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Engineering Sexual Reproduction, Non-Mendelian Style

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An altered mitotic spindle prevents mixing of maternal and paternal DNA, yielding offspring from a single parent. How could this be used to study imprinting and epigenetics? Learn more...

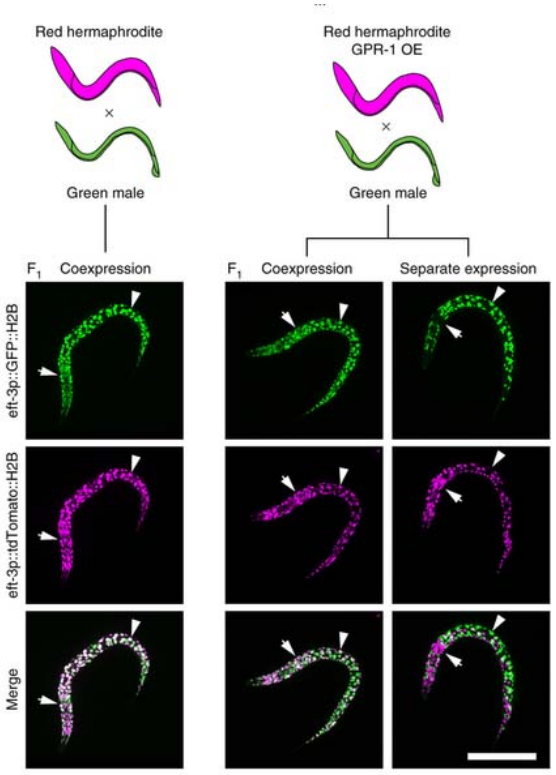
Sex may be mysterious, but what happens next, genetically speaking, is not. Sperm meets egg, genetic mixing ensues, and the next generation—bearing the blended genetic attributes of both mother and father—is born.

That process is called Mendelian inheritance, and it is the way sexually reproducing organisms, from humans to fruit flies to dinosaurs, have reproduced since time immemorial. But as it turns out, they don't necessarily have to.

In a study published in *Nature Biotechnology*, Judith Besseling and Henrik Bringmann of the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, describe a strategy for so-called "non-Mendelian inheritance" in *Caenorhabditis elegans*. Tinkering with the mitotic spindle apparatus disrupts the fusion of maternal and paternal genetic material that occurs prior to the first embryonic cell division. As a result, cells created through this process represent one parent or the other, but not both (1).

"This is very pioneering," said Alexandra Bezler, a postdoctoral fellow at the University of Lausanne, Switzerland, who also studies non-Mendelian inheritance. "Nobody has done this in any organism before."

According to Bringmann, the method has a range of applications, from breeding and epigenetics to synthetic biology. "This is a good proof of principle that by changing cell biological structures, we can generate cells—and ultimately animals—with novel properties."



Increasing microtubule-based pulling forces leads to two monopolar spindles, which segregate chromosomes unequally (1).

Tuning Gene Expression

Mendelian inheritance is far afield of Bringmann's primary research focus. His lab concentrates primarily on sleep regulation and function, but with a background in genetics and cell biology, he also studies the mitotic spindle.

In 2011, Bringmann's team described a method for adjusting exogenous protein expression in *C. elegans* by tweaking codon usage. Basically, by using a cell's tRNA abundance to guide codon usage in a transgene, the team found they could boost or dampen protein expression in nematodes (2). One of the proteins they used to test that method was GPR-1, a component of the mitotic spindle.

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Built of microtubules and molecular motors, the mitotic spindle positions sister chromatids in the center of the dividing cell during metaphase and then pulls the individual chromosomes apart during anaphase, thus ensuring that both daughter cells receive a complete complement of genetic material.

GPR-1 is one of the critical force regulators of that process. Loss of GPR-1 weakens the spindle pulling force; overexpression of the protein can disintegrate the structure, yielding not one bipolar spindle but two monopolar ones. Bringmann wondered what would happen if that disintegration occurred during the first cell division of embryogenesis.

To find out, he and Besseling used codon adaptation to ramp up GPR-1 expression specifically in the *C. elegans* germline. They then allowed a sperm to fertilize an egg and watched what transpired via time-lapse microscopy. As predicted, the two pronuclei, each containing the genetic contribution of one parent, were yanked apart into two daughter cells before they could fuse. As a result, the worms that grow from those embryos contain organs derived either from the father or the mother, but not both.

"That's the cool thing about it, that it actually works," Bringmann said. "There is usually a lethality associated with everything whenever you mess with the mitotic spindle." But in this case, the separation of genetic material is so clean

that the embryos survive.

Multicolored Worms

To demonstrate that point graphically, Besseling and Bringmann tagged GPR-1-overexpressing hermaphrodites with green fluorescent protein (GFP) and normal males with the red fluorescent protein tdTomato. In normal Mendelian inheritance, the resulting offspring would be made entirely of cells expressing both proteins, which appear yellow in dual-color overlays. But when GPR-1 was overexpressed, the animals contained cells that were monochromatic.

C. elegans is unique among model organisms in that it has a defined, and invariant, cellular lineage. Researchers have worked out precisely which cells in the adult arise from which progenitors, and they can trace those lineages all the way back to the two-cell stage. When a fertilized *C. elegans* egg divides, it produces two cells, AB and P1. The former produces specific somatic tissues, including the nervous system; the latter produces the remaining somatic tissues as well as the germline. Normally, that doesn't matter, as every cell is genetically identical. But using Bringmann's technique, if the AB cell is paternally derived, the result would be a worm with a paternal nervous system and maternal gametes; if the AB cell is maternal (as was most frequently the case), the converse would be true.

"Ultimately," Bringmann said, "this is a novel way of cloning animals."

The team reported relatively high lethality rates of 28% to 63% in some crosses, likely stemming from the fact that *C. elegans* males have no Y chromosome— they are "XO." As paternally derived gametes have no X chromosome, a non-Mendelian worm with paternal gametes has a 50/50 chance of producing an embryo that contains no X chromosome at all when crossed to a male. "These cells obviously are inviable," Bringmann said.

The method has several exciting applications, especially for genetics and epigenetics. For instance, it enables the generation of mosaic animals in which different tissues in the same organism arise from parents with different constellations of phenotypes. "Entire genomes, not individual mutations, that is the advantage here," Bringmann said.

Non-Mendelian animals should also provide a platform for studying genetic imprinting, the differential expression of certain genes depending upon whether they are maternally or paternally derived. And they can be used to study the impact of environmental exposures on future generations of animals, a concept called transgenerational inheritance.

According to Bringmann, the non-Mendelian inheritance strategy should work in other, higher eukaryotes, including mice, as GPR-1 is highly conserved. Indeed, preliminary experiments in mammalian tissue culture suggest GPR-1 over-expression leads to similar force amplification, although the team has not yet tried it in the zygote.

That said, however, Bezler thinks the method may prove less useful as researchers move up the evolutionary ladder, since other organisms do not share the rigidly defined cell lineages of *C. elegans*. Although individual cells would indeed be maternal or paternal in origin, entire tissues likely would not be. "It will just be random, and it will be random in each animal." That could complicate large-scale analyses, she said.

But at least in *C. elegans,* Bezler sees considerable promise. And it is one she plans to act on. "We ordered the strains," she said. "We will do something with it."

References

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