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2 Every Cell Has a Sex

ABSTRACT

The biological differences between the sexes have long been recognized at the biochemical and cellular levels. Rapid advances in molecular biology have revealed the genetic and molecular bases of a number of sex-based differences in health and human disease, some of which are attributed to sexual genotype—XX in the female and XY in the male. Genes on the sex chromosomes can be expressed differently between males and females because of the presence of either single or double copies of the gene and because of the phenomena of different meiotic effects, X inactivation, and genetic imprinting. The inheritance of either a male or a female genotype is further influenced by the source (maternal or paternal) of the X chromosome. The relative roles of the sex chromosome genes and their expression explains X-chromosome-linked disease and is likely to illuminate the reasons for heterogeneous expression of some diseases within and between the sexes.

The notion that there are biological differences between the sexes is most evident and comfortable when it is applied to the reproductive system. However, sex differences have been identified or suggested at many levels of biological organization, from biochemical to behavioral. For the majority of the population, as well as a substantial fraction of scientists, not all known differences are obvious, and not all of those that have been suggested or suspected are easily explainable in biological terms.

In terms of genetic mechanisms, two general models attempt to explain how an individual's genes give rise to sex differences (Figure 2–1). In the first model, a series of critical hormone-responsive genes, shared by both males and females, are influenced differently in the alternative hormonal milieus of the male or female throughout their life spans, thus leading to or contributing to the many differences observed between the sexes. In the second model (which is not necessarily exclusive of the first one), one or more genes, located on the sex chromosomes and thus expressed differently in the two sexes, encode proteins involved in ratelimiting or rate-influencing steps in biochemical or physiological pathways that are critical to establishing differences between the sexes.

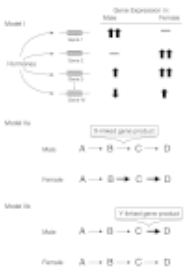

FIGURE 2–1

Schematic representation of two general models used to explain sex differences in gene expression. In Model I, hormones in males and females differentially influence the level of expression of different genes (Gene 1 to Gene *N*) in the genome. Arrows (more...)

The purpose of this chapter is twofold: (1) to describe those differences that exist between males and females at the biochemical and cellular levels and that result directly from the defining genotypic difference between male and female mammals, namely, an XY (male) sex chromosome constitution versus an XX (female) sex chromosome constitution, and (2) to

describe how males and females may transmit to their offspring genetic information that is the same but that is transmitted at different observed phenotypic or genotypic ratios. This information will then serve as a foundation for consideration of the onset of sex differences during development and throughout life in response to both intrinsic and extrinsic exposures.

SEX AND THE HUMAN GENOME

Males and females have partially different genomes. Viewed from a purely reductionist standpoint, many differences between the male and female sexes are predicted to be rooted in differences between the genetic contents of male and female cells and differences in the expression of those genetic contents. As the complete DNA sequence of the human genome has now been determined, it is important to place the discussions of this chapter into the context of the human genome.

The human genome contains, by current measurements, a little more than 3 billion base pairs of DNA (Lander, 1996; National Human Genome Research Institute, 2000). Earlier estimates predicted an estimated 50,000 to 100,000 different genes (National Human Genome Research Institute, 2000). The most recent estimates, based on the current drafts of the human genome sequence, suggest that there are approximately 30,000 human genes (International Human Genome Sequencing Consortium, 2001; Venter et al., 2001). However, this lower figure may be a minimum estimate because it is derived using an algorithm that identifies genes on the basis of their similarity to a modest sized panel of already characterized human genes.

The hallmark of human biology is variation, and much of the observed variation both within and between the sexes is encoded within the human genome. At the DNA level, an estimated 1 of every 1,300 bases on the autosomes (non-sex-determining chromosomes) differs between any two individuals (International SNP Map Working Group, 2001; Nickerson et al., 1998; Venter et al., 2001). In other words, the genomes of individuals may differ at some 4 to 6 million base positions. Some of these differences will lead to gene products that are functionally distinct, for example, receptors that differ in their affinity or rate of turnover, enzymes that differ in their steady-state levels, and genes that differ in their degree of hormone responsiveness. Although ongoing studies of human DNA variation will soon provide a more robust estimate, one can calculate from previous studies of enzyme variation and more recent investigations of gene variation (Zwick et al., 2000) that the precise composition and functioning of thousands of proteins will differ between any two individuals.

Notwithstanding this degree of population-level variation in the DNA sequence, most of the genes in the genome are thought to not differ in either sequence or level of expression as a simple consequence of the sex of the individual. However, as will be illustrated more fully in the following sections, there are three types of genes (see also Box 2–1) in which an individual's sex per se is likely to play a role.

BOX 2–1

Genetic Factors That May Differentially Affect the Basic Biochemistry of Male and Female Cells.

First, genes on the Y chromosome are expressed only in males, and many of these have no counterpart on the X chromosome or autosomes; thus, expression of these genes will be limited to males.

Second, some genes on the X chromosome are expressed at higher levels in females than in males. Although the process of X-chromosome inactivation equalizes the effective dosage of most X-chromosome genes between male and female cells by inactivating one of the two X chromosomes in female cells, not all genes on the inactivated X chromosome respond to this mechanism. The relatively few genes that are not equalized can have significant effects on the phenotypes of cells.

Third, the expression of many genes is likely to be influenced by hormonal differences between the two sexes. For example, some of these may be genes whose expression is limited to sexually dimorphic tissues or cell types (e.g., the ovary, testis, prostate, and breast), whereas others may be globally expressed but subject to hormonal regulation in different tissues or at different times during development (see Chapter 3).

Although only a limited number of genes have been examined to date, from the standpoint of sexual dimorphism, new approaches to quantification of the expression of genes in different samples on a genomewide basis promise to change this. DNA arrays, or "gene chips," containing tens of thousands of human genes can be queried to compare their levels of expression between different tissues or different sexes under a variety of physiological or hormonal conditions (Lander, 1996; Lockhart and Winzeler, 2000). Such studies will yield a large database of gene expression data. More difficult will be determination of the relative effects of differences in gene expression on the characteristic phenotypic differences seen between males and females. Nonetheless, this new technology with DNA arrays promises to provide a comprehensive functional view of the genome in different cellular states, and studies that address differences in expression throughout the male and female genomes should reap a rich harvest.

BASIC MOLECULAR GENETICS: WHAT IS THE POTENTIAL FOR DIFFERENCES BETWEEN THE SEXES?

The issue of whether there should be genetic differences in basic cellular biochemistry between female and male cells (as a direct result of sex chromosome constitution rather than hormonal influences) (see Figure 2– 1 and Box 2–1) is often approached from two opposing perspectives. Geneticist Jacques Monod's famous adage that "What's true of *Escherichia coli* is true of an elephant" represents the point of view that genes have been conserved over time and among species. This view has had extraordinary staying power in molecular biology and genetics, and if "yeast" was substituted for *"E.coli,"* the statement would have even greater vitality. If the basic biochemistries of organisms separated by a billion years of evolution are so similar, then (so goes the logic) why should one expect that males and females within the *same* species should exhibit important differences in their basic biochemistries? An opposing perspective acknowledges that the majority of human disease-causing mutations exhibit dominant or semidominant effects (McKusick, 2000). Thus, a change in the activity of a single gene can have a large effect on the organism that carries that gene. Because the sex chromosomes comprise approximately 5 percent of the total human genome (Figure 2–2), there is the potential for 1 in 20 biochemical reactions to be differentially affected in male versus female cells. From this standpoint, it is difficult to imagine that male and female cells will not differ in at least some aspects of basic biochemistry, given the complexity of most biological pathways.

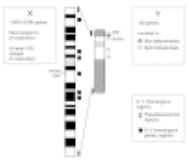

FIGURE 2–2

Comparison of gene contents and gene organizations on the X and Y chromosomes (see text for details).

Males Have a Y Chromosome, Females Do Not

The male genome differs from the female genome in the number of X chromosomes that it contains, as well as by the presence of a Y chromosome. It is the overriding presence of a gene on the Y chromosome *(SRY)* that results in development of the male gonadal phenotype. However, apart from causing the dramatic divergence from the female developmental pathway (which the indeterminate gonad would otherwise follow and which has been discussed in a number of reviews [Hiort and Holterhus, 2000, Sinclair, 1998; Vilain and McCabe, 1998]), it was long considered a valid biological question to ask whether the Y chromosome carried any genes of "importance." The paucity and nature of traits that were thought, by genetic criteria, to segregate with the Y chromosome ("hairy ears," for example [Dronamraju, 1964]) tended to reinforce the notion that the Y chromosome encoded the male gonadal phenotype (Koopman et al., 1991), one or more genes involved in male fertility (Lahn and Page, 1997), the HY male transplantation antigen (Wachtel et al., 1974), and not much else. Surprisingly, recent studies show that the Y chromosome carries some genes that are involved in basic cellular functions and that are expressed in many tissues (Lahn and Page, 1997).

Cytologically, the Y chromosome consists of two genetically distinct parts (Figure 2–2). The most distal portion of the Y-chromosome short arm (Yp) is shared with the most distal portion of the X-chromosome short arm (Xp) and normally recombines with its X-chromosome counterpart during meiosis in males. This region is called the "pseudoautosomal region" because loci in this region undergo pairing and exchange between the two sex chromosomes during spermatogenesis, just as genes on autosomes exchange between homologues. There is also a second pseudoautosomal region involving sequences on the distal long arms of the sex chromosomes (Watson et al., 1992) (Figure 2–2). The remainder of the Y chromosome (the Y-chromosome-specific portion) does not recombine with the X chromosome and strictly comprises "Y-chromosome-linked DNA" (although some of the nonrecombining part of the Y chromosome retains residual homology to X-chromosome-linked genes, reflecting the shared evolutionary history of the two sex chromosomes [see below]). The pseudoautosomal region(s) reflects the role of the Y chromosome as an essential pairing homologue of the X chromosome during meiosis in males (Rappold, 1993), whereas the Y-chromosome-specific region, including the testis-determining factor gene, *SRY,* provides the chromosomal basis of sex determination.

The Y chromosome is one of the smallest human chromosomes, with an estimated average size of 60 million base pairs, which is less than half the size of the X chromosome. Cytologically, much of the long arm (Yq) is heterochromatic and variable in size within populations, consisting largely of several families of repetitive DNA sequences that have no obvious function. A significant proportion of the Y-chromosome-specific sequences on both Yp and Yq are, in fact, homologous (but not identical) to sequences on the X chromosome. These sequences, although homologous, should not be confused with the pseudoautosomal regions. Pseudoautosomal sequences may be identical on the X and Y chromosomes, reflecting their frequent meiotic exchange, whereas the sequences on Yp and Yq homologous with the Y and X chromosomes are more distantly related to each other, reflecting their divergence from a common ancestral chromosome (Lahn and Page, 1999).

Only about two dozen different genes are encoded on the Y chromosome (although some are present in multiple copies). Unlike collections of genes that are located on the autosomes and the X chromosome and that reflect a broad sampling of different functions without any obvious chromosomal coherence, Y-chromosome-linked genes demonstrate functional clustering and can be categorized into only two distinct classes (Lahn and Page, 1997). One class consists of genes

that are homologous to X-chromosome-linked genes and that are, for the most part, expressed ubiquitously in different tissues. Some of these genes are involved in basic cellular functions, thus providing a basis for functional differences between male and female cells. For example, the ribosomal protein S4 genes on the X and Y chromosomes encode slightly different protein isoforms (Watanabe et al., 1993); thus, ribosomes in male cells will differ characteristically from ribosomes in female cells, setting up the potential for widespread biochemical differences between the sexes. The second class of Y-chromosome-linked genes consists of Y-chromosome-specific genes that are expressed specifically in the testis and that may be involved in spermatogenesis (Figure 2–2). Deletion or mutation of some of these genes has been implicated in cases of male infertility, but otherwise, these genes have no obvious phenotypic effects (Kent-First et al., 1999; McDonough, 1998).

Females Have Two X Chromosomes, Males Have One

Male and female genomes also differ in the other sex chromosome, the X chromosome, in that females have twice the dose of X-chromosomelinked genes that males have. The X chromosome consists of approximately 160 million base pairs of DNA (about 5 percent of the total haploid genome) and encodes an estimated 1,000 to 2,000 genes (Figure 2–2). By the nature of X-chromosome-linked patterns of inheritance, females can be either homozygous or heterozygous for X-chromosome-linked traits, whereas males, because they have only a single X chromosome, are hemizygous. Of those X-chromosome-linked genes known to date, most are X chromosome specific; only pseudoautosomal genes and a few genes that map outside of the pseudoautosomal region have been demonstrated to have functionally equivalent Y-chromosome homologues (Willard, 2000).

Products of X-chromosome-linked genes, like those on the autosomes, are involved in virtually all aspects of cellular function, intermediary metabolism, development, and growth control. Although many are responsible for general cellular functions and are expressed widely in different tissues, others are specific to particular tissues or particular time points during development, and several are known to be responsible for steps in gonadal differentiation (Pinsky et al., 1999).

X-Chromosome Inactivation Compensates for Differences in Gene Dosage

The twofold difference between males and females in the dosage of genes on the X chromosome is negated at many loci by the process of X-chromosome inactivation (Figure 2–3). X-chromosome inactivation is, on a cytological level, a large-scale process in which one of the two X chromosomes becomes heterochromatic. The end result of this process can be seen under the microscope as the Barr chromatin body in the nucleus of the female cells. X-chromosome inactivation is associated with extensive silencing of genes on the affected X chromosome and occurs in almost every cell of XX females but does not occur in XY males. The one documented exception to this rule occurs, reciprocally, in reproductive cells; the single X chromosome of males becomes heterochromatic in spermatocytes, whereas both X chromosomes are thought to be active in primary oocytes. This unusual characteristic in which both X chromosomes are active in a single cell also occurs very early in the development of female embryos. Because the process of X-chromosome inactivation is not completed until near the time of implantation (reviewed by Willard [2000]), there is a preimplantation developmental window during which there may be basic differences in cellular chemistry between female and male embryos. It is unknown whether the differences in gene expression that have been shown to occur (Gutierrez-

Adan et al., 2000; Latham et al., 2000) or that may occur during this period influence the establishment of additional differences between the sexes during the postimplantation or postnatal periods.

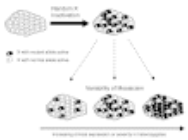

FIGURE 2–3

Schematic representation of X-chromosome inactivation in female somatic cells. Inactivation early in development is believed to be random, with an equal probability a priori that the maternal or paternal X chromosome will be active or inactive. Females (more...)

In any case, the simple fact of X-chromosome inactivation leads to two levels of difference between males and females. The first is that XX cells must operate whatever cellular machinery is required to initiate and establish the inactivation of an X chromosome in all mitotically active cells and also (perhaps) to actively maintain the inactive state of one X chromosome in terminally differentiated cells first. The second level of difference is superimposed on the first and is a property of populations of XX cells: females, by virtue of not inactivating the same X chromosome in every cell, are “epigenetic mosaics.”

X-Chromosome-Based Differences Between Cells

There has been substantial recent progress in understanding the biochemistry and molecular biology of the X-chromosome inactivation process. These advances have been described in detail in several recent reviews (Heard et al., 1997; Willard, 2000), but the overall conclusion relevant to this report is that genes involved in the initiation, establishment, or maintenance of the X-chromosome inactivation process are or have been expressed in every somatic cell of females. Although some of the genes in the X-chromosome inactivation pathway may be expressed at some level or at some time in males (Daniels et al., 1997; Ray et al., 1997), the overall process that results in the cytologically visible heterochromatization of an entire chromosome is a fundamentally “female” characteristic, whether considered in vivo or in vitro. Here, then, is a basic biochemical process that is a fundamental consequence of having two X chromosomes. The biochemical results of the process can be measured and quantified in the tissues of individual females or in cells in culture dishes. The process affects genes that are involved in many important metabolic processes as well as genes that are known to be important in the regulation of expression of other genes (Amir et al., 1999; Melcher et al., 2000).

Because there is a stochastic or random component in the choice of which of the two X chromosomes is inactivated (Puck and Willard, 1998), individual females have two epigenetically distinct populations of cells: those in which the maternally derived X chromosome remains active and those in which the paternally derived X chromosome remains active (Figure 2–3). By contrast, males have only an active maternally derived X chromosome in all of their cells.

This X-chromosome-based, female-specific mosaicism is often invoked as the reason for much of the dramatic sex differences observed in the severities of recessive X-chromosome-linked disease phenotypes (McKusick, 2000). All cells of XY males must suffer the consequences of a mutation in an X-chromosome-linked gene, but only that fraction of a female's cells that carry the mutation on the active X chromosome will be affected. Such situations have resulted, in some cases, in strong somatic selection against cells that bear the mutation on the active X

chromosome and thus avoidance (or minimization) of the disease phenotype (Belmont, 1996; Willard, 2000).

It should be noted that the stochastic nature of the initial choice of which X chromosome to inactivate can be influenced by many factors. Environmental, epigenetic, and genetic factors have all been demonstrated to influence the X-chromosome inactivation pattern (the proportion of a female's cells with a designated active X chromosome) of individual females (Puck and Willard, 1998). The relative importance of each may be different in different individuals, to the extent that all sisters within an individual family may show nearly identical patterns of X-chromosome inactivation, whereas identical twins in other families may exhibit wide variations in the proportions of their cells that have one or the other active X chromosome.

Not all of the genes on the X chromosome respond to the inactivation process by transcriptional silencing (Willard, 2000). This fact may lead secondarily to biochemical differences between XX cells and XY cells. As many as 10 to 15 percent of X-chromosome-linked genes have been identified as being expressed from the inactive X chromosome, at least in cells in culture, and are therefore said to "escape" X-chromosome inactivation (Carrel et al., 1999). Some of these are transcribed from both the active and the inactive X chromosome at similar levels, whereas others appear to be transcribed from the inactive X chromosome at reduced, but still significant, levels (Carrel and Willard, 1999; Fisher et al., 1990). Regardless of the level at which such genes escape X-chromosome inactivation, it is likely that XX cells will produce higher levels of gene product from some of these loci than XY cells will. It has been suggested that some of these differences may lead to sex-specific levels of risk for certain diseases, such as the suspected relationship between gastrin-releasing peptide receptor and smoking-related lung cancer (Shriver et al., 2000). Gastrin-releasing peptide is expressed by both the active and the inactive X chromosomes, and elevated levels of gastrin-releasing peptide are hypothesized to be associated with an elevated risk of lung cancer in women who smoke.

An interesting genomic consideration resulting from the study of genes that escape X-chromosome inactivation is that their distribution along the X chromosome is not random. A higher proportion of the genes on the short arm of the X chromosome than on the long arm of the X chromosome escape X-chromosome inactivation (Carrel et al., 1999). This issue may reflect the different evolutionary histories of the X-chromosome arms (Lahn and Page, 1999) but may also be related to whether particular X-chromosome-linked genes have homologues on the Y chromosome (Jegalian and Page, 1998). It is of some interest that the particular genes that escape inactivation appear to differ among different females (Carrel and Willard, 1999), thus providing additional avenues for differences between and within the sexes. It is unknown whether there are significant population variations in patterns of inactivation and thus X-chromosome-linked gene expression.

The X-Chromosome Dosage Matters

In general, the possible effect(s) of any variant in an X-chromosome-linked gene may differ between the sexes for a variety of reasons, as outlined below.

- **Gene dosage.** For genes that are specific to the X chromosome and that escape X-chromosome inactivation, female cells (with two X chromosomes) may contain higher levels of the gene product than male cells, which have only a single X chromosome. Depending on the cellular role of the particular gene product, this dosage difference may have more pervasive effects on the expression of other genes in the genome. For example,

a twofold change in the level of an X-chromosome-linked transcription factor might lead to dramatic effects on the levels of genes regulated by that transcription factor.

- **Mosaicism.** For genes that are subject to X-chromosome inactivation, most females are mosaics of two cell populations, one expressing alleles on the paternally inherited X chromosome and one expressing alleles on the maternally inherited X chromosome (see below). Thus, expression of an X-chromosome-linked phenotype is often much more variable in females than in males.
- **Hemizygosity.** Because males have only a single X chromosome, functional variants cannot be "masked" by a second X chromosome. Thus, males often demonstrate a clearer, more common, or more extreme version of the variant phenotype than females do.
- **X-chromosome-linked dominant traits.** A dramatic example of the effect of male hemizygosity for X-chromosome-linked traits involves X-chromosome-linked dominant mutations that are lethal in males in utero and that are therefore evident only in females. For example, X-chromosome-linked incontinentia pigmenti is a relatively benign dermatological condition in females, but it is lethal in males who inherit a mutant allele (Smahi et al., 2000).

Differences Between Male and Female Cells That Have Not Been Linked to Sex Chromosomes

The incidence of a number of diseases whose etiologies cannot be traced to the sex chromosomes differ dramatically between males and females (McKusick, 2000). Although the basis for these differences in incidence is most often ascribed to hormonal influences, the possibility that other genetic differences are at fault cannot be discounted.

EFFECTS OF PARENTAL IMPRINTING ON THE EXPRESSION OF GENETIC INFORMATION

The discovery that some genes are expressed only from the maternal allele and that others are expressed only from the paternal allele, a phenomenon called "genomic imprinting" (reviewed by Tilghman [1999]), reinforces the concept that there are multiple biochemical differences between the gametogenic cells of males and females and that these differences may affect the expression of genetic information in the next generation.

Because autosomes are transmitted equally to both sexes, it is not predicted that inheritance of imprinted genetic information on the autosomes should have a differential effect on male versus female offspring. The situation is different for sex chromosomes. Imprinting-related differences between the sexes do exist for the X chromosome. Males have only a maternal X chromosome, but females have both a maternal X chromosome and a paternal X chromosome; therefore, X-chromosome-linked genes that pass through the paternal germ line have the potential to affect the phenotype of female offspring but not that of male offspring. In this regard, there is direct evidence that the imprinting process affects the expression of alleles in females at the *Xist* locus (a gene that is critical to the process of X-chromosome inactivation and that is expressed primarily from the paternal allele in some extraembryonic cells) in females (reviewed by Lyon [1999]).

There is also indirect evidence that imprinting affects the expression of a locus on Xp that has female-specific effects on cognitive and behavioral phenotypes. The latter evidence is derived from studies of patients with Turner syndrome, who have inherited only one X chromosome

(XO) from either the mother or the father (Skuse et al., 1997). These findings may have broader implications for cognitive function or behavior in males and females because males inherit only a maternal X chromosome, whereas females inherit both a maternal X chromosome and a paternal X chromosome.

UNEXPECTED OR NONOBVIOUS SEX DIFFERENCES

Sex-Specific Meiotic Effects

Although the basic mechanism of meiosis, the creation of haploid gametes from diploid precursors, is universal, there are both quantitative and qualitative differences between males and females in the production of gametes. These differences have characteristic effects on the ways that males and females drive the evolutionary process, as well as the mechanisms by which diseases that result from genetic defects are manifest.

The three most important differences between males and females in the gametogenic process are as follows: (1) the number of stem cell divisions that occur to give rise to the germ cell population, (2) the timing of the first and second meiotic divisions, and (3) the number of gametes produced from each primary germ cell.

The male produces billions of sperm from a population of stem cells that continue to divide throughout the entire adult life. In contrast, the female produces a relatively small number of ova (~500) from a limited population of oocytes that arise early in embryogenesis. These oocytes are arrested at the meiotic prophase from fetal life until ovulation, which may occur as many as 50 years after the initiation of meiosis. This simple numerical difference in the number of stem cell divisions between the two sexes dictates that most mutations resulting from errors in DNA replication take place in the male germ line (Haldane, 1935), although the magnitude of this difference and whether additional factors may contribute are subjects of debate (Hurst and Ellegren, 1998). On the other hand, the protracted length of time that an individual ovum may be arrested at the meiotic prophase is correlated with the fact that aneuploidy (gain or loss of one or more chromosomes) resulting from nondisjunction (improper separation of chromosomes at nuclear division) occurs much more frequently through the female germ line than through the male germ line (Hassold et al., 2000).

Although all four products of meiosis in the male have the potential to become functional sperm, each primary oocyte gives rise to only a single ovum. Additional differences in the meiotic process are found in the observed rate of recombination and the consequent length of the human genetic map obtained by measurement with chromosomes from females compared with those obtained by measurement with chromosomes from males. In general, there is more recombination over the autosomes during female meiosis than during male meiosis (Broman et al., 1998). Only the comparatively small, “pseudoautosomal” portion of the X and Y chromosomes recombine during male meiosis, but the rate of recombination in this region is approximately 10 times greater than the rate of recombination in this region during female meiosis (Hunt and LeMaire, 1992; Rappold, 1993).

Sex-of-Offspring-Specific Transmission Ratio Distortion

In a number of instances the inheritance of alleles from heterozygous parents does not appear to be equal between male and female offspring (Naumova et al., 1998; Pardo-Manuel de Villena et al., 2000; Siracusa et al., 1991; see also Sapienza [1994] for a review). Such sex-specific biases in the inheritance of genetic information are not expected per se (especially in the case of

autosomal loci) but may be due to a number of causes, including meiotic drive, preferential cosegregation of sex chromosomes with one of a pair of homologous chromosomes, preferential fertilization, and preferential death of the embryo of one sex. These biases that are specific to the sex of the offspring have been observed as a result of transmission through both male and female parents (reviewed by Sapienza [1994]). As a practical matter, it is important to consider the source and magnitude of any observed inheritance biases because they may affect the mapping and identification of genetic traits that are more prevalent in one sex than the other. An interesting side effect of sex-of-offspring-specific transmission ratio distortion is that the observed frequencies of alleles at some loci may differ between the sexes.

GENETICS AS A TOOL

Genetics has long been an important tool for the dissection of biological mechanisms. Its use, however, has been limited by the investigator's ability to find appropriate mutant phenotypes and then to identify the gene and gene product responsible for producing the phenotype. Recently, an array of genetic techniques has greatly expanded the power of genetics. These techniques exploit the ability to clone specific genes and modify their sequences to destroy or modify the gene product. This modified sequence is then inserted into the genome of an intact animal or cultured cell to determine the effect on the phenotype. The details of introducing transgenes vary with species, but in the most successful cases, the transgene can exactly replace the resident gene. In other cases, transgenes cannot be specifically targeted and the resident gene may still be present; thus, the types of questions that can be asked are more limited.

Transgenic techniques have overcome several problems of conventional genetics. They greatly speed study because there is a direct link between the gene used in the experiment and the phenotype. The investigator can precisely specify the gene modification rather than depend on random mutagenesis, making it possible to focus not only on a specific gene but also on a particular feature of that gene. For example, by removing the domain responsible for phosphorylation, one can study its role in the parent protein.

Two classes of important genes are difficult to study by conventional genetics. The first class is redundant genes, such as families of genes that all fulfill the same function. It is unlikely that random mutagenesis will knock out all members of even a very small family of genes, but targeted transgenes can easily achieve this to allow study of the action of this gene family. A second class of important genes includes those that produce lethal phenotypes or that have effects in one developmental stage that preclude study of their activity in a later stage. Techniques that allow the transgene to be specifically inactivated in a tissue of choice offer ways to bypass these problems because the gene can be expressed normally except in the tissue where it is being studied. Similar techniques can be used to drive inappropriate expression of a gene in specific tissues where the inappropriate expression can be informative.

Transgenic techniques can also be used to construct model systems to meet specific requirements. For example, mouse transgenic systems are being used in many laboratories as models for human genetic diseases and for cancer studies. The models need not be restricted to mouse genes but can also contain transgenes of human origin to study specific interactions. The increasing number of mice being bred to carry transgenes of interest makes it possible to rapidly test gene interactions by breeding different transgenic animals. Despite the power of these new techniques, however, interpretation of experimental results is not always straightforward. For example, the manipulations involved with introducing the new DNA sequence can sometimes introduce unexpected genetic changes, either at the locus under study or at unrelated loci. In

addition, identical transgene or knockout models may have variable phenotypes, depending on the strain's background (just as for “normal” mutant alleles).

FINDINGS AND RECOMMENDATIONS

Findings

Males and females have partially different genomes:

- The Y chromosome carries genes that are involved in basic cellular functions and that are expressed in many different tissues.
- In females, the majority of genes on one of the two X chromosomes are silenced in every cell. This inactivation makes each female a functional mosaic because some cells express one X chromosome and others cells express the other one. The advantages of heterozygosity can be amplified by selection against cells in which the active X chromosome carries a detrimental allele.
- Some genes on the inactive X chromosome are not silenced, leading to higher levels of their products in female cells.
- Female cells must have cellular machinery to establish and maintain the inactivation of the X chromosome.
- Male and female germ cells differentially imprint the genetic information to be transmitted to their progeny.

These findings argue that there are multiple, ubiquitous differences in the basic cellular biochemistry of males and females that can affect an individual's health. Many of these differences do not necessarily arise as a result of differences in the hormonal environment of the male and female but are a direct result of the genetic differences between the two sexes.

Recommendation

RECOMMENDATION 1: Promote research on sex at the cellular level.

The committee recommends that research be conducted to

- **determine the functions and effects of X-chromosome- and Y-chromosome-linked genes in somatic cells as well as germ-line cells,**
- **determine how genetic sex differences influence other levels of biological organization (cell, organ, organ system, organism), including susceptibility to disease, and**
- **develop systems that can identify and distinguish between the effects of genes and the effects of hormones.**

The phenotypic differences between males and females are determined, initially, by genes on the sex chromosomes. Sex chromosome-linked genes can be expressed in both germ-line and somatic cells and could influence an individual's phenotype, including disease susceptibility, at many levels.

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