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LETTER TO THE EDITOR

Stereotyped distribution of midbody remnants in early *C. elegans* embryos requires cell death genes and is dispensable for development

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Dear Editor,

The midbody is a structure formed within the intercellular bridge towards the end of cytokinesis [1]. Microtubules within this bridge are then severed on one side of the midbody during abscission, thus generating a midbody remnant in one of the resulting daughter cells. Midbody remnants persist long after cell division and accumulate preferentially in stem cells, induced pluripotent stem (iPS) cells, and cancer stem cells [2-4]. Upon induction of differentiation, midbody remnants are degraded by autophagy or released into the extracellular milieu in some tissue culture cells, and it has been proposed that such removal is critical for enabling a differentiation program [2-4]. However, the fate of midbody remnants in a developing organism remains elusive, and whether their presence plays a role in cell fate determination *in vivo* is not known.

We addressed these questions in early *Caenorhabditis elegans* (*C. elegans*) embryos. We performed time-lapse differential interfering contrast (DIC) and fluorescence imaging to track the midbody remnant generated during the cleavage of the zygote P_0 into the anterior blastomere AB and the posterior blastomere P_1 (Figure 1A and 1B). We also tracked the midbody remnants generated during the subsequent cleavages of AB into ABa and ABp, as well as of P_1 into EMS and P_2 (Figure 1A). We used GFP-tagged ZEN-4 to track midbody remnants, and either DIC or mCherry fused to a pleckstrin homology (PH) domain to visualize the periphery of cells [5]. Unexpectedly, we found that whereas the P_0 midbody remnant is present initially between the AB and P_1 blastomeres (Figure 1C-0 min and 1D-0 min, red arrowheads), it is then almost invariably internalized into the EMS blastomere or one of its immediate descendants (Figure 1B, 1C-15 min and 1D-20 min, red arrows, Supplementary information, Figure S1A, S1B, Movies S1 and S2). The P_0 midbody remnant is internalized and contains membranous material, as evidenced by the presence of mCherry-PH and the early endosomal marker GFP::RAB-5 (Supplementary information, Figure S1C-S1D), which is consistent with the fact that the midbody is internalized into the *C. elegans* Q neuroblast lineage later in development [5]. Furthermore, we found that the AB midbody remnant is also usually internalized by the EMS lineage in the early embryo (Figure 1C-20min and 1D-20 min, purple arrows, Supplementary information, Figure S1A, S1B, S2G and Movie S1). We also found that the P_1 midbody remnant is typically internalized by the P_2 blastomere (Figure 1C-20 min, yellow arrow, Supplementary information, Figure S1A, S1B and Movie S1). We conclude that midbody remnants have a stereotyped distribution in early *C. elegans* embryos (Figure 1A).

We next investigated the mechanisms directing the internalization of the P_0 and AB midbody remnants almost exclusively into the EMS lineage and not into ABa or ABp, which are also in contact with these midbody remnants. We first tested whether such stereotyped distribution is under the control of anterior-posterior (A-P) polarity cues. We used RNAi to deplete *par-2* and *par-3*, which are essential for the A-P polarity, and found that the P_0 and AB midbody remnants are internalized indiscriminately by either an anterior or posterior blastomere in such embryos (Figure 1B, Supplementary information, Figure S2C, S2D, S2F and S2G). Therefore, A-P polarity contributes to directing midbody remnant segregation in early *C. elegans* embryos.

What aspect of A-P polarity is relevant for the stereotyped internalization of midbody remnants observed in the wild-type? In the one-cell embryo, A-P polarity cues ensure that a larger net pulling force acts on the posterior spindle pole, thus enabling asymmetric spindle positioning and an unequal first cleavage [6]. We reasoned that this might impart a bias during abscission, so that the P_0 midbody remnant is positioned closer to the P_1 blastomere and thus gets internalized by its descendants of the EMS lineage. We set out to test this hypothesis by depleting the partially redundant Gα proteins GOA-1/GPA-16;

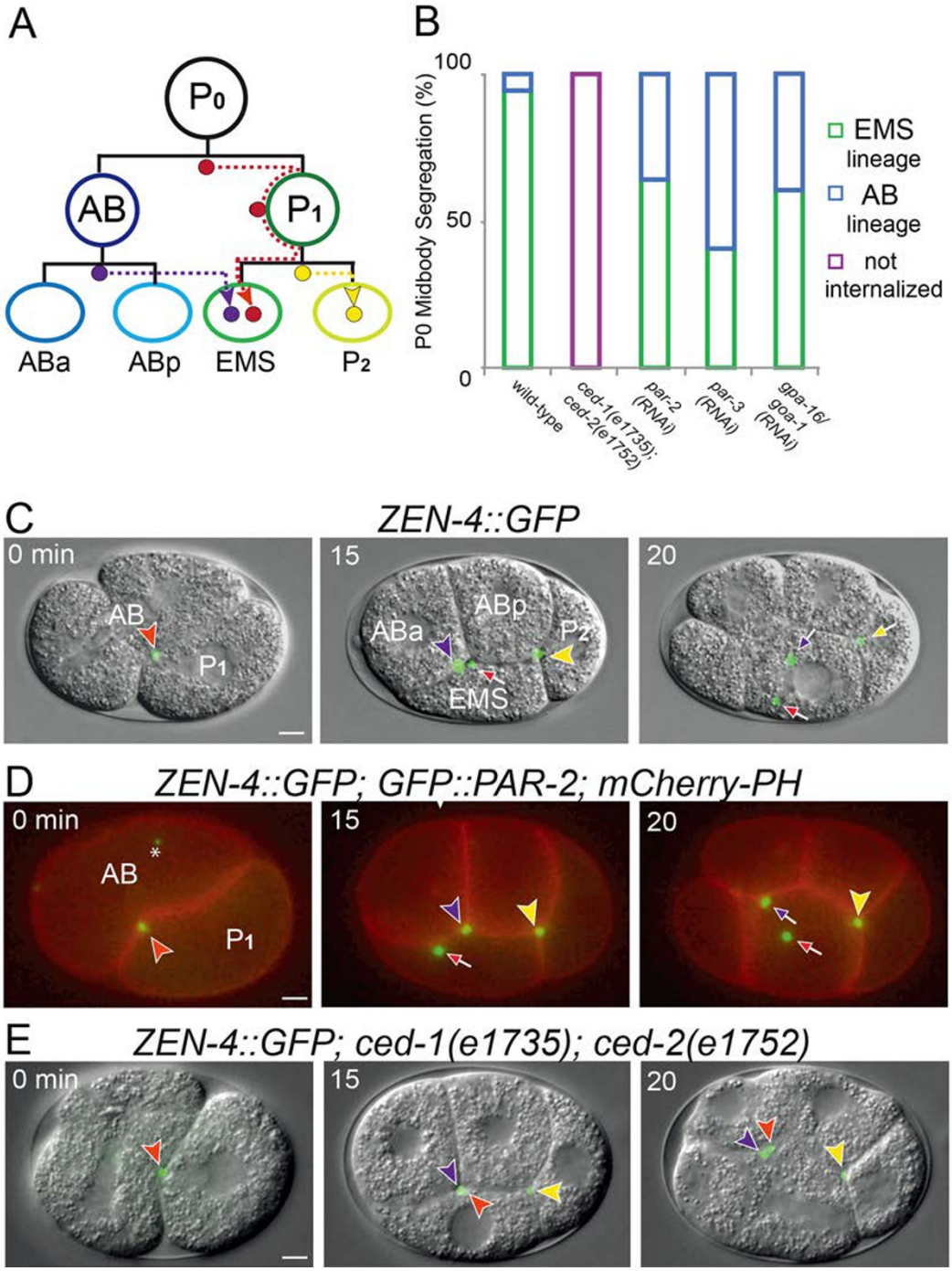

Figure 1 Stereotyped distribution of midbody remnants in early *C. elegans* embryos. **(A)** Schematics of the distribution of midbody remnants in early *C. elegans* embryos, along with the name of blastomeres. Color code of midbody remnants is as in **C**-**E**. **(B)** Distribution of the P_0 midbody remnant in embryos of the indicated genotypes. Color code of blastomeres is as in **A**. Wild-type, *N* = 18; *ced-1*(*e1735*); *ced-2*(*e1752*), *N* = 10; *par-2*(*RNAi*), *N* = 11; *par-3*(*RNAi*), *N* = 16; *goa-1*/*gpa-16*(*RNAi*), *N* = 10. Note that the identity of blastomeres is altered in *par-2*(*RNAi*) and *par-3*(*RNAi*) embryos, hence our referring to them as "anterior" (corresponding to ABa and ABp in the wild type) and "posterior" (corresponding to EMS and P_2 in the wild type) in the main text. **(C**, **E)** Images from dual DIC and time-lapse fluorescence microscopy of otherwise wild-type **(C)** or *ced-1*(*e1735*); *ced-2*(*e1752*) double-mutant embryos **(E)** expressing ZEN-4::GFP. The GFP signal has been overlaid onto the DIC image. See also Supplementary information, Movies S1, S2 and S3. **(D)** Images from time-lapse fluorescence microscopy of wild-type embryo expressing ZEN-4::GFP, GFP::PAR-2 and mCherry-PH. In **C**-**E**, midbody remnants are indicated initially by arrowheads (P_0: red, AB: purple, P_1: yellow). Similarly colored arrows indicate internalization of the corresponding midbody remnant. Blastomere names are indicated in the first two panels in **C**. Asterisk in **D** indicates polar body. Scale bars: 5 μm. Time is indicated in minute, starting from the onset of AB cytokinesis.

this results in one-cell embryos undergoing symmetric spindle positioning despite proper A-P polarity [7]. Remarkably, we found that *goa-1*/*gpa-16*(*RNAi*) embryos exhibit randomized segregation of the P_0 midbody remnant (Figure 1B and Supplementary information, Figure S2E). Distribution of the AB and P_1 midbody remnants is randomized as well in such embryos (Supplementary information, Figure S2G and S2H), perhaps as a consequence of the equal first division. Overall, we conclude that the stereotyped distribution of the P_0 midbody remnant, as well as that of the AB and P_1 midbody remnants, albeit perhaps indirectly, are due to the asymmetric positioning of the first mitotic spindle.

We investigated the mechanisms that permit the internalization of midbody remnants in early *C. elegans* embryos. The fact that the AB midbody remnant is incorporated into a cell from the EMS lineage, and not into one from the AB lineage, implies that it has been released into the extracellular milieu before being internalized. Midbody remnants are also released from *C. elegans* Q neuroblasts before being degraded by the neighboring phagocytic cells; the apoptotic cell corpse engulfment genes *ced-1* and *ced-2* are essential for this process [5]. Since *ced-1* and *ced-2* are expressed in *C. elegans* early embryos (see http://nematode.lab.nig.ac.jp/), we examined whether these genes are required for the engulfment of midbody remnants in the embryo. Importantly, we found that the P_0, AB and P_1 midbody remnants remain outside of cells in *ced-1*, *ced-2* mutant or *ced-1*; *ced-2* double-mutant embryos (Figure 1B, 1E, red, purple and yellow arrowheads, Supplementary information, Figure S2A, S2B, S2F, S2G, S2H and Movie S3). We conclude that *ced-1* and *ced-2* are essential for the internalization of midbody remnants in early *C. elegans* embryos.

In conclusion, we uncovered a remarkably stereotyped segregation of midbody remnants in early *C. elegans* embryos. We showed that such segregation relies on a two-tiered mechanism, whereby midbody remnants are first shed into the extracellular milieu in a biased manner due to asymmetric spindle positioning, and then are internalized in a *ced-1*; *ced-2*-dependent manner. Importantly, it has been known for decades that *ced-1*, *ced-2* or *ced-1*; *ced-2* mutant animals are viable and do not exhibit obvious defects [8] , which we have confirmed by examining *ced-1*; *ced-2* double-mutant embryos expressing ZEN-4::GFP (data not shown). Therefore, our findings suggest that midbody remnants are dispensable for fate determination in an intact organism.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)