

Sex Determination: The Fishy Tale of *Dmrt1* Dispatch

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The idea that the *Dmrt1* gene provides a unifying sex-determining mechanism in non-mammalian vertebrates is left high and dry by recent observations in fish.

The rise to fame of *Dmrt1* in the field of sex determination reads like a classic detective story. Tantalizing evidence obtained over the last 5 years, albeit with intriguing twists of plot, pointed to a conserved role for *Dmrt1* in male sexual development. The discovery of a *Dmrt1* homologue on the Y chromosome of the model fish medaka raised speculation that this might be a male-determining 'switch' gene in all non-mammalian vertebrates. But new work published in this issue of *Current Biology* [1] shows that this gene is absent even from closely related fish species, reinforcing the view that sex determining mechanisms have been reinvented many times during animal evolution.

In the first chapter of the story, decades of elegant genetic studies led to a clear picture of the molecular pathways of sex determination in the model invertebrates *Drosophila melanogaster* and *Caenorhabditis elegans*. Curiously, these pathways bear little resemblance to each other [2]. Meanwhile, efforts were being made to piece together the pathway in mammals, with the discovery of the mammalian Y-linked testis-determining gene *Sry*, and a critical downstream gene *Sox9*, neither of which showed any similarity to sex-determining genes of *Drosophila* or *C. elegans* (Figure 1) [3]. All the evidence suggested that sex determination might flout the conventional rules of evolutionary conservation and that, unlike in other areas of developmental biology, little would be gained by taking genes important in one metazoan taxon and isolating and studying homologues in others.

Chapter 2 blew this idea out of the water, with the startling discovery of a human gene homologous to the *Drosophila* sex regulatory gene *doublesex* and the *C. elegans* sex regulator *mab-3* [4]. All three genes encode proteins related by a common DNA-binding domain, dubbed the DM domain. More intriguingly, this human gene, *DMRT1*, maps to a region of chromosome 9p that, when deleted, is responsible for XY gonadal dysgenesis [5]. It is not possible to point the finger directly at *DMRT1* as the cause of this dysgenesis, as other nearby genes are also deleted in affected patients [6,7]. Nevertheless, the clear implication is that some aspect of *Dmrt1* function might have been conserved from flies and worms through to humans.

This theme is explored further in chapter 3. Not only are chickens found to have a *Dmrt1* homologue, but also this gene maps to the Z chromosome [8]. Birds do things differently in sex determination: unlike mammals, females are the heterogametic sex (ZW), while males are homogametic (ZZ). It is not clear whether this system revolves around a dominant W-linked female-determining gene, or a dosage-sensitive Z-linked male-determining gene. Clearly, *Dmrt1* is a candidate in the latter scenario.

Perhaps the most convincing evidence for an important role of *Dmrt1* in testis differentiation in vertebrates came from expression studies. These studies showed, in a number of species including humans, mice, chickens, alligators and turtles, that *Dmrt1* expression is limited to the gonads [9-13]. This expression is considerably up-regulated in developing testes compared to ovaries. The timing of this up-regulation varies between species, but it generally occurs in the late sex-determining or early testis-differentiation period. These characteristics of *Dmrt1* expression in different species leave little doubt that this gene is specifically involved in the early formation of testes.

The first twist in the story comes from the observation that *Dmrt1* knockout mice do not show sex reversal or dysgenesis of fetal testes; rather, the testes show defects in survival and differentiation of somatic and germ cells after birth [14]. This could mean that *Dmrt1* is not involved in testis determination in mice, or in vertebrates more generally. An alternative explanation is that other, similar genes compensate for the lack of *Dmrt1* in these mice. It is now known that a family of seven DM genes exists in humans and mice [15]. Some of these colocalise with *DMRT1* on human chromosome 9p, and may contribute to the gonadal dysgenesis phenotype of the 9p deletion patients. The expression profiles of all family members are being investigated to assess which gene or genes might compensate for *Dmrt1* in the knockout mice. Until these data become available, and *Dmrt1* knockout mice are crossed with mice deficient in other DM genes, the jury is out as to what role, if any, is played by *Dmrt1* in sex determination in mice.

Meanwhile, hot news came from the study of medaka fish. Medaka, unlike many other fish, use a simple genetic sex determination mechanism similar to that found in mammals, with XX females and XY males. Two groups set out to positionally identify the male sex-determining gene from the medaka Y chromosome. Scharl and colleagues [16] delimited the Y chromosome-specific region to some 260 kilobases, and identified a *Dmrt1* homologue, *Dmrt1bY*, as the only functional gene in this chromosomal segment. Nagahama and colleagues [17] reported similar findings, and went further to analyse two inbred mutant strains, Awara and Shirone, that yield XY females. The Awara mutant was found to carry a mutation causing a frameshift and premature termination of the *Dmrt1bY*

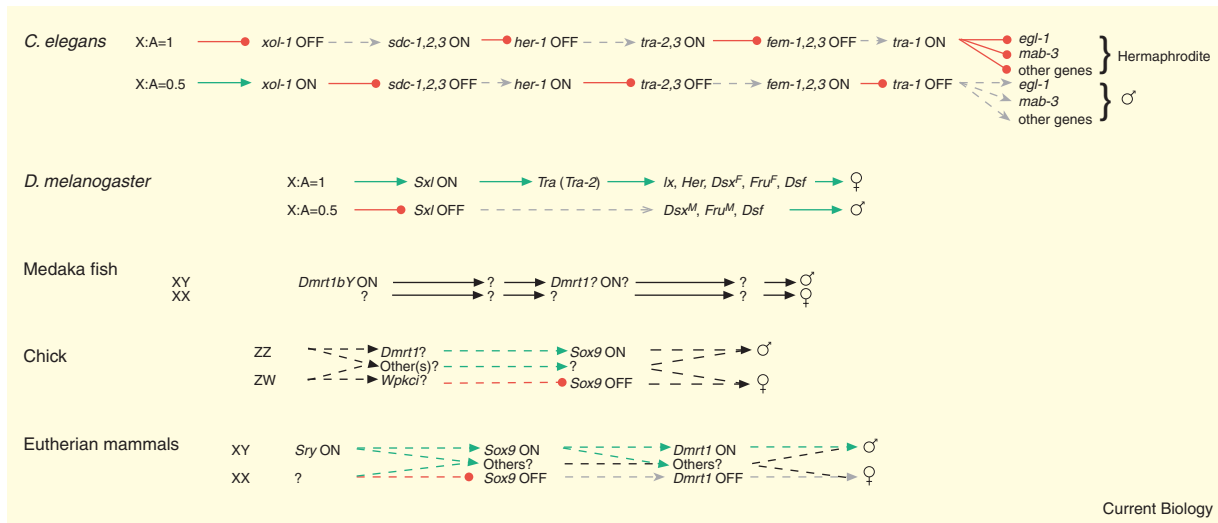


Figure 1. Genetic pathways of sex determination in various metazoan model organisms. In general, there is little obvious similarity between pathways, with the notable exception of the DM genes *Dmrt1*, *mab-3* and *Dsx*. Green arrow, positive regulation; red arrow, negative regulation; grey arrow, conditions permissive for gene expression. Dotted line, inferred regulatory relationship.

protein, while Shirone was found to have reduced *Dmrt1bY* expression, presumably due to an uncharacterized regulatory defect. To date, no evidence has been produced by gain-of-function studies that *Dmrt1bY* can induce male development in XX medaka. However, the identification of *Dmrt1bY* as a gene that maps to the sex-determining region of the medaka Y chromosome and is necessary for male development reawakened the notion that *Dmrt1* is a fundamental male sex-determining gene, the function of which is conserved throughout vertebrates.

The new data from Schartl's group [1] have thrown the field into chaos once more. While *Dmrt1bY* may be an important sex-determining gene in medaka (*Oryzias latipes*), it now appears that the gene is not present in the closely related species *Oryzias curvinotus*. Schartl and colleagues [1] used Southern blotting, the polymerase chain reaction (PCR) and *in situ* hybridization to look for *Dmrt1bY* in *O. curvinotus*, under conditions

clearly capable of detecting the gene in other species, and conclude that it is simply not there. This prompted the authors to look more widely. They looked at another species from the same genus (*O. celebensis*), a species from the same order (guppy), a species from the same superorder (tilapia), and the more distantly related zebrafish. They scoured the recently released genome sequence of the puffer fish *Fugu*. *Dmrt1bY* was nowhere to be found.

It is clear that *Dmrt1* does not have a widespread role as a sex-determining switch gene among vertebrates. But do these observations really debunk *Dmrt1* as an important, conserved effector of testis development? Probably not. It must be remembered that *Dmrt1bY* is a duplicated version of *Dmrt1* that found its way onto the Y chromosome of medaka at some time after the emergence of the genus *Oryzias*. Those fish species that lack *Dmrt1bY* retain *Dmrt1*, which may have a downstream role in testis development analo-

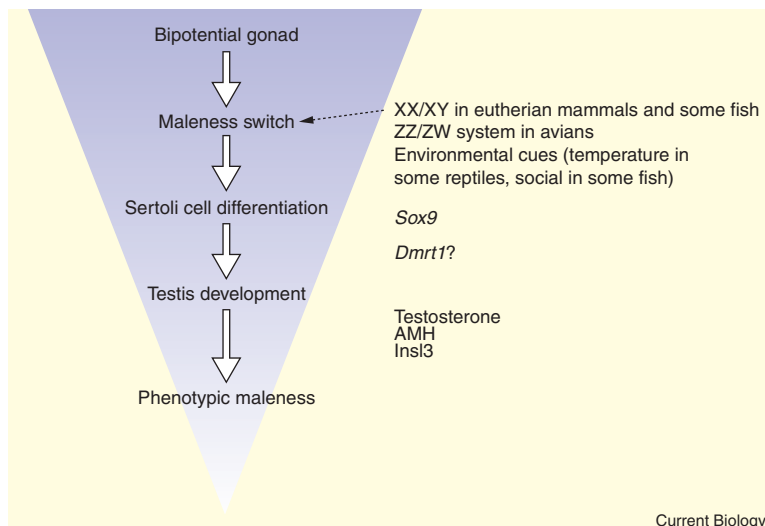


Figure 2. Diversity of switch mechanisms used in vertebrate testis determination.

In most eutherian mammals, male sex is determined by *Sry* on the Y chromosome, but some eutherians lack *Sry* or a Y chromosome altogether, while others have *Sry*-like genes on both X and Y chromosomes. Medaka fish also use an XX/XY system, but maleness is determined by *Dmrt1bY*, not *Sry*. While birds and some reptiles use a ZZ/ZW system, the nature of the sex determining switch mechanism remains unknown. Similarly, other groups use temperature or social cues to determine sex, but the genetic basis of this control is not yet known. It remains possible, however, that the different switch mechanisms converge on a common pathway of testis development that may include genes such as *Sox9* and *Dmrt1*.

gous to its putative role in humans and mice, although this remains to be determined experimentally.

The studies relating to medaka *Dmrt1* illustrate yet again the vast diversity of sex-determining mechanisms that have evolved in the animal kingdom (Figure 1), a diversity that thwarts almost all attempts to distil common themes. Even if a role for *Dmrt1* as a sex-determining switch gene in chickens is established, we will be left wondering whether the same holds true in all bird species, and in the reptilian clades that use a ZZ/ZW sex-determining mechanism (Figure 2). We will also need to continue searching for the elusive genes that underpin sex determination in mammalian species such as monotremes [18] and various mole voles [19] that lack *Sry*, and in vertebrate species that determine sex through environmental cues such as temperature and social situation. It seems *Dmrt1* and *Sry* have many accomplices in animal sex determination. So who dunnit? Be sure to read further chapters.

References

1. Kondo, M., Nanda, I., Hornung, U., Asakawa, S., Shimizu, N., Mitani, H., Schmid, M., Shima, A. and Scharl, M. (2003). Absence of the candidate male sex determining gene DMRT1bY of medaka from other fish. *Curr. Biol.* *13*, this issue.
2. Zarkower, D. (2001). Establishing sexual dimorphism: conservation amidst diversity? *Nat. Rev. Genet.* *2*, 175-185.
3. Koopman, P. (2001). *Sry*, *Sox9* and mammalian sex determination. *EXS* *91*, 25-56.
4. Raymond, C.S., Shamu, C.E., Shen, M.M., Seifert, K.J., Hirsch, B., Hodgkin, J. and Zarkower, D. (1998). Evidence for evolutionary conservation of sex-determining genes. *Nature* *391*, 691-695.
5. Veitia, R.A., Nunes, M., Quintana-Murci, L., Rappaport, R., Thibaud, E., Jaubert, F., Fellous, M., McElreavey, K., Goncalves, J., Silva, M., et al. (1998). Swyer syndrome and 46,XY partial gonadal dysgenesis associated with 9p deletions in the absence of monosomy-9p syndrome. *Am. J. Hum. Genet.* *63*, 901-905.
6. Raymond, C.S., Parker, E.D., Kettlewell, J.R., Brown, L.G., Page, D.C., Kusz, K., Jaruzelska, J., Reinberg, Y., Flejter, W.L., Bardwell, V.J., et al. (1999). A region of human chromosome 9p required for testis development contains two genes related to known sexual regulators. *Hum. Mol. Genet.* *8*, 989-996.
7. Ottolenghi, C., Veitia, R., Quintana-Murci, L., Torchard, D., Scapoli, L., Souleyreau-Therville, N., Beckmann, J., Fellous, M. and McElreavey, K. (2000). The region on 9p associated with 46,XY sex reversal contains several transcripts expressed in the urogenital system and a novel doublesex-related domain. *Genomics* *64*, 170-178.
8. Nanda, I., Shan, Z., Scharl, M., Burt, D.W., Koehler, M., Nothwang, H., Grutzner, F., Paton, I.R., Windsor, D., Dunn, I., et al. (1999). 300 million years of conserved synteny between chicken Z and human chromosome 9. *Nat. Genet.* *21*, 258-259.
9. Raymond, C.S., Kettlewell, J.R., Hirsch, B., Bardwell, V.J. and Zarkower, D. (1999). Expression of *Dmrt1* in the genital ridge of mouse and chicken embryos suggests a role in vertebrate sexual development. *Dev. Biol.* *215*, 208-220.
10. Smith, C.A., McClive, P.J., Western, P.S., Reed, K.J. and Sinclair, A.H. (1999). Conservation of a sex-determining gene. *Nature* *402*, 601-602.
11. De Grandi, A., Calvari, V., Bertini, V., Bulfone, A., Peverali, G., Camerino, G., Borsani, G. and Guioli, S. (2000). The expression pattern of a mouse doublesex-related gene is consistent with a role in gonadal differentiation. *Mech. Dev.* *90*, 323-326.
12. Kettlewell, J.R., Raymond, C.S. and Zarkower, D. (2000). Temperature-dependent expression of turtle *Dmrt1* prior to sexual differentiation. *Genesis* *26*, 174-178.
13. Moniot, B., Berta, P., Scherer, G., Sudbeck, P. and Poulat, F. (2000). Male specific expression suggests role of DMRT1 in human sex determination. *Mech. Dev.* *91*, 323-325.
14. Raymond, C.S., Murphy, M.W., O'Sullivan, M.G., Bardwell, V.J. and Zarkower, D. (2000). *Dmrt1*, a gene related to worm and fly sexual regulators, is required for mammalian testis differentiation. *Genes Dev.* *14*, 2587-2595.
15. Ottolenghi, C., Fellous, M., Barbieri, M. and McElreavey, K. (2002). Novel paralogy relations among human chromosomes support a link between the phylogeny of doublesex-related genes and the evolution of sex determination. *Genomics* *79*, 333-343.
16. Nanda, I., Kondo, M., Hornung, U., Asakawa, S., Winkler, C., Shimizu, A., Shan, Z., Haaf, T., Shimizu, N., Shima, A., et al. (2002). A duplicated copy of DMRT1 in the sex-determining region of the Y chromosome of the medaka, *Oryzias latipes*. *Proc. Natl. Acad. Sci. USA* *99*, 11778-11783.
17. Matsuda, M., Nagahama, Y., Shinomiya, A., Sato, T., Matsuda, C., Kobayashi, T., Morrey, C.E., Shibata, N., Asakawa, S., Shimizu, N., et al. (2002). DMY is a Y-specific DM-domain gene required for male development in the medaka fish. *Nature* *417*, 559-563.
18. Pask, A. and Graves, J.A. (2001). Sex chromosomes and sex-determining genes: insights from marsupials and monotremes. *EXS* *91*, 71-95.
19. Just, W., Rau, W., Vogel, W., Akhverdian, M., Fredga, K., Graves, J.A. and Lyapunova, E. (1995). Absence of *Sry* in species of the vole *Ellobius*. *Nat. Genet.* *11*, 117-118.