these events haven't been narrowed quite enough. Nigel Brown, a teratologist (specializing in the study of congenital malformations) at St. George's Hospital Medical School in London, and Lewis Wolpert, a developmental biologist at University College, London, are enthusiastic about the advance, but say that a 50/50 ratio of heart handedness can result from other influences, such as heat shock, and that Tabin's group should have tried for a 100% flip by implanting wrong-sided activin and *Shh* in the same embryos. Tabin responds: "We think the results are unlikely to be artifactual, because control implants did not cause randomization. However, the experiments Lewis and Nigel suggest are indeed being done."

Brown also notes that the search for equivalent genetic cascades in other organisms could run into "several potential difficulties." Mice lacking activin receptor genes, for example, have normal asymmetry, suggesting that mammals may not require this protein for left-right specification, he and Wolpert note.

Still, Wolpert is confident the researchers are on the right track. "Right now there are puzzles," he says, but left-right specification in vertebrates "will probably all be the same in the end." For an asymmetrical story, that would be a strikingly even-handed conclusion.

–Wade Roush

IMMUNOLOGY

Long-Sought H-Y Antigen Found

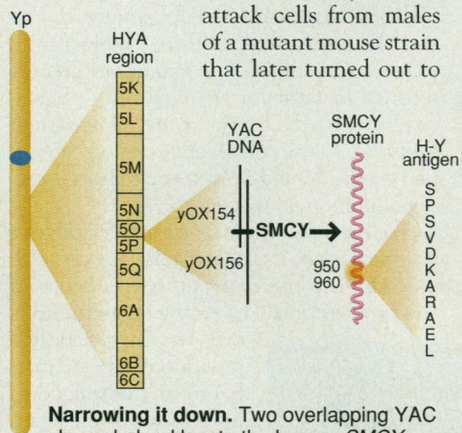
Ever since 1955, transplant surgeons and immunologists alike have been perplexed by a mystery. Although males seem to tolerate transplanted female tissues very well, females sometimes reject transplants from males, even when the tissues are closely matched immunologically and genetically. "It's something that's been driving immunologists crazy for many years," says immunologist Victor Engelhard of the University of Virginia, Charlottesville. But no longer.

Two reports, one in the 24 August issue of *Nature* from a team led by Elizabeth Simpson of the MRC Clinical Sciences Centre at Hammersmith Hospital in London and Michael J. Mitchell of INSERM in Marseilles, France, and the other on page 1588 of this issue of *Science* from Engelhard, Els Goulmy of Leiden University Hospital in the Netherlands, and their colleagues, have now solved at least part of the mystery. Both groups have found an elusive factor that makes male tissue unacceptable to females, the Simpson-Mitchell team in mice and the Engelhard-Goulmy team in humans. It turns out to be a short peptide encoded by a segment of a gene called *SMCY*, which is found on the Y chromosome of males.

Immunologists are relieved, says Polly Matzinger, an immunologist at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland. "Forty years of failure makes people hungry," she declares. But the quest may not be over, as more H-Y antigens may lie waiting in the wings. Even so, the discovery means that researchers can begin to develop ways to avoid or neutralize H-Y rejections and, as a result, allow more frequent male-to-female transplants. In fact, the prospect that H-Y antigens might be clinically useful delayed publication of the *Science* paper as the biotech firm Promega Corp. of Madison, Wisconsin, sought to protect its stake in the finding.

Immunologists learned early on that the antigen at fault in the organ rejection was encoded by a Y chromosome gene, because the problem occurred in inbred mice that were genetically identical except for the fact that the males had a Y chromosome while the females didn't. That's why they dubbed it the H-Y antigen, for histocompatibility antigen from the Y chromosome. But for 2 decades researchers made little headway in further narrowing down H-Y's identity or the gene's location, primarily because they were unable to come up with antibodies to the antigen that could help them in their search. As immunologists learned in the mid- to late 1970s, antigens like H-Y are formed intracellularly and, as a result, do not generally trigger antibody production. Instead, proteins encoded by genes of the major histocompatibility complex (MHC) display the antigen peptide on the cell surface, where it draws attack by the immune system's T cells.

At about the same time, geneticist Simpson established T cell clones that specifically killed cells carrying the H-Y antigen, thereby providing a tool that would help in the hunt. Indeed, in the late 1980s, the cells provided a critical clue when Simpson found that they did not attack cells from males of a mutant mouse strain that later turned out to be missing a piece of the short arm of the Y chromosome. This showed that the H-Y antigen gene was located in the deleted section, thereby narrowing the search down to about 900 kilobases of DNA.



Narrowing it down. Two overlapping YAC clones helped locate the human *SMCY* gene, which codes for the H-Y antigen.

SOURCE: WANG *ET AL.*

By that time, geneticist Colin Bishop, who was then working at the Pasteur Institute in Paris, and his colleagues, including Mitchell, who was in Bishop's lab at the Pasteur, had begun a systematic search through the DNA of the deleted Y chromosome region. But it was not until 1994, after Bishop had moved to Baylor College of Medicine in Houston, that he and his colleagues found a likely looking gene: *SMCY*, which stands for "selected mouse cDNA on Y." Unlike earlier candidates, which were active only in the testis, this gene was expressed in all kinds of tissues. Also, even though *SMCY* has a counterpart, *SMCX*, on the X chromosome, the two genes are only 82% identical—different enough that peptide fragments of the male protein could look foreign to a female immune system.

Simpson and her colleagues built on that work, she says, by combing the *SMCY* DNA for the specific sequence coding for the peptide: "We approached it scissorlike—immunologically and genetically." In collaboration with Mitchell, her group began putting ever smaller pieces of *SMCY* DNA into cells taken from female mice and testing those cells to see if they were attacked by the H-Y antigen–specific T cells. They also compared the DNA pieces that elicited a reaction with parallel sections of *SMCX* to identify the differences. By June, the Simpson-Mitchell team had homed in on their H-Y antigen, an eight-amino-acid peptide located near one end of the SMCY protein. "It's a great relief and pleasure," Simpson says.

The Engelhard team took a totally different tack in their search. Instead of dissecting a promising gene, they pursued the peptide directly. Engelhard and his chemist collaborator Donald Hunt, also at the University of Virginia, had developed a technique for pinning down hard-to-find peptide antigens by pulling them off the MHC molecules that display them on the cell surface and then sorting and testing the peptides. Engelhard says that after he was contacted earlier this year by Goulmy, who had worked with Simpson pursuing H-Y in both mice and humans, "we all agreed that H-Y, because of its history, should be our next [target]."

By June, the team had identified a candidate peptide. When they then searched the databases for genes that could encode a peptide with that sequence, the best they came

up with was an SMCX sequence, which encoded nine of the 11 amino acids. At that point, the SMCY gene was not in the database, but when Engelhard called Bishop about SMCX, he learned about the other gene and in 24 hours had the SMCY sequence in hand to compare. "It was a perfect match," Engelhard says.

The Simpson-Mitchell team submitted its paper to *Nature*, while Engelhard, Goulmy, and their colleagues sent theirs to *Science*. The manuscript editors at the two journals agreed to publish the papers simultaneously. But it was not to be. One of Bishop's colleagues had done some of the SMCY sequencing while at Promega. Thinking it was OK, that colleague and Bishop supplied Engelhard with the entire gene. But as Bishop now says ruefully, "Apparently, I should not have done that." Promega complained and demanded time to file a patent on the gene, thus delaying publication of the *Science* paper.

Meanwhile, onlookers are wondering whether H-Y warrants such a legal frenzy. "The clinical significance [of H-Y] is not so clear," says Harald von Boehmer, an immunologist at the Basel Institute for Immunology in Switzerland and the Institut Necker, INSERM, in Paris. "Nobody really knows how high the risk [of rejection] is with this particular antigen." Attempts to use the peptide to develop anti-rejection therapies could also be complicated, because Simpson has shown there is more than one H-Y antigen. There are even hints that SMCY may not be the only gene encoding them.

A great many other questions also remain to be answered about the SMCY gene. One concerns its normal function. "Nature didn't worry about skin grafts. The true biological role of SMCY can't be in transplant rejection," Bishop quips. Nor is it, as was once proposed, the sex-determining gene, even though it seems to exist in many different species, showing that it has been conserved during evolution.

What else it might do remains unclear, although one clue comes from the structure of the SMCY protein. It resembles that of other proteins known to be transcription factors that turn genes on or off. But at this point, no one has direct proof that H-Y is a transcription factor, never mind what genes it might control.

All in all, the H-Y antigen mystery is beginning to take on the features of a Russian doll: Removing the first layer only reveals the deeper mysteries within, each a little more intricate. But solving the mysteries will be well worth the trouble, von Boehmer predicts: "It must have some important function; otherwise it wouldn't be so conserved."

–Elizabeth Pennisi

Elizabeth Pennisi is a science writer based in Takoma Park, Maryland.

MICROBIOLOGY

Call to Desegregate Microbial Databases

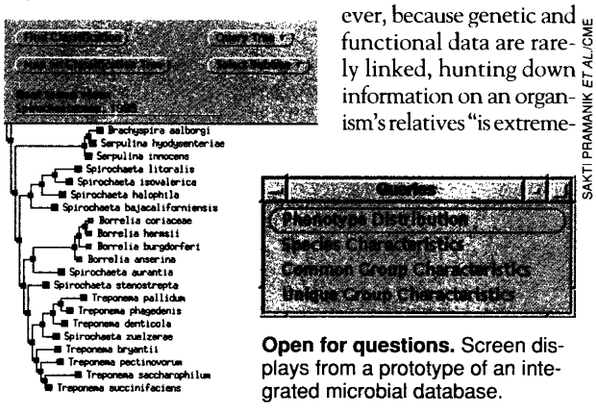
Biological databases can be powerful tools for comparing organisms or pooling different kinds of information about a single species. But when complementary information about an organism is scattered in different databases, they can also become a powerful source of frustration. That's the problem facing microbiologists, who have built a vast array of separate databases on the ecology, biochemistry, physiology, and genetic makeup of microbes—with no easy way to extract data from several sites at once. But relief is on the way: Two weeks ago, at the International Symposium on Microbial Ecology in Santos, Brazil, researchers announced an international effort to end this fragmentation of their field by linking their databases into an integrated web that will be known as the Federated Microbiology Database.

A modest version of this web, connecting three different databases, is already under construction at the National Science Foundation's Center for Microbial Ecology (CME) at Michigan State University in East Lansing. But the larger scheme announced at the Brazil meeting would enable microbiologists to trace both functional and genetic traits through a vast number of microbes without having to consult many databases individually. "The amount of time-saving will be so great," says microbiologist Gary Olsen of the University of Illinois, Urbana-Champaign, "that we'll be able to pose questions we just haven't been able to before."

A major goal of the effort, led by the CME and initiated by its director, Jim Tiedje, is bridging a gap that reflects the field's history. Like botanists, microbiologists originally classified organisms by visible characteristics such as shape and color; later, they took a whole array of physiological characteristics into account as well. Since the 1970s, genetic technologies have also made it possible to study and classify microbes at the genetic level. The result, however, has been two distinct sets of databases.

The federated database project should end this separation, allowing biologists to "understand and explore taxonomic characteristics in terms of an organism's phylogeny," says CME's Larry Forney. As a result, it should help microbiologists explore microbial evolution and deepen their understanding of individual species.

For example, when a researcher isolates a new pathogen, its genetic relatives can provide clues to the new pathogen's behavior and how to control it. At the moment, however, because genetic and functional data are rarely linked, hunting down information on an organism's relatives "is extremely tedious and time-consuming," says Olsen. When the federated database is in place, researchers will be able to automate this kind of complex data search using specialized software designed to interrogate all the relevant databases and report back.



SAKTI PRAMANIK ET AL./CME

Open for questions. Screen displays from a prototype of an integrated microbial database.

Such software tools exist already, says Forney. A more difficult part of the project, he says, will be persuading database managers to open their data collections, many of which are incomplete or proprietary. The first step toward that goal came in early August, when representatives of the world's leading microbial databases met at the CME, agreed on the need to link their databases, and laid plans to link three of the most important ones: the Ribosomal Database (a University of Illinois project that contains sequences of ribosomal RNA genes), a fatty acid methyl ester profile database (a set of data on cellular fatty acids), and the Phenotypic Database in *Bergey's Manuals* (a collection of taxonomic data based at Michigan State). This three-way linkage should be completed within a year, with work being coordinated by the CME.

Expanding that core into the federation proposed in Brazil will take a good deal more effort and expense, says Forney—much of it to complete the data sets in the individual databases and prepare them for open access. The organizers expect that much of the funding will come from the U.S. and other governments, but they are also hoping to attract corporate and foundation sponsorship. If they succeed, the world of microbes may start to look a little less fragmented.

–Margaret Wertheim

Margaret Wertheim's book Pythagoras' Trousers—*a history of the relationship between physics and religion—has just been published by Times Books.*