The elusive action of sex-determining genes: mitochondria to the rescue?

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Abstract

According to the accepted dogma of mammalian sex determination, the Y-linked gene SRY initiates male development by inducing hitherto uncommitted somatic cells of the fetal gonad to develop into Sertoli cells. However, it has become evident that the correct functioning of an increasing number of genes on other chromosomes is required for testicular organogenesis. They include the SRY-related gene, SOX9, which plays important roles in both sex determination and chondrogenesis, as well as genes responsible for the production of growth factors, i.e. fibroblast growth factor 9, platelet derived growth factor A, and the members of the insulin-receptor family of genes. It is known, moreover, that differences between the sexes begin to develop long before the differentiation of Sertoli cells, including an increase in gonadal size and cell proliferation, and accelerated development of XY embryos at early pre-implantation stages. There is also evidence of transcription of Y-linked, and of X-linked, genes and of an enhanced metabolic rate in XY embryos. Furthermore, the condition of true hermaphroditism does not fit into a simple genotype/phenotype relationship. The proposal that “testis-determining” genes act by increasing metabolic rates rather than directly determining Sertoli cell differentiation can account for a number of observations that do not fit the current model, including pregonadal sex differences, the activity of the same gene in different organ systems, and the frequent co-existence of sexual and somatic abnormalities. It also sheds light on the pervasive differences between metabolic rates of mammalian males and females, while the facts of true hermaphroditism can be viewed as remnants of temperature-dependent sex determination in ectothermic vertebrates. Growing interest in mitochondria, which play a central role in the provision of energy to eukaryotic cells, makes a shift of paradigm from gonadal histology to energy metabolism timely, particularly since new techniques have become available for testing the hypothesis, and for widening the experimental approach to sex determination. If the hypothesis is correct, it would mean that male sex is determined by nuclear genes inherited from the father regulating the activity of maternally derived mitochondria.

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1. Introduction

The genetics of sex determination is a child of the 20th century, which overturned the previously held view that sex was determined by the environment of the developing organism. Edmund Wilson, whose views underwent a radical change at the beginning of the new century, was himself one of the pioneers who brought about this change in outlook. His studies of the chromosomes of insects revealed two different dimorphisms in the spermatocytes: either a chromosome was present in one class and absent in the other, or both classes contained a chromosome of unequal size (Wilson, 1905). In the same year Netty Stevens (1905) found that in the common mealworm, Tenebrio molitor, males, but not females had one chromosome that was smaller than the...
and its partner as the “X chromosome” (Wilson, 1909), chromosome became known as the “Y chromosome” and its partner as the “X chromosome” (Wilson, 1909), and both as “sex chromosomes” (Wilson, 1911). During the third quarter of the 20th century, the sex chromosomes of the human and other mammalian species came to the fore, and during the last quarter of the century, there was a hunt for the mammalian sex-determining gene. It ended with the isolation of SRY from a 35kb region of the human Y-chromosome (Sinclair et al., 1990), the cloning of a corresponding gene, named Sry, from the Y chromosome of the mouse (Gubbay et al., 1990), and the demonstration that Sry, when added as transgene to XX embryos, was able to induce male development in some of them (Koopman et al., 1991). The fact that some human XY females were found to have mutations in SRY was further evidence of the male-determining function of the gene (Affara et al., 1993).

However, the expectation that the identification of the gene would lead to an elucidation of a pathway of genes causing the “indifferent” gonadal rudiment to form a testis has not been fulfilled. The SRY gene encodes a DNA-binding protein, but no direct targets have been identified; hence, the mechanism by which it controls the development of the testis and the male phenotype remains essentially unknown. It may be appropriate, therefore, to examine the basis on which the present-day view of sex determination is built.

2. The current dogma

The current model of mammalian sex determination is based on three principles.

First, according to the embryological evidence, the gonads appear at first morphologically identical in both sexes and subsequently develop into ovaries or testes according to the sex chromosome constitution (van Wagenen and Simpson, 1965).

Second, the idea publicized by Alfred Jost (1953), that the difference between males and females begins with the histological differentiation of the hitherto undifferentiated gonad into a testis, which subsequently secretes hormones that masculinize the reproductive tract, and that in the absence of fetal testes, female development occurs by default.

The third principle relates to the agent that causes the differentiation of the testis. Various anomalies of the sex chromosome constitution, particularly in humans, demonstrated the male-determining function of the Y chromosome, which, combined with the Jost principle, led to the conclusion that the Y chromosome determines the differentiation of the testis (Ford, 1970); and so the search for the “testis-determining factor” was initiated. Subsequent studies with the electron-microscope revealed the Sertoli cell as its first visible component (Jost et al., 1981), and this brought about the association of the “testis-determining factor” with the differentiation of Sertoli cells. As already mentioned, the search for the gene encoding the factor ended with the discovery of SRY.

Subsequently, however, exceptions came to light. In humans, some female patients were found in whom an apparently normal SRY gene was present as well as some male patients who lacked the gene (Vilain, 2002). At the same time, a growing number of genes on other chromosomes was identified, whose correct function was also required for testis development. Consequently, the view of a simple pathway leading from SRY to the differentiation of previously uncommitted somatic cells in the genital ridge into Sertoli cells, and hence to the induction of the male phenotype, has been replaced by the idea of a network of interacting genes (Koopman, 2001). Their mode of action, however, remains essentially unknown.

Furthermore, there is a substantial body of data that is at variance with different aspects of the current dogma.

3. When does sex differentiation begin?

Although the pivotal position of the differentiation of the fetal gonad into a testis capable of secreting hormones that masculinize the reproductive tract remains unchallenged, it has become evident that the differentiation of Sertoli cells is not the first phenotypic difference between XY and XX embryos. There are numerous findings suggesting that differences in the rate of development occur prior to the appearance of Sertoli cells and, indeed, the formation of genital ridges; and even though the significance of these early differences on gonadal sex differentiation is not yet known, the increasing evidence of the effects of early growth rates and birth weights on physiology and pathology in later life (Barker, 1998; Leon et al., 1998; Betteridge, 2001; Wells, 2003) demand that these findings can no longer be ignored.

Quantitative studies on mammalian fetuses have shown that faster growth of the male gonad compared with that of the female can be detected before any histological differences are apparent (Lindh, 1961; Mittwoch et al., 1969), which has led to the suggestion that testicular differentiation is dependent on an accelerated growth rate, brought about by an increased rate of cell proliferation (Mittwoch et al., 1969; Mittwoch, 1970, 1986; Hunt and Mittwoch, 1987).
Recent investigations using BrdU (5′-bromo-2′-deoxy-uridine) labelling revealed that Sry induced cell proliferation in the gonads of fetal mice, and that an increase in cell proliferation was the first identifiable effect of the gene’s activity (Schmahl et al., 2000). Further evidence indicated that inhibiting cell proliferation during a critical period of early gonad formation prevents the differentiation of Sertoli cells and the establishment of testicular histology (Schmahl and Capel, 2003).

It has also been shown that in different mammalian species, including humans, bovines, mice and rats, XY conceptuses are developmentally more advanced, and therefore bigger, than XX conceptuses of similar gestational age (see review by Burgoyne et al., 1995). Such differences have been seen at the earliest stages of development. In human embryos produced by in vitro fertilization, male embryos averaged more cells than female ones on day 2 after fertilization, and there was evidence of increased metabolic activity in male embryos between days 2 and 5 (Ray et al., 1995); and transcription of the Y-chromosomal genes, SRY and ZFY has been reported in cleavage embryos of both mice (Zwingman et al., 1993) and humans (Ao et al., 1994; Fiddler et al., 1995). Transcripts of ZFY have also been documented in bovine embryos at the 8–16-cell stage and in blastocysts, whereas levels of transcripts of G6PD and of other X-linked genes where higher in female morulae and blastocysts (Peippo et al., 2002). The way in which these findings relate to gonad differentiation remains to be established. Edwards and Beard (1999) have postulated that the early expression of sex-determining genes could be involved in the allocation of germ cells. Further investigations will clearly be needed to settle this question.

The sum total of the developmental evidence demonstrates that the process resulting in the differences between the sexes begins long before the gonads are formed (Erickson, 1997) and can affect the development of organs other than gonads. Therefore, not all the differences between the sexes can be explained by the presence or absence of testicular hormones (Blecher and Wilkinson, 1989; Fiddler and Pergament, 1997; Mittwoch, 2000). Recently, David Page and collaborators (Skaletsky et al., 2003) arrived at a similar conclusion on the basis of their discovery of a larger than expected number of Y-chromosomal genes.

4. True hermaphroditism, the environment and the control of metabolic rate

Unilateral manifestations in bilateral organs are of particular interest, because they suggest an environmental component in addition to the genetic causation (Mittwoch, 1996). True hermaphroditism is characterized by the presence of ovarian and testicular tissue in the same individual. The majority of patients have XX sex chromosomes, others have mixed cell lines with XX/XY and other combinations, and about 10% of patients are XY. Molecular analysis in most XX patients failed to detect evidence of SRY (Berkovitz et al., 1992). This raises the question as to the causes of testicular development in the absence of SRY, as well as for the reason for ovarian development in the minority of patients who appear to be totally XY.

An additional difficulty comes from the positioning of the gonads in true hermaphroditism, in which large surveys have revealed bilateral asymmetry (van Niekirk and Retief, 1981; Krob et al., 1994). The ovotestis, which can be unilateral or bilateral, is the most common gonad found in patients, followed by a unilateral ovary, while a unilateral testis is the rarest. Ovaries occur twice as often on the left side compared with the right, while testicular tissue is more commonly situated on the right.

The basic asymmetry of the developmental potential of left and right gonads in the absence of an effective genetic mechanism is foreshadowed during the development of normal fetuses, when right gonads are, on average, more advanced than those on the left (Mittwoch and Kirk, 1975; Mittwoch and Mahadevaiah, 1980; Mittwoch, 1986). Recently, it was shown by Fukuda et al. (2000) that in women the dominant follicle develops more often in the right than in the left ovary and that, moreover, right-sided ovulations seem to have a higher implantation rate—evidence that the difference in developmental rates in the fetus exerts an effect in adulthood. Evidently, under exceptional circumstances during fetal development, a minor difference in growth rates between the gonads on the left and the right can be translated into ovarian versus testicular differentiation.

The environmental component in the etiology of human true hermaphroditism may be viewed as a feeble remnant of the prominent role played by the environment, particularly temperature, in earlier vertebrates, such as reptiles (Lance, 1994; Pieau et al., 1999)—a mechanism that is obviously no longer available in mammals. In ectothermic animals, temperature plays an important role in determining, not only developmental rates and final size, but also characters such as vertebral number and certain morphometric traits (Braña and Ji, 2000). That remnants of temperature-dependent sex determination may persist in humans was also suggested by McLachlan and Storey (2003).

The effects of temperature on growth and development raise the question whether the control of metabolic rate could be a common denominator between the effects of sex-determining genes and environmental factors (Kraak and de Looze, 1993). A difference between basal metabolic rates of males and females is particularly evident in humans, for whom most data are available. Surveys of basal metabolic rates have shown
that the average is higher in male infants from 11 months onwards, in children aged 3–16 years, as well as in adults of all ages (Lentner, 1981). Indeed, as already mentioned, there is evidence of a higher metabolic rate of male embryos produced by in vitro fertilization as early as 2 days after insemination (Ray et al., 1995). An increased metabolic rate has also been reported in male pre-implantation bovine embryos (Tiffin et al., 1991).

These facts suggest the intriguing possibility that the higher metabolic rate in human males may be initiated by Y-chromosomal genes and can later be modulated by other genes, by hormones and by environmental factors.

5. The “testis-determining” genes, SRY and SOX9

The role of SRY in testicular development remains unchallenged. However, the view that the activity of the gene is confined to the developing testis is less convincing. As shown by Clépet et al. (1993), SRY transcripts in humans are not confined to the presumptive and mature gonadal tissues in the embryo and adult, but are also found in a variety of other locations, including all fetal (16/17 weeks) tissues examined, i.e. adrenal, brain, liver, pancreas, small intestine, spleen, thymus and heart. In adult tissues, SRY expression was detected in testis, heart, liver and kidney, but not in lung. Nevertheless, the authors reject a general role for the gene, apparently on the basis that XY females with gonadal dysgenesis caused by mutations in SRY do not show obvious somatic malformations; but this hardly precludes subtle effects on growth and development.

In the case of the SRY-related gene, SOX9, which is located on human autosome 17, there is no room for doubting its ability to affect a wide variety of organs. Haplo-insufficiency of the gene, caused either by a mutation in one of its member by a chromosome rearrangement, gives rise to campomelic dysplasia, a severe skeletal malformation syndrome; in addition, the majority of XY patients develop as females (Schafer et al., 1995). Transcripts of SOX9 have been detected in human fetal brain, liver and kidney, and in most adult tissues tested, with the exception of leucocytes, spleen and thymus.

In mouse embryos, Sox9 is expressed during chondrogenesis, and appears to be necessary for the differentiation of chondrocytes from mesenchymal cells (Koopman, 2001). As regards the developing gonads, the gene seems to be expressed initially in the gonads of both sexes, followed by higher levels in male gonads and a reduction in female gonads. An increase in Sox9 expression failed to occur in XY gonads that lacked cords following the application of proliferation inhibitors (Schmahl and Capel, 2003). It is evident that sufficiently high levels of SOX9 protein, as well as of SRY, are required for testis development (Koopman, 2001), although it has recently been shown that SOX8 activity, a product of a related gene, Sox8, can partially compensate for reduced SOX9 activity (Schepers et al., 2003). Furthermore, the introduction of a transgene of Sox9 can induce testis development in XX mice in the absence of Sry (Vidal et al., 2001).

6. Growth factors involved in the differentiation of the testis

There are at least a dozen genes known to play a role in the determination of sex (Lovell-Badge et al., 2002), and their number continues to grow. While the action of many of them is still unknown, they include some coding for known growth factors. One of them, Fgf9, codes for fibroblast growth factor 9, which is expressed in lung and other organs of mouse embryos (Colvin et al., 2001). Mice lacking fibroblast growth factor 9 due to a homozygous deletion of the gene die at birth, probably because of lung hypoplasia, and many of the newborns exhibit male-to-female sex reversal (Colvin et al., 2001).

One of the platelet-derived growth factors (PDGFs) is now known to be necessary for testis organogenesis. Brennan et al. (2003) found that the Pdgf-A gene and its receptor gene, Pdgfr-α, are expressed differentially in XX and XY fetal mouse gonads, and that the absence of the receptor gene, there was a severe reduction in XY cell proliferation, mesonephric cell migration and Leydig cell differentiation. It may be surmised that these processes are dependent on an increased energy metabolism compared with less active tissues. It also seems likely that genes are involved in the production of this energy.

Recently further evidence for the necessity of growth factors in male sexual development has come to light. Nef et al. (2003) showed that XY mouse embryos in which all three insulin receptor genes were non-functioning produced ovaries instead of testes. The genes, known as Ir, encoding the insulin receptor, Igf1r, encoding the insulin-like growth factor-1 receptor, and Irr, which encodes the insulin-receptor related receptor (Koopman, 2003).

7. Sex-determining genes: controllers of energy metabolism?

Differences between metabolic rates occur both within the same organism and between different organisms (Rolfe and Brown, 1997). The causes for such differences have not so far been clarified. Nevertheless, it seems reasonable to assume that for differences within an organism, nuclear genes that are differentially expressed in different organ systems are likely to be
involved, whereas for differences between organisms different genotypes could be responsible in addition.

As regards inter-organismal differences in metabolic rates, it seems to be the rule that in mammals males have higher rates of minimal metabolism than females (Blaxter, 1989). As mentioned above, it is indeed remarkable how pervasive this difference appear to be in our own species, having been recorded in pre-implantation embryos (Ray et al., 1995), as well as in infants and children from 11 months to 16 years, and at all ages of adulthood (Lentner, 1981). I suggest that the sex-determining genes initiate this process of setting a higher metabolic rate in mammalian males.

It needs to be remembered, however, that the mammalian sex-determining system is neither universal nor even widely distributed in the animal kingdom, and the same applies to the concept that males are larger, and more aggressive, than females (Lockshin, 1999). The varying size relationship between the sexes, which is in obvious contrast to the constant dichotomy between testis and ovary, supports the hypothesis by Kraak and de Looze (1993) that the existence of different sex-determining mechanisms in vertebrates relates to whether the male or the female is the larger and stronger sex. At present there is some tantalizing evidence that early fast proliferation of male gonads may be a feature in all vertebrates, but that in birds with ZW sex chromosomes, and in reptiles with high female-producing temperatures, this trend is later replaced by higher proliferation rates in the ovary (Mittwoch, 1998; Schmahl et al., 2003).

8. Hypothesis

I propose that a divergence in energy metabolism is at the root of the difference between the sexes. In mammals, males are metabolically more active than females from fertilization onwards, and this difference is amplified in the developing testes by the production of testicular hormones. The testis-determining genes, SRY and SOX9, effect an increase in metabolic rate in the tissues in which they are active, resulting in an increase of cell proliferation and of other developmental processes, such as cell migration (Buehr et al., 1993). Similar effects are produced by a rise in temperature in ectothermic vertebrates.

9. Testing the hypothesis

The present time is witnessing a rebirth in the study of mitochondria (Kiberstis, 1999; Rich, 2003), which has given rise to many new techniques for studying cellular metabolism. Since mitochondria produce most of the cell’s energy by oxidative phosphorylation (Saraste, 1999), and since metabolically active cells tend to contain more mitochondria than less active ones, the question arises whether there is a difference in the number and activity of mitochondria in developing male and female mammals. The likelihood of this possibility is increased by the finding that reciprocal crosses between two strains of mice provided evidence of a maternal effect on testis weight (Hunt and Mittwoch, 1987), since mitochondria are maternally inherited. Although it has long been known that an alteration in mitochondria can affect the activity of nuclear genes involved in the biogenesis of cell surface components (Wilkie et al., 1983), there seems to be little understanding at present of what causes inter-organismal differences in metabolic rates.

An obvious system to address this question with regard to males and females is to compare the mitochondria in developing gonads of XX and XY mouse embryos, in which there is a very large difference in rates of cell proliferation. The technique of microfluorimetry (Duchen et al., 2001), seems ideally suited for this purpose. Utilizing confocal microscopy, it allows mitochondria to be studied within living cells and, in addition, oxygen consumption to be measured.

10. Conclusion

A shift in the paradigm of sex determination from gonadal histology to energy metabolism could account for a number of observations on normal and abnormal sexual development that do not fit into the current model. These include pregonadal sex differences, the interaction of genes and environment, the frequent co-existence of gonadal and somatic abnormalities, as well as sex biases in congenital anomalies (Lubinsky, 1997). The necessity to set the right metabolic rate also makes sense of the paramount importance of the correct number of copies of sex-determining genes (Vilain, 2002).

The growing interest in mitochondria makes this change particularly timely by providing new techniques with which the hypothesis can be tested. These techniques could add valuable experimental procedures for the study of sex determination.

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References


