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Sex Determination in the Honeybee

Sex determination in honeybees involves a multi-allelic locus, such that homozygotes develop as males and heterozygotes as females. In this issue of *Cell*, Beye and colleagues (2003) report the cloning of the sex-determining gene, *csd*. It codes for an SR protein, and different alleles have very different amino-acid sequences. Inactivating *csd* leads to development as a male.

In 1845, the German apiarist J. Dzierzon proposed that male honeybees arise from unfertilized eggs, while females come from fertilized eggs. It is now known that this sex determination system (haplodiploidy) is probably common to all sexually reproducing members of the *Hymenoptera* (ants, bees, and wasps) and *Thysanoptera* (thrips), as well as being found sporadically in other orders of insects including beetles and *Homoptera* (scale insects and whiteflies), in the *Acarina* (ticks and mites), and in monogont rotifers (Bull, 1983). It is very hard to see how haplodiploidy could evolve from one of the two best-known sex-determining systems, a male-determining Y chromosome (as found in mammals), or X: autosome balance (as in *Drosophila* and *Caenorhabditis*). In the first case, it is impossible for a male to develop without the male-determining region of the Y, and for a female to develop in its presence. In the second case, there is no difference in X: autosome balance between haploids and diploids.

In the few cases (all in *Hymenoptera*) in which a detailed genetic analysis of haplodiploid sex determination has been performed, the mechanism involves what is known as complementary sex determination (Bull, 1983). Females are always heterozygous for a pair of distinct alleles at the sex-determining locus, whereas males are homozygous (if derived from a fertilized egg), or haploid (if derived from an unfertilized egg). Such a sex determination system can perfectly well exist without haplodiploidy if all eggs are fertilized, and evolutionary models of the conversion of diploid complementary sex determination into haplodiploidy can be constructed (Bull, 1983). This removes some of the mystery surrounding the origin of haplodiploidy.

In honeybees, the best-studied example of complementary sex determination, homozygous diploid males can be produced by inbreeeding, but are normally eaten by the workers. The lethality of diploid males means that there is a selection pressure to increase the number of functionally distinct alleles, and indeed in honeybees as many 12 alleles have been detected in a single population (Bull, 1983). This pressure to make the system highly polymorphic is similar that in the self-incompatibility loci of flowering plants and some fungal mating types, where a large number of alleles coexist and successful matings only occur between individuals carrying different alleles (Charlesworth, 2002; Casselton, 2002). Variability with respect to neutral markers at sites closely linked to the sex locus itself has been exploited by Beye et al. to identify, clone, and characterize the sex-determining gene of honeybees, *csd*, in a tour de force of positional cloning (Beye et al., 2003).

Earlier work had identified two genetic markers flanking the sex-determining locus, at distances of 1 and 7 cM. Using the closer marker, they isolated 70 kb of DNA containing the sex locus, using polymorphic markers to orientate a chromosome walk. They found a 13 kb region that was always heterozygous in females in the cross they used. cDNA analyses identified a transcript within this region, and they inferred that this was likely to be the sex-determining gene itself. Sequencing of the corresponding genomic DNA shows that *csd* consists of 1453 bases, with nine exons and a protein of 385 amino acids. The protein is a novel type of arginine-serine rich (SR) protein. Intriguingly, its C terminus has some sequence similarity with the protein coded by the *tra* locus of *Drosophila*, an important player in sex determination (Marín and Baker, 1998). Expression studies showed that *csd* is transcribed in both males and females, starting at 12 hr of development, so that differential expression plays no role in sex determination.

Sequence comparisons were carried out between four different sex-determining alleles, revealing an unusually high level of amino acid sequence differences between alleles, especially toward the C-terminal region. In addition to single substitutions, alleles typically differ with respect to insertions and deletions of amino acids, often in tracts of several at a time. Variation in a hypervariable region mainly involves repeats of asparagine and tyrosine residues. This abundance of amino acid sequence differences among alleles is also seen in self-incompatibility alleles, and presents a considerable problem for identifying sites of functional significance. The magnitude of these differences suggests that the alleles may be rather old, in terms of evolutionary origin; it would be of interest to look for trans-specific polymorphisms of the type sometimes found at self-incompatibility loci (Charlesworth, 2002).

This pattern of variation in itself strongly supports the inference that *csd* is the sex-determining locus. Further evidence is provided by functional studies, using RNA interference. Injection of *csd* dsRNA into developing eggs caused genetic females to develop as male larvae with high probability, but males were unaffected. This indicates that *csd* function is required in females, but not in males. In turn, this implies that its product is nonfunctional when *csd* is transcribed from a single allele. Again, this has some parallels with self-incompatibility and mating-type systems, in which pollination or development of a sexual fusion product cannot proceed if genetically similar partner cells are involved. The fact

that RS proteins typically engage in protein-protein interaction suggests that *csd* may well be interacting with other proteins; in addition, some form of homodimerization is the simplest explanation for the effect of having only a single allele on its functionality. The details of its actions remain to be determined. While it is tempting to speculate that *csd* may be regulating *dsx*, the only known phylogenetically well-conserved component of the sex determination pathway in insects (Marín and Baker, 1998), at present this is just speculation.

In addition to these mechanistic questions, the characterization of *csd* opens the door to asking questions about the evolution of haplodiploidy in the *Hymenoptera* at large, e.g., was there a single origin or multiple origins of complementary sex determination? One problem for the single origin model is that complementary sex determination cannot be the only form of sex determination in *Hymenoptera*. Many species of Hymenopteran parasitoids are regular inbreeders, yet produce an excess of female over male offspring (Bull, 1983). It is clear that this cannot happen with complementary sex determination, since homozygosity for sex-determining alleles produces males. Genetic studies in the parasitoid wasp *Nasonia vitripennis* have confirmed that sex determination works quite differently in this inbreeder, and may involve some form of genomic imprinting (Dobson and Tanouye, 1998). This raises some awkward questions concerning what is going on in these species, and how it relates to the "standard" complementary sex-determining mechanism. Like most good pieces of science, a lot of interesting questions are raised by the characterization of *csd.*

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Cell Proliferation without Cyclin E-CDK2

In this issue of *Cell*, Geng et al. report that mice can undergo embryonic development without cyclins E1 and E2. Fibroblasts derived from the double knockout embryos proliferate normally in culture. However, E type cyclins seem essential for endoreplication and exit from quiescence.

Cyclin-dependent kinases (CDKs) have been likened to the "traffic lights" of the cell cycle (Nasmyth, 2001). They promote and coordinate DNA replication during S phase and chromosome segregation during mitosis. They also ensure that nuclear DNA is replicated once and only once in each cell cycle. In mammalian cells, the D type cyclins (D1, D2, D3) associate with CDK4 and CDK6 kinases, and their primary function is to phosphorylate the retinoblastoma (Rb) family of transcriptional repressors during G1. The E type (E1, E2) and A type (A1, A2) cyclins associate with CDK2 and regulate initiation of DNA replication and progression through S phase. Finally, the B type cyclins (B1, B2, B3) associate with CDK1 to control entry into and exit from mitosis.

It turns out that mouse cells may survive with a couple of "broken lights." Geng et al. (this issue of *Cell*) have generated strains of mice in which cyclins E1 or E2 have been deleted. Both $E1^{-/-}$ and $E2^{-/-}$ mice develop normally and are viable, except that half the $E2^{-/-}$ males are sterile due to incomplete testis development. Crossing of the two transgenic strains did not yield viable double knockout mice. However, $E1^{-/-}E2^{-/-}$ embryos survived until the 10^{th} day of gestation. Prenatal death was caused by the mutant placenta, and some $E1^{-/-}E2^{-/-}$ embryos successfully completed gestation when they developed in a wild-type placenta. In culture, fibroblasts derived from the $E1^{-/-}E2^{-/-}$ embryos were able to undergo several rounds of division before becoming senescent. These studies led to the rather provocative conclusion that cyclin E is dispensable for cell proliferation and development in the mouse.

In an independent study, Ortega et al. (2003) discovered that CDK2, the kinase activated by E type and A type cyclins, is also dispensable for mouse development. $CDK2^{-/-}$ animals are viable and do not display anatomical or behavioral abnormalities, except for severe gonad atrophy. In addition, $CDK2^{-/-}$ embryo fibroblasts proliferate normally in culture, indicating that CDK2 activity is not necessary for mitotic cell division. In contrast, CDK2 appears to be essential for meiosis, for $CDK2^{-/-}$ mice are sterile due to a block in spermatogenesis and oogenesis during prophase I.

The fact that E type cyclins are not essential for fibroblast proliferation is not without precedent. In yeast, the cyclins that promote entry into S phase can be deleted without major consequences because the mitotic cyclins can take over their functions (reviewed by Kelly and Brown, 2000). There are also cases of cyclin complementation in mammalian cells: a previous study from P. Sicinski's laboratory revealed that cyclin E introduced at the endogenous cyclin D1 locus rescued the phenotypic defects of cyclin $D1^{-/-}$ mice (Geng et al., 1999). Therefore, it is a reasonable assumption that cyclin A can complement the lack of cyclin E, at least during mitotic cycles.

In the case of $CDK2^{-/-}$ cells, viability is likely due to surrogate kinases associating with cyclin A and cyclin E. It is known that cyclin A can associate with CDK1, but it is not clear whether cyclin E can associate with a kinase other than CDK2. In fact, an immunoprecipitate