RESEARCH ARTICLE

CELL BIOLOGY

The deubiquitinase USP8 targets ESCRT-III to promote incomplete cell division

Juliette Mathieu*, Pascale Michel-Hissier, Virginie Boucherit, Jean-René Huynh*

In many vertebrate and invertebrate organisms, gametes develop within groups of interconnected cells called germline cysts formed by several rounds of incomplete divisions. We found that loss of the deubiquitinase USP8 gene in *Drosophila* can transform incomplete divisions of germline cells into complete divisions. Conversely, overexpression of USP8 in germline stem cells is sufficient for the reverse transformation from complete to incomplete cytokinesis. The ESCRT-III proteins CHMP2B and Shrub/CHMP4 are targets of USP8 deubiquitinating activity. In *Usp8* mutant sister cells, ectopic recruitment of ESCRT proteins at intercellular bridges causes cysts to break apart. A Shrub/CHMP4 variant that cannot be ubiquitinated does not localize at abscission bridges and cannot complete abscission. Our results uncover ubiquitination of ESCRT-III as a major switch between two types of cell division.

Clonal multicellularity arises by incomplete divisions of single-cell precursors and gives rise to groups of sister cells linked by cytoplasmic bridges. Such mechanisms underlie the formation of colonies of unicellular organisms and the emergence of multicellularity (*1*). It is also a conserved feature of germline cell development, where cysts of sister cells share the same cytoplasm (*2*). During *Drosophila* oogenesis, cells display complete or incomplete cytokinesis. Indeed, germline stem cells (GSCs) first divide completely to produce a single-cell precursor called a cystoblast (CB). Then, each CB goes through four rounds of incomplete division to generate germline cysts made of 16 sister cells, all linked by cytoplasmic bridges called ring canals (RCs) (*3*) (Fig. 1, A and B).

USP8 is necessary and sufficient to promote incomplete divisions

To identify genes involved in cyst formation, we expressed a collection of short hairpin RNAs (shRNAs) and screened for egg chambers with an abnormal number of nuclei. Two independent shRNA lines targeting the deubiquitinating enzyme USP8 (*4*) induced the formation of egg chambers made of eight, four, or two nuclei, instead of 16 (Fig. 1, C and E, and fig. S1, A and B). These egg chambers resulted from partial breaking of cysts due to ectopic cytokinetic abscission in the germarium. Indeed, the fusome, an endoplasmic reticulum (ER)–derived organelle, formed a midbody-like structure, a feature of complete abscission, which is not seen in wild-type (WT) cysts (Fig. 1, D and F, and fig. S1, B and C) (*5*, *6*). We demonstrated that they were complete abscissions by showing that photoactivatable molecules were not able to diffuse between sister cells, when the midbody structure was present (fig. S2, A and B, and movies S1 to S4). Furthermore, using live imaging, we observed the physical separation of the fusome in mutant cysts (fig. S2, C and D, and movies S5 and S6). Depletion of *Usp8* caused similar ectopic abscissions in male germ cells (fig. S1, D and E). Homozygous mutant cysts for a null allele of *Usp8* (*7*) [$Usp8^{KO}$ germline clone (GLC)] exhibited the same complete abscission phenotype, arguing for the specificity of these shRNAs (fig. S1, F to H). To rule out the possibility that complete abscissions of *Usp8*-depleted cysts were caused by dedifferentiation of cysts into GSCs (*8*, *9*), we verified that *Usp8*-depleted cysts still expressed the differentiation marker Bam (fig. S3, A and B). $Usp8^{KO}$ GLC also lacked Nanos expression at four-cell cysts (*10*), similarly to control differentiating cysts (fig. S3C). In addition, we found that mutant cysts could enter meiosis, although an oocyte was never maintained (fig. S3, D and E). Together, these observations indicated that *Usp8* is necessary to maintain incomplete cytokinesis in *Drosophila* germline cysts.

Conversely, we tested whether USP8 could be sufficient to inhibit cytokinesis in cells that normally complete abscission, such as GSCs. We overexpressed a wild-type form of USP8 in these stem cells and observed the formation of stabilized cytoplasmic bridges in stem cells ("stem cysts") (*11*) (Fig. 1H), which led to CBs made of two cells and egg chambers made of 32 rather than 16 cells (Fig. 1G and fig. S4A) (*6*, *11*, *12*). Incomplete cytokinesis in GSCs overexpressing USP8 was not caused by premature differentiation of GSCs, as these GSCs did not express the cyst-specific protein Bam (Fig. 1H). Similarly, we found that overexpressing USP8 in somatic follicle cells surrounding germline cells and in Schneider 2 (S2) cells also led to the formation of ectopic cysts (fig. S4, C to I). Taken together, these results demonstrated that USP8 is necessary and sufficient to promote incomplete divisions in germline and somatic cells.

USP8 deubiquitinates ESCRT-III proteins

Next, we tested whether USP8 deubiquitinase activity (DUB) was required for the regulation of abscission. We expressed a catalytically inactive form of USP8, called USP8-C572A (*7*, *13*, *14*), in $Usp8^{KO}$ cysts and found that, in contrast to wild-type USP8, it was unable to rescue germline cysts from breaking (Fig. 2, A and B, and fig. S5, A to C). Similarly, overexpression of USP8-C572A was not sufficient to arrest cytokinesis or induce the formation of stem cysts in GSCs, follicle cells, or S2 cells (fig. S4, A to I). Hence, USP8 DUB activity is required for the regulation of abscission.

We observed that USP8 localized at sites of cytokinesis in both males and females, that is, in midbodies of GSC/CB pairs, RCs of germline cysts, and follicle cell RCs (Fig. 2, C and D, and fig. S6, A to C). In the absence of *Usp8*, we noticed an accumulation of ubiquitinated proteins at ectopic midbodies (Fig. 2, E and F), in addition to cytoplasmic aggregates, as described in mammals (fig. S7, A to F). These data suggest that the relevant targets of USP8 could be localized at abscission sites.

The N-terminal microtubule interacting and transport (MIT) domain of human USP8 interacts with ESCRT-III proteins, which are thought to be the driving force of several membrane scission events, including abscission (*15*–*18*). We found that three *Drosophila* ESCRT-III proteins, CHMP1 (charged multivesicular body protein 1), CHMP2B (charged multivesicular body protein 2b), and Shrub (the unique *Drosophila* homolog of CHMP4), could immunoprecipitate USP8 (Fig. 3A and fig. S8A) (*19*–*21*). All three proteins were ubiquitinated in *Drosophila* cells (Fig. 3B and fig. S8B). Expressing a wild-type form of USP8 was sufficient to greatly reduce the amount of ubiquitinated CHMP2B and Shrub, but not of CHMP1 (Fig. 3B and fig. S8B). In contrast, the inactive USP8-C572A had no effect on the ubiquitination levels, although it could still bind all three ESCRT-III proteins (Fig. 3, A and B, and fig. S8, A and B). Conversely, knocking down *Usp8* in S2 cells led to increased levels of ubiquitinated Shrub and CHMP2B (fig. S8, C to E). We concluded that, unlike in intralumenal vesicle formation in yeast (*22*), USP8 is necessary and sufficient to deubiquitinate CHMP2B and Shrub.

USP8 regulates ESCRT-III accumulation at the intercellular bridge

We then investigated whether CHMP2B and Shrub could be relevant targets in vivo. Shrub,

Center for Interdisciplinary Research in Biology, Collège de France, PSL Research University, CNRS, Inserm, Paris, France.
*Corresponding author. Email: juliette.mathieu@college-de-france.fr (J.M.); jean-rene.huynh@college-de-france.fr (J.-R.H.)

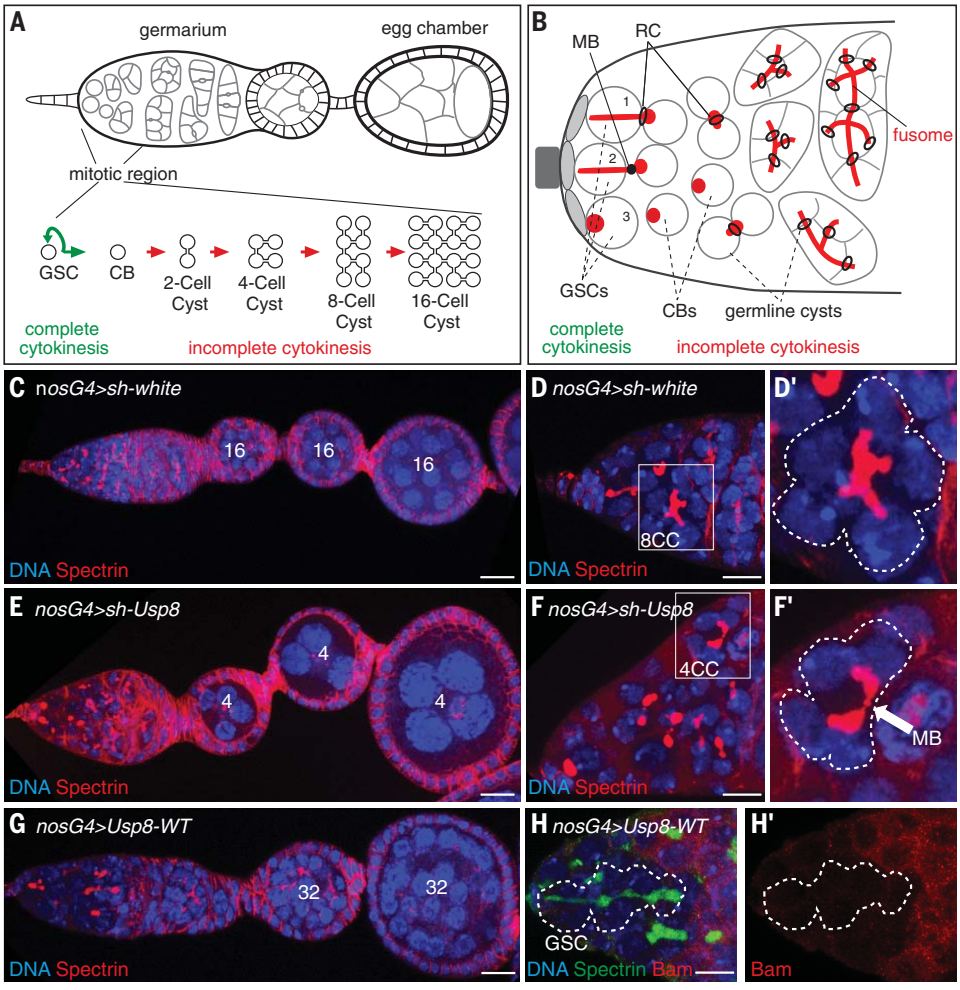

Fig. 1. USP8 is necessary and sufficient to block abscission in the germline lineage. (**A**) (Top) Female ovariole. (Bottom) Cell lineage of the germline stem cell (GSC). (**B**) Mitotic region of the germarium. At the end of mitosis, GSCs are connected to their daughter CBs through a ring canal (RC) (GSC #1) and later by a midbody (MB) (GSC #2). After complete cytokinesis, GSCs and CBs are fully separated (GSC #3). The fusome (red) links the GSC and the CB before abscission and also passes through all RCs of germline cysts. Cap cells, light gray; terminal filament cells, dark gray. (**C** to **H′**) Confocal images of fixed ovarioles [(C), (E), and (G)] and germaria [(D), (F), and (H)] of females expressing *shRNA-w*, *shRNA-Usp8*, or *Usp8-WT* under the control of *nos-GAL4* driver. [(D′) and (F′)] Close-up images of the cysts framed in (D) and (F). Dashed lines surround germline cysts [(D′) and (F′)] or a stem cyst that originates from the GSC [(H) and (H′)]. The white arrow indicates the ectopic MB of a breaking cyst. Scale bars: 20 μm [(C), (E), and (G)] and 10 μm [(D), (F), and (H)]. 4CC, four-cell cyst; 8CC, eight-cell cyst.

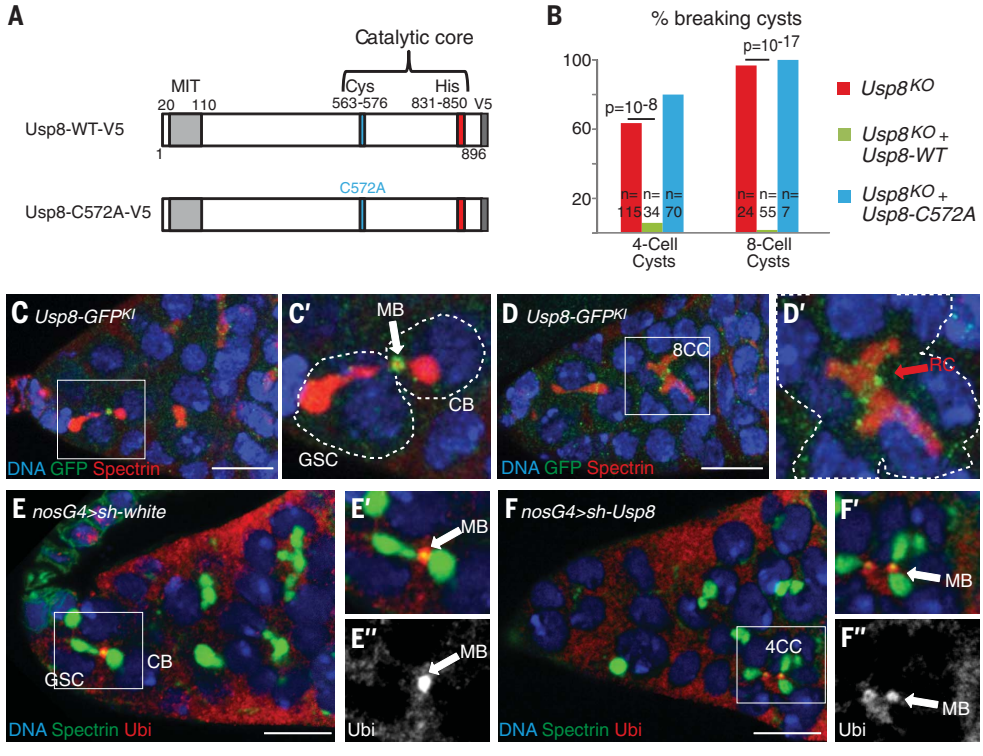

Fig. 2. The deubiquitinating activity of USP8 is essential for its function in germline cysts. (**A**) Scheme of the V5-tagged variants of USP8 proteins. (**B**) Fraction of four- and eight-cell cysts exhibiting a breaking phenotype. *P* values (p) are indicated and derived from Chi square tests. The *n* values indicate the number of cysts analyzed. (**C** and **D**) Confocal images of germaria expressing green fluorescent protein (GFP)–tagged USP8 from the endogenous locus. (**C′** and **D′**) Close-up of the cells framed in (C) and (D). Dashed lines surround a GSC and its daughter CB (C′) and a germline cyst (D′). The white arrow indicates the MB between the GSC and the CB in (C′), and the red arrow indicates the central RC in (D′). (**E** and **F**) Confocal images of germaria expressing *shRNA-w* (E) or *shRNA-Usp8* (F) under the control of *nos-GAL4* driver stained with α-ubiquitin (Ubi) and α-spectrin. (**E′** and **E″**) Close-up views of the GSC/CB pair framed in (E). (**F′** and **F″**) Close-up views of the breaking 4CC framed in (F). White arrows indicate the MB between the GSC and the CB in (E), and the MB of the breaking 4CC in (F). Scale bars: 10 μm.

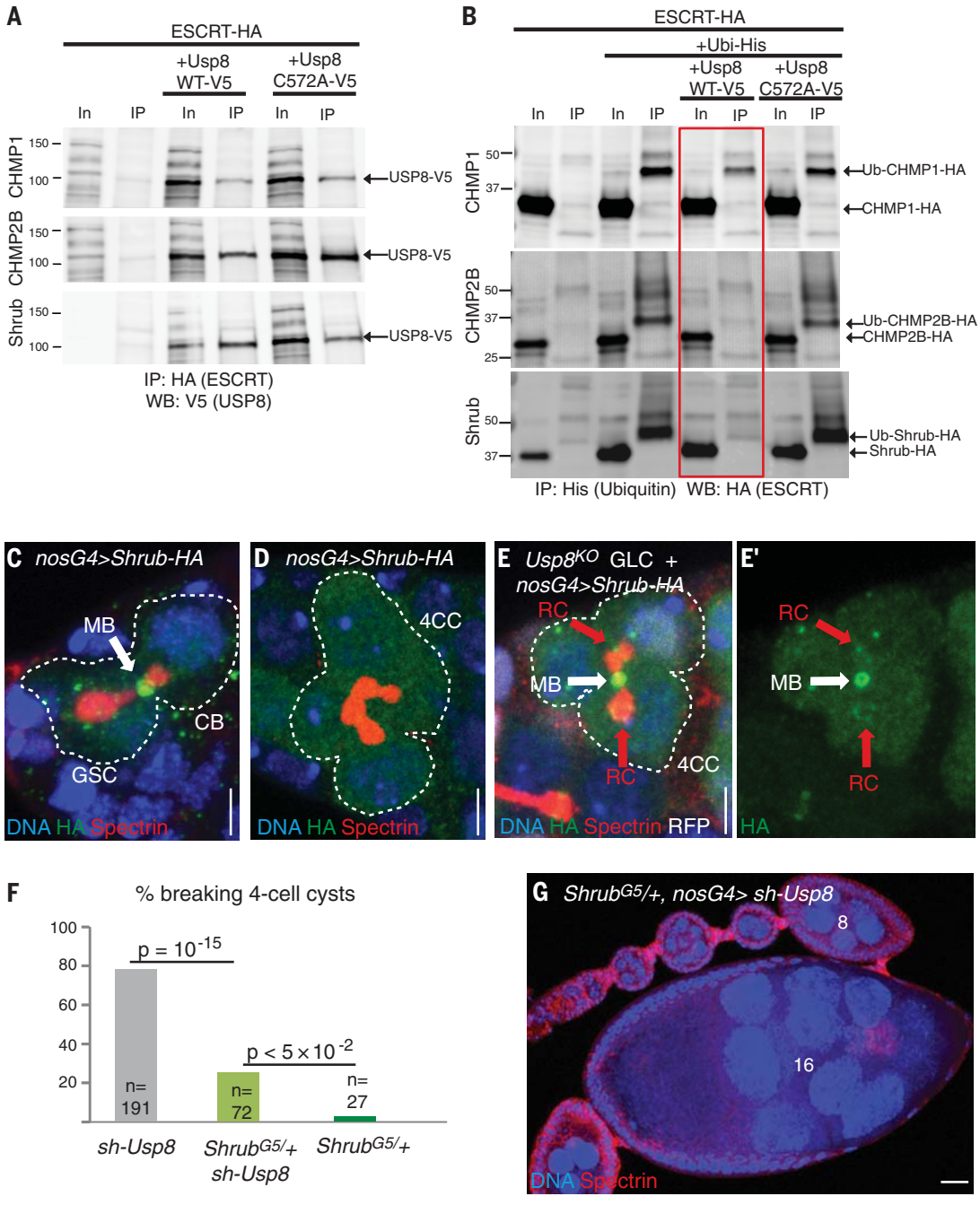

Fig. 3. USP8 regulates CHMP2B and Shrub ubiquitination levels and localization. (**A**) Immunoblots of input ("In," 1/20 of the IP) or immunoprecipitated ("IP") ESCRT-III proteins from S2 cells transfected with ESCRT-HA (hemagglutinin) and V5-tagged USP8 variants. Membranes were blotted with α-V5 antibody. (**B**) Immunoblots of input (1/20 of the IP) or IP ubiquitinated proteins from S2 cells transfected with ESCRT-HA, His-tagged ubiquitin, and V5-tagged USP8 variants. Membranes were blotted with α-HA antibody to reveal ubiquitinated ESCRT-III. USP8 transfection is sufficient to abolish CHMP2B and Shrub ubiquitination (red frame). (**C** and **D**) Confocal images of fixed germaria of females expressing *Shrub-HA* under the control of *nos-Gal4*. A GSC/CB pair (C) and a 4CC (D) are surrounded by dashed lines. The white arrow in (C) indicates the MB between the GSC and the CB. Shrub-HA is not detected at the 4CC RC. Scale bars: 5 μm. (**E**) Confocal images of a fixed germarium of a female expressing *Shrub-HA* under the control of *nos-Gal4* with $Usp8^{KO}$ GLC [red fluorescent protein (RFP)–negative cells]. The white arrow indicates the ectopic MB of the breaking 4CC, the red arrows indicate ectopic Shrub-HA at the RCs of the mutant 4CC. Scale bar: 5 μm. (**F**) Fraction of 4CCs exhibiting a breaking phenotype. *P* values (p) are derived from Chi Square tests. The *n* values indicate the number of cysts analyzed. (**G**) Confocal images of a fixed ovariole of a female expressing *shRNA-Usp8* in a $shrb^{G5}$/+ background. The number of nuclei of some ovarioles are indicated. Scale bar: 20 μm.

CHMP2B, and CHMP1 localized at the RC and midbody of GSC/CB pairs (*6*) but were absent at cyst RCs (Fig. 3, C and D, and figs. S9, A, B, D, F, G, I, and J, and S10, A and C). However, in male and female cysts lacking *Usp8*, Shrub, CHMP2B, and CHMP1 all localized at ectopic midbodies and cyst RCs (Fig. 3E and figs. S9, C, E, H, and K, and S10, B and D). To test whether the ectopic localization of ESCRT-III was responsible for ectopic abscissions seen in the absence of USP8, we reduced Shrub or CHMP2B levels in *Usp8*-depleted cysts. In these contexts, we counted fewer four-cell cysts in the process of breaking apart than in cysts depleted of USP8 alone (Fig. 3F and fig. S11, A to C). In addition, we found that older egg chambers contained more nuclei than did *sh-Usp8* mutant egg chambers (fig. S11, D and E). We also observed complete rescue with egg chambers made of 16 cells and a posterior oocyte, which was not seen with *sh-Usp8* alone (Fig. 3J). Altogether, we conclude that deubiquitination of Shrub and CHMP2B by USP8 is required to block their localization at RCs and subsequent abscissions, allowing the formation of 16-cell germline cysts.

Ubiquitination of Shrub is essential to promote abscission in the GSCs

Next, we assessed the relevance of ESCRT protein ubiquitination during complete abscission by testing the ability of a nonubiquitinable variant of Shrub (Shrub-KR) (fig. S12A) to replace endogenous Shrub in GSCs. When we removed both copies of *Shrub* from GSCs, we found that Shrub-KR only poorly compensated

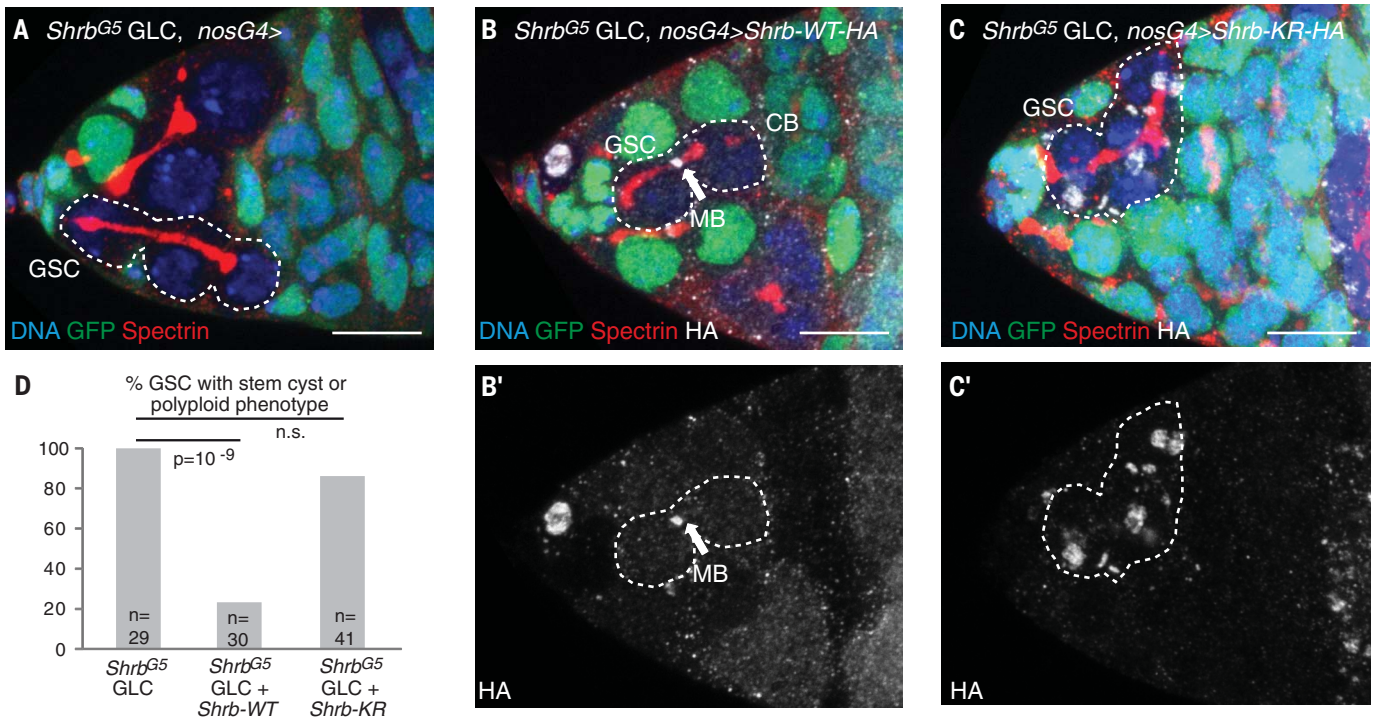

Fig. 4. Ubiquitination of Shrub is essential to promote abscission in the GSCs. (**A** to **C**) Confocal images of fixed germaria with *shrb*^{G5} GLC (GFP negative) expressing *Shrb-WT-HA* (B), *Shrb-KR-HA* (C), or no transgene (A). Dashed lines surround a GSC/CB pair (B) or stem cysts [(A) and (C)]. White arrow indicates the MB positive for Shrb-WT-HA. Scale bars: 10 μm. (**D**) Fraction of GSCs exhibiting a stem-cyst or polyploid phenotype. *P* values (p) are derived from Chi Square tests. ns, not significant (*P* = 0.01). The *n* values indicate the number of GSCs analyzed.

for the absence of Shrub, whereas Shrub-WT rescued *Shrub* mutant germline clones (Fig. 4, A to D, and fig. S12, A and B). In these nonrescued GSCs, Shrub-KR only formed cytoplasmic aggregates and did not localize at RCs (Fig. 4C). Shrub-KR and Shrub-WT were equally able to fully rescue stem-cyst phenotypes when only one copy of *Shrub* was removed in *Shrub*^{G5}/+ females (fig. S12C). Like Shrub-WT, Shrub-KR localized at RCs and midbodies of *Shrub*^{G5}/+ heterozygous GSC/CB pairs (fig. S12, D and E). Furthermore, we could precipitate Shrub-KR and Shrub-WT in *Drosophila* cells, indicating that both proteins could interact physically (fig. S12F). These results showed that Shrub-KR was still functional and able to localize properly when one copy of endogenous Shrub remained in GSCs. Finally, knocking down *Usp8* in *Shrub*^{G5}/+ heterozygous females rescued abscission arrest and the formation of stem cysts, indicating that ubiquitination could also regulate ESCRT-III in GSCs (fig. S12, G to I). Overall, our results show that in both GSCs and germline cysts, USP8 deubiquitinates ESCRT-III proteins to inhibit their localization at intercellular bridges.

A model for the differential accumulation of ESCRT-III at intercellular bridges in GSCs and germline cysts.

Whereas ESCRT proteins are barely detectable in germline cysts, they accumulate at GSC midbodies, despite the expression of USP8 in both cell types (Figs. 2, C and D, and 3, C and D, and fig. S9). This apparent contradiction could be explained by a major difference in cell cycle length between GSCs and germline cysts. GSCs cycle in 24 hours and take ~15 hours to complete abscission (*23*, *24*). In contrast, cysts go through four rounds of mitosis in 24 hours, that is, 6 hours for each cycle. In GSCs, we measured a progressive accumulation of ESCRT-III proteins at the GSC-CB intercellular bridge during the 15 hours after mitosis, whereas ESCRT-III remained barely detectable in cyst cells (Fig. 5A). In contrast, in the absence of *Usp8*, CHMP2B also accumulated progressively in cyst cells with ectopic abscission (Fig. 5A). We noted that the levels of USP8 varied along the cell cycle, peaking during mitosis (Fig. 5B). These observations suggest that in GSCs, ESCRT proteins have time to accumulate to complete abscission before the next mitosis, whereas in cysts, shorter cell cycles and the presence of peak levels of USP8 prevent ESCRT accumulation and stabilize incomplete divisions. In this model, the combination of different cell cycle lengths and USP8 accumulation during mitosis is sufficient to explain the switch from complete to incomplete cytokinesis (Fig. 5E). To test this model experimentally, we reasoned that lengthening the postmitotic phase of germline cysts could be sufficient for ESCRT protein accumulation and completion of cell divisions. We used flies in which the differentiation of germline cysts could be synchronized by the expression of the *bam* gene following a heat shock (HS) (*9*, *25*). At 18 hours after HS, the majority of four-cell cysts in these flies were devoid of ESCRT proteins as in wild-type cysts (Fig. 5, C and D, left, and fig. S13, A, B, D, and E). When we dissected flies 28 hours after HS, most four-cell cysts exhibited CHMP2B-GFP at the RCs (Fig. 5D, middle, and fig. S13, D and E), some of which were even breaking apart, as in *Usp8* mutant cysts (fig. S13F). At 38 hours after HS, we observed an increase in both the amount of CHMP2B-GFP at the RCs and the number of four-cell cysts breaking apart (Fig. 5D, right, and fig. S13, E and F). These results show that when germline cysts were blocked at the four-cell stage for at least 10 hours (from 18 to 28 hours) or 20 hours (from 18 to 38 hours), they accumulated ESCRT proteins at ring canals and completed abscission. These results showed that lengthening the postmitotic phase of four-cell cysts is sufficient for ESCRT proteins to accumulate at ring canals and induce ectopic abscission in germline cysts, as in a *Usp8* mutant (Fig. 5E).

Collectively, our data demonstrate that ubiquitination of ESCRT-III is critical to regulate the switch between complete and incomplete divisions. We propose a model in which USP8 deubiquitinates the ESCRT-III proteins Shrub and CHMP2B, which prevents complete

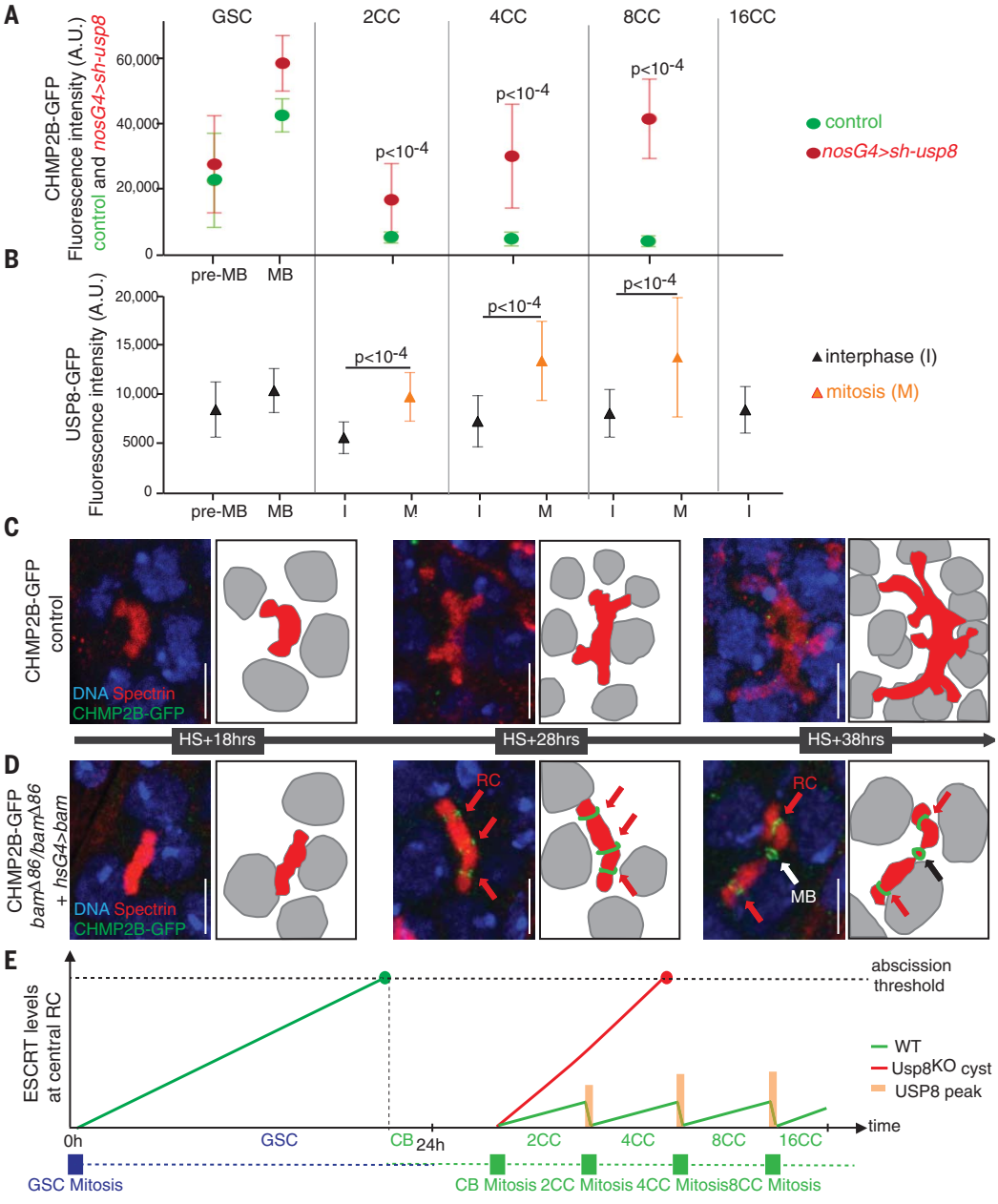

Fig. 5. Differential accumulation of ESCRT-III at intercellular bridges in GSCs and germline cysts. (**A**) Fluorescence intensity [arbitrary units (A.U.)] of CHMP2B-GFP at the RC (pre-MB) or the MB of GSC and at the central RC of cysts (2CC, 4CC, and 8CC) in control (green) or *sh-Usp8* (red)–expressing flies. *P* values (p) between control and shRNA-expressing flies are derived from Mann-Whitney tests. (**B**) Fluorescence intensity (A.U.) of USP8-GFP at the RC (pre-MB) or the MB of GSC and at the central RC of cysts (2CC, 4CC, 8CC, and 16CC) in $Usp8^{KI}$ flies in interphase (black) or mitosis (orange). *P* values (p) between interphase and mitosis are derived from Mann-Whitney tests. (**C** and **D**) Confocal images and schemes of fusomes (stained for α-spectrin, red) in germaria of WT females expressing *CHMP2B-GFP* (C) or of $bam^{\Delta 86}$ females expressing *CHMP2B-GFP* and *HS*-bam (D). Time after heat shock is indicated on the dark arrow between (C) and (D). In (D), 4CCs are observed at 18 hours after HS (left). CHMP2B-GFP is then observed at the RCs (red arrow) of a 4CC (28 hours, middle), and the central RC of a 4CC breaks into a MB (white arrow) at 38 hours after HS (right). Scale bars: 5 μm. (**E**) Schematic model of ESCRT levels over time in the germarium. The GSC has a 24-hour cell cycle. Abscission between the newly formed GSC and its daughter CB occurs late in the cell cycle, before the next mitosis, and thus the GSC/CB pair never experiences a USP8 peak. During this long period, the GSC/CB RC therefore accumulates ESCRT, reaching a high level allowing abscission. The CB becomes a 16CC in 24 hours; therefore each cycle lasts only 6 hours, leaving much less time to accumulate ESCRT at RC at each cycle. In addition, USP8 levels at the RC in the cyst peak at each mitosis (orange bars) and remove (or block the dynamic recruitment of) ESCRTs at the RCs. We propose that short cell cycle duration combined with USP8 peaks in mitosis are responsible for both the low levels of ESCRT at cyst RCs and the absence of abscission. When USP8 is absent (red), ESCRT levels increase at each cyst cycle, allowing abscission.

abscission and allows sister cells to remain linked by stabilized cytoplasmic bridges. Both GSCs and germline cysts express *Usp8* and several ESCRT-III proteins, despite different outcomes for abscission. Complete and incomplete abscission are thus not inherently different in nature. We propose that it is mainly the duration of abscission, regulated by the cell cycle, that differs in the two cases (Fig. 5E).

A major question arising from our work is the evolutionary relationship between complete and incomplete division, and whether USP8 played a role in the emergence of one from the other. We observed the most drastic consequences of *Usp8* loss in germline cysts, not in stem cells, indicating that the main function of USP8 is to promote incomplete division and that it seems dispensable for complete cytokinesis. Besides germ cells, unicellular organisms such as the choanoflagellates can also form colonies of sister cells by incomplete divisions. These colonies, considered a first step toward multicellularity, are linked by cytoplasmic bridges that resemble metazoan ring canals (*1*). Given that ESCRT-III function in abscission is conserved from archaebacteria to vertebrates (*26*), it will be interesting to investigate whether ubiquitination of ESCRT-III plays a role in the emergence of these incomplete divisions.

REFERENCES AND NOTES

1. S. R. Fairclough, M. J. Dayel, N. King, *Curr. Biol.* **20**, R875–R876 (2010).
2. M. E. Pepling, M. de Cuevas, A. C. Spradling, *Trends Cell Biol.* **9**, 257–262 (1999).
3. J. R. Huynh, D. St Johnston, *Curr. Biol.* **14**, R438–R449 (2004).

4. N. Loncle, M. Agromayor, J. Martin-Serrano, D. W. Williams, *Sci. Rep.* **5**, 8461 (2015).
5. J. Mathieu, J. R. Huynh, *Methods Cell Biol.* **137**, 105–118 (2017).
6. N. R. Matias, J. Mathieu, J. R. Huynh, *PLOS Genet.* **11**, e1004653 (2015).
7. A. Mukai *et al*., *EMBO J.* **29**, 2114–2125 (2010).
8. D. McKearin, B. Ohlstein, *Development* **121**, 2937–2947 (1995).
9. T. Kai, A. Spradling, *Nature* **428**, 564–569 (2004).
10. L. Gilboa, R. Lehmann, *Curr. Biol.* **14**, 981–986 (2004).
11. J. Mathieu *et al*., *Dev. Cell* **26**, 250–265 (2013).
12. A. H. Eikenes *et al*., *PLOS Genet.* **11**, e1004904 (2015).
13. E. Mizuno *et al*., *Mol. Biol. Cell* **16**, 5163–5174 (2005).
14. W. Luo *et al*., *Genes Dev.* **26**, 2536–2549 (2012).
15. P. E. Row *et al*., *J. Biol. Chem.* **282**, 30929–30937 (2007).
16. J. Guizetti *et al*., *Science* **331**, 1616–1620 (2011).
17. J. Schöneberg, I. H. Lee, J. H. Iwasa, J. H. Hurley, *Nat. Rev. Mol. Cell Biol.* **18**, 5–17 (2017).
18. B. Mierzwa, D. W. Gerlich, *Dev. Cell* **31**, 525–538 (2014).
19. E. Nikko, B. André, *Traffic* **8**, 566–581 (2007).
20. N. Johnson, M. West, G. Odorizzi, *Mol. Biol. Cell* **28**, 661–672 (2017).
21. X. Crespo-Yáñez *et al*., *PLOS Genet.* **14**, e1007456 (2018).
22. D. Buysse, A. K. Pfitzner, M. West, A. Roux, G. Odorizzi, *J. Cell Sci.* **133**, jcs241455 (2020).
23. G. Villa-Fombuena, M. Lobo-Pecellín, M. Marín-Menguiano, P. Rojas-Ríos, A. González-Reyes, *Development* **148**, dev199716 (2021).
24. L. X. Morris, A. C. Spradling, *Development* **138**, 2207–2215 (2011).
25. B. Ohlstein, D. McKearin, *Development* **124**, 3651–3662 (1997).
26. G. Tarrason Risa *et al*., *Science* **369**, eaaz2532 (2020).

ACKNOWLEDGMENTS

We thank M. O. Fauvarque, S. Goto, F. K. Teixeira, M. Furthauer, P. Thérond, K. Haglund, the Bloomington Stock center, and the Developmental Studies Hybridoma Bank for reagents used in this study. We thank A. Echard for discussions and suggestions and the Huynh lab, M. H. Verlhac, D. St Johnston, and E. Montembault for helpful comments on the manuscript. We are grateful to the Orion Imaging Facility (Collège de France) and BDD Curie Imaging facility. **Funding:** The J.-R.H. lab is supported by CNRS, INSERM, Collège de France, La Fondation pour la Recherche Médicale (FRM) (Equipes FRM DEQ20160334884), Agence Nationale de la Recherche (ANR) (ANR-15-CE13-0001-01, AbsCyStem), and the Bettencourt Schueller Foundation. **Author contributions:** J.M. and J.-R.H. designed the project, analyzed the data, and wrote the manuscript. J.M., P.M.-H., and V.B. produced reagents and performed experiments. **Competing interests:** The authors declare no competing or financial interests. **Data and materials availability:** All materials are available upon request.

SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.abg2653
Materials and Methods
Supplementary Text
Figs. S1 to S13
List of Genotypes
References (*27*–*40*)
MDAR Reproducibility Checklist
Movies S1 to S6

View/request a protocol for this paper from *Bio-protocol*.

Submitted 22 December 2020; accepted 12 April 2022
10.1126/science.abg2653