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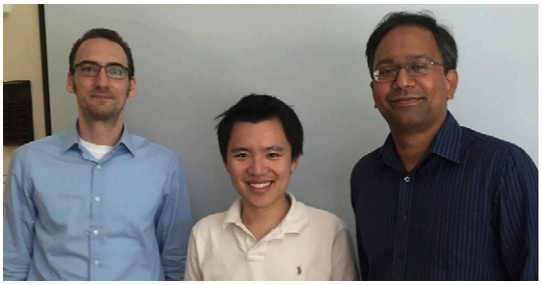
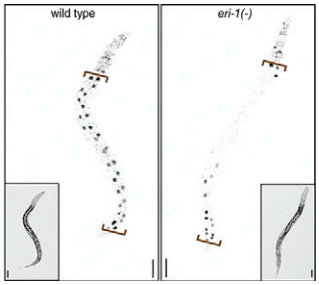
A program to reduce inequality

Study explores how developing organisms maintain tissue homogeneity.

FOCAL POINT





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(Left to right) Michael Bloodgood, Hai Le, Antony Jose, and colleagues investigate how *C. elegans* maintains tissue homogeneity by examining why, in the absence of the exonuclease ERI-1 (right), only some intestinal cells silence the expression of nuclear-localized GFP from a repetitive transgenic array. The researchers find that, in the absence of ERI-1, dsRNA produced from the transgene can stably silence GFP expression via the canonical RNAi pathway. This dsRNA is unequally segregated during the division of the blastomere that gives rise to intestinal cells, leading to the variation in transgene expression. ERI-1 maintains homogenous expression across the tissue by lowering dsRNA levels below a threshold required to initiate silencing.

During development, cells must differentiate to form diverse tissues, a process that is often set in motion by the unequal segregation of key factors during mitosis. Once a tissue has been specified, however, its constituent cells must remain similar to each other, even as they continue to divide and, by chance, segregate their components unequally to their daughter cells. Le et al. now identify a mechanism that helps maintain tissue homogeneity during *C. elegans* development (1).

Little is known about the factors that threaten or maintain tissue homogeneity, largely because it is difficult to measure the levels of different components in each cell of a particular tissue. But Antony Jose and colleagues at the University of Maryland stumbled on a system to investigate tissue homogeneity while studying the expression of a repetitive DNA transgene in *C. elegans*. The transgene in question consists of over 200 copies of the *sur-5::gfp* plasmid and, because the copies are arranged in different orientations, it produces double-stranded RNAs (dsRNAs) capable of silencing GFP expression by RNA interference (RNAi). Nevertheless, GFP is highly expressed—particularly in intestinal cells—because proteins such as the exonuclease ERI-1 inhibit the RNAi pathway (2). Deleting the *eri-1* gene allows the RNAi pathway to silence the *sur5::gfp* transgene in some cells, but other cells continue to express GFP at high levels (2, 3). "You see intestinal cells that are up to 100-fold different from each other with respect to GFP expression," Jose says. "So we wondered if we could use this system to study tissue homogeneity."

Jose and colleagues, led by undergraduate Hai Le, first investigated how the *sur-5::gfp* transgene is silenced, and found that dsRNA produced from the repetitive array is processed through the canonical RNAi pathway and the nuclear Argonaute protein NRDE-3 (1). The silencing was locus specific: the *sur-5::gfp* array could be silenced in intestinal cells that still showed expression from other repetitive transgenes or single-copy GFP inserts.

NRDE-3 promotes the deposition of repressive chromatin modifications at sites targeted by dsRNAs (4, 5), suggesting that the *sur-5::gfp* transgene can be stably silenced. Indeed, when Le et al. followed worms over several days of development, they saw that silencing was initiated in some intestinal cells before the larval stage, and that GFP expression then remained either on or off, even as the cells underwent DNA endoreduplication and nuclear division.

> **"ERI-1 maintains tissue homogeneity by keeping dsRNA levels below a threshold required to trigger silencing."**

Le et al. were also able to compare the intestines of different animals to determine whether particular cells always silenced, or always expressed, *sur-5::gfp* in the absence of *eri-1*. The transgene's expression pattern varied between animals, the researchers found, indicating that no cell is particularly susceptible or resistant to silencing.

This comparison was possible because the worm intestine arises via a stereotypical pattern of development that starts with the division of a single embryonic blastomere into anterior and posterior daughter cells. Using machine learning algorithms similar to those used by the linguist Michael Bloodgood, Le et al. determined that lineally related intestinal cells tended to regulate the *sur5::gfp* transgene similarly. If a descendent of the anterior progenitor cell showed *sur5::gfp* silencing, other anterior descendants were also likely to have turned off the transgene, whereas posterior descendants were more likely to continue expressing GFP. The converse was also true, suggesting that, in the absence of *eri-1*, heterogeneity arises when dsRNA, or another initiator of transgene silencing, is unevenly segregated between the daughters of the intestinal blastomere. "That causes silencing in some intestinal cells, which is stably maintained by a chromatin-based mechanism," Jose explains. "The exonuclease ERI-1 maintains tissue homogeneity by keeping dsRNA levels below a threshold required to trigger silencing."

Jose and colleagues now want to identify endogenous DNA repeats that are regulated similarly to the *sur-5::gfp* transgene. The mechanism could be crucial for maintaining overall tissue homogeneity, not only in worms but also in humans, whose genome consists of large amounts of repetitive DNA.

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3. Jose, A.M., et al. 2009. *Proc. Natl. Acad. Sci. USA*. 106:2283–2288.
4. Guang, S., et al. 2010. *Nature*. 465:1097–1101.
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