Global Tissue Revolutions in a Morphogenetic Movement Controlling Elongation

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Materials and Methods
SOM Text
Tables S1 to S4
References

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Global Tissue Revolutions in a Morphogenetic Movement Controlling Elongation

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Polarized cell behaviors drive axis elongation in animal embryos, but the mechanisms underlying elongation of many tissues remain unknown. Eggs of *Drosophila* undergo elongation from a sphere to an ellipsoid during oogenesis. We used live imaging of follicles (developing eggs) to elucidate the cellular basis of egg elongation. We find that elongating follicles undergo repeated rounds of circumferential rotation around their long axes. Follicle epithelia mutant for integrin or collagen IV fail to rotate and elongate, which results in round eggs. We present evidence that polarized rotation is required to build a polarized, fibrillar extracellular matrix (ECM) that constrains tissue shape. Thus, global tissue rotation is a morphogenetic behavior that uses planar polarity information in the ECM to control tissue elongation.

Elongation of a tissue along a major body axis is a central and conserved feature of animal development (*1*, *2*), and defects in this process cause human developmental abnormalities (*3*). Studies of elongating tissues have uncovered morphogenetic behaviors such as convergent extension, part of a small repertoire of cell movements known to shape animal body plans (*4*). However, for many tissues, the mechanism underlying their elongation is unknown.

The development of the ellipsoid *Drosophila* egg is an elegant case of tissue elongation. *Drosophila* eggs develop from individual follicles, each consisting of a somatic follicle cell epithelium that surrounds the germline. Follicles are initially spherical and grow isotropically but acquire anisotropic growth along the antero-posterior (A-P) axis from stage 4 of oogenesis to form a mature (stage 14) egg (Fig. 1, A and B) (*5*, *6*). Seventy-four percent of this 2.5-fold elongation is achieved in 20 hours between stages 5 and 9 (Fig. 1B). How the developing follicle breaks symmetry to channel a 24-fold increase in volume during these stages (*7*), from a sphere to an ellipsoid, is poorly understood. Evidence indicates that egg shape requires activities within the follicle epithelium (*8*), specifically from proteins linking intracellular actin to the extracellular matrix (ECM) (*6*, *9*–*14*), but how follicle cells confer egg shape has remained elusive.

Our analysis of fixed samples suggested that polarized cell divisions and cell shape changes, which are associated with elongation of other tissues (*15*–*18*), are not readily apparent in elongating follicles. To determine whether dynamic cell behaviors are involved, we used live imaging of elongating follicles (fig. S1) (*19*–*21*). This analysis revealed a morphogenetic behavior (fig. S1 and movie S1). The entire follicle epithelium undergoes a dramatic migration, in a circumferential direction around the elongating A-P axis, which leads to global rotation of this geometrically continuous tissue (Fig. 1C and movie S2).

Polarized rotation is observed in >95% of wild-type (WT) follicles (n >100) with a velocity of either 0.26 or 0.78 μm/min and both left- and right-handed chirality (Fig. 1C and movie S2). Polarized rotation is developmentally regulated and occurs predominantly between stages 5 and 9, which parallels the major phase of follicle elongation (Fig. 1B). The data suggest that a developing follicle undergoes approximately three revolutions during elongation.

Visualization of germline nuclei revealed rotation in concert with follicle cells, both in direction and angular velocity (Fig. 1C and movie S2). By contrast, follicle cells move across static collagen IV fibrils (Fig. 1D and movie S3), which demonstrates active rotation over an ECM substrate. "Follicle rotation" therefore involves global polarized revolutions of each developing egg within the basement membrane that encases each follicle.

The strong correlation between the phases of follicle rotation and egg elongation suggests that this behavior might play a role in morphogenesis. We analyzed follicles mosaic for null mutants in the integrin β_{PS} subunit (*myospheroid*; *mys*), which is required for egg elongation (*10*, *22*). *mys* mosaic follicles are significantly rounder than WT controls from stage 5 (Fig. 2, B and D, and fig. S2), the time when follicle rotation normally occurs. Live imaging of round *mys* mutant follicles revealed failure to rotate or off-axis rotation in most samples (Fig. 2, F and H; fig. S3; and movies S4 to S6).

The requirement for β_{PS} integrin in follicle shape and rotation suggests that cell-ECM interactions link both processes. Unique among ECM components, collagen IV forms circumferentially planar polarized fibrils around the follicle during the entire elongation phase (Fig. 3, A to E, and fig. S4). This led us to hypothesize that collagen IV may control egg shape, as do laminin and perlecan (*6*, *14*). Indeed, follicles with epithelia entirely mutant for collagen IV α2 (*viking; vkg*) deviate in shape at stage 8 (Fig. 2, C and D, and fig. S2) and ultimately form round eggs. Live imaging revealed polarized rotation until stage 7, when there is a notable breakdown in rotation (Fig. 2, G and H, and movies S7 and S8). These data demonstrate that mutations in genes that block follicle rotation also block elongation in a similar time frame, which suggests that these two processes are tightly coupled.

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What mechanism might link follicle rotation to egg shape? It is intriguing that collagen IV fibrils increase in length and density during follicle rotation (Fig. 3, A to F, and fig. S4) and change their organization. Collagen IV forms an initial basal matrix around young follicles (Fig. 1A), with distinct puncta that mature into circumferentially oriented fibrils from stage 5 onward (Fig. 3, A to E, and fig. S4). Fibril orientation is tightly regulated, in the same orientation (Fig. 3F) that the follicle rotates. This suggests that coordinated migration of follicle cells during global rotation may direct the polarization of the fibrillar ECM.

To test this in vivo, we carried out clonal analysis with a chromosome in which collagen IV–GFP [into which green fluorescent protein (GFP) is incorporated] is genetically linked to a nuclear red fluorescent protein (RFP) marker (Fig. 3G), which allows the position of RFP-marked follicle cells to be compared with the distribution of collagen IV–GFP that they have secreted. We observed that collagen IV–GFP fibrils are present in unmarked domains of the follicle epithelium, which indicates that the cells that produced them moved relative to these fibrils. This was not observed when follicle rotation was blocked (fig. S6). Moreover, when cells that produce collagen IV–GFP are distributed across the A-P axis of the follicle, they distribute marked fibrils across this axis (Fig. 3H). In contrast, if follicle cells that produce collagen IV–GFP occupy primarily the anterior or posterior half of the follicle, marked fibrils are associated only with that region of the follicle (Fig. 3I). These data are consistent with a model in which global rotation builds the polarized basement membrane that surrounds developing follicles.

What is the role of the polarized basement membrane produced by follicle rotation? One model is that of a "molecular corset," which could control egg shape by constraining growth along the dorso-ventral axis. Planar-polarized basal actin filaments of the follicle epithelium have been proposed to form a corset (*6*, *10*, *13*, *23*, *24*). However, acutely disrupting actin filaments in elongated follicles with latrunculin A did not perturb follicle shape (Fig. 4, B and D).

Collagen IV organization made it an attractive alternative for a molecular corset. We found that basement membrane integrity was compromised in *vkg* mutant follicles (fig. S6), consistent with vertebrate studies showing that type IV collagens maintain, but do not establish, ECM organization (*25*). We reasoned that if the fibrillar collagen IV matrix acts as a molecular corset, then its acute loss should affect the shape of elongated follicles. We therefore treated stage 12 follicles, which have completed global revolutions and display a polarized collagen IV matrix, with collagenase. Acute loss of collagen IV rounds these follicles (Fig. 4, C and D; and fig. S5), which supports the idea that the collagen IV matrix functions as a molecular corset.

Finally, when follicle rotation is blocked (in round *mys* mosaic follicles) the collagen IV matrix is present, but its organization is perturbed (Fig.

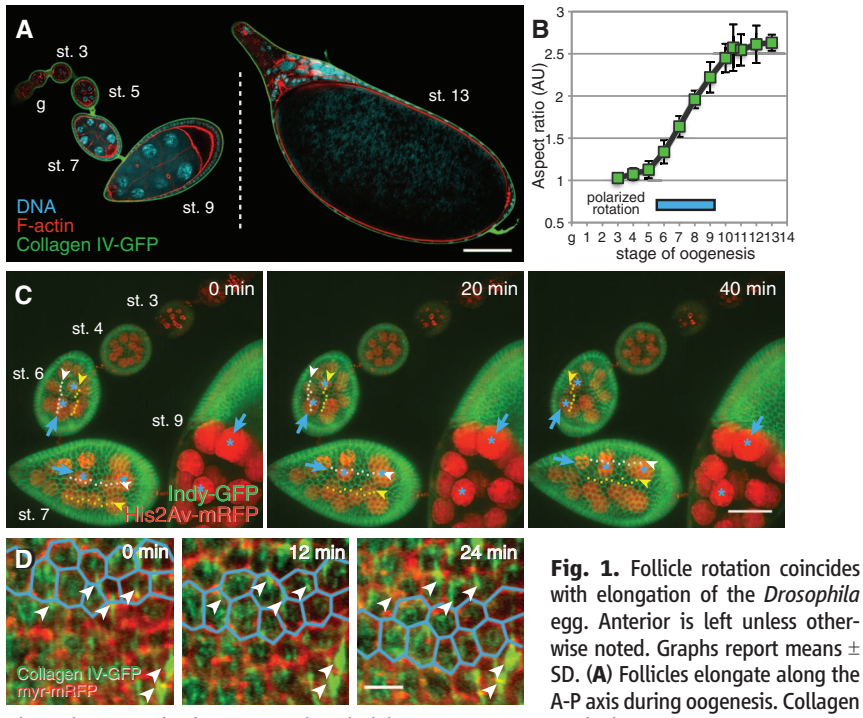
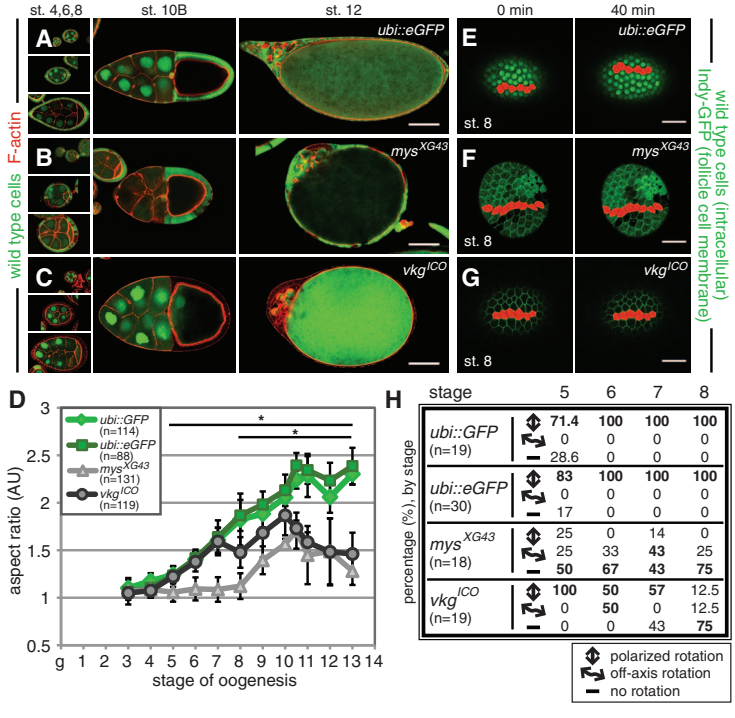


Fig. 1. Follicle rotation coincides with elongation of the *Drosophila* egg. Anterior is left unless otherwise noted. Graphs report means ± SD. (**A**) Follicles elongate along the A-P axis during oogenesis. Collagen IV–GFP (green), F-actin (red), and DNA (cyan). (**B**) Follicle aspect ratio (AR) quantification reveals that elongation occurs primarily between stages 5 and 9. AU, arbitrary units. Solid blue line indicates stages during which polarized rotation is observed. (**C**) Tracking of follicle cells (white and yellow dots, arrowheads) and germline nuclei (red; blue asterisks, arrows) rotate together. Note that the stage 9 follicle does not rotate. (**D**) Follicle cells (red; tracked cells outlined in blue) move against static collagen IV–GFP fibrils (green, arrowheads). Scale bars in (A), 100 μm; (C), 50 μm; (D), 10 μm.



		5	6	7	8
ubi::GFP (n=19)	polarized	71.4	100	100	100
	off-axis	0	0	0	0
	no rotation	28.6	0	0	0
ubi::eGFP (n=30)	polarized	83	100	100	100
	off-axis	0	0	0	0
	no rotation	17	0	0	0
mys XG43 (n=18)	polarized	25	0	14	0
	off-axis	25	33	**43**	25
	no rotation	**50**	**67**	**43**	**75**
vkg ICO (n=19)	polarized	**100**	**50**	**57**	12.5
	off-axis	0	**50**	0	12.5
	no rotation	0	0	43	**75**

Fig. 2. *mys* or *vkg* mosaic follicles do not undergo polarized rotation and do not elongate. (**A** to **D**) Follicles with epithelial clones of *mys* or *vkg* (GFP-negative) show shape defects during rotation. *mys* mutant follicles (B) are rounder from stage 5; *vkg* mutant follicles (C) are rounder from stage 8. AR is displayed in (D). Asterisks indicate $P < 0.05$. (**E** to **G**) Round follicles with *mys* (F) and *vkg* (G) clones are defective in rotation compared to control (E). WT cells are shown as green intracellular, Indy-GFP, as green membrane; tracked cells are pseudocolored red. (**H**) Percentage of follicles undergoing polarized, off-axis, or no rotation from live imaging. Scale bars in (A to C), 100 μm; (E to G), 25 μm.

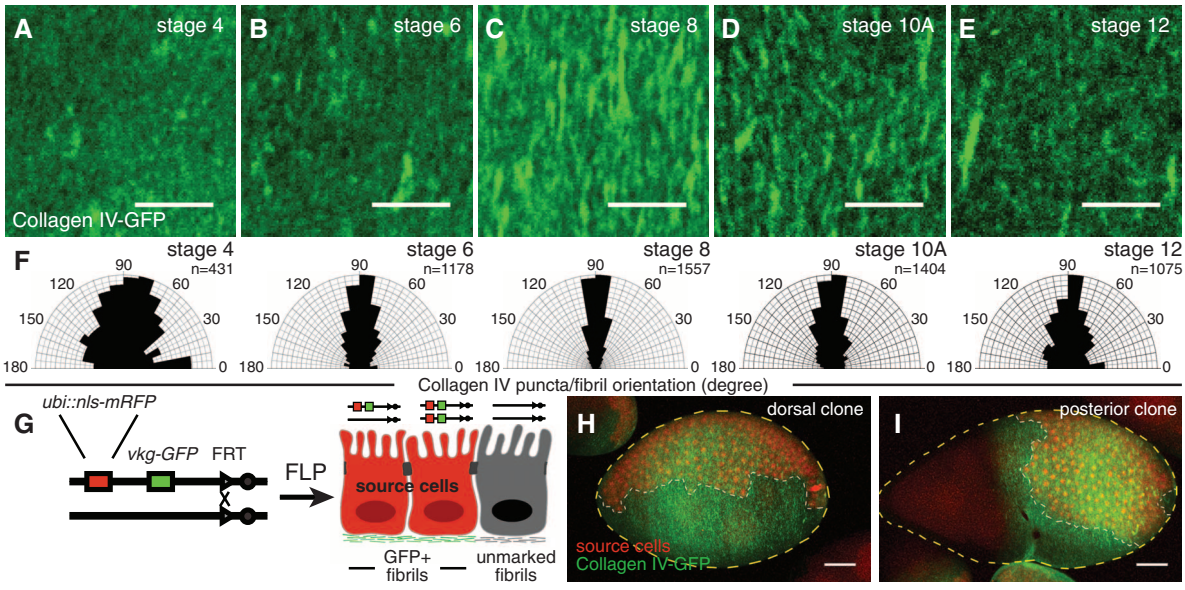
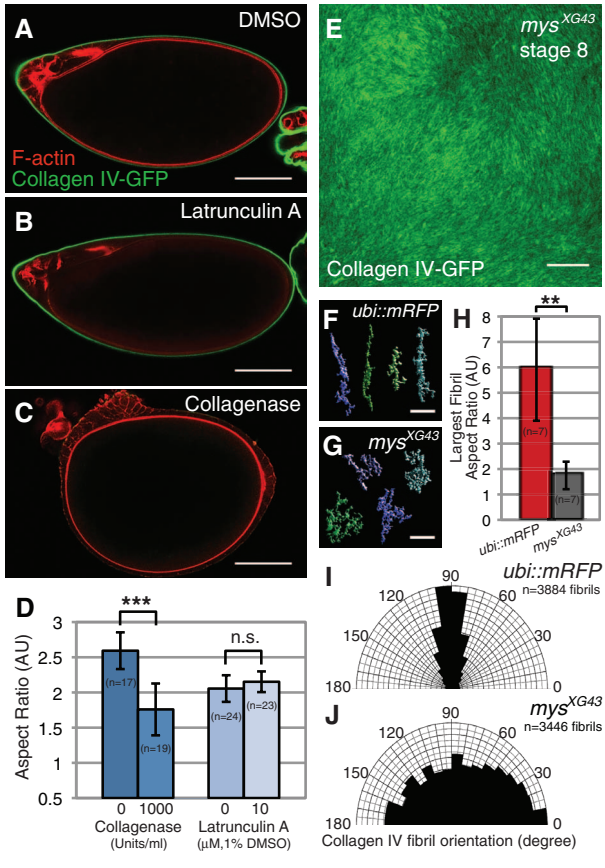


Fig. 3. A polarized fibrillar collagen IV matrix is built during follicle rotation. (**A** to **F**) Length and density of collagen IV fibrils increase during follicle rotation. Collagen IV–GFP (green) puncta at stage 4 (A) mature into polarized fibrils (B to E). (F) Collagen fibrils orient perpendicular to the A-P axis. (**G**) Strategy to create RFP-marked follicle cells producing collagen IV–GFP in genetic mosaics. Source cells distributed across the A-P axis (**H**) produce a uniform distribution of collagen-GFP fibrils, whereas posteriorly restricted source cells (**I**) produce only posterior labeled fibrils in stage 8 follicles. Scale bars in (A to E), 5 μm; (H to I), 25 μm.

Fig. 4. A polarized fibrillar collagen IV matrix maintains follicle shape. (**A** to **C**) Acute drug treatment of elongated stage 12 follicles. Collagenase (C), but not latrunculin A (B), perturbs follicle shape, quantified in (**D**). (**E** to **J**) The Collagen IV matrix (green) is present but disorganized in round *mys* mutant follicles [compare (E) with Fig. 3C]. Fibrils normally elongated in WT (F) fail to elongate in *mys* mutants (G); largest fibril shapes quantified in (H). (I and J) Collagen fibril orientation is lost in *mys* mutant follicles. Double asterisks indicate $P < 0.01$; triple asterisks indicate $P << 0.001$. Scale bars in (A to C), 100 μm; (E), 10 μm; (F to G), 3 μm.



4E). Although fibril density and length of the longest fibrils was unchanged, the shape of individual fibrils was significantly altered (Fig. 4, F to H). Most important, the uniform orientation of fibrils was completely lost (Fig. 4, I and J). Together, these results suggest that the polarization of the collagen IV matrix, via global tissue revolutions, governs elongation of the *Drosophila* egg (fig. S7).

In this work, we expand the repertoire of known morphogenetic behaviors by identifying a morphogenetic movement that elongates a developing tissue. As do many other collective cell migrations (*26*, *27*), basal cell–ECM focal contacts provide the motile force for follicle rotation, but the follicle's unique closed topology, with no leading edge, results in a treadmill-like migration with no net translocation. As in convergent extension, cells move orthogonally to the axis of elongation to generate a more than twofold elongation of the tissue. However, in the radially symmetric follicle epithelium, no axis of convergence is evident. Engagement of all cells of the tissue in multiple revolutions distinguishes follicle rotation from known phenomena involving partial and local rotation of cell clusters within a tissue (*28*, *29*). What signal(s) dictate the chirality, onset, and cessation of rotation remains as interesting unanswered questions.

Polarized cell movements involve planar cell polarity (PCP). PCP in the follicle was first noted two decades ago (*23*), and whereas follicle PCP and egg shape are independent of the core PCP signaling pathway (*24*, *30*), they do require proteins that link the actin cytoskeleton and ECM (*6*, *9*–*14*). Early work proposed that polarized basal actin mechanically constrains egg shape (*23*), but the discovery of follicle rotation suggests an alternative, in which actin filaments are required for polarized cell motility during egg elongation. Our data indicate that polarized, global follicle rotation directs polarization of the collagen IV matrix, which echoes other systems where migrating cells influence surrounding ECM structure. Notably, the polarized ECM can communicate PCP information (*6*, *31*) and also feed back to promote directed tissue

migration. Because individual follicle cells move over ECM fibrils previously oriented by neighbors, global rotation can both amplify and reinforce coordination of PCP across the entire tissue. We suggest that PCP coordination in the follicle may thus result from dynamic movement of an epithelium across a static cue—the ECM—rather than propagation of a cue through a static epithelium.

Finally, in providing a specific mechanism for the control of *Drosophila* egg shape, our work sheds light on the general role of the ECM in sculpting tissues. The circumferential collagen IV fibrils formed by follicle rotation recall the circumferential cellulose fibrils formed by rotating the cellulose synthase complex within the plasma membrane during elongation of stationary plant cells (*32*). Whereas our proposed mechanism can account for the majority of follicle elongation, data from *Drosophila* mutants suggest that it is only one of several mechanisms that establish final egg shape.

To the best of our knowledge, a morphogenetic movement with the attributes of follicle rotation has not been described in other animal tissues. We note that the existence of follicle rotation was not anticipated, despite a rich history of studies on *Drosophila* oogenesis from fixed specimens. Live imaging of other morphogenetic events may uncover additional instances where polarized tissue rotation influences tissue and organ shape.

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Materials and Methods
Figs. S1 to S7
References
Movies S1 to S8

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Development of Transgenic Fungi That Kill Human Malaria Parasites in Mosquitoes

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Metarhizium anisopliae infects mosquitoes through the cuticle and proliferates in the hemolymph. To allow *M. anisopliae* to combat malaria in mosquitoes with advanced malaria infections, we produced recombinant strains expressing molecules that target sporozoites as they travel through the hemolymph to the salivary glands. Eleven days after a *Plasmodium*-infected blood meal, mosquitoes were treated with *M. anisopliae* expressing salivary gland and midgut peptide 1 (SM1), which blocks attachment of sporozoites to salivary glands; a single-chain antibody that agglutinates sporozoites; or scorpine, which is an antimicrobial toxin. These reduced sporozoite counts by 71%, 85%, and 90%, respectively. *M. anisopliae* expressing scorpine and an $[SM1]_8$:scorpine fusion protein reduced sporozoite counts by 98%, suggesting that *Metarhizium*-mediated inhibition of *Plasmodium* development could be a powerful weapon for combating malaria.

Nearly half of the world population is at risk of contracting malaria, and over one million people, mostly African children, die of the disease every year. Efforts to control the disease are hampered by increased resistance of parasites and vectors to drugs and insecticides (*1*). Emergence and spread of pyrethroid-resistant mosquitoes is a particular threat, because pyrethroid-treated bed nets are the mainstay of malaria control programs and there are no immediate prospects for new chemical insecticides (*2*, *3*). There is consequently a pressing need for practical alternatives for malaria control (*1*). Several field and laboratory studies have used fungi, such as *Metarhizium anisopliae*, that are pathogenic to adult mosquitoes. Unlike bacteria and viruses, fungal pathogens infect mosquitoes through direct contact with the cuticle and so lend themselves to strategies currently used for delivery of chemical insecticides, for example, being sprayed on indoor surfaces of houses, cotton ceiling hangings, curtains, and bed nets (*4*, *5*) or used in outdoor odor-baited traps (*6*). Fungal spores persist on some treated surfaces for months (*5*) and can be used in insecticide-resistance management or integrated vector management because fungal infections act synergistically with various insecticides [including pyrethroids and dichlorodiphenyltrichloroethane (DDT)], and fungi are equally effective against insecticide-resistant and insecticide-susceptible mosquitoes (*7*, *8*).

Using currently available fungal strains mosquito death is slow, but it takes about 12 to 14 days for *Plasmodium falciparum*, the causative

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