same stage.

[8]Nuclei have progressed into the next cell cycle, indicative of a failure in cytokinesis.

aberrant mitoses maintain their cellular identity. Therefore, we examined whether P-granules are segregated properly in these embryos. P-granules are small proteinaceous particles consisting of RNA and several recently identified proteins, many of which have been implicated in RNA metabolism (Seydoux and Fire, 1994; Draper et al., 1996; Gruidl et al., 1996; Guedes and Priess, 1997). P-granules segregated to the posterior end of the embryo prior to the first mitotic division are retained at the posterior cortex of the P_1 cell, and continue to be partitioned to the germ lineage in each subsequent division (Strome and Wood, 1983; Hird et al., 1996). Staining of both wild-type and *air-1*(*RNAi*) embryos with an antibody specific for P-granules (OIC1D4; Strome and Wood, 1983) revealed that these particles were often mislocalized in *air-1*(*RNAi*) embryos (Fig. 6). As expected, P-granules were localized in the posterior blastomere of each wild-type embryo (Fig. 6A). In contrast, P-granules in *air-1*(*RNAi*) embryos were often found at both poles of the embryos (Fig. 6B, arrows), or clumped around nuclei in the middle of embryos (Fig. 6B, arrowheads). The bipolar localization was observed in younger embryos (1- and 2-cell stage), whereas the clumping of P-granules with nuclei tended to occur in older embryos (see below).

Given that P-granule segregation is defective in *air-1*(*RNAi*) embryos, we examined the localization of another germline factor, the PIE-1 protein. PIE-1 is localized to the germ lineage in *C. elegans* embryos and is transiently associated with P-granules in these cells (Mello et al., 1996). In wild-type animals, PIE-1 is localized to the posterior in postmeiotic 1-cell embryos and is segregated specifically to P_1 at the first division. During this division, PIE-1 staining is apparent on the posterior centrosome. As the P_1 cell begins to divide, PIE-1 again becomes localized to the centrosomes. As mitosis proceeds, the PIE-1 protein present on the centrosome destined for the somatic daughter disappears, whereas that on the germline destined centrosome persists. Concurrently, PIE-1 is specifically localized to the P_2 daughter cell. The same set of events occurs in subsequent divisions as PIE-1 is specifically localized to the germ lineage blastomeres P_3 and P_4. It has been proposed that this differential association of PIE-1 with the germline destined centrosome is involved in the specific localization of PIE-1 to the P-lineage blastomeres (Mello et al., 1996).

To address how PIE-1 and P-granules behaved in the same embryo, wild-type and *air-1*(*RNAi*) embryos were double-stained with a P-granule-specific rabbit polyclonal antibody and a mouse monoclonal antibody specific for PIE-1. In wild-type embryos, both PIE-1 and P-granules were localized to a

Table 2. Spindle and microtubule defects in meiotic, pronuclear, 1-cell mitotic and 2-cell *air-1(RNAi)*

	Meiotic		Pronuclear		1-cell mitotic		2-cell			
DNA	Total	%	Total	%	Total	%	Total		%	
Normal	20/20	100	17/17	100	1/16	6	1/32		3	
Abnormal	0/20	0	0/17	0	15/16	94	31/32		97	
							AB		P_1	
							Total	%	Total	%
-small spindle[1]					7/16	44%	7/32	22%	7/32	22%
-inappropriate interphase array[2]					7/16	44%	9/32	28%	10/32	31%
-multiple centrosomes[3]					2/16	13%	1/32	3%		
-fewer microtubules[4]					7/16	44%	7/32	22%	7/32	22%
-stretched midbody[5]					4/16	25%	1/32	3%	2/32	6%
							Total		%	
-both spindles in same orientation							2/32		6%	

Fixed *air-1(RNAi)* embryos were stained with DAPI and α-tubulin antibodies. All embryos were staged as meiotic, pronuclear, 1-cell mitotic, or 2-cell embryos as described in the text. Embryos were then scored for the microtubule defects listed at left. Most embryos had multiple defects, therefore the percentages in each column do not add up to 100%.

[1]Spindles were designated as 'small' if they had smaller centrosomes and/or shorter microtubules (microtubules do not reach the cell cortex) as compared to wild-type spindles at similar stages of the cell cycle (examples are shown in Figs 4 and 5).

[2]An interphase-like array was judged as inappropriate if it was found in a cell with condensed chromatin.

[3]Cells designated as having multiple centrosomes had more than two centrosomes per cell.

[4]Spindles were designated as having fewer microtubules if the microtubule density (as assessed by eye) was much less than a wild-type spindle (compare Fig. 4L,P with Fig. 4J,N).

[5]Anaphase spindles that were longer than wild type appeared to have 'stretched midbodies' as compared to wild-type controls (see Fig. 4T).

single posterior blastomere and PIE-1 staining of P-granules was seen (Fig. 7A-F). Strong staining of centrosomes was observed with the PIE-1 antibody (arrowhead, Fig. 7C). Increased PIE-1 staining of centrosomes was also apparent along with increased concentrations of P-granules at the future sites of germline blastomeres (arrowheads, Fig. 7E,F).

In *air-1*(*RNAi*) embryos, P-granules and PIE-1 were localized to a single blastomere in approximately half of the examined embryos (Table 3; Fig. 7G-I). In some cases, the staining blastomere was completely anucleate (data not shown). Thus, despite very significant defects in chromatin distribution, PIE-1 can still be localized to a single cell. Furthermore, although PIE-1 was readily apparent on mitotic centrosomes in wild-type embryos (Fig. 7C,F), it was never detected on centrosomes in *air-1*(*RNAi*) embryos (n>200 embryos) (Fig. 7G-O and data not shown). This result suggests that: (1) AIR-1 is required for PIE-1's association with centrosomes, and (2) this association is not absolutely required for PIE-1 localization to germline blastomeres. However, our results do not exclude the possibility that the PIE-1 protein was masked or present at undetectable levels on centrosomes in *air-1*(*RNAi*) embryos.

Although both P-granules and PIE-1 can be localized to a single blastomere in the absence of AIR-1, single-cell localization was most often seen in younger embryos, and was less common in older embryos (Table 3). Defects in P-granule and PIE-1 localization that were observed in 1-cell and 2-cell *air-1*(*RNAi*) embryos included the bipolar localization of both P-granules and PIE-1 (Fig. 7J-L), or bipolar P-granules with PIE-1 localized to only one pole (data not shown). In older embryos, P-granules were often mislocalized to more than one blastomere and, in the majority of these cases, the PIE-1 protein

Table 3. P-granule and PIE-1 localization in *air-1(RNAi)* embryos

P-granules	PIE-1	1-cell	2-cell	4-cell	>8-cell	Total No.	Total %
Localized	Localized	10	37	6	4	57	43
Localized	Absent	0	0	1	9	10	8
Localized	Mislocalized	0	1	2	0	3	2
Mislocalized‡	Localized*	3	6	2	0	11	8
Mislocalized‡	Absent	1	2	2	38	43	33
Mislocalized‡	Mislocalized*	3	1	0	0	4	3
Absent	Localized	0	3	0	0	3	2
Absent	Mislocalized	1	0	0	0	1	1
	Total:	18	50	13	51	132	100

air-1(RNAi) embryos were fixed and co-stained with DAPI, a P-granule-specific antibody, and antiserum specific for PIE-1. Embryos were scored for cell or nuclei number and the localization of P-granules and PIE-1. Localized indicates that P-granules or PIE-1 are found in a single blastomere at one end of the embryo. Mislocalized indicates that P-granules and/or PIE-1 are found in multiple blastomeres or are found in the middle of the embryo.

*PIE-1 remains localized with at least a portion of the P-granules

‡7/7 1-cell and 5/9 2-cell *air-1(RNAi)* embryos with mislocalized P-granules had P-granules present at both poles of the embryo.

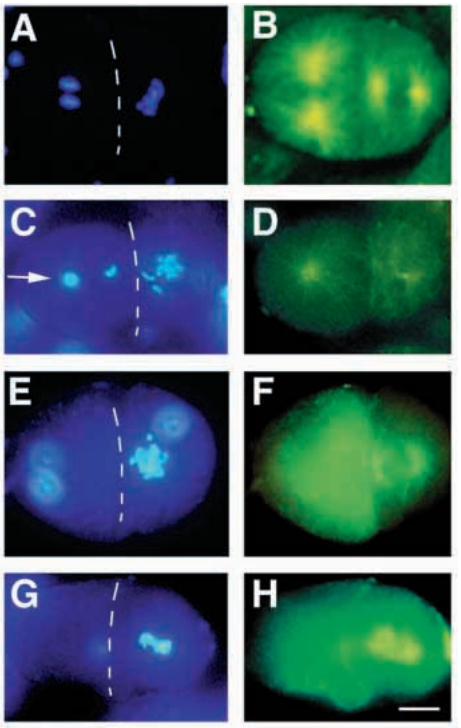

Fig. 5. 2-cell stage wild-type and *air-1(RNAi)* embryos were dissected from adult hermaphrodites, fixed and stained with DAPI (A,C,E,G) and an α-tubulin-specific antibody (B,D,F,H). The anterior AB cell is to the left and the posterior P_1 cell is to the right in each panel (cells are separated by a dashed line in A,C,E,G). In addition to the size differences between the two cells and the position of the polar bodies, embryos were counterstained with P-granule-specific antibodies to assist in the identification of the P_1 blastomere. (A,B) A wild-type embryo. The AB cell at left is in anaphase, P_1 is in prometaphase. Note that the spindles in AB and P_1 are perpendicular to one another. (C-H) *air-1(RNAi)* embryos showing various chromatin and microtubule defects. (C,D) An *air-1(RNAi)* embryo in which the AB nucleus is hypercondensed (arrow). Free chromosomes are apparent in both AB and P_1. A spindle is not apparent in any focal plane of P_1 although the chromatin appears to be in prometaphase. There are two separated centrosomes in AB (one is out of the focal plane) surrounding the hypercondensed chromatin in this cell. (E,F) An *air-1(RNAi)* embryo in which all of the chromosomes have been segregated to P_1. Out-of-focus DAPI staining at anterior are the polar bodies. (F) An abnormal bipolar spindle is apparent around the polyploid prometaphase nucleus in P_1 (compare to the P_1 spindle shown in B). The spindle has rotated to lie in the correct orientation. (G,H) An *air-1(RNAi)* embryo in which all the chromatin has been segregated to P_1. P_1 is in anaphase. Although the chromatin and mitotic spindle are both abnormal, the division is occuring in the correct orientation for P_1. Bar, approx. 10 μm.

was undetectable (Table 3). P-granules were often clumped around many nuclei in older embryos while PIE-1 staining was absent (Fig. 7M-O). These results suggest that mislocalized P-granules can persist more readily than mislocalized PIE-1 and that mislocalized PIE-1 may be preferentially degraded.

In summary, both P-granules and PIE-1 could be localized to a single blastomere in early *air-1*(*RNAi*) embryos. However, in older embryos, P-granule mislocalization became apparent and PIE-1 was not usually detectable. This contrasts with observations in wild-type embryos, where PIE-1 can be detected up to the 100-cell stage (Mello et al., 1996), and P-granules persist in the germline precursor cells (Z2 and Z3) throughout embryogenesis and are readily visible at all stages of postembryonic development (Strome and Wood, 1983). Ectopic P-granules were found in the absence of PIE-1, but PIE-1 was rarely found in the absence of P-granules. Although PIE-1's association with P-granules was apparent in these embryos, PIE-1 staining of centrosomes was never detected in the absence of AIR-1.

DISCUSSION

AIR-1 is associated with mitotic centrosomes

Here we have described the subcellular location and the consequences of disrupting the expression of the *C. elegans* AIR-1 protein, a member of the Aurora and Ipl1 family of protein kinases. To date, the subcellular location of only one other member of this protein kinase family has been described (Gopalan et al., 1997; Kimura et al., 1997). The mammalian protein IAK-1 has been shown to be associated with the duplicated centrosomes and microtubules of the mitotic spindle in tissue culture cells. AIR-1 is localized to mitotic centrosomes throughout *C. elegans* embryogenesis and is also present on the centrosomes of dividing cells in the adult, including the mitotic germ cells of the distal gonad (data not shown). These data suggest that other members of this protein kinase family, including Ipl1 and Aurora, may also be associated with microtubules, centrosomes and/or other microtubule organizing centers.

Disruption of AIR-1 expression results in severe aneuploidy

Injection of antisense RNA corresponding to the entire *air-1* cDNA into the gonads of adult hermaphrodites caused fully

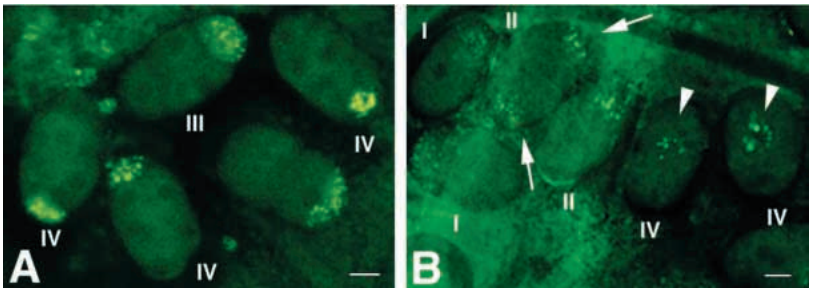

Fig. 6. Wild-type and *air-1(RNAi)* embryos were stained with the P-granule-specific OIC1D4 antibody. Roman numerals represent the following stages: (I) 1-cell embryo, (II) 2-cell embryo, (III) 4-cell embryo, (IV) 8-cell and older embryos. (A) P-granules in wild-type embryos are well localized to a single posterior blastomere. (B) P-granules in *air-1(RNAi)* embryos are often localized to a single blastomere in younger embryos (the 2-cell embryo (II) in the middle of B), although several 1- and 2-cell embryos revealed the presence of P-granules at both poles (2-cell embryo with arrows). P-granules tended to be mislocalized in older embryos, usually 'clumping' together in the middle of an embryo (arrowheads). Bar, approx. 10 μm.

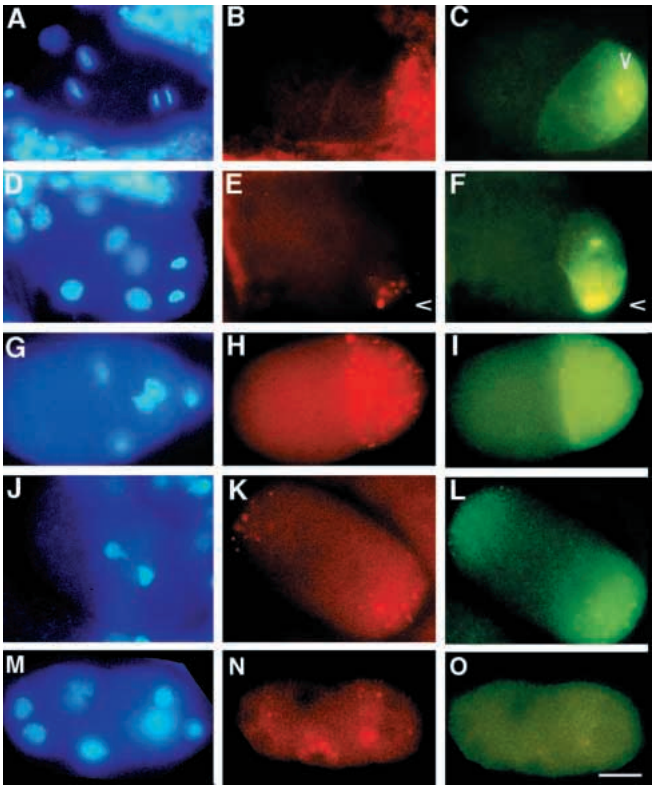

Fig. 7. Wild-type (A-F) and *air-1*(*RNAi*) embryos (G-O) stained with DAPI (left panel), a P-granule-specific antibody (middle panel) and a PIE-1-specific antibody (right panel) are shown. PIE-1 staining of centrosomes is apparent in C (arrowhead) and F. Arrowheads in E and F point out P-granule and PIE-1 accumulation at the future site of P_3. (G-I) An *air-1(RNAi*) embryo in which P-granules and PIE-1 are localized to a single blastomere. Note the very abnormal chromatin distribution in this embryo (G). (J-L) A 1-cell mitotic *air-1(RNAi)* embryo with (K) bipolar P-granules and (L) bipolar PIE-1. Note the anaphase chromatin bridge in J. (M-O) An example of an *air-1(RNAi)* embryo in which P-granules are 'clumped' around nuclei (N) and PIE-1 is not detectable (O). Bar, approx. 10 μm.

penetrant embryonic lethality in the resultant brood. Several studies in *C. elegans* have shown that RNA-mediated interference (RNAi) predictably reproduces phenotypes that are known to result from severe loss-of-function or null alleles (Guo and Kemphues, 1996b; Lin et al., 1995; Powell-Coffman et al., 1996; Rocheleau et al., 1997; Guedes and Priess, 1997). Most importantly, we and others have found that RNAi results in the loss of detectable protein expression in the affected embryos (Lin et al., 1995; Powell-Coffman et al., 1996).

The terminal phenotype of embryos produced by *air-1*(*RNAi*) was characterized as severe aneuploidy. Younger embryos displayed incomplete pronuclear fusion, numerous anaphase chromatin bridges, chromosome segregation defects and polyploid cells apparently arising from a failure to complete mitosis and/or cytokinesis. Mutations in both *ipl1* and *aurora* also result in chromosome segregation defects and polyploidy (Chan and Botstein, 1993; Glover et al., 1995). However, unlike *aurora*, the defects seen in *air-1*(*RNAi*) embryos do not arise from a failure in centrosome separation (Glover et al., 1995). Bipolar spindles are found in *air-1*(*RNAi*) embryos, as they are in *ipl1* mutant yeast cells (Francisco et al., 1994). Thus, AIR-1, like Ipl1, does not appear to be absolutely required for centrosome duplication, centrosome separation or centrosome migration to opposite sides of the nucleus.

Like *air-1*(*RNAi*) embryos, mutations in a gene encoding another component of mitotic centrosomes in *C. elegans*, *zyg-9*, also result in defects in pronuclear migration and fusion (Albertson 1984; Matthews et al., 1998). Zyg-9 appears to be involved in the control of microtubule length since microtubules in *zyg-9* mutants are much shorter than those in wild-type cells (Albertson 1984). Thus, other proteins like AIR-1 that have been implicated in microtubule dynamics at centrosomes also affect the premitotic events of pronuclear migration and fusion.

AIR-1 affects the formation of mitotic centrosomes and spindles

Although bipolar spindles are found in the absence of AIR-1, they appear to have fewer and shorter microtubules than normal (the microtubules do not reach the cell cortex as they do in wild-type spindles), and are found in cells that still contain an interphase-like array of microtubules. The centrosomes nucleating these mitotic spindles are also small and ill-defined. Our results are consistent with AIR-1 having a role in the transition from interphase microtubule arrays to the dynamic microtubules that are nucleated by mitotic centrosomes. Defects in this transition are likely to be the primary cause of the mitotic spindle and chromosome segregation abnormalities that we have described in *air-1*(*RNAi*) embryos. A phosphorylation cascade dependent on $p34^{cdc2}$ function has been shown to be necessary for this transition (Verde et al., 1990), and phosphorylation of centrosomal components is required for the increased nucleation of microtubules from mitotic centrosomes (Karsenti, 1991; Nigg et al., 1996). Our results suggest that AIR-1 may be an important structural component of centrosomes and its kinase activity may function in the phosphorylation cascades that are required for the recruitment of other centrosomal proteins and for the formation of the mitotic spindle.

The defects observed in *air-1*(*RNAi*) embryos are reminiscent of the defects seen in the *Drosophila* mutants *abnormal anaphase resolution (aar)* and *microtubule star (mts)* (Gomes et al., 1993; Mayer-Jaekel et al., 1993; Snaith et al., 1996). *aar* mutants are characterized by anaphase chromatin bridges and the presence of apparently intact, 'lagging' chromosomes similar to those seen in *air-1*(*RNAi*) embryos (Gomes et al., 1993; Mayer-Jaekel et al., 1993). *mts* mutant embryos die at the time of cellularization and are characterized by a block in anaphase progression (Snaith et al., 1996). Multiple centrosomes are seen in *mts* cellularized embryos, showing that, as in *air-1*(*RNAi*) embryos, centrosome duplication and DNA replication can be uncoupled from the completion of mitosis (Snaith et al., 1996). A similar uncoupling has also been noted in mutations at the *Drosophila gnu* locus (Freeman et al., 1986).

Both the *aar* and *mts* loci encode subunits of the *Drosophila* protein phosphatase 2A (PP2A; Mayer-Jaekel et al., 1993; Snaith et al., 1996). Additionally, mutations in one of the *Drosophila* isozymes of protein phosphatase 1 (PP1) are also characterized by mitotic defects, including abnormal sister

chromatid segregation, excessive chromosome condensation and polyploid cells with multiple centrosomes (Axton et al., 1990). Since mutations in these essential *Drosophila* phosphatase subunits phenocopy many of the defects seen in *air-1*(*RNAi*) embryos, AIR-1 is likely to be acting in parallel with mitotically active protein phosphatases in *C. elegans.* Interestingly, mutations at the *glc7* locus, which encodes the PP1 of *S. cerevisiae*, can suppress the mitotic defects of a temperature-sensitive allele of *ipl1* (Francisco et al., 1994). These experiments suggest that PP1 acts in opposition to the function of the Ipl1 kinase (Francisco et al., 1994).

Studies in *Xenopus* cell-free extracts have shown that PP1 and PP2A both play distinct roles in controlling mitotic microtubule dynamics (Tournebize et al., 1997). Possible substrates of these phosphatases include OP18 (Tournebize et al., 1997; Belmont and Mitchison, 1996) and microtubule-associated proteins (MAPs) whose binding to microtubules is negatively regulated by phosphorylation (Sorger et al., 1997). A number of motor proteins necessary for mitotic progression and chromosome segregation are also regulated by phosphorylation, including the centrosome-associated kinesin, Eg5 (Barton and Goldstein, 1996; Nigg et al., 1996; Blangy et al., 1995). The activity of such proteins may be modulated by the combined or opposing activities of protein kinases like AIR-1 and as yet unidentified protein phosphatases.

The localization of P-granules and PIE-1 is defective in *air-1*(*RNAi*) embryos

In addition to a requirement during mitosis, AIR-1 has a probable role in microtubule-based developmental processes as well. P-granules and PIE-1 are both germline factors that are thought to be partially dependent on microtubules for their localization. The initial posterior localization of P-granules in 1-cell embryos is dependent on a cytoskeletal-based streaming of P-granules and cytoplasm to the posterior, with a concomitant degradation of those granules that remain at the anterior (Strome and Wood, 1983; Hird et al., 1996). In 4-cell and older wild-type embryos, P-granules normally associate with the nuclear envelope and move with the nucleus to the future germline side of the cell. This process has been shown to be microtubule-dependent (Hird et al., 1996).

P-granules are localized to a single blastomere in over 50% of the 1-cell and 2-cell stage *air-1*(*RNAi*) embryos examined. This localization occurs despite gross abnormalities in chromatin distribution. However, segregation defects are apparent in older embryos (>8 nuclei), with the P-granules becoming clumped around nuclei that are not in the posterior of the embryo. The mislocalization that is seen in a portion of the younger embryos is usually characterized by P-granules being present at both poles of the embryo. Since P-granules are not found throughout *air-1*(*RNAi*) embryos, it appears that this early defect may not be in segregation per se, but rather may be due to defects in the proposed anterior degradation machinery. Indeed, segregation of P-granules in the 1-cell embryo has been shown to be dependent on microfilaments and is microtubule independent (Strome and Wood, 1983; Hill and Strome, 1988). However, in these experiments, embryos were treated with microtubule inhibitors at the pronuclear stage, thus they did not address whether there is an earlier requirement for microtubules in the establishment or maintenance of the proposed anterior degradation machinery (Hird et al., 1996). Since the mechanism by which the anterior degradation machinery functions is entirely unknown, the possible role of AIR-1 and/or microtubules in this process remains to be determined.

In P_2 and P_3, P-granules associate with germline-destined nuclei in a process that is microtubule-dependent (Hird et al., 1996). Hence, the mislocalization of P-granules that we find in older *air-1*(*RNAi*) embryos could be due to a primary defect in nuclear movement. Alternatively, since we have noted cases in which P-granules are present in anucleate cells, AIR-1 may be affecting a microtubule-based P-granule segregation system that is independent of nuclear behavior. Whether the two processes are coupled through a common regulatory mechanism that affects microtubule dynamics and whether AIR-1 is involved in a such a mechanism are important questions worthy of further investigation.

In addition to P-granules, the localization of another germline factor, PIE-1, is also affected in *air-1*(*RNAi*) embryos. PIE-1 represses transcription in the germline precursor cells, thus preventing somatic differentiation in these cells (Mello et al., 1992; Seydoux et al., 1996; Seydoux and Dunn, 1997). The localization of PIE-1 to the germline blastomeres has been thought to be dependent on its association with centrosomes and its perdurance on the centrosome that is destined for the germline daughter in each division (Mello et al., 1996). Although centrosome-staining of PIE-1 is readily apparent in wild-type embryos, we have never observed PIE-1 staining of centrosomes in *air-1*(*RNAi*) embryos. This suggests that the presence of AIR-1 on centrosomes is necessary for the association of PIE-1 with centrosomes. However, PIE-1 is still localized to a single blastomere in over 50% of the 1-, 2- and 4-cell embryos that we have examined. Thus, the association of PIE-1 with centrosomes does not appear to be essential for the localization of PIE-1 to germline blastomeres.

Our results instead suggest that the localization of PIE-1 is linked to its association with P-granules. Ectopic P-granules can exist in the absence of PIE-1, but the converse is very rarely true. Similar conclusions were reached in a recent study where the localizations of PIE-1 and P-granules were examined in a number of mutants in which P-granules are known to be mis-segregated (Tenenhaus et al., 1998). Immunofluorescence analysis of *mex-1* mutants also suggested that PIE-1 localization is linked to the segregation of P-granules (Guedes and Priess, 1997). However, neither of these studies addressed whether PIE-1's centrosome-association is necessary for its localization to germline blastomeres.

AIR-1 is involved in multiple microtubule-based processes

AIR-1 is a highly conserved centrosomal protein whose function is required during *C. elegans* embryogenesis. In the absence of AIR-1 protein expression, mitotic centrosomes are ill-defined and nucleate abnormal mitotic spindles that do not properly segregate chromosomes. Furthermore, P-granules are not properly localized at stages when P-granule segregation has been shown to be microtubule-dependent and the PIE-1 protein no longer associates with AIR-1-deficient mitotic centrosomes. In addition, a small fraction of *air-1(RNAi)* embryos also had defects in the orientation of cleavage axes.

Centrosomes are involved in establishing the orientation of the division axes in the early cleavages of *C. elegans* embryos

(Hyman and White, 1987; Hyman, 1989). The reorientation of the mitotic spindle that occurs in these divisions is influenced by microtubules that emanate from the centrosome to the cell cortex (Hyman and White, 1987). A number of mutations that affect the orientation and timing of these early cleavages have been identified. They are called *partitioning-defective*, or *par* mutants, and many of them are defective in the segregation of P-granules (Guo and Kemphues, 1996a; Nelson and Grindstaff, 1997; Cheng et al., 1995). Many of the *par* protein products have been identified and some have been shown to be asymmetrically distributed on the cell cortex (Guo and Kemphues, 1995; Boyd et al., 1996; Etemad-Moghadam et al., 1995; Levitan et al., 1994; Watts et al., 1996). PAR-1 is asymmetrically localized in the early embryo and is homologous to the mammalian MARK (MAP/microtubule affinity-regulating kinase) family of protein kinases (Guo and Kemphues, 1995; Drewes et al., 1997). The MARK protein kinases phosphorylate MAPs, and MARK overexpression results in the hyperphosphorylation of MAPs and in the disintegration of microtubule arrays in tissue culture cells (Drewes et al., 1997). Although the PAR proteins clearly influence the orientation of the early cleavage axes and may do so by affecting microtubule behavior, none of the PAR proteins have been shown to be associated with centrosomes. This is surprising given the central role of centrosomes in the orientation process (Hyman and White, 1988; Hyman, 1989). Perhaps the pleiotropic nature of the defects seen in *air-1(RNAi)* embryos is characteristic of other centrosomal proteins that influence microtubule dynamics, and thus may have prevented their identification in genetic screens for PAR phenotypes.

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