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The Eutherian Pseudoautosomal Region

Terje Raudsepp[a] Bhanu P. Chowdhary[b]

[a]Department of Veterinary Integrative Biosciences, CVM, Texas A&M University, College Station, Tex., USA;
[b]New Research Complex, Qatar University, Doha, Qatar

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Abstract
The pseudoautosomal region (PAR) is a unique segment of sequence homology between differentiated sex chromosomes where recombination occurs during meiosis. Molecular and functional properties of the PAR are distinctive from the autosomes and the remaining regions of the sex chromosomes. These include a higher rate of recombination than genome average, bias towards GC-substitutions and increased interindividual nucleotide divergence and mutations. As yet, the PAR has been physically demarcated in only 28 eutherian species representing 6 mammalian orders. Murid rodents have the smallest, gene-poorest and most diverged PARs. Other eutherian PARs are largely homologous but differ in size and gene content, being the smallest in equids and human/simian primates and much larger in other eutherians. Because pseudoautosomal genes escape X inactivation, their dosage changes with sex chromosome aneuploidies, whereas phenotypic effects of the latter depend on the size and gene content of the PAR. Thus, X monosomy is more viable in mice, humans and horses than in species with larger PARs. Presently, little is known about the functions of PAR genes in individual species, though human studies suggest their involvement in early embryonic development. The PAR is, thus, of evolutionary, genetic and biomedical significance and a 'research hotspot' in eutherian genomes.

The pseudoautosomal region (PAR) is a segment of true sequence homology between differentiated sex chromosomes where the sex chromosomes synapse and recombine during meiosis. The PAR has unique structural and functional properties not found in other parts of the genome. The present review aims to give a comprehensive overview of the current knowledge about the PAR in eutherian mammals: (1) to describe the unique properties of the region, (2) to provide an overview about the comparative organization and evolutionary dynamics of the PAR in eutherian mammals, and (3) to discuss the functions of PAR genes with regards to their implication on eutherian biology, health and disease.

Terje Raudsepp
Department of Veterinary Integrative Biosciences
College of Veterinary Medicine and Biomedical Sciences
Texas A&M University, College Station, TX 77843-4458 (USA)
E-Mail TRaudsepp@cvm.tamu.edu

Bhanu P. Chowdhary
Office of VP for Research, Qatar University
Al Tarfa, PO Box 2713, Doha (Qatar)
E-Mail bchowdhary@qu.edu.qa

1424–8581/16/1473–0081$39.50/0

E-Mail karger@karger.com
www.karger.com/cgr

The PAR and the Sex Chromosomes

The PAR is directly associated with the emergence of differentiated sex chromosomes. The latter happened independently in a diverse range of organisms during evolution giving rise to 2 principal sex chromosome systems: the XY-system with heterogametic males and the ZW-system with heterogametic females [Iwase et al., 2003; Charlesworth et al., 2005; Graves, 2006, 2010; Jamilena et al., 2008; Bellott et al., 2010; Ellegren, 2011]. It is noteworthy that even though there is no homology between the sex chromosomes in different taxa, molecular mechanisms that led to their formation are surprisingly similar.

As first proposed for the fruit fly [Muller, 1914] and now generally accepted for all other organisms, the sex chromosomes originate from a pair of autosomes (different pairs in different taxa) that progressively differentiated from each other during the course of evolution. The key factor triggering this process has been gradually increased in mutation-led sequence differences that eventually hampered pairing and suppressed recombination around a sex-determining or sex-benefit locus, later spreading over other regions of the 2 chromosomes [Ohno, 1967; Charlesworth et al., 2005; Graves, 2006; Ming and Moore, 2007; Kaiser and Bachtrog, 2010; Ellegren, 2011; Otto et al., 2011; Smeds et al., 2014]. Suppressed recombination, in turn, led to genetic reduction and degeneration of the sex-specific chromosome, resulting in structurally and molecularly distinct sex chromosomes – the X and the Y in, for example, mammals, fruit flies and cucumbers, and the Z and the W in butterflies, lizards and birds [Jamilena et al., 2008; Kaiser and Bachtrog, 2010; Otto et al., 2011; Smeds et al., 2014].

In many species, sequence divergence between the sex chromosomes has not extended to the entire chromosome, retaining defined segment(s) of true sequence homology where meiotic synapsis, homologous pairing and recombination persist. Such regions that behave similar to autosomes in pairing, synapsis and recombination are known as PARs – a term coined to underscore that despite being born on sex chromosomes, the region behaves like autosomes [Burgoyne, 1982].

One or more PARs have been found in diverse organisms including plants, insects, crustaceans, fish, birds, and mammals [Otto et al., 2011]. However, while PARs are typically associated with differentiated sex chromosomes, not all organisms with sex chromosomes necessarily have it. For example, both the eutherian and marsupial mammals have the XY sex chromosome system, but the PAR is found only in eutherians and not in marsupials [Toder and Graves, 1998]. The status is even more puzzling in monotremes – the platypus and echidnas. These egg-laying mammals have a complex X1Y1-X5Y5 sex chromosome system with 9 PARs that undergo directional and ordered meiotic pairing known as 'platypus chain reaction' [Daish et al., 2009]. Notably, none of the monotreme sex chromosomes or PARs share homology with marsupial or eutherian sex chromosomes [Daish and Grützner, 2009], illustrating the diversity of sex chromosome evolution even within the same class (Mammalia) of organisms.

The Eutherian PAR

The PAR was first discovered by Koller and Darlington [1934] who studied cytological preparations of male rats and noticed that in the first meiotic division 1 or 2 chiasmata are formed between the X and the Y – an indication of crossing over. It took another 45 years to confirm the presence of synaptonemal complexes between the sex chromosomes in other eutherian species, such as mouse, hamster, baboon, bull [Pathak and Hsu, 1979], and man [Pathak and Elder, 1980]. In the following decades, these observations were validated and further information regarding the structure/gene content was obtained in mice and men through studies of male meiosis [Oliver-Bonet et al., 2006; Kauppi et al., 2011], linkage analysis, gene mapping [Ellis and Goodfellow, 1989; Perry et al., 2001], and sex chromosome sequencing [Skaletsky et al., 2003; Ross et al., 2005; Soh et al., 2014]. The presence of the PAR has also been acknowledged in all domestic animals and a few wild mammals [Van Laere et al., 2008; Raudsepp et al., 2012; Das et al., 2013] and is thought to be a common feature of eutherian sex chromosomes. The presently known exceptions are some species of arvicoline and gerbilline rodents, such as the Mediterranean pine vole and Mongolian gerbil that, like in marsupials, have asynaptic and achiasmatic sex chromosomes with no PAR [de la Fuente et al., 2012].

Molecular and Functional Features of the PAR

The PAR is characterized by distinct and interrelated structural and functional features (fig. 1). Structurally, the PAR has 96–100% sequence homology between the X and the Y chromosomes. Hence, loci located within the PAR are diploid, undergo recombination in males and females [Galtier, 2004; Ross et al., 2005; Blaschke and Rappold,

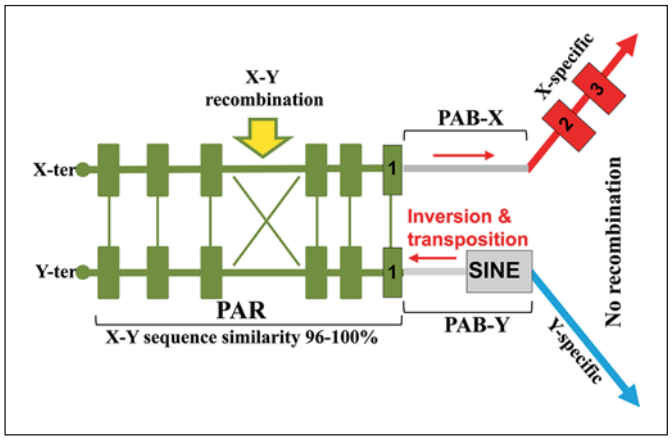

Fig. 1. Schematic organization of the PAR. The PAR is shown in green, the PAB in gray, the X chromosome in red, and the Y chromosome in blue colors. Vertical lines between the X and Y chromosomes in the PAR indicate sequence homology and a cross denotes a recombination site. Filled rectangles stand for genes/gene exons; filled rectangles with numbers 1, 2, 3 denote 3 exons of a gene that starts in the PAR, continues in the X chromosome but is truncated in the Y chromosome. Insertion of a SINE element and an inversion (arrows) define the PAB and the end of X-Y sequence homology and recombination. The figure describes general features of the PAR and it does not represent the PAR of any particular species.

2006; Flaquer et al., 2008], and are not subjected to dosage compensation by X inactivation (XCI) in females [Ellis and Goodfellow, 1989; Brown and Greally, 2003; Ross et al., 2005; Prothero et al., 2009; Deng et al., 2014]. It is suggested that recombination is necessary to maintain homology between the sex chromosomes [Ross et al., 2005]. The physical domain of the PAR typically lies between the terminal ends of the sex chromosomes and the pseudoautosomal boundary (PAB) – a border across which sequence homology between the X and the Y decreases, recombination ceases, and sex chromosome-specific sequences begin [Galtier, 2004; Ross et al., 2005].

Recombination

It is thought that the PAR exists because recombination is one of the essential mechanisms to ensure proper pairing and segregation of sex chromosomes during meiosis [Otto et al., 2011; Smeds et al., 2014]. This is consistent with studies in humans and mouse showing that the inhibition of recombination in the PAR is associated with an increased frequency of XY aneuploidy and male infertility [Kauppi et al., 2012; White et al., 2012].

Requirement for at least one crossover in the small PAR significantly elevates the recombination rate in the region. For example, in human PAR1, which is 2.7 Mb in size, recombination is 10- to 20-fold higher than the genome average [Filatov, 2004; Flaquer et al., 2008, 2009; Hinch et al., 2014]. Likewise, the recombination rate in the mouse PAR is 7-fold higher in male meiosis than in female meiosis [Soriano et al., 1987]. The possibility of recombination in the tiny 700-kb murine PAR has been a true mystery because in the mouse genome, on average, one double-strand break forms only at every 10 Mb [Kauppi et al., 2012]. It appears that the dynamics of recombination in the mouse PAR is temporally and genetically distinct from the rest of the genome. The murine PAR has an unusual higher-order chromatin structure and packaging, characterized by several-fold smaller chromatin loops than in autosomes, providing increased opportunity for cutting to promote high-frequency double-strand break formation [Kauppi et al., 2012].

Though it is not known whether these unique features of PAR chromatin are limited to mouse/murids or are common to all eutherians, elevated recombination rates have also been recorded in other eutherian PARs. For example, while the genome-wide recombination rates in pigs are overall higher in females, its frequency in the PAR during male meiosis is almost 3-fold higher compared to female meiosis [Guo et al., 2009]. Similarly, genetic distances between the same PAR markers in cattle are 6 times longer in male meiosis than in female meiosis [Simianer et al., 1997], suggesting a higher recombination rate in this region in males.

Further, recombination events are not evenly distributed in the PAR. A progressive increase in the number of recombination hotspots has been observed in the region as one moves from the PAB towards the telomere [Filatov, 2004; Bussell et al., 2006; Myers et al., 2008]. Interestingly, hotspot activities in the human PAR1 differ significantly among populations. Also, none of the human PAR1 recombination hotspots are shared with those of the chimpanzee PAR [Hinch et al., 2014]. Next, recombination hotspots have been found to be associated with methylation-related single nucleotide polymorphisms (SNPs) and genomic imprinting [Sigurdsson et al., 2009], suggesting that some PAR sequences might be regulated in a parent of origin-specific manner. An evidence for this is the presence of 2 imprinted quantitative trait loci in the pig PAR [Duthie et al., 2009].

Properties of the PAR Sequence

The PAR is characterized by molecular patterns that are distinct from the rest of the X chromosome. These include GC bias in nucleotide substitutions and high rates

of nucleotide divergence. Significantly higher GC base pair content has been observed in the PAR of humans [Ross et al., 2005; Hinch et al., 2014], mouse [Perry and Ashworth, 1999], horse [Raudsepp and Chowdhary, 2008], and cattle [Van Laere et al., 2008; Das et al., 2009] and is explained by a combined effect of biased gene conversion, DNA methylation and recombination [Filatov, 2004; Galtier, 2004; Chen et al., 2006]. Because recombination is mutagenic, the PAR is expected to be enriched with SNPs, CNVs and segmental duplications [Perry and Ashworth, 1999; Filatov, 2004; Bussell et al., 2006]. Indeed, the well-studied human PAR1 is characterized by genomic instability, high rate of interindividual variation, and the presence of segmentally duplicated gene families [Meroni et al., 1996; Ried et al., 1998; Ross et al., 2005; Bussell et al., 2006].

Recombination also triggers nucleotide divergence between the PARs of closely related species as illustrated by sequence comparison between the human PAR1 and the PARs of chimpanzee [Bussell et al., 2006] and orangutan [Filatov and Gerrard, 2003]. Both studies observed that the rate of noncoding nucleotide substitutions *(K)* between human-chimpanzee/human-orangutan genes from the distal portion of the PAR, viz., introns of *SHOX*, *PPP2R3L* and *ASMT*, is significantly elevated (K = 5.7–8.7%) compared to the average human-ape noncoding divergence (K = 3%) or the rate observed for X-specific genes (K = 2.7%) [Filatov and Gerrard, 2003]. Though similar studies are not available for other eutherian groups, it is tempting to speculate that rapid divergence of the PAR sequence may be one of the molecular mechanisms for establishing reproductive barriers between species and contributing to the sterility of hybrid males.

Organization of the PAR in Eutherian Mammals

The fundamental body of knowledge about the structure and function of the eutherian PAR is almost exclusively based on the studies in humans [Ellis and Goodfellow, 1989; Skaletsky et al., 2003; Ross et al., 2005; Hinch et al., 2014] and mice [Ellison et al., 1996; Palmer et al., 1997; Gianfrancesco et al., 2001; Perry et al., 2001]. In other eutherians, information about the PAR started to emerge only during the past decade [Raudsepp et al., 2012]. Despite the availability of detailed whole-genome linkage, radiation hybrid and/or cytogenetic maps for all domestic species [Raudsepp et al., 2012] and reference genomes for over 50 eutherian mammals (UCSC Genome Browser: http://genome.ucsc.edu/; Ensembl Genome Browser: http://www.ensembl.org/index.html; NCBI: http://www.ncbi.nlm.nih.gov/), knowledge about the PAR is limited to just a few eutherian species. The PAR is not demarcated in most species with reference genomes because female individuals were sequenced providing diploid data for the X chromosome, but no sequences for the Y chromosome. This sets limitations to properly delineating XY sequence homology and the PAR. Further, in species where the PAR is defined, the sequence of the region is incomplete, with gaps and misassemblies due to technical difficulties to sequence and assemble GC-rich complex sequences [Huddleston et al., 2014]. Hence, the PAR remains among the least characterized and the most poorly assembled euchromatic region in eutherian genomes after the Y chromosome. However, because the eutherian X chromosome is, in general, highly conserved in size, gene order and content across species [Raudsepp et al., 2004; Waters and Robinson, 2013], the majority of eutherian PARs studied so far are largely collinear with the human PAR1 (fig. 2). Notable exceptions are murine rodents, where the X chromosome has undergone extensive evolutionary rearrangements, and the PAR shares no homology with other eutherians.

Human

The human PAR is indisputably the best characterized among mammals, essentially thanks to the availability of finished sequence for both the X [Ross et al., 2005] and the Y chromosomes [Skaletsky et al., 2003]. As far as known, human is the only eutherian species to have 2 PARs [Hughes and Rozen, 2012] (fig. 2). The PAR1 is located at the termini of Xp/Yp [Ellis and Goodfellow, 1989; Ellis et al., 1989] and is similar in gene content to other eutherian PARs [Raudsepp et al., 2012], while the PAR2 at the termini of Xq/Yq is strictly human specific [Ciccodicola et al., 2000; Charchar et al., 2003; Hughes et al., 2010]. Recently, it was claimed that humans may even have PAR3, which is located at Xq21.3/Yp11.2 [Veerappa et al., 2013] and corresponds to the known X-transposed region in the Y chromosome [Skaletsky et al., 2003]. However, since the X-transposed region typically does not undergo recombination [Skaletsky et al., 2003] and unequal allelic exchange in this region has been found only in a few cases of pathology and in less than 2% of normal population [Veerappa et al., 2013], it might be premature to consider the region as a true PAR.

The PAR1 contains at least 25 genes (16 protein-coding and 9 RNA genes; UCSC) and has a relatively higher gene density (10 genes/Mb) than the rest of the X chro-

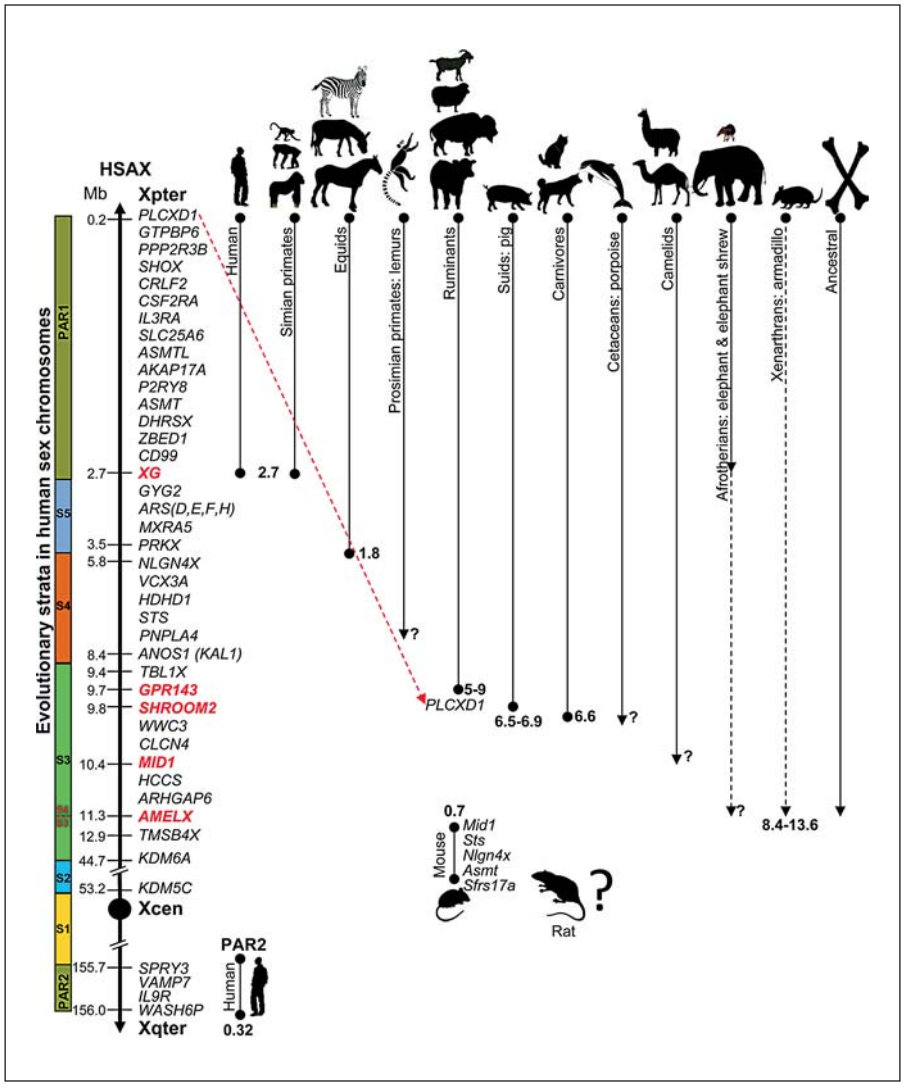

Fig. 2. Comparative organization of the eutherian PARs. A gene map of human Xp (left) serves as a reference for the PAR gene content in all eutherians. The exception is the mouse PAR which is shown separately (middle). Genes in red font demarcate known eutherian PABs. Evolutionary strata (S1–S5) of human sex chromosomes [Ross et al., 2005; Lemaitre et al., 2009] are shown at the far left. Earlier proposed S3/S4 boundary which aligns with *AMELX* [Lahn and Page, 1999; Iwase et al., 2003] is marked in red. The PAR size and gene content in different eutherian groups are shown with vertical lines starting with species images and names, and ending with numbers if the PAR size (Mb) is known, or with '?' if the size estimate is approximate. A red dotted arrow indicates the rearranged location of ruminant *PLCXD1* proximal to the PAR.

mosome (7 genes/Mb) [Ross et al., 2005]. The physical boundary of the PAR1 is demarcated by the *XG* blood group gene at 2.7 Mb [Pritchard et al., 1987; Goodfellow et al., 1988; Ellis et al., 1989; Galtier, 2004; Ross et al., 2005]. Although over 99.3% of the sequence of the euchromatic region of the human X chromosome has been determined [Ross et al., 2005], the PAR1 sequence is still incomplete and has 5 gaps with an approximate size of 350 kb in the GRCh38 assembly (UCSC) – a testimony to the complexity of the region and the challenges it poses for assembly.

The PAR2 emerged in the human lineage after divergence from the great apes and is proposed to be a result of an L1-mediated ectopic recombination event that transferred the terminal region of the X to the Y chromosome [Charchar et al., 2003]. The PAR2 is 320 kb in size and contains 4 genes (fig. 2) of which the proximal *SPRY3 and VAMP7* undergo XCI, while the terminal *IL9R* and *WASH6P* escape it [De Bonis et al., 2006]. In contrast to PAR1, the PAR2 undergoes recombination only occasionally [Charchar et al., 2003]. Interestingly, in eutherians other than primates, only the 2 proximal PAR2 genes, *SPRY3* and *VAMP7,* are X linked. Of the PAR2 distal loci, *IL9R* maps to autosomes and *WASH6P* has not been annotated in other eutherian genomes (UCSC).

Primates

The single PARs in the chimpanzee, gorilla and rhesus macaque Xp/Yq largely correspond to human PAR1 in size and gene content and the location of the PAB in *XG*

[Ellis et al., 1990; Hughes et al., 2012] (fig. 2). The few differences include the deletion of the *GTPBP6* gene in chimpanzee; the gene is located on the distal part of human PAR1 [Filatov and Gerrard, 2003; Bussell et al., 2006]. Also, there is an *Alu* repeat inserted at the PAB in human and chimpanzee Y chromosome (PAB-Y), but not in Old World monkeys [Ellis et al., 1990]. Currently, a finished and annotated sequence assembly is available for human PAR1 only, while sequences of other primate PARs are incomplete: the terminal 2.6 Mb of chimpanzee Xp comprises multiple contigs and gaps, and the assembly of the terminal Xp in macaque is just 300 kb with 3 genes (UCSC). Hence, estimation of the actual degree of similarity between primate PARs will require an improvement of sequence information.

Major differences have been observed between the PARs of simian ('higher' primates) and prosimian primates (lemurs) that separated about 52 million years ago [Gläser et al., 1997]. In lemurs, the PAR is at the tip of Xq (compared to Xp in other primates) and is probably larger because it contains *PRKX* and *STS*, which are X-specific genes in simian primates [Gläser et al., 1997, 1999]. The location of prosimian PAB, however, has not yet been determined.

Rodents

The PAR of the house mouse *(Mus musculus domesticus)* is the second best studied eutherian PAR after human and, remarkably, shares little homology with the human PAR1 or any other non-murine eutherian PAR. In fact, the ~700-kb mouse PAR at Xq/Yp is the smallest known in eutherians, contains 5 protein-coding genes, *Sfrs17a*, *Asmt*, *Nlgn4x*, *Sts*, and *Mid1* [Mueller et al., 2013; Bellott et al., 2014], and has the PAB located in *Mid1* intron 3–4 [Palmer et al., 1997; Perry and Ashworth, 1999; White et al., 2012]. Studies in related murine species show that the PAR has undergone rapid evolution: in the subspecies *M. m. castaneus,* the PAB is 430 kb proximal to the location seen in *M. m. domesticus*, leading to all 10 exons of *Mid1* being part of the PAR; in *M. spretus*, a species that diverged from the house mouse 2–4 million years ago, the PAR extends proximally even further, but does not include *Mid1,* as the latter is located in the X-specific region due to chromosome rearrangements [Perry and Ashworth, 1999; White et al., 2012]. These differences in closely related species suggest that the murine PAR, indeed, has evolved rapidly.

Curiously, despite early discovery [Koller and Darlington, 1934], the PAR in rats has been poorly characterized. While location of the PAR in rats is still a matter of debate, molecular constitution (genes/sequence) of the region is also sparsely known. One meiotic study suggests that the short arm of the acrocentric X chromosome pairs with the long arm of the Y chromosome [Joseph and Chandley, 1984]. Despite the lack of consensus about at which end of the X chromosome the rat PAR is located, sequence maps of the termini of the rat X chromosome, viz., Xpter-*Zfp182-Spaca5* and Xqter-*Arhgef6-Rbmxl1-Rbmx* (UCSC) share no homology with the mouse or human PARs, suggesting that the rat PAR may have a different constitution compared to the 2 species.

Horse and Equids

Horse was the first non-primate/non-murine eutherian species where the size, gene content and the PAB were determined [Raudsepp and Chowdhary, 2008] before the completion of the reference genome assembly [Wade et al., 2009]. This was accomplished by constructing a high-resolution contig map of large insert clones (BACs) over the region. Physical mapping of selected BACs by FISH to male metaphase spreads and amplifying BAC-end sequences by PCR from male and female horse DNA, allowed clear distinction between the PAR- and the X-/Y-specific regions.

The horse PAR contains at least 18 coding genes and is ~1.8 Mb in size, as estimated from a tiling path of overlapping BAC clones. Notably, the horse PAR is almost a million base pairs smaller than human PAR1 but extends proximal to the *XG* gene (that forms the human PAB) and contains genes that are X specific in humans (fig. 2). As the X chromosomes of human and horse are largely collinear for their genes [Raudsepp et al., 2004], the abovementioned differences are likely the result of differences in the content of repetitive sequences [Raudsepp and Chowdhary, 2008]. As shown in figure 2, the equine PAB is proximal to *PRKXY* and distal to *NLGN4X* in the X chromosome. The location of the PAB in other equids, viz., the Przewalski's horse, donkey, onagers and zebras, is also in the same region and is flanked by the 2 genes (our unpublished data). Cytogenetically, the PAR is located at Xp in all equids, while more rearrangements have shaped the Y chromosome where the PAR is at Yq in the horse, donkey and Hartmann's mountain zebra but at Yp in Burchell's zebra (fig. 3). An unusual feature of the horse PAR is that it shares an ~200-kb SD with the male-specific region of the Y chromosome [Raudsepp and Chowdhary, 2008]. Whether this duplication is present in other equids needs further investigation.

Compared to the BAC contig map, the reference assembly (EquCab2) of the PAR in the horse X chromo-

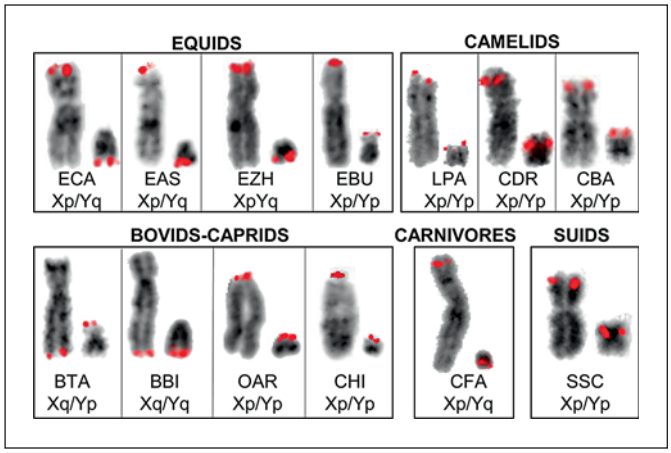

Fig. 3. Cytogenetic localization of the PAR by FISH (red signals) in eutherian sex chromosomes. Equids: ECA = Horse; EAS = donkey; EZH = Hartmann's mountain zebra; EBU = Burchell's zebra. Camelids: LPA = Alpaca; CDR = dromedary; CBA = Bactrian camel. Bovids/caprids: BTA = Taurine cattle; BBI = American bison; OAR = sheep; CHI = goat. Carnivores: CFA = Dog. Suids: SSC = Pig [Raudsepp and Chowdhary, 2008; Das et al., 2009; Li et al., 2013; Avila et al., 2014; our unpublished data].

some spans only 1.1 Mb with multiple gaps. Moreover, the sequence data includes only 12 genes (UCSC) compared to 18 reported in the contig [Raudsepp and Chowdhary, 2008]. Notably, both the contig map and reference assembly do not show the *SHOX* gene – a biomedically important gene in the human PAR1 (see below) and a known PAR locus in pigs [Das et al., 2013] and carnivores [Li et al., 2013]. Currently, efforts are underway to reconstruct the equine PAR by long-read next-generation sequencing using the minimum BAC tiling path of the region (our unpublished data). This may shed light on the status of *SHOX* and perhaps help improve the alignment between the BAC and sequence maps.

Cattle, Goat, Sheep, and Other Ruminants

Compared to human and many other eutherian species, the X chromosome in bovids/ruminants is rearranged [Iannuzzi et al., 2000]. Due to this, the PAR is located at qter in the submetacentric X chromosome of taurine and zebu cattle, at qter in the acrocentric X chromosome of river buffalo, and at pter in the acrocentric X chromosomes of sheep and goats [Das et al., 2009; Raudsepp et al., 2012] (fig. 3). Despite these rearrangements, cytogenetic mapping of 20 PAR and 5 X-specific genes in cattle, sheep and goats shows that the ruminant PAR contains the same genes as the human PAR1 and proximal Xp sequences, and the linear order of the genes is largely maintained [Das et al., 2009] (fig. 2). The only notable difference between cattle/ruminants and other eutherian species is the position of the *PLCXD1* locus which is the most terminal PAR gene in humans, horses, pigs, dogs, and cats but is X specific in ruminants, located between *SHROOM2* and *WWC3* [Das et al., 2009] (fig. 2). The physical domain of cattle/ruminant PAR is demarcated by the *GPR143* gene that spans the PAB in cattle, zebu, bison, yak, banteng, and sheep [Van Laere et al., 2008]. Based on comparative gene mapping and reference genome analysis, the size of the bovine/ruminant PAR is thought to be 5–9 Mb [Das et al., 2009]. A more accurate estimate requires improvements (closing gaps) in the current assembly of the terminal part of cattle Xq.

Pig

Initial physical location of the PAR in pigs was determined by FISH mapping *STS*, *KAL1* (alias *ANOS1*) and *PRKX* to Xpter/Ypter [Quilter et al., 2002] (fig. 3). However, the region was properly demarcated and mapped only recently by using array comparative hybridization and FISH analysis of a pig X-chromosome BAC tiling-path microarray [Skinner et al., 2013], and by FISH mapping and sequence analysis of 20 BAC clones containing 12 porcine orthologs of eutherian PAR genes [Das et al., 2013]. Both studies show that the pig has a single PAR that has similar gene content and order as other eutherian PARs or Xp terminal regions (fig. 2). The pig PAB is located in *SHROOM2* [Das et al., 2013], and the size of the PAR is 6.5–6.9 Mb [Das et al., 2013; Skinner et al., 2013]. Since Xpter sequence assembly in the pig reference genome susScr3 (UCSC) has yet to be finalized, this estimate is approximate.

Dog and Cat

As in other domestic species, the cytogenetic location of the PAR in dogs was initially determined by FISH. Mapping cosmid clones for *SLC25A6*, *PRKX* and *STS* to Xp/Yq indicated that the canine PAR is larger than the human PAR1 and contains genes that are X specific in humans [Toder et al., 1997] (fig. 2). Later it was shown that both the dog and cat PABs are located inside or near the *SHROOM2* locus [Murphy et al., 2007; Van Laere et al., 2008; Young et al., 2008]. These observations were recently confirmed and refined by sequence analysis of dog and cat Y chromosomes showing that the carnivore PAR extends to the terminal end of *SHROOM2* [Li et al., 2013] and is 6.6 Mb in size. Based on the dog X chromosome sequence assembly canFam3 (USCS, Ensembl), the PAR in dogs contains over 40 protein-coding genes, which is

substantially more than in human or equid PARs (fig. 2). Orthologs of almost half of canine PAR genes are X specific in humans (23 genes) and horses (14 genes; Ensembl), suggesting that they are exposed to different molecular forces (recombination) and regulation (XCI) in different species.

Alpaca and Dromedary

Preliminary data are available for the PAR in camelids. Comparison of female-to-male copy number ratios for putative PAR genes by quantitative PCR initially located the alpaca PAB between *SHROOM2* and *CLCN4* in the X chromosome [Raudsepp et al., 2012]. This was followed by FISH mapping 10 eutherian X-linked genes *(CSF2RA, ARSF, STS, PNPLA4, KAL1, GPR143, SHROOM2, WWC3, CLCN4,* and *MID1)* to Xp/Yp in alpaca and dromedary [Avila et al., 2014] (fig. 3), indicating their PAR status in camelids. These findings suggest that the camelid PAR may be larger than that of pigs and carnivores and includes genes proximal to *SHROOM2* (fig. 2). However, recent experiments with additional BACs for *MID1* map the gene only to the X chromosome in alpacas, dromedaries and Bactrian camels and disagree with previous data (our unpublished results). Thus, more studies are needed to properly verify the gene content and size of camelid PAR and define the PAB.

Other Eutherian Species

Information about the size, contents and organization of the PAR in other eutherian groups is limited. It has been shown that *GPR143* and *SHROOM2* are PAR genes in the porpoise (Cetacea) [Van Laere et al., 2008]; however, the PAB has not been defined in the species. The PAR is also reported in some of the species of Afrotheria and Xenarthra – the 2 basal eutherian orders [Meredith et al., 2011]. This is evidenced by immunocytological analysis of meiotic prophase chromosomes in the spermatocytes of Cape rock elephant shrew [Waters et al., 2007a] and 3 armadillo species [Sciurano et al., 2012] and by mapping the *XG* locus to both sex chromosomes in elephants [Delgado et al., 2009]. Finally, based on the size of XY synaptonemal complex in armadillos and the fact that almost the entire Y chromosome synapses with the X, it is thought that Xenarthrans may have the largest known PAR (8.4–13.6 Mb) among eutherians [Sciurano et al., 2012]. If this is so, the Xenarthran PAR may be the most similar known PAR to ancestral eutherian PAR, which is thought to have extended up to the amelogenin *(AMELX)* locus [Iwase et al., 2003] that maps at 11.3 Mb in the human X chromosome (fig. 2).

Evolution of the PAR

Autosomal location of all eutherian pseudoautosomal and most of other Xp genes in marsupials suggests that the eutherian PAR was not part of the ancestral sex chromosomes, and it originated from a large autosomal addition to eutherian proto-sex chromosomes ~80–130 million years ago [Park et al., 2005; Waters et al., 2007b; Marshall Graves, 2008]. The original eutherian PAR was probably large and extended up to *AMELX* [Iwase et al., 2003] or even further up to *ZFX* or *KDM6A* (*UTX)* [Graves et al., 1998; Park et al., 2005] (fig. 2). Since then, molecular events such as inversions and transpositions have shaped the PAR by reducing XY homology, introducing new barriers to recombination, and moving the PAB [Iwase et al., 2003; Marais and Galtier, 2003; Park et al., 2005]. Overall, the PAR is a dynamic entity with a tendency to shrink as recombination suppression spreads [Graves et al., 1998; Otto et al., 2011; Smeds et al., 2014]. Perhaps the only way to salvage this process is to have new autosomal additions to the sex chromosomes, as it has happened in collared lemmings, where a pair of autosomes has translocated to the X and Y creating a neo-XY sex chromosome system with a neo-PAR [Berend et al., 1997].

The PAR in murid rodents seems to have undergone the most extreme set of changes that resulted in loss of similarity with other eutherian PARs. The PARs in ruminants, pigs, carnivores, camelids, cetaceans, and prosimian primates resemble each other and the putative ancestral configuration. Compared to this, more changes have shaped the PAR in simian primates, humans and equids reducing the size of the region by about 3- to 5-fold compared to other eutherians or the likely ancestral form (fig. 2). Interestingly, though a variety of evolutionary rearrangements in the eutherian PAR have shifted the position of the PAB, the overall gene content and order in the X chromosome has largely remained conserved [Ross et al., 2005].

The rate of divergence of XY gene pairs in humans have allowed delineation of 4–12 distinct evolutionary strata [Lahn and Page, 1999; Skaletsky et al., 2003; Ross et al., 2005; Lemaitre et al., 2009; Pandey et al., 2013], each demarcating historic XY recombination suppression event(s). Three of these strata are collinear with non-primate PARs (fig. 2), and notably, the boundaries of each of these human strata largely coincide with demarcated PABs in other eutherians [Van Laere et al., 2008; Das et al., 2009], suggesting that some rearrangement hotspots might be shared between species.

The Pseudoautosomal Boundary

Even though the details of the PAB sequence are known for only a few species, viz., humans [Ellis et al., 1994; Skaletsky et al., 2003; Ross et al., 2005], mouse [Palmer et al., 1997; Perry et al., 2001], cattle/ruminants [Van Laere et al., 2008], pigs [Das et al., 2013], and carnivores [Li et al., 2013], it is notable that the molecular signatures of the boundaries are very similar. The PAB is typically defined by an insertion of a transposable element, which initiates suppression of recombination. Also, the PAB frequently spans a protein-coding gene, so that the gene remains intact in one sex chromosome and gets truncated in another (fig. 1).

The PAB of human PAR1 and chimp PAR is defined by an *Alu* (SINE) element in intron 3–4 of the *XG* gene, so that the gene remains intact in the X but is truncated in the Y chromosome [Ellis et al., 1994; Weller et al., 1995; Galtier, 2004]. However, a more detailed analysis of human *XG* indicates that the ancestral inversion that defined the current human PAB was preceded by *XG* duplication in the Y chromosome. As a result, humans have 3 copies of *XG*: one functional copy in the X chromosome, a truncated copy in PAR-Y, and a pseudogenized and inverted copy in proximal Yp at 12.4 Mb [Weller et al., 1995; Lemaitre et al., 2009].

The mouse PAB is located in intron 3–4 of *Mid1* and truncates the gene in the Y chromosome [Palmer et al., 1997; Perry et al., 2001]. In bovids/ruminants, the PAB coincides with a Bov-tA1 SINE element in the X and a closely related Bov-tA2 element in the Y chromosome and truncates at least two 5′ exons from the *GPR143* gene in the Y chromosome [Van Laere et al., 2008]. In pigs, the PAB is in *SHROOM2* intron 3–4, contains a tRNAGlu/SINE and truncates all seven 5′ exons of the gene in the Y chromosome [Das et al., 2013]. The carnivore PAB is also demarcated by *SHROOM2*, but the boundary is located at the end of the gene leaving it intact on both sex chromosomes [Li et al., 2013]. Sequencing and analysis of PAB-spanning BACs in horses [Raudsepp and Chowdhary, 2008] indicates that the equine PAB is also defined by a SINE insertion and spans a coding gene, tentatively identified as an orthologue of the human *XKR3* (our unpublished data).

Finally, analysis of amelogenin *(AMELX* and *AMELY)* sequences in eutherian species shows that the 5′ and 3′ portions of the gene belong to different evolutionary strata (S3 and S4 according to Lahn and Page [1999]) – an indication that *AMELX/Y* might span an ancestral eutherian PAB which is located in intron 2–3 and defined by a MER-transposon in all species studied [Iwase et al., 2003].

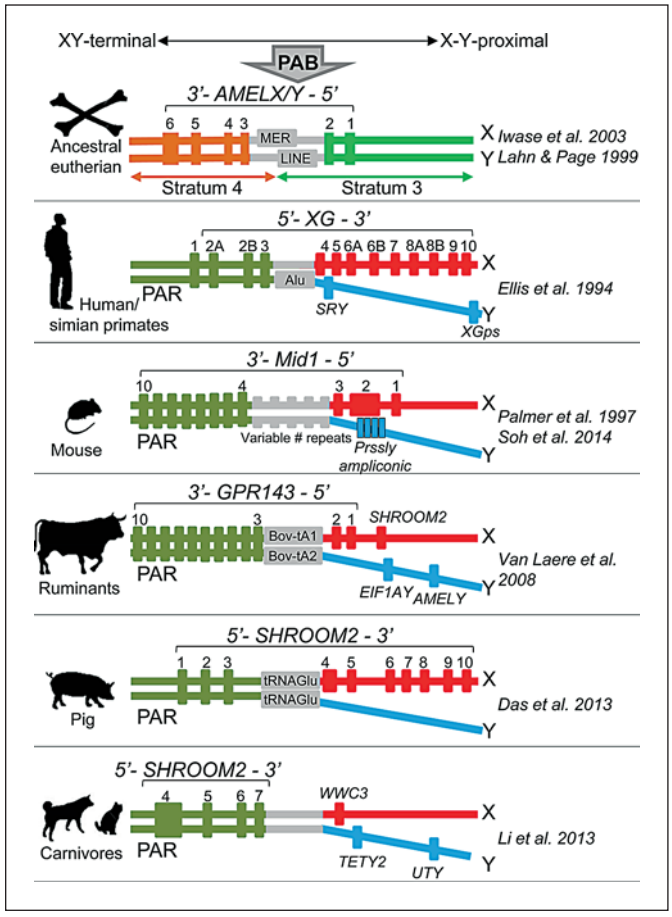

Fig. 4. Comparative organization of extant and putative ancestral eutherian PABs. The figure includes only the species where the PAB (gray) has been defined at sequence level. Where available, the closest genes to the PAB in the X (red) and the Y (blue) chromosomes are shown. Numbers denote gene exons. Note that in all species, except carnivores, the PAB spans a gene and is defined by retroposon insertions.

However, more recent studies place the border between strata S3/S4 distal to *TBL1X* at 9.4 Mb [Ross et al., 2005; Lemaitre et al., 2009; Pandey et al., 2013] (fig. 2), thus challenging this theory. Molecular features of currently known eutherian PABs are summarized in figure 4.

The PAR Genes and Genetic Disorders

Several unique features distinguish the PAR genes from other sex-linked as well as autosomal loci. Differently from classical sex-linked genes, the PAR genes are diploid, having 2 alleles at a locus. As PAR genes escape XCI in eutherian females, both alleles are expressed in

males and females [Brown and Greally, 2003; Prothero et al., 2009; Deng et al., 2014]. In contrast to autosomal genes, the PAR genes are sex linked and their variants can become associated with gender. Further, the PAR genes are situated in an unusual genomic environment characterized by an elevated rate of recombination, genome instability and mutations [Ried et al., 1998; Filatov and Gerrard, 2003; Filatov, 2004; Bussell et al., 2006]. Most interestingly, since the PAR varies in size and gene content between eutherian species (fig. 2), different sets of genes are exposed to this environment in different species, whereas orthologs of the same gene can be pseudoautosomal in one species and X specific in another. It is therefore of great interest how the distinct features of PAR genes correlate with their functions and phenotypic effects in different eutherian species.

Sex Chromosome Aneuploidies

Functional diploidy of PAR genes has been of interest for dissecting the molecular basis of sex chromosome aneuploidies, particularly X monosomy [Raudsepp et al., 2012]. It appears that the prevalence of X monosomy is directly related to the size of the PAR: viable individuals with X monosomy are commonly found in species with small PARs, such as horses, humans and mice but are very rare in cattle/ruminants, camelids, carnivores, and pigs, in which the PAR is substantially larger (fig. 2) [Raudsepp et al., 2012]. The plausible explanation is that haploinsufficiency is better tolerated if fewer genes are involved. The matter gets further complicated because, in addition to the PAR genes, about 15% of other X-linked genes in humans and 3% in mice also escape XCI [Mueller et al., 2013; Bellott et al., 2014; Deng et al., 2014], thus contributing to the cumulative effect of haploinsufficiency. The expression profile of human and murine non-PAR escapees is broader than that of other X-linked genes extending to the earliest stages of development right after the onset of zygotic gene activation [Bellott et al., 2014]. Knowledge about non-PAR XCI escapee genes in other eutherian species, however, is very limited.

While there seems to be a direct link between the PAR size and embryonic viability of X monosomy, no similar correlation has been found for aneuploidies with extra X chromosomes, such as X trisomy [Raudsepp et al., 2012]. Apparently, an overdose for XCI escaping genes has a milder genetic effect than haploinsufficiency.

The Functions of PAR Genes

The involvement of PAR genes has been suggested for a variety of human disorders, such as short stature, asthma, psychiatric disorders, and leukemia. Despite this, surprisingly little is known about the identity and functions of these genes. Perhaps the short stature homeobox gene, *SHOX,* known to regulate skeletal development, is the only one that has unambiguously been associated with clinical conditions [Rao et al., 1997; Flaquer et al., 2008; Fukami et al., 2015]. Haploinsufficiency or dysregulation of *SHOX* due to X monosomy, microdeletions, or disruption of *cis*-regulatory elements around the gene are known to cause idiopathic short stature, Léri-Weill dyschondrosteosis and other skeletal changes in humans [Benito-Sanz et al., 2012; Fukami et al., 2015]. Short stature and minor skeletal deformities are also characteristic to X monosomy in animals [Raudsepp et al., 2012; Romano et al., 2015], but not in mice, where *Shox* is autosomal (chr. 3; UCSC). On the other hand, a *SHOX* overdose in human XXY Klinefelter syndrome has been associated with tall stature [Tüttelmann and Gromoll, 2010].

Human stem cell studies suggest that pseudoautosomal and other XCI escaping genes may have an important role in placental functions and early development [Urbach and Benvenisty, 2009; Li et al., 2012; Bellott et al., 2014]. This explains the 70–99% lethality of non-mosaic 45,X human embryos [Urbach and Benvenisty, 2009; Berletch et al., 2010; Hook and Warburton, 2014] as well as the almost complete unviability of X monosomy in species with large PARs [Raudsepp et al., 2012]. A proposed candidate gene for embryonic lethality in X monosomy is colony-stimulating factor 2 receptor alpha, *CSF2RA,* which is essential for normal placental development [Urbach and Benvenisty, 2009; Hook and Warburton, 2014]. The involvement of PAR genes in early development is also supported by studies in pigs showing that some PAR loci are imprinted [Duthie et al., 2009] and that in day 26 and day 60 embryos, PAR genes tend to be expressed at higher levels compared to X-specific genes [Das et al., 2013].

In addition, PAR1 has been considered as a candidate region for bipolar affective disorder in humans [Flaquer et al., 2010]. Also, co-occurrence of deletions in the autosomal *IKZF1* gene with deletions in PAR1, resulting in *P2RY8-CRLF2* fusion, has been associated with poor prognosis in children with B-cell acute lymphoblastic leukemia [Olsson et al., 2015].

Finally, recombination between the sex chromosomes in the PAR can expose genes near PAB-Y to meiotic errors. In humans and simian primates, *SRY* – the mammalian male sex-determining gene, is located just 35 kb proximal to the PAB [Skaletsky et al., 2003]. Thus, a crossover error can easily move *SRY* from the Y to the X

chromosome and give rise to XX males [Ergun-Longmire et al., 2005]. However, *SRY* is not close to the PAB in any other eutherian species studied so far [Raudsepp et al., 2010, 2012; Das et al., 2012], explaining why *SRY*-positive XX males are very rare or not found in other eutherian species [Raudsepp et al., 2010]. Therefore, a recent report about an *SRY*-positive 38,XX virilized tortoiseshell cat with Xp;Yp translocation [Szczerbal et al., 2015] is exceptional, particularly because the feline *SRY* is located 1.7 Mb proximal from the PAB [Li et al., 2013].

Summary

The PAR is perhaps one of the most poorly studied euchromatic regions in eutherian genomes, being less attended by whole-genome gene-mapping projects and having patchy assemblies in most reference genomes. Despite this, the body of knowledge about the eutherian PAR is gradually improving, revealing new interesting evolutionary and functional aspects of the region. It appears that the majority of eutherian PARs are more alike than previously thought and resemble the putative ancestral form (fig. 2). On the other hand, the small PARs in murid rodents, simian primates and equids have evolved rapidly and probably represent more advanced evolutionary stages of the region. However, as the current knowledge of eutherian PARs is based on less than 30 species from just 6 eutherian orders (primates, rodents, Perissodactyla, Cetartiodactyla, Afrotheria, and Xenarthra; fig. 2), a comprehensive view about the comparative organization of the region will need inclusion of more diverse species.

Knowledge about the PAR genes and their functions is gradually growing, thus providing evidence that subtle differences in the size and gene content of the region may have critical implication of sex chromosome aneuploidies on embryonic survival and development. Though very little is known about the functions of individual PAR genes, recent studies indicate that the PAR and other XCI escapee genes might be important for placenta formation and early embryonic development, implying that functional significance of the PAR is not limited to sex chromosome segregation in male meiosis.

In conclusion, there are many good reasons to further investigate the PAR as it is a unique and relatively unexplored part of eutherian genomes. Sequencing, reconstructing and annotating the PAR sequence in reference genomes using advanced next-generation sequencing and bioinformatic approaches would be an excellent systematic approach for mining the wealth of information on diverse PARs, which, in turn, will allow an organized analysis of the region.

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The authors have no conflicts of interest to disclose.

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