

Research news

Getting the right dose of sex (chromosomes)

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Gene-expression analysis provides evidence for dosage compensation of the X chromosome in flies, mice and worms.

Ever since the Garden of Eden, men have been fascinated by what makes them different from women. This interest has focused recent attention on the sex chromosomes X and Y. In mammals and *Drosophila*, males are XY and females XX. In nematodes, males are XO (as there is no Y) and hermaphrodites (or self-fertile females) are XX. The Y chromosome is relatively gene poor, so X-chromosome dosage appears to be critical for sex determination. But this set-up poses two problems: how do females deal with having a double dose of X-linked genes, and how do males deal with being **aneuploid** for the X chromosome? Now, in *Journal of Biology* [1], Vaijayanti Gupta and colleagues describe microarray studies to investigate how gene expression from the X chromosome or the **autosomes** is equilibrated in flies, worms and mice (see 'The bottom line' box for a summary of the work and the 'Background' box for definitions).

Sex equality

Different species have developed strikingly different strategies to deal with disparities in the dose of X chromosome between males and females: in XX female mammals, one of the two X

chromosomes is randomly inactivated; XX hermaphrodite nematodes halve the expression from each X chromosome; and male *Drosophila* double expression from their single X chromosome in somatic cells [2,3]. These dosage compensation mechanisms serve to balance the differences between the number of copies of X-linked genes in somatic tissues of the two sexes.

"Although we now know that these species use different approaches to achieve dosage compensation, this amounts mainly to playing differently with a limited panoply of chromatin-based modifications," says Philip Avner from the Pasteur Institute in Paris, France. X inactivation in

mammals requires expression of the *Xist* gene, which produces a large, non-coding RNA that coats the inactive X chromosome [3]. The inactive X is characterized by DNA methylation, histone hypoacetylation, late replication and enrichment in the variant histone macroH2A. The hypertranscription of the *Drosophila* X chromosome in somatic cells is dependent on the 'male specific lethal' (*msl*) loci, which encode a histone-modifying MSL complex that acetylates histone H4 on lysine 16 (H4 K16) [4].

"Much less attention has been paid to the question of X/autosome dosage," says Avner. "X inactivation would be expected to lead to halved

The bottom line

- Microarray analysis of expression of genes on the *Drosophila* X chromosome provides the first evidence for dosage compensation in germline cells.
- Upregulation of expression from the X chromosome ensures balanced expression between a single X chromosome and two autosomes.
- Increased expression of the X chromosome occurs in flies, nematodes and mice.

Background

- **Dosage compensation** is a genetic regulatory mechanism that equalizes the expression of genes on the X chromosome, so that, for example, they are equally expressed in XY males and in XX females.
- Males have one fewer X chromosome than they have the other chromosomes (the **autosomes**); they are **aneuploid** with respect to the X chromosome. The consequence is that all genes on the sex chromosomes exhibit **haploinsufficiency**, as they exist in only copy compared to the two copies of genes present on the autosomes. Males have a genotype that can be written **X;AA**, whereas females are **XX;AA**.

quantities of X-linked gene products compared to autosomal gene products. **Haploinsufficiency** for the entire X would *a priori* be expected to be catastrophic to the organism and lead to lethality during early embryonic development." Haploinsufficiency was the issue that Gupta and colleagues set out to address. "There was a lack of evidence for a germline dosage compensation machinery," notes Gupta, citing studies showing that in *Drosophila* the X chromosomes are not coated with MSL complexes or hyperacetylated on H4 K16 in male germ cells [5] (see the 'Behind the scenes' box for more on the rationale for the work). "Also, Parisi *et al.* [6] showed that a subset of ribosomal protein-encoding genes are equally expressed in both testis and ovaries and we had seen that X;AA and XX;AA tumors showed very similar gene expression profiles," adds Gupta. "I've been thinking about this problem since I was a graduate student in the late 80s," recalls Brian Oliver who heads the research group at the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, USA. "Until microarrays appeared, we didn't really see a good way to do a convincing experiment."

Testing testes and ovaries

Microarray chips covering virtually the entire *Drosophila* genome offered

Oliver's group the chance to look simultaneously at thousands of genes and their response to changing gene doses. "It would be extremely difficult to draw meaningful conclusions about the dosage compensation of an entire chromosome based on just a handful of genes," notes Gupta. They used genetic tricks to remove the influence of sex-biased expression and included many replicate hybridizations for every sample. "We ensured that the X;AA and XX;AA matched tissue samples were compared directly against each other, by hybridizing to the same array. By including the loop design, we could compare any sample in the design to any other. This increased our total number of replicates (both direct and indirect). For example, we have 7 X;AA *hs-tra* tumors and 17 XX;AA *otu* and *Sxl* tumors [these are the genotypes used]. There were 6 comparisons by direct hybridizations between the X;AA and XX;AA tumors. But we could additionally compute 113 X;AA versus XX;AA tumor comparisons indirectly ((17 x 7) - 6)."

The results were strikingly clear: the expression ratios between X chromosome and autosome genes were tightly centered on 1-fold, indicating that dosage compensation occurs in the germline. Gupta and colleagues performed a large number of controls using flies with deleted or duplicated

autosomal segments to be sure that they could detect gene dosage effects on other chromosomes [1]. "We were able to detect gene expression changes for our control autosomal aneuploidy corresponding to as small as 1.5-fold gene dose change, which was actually a more stringent dose difference, compared to the 2-fold dose difference in X chromosome," says Gupta.

Not content with their impressive results in flies, Oliver's group went on to reanalyze microarray data from *Caenorhabditis elegans* and mice. Their results led them to a similar conclusion: that the single X chromosomes of X;AA nematodes and mice were expressed at similar levels to two autosomes. Di Nguyen and Christine Disteche, at the University of Washington, Seattle, USA, recently reported a similar study of gene expression in a range of human and mouse somatic tissues [7]. They also found that doubling of the global expression level of the X chromosome leads to dosage compensation in mammalian somatic tissues. "Interestingly, X-chromosome expression levels appear even more markedly increased in the brain," comments Avner. "Dare one suggest that the X chromosome has become specialized in cognitive function and sex? Also, although some X-linked genes are expressed in post-meiotic cells of the male and female germlines, upregulation is absent, allowing X/A ratios to be maintained in an equivalent fashion to somatic tissues."

How exactly do they do it?

"Taken together, these experiments suggest that, with the development of heteromorphic sex chromosomes, the driving and unifying force may have been to maintain X/autosome levels within relatively strict limits to avoid haploinsufficiency, and that mechanisms to ensure dosage compensation may well have been added in later," suggests Avner. "But these results leave us with the conundrum of how, and exactly when, upregulation of the

Behind the scenes

Journal of Biology asked Vijayanti Gupta and Brian Oliver about their dosage compensation study.

What prompted you to embark on a study of X-chromosome dosage compensation?

Given that aneuploidy is bad, either germ cells need a mechanism that helps them cope with reduced X-chromosome expression, or they need a compensation mechanism to equilibrate X-chromosome expression. So, no matter what we found, it would be important. We undertook detailed analyses of this phenomenon using whole-genome arrays for comparing gene expression of X;AA and XX;AA tissues and, importantly, using autosomal aneuploidies as necessary controls, to see if there were expression changes in the absence of specialized X-chromosome compensation machinery.

How long did the study take and what were the difficult steps you encountered?

First, we had to wait 20 years for the technology to arrive! We took approximately 8 months to complete setting up crosses, growing flies, dissecting the tissues, and running the microarray hybridization experiments. It took approximately 10-12 months to complete all the data analyses and statistical tests. Several key steps ensured that our experiments would give us meaningful data. These included the use of large numbers of genome-wide microarrays and *Drosophila* genetics, which allowed us to remove sex-biased expression from X;AA and XX;AA tissues and compare only dose differences.

What was your initial reaction to the results and how were they received by others?

Needless to say, we were very excited when we saw that by using microarrays we were able to detect dose changes as small as 1.5-fold on autosomes. This gave us the first indication that we would perhaps be able to draw inferences about germline dosage compensation conclusively. When we saw that the effects of 2-fold dose differences of the X chromosome were far less than even 1.5-fold autosomal dose change, we were very excited, since this would be quite contrary to current ideas about dosage effects in the germline.

The results have been received with a lot of enthusiasm by many people in the field. It is a very old problem, but with very little experimental evidence. These results should lead to some rethinking about dosage compensation. Virtually everyone in the field thinks of dosage compensation as operating to equilibrate X chromosome expression between the sexes.

What are the next steps?

An obvious area of interest is finding what constitutes the germline dosage-compensation mechanism. It does not involve the MSL complex that functions in the soma. Dosage compensation should be marked by a change in the modifications of core histone proteins that are bound to the chromosome. So identifying these modifications will be of key interest. But the mechanism could come from an unexpected direction. We have also studied steady-state message pools. Anything from chromosome structure to mRNA stability could give the result.

thousand or so genes on the X chromosome is put in place during early development alongside X-inactivation. Or alternatively, when and how down-regulation is achieved specifically in post-meiotic cells."

Gupta thinks that studying the germline X-chromosome gene expression in other organisms (such as mice and *C. elegans*) using a similar approach might bring new insights.

Oliver is very keen to get a handle on the mechanism. "We'll need to probe chromatin structure in the germline by CHIP-chip [chromatin immunoprecipitation] type methods," he says. He cites recent studies investigating how many of the X-chromosome genes might be regulated by the MSL complex [8,9]. "There may be other players besides the MSLs in the soma," says Oliver. "This supports our observation

of MSL-independent dosage compensation in the germline." But he is also keeping an open mind about dosage compensation. He is interested in how the apparent moderate dosage compensation on the autosomes works and what this can tell us about general properties of gene expression networks. "And what is the best reference for measuring dosage compensation?" asks Oliver provocatively. "It is still

possible, as long suggested by Jim Birchler, that autosomal expression is down in X;AA individuals. $2X = AA$ or $X = (AA)/2$?" Whether it is indeed the expression on the X chromosome that goes up or expression of the autosomes that goes down, the mysteries of the X will continue to fascinate both sexes for many years to come.

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