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REVIEW

The origin and evolution of the pseudoautosomal regions of human sex chromosomes

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The human X and Y chromosomes share two homologous pseudoautosomal regions (PARs) which pair and recombine at meiosis. PAR1 lies at the tips of the short arms, and the smaller PAR2 at the tips of the long arms. PAR1 contains several active genes, and has been thought to be critical for pairing and fertility. The inconsistent gene content of the PARs between different species of eutherian ('placental') mammals suggests that gene content is immaterial to function, and the failure to detect a PAR at all in some rodents and all marsupials implies that homologous pairing is not universally essential for fertility. The autosomal localization of marsupial homologues of human PAR1 genes and their co-localization with human Xp22 genes implies that the human PAR1 represents a relic of part of an autosomal region added to both X and Y chromosomes between 80 and 130 MYrBP. The same argument may be made for part of PAR2. Independent additions to the sex chromosomes of macropodid marsupials and monotremes can also be inferred from comparative mapping. We conclude that the PARs are relics of differential additions, loss, rearrangement and degradation of the Y chromosome in different mammalian lineages.

THE HUMAN PSEUDOAUTOSOMAL REGIONS

The human X and Y chromosomes are morphologically and genetically distinct. The X is large and euchromatic, and on the basis of its size would be expected to contain 3000–4000 genes, whereas the Y is small and heterochromatic, and contains, at last count, ~30 (1,2). However, at either end of the sex chromosomes are X–Y homologous regions. These pseudoautosomal regions (PARs) pair regularly at male meiosis and undergo recombination. PAR1 at the tips of the short arms, is 2.6 Mb long and contains eight genes (*PGPL*, *SHOX*, *CSF2RA*, *IL3RA*, *ANT3*, *ASMT*, *XE7* and *MIC2*) and the 5′ region of a ninth (*XGA*) (3, others reviewed in ref. 4). Only two known genes, *SYBL1* and *IL9R*, have so far been discovered on the shorter (320 kb) PAR2 at the tips of the long arms (5–7).

The part of the differential region in Xp22.3 just proximal to PAR1 also contains numerous genes with active or inactive homologues on the Y (X–Y shared genes). *STS*, *ARSD* and *ARSE*, *PRKX*, *GS1*, *KAL* and *GS2* all detect closely related pseudogenes on the Y whereas *AMELX*, and the more proximal *ZFX*, have active homologues (8). Not surprisingly, since their dosage is equal in males and females, PAR and active X–Y shared genes are not subject to X chromosome inactivation in females. Even X-linked genes, like *STS*, which recognize only pseudogenes on the Y, are exempt from X inactivation. The PAR2 gene *IL9R*, which is expressed from the Y copy, also escapes inactivation on the X (9). However, the Y copy of *SYBL1* is not expressed, and the X copy is subject to inactivation (10).

Because deletion of PAR1 results in failure of pairing and male sterility (11), PAR1 has been thought to be essential for meiotic pairing and male fertility. To discover whether this requirement is universal, and to what extent the gene content of the PAR is relevant to its function, it has been instructive to compare the PARs of different mammal species.

GENE CONTENT OF THE PAR IN OTHER EUTHERIAN MAMMALS

The PAR of eutherian ('placental') mammals has a surprisingly variable gene content for a region credited with such a critical function (Fig. 1). There is variation even within primates, and the mouse and human PARs are completely non-homologous.

However, the overlapping sets of genes within and near the PARs in different eutherian species suggest that they all represent relics of a larger ancestral PAR which once extended from Xp22 to Xpter. It included human X–Y shared genes such as *ZFX*/*Y*, *AMELX*/*Y*, *PRKX*/*Y* and *STS*/*STSP*, as well as the present human PAR genes. This explains why the pairing segments of carnivore and artiodactyl sex chromosomes are larger than the human PAR

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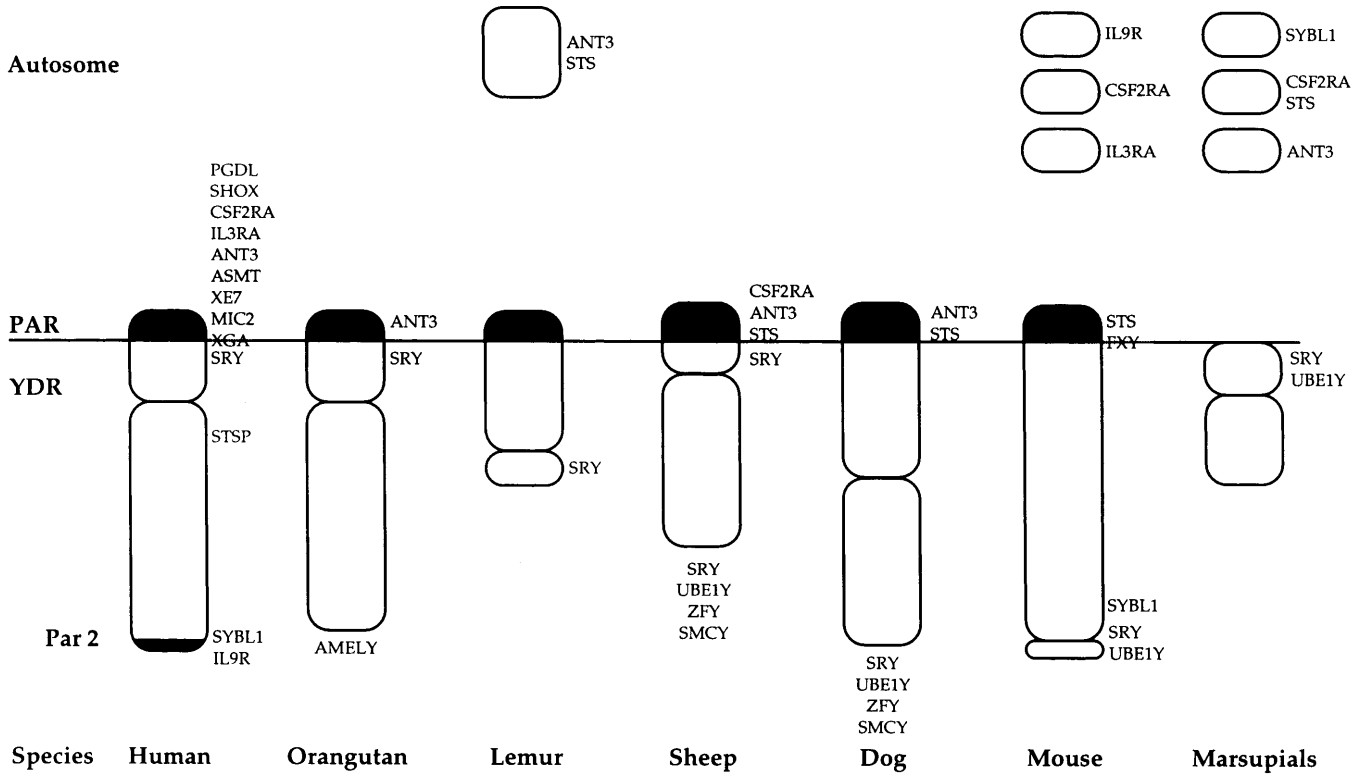

Figure 1. Representation of the gene content of the pseudoautosomal regions (PARs) of the Y chromosomes of different therian mammals: eutherians (human, orang-utan, lemur, sheep, dog, mouse) and marsupial (tammar wallaby). With the exception of the *SRY* gene which determines male sex, only genes which are present in the PAR of at least one therian species are shown.

and apparently include *STS* and *PRKX* as well as *ANT3* and *CSF2RA* (12).

The ~2.0 Mb PAR at the ends of the mouse Xq and Yq contains none of the human PAR genes. Only one active gene, *Sts*, has been detected in the PAR (13,14). The newly recognized *Mid1(Fxy)* gene (whose human homologue lies near the PAR on the X and has no Y partner) spans the pseudoautosomal boundary on the mouse X and detects a truncated partner at the boundary of the PAR on the Y (15–18). In mouse, as in human, *Zfx* has homologues on the Y. All these genes, as well as other human Xp genes such as *AMELX* and *CLC4*, lie in the same order on the human and mouse X. These observations can also be explained by the hypothesis that the whole human Xp22–Xpter region was once part of an ancient PAR, whose boundary has moved to different extents in different species.

However, other observations require gain or loss of regions from the PAR to autosomes. The autosomal location of *ANT3* and *STS* in prosimians (19), and of *IL3RA* and *CSF2RA* in mouse (20,21), could be explained either by differential addition or loss from this ancestral PAR. The absence of these genes from the X and Y in prosimians and mice, and doubts about the homology of human and mouse *Sts*, initially suggested that the whole *CSF2RA–STS* region might have been added only recently to the primate sex chromosomes. However, the recent finding of human PAR and Xp22.3 genes within the pairing segment on the tips of the X and Y in carnivores and ungulates, and the demonstration that the cloned mouse *Sts* is, after all, homologous to human *STS*, means that this region must have been a part of the sex chromosomes for at least 80 million years (12,14). Parts of the PAR have therefore been lost independently in prosimians and rodents.

The question of the origin of the human PAR2 has been addressed recently by comparative mapping of PAR2 sequences and genes in other mammals. A sequence now known to lie within human PAR2 was shown by Southern analysis to be X-specific in great apes (22), and the sequence at the PAR2 boundary on the X and Y suggests that illegitimate recombination between LINEs resulted in a transposition from the X to the Y (23). A mouse homologue of *SYBL1* maps close to the centromere of the mouse X (10). These data imply that PAR2 was part of the original eutherian X, but recently was transposed to the Y in the primate lineage. However, the more distal *IL9R* is autosomal in mouse (9), so it may also have been part of an ancient X chromosome and subsequently been lost in mouse, or may be part of a very recent addition to both sex chromosomes in the primate lineage.

Thus the variation in PAR gene content reflects the stochastic processes of differential addition, rearrangement and Y degradation.The inconsistent gene contents of the PAR in different eutherian species argues that the PAR plays no sequence-dependent role in fertility.

DO WE NEED A PAR AT ALL?

Since the suggestion of Koller and Darlington (24) that the mammalian X and Y chromosomes share a pairing region and the demonstration of pairing between the human X and Y (25), it has been expected that the PAR serves a critical function in mammalian spermatogenesis. Indeed, there is much evidence that XY pairing is essential for spermatogenesis in mice and humans (11,26), and non-homology of this region may contribute to male infertility in species hybrids which obey Haldane's rule (27). However, there are some rodent species which apparently accomplish meiosis without the benefit of homologous pairing (e.g. ref. 28), and the necessity for homologous pairing has been challenged even for mouse and man (29).

The absence of a PAR is most striking in marsupials. Cytological work first led to the conclusion that the small X and Y of Australian and American marsupials failed to undergo homologous pairing at male meiosis. The condensed X and Y remained separate until mid-pachytene, when they formed end-to-end attachments but never underwent synapsis or crossing over (30,31).

Since it remained possible that chiasmata in the condensed sex chromosomes evaded detection, X–Y homology in marsupials recently was re-investigated using chromosome painting in a dasyurid species which has a minimal X and a tiny Y. When DNA from a female *Sminthopsis macroura* was painted onto a male chromosome spread, the X chromosome and autosomes labelled strongly, but the Y remained completely unlabelled. Conversely, painting with the microdissected Y produced no signal over the X or autosomes (Toder *et al*., in preparation). Thus there is no molecular evidence for a PAR in this species. Even in kangaroo species whose X and Y share recently added heterochromatic regions (32), these do not pair.

The absence of a PAR and of homologous pairing between the marsupial X and Y apparently causes no disruption to regular segregation at meiosis, implying that a PAR is not a universal necessity for mammalian sex chromosome pairing and male fertility.

THE ORIGIN OF THE HUMAN PARS

Pursuing the origin of the human PAR into even earlier mammalian ancestors requires comparative mapping between more distantly related mammal groups. Marsupials and monotremes diverged independently from eutherian mammals ~130 and 170 MYrBP respectively.

Comparisons have already provided valuable insights into the origins of regions of the human sex chromosomes. Marsupials have a small basic X and a tiny Y, which, as we have seen, do not undergo homologous pairing. Monotremes are just the opposite, having large sex chromosomes which pair over the entire short arm of the X and long arm of the Y (33). Genes on the long arm and pericentric region of the human X lie on the X in all three mammal groups (8). This X conserved region (XCR) therefore represents an ancient X in a common ancestor at least 170 million years ago. The presence of three human and/or mouse X–Y shared genes (*RPS4X*/*Y, SMCX*/*Y* and *Ube1x*/*y*) on the X and Y in marsupials implies that at least part of this ancient X was also shared with the ancient Y. The large pairing region of the monotreme X and Y chromosomes contains several classic human X-linked genes with no Y homologues in any eutherian species (34), as well as the *UBE1Y* gene (35) which is present on the differentiated regions of the X and Y in all therian mammals except primates. This implies that at least some of the conserved region of the X was originally pseudoautosomal in an ancestral mammal.

In contrast, genes on the differential region of Xp distal to the fusion point at Xp11.23 (36) are autosomal in marsupials and monotremes, lying in at least two conserved clusters. Since marsupials and monotremes diverged independently from eutherians, this implies that most of the human Xp was added to the eutherian X after the divergence of eutherians and marsupials 130 MYrBP, but before the eutherian radiation 80 MYrBP. This X added region (XAR) harbours most of the X–Y shared genes, implying that addition must have been made to both the X and Y. The human X and Y is proposed to have evolved by cyclical additions to the PAR, offset by progressive degradation of the Y chromosome (8).

The demonstration that the eutherian X is composed of ancient and recently added regions raises questions concerning the origin and the function of the human PARs. Are the PARs merely parts of the XAR which, for reasons of their recent addition, have not yet been degraded on the Y? Alternatively, are the PARs ancient features of the mammalian X and Y chromosomes, conserved through evolution because they maintain equal dosage of some critical PAR gene(s) between males and females, or sequences which perform a function in meiotic pairing and male fertility?

The recent finding that cloned marsupial homologues of human and mouse pseudoautosomal genes *CSF2RA*, *ANT3* and *STS* are autosomal in the tammar wallaby (37) implies that the eutherian PAR is a recent addition. Marsupial *CSF2RA* and *STS* co-localized with seven other human Xp genes within a single autosomal cluster in marsupials, implying that the human PAR was part of the larger autosomal addition to the X and Y 130–80 MYrBP. Marsupial *ANT3* maps separately, so it may represent a region independently added to the eutherian PAR, or rearranged in marsupials.

We conclude, therefore, that the human PAR1 was part of the autosomal region(s) transferred relatively recently to the eutherian X and Y chromosomes.

The origin of PAR2 is more ambiguous. *SYBL1* recently was mapped to the distal region of Xq in the marsupial *Potorous tridactylis* (10), and it was concluded that the PAR2 was part of the XCR that represents the original mammalian sex chromosomes. However, unbeknown to the authors of this interesting work, the long (satellited) arm of the potoroo X is actually an autosome in disguise. The potoroo is one of several marsupials in which a recent X–autosome fusion has produced a classic XY_1Y_2 sex chromosome system (38). Xq pairs with Y_2, which represents the original autosome (Fig. 2). An autosomal location of *SYBL1* in marsupials could mean that PAR2, like PAR1, was not a part of the original mammalian X, but was added to the X and Y 130–80 MYrBP. Alternatively, the region could have been part of the original X, which subsequently was lost in the marsupial lineage. Its presence on the human Y may derive from an ancient paired X and Y, or may be the result of more recent illegitimate recombination between the primate X and Y (23).

INDEPENDENT PSEUDOAUTOSOMAL ADDITIONS TO SEX CHROMOSOMES

Comparisons between the most distantly related mammals also provide evidence that different additions to sex chromosomes have occurred independently in different lineages.

The PAR of platypus and echidna sex chromosomes contains, as well as genes which are on the conserved XCR, a large euchromatic region to which no genes have yet been mapped. This presumably contains genes that are autosomal in eutherians, and represents an independent addition to both sex chromosomes in an ancestral monotreme.

Another independent addition is represented by relics of a nucleolus organizer region (NOR)-bearing segment on the sex chromosomes in kangaroo species. Macropodid X chromosomes all contain ribosomal DNA on a heterochromatic short arm. The Y chromosome in at least one kangaroo species (*Macropus parryi*) retains an active NOR (39), and chromosome painting reveals sequences shared between the heterochromatic tammar wallaby Xp and the Yq (32). This suggests that the NOR-bearing region was added to the X and Y in an ancestral macropodid, and was originally pseudoautosomal, but that the rRNA genes have degraded in most species and the heterochromatin on the X and Y has lost homology.

The process of sequential addition was taken to extremes by another kangaroo species, in which an autosomal region representing ~20% of the genome was added to both the X and Y chromosomes atop the NOR addition. The swamp wallaby, *Wallabia bicolor*, has what looks like a classic XY_1Y_2 system in which a large autosome was added to the NOR-containing arm of the original X. The Y_2 represents the second copy of this autosome (itself a fusion of chromosomes 2 and 7 in other kangaroo species). However, chromosome painting reveals that the Y_2 also contains sequences from the shared region of the X and Y, implying that originally the autosome was added to both sex chromosomes (within the PAR provided by the NOR addition), then subsequently lost from the Y_1 (40).

The inconsistent gene contents of the PAR in different mammal groups therefore reflect differential addition, as well as differential loss, rearrangement and degradation of the Y chromosome.

MODEL FOR THE EVOLUTION OF THE EUTHERIAN PARS

The presence or absence of a PAR, its gene content and its function, can be easily understood in terms of the evolutionary forces that have shaped the mammalian sex chromosomes: addition of autosomal segments to the shared region of proto-X and -Y chromosomes, and progressive degradation of the Y chromosome (8). This addition–attrition hypothesis predicts three sources of variation of the PAR between species (Fig. 3).

Lineage-specific addition or loss of PAR genes may occur by translocations between the PAR and an autosome. Such exchanges would cause minimal disruption to fertility, and do not really constitute transgressions of Ohno's law that the X chromosome is conserved (41,42). Independent additions to the PAR evidently have occurred in eutherians, macropodid marsupials and monotremes. Lineage-specific loss of PAR genes is evident in mouse and prosimians. In addition, the recent finding that *Clcn4*, lying in the differential region of the X distal to *ZFX* and *AMELX* in human and *Mus spretus*, is autosomal in *M.musculus* (43,44) is understandable if this gene were part of an ancestral PAR which could be exchanged with autosomes without causing sterility (45).

Another source of PAR variation is the different extent of degradation of the Y in different lineages, either by accumulation of small changes at the boundary or by major rearrangement.

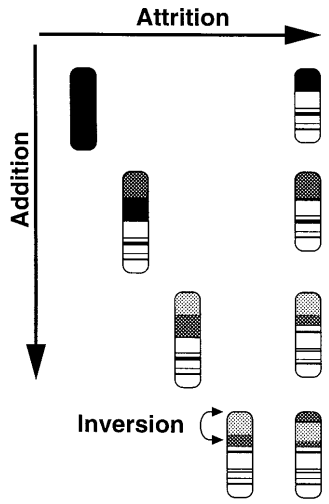

Figure 2. Variation of the gene content of the PAR predicted by the addition–attrition hypothesis for the evolution of mammalian sex chromosomes. (i) Attrition. The original Y (black) was homologous to the X except at a male sex-determining locus, around which recombination was suppressed. Deletion and mutation within this genetically isolated region progressively reduced the coding region of the Y, leaving a few functional male-specific genes (black stripes on a background of white non-coding DNA) and a terminal PAR (black) still homologous and recombining with the X. (ii) Addition of an autosomal segment (dark grey) to the PAR of one of the sex chromosomes, followed by recombination onto the other, results in different terminal sequences. Attrition of both the original and the added region leaves a few active genes (black and grey stripes) and a different PAR (dark grey terminal region). This process can be repeated, adding another (light grey) terminal region which ultimately becomes the new PAR. By the same mechanism, genes may be lost from the PAR to an autosome. Different autosomal segments could be added to the PAR independently in different lineages, and attrition would lead to PARs with different gene contents in different lineages. (iii) Rearrangement (in this case inversion) within the paired region of the partially differentiated Y may change the gene order (dark and light grey regions) in some lineages. Progressive attrition leaves a PAR with the gene content of the inverted region.

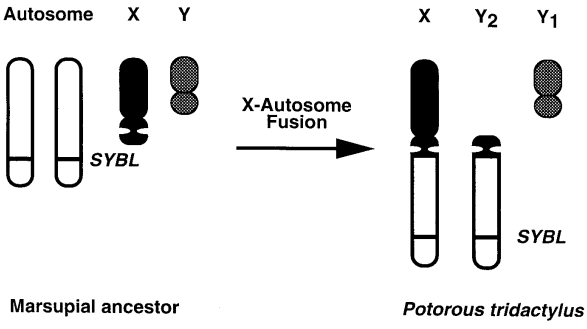

Figure 3. Autosomal location of the human PAR2 gene *SYBL1* in marsupials. The potoroo has an XY_1Y_2 sex chromosome system. The short arm and proximal part of the long arm (carrying the NOR) are derived from the original X, and the remainder of the long arm, bearing *SYBL1*, was originally autosomal.

Gradual loss of homology with the X has been demonstrated by sequence comparisons of the pseudoautosomal boundary in human and great apes (46). Cataclysmic loss of homology

between human Xp22.3 and its Y homologue is ascribed to one or probably more inversions in the primate Y chromosome (47,48). This removed part of the original PAR to Yq11.2, including half of the unfortunate *XGA* gene (49). In their new site, genes such as *STS* degraded rapidly because they were now isolated from recombination.

A third potential source of variation would be an inversion contained within the PAR. This would not, of itself, disrupt homology, but would change gene order so that after degradation, different terminal sequences would remain pseudoautosomal in different lineages.

THE FUTURE OF THE PAR

The end-point in Y attrition would be expected to be the complete differentiation of the X and Y, and the disappearance of the PAR.

This point evidently has been reached by marsupial X and Y chromosomes. The tiny dasyurid X and Y lack any homology (Toder *et al*., in preparation). Even the larger macropodid X and Y, with their recent additon of the NOR region (32), show no homologous pairing (31). In most species, there is no active NOR on the Y (39), suggesting that the sequences on the Y have been inactivated and degraded. A new mechanism for X–Y recognition, association and segregation, perhaps involving telomere sequences, must have replaced homologous pairing and recombination in marsupials.

The mouse PAR also seeems to be in the last throes of degradation. Although it still contains an active *Sts* gene, the atypical GC-rich base composition of the distal 250 kb of the PAR, *Sts* included (14), suggests that it is now being selected for another role (pairing?) at the expense of activity of the genes it bears. The loss of a degraded PAR in the Akodont rodents, and the evolution of other mechanisms to ensure meiotic segregation, may provide a vision of what is in store for the sex chromosomes of other rodents—and maybe primates—in the 100 million years to come.

The only salvation for the PAR will be a further addition to both X and Y, as evidently has occurred in some rodent lineages [e.g. lemmings (50)]. Continued addition to the X and degradation of the Y would produce species in which males are haploid and females diploid for a large fraction of the genome. Taken to extremes, this could eventually lead to a haplodiploid mode of sex determination such as that in some insects.

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