Imprinting in Prader–Willi and Angelman syndromes

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Imprinted genes are marked in the germline and retain molecular memory of their parental origin, resulting in allelic expression differences during development. Abnormalities in imprinted inheritance occur in several genetic diseases and cancer, and are exemplified by the diverse genetic defects involving chromosome 15q11–q13 in Prader–Willi (PWS) and Angelman (AS) syndromes. PWS involves loss of function of multiple paternally expressed genes, while mutations in a single gene, UBE3A, which is subject to spatially restricted imprinting, occur in some AS patients. Identification of mutations in the imprinting process in PWS and AS has led to a definition of an imprinting center (IC), involving the promoter (in PWS) or an alternative transcript of the SNRPN gene (in AS). The IC regulates initiation of imprint switching for all genes in a 2 Mb imprinted domain during gametogenesis. Imprinting mutations define a novel mechanism of genetic disease because they have no direct effect in the affected patient but, rather, it is the parental germline effect of an IC mutation that leads to disease in the offspring.

Genomic imprinting refers to the differential marking of maternally and paternally inherited alleles of specific genes or chromosome regions during gametogenesis, leading after fertilization to differential expression during development[1]. In imprinting, this mark (or imprint) allows the cell to discern the parental origin of each allele, where one allele of an imprinted gene is usually active and the other is silenced in normal development[2]. The loss of imprinted-gene expression has a significant role in human genetic disease, leading to disorders such as Prader–Willi and Angelman syndromes (PWS and AS)[3], Beckwith–Weidemann syndrome (BWS)[4], a variety of pediatric tumors and adult cancers[5,6] and others[6].

PWS and AS are devastating conditions, each occurring in about 1/15 000 births and associated with significant developmental, behavioral and mental problems. PWS patients have neonatal hypotonia with failure to thrive, hyperphagia and severe obesity, hypogonadism, short stature, small hands and feet, mental retardation with learning disabilities, and obsessive-compulsive disorder[6] (Fig. 1a). AS is characterized by ataxia, tremulousness, seizures, sleep disorder, hyperactivity, severe mental retardation with lack of speech, and a happy disposition with paroxysms of laughter[6] (Fig. 1b). PWS and AS are caused by defects in imprinted-gene inheritance in a region of chromosome 15q11 to q13. Here, we review the progress in identifying novel genes in the 2 Mb imprinted PWS and AS region, and the advances in elucidating the mechanism by which the coordinate imprint switch of these genes is regulated in the germline.

Molecular classes of PWS and AS

There are multiple genetic mechanisms that can lead to PWS and AS, but despite this complexity each leads to a common gene deficit[3]. Each molecular mechanism in PWS and AS abolishes imprinted (parent-of-origin-specific) expression, such that paternal gene expression is silenced in PWS, and maternal gene expression is abolished in AS (Fig. 2). The most common genetic mechanism in PWS and AS is a large chromosomal deletion that is the same size in the majority of deletions in these syndromes[3,7]. However, all deletions in PWS are paternal in origin and all deletions in AS are maternal in origin[3,6]. Furthermore, maternal uniparental disomy (UPD) is common in PWS (Refs 3, 6, 8; Fig. 2). In UPD, the presence of a second structurally normal maternal chromosome 15 cannot complement the missing paternal chromosome, and produces the same PWS phenotype, as do large deletions. This suggests that the maternally inherited PWS gene(s) are normally silent and that it is only the paternally inherited PWS gene(s) that are expressed. Paternal UPD occurs rarely in AS, but indicates that the AS gene(s) are active only when maternally inherited[3,6]. Therefore, the parental origin determines the clinical nature of the syndrome and both parental contributions of chromosome 15q11–q13 are required for normal development.

About 5% of PWS and AS patients inherit a copy of chromosome 15 from each parent, but they have abnormal DNA methylation and gene expression, typical for the syndrome, throughout the imprinted 15q11–q13 region[3,6,9–15] (T. Ohta *et al.*, unpublished) (Fig. 2). This suggests that such patients have a mutation in the imprinting process itself. About a half of the PWS and AS imprinting-mutation patients have a detectable microdeletion[3,9–15] (T. Ohta *et al.*, unpublished) and half do not[12,16] (T. Ohta *et al.*, unpublished; B. Horsthemke *et al.*, unpublished). However, mutation analysis in the non-deletion cases has not identified any mutations. These non-deletion PWS and AS imprinting-mutation cases might arise from a developmental or stochastic failure to switch the imprint in the germline (T. Ohta *et al.*, unpublished) (see below). Consistent with this hypothesis, all non-deletion cases are sporadic, whereas the microdeletion cases are familial[12,16] (T. Ohta *et al.*, unpublished).

In PWS, a few rare cases have a balanced translocation in 15q11–q13 (Ref. 17; Fig. 2), although the mechanism of PWS is unknown in these cases and probably involves a disruption of chromatin structure. In AS, about 20% of patients do not have a deletion, UPD, nor imprinting mutation, and inherit a copy of chromosome 15 from each parent (Fig. 2). Such cases were predicted to have an AS gene mutation and have recently been shown to result from mutations in the *UBE3A* gene[18,19]. This molecular class is absent in PWS, suggesting that PWS results from the loss of expression of two or more paternally expressed genes. Patients with mutations in a single gene would have only a partial PWS phenotype and be unrecognized clinically.

The AS gene (*UBE3A*) encodes a ubiquitin-protein ligase

The location of the AS gene was initially narrowed down by the study of rare patients with atypical deletions[3] and the identification of an inversion breakpoint within

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a candidate gene[18]. This was the previously characterized *UBE3A* gene, which has been shown to have mutations in four AS patients, including familial cases[18,19]. These include a *de novo* 5 bp duplication and a maternally inherited splice mutation, both leading to frameshifts and premature translation termination[18], as well as two additional *de novo* events, a missense mutation and a 2 bp deletion leading to a frameshift[19]. Nevertheless, until mutations in *UBE3A* are identified in many other such cases there is a formal possibility that other maternally expressed genes could contribute to the AS phenotype. The *UBE3A* gene encodes an E3 ubiquitin-protein ligase (UBE3A, formerly E6AP)[20–22], that probably functions to ubiquitinate a diverse range of protein substrates in targeting those proteins for rapid turnover in the cell[21–24] and, thus, might explain the pleiotropic clinical features of AS.

Initial studies demonstrated that the human *UBE3A* gene was not imprinted in the tissues analyzed[18,19,25]. Nevertheless, genetic evidence clearly indicates that the AS gene must be imprinted. Recent studies in the mouse[26] (D.K. Johnson *et al.*, unpublished) and human[27,28] have shown imprinting and maternal-only expression of the *UBE3A* gene in specific regions of the brain, particularly in the hippocampus and cerebellum. The molecular basis for spatially restricted imprinting of *UBE3A* is unknown, but could involve differentially regulated promoters[26,28] or isoforms produced by splicing[18,29], or mechanisms directly inhibiting expression of the paternal allele, such as overlapping or antisense gene regulation. Incomplete imprinting of *UBE3A* might also explain the milder AS phenotype associated with UPD (Ref. 30) and imprinting mutations[15].

Candidate genes for PWS

Chromosome 15q11–q13 has three subregions (Fig. 3a), including a distal non-imprinted region, a central region containing a gene expressed from the maternally inherited chromosome only, involved in AS, and a proximal region containing paternal-only expressed genes, at least some of which are involved in PWS. At least seven genes and ESTs expressed from the paternal chromosome only have been identified in 15q11–q13 (Refs 3, 11, 31–34; Fig. 3a) (M.T.C. Jong *et al.*, unpublished; T.C. Gebuhr *et al.*, unpublished), each a candidate for involvement in PWS. It is likely that additional paternal-only imprinted genes will be found in uncharacterized regions between *ZNF127* and *UBE3A* (Fig. 3a). Should mutations not be found in a major PWS gene in PWS-like patients, identification of the critical PWS genes will require transgenic mouse models. While many clinical features of PWS can be attributed to a hypothalamic defect, the pathology is often more limited than the expression patterns of an etiological gene in disease, making limited hypothalamic expression of a key gene unlikely. *IPW* does not encode a protein, similar to two other imprinted non-translated RNAs implicated in regional regulation, *H19* and *Xist* (Ref. 33); however, mutations in the imprinting process do not map to this location (Fig. 3a, c). A transcript in the imprinting center (IC), or regulatory region, of 15q11–q13 (Ref. 13) is discussed below. While X-inactivation has some similarities to imprinting, X-inactivation leads to chromosome-wide silencing in somatic cells[35], whereas the IC controls imprint switching in the germline (see below); thus, the processes are quite distinct.

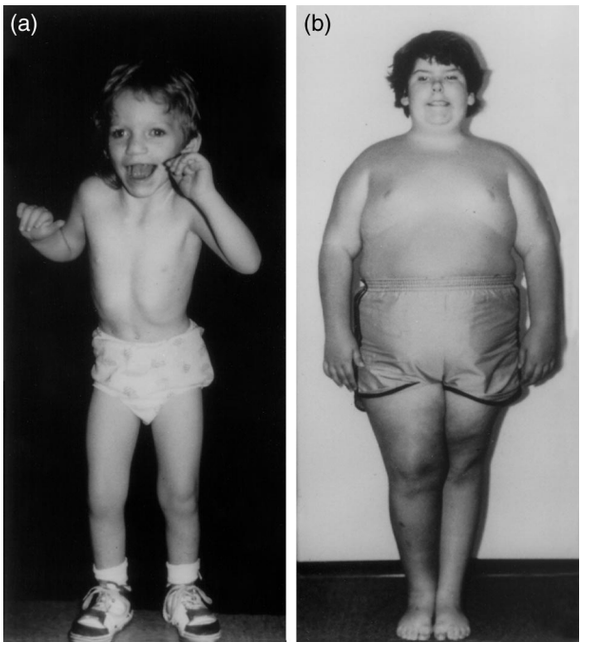

FIGURE 1. Clinical phenotype of imprinted disorders. (a) Angelman syndrome. The characteristic happy disposition, laughter, widely spaced teeth and wide mouth, and stiff, upheld arms and broad stance are exhibited. (b) Prader–Willi syndrome. This patient displays the typical central obesity, short stature, small hands and feet, and mild facial dysmorphism.

Intriguingly, each of the imprinted genes in 15q11–q13 appears to be brain-specific in expression or imprinting in human or mouse[3,11,26–28,31–34] (M.T.C. Jong *et al.*, unpublished), and are associated with neurobehavioral syndromes (PWS and AS). As discussed below, each such gene is coordinately controlled by imprint switching in the germline. Together, these findings imply the evolution and function of a developmentally linked domain of imprinted genes, a situation similar to that in BWS (Ref. 4).

Imprinting mutations in PWS and AS

During the life cycle, imprinting must switch at each generation and be reset in developing germ cells, so that the maternal or paternal imprint is specific for the sex of the individual. Perhaps one of the most remarkable findings to come from study of PWS and AS is that of patients with mutations in this imprint-switch process. This is critical not only for a molecular understanding of imprinting in 15q11–q13, but potentially for other imprinted chromosomal regions because similar mutations in the imprinting process also occur in multiple types of pediatric and adult cancers, and BWS (Refs 4–6). These PWS and AS patients have the classical clinical phenotype[15], and about half the cases are familial[10,12–15] (R.D. Nicholls *et al.*, unpublished).

Approximately half of the cases with imprinting mutations, including all familial cases, have an inherited microdeletion overlapping the first exon (PWS)[10–12] (T. Ohta *et al.*, unpublished) or upstream of the first exon (AS)[12–14] of the *SNRPN* gene (Fig. 3c). The microdeletions define the IC (Fig. 3c) and examination of the inheritance patterns of IC microdeletions have shown

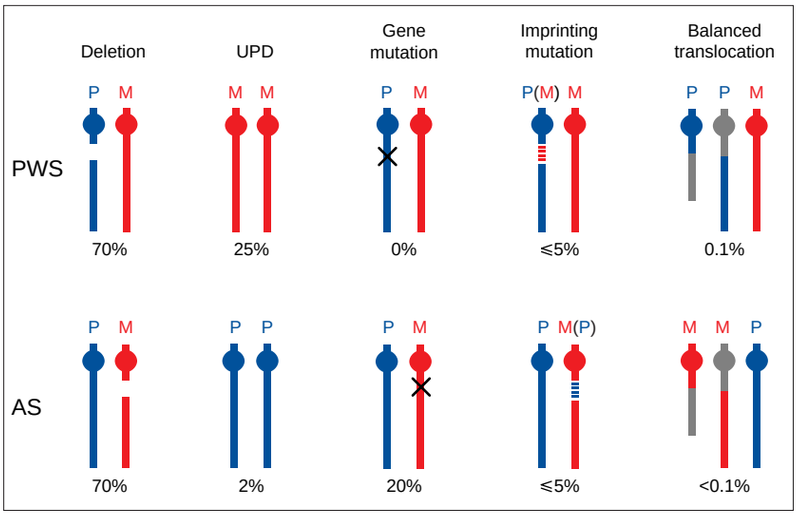

FIGURE 2. Molecular classes of Prader–Willi and Angelman syndrome. Each genetic event is parental-specific in PWS and AS. Deletions and imprinting mutations occur with equal frequency in both syndromes, whereas UPD is more common in PWS than AS because of higher rates of maternal nondysjunction. The gene mutation class in AS appears to be lacking in PWS, which probably indicates that PWS represents a contiguous gene syndrome. Abbreviations: hatched chromosome, non-chromosome 15 in rare balanced translocations; M, maternal (red); M(P), maternal inheritance of imprinting center (IC) mutations with a fixed paternal epigenotype (horizontal lines); P, paternal (blue); P(M), paternal inheritance of IC mutations with a fixed maternal epigenotype; UPD, uniparental disomy.

that these can transmit silently through many generations, as long as the transmitting sex does not change. Because imprinting is a reversible process, in which the germline imprint must be erased and reset each generation, this provides an explanation for the inheritance patterns in these PWS and AS families (Fig. 4).

When a maternally derived imprinting mutation is transmitted through a male, the maternal epigenotype* cannot be reset in his germline to the normal paternal epigenotype (Refs 10, 12–15, T. Ohta *et al.*, unpublished; Fig. 4a). Thus, this mutation blocks the ability to perform the maternal-to-paternal (mat→pat) imprint switch (Fig. 3c) in the male germline, resulting in transmission of a maternal imprint to half his gametes. Offspring inheriting the abnormal gametic epigenotype from their father also inherit a normal maternal epigenotype from their mother. Thus, they are homozygous for a maternal epigenotype (Fig. 4a) and PWS develops. Similarly, transmission of a paternally derived imprinting mutation through a female results in failure to switch the imprint and inheritance of an abnormal paternal epigenotype from the mother, along with a normal paternal imprint (Fig. 4b). The resulting offspring are homozygous for a paternal imprint and hence develop AS. These latter mutations, therefore, block the pat→mat imprint switch in the female germline (Fig. 3c).

In such PWS and AS patients, the mutation has no direct effect in the patient. Rather, the mutation blocks the switch of the grandparental imprint in the parental germline and it is a consequence of this effect that genetic disease arises in half the offspring. This clearly represents a new paradigm as a mechanism of inherited genetic disease.

To define a critical IC element in AS imprinting mutation patients, the shortest region of microdeletion overlap (SRO) has been defined which is 2 kb (Ref. 14; Fig. 3c). Interestingly, this 2 kb AS-SRO region is inside intron 3 of a novel transcript in the IC, representing alternative 5′ exons of the *SNRPN* gene[13] (Fig. 3b). These 5′ exons are expressed about 100-fold lower in somatic cells than the *bona fide SNRPN* transcript initiating at a CpG-island[13] (Fig. 3b). The 5′ exons are now referred to as the *IC* transcript, because it is postulated to have a different function than that of the protein-coding function of *SNRPN*. Like *SNRPN*, the *IC* transcript is imprinted and paternally expressed only, in brain and heart[13]. In PWS, an SRO of 4 kb is defined by analysis of seven microdeletions[12] (T. Ohta *et al.*, unpublished). This region defines the critical mat→pat imprint-switch element in the male germline. The PWS-SRO includes exon 1 and the CpG-island promoter of the *SNRPN* gene.

The imprinting center and mechanisms of imprinting

Because all imprinted genes studied have been associated with differential DNA methylation (see Fig. 3b), models have been invoked in which DNA methylation is the key or only regulator of imprinting. Inherent in such models is the idea that DNA methylation represents the gametic mark that is inherited, and imparts the maternal- or paternal-specific allelic information to cells of the embryo and adult[3,31,36,37]. DNA-methyltransferase-deficient mice have been produced that have loss of imprinted gene expression[38], which is consistent with an important role for methylation in imprinting. Nevertheless, these experiments do not distinguish between a role in maintenance or establishment of imprinting.

The *SNRPN* gene is very tightly regulated by imprinting, showing paternal-only expression and complete differential methylation in the promoter in all studied somatic tissues[31,39–41]. Methylation status at *SNRPN* (Fig. 3b) can be assessed by two independent methylation-PCR methods[42,43] (S. Saitoh *et al.*, unpublished), and represents the best single diagnostic tool for AS and PWS, because methylation identifies all such patients with a deletion, UPD, or imprinting mutation[3,9,11,15,31,44]. *SNRPN* contains two regions with DNA-methylation imprints[31,41]: the exon 1 region, which is unmethylated on the expressed paternal allele and methylated on the silenced maternal allele; and a region in intron 7, which is methylated on the paternal allele and unmethylated on the maternal allele (Fig. 3b). Both methylation imprints appear to be present in the gametes (sperm and oocytes), suggesting that one or both sites represent the gametic imprint for this gene[31,41]. Similarly, the mouse *Igf2r* gene has the gametic

* The term epigenotype refers to information that is heritable and alters the phenotype of offspring, but is not encoded specifically in the primary sequence of the DNA.

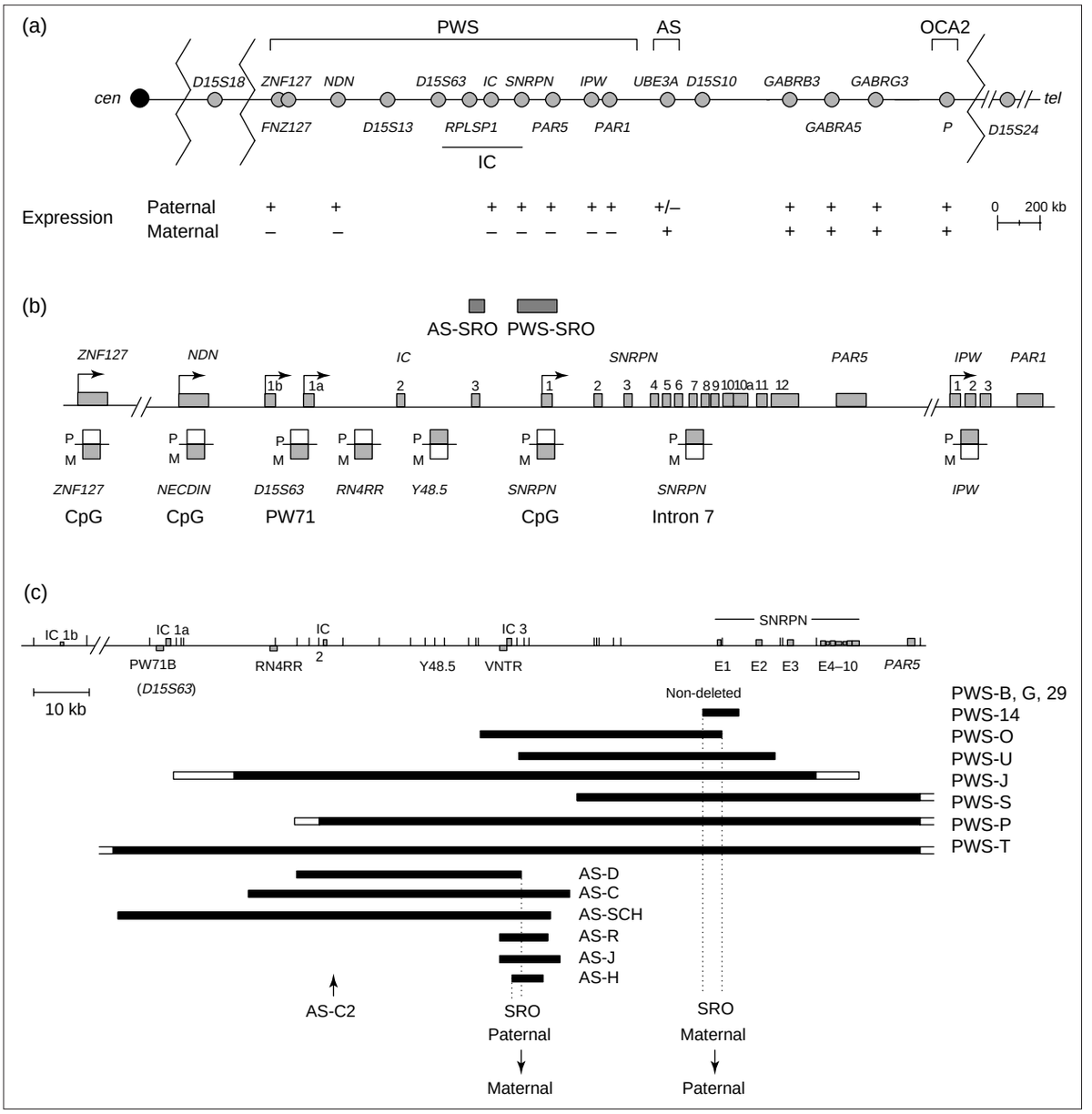

FIGURE 3. Genetic maps of human chromosome 15q11–q13. (a) Genes and imprinting. The position of genes and reference markers (gray circles), transcription from paternal or maternal alleles (+, expressed; –, not expressed), the common cytogenetic deletion breakpoints (zigzag lines), and critical regions involved in PWS, AS, oculocutaneous albinism (OCA2; Ref. 59), and the imprinting center (IC) are shown. (b) DNA methylation patterns of imprinted genes. Shown are exons of genes (gray boxes), transcription start site of each gene (arrows) where the *IC* transcript has two alternative start sites, and unmethylated (open boxes) or methylated (filled boxes) sequences. Abbreviations: CpG, CpG-island; M, maternal allele; P, paternal allele. (c) Structure of the imprinting center (IC). The IC spans the 150 kb region including exons of the *IC* transcript through the *SNRPN* CpG-island at exon 1 (black boxes). The shortest region of microdeletion overlap (SRO) in a series of AS and PWS patients with an imprinting mutation is illustrated[12–14] (T. Ohta *et al.*, unpublished). These deletions lead either to failure to switch the pat→mat imprint in the female germline and, hence, to AS in offspring inheriting the mutation, or to failure to switch the mat→pat imprint in the male germline, leading to PWS in offspring. A point mutation in family AS-C2 occurs in the donor splice site of the *IC* transcript[13]. VNTR, variable number of tandem repeat sequence[14].

methylation imprint on the expressed maternal allele, but only in the body of the gene, and in this case it has been shown that the promoter of the paternal allele is methylated and inactivated solely as a postimplantation event[36]. Thus, a repressor might bind the allele that is parentally unmethylated, inactivating that allele and locking in the imprinted state[36]. This event now appears to involve a paternally expressed, noncoding antisense RNA (Ref. 45).

But how are the correct DNA-methylation patterns established in human chromosome 15q11–q13? It has been shown that imprinted chromosomal regions show homologous association[46], in addition to asynchronous replication[47]. These phenomena are not required to establish imprinting of *Igf2r* or *Igf2-H19* in hemizygous YAC transgenes[45,48] and, therefore, might not have a mechanistic role but arise simply as a consequence of

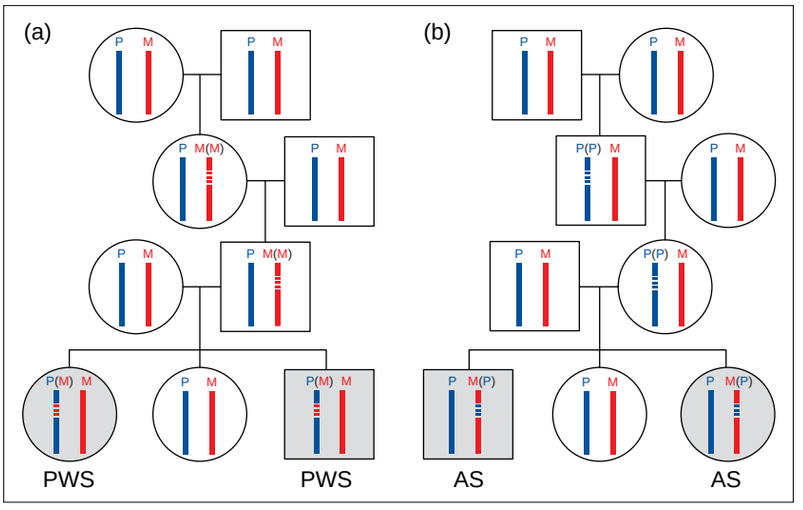

FIGURE 4. Inheritance of imprinting mutations: failure of imprint switching in the parental germline. The inherited chromosome 15 imprint (epigenotype) in somatic cells is shown inside the female (circle) and male (square) symbols, whereas the germline imprint is discussed below and in the text. (a) PWS: if a mutation arises in the germline of an ancestral female, it is postulated to fix the maternal imprint into the chromosome [M(M)]. Because it has a maternal imprint, it transmits silently (normal phenotype) to the next generation. However, in a male, the mutation blocks resetting of the maternal imprint, such that a paternal chromosome with a maternal imprint [P(M)] is transmitted. Consequently, 50% of children have inherited maternal imprints only, and so develop PWS. (b) AS: a paternal imprint fixed into the chromosome in an ancestral male [P(P)] can be transmitted silently through males, but in the female germline, the mutation blocks the imprint switch from a paternal imprint. A female then transmits a maternal chromosome with a paternal imprint [M(P)], and because 50% of her children inherit this chromosome, they develop AS.

differences in chromatin structure between parental alleles. On the other hand, genetic data, as discussed above, suggests that there is an IC that plays an important role in the control of imprinting in the PWS and AS region. Models have been proposed to account for the behavior of the IC. For example, expression of the *IC* transcript on the paternally derived chromosome might be integrally involved in the pat→mat imprint switch in the female germline[13]. Expression of the *IC* transcript, which splices to *SNRPN* exon 2, might prevent expression of *SNRPN* transcripts from the CpG-island promoter. How the switch is completed is unknown, but it might involve *trans*-acting factors binding to specific sites in the IC region[13], or the propagation of heterochromatic-like chromatin states[12]. A point mutation in the splice donor site of the *IC* transcript exon 2 in one AS family (Fig. 3c) provides supporting evidence for a role of the *IC* transcript in the pat→mat switch in the female germline[13], as does deletion of at least one exon in all but one AS microdeletion[13,14]. Nevertheless, this exceptional microdeletion maps within an intron (Fig. 3c), which could affect a splice or transcriptional enhancer of the *IC* transcript. Alternatively, this region might represent a binding site for a *trans*-acting factor required to complete the pat→mat switch. The mechanism by which the IC signal is transmitted *in cis* to the *UBE3A* gene promoter located several hundred kilobases away is unknown.

Several models for the molecular basis for initiation of imprint switching in the male germline can be considered. In one model, the binding of a transcription factor (TF) to the methylated maternal chromosome initiates *SNRPN* transcription from the promoter (T. Ohta *et al.*, unpublished). The binding of a TF and activation of transcription might serve to initiate the imprint switch, because expression of *SNRPN* is associated with the paternal imprint of this gene. Transcription could be coupled to concomitant or subsequent demethylation of the promoter and methylation of intron 7 (see Fig. 3b) in the germline[41]. The IC signal then transmits bidirectionally *in cis* to genes located up to 1 Mb away (Fig. 3a).

A second, but not mutually exclusive, model proposes that the *SNRPN* exon 1 region represents a nucleation center for heterochromatin assembly and disassembly, which is made accessible by the *IC* transcript. Similar mechanisms have been proposed for the role of *Xist* in X-inactivation[49], and for the inheritance of active and inactive patterns of gene expression during somatic cell divisions in *Drosophila*, which involve higher-order chromatin structures[50]. Here, the Polycomb-group (Pc-G) factors interact with Pc-G-responsive elements to induce heterochromatin-like structures, which are counteracted by the trithorax-group (trx-G) factors. Thus, regions involved in the control of imprinting, such as the promoter/exon 1 region of *SNRPN* might contain a Pc-G/trx-G responsive element, which can be tested in transgenic flies because Pc-G regulated chromatin can silence reporter genes by inhibiting the accessibility of a *trans*-activator to its target DNA (Refs 50–52). Using this system, strong silencing was observed in constructs containing the *SNRPN* exon 1 region, extending from 200 bp upstream to 500 bp downstream[52]. Silencing was bidirectional and effective over more than 1 kb. These findings suggest that a putative silencer element participates in the somatic repression of the maternal *SNRPN* allele or in the long-range regulation of the imprinted 15q11–q13 domain, the latter requiring additional interacting secondary sites. Erasure of this repressive chromatin imprint in primordial male germ cells would then require an activating factor, as described above, which interacts with the silencer to displace the silencing proteins. A maternally derived deletion of this target sequence, as observed in fathers of PWS imprinting-mutation patients would, therefore, result in a failure to erase the maternal imprint in the male germline and, consequently, in the transmission of a paternal chromosome with a (grand)maternal imprint (Fig. 4a). Conversely, the pat→mat element, mutated in AS imprinting mutation patients, might be required to erase the paternal imprint in the female germline to re-establish silencing[13].

Evolutionary conservation of imprinting

Genes from human chromosome 15q11–q13 have homologs in mouse chromosome 7C (Refs 3, 34, 53–57; M.T.C. Jong *et al.*, unpublished) (Fig. 5). Genetic breeding

of chromosome rearrangements has led to animals with maternal or paternal UPD for the central region of chromosome 7 (Fig. 5), which have an imprinted phenotype[53,57]. The maternal UPD is a genetic model of PWS, but the animals die postnatally before weaning[53]. This might be a consequence of hypotonia and failure to thrive, as with PWS neonates[3,6]. In contrast, the paternal UPD phenotype could represent either the effect of the *Ube3a* gene[57] or imprinted genes near the imprinted *Peg3* gene[59] in the centromeric portion of chromosome 7. Nevertheless, the paternal UPD mice have phenotypic similarities to AS (Ref. 57), although improved models will be generated by targeted mutagenesis of *Ube3a* or the imprinting process. Imprinted genes in the mouse include at least five conserved, paternally expressed genes[3,34,53,54,56] (T.C. Gebuhr *et al*., unpublished; M.T.C. Jong *et al*., unpublished), and the limited maternal expression of *Ube3a* (Ref. 26; D.K. Johnson *et al*., unpublished) (Fig. 5).

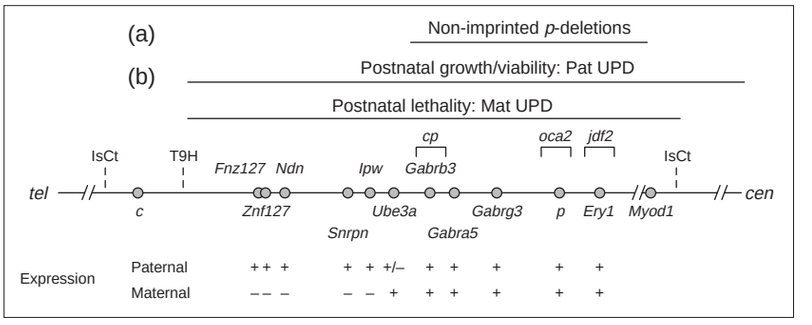

FIGURE 5. Genetic map and imprinting in mouse chromosome 7C. Illustrated are the position of genes and genetic markers (circles), transcription from paternal or maternal alleles (+, expressed; –, not expressed), and genetic regions involved in (a) non-imprinted, radiation-induced deletions at the *p*-locus[54,55] and (b) imprinted uniparental disomy (UPD) phenotypes[53,57]. UPD is generated by breeding and segregation of the balanced T50H (not shown) or T9H translocations, or the IsCt inversion/insertion[53,57] (dashed lines). The UPD phenotypes represent genetic mouse models of PWS (Mat UPD) and AS (Pat UPD).

Recently, we have shown that expression and methylation imprints for imprinted genes in 15q11–q13 and 11p15 are maintained in rodent–human somatic cell hybrids, demonstrating that the somatic imprint signals are recognized when human chromosomal DNA is repackaged with mouse chromatin proteins (J.M. Gabriel *et al*., unpublished). In addition, because each imprinted gene in mouse 7C and human 15q11–q13 are conserved in relative chromosomal position, gene structure, sequence and imprinted status, the mechanism of imprinting is probably conserved in the evolutionary distance between these two mammalian species.

The molecular life cycle of imprinting

The mechanism by which imprinting is set in the germline, maintained in embryogenesis and postnatal development, and reversed in the germline, is largely unknown. However, based on the descriptions above of gene-specific DNA-methylation imprints and of an IC involved in germline switching of the imprint over a 2 Mb imprinted domain, we can formulate a model for the regulation of imprinting in 15q11–q13 throughout the mammalian life-cycle. The primary step in initiation of imprint switching in the mammalian germline is the IC function. This is followed by spreading of the IC signal bidirectionally across the 2 Mb imprinted domain, and recognition of this signal in the germline by all target imprinted genes (Fig. 3a; Fig. 5), with subsequent setting of gene-specific DNA methylation that is distinctive for the male or female germline. After fertilization, some genes are regulated by the gametic imprint in early preimplantation development (B.M. Cattanach *et al*., unpublished), whereas other genes carry the gametic methylation imprint in the body of the gene and undergo subsequent promoter methylation in order to lock in the imprinted state, which in turn dictates monoallelic expression of the gene. Further understanding of the mechanisms of imprinting and pathophysiology of PWS and AS will come not only from continued analyses of these patients, but also rely upon the development of mouse models in which to study the critical germline and brain tissues.

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Something from nothing: the evolution and utility of satellite repeats

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Large blocks of tandemly repeated sequences, or satellites, surround the centromeres of complex eukaryotes. During mitosis in Drosophila, satellite DNA binds proteins that, during interphase, bind other sites. The requirement for a repeat to borrow a partner protein from those available at mitosis might limit the spectrum of repeat units that can be expanded into large blocks. To account for the ubiquity and pericentric localization of satellites, we propose that they are utilized to maintain regions of late replication, thus ensuring that the centromere is the last region to replicate on a chromosome.

Tandemly repetitive sequences have long presented a genomic puzzle: they are ubiquitous, yet they are conserved in neither content nor occurrence. These sequences were originally identified as satellite bands in ultracentrifuge density gradients of complex eukaryotic genomes, but now the term 'satellite DNA' is applied to any tandem repetitive sequence[1]. Satellite DNA units can be divided into two classes: short sequence repeats consisting of 2–20 bp units, and more-complex sequence repeat units of a few hundred base pairs. Repeats are present in blocks of hundreds to thousands of tandem units. On a typical chromosome, larger repeat blocks reside primarily in the vicinity of the centromere, whereas 'microsatellite' repeat arrays are more uniformly distributed[2–4]. The sequence biases of repeats give distinct chromosomal banding patterns when mitotic chromosomes are stained with cytological dyes, such as Quinacrine and Giemsa. Blocks of satellite DNA around the centromere are constitutively heterochromatic, remaining condensed and transcriptionally inactive during interphase, unlike repetitive portions of the euchromatic chromosome arms. Despite extensive characterization of satellite sequences in mammals and *Drosophila* over the past ~30 years, no sequence-specific role has been definitively established.

Satellite-binding proteins

Recently, proteins have been described that interact with pericentric heterochromatin in mitotic chromosome spreads, leading to renewed speculation concerning the function of these sequences. Some patterns of interaction have been interpreted as specific binding to a subset of simple-sequence repeats in heterochromatin (Table 1), while others have been interpreted as binding to heterochromatin in general, or to the centromere in particular. One of these proteins is *Drosophila* GAGA factor, which has been extensively studied because of its involvement in the regulation of transcription of several genes, including *hsp70*, *hsp26*, *actin 5C* and *Ubx* (Ref. 5). At least in some cases, GAGA factor is thought to work by facilitating the repositioning of nucleosomes[6]. As its name implies, GAGA factor has affinity for binding to sites of high GA content in the regulatory regions of genes. Binding of anti-GAGA antibody to the interphase polytene nuclei of *Drosophila melanogaster* larvae detects

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