that the diagnosis of PWS was correct. PWS has many symptoms, some of which are non-specific and subtle. The individual examined had a combination of key features which, taken together, are typical of PWS[3], but the individual was relatively tall for a person with PWS and had a head circumference greater than the normal range.

Can mouse models help?

Many mouse deletion mutants have been generated for investigations of PWS (summarized in ref. 6). The earliest model, with maternal uniparental disomy of central mouse chromosome 7 (ref. 7), and later models with paternal deletions of the entire region or the imprinting center all fail to thrive and die within a few days of birth. Paternal deletion of individual genes, for example, *Snrpn*[8,9] or *Magel2* (ref. 10), is compatible with adult survival in mice, but hyperphagia and obesity, classic features of PWS, have not been observed.

Recently, two mouse models have been described in which the cluster of MBII-85 snoRNAs (orthologous with HBII-85) was deleted[6,11]. Both models show pre-weaning growth retardation, which is an indication of failure to thrive, and both are adult viable, but neither are obese. Full investigation of one model has revealed adult hyperphagia[11]. Thus, one hallmark feature of PWS occurs in mice deficient for the MBII-85 cluster. The lack of obesity may just reflect species differences. Investigation of hyperphagia in the second mouse model is needed, and a full investigation of milk intake in relation to pre-weaning growth retardation is needed for both models. The mouse work is broadly supportive of the involvement of HBII-85 snoRNAs in PWS, but does not exclude involvement of other genes in minor features of the syndrome.

Role of snoRNAs

The HBII-85 cluster is made up of C/D box snoRNAs that are predominantly expressed in brain. The role of most C/D box snoRNAs is to base pair with their target sequences in ribosomal RNA (rRNA) or small nuclear RNA (snRNA) and to methylate the ribose 2′-hydroxyl group of specific nucleotides[12]. But so far, no targets have been found for HBII-85. Notably, three groups of expressed snoRNAs in the HBII-85 cluster can be distinguished on the basis of sequence. It is therefore possible that each group has a distinct target, or that only one or two groups are functional and the others are not[13]. There is now a real urgency to identify the target(s) of this cluster and the type of modification catalyzed. This is a worthwhile challenge, because identification of targets could raise the possibility of developing therapies.

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The not-so-silent X

Christine M Disteche

The X chromosome has been thought to be mostly silent after meiosis in males. A new study reports that this is not the case for a set of multicopy genes often arranged in giant palindromes and highly expressed in spermatids.

Unpaired chromosomes become silenced at meiosis by a mechanism known as meiotic silencing of unsynapsed chromatin[1]. The mammalian X and Y chromosomes, mostly unpaired at male meiosis, are silenced by this mechanism (meiotic sex chromosome inactivation, or MSCI). A lingering question has been: what happens after meiosis? Are X-linked genes permanently silenced, or do they reactivate? A new comprehensive study by Jacob Mueller and colleagues[2] on page 794 of this issue addresses this question by demonstrating that a subset of X-linked genes is reactivated after meiosis. What is noteworthy is that many of the genes with significant expression in spermatids are members of large gene families with multiple copies on the X chromosome that are often organized into giant palindromic structures. Thus, amplification of X-linked genes may have evolved as a way to restore gene expression from the meiosis-repressed X chromosome (**Fig. 1**).

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Be fruitful and multiply

The sex chromosomes have accumulated genes that function in female and male sexual reproduction[3,4]. For example, the X chromosome is enriched in genes expressed in spermatogonia[5]. However, the X was thought to be depleted in genes expressed after meiosis[3]; this was attributed to the effect of MSCI. The new study by Mueller *et al.*[2] shows that many X-linked genes are specifically expressed in spermatids. Thus, the X chromosome is enriched in genes important for several stages of sperm development, both pre- and postmeiosis. Why has the X chromosome become a repository of genes with multifaceted roles in sexual reproduction? The sex-specialization of the X stems from its hemizygous state in males, which provides an opportunity for the evolution of male-advantageous genes[6]. Thus, it makes perfect sense that the X chromosome has found a way to be involved in spermiogenesis by multiplying copies of some genes.

In somatic cells, dosage compensation between the X and autosomes is achieved by a doubling of gene expression from the X chromosome. The X chromosome is then repressed in haploid germ cells, where the average X-linked gene expression is low, but not absent[7,8]. Expression analysis of individual X-linked genes also indicated reactivation after male meiosis, and cytological studies showed that RNA polymerase II was associated with the sex chromosomes after meiosis[1,9]. Mueller *et al.*[2] set out to examine the expression of multicopy genes. First, they searched for ampliconic structures on the mouse X chromosome and found 22 regions organized into either palindromes or tandem

repeats, covering as much as 12% of the X. Amazingly, almost all amplicons contained genes expressed in testis. An additional ten multicopy but nonampliconic genes also had testis-biased expression. Considered together, these multicopy genes show higher expression than autosomal genes in spermatids. In contrast, reactivation of single-copy genes is variable, and for some genes, it occurs in a relatively small subset of spermatids as detected by RNA-FISH.

Spermatids are organized in tetrads so that the Y-bearing spermatids have access to X-linked products from the X-bearing spermatids. This very special organization alone suggested that expression of some X-linked genes would be essential for spermatid survival. MSCI is partially rescued by expression of retrogenes transposed from the X chromosome to autosomes[10]. Such genes are usually housekeeping genes whose products must be available throughout meiosis. Retrogenes may also function after meiosis to compensate for partial repression of single-copy X-linked genes. Furthermore, genes with up to 25 copies on the X chromosome were evidently selected in order to provide sufficient product to be shared between X- and Y-bearing spermatids. One of the multicopy gene families included in a palindromic structure found by Mueller *et al.*[2] is the homeobox gene cluster *Rhox*. Loss of one member in mutant mice apparently results in increased expression from other members, suggesting that *Rhox* copies are interchangeable[11]. Another family comprises the *Slx* genes, whose protein product is cytoplasmic and thus could easily be shared by spermatids in a tetrad[12]. Thus, the multiplicity of copies may allow for sufficient additive expression to provide for both types of spermatids.

Expressed on the X

Multicopy genes are prevalent on both sex chromosomes. On the human X chromosome and now on the mouse X chromosome, such palindromic structures were found to predominantly contain testes-specific genes[13]. The gene families only partially overlap between species, suggesting that gene amplification has occurred independently in the rodent and primate lineages. The palindromic structures could self-pair and therefore be protected from silencing by MSCI. Similar palindromic structures are prevalent on the human Y chromosome, which may self-recombine to protect against MSCI and degradation due to lack of recombination between the sex chromosomes[14]. Some of the X-linked multicopy gene families, such as *Slx*, have paralogs on the Y chromosome (*Sly*). It remains to be determined whether these paralogs have similar functions.

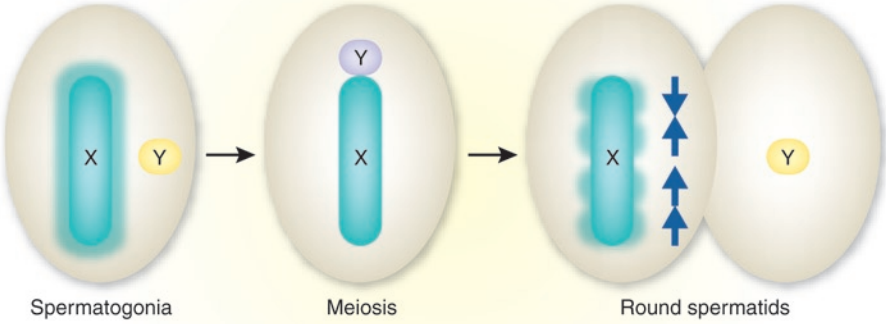

Figure 1 X chromosome expression in male meiosis. Before meiosis, the X chromosome is highly expressed in spermatogonia. After meiotic sex chromosome inactivation, the X and Y chromosomes are turned off. In X-bearing spermatids, reactivation of X-linked genes occurs, mostly at loci with multiple copies arranged either in palindromes (head to head arrows) or in tandem (head to tail arrows). Attached Y-bearing spermatids could receive products from these genes.

In mature sperm, most of the genome is silenced—autosomes and sex chromosomes alike—because of its assembly into a different type of chromatin that contains protamines rather than histones. The early paternal X inactivation upon fertilization observed in mice has been interpreted as either a continuation of the meiotic silencing of the X chromosome, or as a *de novo* silencing event induced by *Xist*[15]. The new data show that the X chromosome partially reactivates in spermatids. Not only are multicopy genes highly expressed, but a number of single-copy genes are also reactivated, albeit at low level. Even if partial silencing persists through fertilization, *de novo Xist* expression seems to be necessary to initiate early paternal X inactivation.

Sex chromosome inactivation at meiosis employs a complex set of chromatin modifications: ATR, BRCA1, γH2AX become associated with the sex chromatin body[1]. Any unpaired autosome becomes silenced by the same mechanism, suggesting that this is a universal silencing mechanism that may exist to abort failed meioses. Postmeiotic sex chromatin is associated with the chromocenter, suggesting that the sex chromosomes are sequestered in a repressive nuclear compartment[7]. It will be interesting to determine how multicopy genes escape this repression. Specific enzymes, yet to be defined, must remove repressive chromatin marks to achieve reactivation. The study by Mueller *et al.* provides a new example of one more step in the complicated saga of sex chromosome expression. This complexity is tied to the evolution of genes with important roles in sexual reproduction, some perhaps shared between males and females. Intriguingly, several of the multicopy genes (*Ott*, for example) expressed in spermatids are also expressed in oocytes.

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