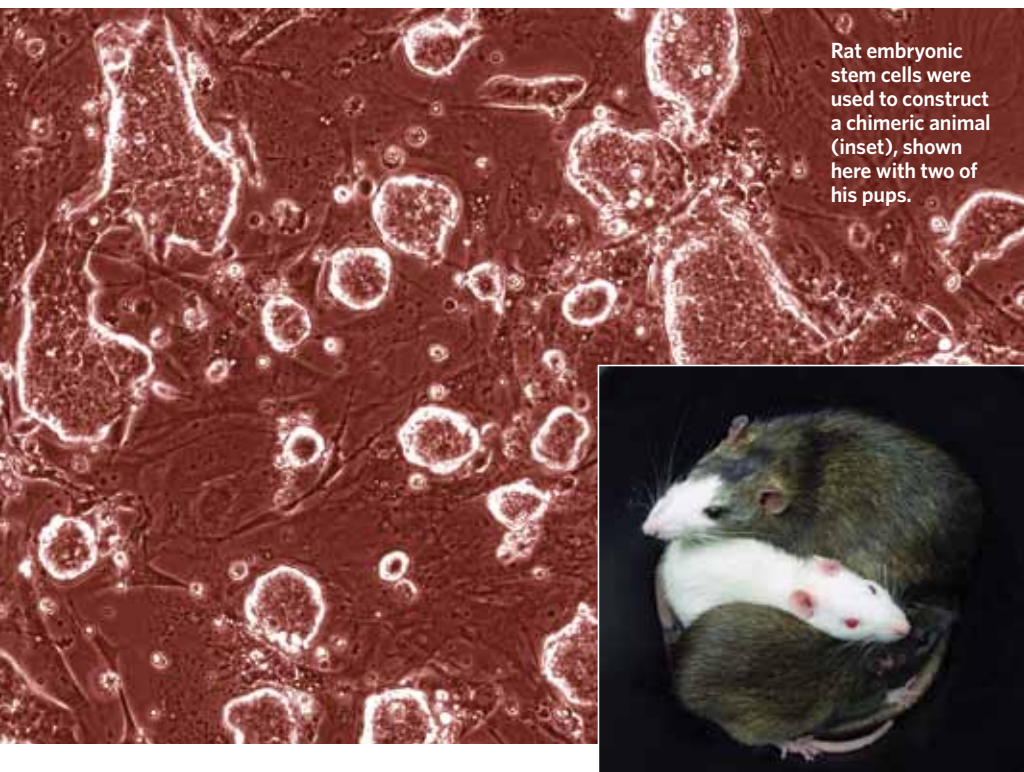


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# Notebook



Rat embryonic stem cells were used to construct a chimeric animal (inset), shown here with two of his pups.

## Stem cell rat race

In 1981, Martin Evans and Matthew Kaufman, working at the University of Cambridge, UK, and Gail Martin, working at the University of California, San Francisco, independently and simultaneously discovered methods to isolate mouse embryonic stem (ES) cells and grow them in culture. The hard part, they assumed, was over. “Everyone thought that within five minutes we’d have embryonic stem cells from everything,” including the mouse’s cousin, the rat, says Mia Buehr, a postdoc at the University of Edinburgh. But that achievement would take another 27 years.

By the early 1990s, researchers were routinely altering the DNA in mouse ES cells to create genetically engineered mice with missing, added, or modified genes. But the same techniques did not work in rats.

Buehr joined Austin Smith’s Edinburgh lab in 1994 tasked with smoking out stem cells from the impervious rat, whose larger size and behavioral sophistication make them more effective animal models than mice for studying many human diseases.

For over a decade, Buehr meticulously tried everything and anything that worked for deriving mouse ES cells to nail down the necessary culture conditions for obtaining rat ES cells. This line of attack was fairly standard, but it wasn’t up to snuff for obtaining rat ES cells. “There’s just a lot of species specificity,” says John Critser, the principal investigator of the Rat Resource and Research Center at the University of Missouri in Columbia.

Buehr and others successfully isolated embryonic stem-like cells, which had some cellular markers consistent with stem cells. However, they couldn’t create a chimeric animal from two cell sources once the stem cells were injected

into a developing blastocyst—the hallmark of an ES cell and a prerequisite for crafting knock-out and knock-in rats.

In 2006, Smith’s team finally had a breakthrough. Qi-Long Ying, another postdoc, discovered the “3i” medium, a cocktail of compounds with three inhibitors of transcription factors involved in cell differentiation. Buehr then helped craft a “2i” formula that included only two inhibiting factors. At long last, she and Ying reported last December that they had successfully derived true rat ES cells that could be genetically manipulated (*Cell*, 135: 1287-98 & 1299-1310, 2008).

Ying, now at the University of Southern California in Los Angeles, suspects that the reason it took so long to obtain rat ES cells was that researchers were stuck on using leukemia inhibitory

factor (LIF), a cytokine that blocks differentiation and maintains pluripotency. LIF is used near-universally for deriving mouse ES cells, but did nothing for the rat ES cell culture media, the researchers found.

Generating transgenic rats with targeted mutations is now “our top priority,” says Ying. Although no one has yet successfully made a rat genetically engineered from ES cells, Ying believes this could happen as soon as this summer, “if all goes well.”

Stem Cell Sciences, a Cambridge-based company that Smith co-founded, is already “in discussions with a number of animal houses and pharmaceutical companies that are interested in the development of genetically engineered rats,” says the company’s CEO Alastair Riddell.

ES cells might not be the only route to genetically engineered rats, however. In January, two research groups reprogrammed adult rat cells—including from liver, skin, and bone marrow cells—to

**Why it took nearly 30 years to isolate ES cells from rats.**

produce rat induced pluripotent stem (iPS) cells (*Cell Stem Cell* 4:11-5 & 16-9, 2009). The team led by Sheng Ding of the Scripps Research Institute in La Jolla, Calif., also successfully generated chimeras from the reprogrammed cells. These cells “can be now used to actually make transgenic animals,” says Ding, who licensed the iPS technology to San Diego-based Fate Therapeutics, a company he helped co-found. “It opens the door to additional model systems that are more feasible in rats than in mice, which is a big deal,” says Paul Grayson, Fate’s president and CEO.

Philip Iannaccone, a developmental biologist at Northwestern University Medical School in Chicago, suspects that it’s now only a matter of time before the first transgenic rats are born. “The combination of Austin Smith’s inhibitors and the iPS approaches are going to prove to be very powerful,” he says.

In addition, Buehr and Ying say that they are each independently now trying to use the 2i and 3i media to isolate ES cells from larger animals, including pigs, cows, and sheep. “This same story has to be repeated in other animals,” Buehr says. “It doesn’t stop with rats.” —**Elie Dolgin**

## Septic sperm

In 2006, Hannah Seidel, a graduate student in Leonid Kruglyak’s lab at Princeton University, performed an experiment that hundreds of *C. elegans* biologists had done before: She crossed two common worm strains, and looked at the progeny. Only this time, unlike previous experimenters, Seidel inspected the Petri plates a bit more carefully. And in the second generation, she noticed scores of dead eggs.

Seidel had decided to perform the experiment after evolutionary geneticist Matt Rockman, then a postdoc working with Kruglyak, had crossed the same parental strains to construct more than 200 recombinant inbred worms for mapping genes. He was inspecting the resulting worms’ genotype data when he noticed that on the left arm of chromosome I, one of the parental strains had

contributed far more than its fair share of DNA: The SNP ratio was nearly 50:1, not 50:50, as he expected. Rockman “basically gave me some worms and said ‘See if you can figure out what’s going on,’” Seidel says.

Next, the two of them were staring at a collection of dead worms. How had Rockman—not to mention the hundreds of *C. elegans* biologists before him who had also crossed the same two strains for basic gene mapping and slews of other

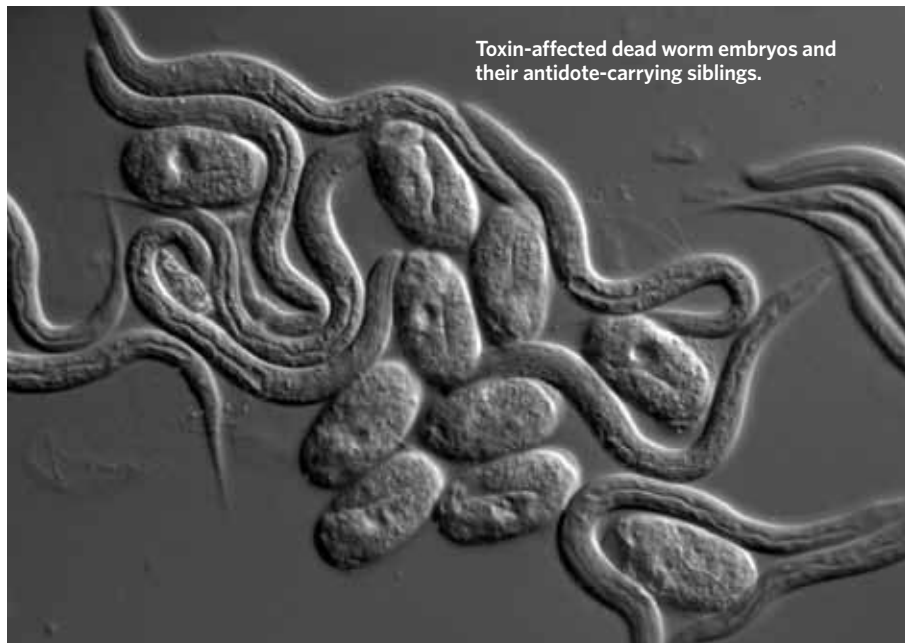
### One quarter of the F2 offspring died.

experiments—missed such an obviously deadly incompatibility? “It’s because the

the offender: It was, indeed, a gene with cross-generational parental effects. But it was a paternal-, not a maternal-effect gene, that operated through the fathers—an extremely rare type of gene and only the second one discovered in *C. elegans*.

The gene—named *peel-1*—bestowed fathers with baby-killing, toxic sperm. Additional fine mapping also revealed a second gene, *zeel-1*, which was tightly linked to *peel-1* and acted as an antidote, making offspring immune to their father’s lethal sperm (*Science* 319:589-94, 2008).

In their cross, one of the parental strains had the toxin and the antidote alleles, while the other strain lacked both.



Toxin-affected dead worm embryos and their antidote-carrying siblings.

worms live so fast,” Rockman, now at New York University, professes. “Unless you count embryos as soon as they’re laid, the plate becomes covered with worms.”

Seidel crunched the numbers and realized that almost exactly one-quarter of the F2 offspring died. According to Mendelian theory, however, basic incompatibility genes should only produce a maximum lethality of three-sixteenths. She did some back-of-the-envelope calculations and realized that a maternal gene that affects developing embryos through secreted proteins in the egg—a so-called maternal-effect gene—could explain the data. She set up a couple more crosses, and pinpointed

Put them together, and toxic F1 fathers were killing off susceptible F2 offspring, but in a genotypically-biased fashion, with the offspring of one strain much more likely to die. The legacy of this one-sided infanticide was what Rockman had stumbled upon in the genomes of his recombinant inbred worms.

What’s more, the deadly one-two genetic punch was not restricted to the worms used in the initial cross. Rockman and Seidel tested more than 50 different strains from around the world (including strains collected by this author for his PhD), and discovered both haplotypes in equal abundance.

Two big outstanding questions remain, says Rockman: Why have both allelic versions been preserved, and what's the modus operandi of the toxin and antidote? "It continues to be mysterious," he says.

"We can see these signatures of what must be selection and the population genetic process," says Patrick Phillips, an evolutionary geneticist at the University of Oregon. "But without understanding the ecology it will be difficult to get at the causation."

Seidel is making headway with the problem, though. She showed that the toxin encodes a small protein that is localized to the sperm cell membrane, while the antidote is activated at mid-embryogenesis, around six hours after the toxic sperm enters the egg. Without the antidote, however, embryos die around two hours later.

Importantly, this implies that the sperm's toxin protein stays intact for at least eight hours. So the big puzzle now, Seidel says, is how the protein remains latent but not broken down for several hours. She suspects that the toxin might be highly resistant to degradation; enough so that it manages to disrupt muscle development many hours later. "It's not impossible," she says, "but no one has ever seen a sperm protein stick around for that long." —**Elie Dolgin**

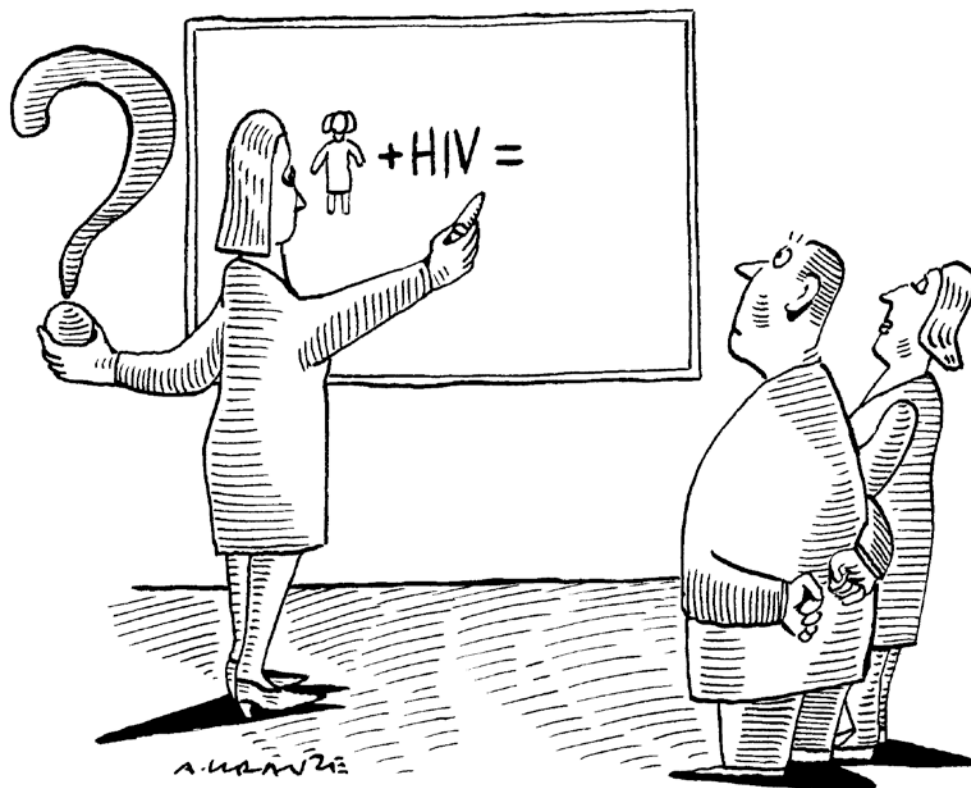
a disease from one place to another. She just laughed at me," Steward recalls.

That's because the crux of tracking disease lies in relatively simple equations, which Blower drew out and explained for Steward on a large white board.

Steward was "blown away" by Blower's thoroughness and enthusiasm. But

models "has been very satisfying," Blower says. But not all scientists side with Blower's models, arguing that reality (and not just TV) is significantly more complex than what she presents in her equations.

Last July, Blower published a model showing that widespread use of vaginal microbicides—an antiretroviral therapy



## Working modeler

One day in late 2004, television art director Karen Steward visited the penthouse floor of a glass office building in Los Angeles to sit down with UCLA epidemiologist Sally Blower and the half dozen members of Blower's Disease Modeling Group and talk about television. Steward was enlisting Blower's scientific expertise for the third episode of the CBS drama *NUMB3RS*, in which an FBI agent's brother uses mathematical models to determine the origin of a mysterious outbreak of Spanish flu.

Presenting the multicolored script, "I asked her to show me graphics on her computer screen that show how to track

the equations—which include parameters such as how long the infection lasts in one person and how many susceptible people that person contacts—were apparently too simple for Hollywood.

"She wanted pretty math," Blower recalls. "So we ended up writing down equations for them that really didn't have anything to do with what they were saying."

For the last two decades, Blower has applied her predictive models to a diverse array of disease scenarios, including a recent, controversial paper that suggested a vaginal microbicide against HIV could surprisingly benefit men more than women. That an increasing number of public health experts are starting to welcome the predictions of mathematical

that was designed to help prevent HIV infection in women—could do more for men than women, and increase the risk of drug resistance in women who are already infected with the virus (*PNAS*, 105:9835-40, 2008).

Microbicide experts took issue with the model's real-world relevance. "In some way, we felt like they slightly missed the point," says Lori Heise, director of the Global Campaign for Microbicides, in Washington, D.C., who published these concerns in a November letter to *PNAS* (105:E73, 2008). It's not clear whether the microbicides are absorbed in the bloodstream, where drug resistance would occur, Heise says. Plus, in order for men to benefit from the microbicide, it would have to protect them from an HIV- ▶