Understanding the Sexome: Measuring and Reporting Sex Differences in Gene Systems

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The current male bias in biomedical research should be eliminated. The large sex differences in incidence and progression of diseases mean that sex-biased factors are an untapped source of factors that protect from disease. Greater understanding will come from intensified study of the "sexome," which is the sum of sex-biased effects on gene networks and cell systems. The global search for sites and mechanisms of sex-specific regulation in diverse tissues will provide unanticipated insights into physiological regulation and targets for novel therapies. **(*Endocrinology* 153: 2551–2555, 2012)**

Imagine a world in which the majority of scientists are women, and the majority of experimental subjects are female. Principles of physiology would be defined first in females and assumed to apply without modification to males. This brave new world sounds out of balance, unreasonable, and perhaps threatening to the average (male) biomedical scientist. Yet, such a world is simply the inverse of that in which we live. Most (68–76%) preclinical scientific publications in major biomedical fields (immunology, neuroscience, physiology, pharmacology, and endocrinology) use only males for subjects or do not report the sex of the animal (1, 2). In some fields, studies of males outnumber studies of females by 3- to 5-fold. This bias threatens to make biomedical science much less relevant to the female half of the population and probably has deep-seated causes. Social biases concerning the role of women no doubt play a role. But even when such biases are recognized, there are other factors that maintain the excessive focus on males. One purported rationale for studying mostly males is that sex differences outside of reproductive physiology are small and unimportant (but see below). Another rationale is that greater variability of traits in females gets in the way of measuring experimental effects, even though the assumption of greater variability is not always supported by data (3). Study of two sexes is more expensive than studying one sex, so cost is an issue. Once scientific paradigms are founded on the exclusive study of males, the bias is perpetuated, because future studies build on past results. Using females in a field dominated by studies of males could mean introducing an unwanted new variable that could lead to unpredictable experimental results. Note that some of these ideas seem to assume that studying females would be "problematic" precisely because females differ significantly from males. Whatever the causes, currently the group behavior of the biomedical research community tends to leave females out. If biomedical science is to be relevant to everyone, however, it is beyond obvious that greater emphasis must be placed on studying females and sex differences.

How are we to remedy this situation? First, it is critical to fight complacency that study of males is enough. We need to perform more studies on females. An important point, however, is that separate and equal study of males and females is not sufficient. The two sexes need to be

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Abbreviations: QTL, Quantitative trait locus.

compared directly. Understanding the physiology of one sex gives important perspectives on the function of the other sex. The comparison raises novel questions about the factors that cause the difference, which leads to a deeper understanding of the variables controlling physiology and disease. For example, if one sex is protected from disease, what sex-specific factors cause the protection? Can the sex-biased protective forces be targeted to develop novel therapies (4)? In some cases, a sex-specific process can only be understood because of the direct comparison of the sexes. For example, X-inactivation, the epigenetic silencing of one X chromosomes in nearly every adult XX cell, never occurs in XY male cells. The evolution and function of female-specific X-inactivation can only be explained by comparison of the sexes (5, 6).

Conceptual Framework for Investigating the Sexome

The function of cells and tissues can be envisioned as the output of a network of molecular interactions. The nodes in the network are gene products, which increase or decrease the activity of other gene products in a complex meshwork. The connections in the network are multidimensional, linear or nonlinear, and complex. Some nodes are more important than others, because of their larger impact on other nodes. Sex differences in gene networks occur because some nodes are sensitive to sex-biased or sex-specific factors, which act on those nodes to modulate their activity. We define the "sexome" as the sum of all sex-specific and sex-biased modulatory interactions that operate within the networks, creating sex differences in connectivity and activity of nodes (Fig. 1). Ultimately, the sexome produces sex differences in emergent phenotypes, such as blood pressure, adiposity, and aggression, to name just a few (7–9). In this model, explaining the sexome requires 1) identifying the primary and downstream (secondary, tertiary, *etc.*) sex-biased factors that act on the network, 2) determining where in the network the biasing factors act and how the sex bias is transmitted through the net, and 3) determining how the sex-biased network interactions give rise to sex differences or sexual equality in emergent phenotypes.

Solving the first goal of sexomics, to identify sex-biased factors, starts with a consideration of the general theory of sexual differentiation, built over the last 100 yr, which has identified three major classes of sex-biased factors that cause sex differences (10, 11). A modern version of this theory states that the primary sex-biased factors are encoded by the sex chromosomes (X and Y), which are the only factors thought to be inherently unequal at the beginning, in male *vs.* female zygotes. These factors include Y genes that have effects only in males, and X genes (and perhaps X nongenic regions) that differentially affect male and female cells, because of the number of copies of X genes, their imprint, or the presence of X heterochromatin only in XX female cells (11). The most important sex-biasing primary Y factor is *Sry*, the gene that causes testes to develop in XY males, and which therefore leads eventually to the secretion of testicular hormones. In females, autosomal or X genes initiate the differentiation of the embryonic ovary and lead to the onset of ovarian secretions. The different hormones secreted by the gonads are so important that they comprise two of the three classes of secondary sex-biasing factors in the sexome (organizational and activational). The organizational effects are hormonal actions early in development to cause permanent sexual differentiation of the genitalia, brain, and other organs (10, 12). The activational effects are reversible effects of female and male gonadal hormones that act at different network nodes, to turn on or modulate diverse sex differences in cell function. Thus, the hormonal sex differences downstream of the developmental switches controlled by *Sry* are very potent components of the sexome. In addition to these hormonal effects, a third class of sex-biased proximate factors are sex chromosome effects. This class includes other X and Y primary sex-determining

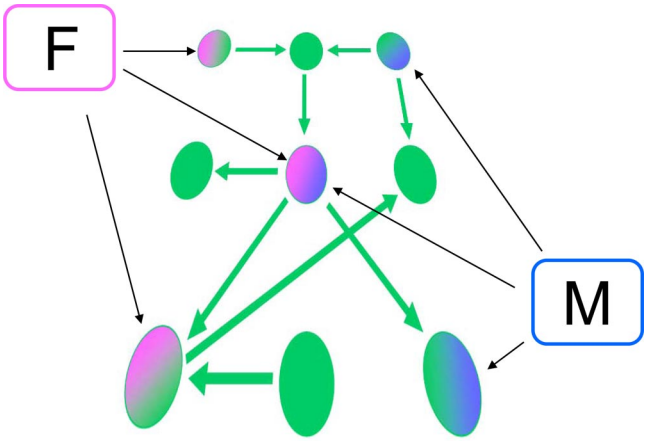

FIG. 1. The sexome. *Green circles* are gene products connected in a network, with *green arrows* indicating interactions between network nodes. The female (F) and male (M) *boxes* show the origins of sex-biasing actions in the network. Female-biasing factors include two X chromosomes, ovarian secretions, *etc.* Male-biasing factors include a single X chromosome, the Y chromosome, testicular secretions, *etc.* The sex-biasing influence of these factors on the network is illustrated by the *pink or blue shading* of gene product nodes. Gene products influenced both by female-biasing and male-biasing factors show *pink and blue shading*. The sex-biasing factors can increase or decrease activity at specific sites in the network, and their effects can propagate through the network to sex-bias various gene modules (data not shown). The sexome is defined as the total of all sex-biasing actions within the network.

gene products that act via nongonadal pathways to exert sex-biasing effects on networks (11).

A newly evolving but game-changing part of the general theory of sexual differentiation is that sex-biasing factors are not always synergistic but are sometimes antagonistic (13). One female-specific process, for example, can counteract another to reduce the female bias and make female cells more similar to male cells than they would otherwise be. The female-biasing effect of a double representation of X genes in XX females is counteracted by the female-specific expression of *Xist*, which causes inactivation of one of the two X chromosomes so that female cells have one active X chromosome, like males. Thus, similarities in function of male and female cells may mask an underlying sex difference in the operation of gene networks (11). Stated differently, male and female cells may get to the same place via different paths. When an external factor, for example a disease or environmental event, impacts the network, it can affect a sex-specific factor that inhibits another, and therefore unmask a sex difference that was previously hidden. Similarly, when we experimentally manipulate the network by removing or enhancing one sex-biased factor, we may unbalance compensatory interactions and reveal underlying sex differences that are not present when all sex-biased factors are operating.

How Important Is the Sex of Cells to Cell Function and Disease?

Large sex differences in tissues directly involved in reproduction have been studied for decades and have provided the main tenets of the general theory of sexual differentiation. Studies of nonreproductive tissues, as noted in the first paragraph, lag behind. Most studies have measured a small number of phenotypes or small regions of the gene network, based on tests of focused hypotheses about sex differences related to specific traits. This is an important start. To date, however, there has been relatively little integration of high-throughput measurements of molecular networks with experimental approaches (manipulation of hormones or sex chromosomes) rationalized by the theory of sexual differentiation. It will be important to perform global studies of sex differences in the transcriptome, proteome, metabolome, epigenome, *etc.* in various tissues to understand the sexome as defined above. To understand the sexome fully, however, it will also be necessary to manipulate important sex-biased factors, such as gonadal hormones, while performing global measurements, to determine where and how the sex-biased factors act globally.

There have been some important first glimpses into the sexome. Numerous investigators have studied sex differences or gonadal hormone effects on the transcriptome using microarrays (*e.g.* Refs. 14–22). When these studies on sex differences are well powered, they uncover a surprising degree of sexual bias (Fig. 2A) (23). In tissues not normally thought to be very sexual, such as liver, adipose tissue, and muscle, half to three-quarters of genes show consistent sex bias in mice (23). Although the average sex difference in level of expression of genes is modest (~9%), the inequality of expression of so many transcripts suggests that sex is an important variable. Sex differences are also found in parent-of-origin effects on the transcriptome (24, 25). Studies comparing the proteome of females and males confirm important sex differences in numerous tissues (26–30). A recent study revealed striking sex differences in human serum metabolite concentrations, with 102 out of 131 metabolites differing significantly between males and females (Fig. 2B) (31). Another study reported large sex differences in aging-related changes in gene expression in specific areas of the human brain, measured globally (32). Although global studies of sex effects on the epigenome have not been reported, there is emerging evidence for sex differences and sex hormone effects on DNA methylation and histone modifications (33). Investigations that identify quantitative trait loci (QTL), re-

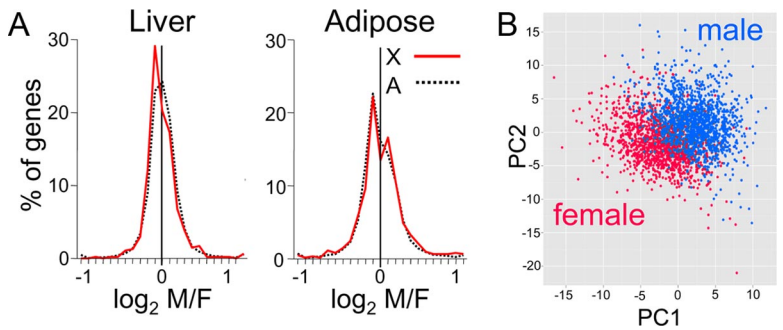

FIG. 2. A, Global sex differences in the mouse transcriptome of tissues not directly involved in reproduction. Histograms show male (M):female (F) ratios of expression of all mRNA measured by microarray profiling in mice (*i.e.* genes with 2-fold higher expression in males are +1 and those with 2-fold higher in females are −1). Sex differences were found in 72% of liver genes and 68% of adipose. Over 70% of genes showing sex bias have less than 20% greater expression in one sex relative to the other, and sex differences of 2-fold are rare. X-inactivation prevents strong female bias in expression of X genes (*red curve*), and results in sex bias in X genes that roughly matches, tissue for tissue, the global sex bias of autosomal genes (*black curve*) with which they interact in each tissue. Republished from Ref. 43 based on data of Ref. 23. B, Sex differences in the human plasma metabolome; 131 metabolites are graphed by the first two principal components (PC) in a partial least square analysis of levels of metabolites in human plasma; 78% of metabolites showed statistically significant sex differences. Reprinted with permission from Ref. 31.

gions of the genome that control complex traits, show important sex differences (24, 34–39). Numerous QTL are only found in one sex, so that isolated study of each sex gives an incomplete perspective on the genetic control of gene networks. In one study, five of five significant QTL for fat mass were sexually dimorphic in their effect, and the relationship between QTL genotype and fat mass was stronger in females (40). The same study found that among liver genes that correlate in their expression with fat mass, 98% showed sex differences in correlation. When modules of genes are recognized within gene networks (in liver, fat, and brain), based on their common patterns of expression across animals, specific modules are found to be different in the two sexes (41). Within the modules are groups of genes showing special sensitivity to gonadal hormones (41). Other studies have integrated transcriptional profiling of time-dependent estrogen effects on breast cancer cell lines with global analysis of estrogen receptor and transcription factor binding sites and estrogen response elements, to construct a model of estradiol regulation of a limited group of transcription factors that mediate downstream effects on diverse molecular pathways (42). Thus, excellent bioinformatic methods are currently available for further extensive dissection of the sexome under normal and disease conditions.

Conclusions

The general theory of sexual differentiation has yet to intersect significantly with the exploding field of systems biology, which uses large orthogonal datasets to measure the aggregate behavior of systems within cells and individuals, viewed from multiple perspectives. These methods allow new opportunities to assess the overall importance of sex on cell and tissue functions, to measure the sexome in health and disease, and to define where in gene networks sex makes a difference. Many diseases affect females and males differently, and the comparison of the sexes therefore offers the chance to find protective agents acting within gene networks that could help protect from disease (4). Understanding the sexome requires both deconstructing of the influences of individual sex-biasing factors, such as hormones and the downstream factors that they affect, as well as integrating the synergistic and compensatory impact of those factors within cell networks and systems. The time to investigate the sexome is now, not after the biology of males is perfectly understood.

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