APPENDIX A
CHROMOSOME FUNCTION: SEX DIFFERENCES[1]

Abstract

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

Chromosomal Mechanisms for Sex: Diversity and Plasticity

Though most animals come in two sexes, the basis for sexual development is diverse. In many genera, females and males have different sex chromosome constitutions. In mammals and fruitflies, the female has a pair of X chromosomes whereas the male is heterogametic, possessing one X and one Y chromosome per cell. In birds, some reptiles and butterflies, the heterogametic sex is female (ZW; males are ZZ). *Caenorhabditis elegans* has only one kind of sex chromosome: XX nematodes are female; XO are male. Sex-defining chromosomes are not universal: some reptiles and fish use environmental cues like temperature to determine the animal's sex.

When sex chromosome constitution determines sexual phenotype, the presence or number of particular chromosomes usually causes the sex-specific production of a protein that determines sex. This protein heads a cascade of gene activities that causes at least some cells to develop as female or male. These pathways and genes that govern sex determination often evolve rapidly. However a few elements, such as the DM (*d*oublesex and *m*ab-3-domain) transcription factors, can be conserved in diverse taxa. We describe here three well-studied systems that illustrate the range of sex-

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determining mechanisms. Note that sexual phenotype includes both the sex-specific development of somatic structures and the differentiation of germ cells into eggs or sperm. Sex-specific regulation of somatic and germline processes are interdependent but not identical.

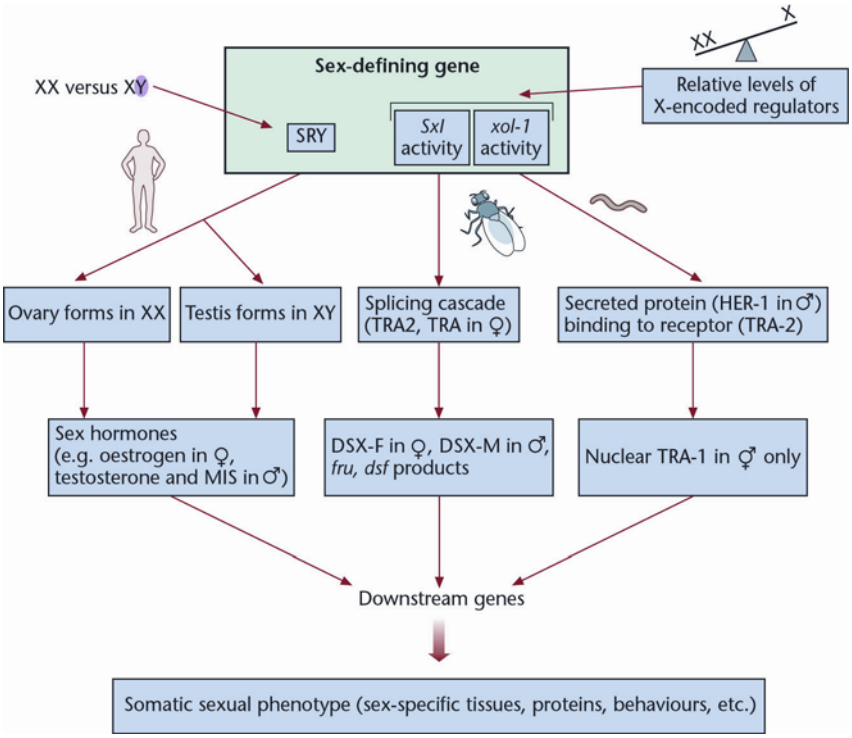
In eutherian mammals, a Y-linked gene determines maleness

Sexual phenotype in eutherian mammals (Figure A.1) often reflects the presence or absence of a Y chromosome. Mammals develop male features if their cells contain a Y chromosome, regardless of the number of X chromosomes; mammals with one X and one Y per cell develop into normal fertile males. The Y chromosome is male-determining because it carries a crucial gene called *Sry* (*S*ex-determining *R*egion on the *Y: SRY* in humans and *Sry* in mice) that determines maleness. *SRY* was identified by mapping the deletions on abnormal Y chromosomes in rare XY women and rare *SRY* translocations in XX men. Its male-determining role was confirmed by the discovery of XY women carrying inactivating point mutations in *SRY*, and by the male development of XX mice carrying a mouse *Sry* transgene (Koopman et al., 1991). Interestingly *Sry* homologues are diverging quickly enough that a human *SRY* transgene does not masculinize XX mice.

Two models have been suggested for *Sry*'s action. *Sry* is a member of the high-mobility group (HMG) family of chromatin-binding proteins. Given its ability to bind DNA, *Sry* might modify chromatin structure to regulate transcription of downstream genes in a sex-specific manner, or to induce an intermediary transcriptional regulator. *Sox9* (*S*ry-like HMG b*ox* 9) is likely to be such an intermediary protein, since its overexpression is necessary and sufficient to cause sex reversal of XX mice. A second model for *Sry* action proposes that it regulates sex-specific alternative splicing. Supporting this idea, *Sry* can bind RNA, rescue the

Figure A.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.


XX versus XY
Sex-defining gene
SRY
Sxl activity
xol-1 activity
XX
X
Relative levels of X-encoded regulators
Ovary forms in XX
Testis forms in XY
Splicing cascade (TRA2, TRA in ♀)
Secreted protein (HER-1 in ♂) binding to receptor (TRA-2)
Sex hormones (e.g. oestrogen in ♀, testosterone and MIS in ♂)
DSX-F in ♀, DSX-M in ♂, fru, dsf products
Nuclear TRA-1 in ♂ only
Downstream genes
Somatic sexual phenotype (sex-specific tissues, proteins, behaviours, etc.)

splicing activity of *Sox6 in vitro*, and localize to splicing factor compartments in the nucleus.

During mouse development, the male gonad undergoes two transformations that depend on *Sry*. First, there is an increase in gonadal cell proliferation, so early male gonads are larger than early female gonads. If this early male gonad cell proliferation is inhibited, males do not form testes and male-specific genes are repressed (Schmahl and Capel, 2003). Second, mesonephric cells migrate into the male gonad. *Sry* probably induces a signal, such as *Fgf9* (*F*ibroblast *g*rowth *f*actor-*9*), that stimulates this migration. If mesonephric cells do not migrate into the gonad, then Sertoli cells (see below) will not differentiate, a testis will not form and thus male development will not occur.

Sry is not the sole determinant of sex: occasional XY animals have been found with normal *Sry* expression, but overt female development. This allowed the identification of other genes necessary for testis, and hence male development. They include: *Wt1* (*W*ilms' *t*umour associated gene *1*), *Sf1* (*S*teroidogenic *f*actor *1*), *Dmrt-1* (*d*oublesex *m*ab-3 *r*elated *t*ranscription factor 1), *Sox9*, and *Dax1* (*D*osage sensitive sex reversal-*a*drenal hypoplasia congenital-critical region of the *X* chromosome, gene *1*) (Morrish and Sinclair, 2002). WT1 and SF1 direct an early non-sex-specific step in gonadal development. They also function later in male development by forming heterodimers with *Sox9* to activate downstream target genes. *Dmrt-1* knockouts in XY mice cause testis defects, but *Dmrt-1's* exact function in male sexual development remains unclear. *Dax1*, a nuclear hormone receptor, is also required for testis development. However, overexpression of *Dax1* can, paradoxically, lead to male to female sex reversal. DAX1 is probably an antagonist of *Sry*, because both genes are expressed in the same tissues and because *Dax1* null mice show reduced expression of *Sox9*, a gene normally activated by *Sry* (Swain et al., 1998). Antagonistic action

between these two genes may 'tip the balance' in favour of testis development by the gonad; the testis produces hormones that result in male development. If the balance tips the other way, an ovary develops, and produces the hormone oestrogen, resulting in female development.

These pathways are important for somatic cell development in the gonad. Somatic Sertoli and Leydig cells, which develop in the testis, produce the hormones that direct male development. Leydig cells produce testosterone which causes male differentiation; in the absence of testosterone signaling, XY mammals develop as females. Sertoli cells produce MIS (*M*ullerian *i*nhibiting *s*ubstance), a TGF-β family member, that causes degeneration of the ducts that would otherwise make female internal reproductive structures.

The ratio of X chromosomes to autosomes determines sex in fruitflies

The sex of *Drosophila melanogaster* is not controlled by the presence or absence of a Y chromosome, but instead depends on the number of X chromosomes relative to the ploidy of its cells (Figure A.1). Two X chromosomes in a diploid cell (X:autosome (X:A) ratio of 1) triggers female development; one X in a diploid cell (X:A = 0.5) specifies maleness. The sexual phenotypes of flies with unusual sex chromosome complements show 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; thus sex is determined on a cell-by-cell basis rather than by a circulating hormone, although secreted factors regulate the sexual development of some parts of the genital disk (Christiansen et al., 2002).

The X:A ratio of a fly embryo cell determines whether it will produce the female-specifying protein SXL (*Sex-l*ethal). Some X-encoded transcription factors are needed to activate the *Sxl* gene. These proteins form complexes with autosomally-

encoded or maternally-derived factors to regulate *Sxl*'s sex-specific promoter. XX embryo cells have two doses of each X-linked transcription factor subunit gene; these produce sufficient protein to activate *Sxl* transcription (Schutt and Nothiger, 2000). In contrast, XO or XY cells, with only one copy of X-linked genes, produce insufficient amounts of the complex needed to activate *Sxl* transcription. Thus, SXL is produced in female but not in male embryos.

After the initial female-specific burst of *Sxl* transcription, *Sxl* transcription becomes driven by a sex-independent promoter. Transcripts from this later promoter must be properly spliced to encode SXL protein. This splicing requires SXL protein (an RNA binding protein). Since only female cells produced SXL protein, they are the only cells that can productively splice the later *Sxl* transcripts. The expression of SXL is therefore set during the first 2 hours of embryonic development and fixes a cell's sexual identity thereafter.

SXL works through a cascade of other genes. In fly somatic cells, SXL causes productive splicing of the *tra* (*tra*nsformer of sexual phenotype) gene's primary transcript. The TRA protein, which is a splicing factor subunit, is thus produced only in females. TRA, in concert with TRA2 protein, catalyzes alternative splicing of genes that regulate sexual characteristics. The best characterized of these downstream genes is *dsx* (*d*ouble-se*x*), a DM-domain transcription factor. Females and males produce different versions of the DSX DNA-binding protein (DSX-F and DSX-M, respectively) owing to alternative last-exon use. To produce DSX-F, TRA-TRA2 must activate a cryptic splice site in the *dsx* transcript; males, lacking TRA, instead perform an alternative 'default' splice that produces DSX-M.

DSX-M, or DSX-F in concert with accessory factors such as *intersex*, binds to the regulatory regions of genes encoding sex-specific RNAs (Christiansen et al., 2002). DSX-F and DSX-M activate transcription of sex-appropriate RNAs, and

interfere with transcription of the sex-inappropriate RNAs. Some of these targets encode the proteins that characterize a male or female fly, such as yolk proteins in females. Other targets of *dsx* include major developmental pathways that must be integrated with sexual development (Christiansen et al., 2002).

As noted above, *dsx* is not the only gene that transduces the sex-determination signal set by SXL and transduced by TRA–TRA2. In some cells within or near the nervous system, at least two other genes, the nuclear receptor-encoding *dsf* (*d*issatis*f*action) gene and the transcription factor *fru* (*fru*itless), are regulated by TRA-TRA2 to control sexual development (Christiansen et al., 2002).

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 spermatogenesis, they also need a Y chromosome, which contains male fertility genes). 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 and eggs later. Diploid male worms are XO; they can arise as the result of X-chromosome meiotic nondisjunction. The number of X chromosomes sets the sex-specific transcription of the sex-specifying gene, *xol-1* (*XO l*ethal-1) (Figure A.1). *xol-1* is normally expressed only in males, apparently as the result of a balance between products of the X-linked genes *fox-1* (*f*eminizing *o*n *X, 1*) and *sex-1* (signal *e*lement on *X, 1*) against unknown autosomal factors. An excess of *fox-1* and *sex-1* (as in XX

hermaphrodites) represses *xol-1's* expression, while in XO males, *xol-1* remains expressed. *xol-1* negatively regulates the expression of *sdc1*, *sdc2* and *sdc3* (*s*ex determination and *d*osage *c*ompensation), which encode factors that transduce the sex-specifying signal to three downstream genetic cascades. These carry out sexual development in the soma, and germline, and also mediate dosage compensation.

In somatic cells, the *xol-1* signal indirectly affects the activity of the transcription factor TRA-1. TRA-1 turns on genes required for hermaphrodite development. Although TRA-1 is present in both XX and XO animals, in the latter it is sequestered by a complex of cytoplasmic proteins and thus cannot access its targets. This sequestration requires the male-specific HER-1 (*her*maphrodite-1) protein. HER-1 binds to the transmembrane protein TRA-2 (note: unrelated to fruitfly TRA2), which releases the cytoplasmic complex. The complex then sequesters TRA-1 and prevents it from activating hermaphrodite genes. The TRA-1 protein acts through additional regulatory molecules. For example, TRA-1 suppresses the gene that encodes MAB-3 a DM domain transcription factor in the DSX and DMRT-1 family. MAB-3 specifies aspects of male development such as sensory sex organ differentiation (Goodwin and Ellis, 2002). Additional twists to the sex determination pathway occur within the germline of hermaphrodites, which must switch from spermatogenesis to oogenesis.

Sex determination pathway conservation and diversification

Vast differences exist in the sex chromosome make-up and sex determination pathways between species. This diversity is far greater than that seen in other developmental pathways, such as those that specify body 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 evolve rapidly.

One of the few conserved sex-determining genes is found at the bottom of the regulatory cascades: the *Drosophila dsx* gene is related to a nematode sex-determination gene (*mab-3*) and to the human testis-development expressed gene, *Dmrt-1* (Raymond et al., 1998). In alligators, turtles, and chickens, *Dmrt-1* shows male-specific expression in the genital ridge, suggesting that the role of DM-domain proteins in sex determination are conserved in most vertebrates. Additionally *DMY*, a gene duplicate of *Dmrt-1*, occurs within the male-determining region of the medaka fish's Y chromosome; mutation of *DMY* causes sex reversal (Matsuda et al., 2002). Taken together, the data suggest conservation of a final downstream regulator of sexual development, but plasticity in the choice of upstream control pathways and the targets of those downstream regulators. Interestingly, several upstream control genes in fruitfly sex-determining cascades also regulate other developmental phenomena (e.g. nervous system development), suggesting that regulators were co-opted from or to sex-determination cascades.

The diversity in sex-determining mechanisms is not all due to large-scale replacement of upstream regulatory cascades: smaller changes can easily alter 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 non-functional and constitutive alleles of *tra-1* to determine maleness and femaleness, respectively. Similarly, a temperature-sensitive mutation in *her-1* can convert the nematode's chromosome-based mechanism to a temperature-based one, analogous to those in some reptiles.

The Gene Dosage Problem

Since most sex-determining mechanisms rely on the presence or absence of a special sex chromosome, or on a special number of sex chromosomes, males and

females thus have different numbers of copies of the genes on sex chromosomes. For individuals to be viable, the products of many genes on sex chromosomes must be found at a particular level relative to those of autosomal genes. Thus, males and females must equalize their amounts of many sex chromosome gene products, despite the sex-based differences in gene copy number. Some formal molecular parallels have become apparent between the mechanisms that regulate this "dosage compensation" in mammals, flies and worms.

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 per 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 homologues of those genes on the Y chromosome's pseudoautosomal region. Thus, those genes 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 cytologically by the presence of a heterochromatic 'Barr body'; there is one fewer Barr body per cell than the number of Xs.

The choice of which X to inactivate is made cell-by-cell early in development. The decision depends on the production of two noncoding RNAs, *Xist* and its antisense counterpart *Tsix*, encoded by the *X-inactivation centre* (*XIC*) of the X chromosome (Avner and Heard, 2001). In a female embryonic cell, initially both X chromosomes produce *Xist* and *Tsix*. *Tsix* transcription interferes with *Xist* accumulation; thus the chromosome that expresses less *Tsix* begins to accumulate more *Xist* RNA. *Xist* RNA coats the length of the X chromosome from which it was derived. That X chromosome becomes heterochromatic and transcriptionally inactive (due in part to DNA methylation, accumulation of macroH2A, and histone

hypoacetylation), except for its *Xist* gene and the genes in its pseudoautosomal region. The other X chromosome eventually stops producing *Xist*, and remains transcriptionally active. The choice of which X chromosome becomes inactivated is usually random. 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.

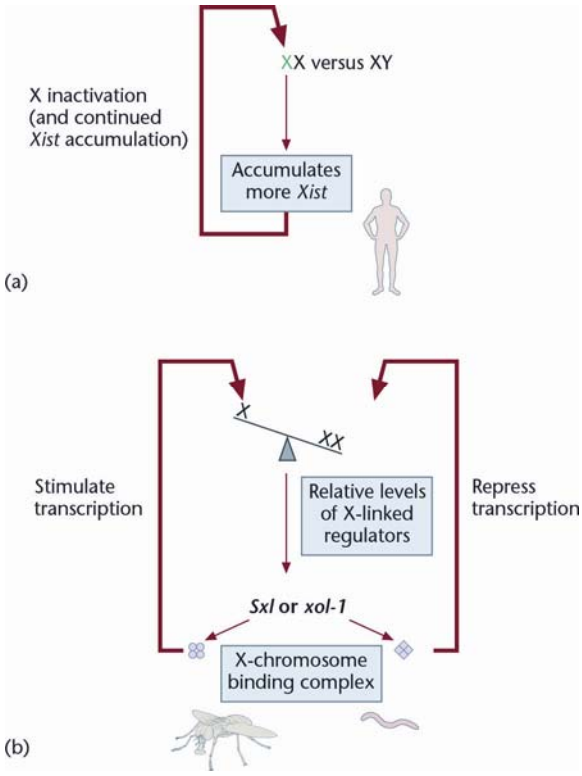
Once a cell has inactivated an X chromosome, all of that cell's descendants inactivate the same X (with the exception of germline cells, which reactivate their inactive X chromosome during gametogenesis). As a result, female mammals are somatic mosaics; some cells activate one X chromosome and other cells activate the other X. Thus, heterozygous females usually show allelic traits from both X chromosomes, as is seen in 'calico' cats, which carry two different alleles of an X-linked coat colour gene. However, in the rare event that all progenitor cells of a particular tissue have the same X chromosome inactive, a heterozygous female would display the phenotype of only one allele in that tissue. A particularly clear case of such an X inactivation bias exists in the extraembryonic tissues of rodents. There the paternal X is always inactivated; this is thought to be controlled by reciprocal imprinting (see below). *Xist* is maternally silenced and *Tsix* is paternally silenced; thus *Xist* accumulates only along the paternal X chromosome, which thus becomes inactive.

Global regulation of X transcription in fruitflies and nematodes

In *D. melanogaster*, both X chromosomes in each female cell are transcriptionally active. 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 A.2b). This elevation is mediated by a dosage compensation complex (DCC) that binds to the male's X chromosome (Amrein, 2000). Five proteins in the DCC –

Figure A.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 DCC 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 A.1, autoregulation of *Sxl* is not shown.


XX versus XY
X inactivation
(and continued
Xist accumulation)
Accumulates
more *Xist*
(a)
X
XX
Stimulate
transcription
Relative levels
of X-linked
regulators
Repress
transcription
Sxl or *xol-1*
X-chromosome
binding complex
(b)

MSL1, MSL3 (*m*ale-*s*pecific *l*ethal), the putative helicase MLE (*m*ale*le*ss), the putative histone acetyltransferase MOF (*m*ale absent *o*n *f*irst (chromosome)) and the protein kinase JIL-1 – are present in females as well as males but do not coat females' X chromosomes. A sixth protein in the DCC, MSL2, is produced only in the absence of *Sxl* function; that is, only in cells with a single X chromosome. All six proteins must be present to form a functional DCC; thus this complex is only present in males (Meller and Kuroda, 2002). The DCC alters the histone-acetylation pattern of the male's X chromosome, resulting in elevated transcriptional activity. The noncoding RNAs *roX1* and *roX2* (*R*NA *o*n the *X* chromosome-1 and 2) also coat the only male X chromosome in a SXL- and MSL2-dependent fashion, and interact with the DCC. *roX* RNAs and the DCC appear to coat the entire X chromosome of males. The *roX1* and *roX2* genes' chromosomal regions are two sites where spreading of the DCC complex initiates and proceeds in *cis* along the X, regulating neighbouring genes.

Though different in molecular detail, dosage compensation in nematodes has parallels to the *D. melanogaster* system (Figure A.2b). Here, too, a macromolecular complex dependent on the sex chromosome make-up of the cell binds to X chromosomes and regulates their transcription. However, opposite to the situation in fruitflies, the *C. elegans* complex binds to both hermaphrodite X chromosomes and downregulates their expression by half. The expression levels of X chromosome genes are thus equal in XX and XO cells. The complex in nematodes is also comprised of multiple proteins functioning in only one sex: SDC-1, SDC-2, SDC-3, MIX-1 (*mi*tosis and *X* associated protein *1*), and several DPY (*d*um*py*) proteins (Meyer, 2000). Although this complex globally regulates X expression, it also regulates expression of specific autosomal genes, in contrast to the situation in *Drosophila* and mammals. For example, in XX nematodes the dosage compensation complex represses expression of the autosomal male sex determination gene *her-1*.

Complex Patterns of Inheritance Conditional on Sex

Sex-linkage

Some patterns of inheritance conditional on sex reflect the fact that sex chromosome complements differ in the two sexes. For example, in mammals, Y-linked mutations will display phenotypes only in males, and are inherited father-to-son. Recessive X-linked syndromes such as hemophilia are seen much more often in males than in females. This is because an XX female can be normal in phenotype while carrying a recessive mutation, but half her sons will be hemizygous for the mutation and display the phenotype.

Sex-limited inheritance

Other patterns of inheritance conditional on sex reflect the fact that phenotypes can depend upon a particular sex-specific milieu, such as the presence of a particular sex hormone. 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), probably because the loss of BRCA-1 is exacerbated by the female-specific proliferation of breast tissue or the female hormonal milieu. Sex-limited inheritance also occurs in other organisms. For example, mutations of the *D. melanogaster* genes *vismay* and *achintya* show meiotic arrest phenotypes only in males, since these genes act only during spermatogenesis.

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 progeny of a heritable trait wholly dependent the 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 mitochondria whose characteristics depend on her genotype. Mitochondria contain their own genomes, so the resulting embryo will retain its mother's mitochondrial genotype. Maternal inheritance of mutant mitochondria means that progeny of affected mothers, but not progeny of affected fathers, will show the mutant trait. An example is a streptomycin-dependent deafness in humans, caused by mutation of a maternally inherited, mitochondrially-encoded ribosomal RNA (Jacobs, 2003).

So-called "maternal effects" also depend on the mothers's genotype, but in contrast to maternal inheritance these effects are not heritable beyond the affected generation. Maternal effects are due to molecules that are donated to the egg by the mother, and whose characteristics are thus dictated by the mother's genotype. Deficiency of these maternally-provided molecules can cause abnormalities in the developing embryo, but these abnormalities are not passed on to the next generation, even if 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. In mammals, passage through the female or the male germline causes 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. One example was noted earlier – the *Tsix* and *Xist* genes in extraembryonic rodent tissues are differentially imprinted by the maternal and paternal germline. Some genetic disorders also have their origins in gene

imprinting. 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.

Pre-existing imprints are usually erased and reset during passage through the 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 an imprint placed by their parent. In such a case, the imprinting that occurred in a grandparent's germline can affect the phenotype of their grandchild, as is seen for some cases of Prader-Willi syndrome.

Sex Chromosome Evolution

The two most common sex chromosome systems are (female/male) XX/XY and ZW/ZZ. The Y and W chromosomes are typically: morphologically smaller than the X or Z, comprised of a few genes which are largely involved in sex-specific processes, rich in heterochromatic regions, composed mainly of non-recombining DNA, and inherited paternally or maternally, respectively.

The exact evolutionary route leading to the divergence of the X and Y chromosomes is unclear. The X and Y are thought to have evolved from homologous autosomes (Charlesworth and Charlesworth, 2000). Early differentiation of sex chromosomes probably initiated with the acquisition of a sex-determining locus (possibly *Sry* in mammals) on one of these autosomes. Such a chromosome would function like a proto-Y, being inherited in a sex-limited manner through the male. Suppression of recombination around the male sex-determining region of the proto-Y would then be likely to arise, because expression of this gene in an XX animal could

lead to germline/soma incompatibilities, causing sterility. Since the proto-Y is found only in males, loss of function mutations could accumulate on its non-recombining region, leading to its divergence and degeneration from the X chromosome. Multiple evolutionary forces may govern this divergence and degeneration (Charlesworth and Charlesworth, 2000). In support of this scenario, the mammalian Y chromosome still retains vestiges of its membership in an original autosomal pair. These vestiges can be seen as four evolutionary strata when sequences of X- and Y-linked homologues are compared. In *Drosophila*, the story is different, because no Y-linked genes have a closest homolog on the X.

When the mammalian Y first began to diverge from the X, it is thought to have contained approximately 1500 genes. Over the past 300 million years, gene loss occurred from the proto-Y at an estimated rate of 5 genes per million years. However, a mechanism evolved that slowed or possibly stopped this decay of the Y. The human Y contains 8 large palindromic repeats within which 15 testis-specific genes are present in multiple copies (comprising 60 of the 78 protein-coding genes on the Y) (Skaletsky et al., 2003). Recombination or gene conversion between such repeats will prevent Y-chromosome genes from accumulating inactivating mutations, by replacing non-functional alleles with functional copies.

As the Y chromosome accumulates loss of function mutations, the relative concentration of X chromosomal genes in males and females will differ by a factor of two, leading to the need for a dosage compensation system. This, and possibly the presence of sexually antagonistic alleles on the X, can also lead to a difference in predominance of certain types of genes on sex chromosomes (Rice, 1984). For example, the X chromosomes of *C. elegans* and *D. melanogaster* carry fewer male-specific genes than expected. One mechanism by which male-specific genes leave the X is by retrotransposition, as is suggested for some spermatogenesis genes in

Drosophila (Parisi et al., 2003). Interestingly, though there is also a bias in gene placement in humans, it is the opposite of what is seen in flies and worms. The human X chromosome is enriched for male-specific genes and shows a shortage of female-specific genes.

Sex Chromosome Aneuploidy Syndromes in Flies and Humans

Because of dosage compensation, sex chromosome aneuploidy is generally tolerated more than autosomal aneuploidy, though some sex aneuploidies do 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 disorders of 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 lowered dosage of the small group of genes normally active on both X chromosomes in a normal female. Females with Turner syndrome are almost always sterile; this probably occurs because inactivated X chromosomes are reactivated early in oogenesis, so that oogonia of XX women have 2 active doses of genes needed for later aspects of oogenesis while XO oogonia have only one. There is significant variability in the phenotype of individuals with Turner syndrome, because most or all of these people are mosaics of XO and XX (or XY) cells. In fact, because 45, XO karyotypes comprise a large proportion of spontaneous miscarriages, fully XO humans are probably inviable. The occasional fertility of individuals with Turner syndrome might be explained by some XX cells in their germlines.

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 can result in lowered intelligence.

D. melanogaster XO animals are phenotypic males, but they are sterile because they lack the Y-linked genes essential for spermatogenesis. XXY fruitflies 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 are 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 this important distinction. These investigations have uncovered novel and important mechanisms for general gene control, and have clarified many seemingly complex patterns of inheritance.