

Meeting report

Eleven years of sexual discovery

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A report on Novartis Foundation Symposium 244 "The Genetics and Biology of Sex Determination", London, UK, 1-3 May 2001.

Eleven years after the discovery of the Y-linked testis-determining gene, *SRY*, 'sex workers' gathered at the Novartis Foundation in London to discuss the state of the field. Novartis Foundation Symposia differ from many other types of meeting in being closed, small (only 15 speakers and 15 observers), with extensive discussion, and focusing primarily on gaps in our knowledge - which in this case were many.

The key step in mammalian sex determination is the development of the undifferentiated embryonic gonads into either testes or ovaries. We have known since 1990 that the *SRY* gene is the critical switch leading to testis development. *SRY* encodes a member of the SOX family of transcription factors and its name denotes 'sex-determining region of the Y chromosome'. Mutations in *SRY* result in XY individuals developing as females, and transgenic XX mice ectopically expressing *Sry* develop into sex-reversed males. This means that among all the Y-chromosome-derived sequences, *SRY* is the only one that is both required and sufficient to initiate male sex determination.

***SRY* is the critical switch**

As *SRY* is clearly critical in this process, much discussion revolved around its possible function. Robin Lovell-Badge (National Institute for Medical Research, London, UK) took us through the key experiments in mice that defined the function of *SRY* and pointed out the many structural and regulatory differences between *Sry* in mouse and *SRY* in humans. Vince Harley (Prince Henry's Institute, Melbourne, Australia) reviewed mutation and gel-shift (protein-DNA interaction) data confirming the critical role of one portion of *SRY*, namely the HMG box motif. The HMG box is essential for

SRY to bind and bend DNA as well as for transporting the protein into the nucleus. Among mammals, *SRY* is poorly conserved outside the HMG box. Jenny Graves (Australian National University, Canberra, Australia) confirmed the long-held suspicion that *SRY* is not present in the monotremes (egg-laying mammals: platypus and echidna). This means that *SRY* is a relatively recent mammalian invention, appearing approximately 130 million years ago. Graves proposed that *SRY* arose when another *SOX* gene 'landed' on the Y chromosome and this translocation became fixed. This proposal led, in subsequent discussions, to the hypothesis that *SRY* is "just an HMG box" that simply mops up some other factor by binding to it. Is it possible that any HMG box could substitute for *SRY*? An alternative to this model was suggested by work from Peter Koopman's group (Institute for Molecular Bioscience, Brisbane, Australia), showing that in transgenic mice expressing *Sry*, the HMG box and some non-HMG-box carboxy-terminal regions are required to cause sex reversal. This led to the "*SRY* is an HMG box with some dangly bits" hypothesis. Despite extensive discussion, no experimental approach could be devised to distinguish between the two hypotheses.

Blanche Capel (Duke University Medical Center, Durham, USA) stressed the critical role of cell migration in testis determination. *Sry* induces proliferation of a cell population that gives rise to Sertoli cells, one of the major cell types in the testis. *Sry* also induces peritubular myoid cells to migrate into the testis, where their role seems to be to physically partition the Sertoli cells from the germ cells. She has also shown that inhibition of germ-cell meiosis appears to result from formation of the testis cords. If *Sry* is absent then germ cells enter meiosis and the gonad becomes an ovary; germ cells play a critical role in ovarian development. Capel proposed that testis cord formation and entry of germ cells into meiosis are competing gonadal pathways.

Other critical questions surrounding *SRY* were discussed but remained unanswered. It is believed that *SRY* acts as an

architectural transcription factor by changing chromatin structure and bringing otherwise widely separated components for transcription into juxtaposition. How is *SRY* regulated? What proteins does *SRY* interact with? Most importantly, what is the direct *in vivo* target of transcriptional regulation by *SRY*? Eleven years on, we still do not know the answers to these fundamental questions.

SOX9 is a pivotal player

The *SRY*-related HMG-box gene *SOX9* clearly has ‘most favored sex gene’ status; every speaker returned to it time and again. Mutations in *SOX9* cause campomelic dysplasia - a syndrome including skeletal malformations - and sex reversal of XY patients to females, firmly implicating it in the testis sex-determining pathway. In mice, *Sox9* is upregulated in the embryonic male gonad shortly after *Sry* is expressed. Lovell-Badge argued that *Sox9* is the only critical gene needed downstream of *Sry*, because duplications of *SOX9* in humans and the *Odsex* mutation in mice (which upregulates *Sox9*) both result in sex-reversed XX males. He further proposed that *Sry* directly targets *Sox9 in vivo*, because of the overlapping expression profiles of the two proteins and because they colocalize in embryonic Sertoli cells. Unfortunately, there is only circumstantial evidence for a direct link between *Sry* and *Sox9*. The exact regulatory sequences of *SOX9* are still unknown, but they appear to span megabases in humans. By contrast, Lovell-Badge presented evidence that, in the mouse, the critical *Sox9* regulatory region is restricted to a more tractable 70 kb region upstream of the gene.

Connections in the sex-determining network

Anti-Müllerian hormone (AMH), as the name suggests, results in the destruction of the embryonic female Müllerian ducts; it is produced by Sertoli cells in the testis. Richard Behringer (University of Texas, Houston, USA) presented extensive data on AMH signaling via its type II receptor and the newly discovered type I receptor. He and other speakers demonstrated that *SOX9* is required for the initiation of *AMH* expression. Other factors that are involved are also being identified. Steroidogenic Factor 1 (SF1) is an orphan nuclear receptor and a key regulator of steroidogenic enzymes. SF1 physically interacts with *SOX9*, which binds next to it on the promoter, to upregulate *AMH*. Andreas Schedl (Max Delbrück Center for Medicine, Berlin, Germany) discussed the multiple role of the Wilms’ tumor 1 (*WT1*) gene in gonad development. His data indicate that in mice, *Wt1* - in particular the *Wt1-KTS* isoform, which lacks three alternatively spliced amino acids - plays a critical role, along with *SF1*, in the formation of the early bipotential gonad. XY mice that lack the *Wt1+KTS* isoform (and so express only the *Wt1-KTS* isoform) develop ovaries. Furthermore, the *Wt1-KTS* isoform has been shown to interact physically with SF1 to synergistically upregulate *AMH* expression levels.

The X-linked *DAX1* (DSS-AHC critical region of the X chromosome,¹) gene plays a role in gametogenesis and endocrine development, but its role in sex determination is unclear. Abnormal duplication of *DAX1* can result in sex reversal of XY individuals to give females, suggesting that *DAX1* has an ‘anti-testis’ action. *In vitro* experiments have shown that *DAX1* can repress the synergistic action of *SF1* and *WT1* on the *AMH* promoter, resulting in down-regulation of *AMH*. Eric Vilain (University of California Los Angeles Medical School, USA) discussed work on *WNT4*, newly discovered to be a sex-determining gene. All other genes involved with gonad development (*SRY*, *SOX9*, *DAX1*, *WT1* and *SF1*) encode transcription factors, but *WNT4* encodes a signaling molecule. Deletions of *Wnt4* in mice result in masculinization of XX individuals; duplication of *WNT4* or its overexpression in humans can cause XY female sex reversal. Ken-ichirou Morohashi (National Institute of Basic Biology, Okazaki, Japan) showed that, in mice, *Wnt4* acts to upregulate *Dax1* transcription. An amino-terminal repeat sequence in *Dax1* interacts with SF1 and represses transcription of *Sf1*. In a female gonad, this will result in suppression of genes downstream of *Sf1*, such as *Amh* and other steroidogenic genes.

Sex across the phyla

David Zarkower (University of Minnesota, Minneapolis, USA) spoke about one of the most conserved elements in sex determination - the DM-domain family of genes, which appear to play a sex-specific role in several phyla. The *Drosophila* Doublesex (*dsx*) and *Caenorhabditis elegans* Mab-3 proteins each have a DM domain, and both are involved in differentiation of sex-specific structures. A search for DM-related genes in humans revealed *DMRT1* (DM-related transcription factor 1), which maps to human chromosome 9p; deletions of this region are associated with XY sex reversal. Both David Zarkower and I presented evidence showing that in mouse, *Dmrt1* is upregulated in the Sertoli cells of the embryonic male gonad. Previous data from Zarkower’s group showed that *Dmrt1* knockout mice are not sex-reversed, but *Dmrt1* may be responsible for Sertoli-cell maintenance. At this meeting, he presented preliminary data suggesting that, on a different genetic background in combination with a weak *Sry* allele, a *Dmrt1* knockout could produce sex-reversed mice. Maybe the gene does have a role in sex determination, or perhaps one of the related DM genes is involved?

Although *SRY* is clearly the sex-determining switch in mammals, what happens in other vertebrates? I discussed the situation in birds, where the females are the heterogametic (ZW) sex and males are homogametic (ZZ). The Z chromosome in many respects resembles the mammalian Y, but the avian and mammalian sex chromosomes are thought to have evolved from different autosomes. Either a dominant W-linked ovary-determining gene or a Z-linked testis-determining gene determines sex in birds. Unfortunately, no

sex-chromosome aneuploid chickens are known; these (particularly a ZO animal) would allow us to distinguish between these two possibilities. Although the sex-determining switch is different, many of the same genes (*SOX9*, *AMH*, *DMRT1*, *SF1*, *WT1* and *DAX1*) play a role in avian gonad development as they do in mammals. It seems the main difference is that the order in the pathway differs. I presented data showing that *AMH* is expressed before *SOX9* in the chicken and alligator, suggesting that in these species, *SOX9* cannot initiate *AMH* expression and Sertoli cell differentiation as it does in mammals. In birds, hormones play an important early role; also, the ovary is more steroidogenically active than the testis, whereas in mammals the reverse is the case. *DMRT1* is particularly interesting in this context, as it maps to the Z chromosome in chickens. I suggested that *DMRT1* may be the critical switch gene in non-mammalian vertebrates. In some reptiles, such as the alligator, the temperature at which eggs are incubated determines the sex, but little information is available to explain how this temperature switch might exert its effect. Russell Fernald (Stanford University, USA) presented a brilliant and entertaining account of the social regulation of sex in cichlid fish. This involves the fish converting behavioral signals in the visual cortex into endocrine and gonadal changes. We have little idea, however, of how social information is transduced through touch and vision into molecular signals that can control sex.

Eleven years on

Since the discovery of *SRY*, what have we learned about sex determination? As our field has matured we have had to discard simple ideas of linear developmental pathways and invoke complex networks. We have several key pieces of the jigsaw puzzle, such as *SRY* and *SOX9*, but much else remains nebulous. Apart from the regulation of *AMH*, we have little idea of how these components interact. According to Gerd Scherer (University of Freiburg, Germany), only 20% of XY sex-reversed human females can be accounted for by defects in the known sex-determining genes. This suggests that many more genes remain to be found if we are to complete the jigsaw puzzle. One approach being taken by Peter Koopman, Andreas Schedl and my lab is to isolate differentially expressed transcripts from the embryonic mouse and also the chicken testis. These genes will then be subjected to rapid screening and functional assays to determine their role in sex determination. Other approaches discussed include the use of large scale chemical (*N*-ethyl-*N*-nitrosourea, ENU) mutagenesis screens for sex-reversed phenotypes in mouse. Both approaches should yield many more pieces of the jigsaw that are presently missing. It is hoped that by the next 'sex-workers' meeting, we will have a clearer picture of the complex developmental network that is sex determination.

The proceedings of this symposium (papers and edited discussions) will be published in February 2002 by John Wiley & Sons Ltd.