Chromosome Function: Sex Differences

Mariana F Wolfner, *Cornell University, Ithaca, New York, USA*

The distinct development of females and males is usually the consequence of differences in their sex chromosome constitution. To compensate for differences in sex chromosome make-up, the sexes adjust the expression of sex-linked genes. Some inheritance patterns depend on the sex or sex chromosome make-up of a parent.

Secondary article

Article Contents

Chromosomal Mechanisms for Sex: Diversity and Plasticity

Though the existence of two sexes is common in animals, the chromosomal basis for sexual development is diverse. In many genera, females and males have different sex chromosome constitutions. In mammals and fruitflies, for example, the female normally has a pair of X chromosomes whereas the male is heterogametic, possessing one X chromosome and one Y chromosome. In birds and some reptiles, the heterogametic sex is female (ZW; males are ZZ). In the nematode *Caenorhabditis elegans* there is only one kind of sex chromosome (X). The existence of sex-defining chromosome constitutions, while very widespread, is not universal: in some strains of houseflies there are no visibly distinct sex chromosomes, and in some reptiles and some fish environmental cues such as temperature determine the animal's sex.

Despite these differences, some generalizations can be made about how sex chromosome constitution usually determines sexual phenotype. In most cases the presence or number of particular chromosomes causes sex-specific production of a protein that determines sex. This protein's action heads a cascade of gene activities that causes cells to develop as female or male, and/or to produce sex-specifying hormones. Sexual phenotype includes sex-specific development of the somatic portion of the animal, as well as the differentiation of germ cells into eggs or sperm. Though somatic and germline sexual development are usually both regulated by sex chromosome constitution, the genetic cascades that specify them can be different, though often interdependent. Below, three well-studied examples are used to illustrate a range of sex-determining mechanisms.

In eutherian mammals, a Y-linked gene determines maleness, opposing an X-linked gene whose action results in female development

Sexual phenotype in mammals (**Figure 1**) was originally interpreted as being due simply to the presence of a Y chromosome in males and its absence in females. Mammals develop male features if their cells contain a Y chromosome, regardless of the number of X chromosomes they possess; mammals whose cells contain a single X and a single Y develop into normal fertile males. The presence of the Y chromosome is male-determining because a crucial gene that determines maleness is located on this chromosome. This gene, *SRY* (*S*ex-determining *R*egion on the *Y*) encodes a member of the high mobility group (HMG) family of chromatin-binding proteins. *SRY* was identified by deletion mapping of abnormal Y chromosomes from rare human males who are XX and rare human females who are XY. Its male-determining role was confirmed by the discovery of XY females carrying inactivating point mutations in *SRY*. Mice have a related gene, *Sry*, on their Y chromosome. That *Sry* specifies maleness was shown by the male development of XX mice carrying an *Sry* transgene. Interestingly, the *SRY*/*Sry* family is diverging quickly enough that the human *SRY* gene did not cause male development of XX mice that carried it as a transgene (Koopman *et al*., 1991).

Sry acts via effects on cells, gene products and hormones. In mouse embryos, *Sry* is expressed in the genital ridge before the developing gonad (or the embryo as a whole) displays overt morphological sex differentiation. As a result of this expression, Sertoli cells develop in the gonadal soma; presence of these cells correlates perfectly with subsequent male development. SRY could act by modifying chromatin structures to allow or prevent transcription of genes characteristic of, or allowing, Sertoli cell development. Alternatively it could act indirectly by inducing an intermediary protein that carried out that function. A candidate for such an intermediary factor is SOX-9, another member of the HMG protein family. DNA

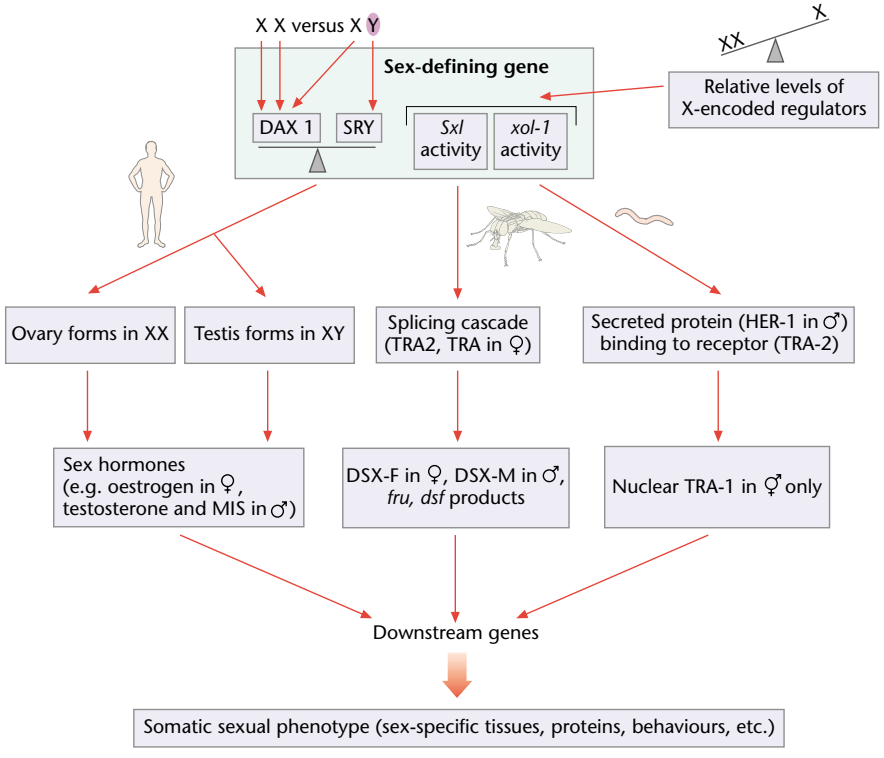

Figure 1 A summary of the sex-determining mechanisms of humans, flies and worms as described in the text. Some details have been deleted for simplicity, including autoregulation of *Sxl* expression that maintains it in the mode set by the X:A ratio, and some of the genes in the regulatory cascades.

sequences that can bind SRY and SOX proteins are found upstream of several genes that encode male-specific RNAs.

Somatic cells in the male gonad (Sertoli and Leydig cells) produce hormones that cause subsequent male development. Testosterone causes the animal to differentiate as male; in the absence of receptors for testosterone, XY mammals develop as females. Another hormone important in an aspect of male development is AMH (antimullerian hormone). This TGF-β family member causes degeneration of the ducts that would otherwise make female internal reproductive structures.

Thus, the signal for maleness is the presence of the SRY protein, encoded on the Y chromosome. Recently, an X-linked gene with a formally analogous genetic position specifying female determination, but in a dose-responsive manner, has been discovered. This gene, *DAX1*, encodes a nuclear hormone receptor (Swain *et al.*, 1998). XX animals possess two doses of *DAX1*, and this instructs their developing gonad to become an ovary. The ovary then produces oestrogen, resulting in female development. XY animals possess one copy of *DAX1* and one of *SRY*. Antagonistic action between these two genes 'tips the balance' in favour of testis development by the gonad; the testis produces testosterone resulting in male development.

The ratio of X chromosomes to autosomes determines sex in fruitflies

The sex of a *Drosophila melanogaster* depends on the number of X chromosomes relative to the ploidy of each of its cells, and is not influenced by the presence or absence of a Y chromosome (**Figure 1**). Presence of two X chromosomes in a diploid cell (X:autosome (X:A) ratio of 1) triggers female development; presence of one X in a diploid cell (X:A = 0.5) specifies maleness. The sexual phenotype of flies with extra X chromosomes and/or autosomes shows that sex determination has only two 'settings' (X:A ≥ 1 = female; X:A ≤ 0.5 = male). Flies with an intermediate X:A ratio of 0.67 develop as mosaics of male and female cells. Each cell 'decides' independently whether to count this ratio as 0.5 or 1; in flies sex is determined on a cell-by-cell basis rather than by a circulating hormone derived from the gonad.

The X:A ratio of a fly embryo cell determines whether or not it will produce the female-specifying protein SXL (*Sex-lethal*). Some subunits of bHLH, bZip and other transcription factors needed to activate the *Sxl* gene are encoded on the X chromosome. The subunits may form a complex, or act cooperatively, with autosomally encoded or maternally derived activational or inhibitory subunits to activate transcription of the *Sxl* promoter. XX embryo

cells have two doses of each of the X-linked transcription factor subunit genes. This is sufficient to activate *Sxl* transcription. In contrast, XO or XY cells produce half this level of X-linked gene products. This results in insufficient transcription factor subunit levels to activate *Sxl* transcription (Erickson and Cline, 1993). Thus, SXL, which is an RNA binding protein, is produced in female but not in male embryos; its presence is essential and instructive for female development.

Shortly after the initial female-specific burst of *Sxl* transcription, *Sxl*'s X:A-dependent promoter is silenced and *Sxl* transcription becomes driven by a sex-independent promoter. However, the transcripts produced from the later promoter require regulated splicing to encode SXL protein. This regulated splicing requires SXL protein. Only female cells contain SXL protein, owing to their early sex-specific transcription of the *Sxl* gene. Thus, only females can produce SXL protein from the later *Sxl* transcripts. This regulation maintains production of SXL in all female cells, and prevents SXL from being produced in male cells. As a consequence of this regulation, the sex of a *D. melanogaster* cell is set during the first 2 h of embryonic development and remains fixed thereafter.

SXL causes female sexual development through a cascade of other genes' activities. In fly somatic cells, SXL causes productive splicing of the primary transcript of the *tra* (*tra*nsformer) gene. (Many gene names describe the phenotype of animals lacking that gene's activity, since that is how the gene was discovered. For example, XX flies mutant in the transformer gene are 'transformed' into phenotypic males, because they lack the *tra* activity that is essential for female development). This results in production of the TRA splicing factor subunit in females only. TRA, in concert with another protein (TRA2), catalyses alternative splicing of genes that regulate sexual characteristics. The best characterized of these downstream genes is the *dsx* (*d*ouble-*sex*) gene. Females and males produce different versions of the DSX DNA-binding protein owing to alternative last-exon use. Production of the female version of DSX protein (DSX-F) requires activation of a cryptic splice site by the TRA–TRA2 splicing factor; males, lacking TRA, do not carry out this splice and instead perform an alternative 'default' splice resulting in the production of the DSX-M protein. DSX-F and DSX-M bind to the regulatory regions of genes encoding sex-specific RNAs, such as those encoding female-specific yolk proteins (Burtis *et al*., 1991). They activate transcription of sex-appropriate RNAs (e.g. female RNAs for DSX-F), and interfere with transcription of the sex-inappropriate RNAs (e.g. male RNAs for DSX-F). As noted above, *dsx* is not the only gene that mediates the sex-determination signal set by SXL and transduced by TRA–TRA2. In some cells of the nervous system, this action appears to be carried out by at least two other genes, the nuclear receptor-encoding *dsf* (*d*issatis*f*action) gene and *fru* (*fru*itless); the latter undergoes TRA–TRA2-dependent alternative splicing (Heinrichs *et al*., 1998).

The *Drosophila* germline also undergoes male- or female-specific differentiation. Diploid *D. melanogaster* germline cells must have two X chromosomes to make eggs. They must have no more than one X chromosome to become sperm (and to complete this process, they also need a Y chromosome, which contains genes needed only for spermatogenesis). Proper sexual development of the germline depends on a cascade of gene activities, most of which differ from those of the somatic sex-determination cascade. It also requires a soma with the proper sexual phenotype to provide sex-appropriate signals to the developing germ cells.

In nematodes, the cellular ratio of X chromosomes to autosomes also determines sex

C. elegans do not contain a Y chromosome. Instead diploid XX animals are hermaphrodites – essentially females that make sperm early in development before switching their germlines to oogenesis. Diploid male worms are XO (they usually arise as the result of X-chromosome nondisjunction during meiosis). The number of X chromosomes in a nematode cell sets the sex-specific transcription of a sex-specifying gene, *xol-1* (*XO l*ethal-1) (**Figure 1**). *xol-1* is normally expressed only in males, apparently as the result of a balance between X-encoded and autosomal factors; an excess of X-encoded factors represses the gene's transcription and translation in hermaphrodites. *xol-1* regulates the expression of several intermediary genes *sdc1*, *sdc2* and *sdc3* (sex determination and dosage compensation), which transduce the sex-specifying signal to the downstream genetic cascades that carry out sexual development.

A cascade of gene activities transduces the *xol-1* signal into effects on the activity of a transcription factor, TRA-1. TRA-1 causes the expression of genes required for hermaphrodite development. Although TRA-1 is present in both XX and XO animals, in the latter it is prevented from entering the nucleus, and hence accessing its targets, by being bound to a complex of cytoplasmic proteins. This in turn is regulated by a secreted protein, HER-1 (*her*maphrodite-1), which is produced only in males, as a consequence of *xol-1* activity. HER-1 protein binds to a transmembrane protein, TRA-2 (note: not the same protein as the fruit fly TRA2), causing the release of the TRA-1-sequestering complex. This complex then prevents TRA-1 from activating hermaphrodite genes, resulting in male development (Kuwabara *et al*., 1992).

The basic determinant of sex in all of the examples described above is sex chromosome composition, which leads to the sex-specific production of a protein that determines sex. Yet there are vast differences in the ways in which chromosome make-up is evaluated and transduced

into the two alternative sexual states. Diversity in sex-determining mechanisms and the gene products that carry them out is far greater than that seen in many other developmental pathways such as segmentation. Though many sex-determining genes are members of conserved gene families, the sex-specifying function of those members is not conserved across phyla, and many sex-determining genes are rapidly evolving. A rare example of conservation among sex-determining genes occurs among downstream members of the regulatory cascades: the *Drosophila dsx* gene is related to a nematode sex-determination gene (*mab-3*) and to a human testis-expressed gene (*DMRT-1*), which lies in a sex-determining region of chromosome 9 (Raymond *et al*., 1998). Taken together, the data suggest conservation of the final downstream regulators of sexual development, but plasticity in the choice of upstream control pathways that generate those regulators. Once chosen, however, the upstream pathways may need to be fairly tightly 'fixed' since their modification could cause sterility. Interestingly, several upstream control genes in fruitfly determining cascades also function in other developmental phenomena (e.g. nervous system development in *D. melanogaster*, Caudy *et al*., 1988), suggesting that regulators or 'cassettes' of regulators were co-opted from or to the sex-determination cascade. Yet the apparent diversity in sex-determining mechanisms need not all be due to large-scale replacement of upstream regulatory cascades: smaller changes can cause large apparent differences in sex-determining mechanisms. For example, the *C. elegans* X-chromosome-based sex-determination mechanism can be converted to a single locus-based (or ZZ/ZW-like) mechanism by only two mutations, which create nonfunctional and constitutive alleles of *tra-1* to determine maleness and femaleness, respectively (Hodgkin, 1983). In an analogous manner, a temperature-sensitive mutation in a control protein could be a simple step towards converting a chromosome-based mechanism to a temperature-based one.

The Gene Dosage Problem and Some Solutions

Most sex-determining mechanisms rely on the presence or absence of a special sex chromosome, or on a special number of sex chromosomes. This results in different numbers of copies of sex chromosome genes in females as opposed to males. Most sex chromosomes carry genes whose expression at a particular level relative to autosomal gene expression is essential for viability. (The *D. melanogaster* Y chromosome, whose genes function only in spermatogenesis, is an exception. These genes also provide useful 'footprints' for the evolutionary distancing between the sex chromosomes. Four 'evolutionary strata' are seen when sequences of X- and Y-linked paralogues are compared (Lahn and Page, 1999).) Thus, there must be compensation at the gene expression level for differences in gene dosage due to sex chromosome constitution between females and males.

X-inactivation in eutherian mammals

In humans and other eutherian mammals, the dosage problem is solved by inactivating genes on all but one X chromosome in each cell. Thus, XX females, like XY males, have a single X active in each cell. (A few X-linked genes are not inactivated, but they are the exception that proves the rule since there are paralogues of those genes on the Y chromosome. Thus, they are present in two copies in an XX female cell and in an XY male cell, and there is no need to adjust their dosage.) X inactivation can be seen

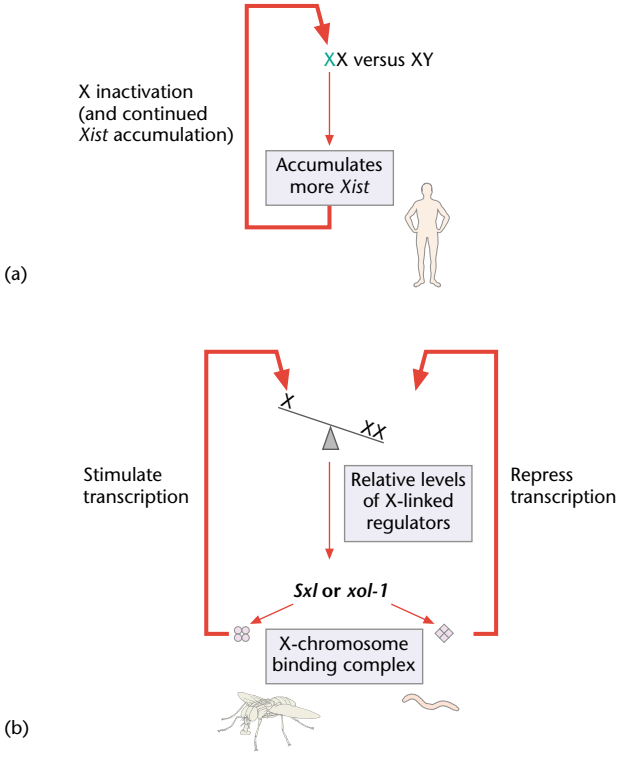

Figure 2 A summary of some solutions to the 'gene dosage problem', as described in the text. Part (a) shows X inactivation control in mammals, simplified for clarity by not showing that initially both X chromosomes accumulate *Xist* RNA. The X chromosome drawn in green has been inactivated, except for its *Xist* locus and its pseudoautosomal region. Part (b) summarizes the situation in flies (the MSL complex is shown schematically as a clump of circles) and nematodes (the chromosome binding complex is shown schematically as a clump of diamonds). As in **Figure 1**, autoregulation of *Sxl* is not shown.

cytologically by the presence of a heterochromatic 'Barr body'; there is one fewer Barr body per cell than the number of X chromosomes in that cell.

The choice of which X to inactivate is made cell-by-cell early in development. The decision depends on the production of a noncoding RNA, *Xist*, by the X chromosome (Brown *et al*., 1991; **Figure 2a**). In a female diploid embryonic cell, initially both X chromosomes produce *Xist*. However, one X chromosome begins to accumulate more *Xist* RNA. This *Xist* RNA coats the X chromosome from which it was derived. That X chromosome becomes heterochromatic and transcriptionally inactive except for its *Xist* gene. The other X chromosome eventually stops producing *Xist*, and remains active. The choice of which X chromosome becomes inactivated is usually random – one X 'wins' by accumulating higher levels of *Xist*. However, in some cases particular X chromosomes are 'stronger' or 'weaker' in terms of *Xist* accumulation, and this skews the probability that those particular chromosomes will be activated.

Once a cell has inactivated an X chromosome, all of its descendants retain the same X as inactive (with the exception of oogenic cells, which appear to reactivate their inactive X chromosome). As a result, female mammals are mosaics, composed of a mixture of cells with one X chromosome active and cells with the other X chromosome active. Most tissues and cell types arise from mixed populations of founder cells and thus are likely to have cells of both X chromosome activity classes. Thus, heterozygous females usually show allelic traits from both X chromosomes. Rarely, all progenitor cells of a particular tissue or cell type have the same X chromosome inactive. In such cases a heterozygous female will display the phenotype of only one allele in that tissue. If expression of a particular X-linked gene in that tissue is deleterious to the point of lethality, a heterozygous female who had only the deleterious X chromosome active in the critical cells or tissue would not survive.

Global regulation of X transcription in fruitflies and nematodes

In *D. melanogaster*, both X chromosomes in each female cell are transcriptionally active. This can be seen by the cellular phenotypes of females heterozygous for X-linked mutations, as well as by direct observation of X-chromosome transcription *in situ* or on Northern blots. Half as much X chromosome expression is insufficient for viability. Therefore, to compensate for its lack of a second X chromosome, a male cell elevates the transcription level of its lone X chromosome approximately two-fold (**Figure 2b**). This process of 'dosage compensation' is mediated by a complex of proteins that binds to the male's X chromosome. Production of the complex depends on lack of activity of *Sxl*, the same initial signal that determines the phenotypic sex of the cell (Kelley *et al*., 1995). Four proteins in the complex – MSL1, MSL3 (*m*ale-*s*pecific *l*ethal), a putative helicase MLE (*m*ale*le*ss), and a putative histone acetyltransferase MOF (*m*ale absent *o*n *f*irst (chromosome)) – are present in females as well as males but do not coat females' X chromosomes. A fifth protein in the complex, MSL2, is produced only in the absence of *Sxl* function; that is, in cells with only a single X chromosome. All five proteins must be present to form a functional complex; thus such a complex is only present in males. Presence of the complex alters the histone-acetylation pattern of the male's X chromosome, resulting in elevated transcriptional activity. A noncoding RNA, *roX1* (*R*NA *o*n the *X* chromosome-1) also coats the only male X chromosome in an SXL- and MSL2-dependent fashion, but a role for this RNA in dosage compensation has not yet been determined. Though *roX1* and the MSL–MLE–MOF complex appear to coat the entire X chromosome of males, unlike the case in mammals, dosage compensation appears to occur on a gene-by-gene basis owing to regulatory elements in the genes. Thus, for example, the X-linked yolk protein genes, which are normally not expressed in males, are not dosage compensated when their expression is forced in males.

Though different in detail, dosage compensation in nematodes has parallels to the *D. melanogaster* system (Chuang *et al*., 1996; **Figure 2b**). Here, too, a macromolecular complex dependent on the sex chromosome make-up of the cell (via *xol-1*) binds to X chromosomes and regulates their transcription. However, in contrast to the situation in fruitflies, the *C. elegans* complex binds to hermaphrodite X chromosomes and downregulates their expression to equal, in total, the expression of the single X chromosome in the male cell.

Complex Patterns of Inheritance Conditional on Sex

Sex-linkage

Some patterns of inheritance conditional on sex reflect the fact that sex chromosomes differ in number or presence in the two sexes. For example, in humans and *D. melanogaster*, the Y chromosome is normally inherited from the father, and a male's X chromosome comes from his mother. Though a female, having two X chromosomes, can be normal in phenotype while carrying (in heterozygous form) a recessive mutation, half her sons will show the phenotype of the recessive mutation since they received no X chromosome from their father to provide a wild-type allele. As a result, certain deleterious conditions, such as haemophilia in humans, are seen only or much more often in males. Conversely, Y-linked mutations in mammals will

display phenotypes only in males, and are inherited father-to-son.

Sex-limited inheritance

Other patterns of inheritance conditional on sex are not caused by sex linkage, but rather by the fact that phenotypes can be conditional upon a particular sex-specific milieu, such as the presence/absence of a particular sex hormone in mammals. These traits are considered sex-limited, since they manifest themselves only in individuals of one sex. There are several examples in humans. Patterned baldness manifests in males because of its testosterone dependence. BRCA-1 (*br*east *ca*ncer-associated gene-1) mutations are associated with some breast cancers in females but only rarely in males (Stratton *et al*., 1994), suggesting that the loss of this gene's action is exacerbated by the female-specific proliferation of breast tissue. Sex-limited inheritance also occurs in other organisms. For example, mutations on the *D. melanogaster* Y chromosome mutations show phenotypes only in males, since only male flies carry out spermatogenesis and this is the only process in which the Y-linked genes act.

Uniparental inheritance/determination

Other traits that are conditional on sex have to do with the differences in development, behaviour and function of the germlines of males and females. One example is uniparental inheritance; its more common form, maternal inheritance, is described immediately below.

Maternal inheritance and maternal effect

Maternal inheritance refers to the appearance in the zygote of a heritable trait wholly dependent on its mother's genotype. In most animals, the zygote derives most of its cytoplasm from its mother. The mother thus provisions the egg with molecules and organelles, whose production and characteristics depend on her genotype. For organelles such as mitochondria that contain their own genomes, inclusion in the egg means that the resulting embryo will retain its mother's genotype for those organelles. Maternal inheritance of mutant mitochondria means that progeny of affected mothers, but not progeny of affected fathers, will show the mutant trait. A number of syndromes showing this pattern of inheritance have been confirmed as due to mutations in the mitochondrial genome. An example is a streptomycin-dependent deafness in humans, caused by mutation of a maternally inherited, mitochondrially encoded ribosomal RNA (Reid *et al*., 1994).

Other effects on progeny also depend on the parent's genotype and the sex of the parental germline, though they are not heritable beyond the affected generation. In addition to organelles containing their own genomes, molecules are donated to the egg by the mother. As in maternal inheritance, the fact that the mother provisions the egg means that her genotype determines whether the embryo contains all the molecules it needs to survive and develop until its own genome is fully activated. Deficiency of any of these maternally provided molecules can cause abnormalities in the embryo, in a phenomenon known as maternal effect. This differs from maternal inheritance since the abnormalities are not passed on to the next generation, assuming the embryo survives to reproduce.

Parental and grandparental determination via genomic imprinting

Genomic imprinting represents another set of effects with inheritance limited by the genotype of the parent and the sex of the parental germline. Passage through the female or the male mammalian germline causes modifications to some genes, including characteristic DNA methylations that can silence genes (Tilghman, 1999). In a few cases, genes inherited through the maternal germline are modified ('imprinted') differently from those inherited through the paternal germline. For example, in human Prader–Willi syndrome, a particular region of chromosome 15 is silenced by imprinting in the maternal germline. Thus, only the paternally derived Prader–Willi region contributes to phenotype. If an individual inherits a mutant Prader–Willi region from her/his father, that individual will have Prader–Willi syndrome even if she/he inherited a normal allele from her/his mother, since the maternal allele will have been silenced by imprinting.

Imprinting is usually reversed during passage through the progeny's germline. Thus a phenotype caused by imprinting is not usually heritable beyond the affected individual. In a few cases, a person's germline is unable to reverse the imprint placed on a gene by their parent. In such a case, the imprinting that occurred in a grandparent's germline can affect the phenotype of their grandchild. For example, in a small number of cases, males are unable to reverse in their germline the maternal imprint placed on the Prader–Willi region by their mothers. Half the children of such men inherit a maternally imprinted Prader–Willi region derived from their paternal grandmothers. These children also receive a maternally imprinted Prader–Willi region from their mothers. Since the children thus have both copies of their Prader–Willi region silenced by oogenesis-type imprints, they display Prader–Willi syndrome.

There May Be a Tendency Toward Higher Point Mutation Rates in Males of Some species

When rates of nucleotide divergence were compared among different chromosomes, mammalian sex chromo-

somes showed differences from one another and from autosomes. Y-linked genes often showed more divergence, and X-linked genes always less, than autosomal genes. The three types of chromosome differ in the extent to which they are passaged through the male and female germlines. The Y chromosome is inherited exclusively through the male. Autosomes are transmitted equally by males and females, whereas only one-third of X chromosome transmission is through the male germline. That their sequence divergence parallels the extent to which the chromosomes are inherited from the male prompted the idea that passage through the male germline is more mutagenic than passage through the female. Sex-specific biochemical differences, such as ones leading to differences in imprinting, could conceivably contribute to this. But an intriguing hypothesis posits that the apparent male mutagenicity relates to differences in the number of cell divisions undertaken by a germline cell prior to becoming a functional gamete. Assuming that mutation frequency increases with each round of chromosome replication due to errors in replication fidelity, germ cells that divide more would be expected to have higher mutation rates. Consistent with the hypothesis, human and mouse male germ cells divide more than their female counterparts. In addition, mammalian male, but not female, germline stem cells continue dividing for the life of the animal. Mammals whose males reproduce later after sexual maturity show exaggerated interchromosomal sequence divergence, consistent with their male germlines having undertaken more cell divisions before becoming gametes. In a contrasting example that is also consistent with the hypothesis, no apparent difference between divergence rates on the X and autosomes is seen in *D. melanogaster*, an organism in which the contribution of pregametic germ cell divisions is predicted to be very similar.

A recent study failed to find an elevated mutation rate on the Y chromosome, but still found mammalian autosomes to be more divergent than the X chromosome. Its authors have proposed that differences in divergence might reflect chromosome-specific issues rather than sex-specific differences in germline cell division, for example that biochemical changes accompanying X inactivation or reactivation might make the X chromosome less prone to mutation.

Sex Chromosome Aneuploidy Syndromes in Flies and Humans

Likely because of dosage compensation, aneuploidy in sex chromosomes is generally tolerated more than aneuploidy for autosomes, though some sex aneuploidies have deleterious consequences. Because sex chromosomes carry genes other than those that determine sex, an imbalance in the number of sex chromosomes can cause developmental problems beyond those specific to sexual phenotype.

Humans whose cells contain only one X chromosome and no Y chromosome (XO) have Turner syndrome. This results in some physical abnormalities, likely due to the presence of only one sex chromosome and thus a lowered dosage of the small group of genes normally present on X and Y (and not inactivated on the X). Females with Turner syndrome are almost always sterile; apparently the human germline, like that of *D. melanogaster*, requires two X chromosomes per cell for egg production. However, there is significant variability in the phenotype of individuals with Turner syndrome. This, and the fact that 45, XO karyotypes comprise a large proportion of spontaneous miscarriages, has led to the hypothesis that a fully XO human is inviable as an embryo, and that individuals with Turner who live are mosaics of XO and XX (or XY) cells; non-XO cells in critical tissues allowed them to survive. A related hypothesis may explain the occasional fertility of individuals with Turner syndrome – in those cases, their germline may have included XX cells. Individuals with two X chromosomes and one or two Y chromosomes display Klinefelter syndrome. These individuals have male morphology, but do not show fully male secondary sexual characteristics. They are infertile, presumably because their two rather than one X chromosome per germline cell is incompatible with formation of functional sperm. Individuals with three X chromosomes but no Y chromosome are relatively normal females, most likely due to inactivation of all but one X chromosome in their somatic cells. They are frequently fertile. Individuals with one X chromosome and two Y chromosomes are fertile males, though often taller than XY males. Some of these sex-chromosome aneuploidies result in lowered intelligence. It should be borne in mind when considering these phenotypes that fertile people who show a generally normal phenotype are less likely to present at clinics in circumstances leading to determination of chromosome make-up. This may lead to an overestimate of the extent of deleterious effects of sex aneuploidies, since minimally affected or unaffected individuals might not be detected.

D. melanogaster XO animals are phenotypic males, but they are sterile because they lack the Y-linked genes essential for spermatogenesis. XXY animals are phenotypically normal, fertile females. Flies in which the X:A ratio is disrupted beyond normal, e.g. triploid animals with only one X chromosome per cell (1X:3A) or diploid animals with three X chromosomes (3X:2A), are poorly viable. One theory holds that the *Drosophila* X chromosome can only be transcribed at two levels – that normally found in a female and that normally found in a male. Under this hypothesis, a 1X:3A animal would not produce enough X-encoded product to balance his autosomal gene product levels, and a 3X:2A animal would produce too much X chromosome products relative to levels of her autosomal products; either imbalance is essentially lethal. Animals with an intermediate 2X:3A sex chromosome constitution

are viable mixtures of female and male cells, as discussed earlier.

Conclusion

The phenotypic differences between females and males is a fundamental distinction in biology. The ability to analyse separately pure populations of each 'state' (female or male) and the identification of chromosome constitutions that control the choice between the sexes has advanced the study of the basis of this important distinction. In addition to identifying novel and important mechanisms for general gene control, such as dosage compensation and X inactivation, studies of how sex chromosome constitution translates to two alternative phenotypes has been helpful in elucidating basic biochemical mechanisms (splicing, hormone action, etc.) and in clarifying some complex patterns of inheritance.

References

Brown CJ, Ballabio A, Rupert JL *et al*. (1991) A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. *Nature* **349**: 38–44.

Burtis KC, Coschigano KT, Baker BS and Wensink PC (1991) The doublesex proteins of *Drosophila melanogaster* bind directly to a sex-specific yolk protein gene enhancer. *EMBO Journal* **10**: 2577–2582.

Caudy M, Grell EH, Dambly-Chaudiere CA, Jan LY and Jan YN (1988) The maternal sex determination gene daughterless has zygotic activity necessary for the formation of peripheral neurons in *Drosophila*. *Genes and Development* **2**: 843–852.

Chuang PT, Lieb JD and Meyer BJ (1996) Sex-specific assembly of a dosage compensation complex on the nematode X chromosome. *Science* **274**: 1736–1739.

Erickson JW and Cline TW (1993) A bZIP protein, sisterless-a, collaborates with bHLH transcription factors early in *Drosophila* development to determine sex. *Genes and Development* **7**: 1688–1702.

Heinrichs V, Ryner LC and Baker BS (1998) Regulation of sex-specific selection of fruitless 5′ splice sites by transformer and transformer-2. *Molecular and Cellular Biology* **18**: 450–458.

Hodgkin J (1983) Two types of sex determination in a nematode. *Nature* **304**: 267–268.

Kelley RL, Solovyeva I, Lyman LM, Richman R, Solovyev V and Kuroda MI (1995) Expression of *msl-2* causes assembly of dosage compensation regulators on the X chromosomes and female lethality in *Drosophila*. *Cell* **81**: 867–877.

Koopman P, Gubbay J, Vivian N, Goodfellow P and Lovell-Badge R (1991) Male development of chromosomally female mice transgenic for Sry. *Nature* **351**: 117–121.

Kuwabara PE, Okkema PG and Kimble J (1992) tra-2 encodes a membrane protein and may mediate cell communication in the *Caenorhabditis elegans* sex determination pathway. *Molecular Biology of the Cell* **3**: 461–473.

Lahn BT and Page DC (1999) Four evolutionary strata on the human X chromosome. *Science* **286**: 964–967.

Raymond CS, Shamu CE, Shen MM *et al*. (1998) Evidence for evolutionary conservation of sex-determining genes. *Nature* **391**: 691–695.

Reid FM, Vernham GA and Jacobs HT (1994) A novel mitochondrial point mutation in a maternal pedigree with sensorineural deafness. *Human Mutation* **3**: 243–247.

Stratton MR, Ford D, Neuhasen S *et al*. (1994) Familial male breast cancer is not linked to the BRCA1 locus on chromosome 17q. *Nature Genetics* **7**: 103–107.

Swain A, Narvaez V, Burgoyne P, Camerino G and Lovell-Badge R (1998) Dax1 antagonizes Sry action in mammalian sex determination. *Nature* **391**: 761–767

Tilghman SM (1999) The sins of the fathers and mothers: genomic imprinting in mammalian development. *Cell* **96**: 185–193.

Further Reading

Bauer VL and Aquadro CF (1997) Rates of DNA sequence evolution are not sex-biased in *Drosophila melanogaster* and *D. simulans*. *Molecular Biology and Evolution* **14**: 1252–1257.

Cline TW and Meyer BJ (1996) Vive la difference: males vs. females in flies vs. worms. *Annual Reviews of Genetics* **30**: 637–702.

Goodfellow PN and Camerino G (1999) DAX-1, an 'antitestis' gene. *Cellular and Molecular Life Sciences* **55**: 857–863.

Jegalian K and Page DC (1998) A proposed path by which genes common to mammalian X and Y chromosomes evolve to become inactivated. *Nature* **349**: 776–780.

Kuroda MI and Meller VH (1997) Transient Xist-ence. *Cell* **91**: 9–11.

Lightowlers RN, Chinnery PF, Turnbull DM and Nowell N (1997) Mammalian mitochondrial genetics: heredity, heteroplasmy and disease. *Trends in Genetics* **13**: 450–455.

Lucchesi JC (1998) Dosage compensation in flies and worms: the ups and downs of X-chromosome regulation. *Current Opinion in Genetics and Development* **8**: 179–184.

Marin I and Baker BS (1998) The evolutionary dynamics of sex determination. *Science* **281**: 1990–1994.

McVean GT and Hurst LD (1997) Evidence for a selectively favourable reduction in the mutation rate of the X chromosome. *Nature* **386**: 388–392.

Meyer BJ (2000) Sex in the worm – counting and compensating X-chromosome dose. *Trends in Genetics* **16**: 247–253.

Morais da Silva S, Hacker A, Harley V *et al*. (1996) Sox9 expression during gonadal development implies a conserved role for the gene in testis differentiation in mammals and birds. *Nature Genetics* **14**: 62–68.

Nicholls RD, Saitoh S and Horsthemke B (1998) Imprinting in Prader–Willi and Angelman syndromes. *Trends in Genetics* **15**: 194–200.

Panning B and Jaenisch R (1998) RNA and the epigenetic regulation of X chromosome inactivation. *Cell* **93**: 305–308.

Schafer AJ and Goodfellow PN (1996) Sex determination in humans. *Bioessays* **18**: 955–963.

Werren JH and Beukeboom LW (1998) Sex determination, sex ratios and genetic conflict. *Annual Review of Ecology and Systematics* **29**: 233–261.

Wilkins AS (1995) Moving up the hierarchy: a hypothesis on the evolution of a genetic sex determination pathway. *Bioessays* **17**: 71–77.