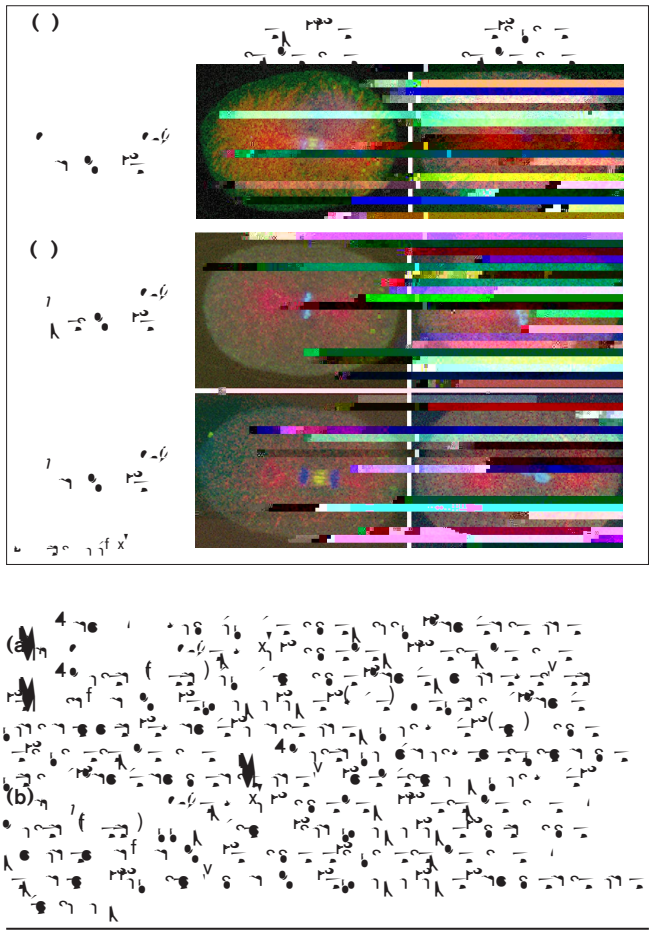
segregation and anaphase spindle elongation occur normally, ZEN-4 may be required specifically for cytokinesis.

While it is clear that MKLP-1 family members play an essential role in cytokinesis, little is known about the mechanisms that control their localization and activity.

F 2



Because *en-4* mutants did not share this defect (Figures 1 and 2a), ZEN-4 cannot mediate all AIR-2 functions.

AI -2 h — h

AIR-2 is required for chromosome segregation and ZEN-4 localization, which occur before cytokinesis. For this reason, we determined when, during the first cell cycle, AIR-2 was required for normal cytokinesis. When *air-2(or207 s)* mutant embryos were shifted to the restrictive temperature after the onset of anaphase, but before the initiation of a visible cleavage furrow, DNA segregation and cytokinesis occurred normally (Figure 3a,d; 7/7 embryos). Only when embryos were shifted to the restrictive temperature before anaphase did cytokinesis fail (Figure 3d; cytokinesis failed in 8/11 embryos shifted 2 minutes or more before anaphase). Conversely, downshift experiments showed that AIR-2 is not required during pronuclear migration, but first becomes necessary when the pronuclei meet, during prometaphase or metaphase (Figure 3f). Incomplete cytokinesis always coincided with defects in DNA segregation in these experiments (upshift, 15/15 embryos; downshift, 12/12 embryos). We conclude that AIR-2 functions at metaphase or early anaphase, and that the cytokinesis defect may result from an earlier requirement for AIR-2 in DNA segregation or ZEN-4 localization.

-4 —

To determine when ZEN-4 functions relative to AIR-2, we performed temperature-shift experiments using *en-4(or153 s)* mutant embryos (Figure 3). Downshift experiments established that ZEN-4 is dispensable until cytokinesis has begun: embryos maintained at the restrictive temperature, then shifted to the permissive temperature before or shortly after the initiation of cytokinesis, divided normally (Figure 3e; 5/5 embryos). Only when the downshift occurred late in cytokinesis were defects apparent (Figure 3e; cytokinesis failed in 11/19 embryos). Thus, ZEN-4 is required after AIR-2, consistent with ZEN-4 functioning downstream of AIR-2 in a pathway.

While we expected to find a requirement for ZEN-4 during cytokinesis, we were surprised to find that ZEN-4 is also required to maintain the separation of daughter cells well after the apparent completion of cytokinesis. If two-cell stage embryos were shifted to the restrictive temperature more than 4 minutes before nuclear envelope breakdown during the ensuing mitosis, the plasma membrane regressed to reform a single cell (Figure 3a,c; 6/6 embryos). Only when shifted within 4 minutes of nuclear envelope breakdown — over halfway through interphase — were cleavage furrows maintained (Figure 3c; 7/7 embryos). We conclude that ZEN-4 acts late in cytokinesis, and to maintain cell separation.

AI -2 , , -4 h —

ZEN-4 accumulates at the spindle midzone during metaphase and anaphase, when AIR-2 is required for cytokinesis. We therefore determined whether AIR-2 is required for ZEN-4 localization at the same time it is required for cytokinesis. When we shifted *air-2(or207 s)* mutant embryos to the restrictive temperature before or during pronuclear migration (Figure 4a; 8/8 embryos), or during metaphase (Figure 4b; 3/3 embryos), chromosome segregation was defective and ZEN-4 was not detectable in the central spindle. In contrast, when *air-2(or207 s)* embryos were shifted to the restrictive temperature in early anaphase, then fixed approximately 2–3 minutes later, after cleavage furrows had ingressed over 50% of the diameter of the embryo, ZEN-4 localization and DNA segregation both appeared normal (Figure 4b, 3/3 embryos). Therefore, AIR-2 may be required only for the initial localization of ZEN-4 at the mitotic spindle but not for its maintenance (see Discussion). Furthermore, the temporal requirement for AIR-2 in ZEN-4 localization coincides with the requirement for AIR-2 in cytokinesis, consistent with AIR-2 functioning through the localization of ZEN-4.

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-4 — —

Although AIR-2 may act in a pathway with ZEN-4, the defects in ZEN-4 localization and in cytokinesis could also result indirectly from the failure of *air-2* mutants to segregate DNA. To address this issue, we observed cytokinesis in embryos lacking the *C. elegans* centromere protein-A (CENP-A) homolog HCP-3, which localizes to kinetochores and is required for chromosome segregation [17]. Cleavage furrows ingressed successfully through reforming nuclei in these embryos, resulting in the formation of closely opposed daughter nuclei connected by a DNA bridge (Figure 5a; 5/5 embryos). Furthermore, ZEN-4 localized normally to the central spindle in *hcp-3(RNAi)* embryos (Figure 5b; 7/7 embryos), indicating that proper DNA segregation was not required for ZEN-4 localization or the formation of interzone microtubules. To compare the segregation defects in *air-2* and *hcp-3* mutant embryos in the absence of cytokinesis, we examined *hcp-3(RNAi); en-4(or153 s)* double mutant embryos. A single, large nucleus reformed in these embryos (5/5 embryos), as occurred in

AIR-2 and ZEN-4 and the FH protein CYK-1. CYK-1 is required for cleavage furrow ingression early in cytokine-

Alternatively, the cytokinesis defects in *air-2* embryos may result indirectly from the failure to segregate DNA [13]. Here, we have established that AIR-2 is required at metaphase or early anaphase for ZEN-4 localization, whereas ZEN-4 first acts late in cytokinesis. Mutations in either gene enhanced the cytokinesis defects in *cyk-1* mutant embryos. Because defects in DNA segregation were not sufficient to disrupt cytokinesis in other mutants, we conclude that AIR-2 and ZEN-4 function sequentially in a linear pathway that is specifically required for the completion of cytokinesis.

Our analysis of ZEN-4 suggests a role for this MKLP late in cytokinesis, after cleavage furrows have already ingressed extensively. In contrast, other MKLPs are required earlier in mitosis. For example, *Drosophila* Pav is required for the formation of the contractile ring in embryonic cytokinesis

2–3 minutes before the metaphase to anaphase transition failed to segregate DNA (Figure 3d), suggesting that AIR-2 activity is severely compromised under similar conditions. Alternatively, transient ZEN-4 localization may result from overexpression of ZEN-4–GFP, or from incomplete elimination of AIR-2 from the interzone of *icp-1(RNAi)* embryos. Although these issues remain unresolved, AIR-2 is clearly a key regulator of ZEN-4 localization during cytokinesis.

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In contrast to AIR-2, most Aurora-like kinases described to date appear to be dispensable for cytokinesis. *Drosophila* Aurora and *Xenopus* Eg2 are required for centrosome separation and formation of a bipolar spindle [25,26], whereas *S. cerevisiae* Ipl1p is required for chromosome segregation and bipolar spindle assembly [27,28]. Finally, a second *C. elegans* Aurora homolog, AIR-1, is required for formation of a normal spindle and the proper partitioning of developmental factors [29]. The mammalian Aurora-like kinase AIM-1, however, is required specifically for cytokinesis

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The mechanism and control of cytokinesis. 9:

Cytokinesis in eukaryotes: a mechanistic comparison. 11:

Towards a molecular understanding of cytokinesis. 10:

Establishment of the mechanism of cytokinesis in animal cells. 109: