

## SEX DETERMINATION

## Y is the answer

Few tasks are sex-specific these days, but biology makes some exceptions. Possibly the most fundamental example of these is the molecular route by which sex is determined. In flies, this involves two crucial events: the decision of which of the two sexual development pathways to follow, and the adjustment of X-chromosome expression to the level appropriate to that sex. Critical genes are those that lie upstream of these two processes, and can be identified by searching for mutations the lethal effects of which depend on the X-chromosome dose (known as sex-specific lethals). The gene *flex* (*female-specific lethal on X*) was thought to be among these, but a recent study questions previous conclusions and shows that the female lethality caused by *flex* depends on the absence of the Y chromosome, rather than on the presence of two X chromosomes.

The *Drosophila* gene *Sxl* (*Sex lethal*) lies at the heart of sex determination — *Sxl* protein is activated (by the double X dosage) only in females, in which an autoregulatory loop involving pre-mRNA splicing ensures continued expression of the female-specific protein isoforms. In males, which are XY, no active *Sxl* exists. *Sxl* is crucial for female development and dosage compensation; moreover, the presence of *Sxl* in males is lethal. An earlier study had proposed *flex* to be a positive regulator of *Sxl* on the basis of, among other things, the ability of a *flex* mutant to rescue the male

lethality of the *Sxl<sup>M</sup>* gain-of-function alleles, which cause the female isoform of *Sxl* to be expressed in males. In his study, Tom Cline witnessed no phenotypic suppression by *flex* of *Sxl<sup>M</sup>* alleles — not even weak ones — and so sought to find a more convincing explanation for the *flex* phenotype itself. This came from an often neglected source: the Y chromosome. This chromosome rescues the *flex*-induced female lethality, whereas *flex* males that lack the Y chromosome (that is, are XO) do not survive.

Which gene(s) on the Y chromosome could be responsible for such an effect? The most likely candidate is the *bb* (*bobbed*) locus (which encodes ribosomal RNAs (rRNA)) — the only fly locus that has alleles on both the X and Y chromosomes. Indeed, the lack of complementation between *flex* and a *bb<sup>-</sup>* Y chromosome, together with the revised map position of *flex*, might just prove the allelism between *flex* and *bb*.

There are two possible conclusions to this story — either *flex* has nothing to do with *Sxl* (or sex determination) and is yet another rRNA locus; or this rRNA molecule has an important function early in sex determination. Either way, this work alerts researchers that the Y chromosome is ignored at their peril.

Tanita Casci

### References and links

**ORIGINAL RESEARCH PAPER** Cline, T. W.

A lesson from *flex*: consider the Y chromosome when assessing *Drosophila* sex-specific lethals. *Development* **128**, 1015–1018 (2001)

**FURTHER READING** Bhattacharya, A. *et al.* *flex*, an X-linked female-lethal mutation in *Drosophila melanogaster* controls the expression of *Sex-lethal*. *Development* **126**, 5485–5493 (1999)

**WEB SITE** Tom Cline's lab



Fly images courtesy of Tom Cline, University of California, Berkeley, USA.



Unpigmented, chimeric zebrafish (top) and its pigmented transgenic offspring. Courtesy of Paul Collodi, Purdue University, Indiana, USA.

## TECHNOLOGY

## Fishing for new tools

One gadget noticeably absent from the zebrafish geneticist's toolkit is an embryonic cell line to use for gene-targeting experiments. But the wait for this vital tool might now be over, with the publication of a technique that maintains undifferentiated, zebrafish embryonic cells in culture, which — when transplanted into embryos — can contribute to the zebrafish germ line.

The secret to the authors' success was their use of a rainbow trout spleen cell line (called RTS34st) to create a monolayer of feeder cells on which they cultured cells derived from gastrula-stage zebrafish embryos. When these embryo cells are cultured without feeder cells, they cease to express the primordial germ-cell marker *vasa* and begin to differentiate after five days. By contrast, when cultured on this feeder layer, embryo cells remain undifferentiated and express *vasa* for at least 25 days. Embryo cells cultured without feeder cells but with RTS34st-conditioned medium also express *vasa* but show earlier signs of differentiation.

But can these cultured cells contribute to the zebrafish germ line — something previous embryonic cell lines have failed to do? To test this, Ma *et al.* injected cultured embryo cells derived from a pigmented, *neo*-expressing transgenic zebrafish strain into unpigmented (GASSI) zebrafish embryos. Of the embryos that survived injection, four produced pigmented, *neo*-positive offspring when crossed to GASSI mates (see picture), indicating that they were germline chimeras. Although the frequency and the degree of germline chimerism are low among the fish that survived injection, the authors reason that these potential barriers to success are offset by the large numbers of offspring that fish produce — this provides many embryos for injection and means that fish with only low rates of chimerism can still produce transgenic offspring.

The next step for Ma *et al.* is to establish longer-term cultures to allow time for gene targeting and selection. This might require further investigation into what the feeder cells provide to embryo cells to keep them undifferentiated.

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### References and links

**ORIGINAL RESEARCH PAPER** Ma, C., Fan, L., Ganassin, R., Bols, N. & Collodi, P.

Production of zebrafish germ-line chimeras from embryo cell cultures. *Proc. Natl Acad. Sci. USA* **98**, 2461–2466.

**WEB SITE** Paul Collodi's lab