

concluded that "this method may be promising for industrialization."

Opinion is divided over the question of how much further progress is dependent on solving the mechanism of the deposition process. The basic issue is the prevention of the formation of the double bonds between carbon atoms that are slightly favored energetically and that lead to the formation of graphite (the carbon atoms in diamond are linked entirely by single bonds). The presence of atomic hydrogen favors the breaking of double bonds and the formation of single bonds (sp^3 hybridized orbitals), which accounts for its solvent effect on graphite. The presence of the methyl radical with its sp^3 orbitals is also thought to be helpful, which effects the choice of starting material. Pinneo at Crystallume thinks, for example, that growth rates can be substantially enhanced if more efficient ways of generating the methyl radical are found. But the details of how all this fits together are not well understood.

Another growth method may also work better. There is some interest in a so-called remote plasma-enhanced chemical vapor deposition technique in which a molecular gas is dissociated in a location away from the substrate. The resulting monomers and an organic gas then flow through a heated region toward the substrate, where pyrolysis and deposition occur as before. Gerald Lucovsky and several colleagues at North Carolina State University and Markunas developed this method for the deposition of insulating materials, such as silicon nitride, on advanced semiconductor structures. It is said that the Research Triangle group used a variation of this method with plasma-excited helium to make its diamond films. That the method works without hydrogen suggests that it is the energy of the plasma-excited species rather than its chemical properties that facilitates diamond formation.

The development of techniques for the rapid manufacture of large quantities of diamond film is only one of the numerous tasks yet to be mastered before potential applications can be actualized. It is not certain, for example, how to bond diamond films to tools. Comparable issues arise in other applications. While the excitement over diamond may be merited by the potential of the material, so far it is only potential. ■ **ARTHUR L. ROBINSON**

ADDITIONAL READING

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Maleness Pinpointed on Y Chromosome

The sex-determining section of the Y chromosome is on the short arm—not where everyone thought it would be

No one really knows what makes a male a male or a female a female. But researchers thought they at least had a hint. Their hypothesis was that the mysterious H-Y antigen somehow determined maleness in mammals. Femaleness was determined by default; those who lack the H-Y antigen become females. Now, however, the H-Y antigen has fallen out of favor as new studies that use the sophisticated tools of molecular biology point to an entirely different chromosomal location for the sex-determining factor. The evidence was discussed on 9 to 10 October at a workshop sponsored by the National Institute of Child Health and Human Development.

There are practical as well as theoretical reasons for wanting to know how the two sexes exist. For instance, animal breeders want to establish the sex of embryos before transferring them to surrogate mothers. Reproductive specialists would like to understand the basis of sex determination because mutations or deletions of sex-determining chromosomal regions have been associated with human infertility.

Ernst Eichwald of the University of Utah, who first discovered the H-Y antigen in the 1950's, told the meeting participants that doctors call him virtually every month asking whether he does H-Y testing to establish the sex of babies born with ambiguous genitalia. Eichwald does not offer this service, and many researchers think that H-Y antigen testing would not resolve the question of sexual ambiguity anyway, since frequently what is wrong is an inappropriate amount of or response to sex hormones.

For decades, researchers have known that some sequence on the Y chromosome determines maleness, at least in mammals. In the 1950's, Eichwald serendipitously discovered the H-Y antigen when he tried to transplant the skin of male mice onto females of the same inbred strain. Ordinarily, mice of the same inbred strain accept skin transplants. But the female mice, Eichwald discovered, rejected the male skin, indicating that there is an antigen that is characteristic of males. He called it the H-Y antigen because it is a histocompatibility antigen and an antigen

that seems unique to the Y chromosome. In fact, he suggested, it could actually be two separate antigens.

Years later, Ellen Goldberg of the University of New Mexico learned that the H-Y antigen, as defined by antisera rather than transplantation, is conserved in evolution and that it is present at the earliest stages of embryo development—in an eight-cell mouse embryo, for example. Finally, in 1975, Susumu Ohno of the City of Hope Research Institute in Duarte, California, proposed the H-Y hypothesis: The H-Y antigen is the diffusible substance that results in testes formation in mammals. It acts like a cell adhesion molecule for the formation of the testes cords.

A few years ago, however, Ann McLaren of the Medical Research Council in London reported some contrary findings. She found a mouse strain that had two X chromosomes yet was male because part of a Y chromosome was translocated onto one of the X chromosomes. These mice, however, do not make the H-Y antigen. McLaren concluded that the H-Y antigen does not determine testes formation but might instead have something to do with spermatogenesis because the mice do not produce sperm.

Michael McClure of the child health institute notes that some people still are trying to use the H-Y antigen to determine the sex of animal embryos. "Some say it works and some say it doesn't," he remarks. "But many people are nervous about using the H-Y antigen now." One difficulty, says David Page of the Whitehead Institute, is that the term H-Y antigen might convey different things to different people. Those, like Eichwald, McLaren, and himself, who define it as a transplantation antigen, are looking at a T-cell response. Those, like Goldberg and Ohno, who define it serologically, are looking at a B-cell response. "There is considerable debate about whether all these investigators are talking about the same thing," Page says.

Recently, Page brought molecular biology to the field. He began by searching for "cases in which the chromosomes and the gonads don't match or in which there is an abnormal Y chromosome." And he found

what seems to be the human equivalent of McLaren's mice by studying patients who were referred to him by infertility clinics. So far, out of 80 patients that he has studied, he has found six XY females who have a deletion of the male-determining portion of the Y chromosome. And he has found 20 normal-looking men who have two X chromosomes and yet are male because a piece of a Y chromosome is translocated onto one of their X chromosomes. Like McLaren's mice, these men produce no sperm.

The men came to infertility clinics in the first place, says Page, either because they appeared to be sterile or because they have small testes. "Why are they sterile? Apparently, they have two strikes against them. Two X chromosomes are incompatible with male fertility. Men with two X's and one Y are infertile and the only thing wrong with them is the two X's. Also, the XX men do not have an intact Y chromosome. The long arm of the Y, which is missing in these men, might play a role in spermatogenesis." Still, Page recognizes, his explanation for these men's infertility, "is an answer but not really an answer."

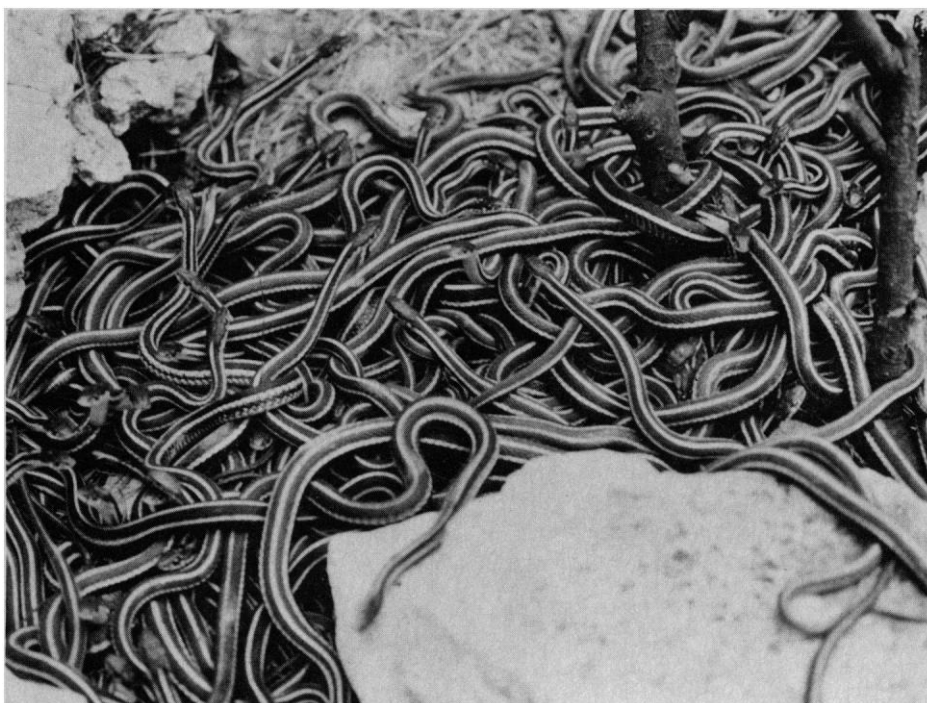
By doing chromosome mappings, Page determined that all the XX men have in common deletion interval 1A, which is on the short arm of the Y chromosome and that the XY women all lack this region. Moreover, he and Elizabeth Simpson of the Medical Research Council in England find that the gene for the H-Y antigen is near the centromere or on the long arm of the Y chromosome—"far from 1A."

Page notes that most DNA in the vicinity of the male-determining gene of humans was recently acquired by the Y chromosome and has no role in sex determination. "Most DNA sequences near 1A are homologous to the long arm of the X chromosome. As much as one-half of the short arm of the Y chromosome is the result of a single huge transposition of the X chromosome that occurred in evolution after the human line diverged from the chimpanzees. On the contrary, one would expect the testes-determining factor itself to be conserved on the Y."

It is an intriguing lead and everyone expects that it will not be long before molecular biologists actually isolate the gene or genes that cause testes to form. But, said the researchers at the meeting, there is, of course, far more to sex than testes or ovaries and, as David Crews of the University of Texas at Austin illustrated, there are examples of animals that confound many common assumptions about sex. For example, it often is assumed that males are the so-called organizing sex because they have two different sex chromosomes. But in birds, females

are the organizing sex—they have two different sex chromosomes whereas males have two identical sex chromosomes. "Birds are the mirror image of mammals," Crews says.

Another common assumption is that sex is determined by sex chromosomes. But leopard geckkos turn out to be males or females depending on the temperature at which they are incubated. At 25°C, only females hatch out of the eggs. At 32°C, virtually every egg hatches into a male. At an intermediate temperature, half the eggs hatch females and half hatch males. The few females that do hatch when the eggs are incubated at high temperatures act like males during courtship and never have been observed to lay eggs or to mate, even though they seem to be physically normal females.



Breeding with old sperm. Red-sided garter snakes breed in the spring, but the males use sperm that they made the year before and stored during the winter. They produce new sperm in the summer after they breed. [Source: David Crews, University of Texas at Austin]

Then there is the case of the animal that is neither male nor female. Of the 45 species of *Cnemidophorus*, a 10-gram lizard that lives in the southwestern United States, 15 reproduce by parthenogenesis. Here, says Crews, is "an organism that has lost the need for sex, yet still exhibits male-like and female-like behavior." As a result of this pseudo-sexual behavior, the animals lay many more eggs than otherwise. So when the animals are not isolated from each other, they court, taking turns playing the male or female role. Whoever is playing the role of the female has her ovaries stimulated and lays eggs. Once she ovulates, she plays the male role,

allowing another lizard to be stimulated.

Then there is the story of the red-sided garter snake that lives in the far north and in which "gonadal activity is completely dissociated from mating behavior," according to Crews. The animals spend as much as 6 months underground during the harsh winter. Then they emerge and mate. But, says Crews, "at the time of mating, the gonads are completely regressed and the sex steroid levels are low." The male snakes "do not produce sperm until the summer, when all breeding has ended. They store this sperm for the next breeding season."

Crews drew a triangle, with behavior at one point, steroids at another, and gametes at the third. Then he drew two-headed arrows along the edges. All the possible connections suggested by the arrows are

ones biologists have sometimes assumed to be true because of their experience with humans and other mammals. But, Crews says, "only the relation that gametes induce steroids is constant. All the other relations have occurred and been lost many times during evolution."

What all this means, says Florence Haseltine of the child health institute, is that "sex differentiation is a lot more complex than laying down just one set of rules." But the first step in mammals—the decision that a fertilized egg will become a male or a female—seems at last within researchers' grasp. ■ GINA KOLATA