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Retention of Latent Centromeres in the Mammalian Genome

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Abstract

The centromere is a cytologically defined entity that possesses a conserved and restricted function in the cell: it is the site of kinetochore assembly and spindle attachment. Despite its conserved function, the centromere is a highly mutable portion of the chromosome, carrying little sequence conservation across taxa. This divergence has made studying the movement of a centromere, either within a single karyotype or between species, a challenging endeavor. Several hypotheses have been proposed to explain the permutability of centromere location within a chromosome. This permutability is termed "centromere repositioning" when described in an evolutionary context and "neocentromerization" when abnormalities within an individual karyotype are considered. Both are characterized by a shift in location of the functional centromere within a chromosome without a concomitant change in linear gene order. Evolutionary studies across lineages clearly indicate that centromere repositioning is not a rare event in karyotypic evolution and must be considered when examining the evolution of chromosome structure and syntenic order. This paper examines the theories proposed to explain centromere repositioning in mammals. These theories are interpreted in light of evidence gained in human studies and in our presented data from the marsupial model species *Macropus eugenii*, the tammar wallaby.

Introduction

The ectopic emergence of a neocentromere occurs most frequently to provide mitotic stability on otherwise acentric chromosome fragments resulting from rearrangement (Amor and Choo 2002; Warburton 2004). Approximately 70 described cases of neocentromeres have been identified on 19 human chromosomes (Warburton 2004). Almost 10% of these cases are meiotically stable and heritable (Amor et al. 2004; Knegt et al. 2003). This has implications for the role that neocentromeres may play in creating karyotypic diversity and potential repositioning of a centromere. Three clear hot spots for neocentromeres have been identified within the human karyotype (3q26–qter, 13q21–32, and 15q24–26) (Amor and Choo 2002), implying a nonrandom mechanism for their appearance. The appearance of centromere repositioning in several primate taxa, exemplified in the shifts identified between Old World monkeys and New World monkeys (Eder et al. 2003)—as well as in cattle (Band et al. 2000), mouse (Armengol et al. 2003), and several marsupial lineages (Eldridge and Close 1993)—indicates this type of chromosome rearrangement may be a significant feature of chromosome evolution in mammals.

Methods

Fluorescence In Situ Hybridization

Bacterial Artificial Chromosome (BAC) fluorescence in situ hybridization was performed as described previously (Ferreri et al. 2004) with modifications. Briefly, BAC DNA was labeled by nick translation, incorporating either a biotin or digoxigenin labeled nucleotide as per standard protocols (Invitrogen). Labeled DNA was hybridized to metaphase chromosomes of *M. eugenii* in the presence of 6 μg sonicated *M. eugenii* DNA under stringent conditions, and posthybridization washes were performed at 45°C in 50% formamide, 2× SSC, and 0.1× SSC (prewarmed to 60°C). Hybridization to *Petrogale assimilis* chromosomes was performed as above without blocking DNA and with a low stringency wash series of 50% formamide, 2× SSC, and 2× SSC at 45°C. After blocking with 5% bovine serum albumin in 4× SSC, 0.2% Tween 20, detection was performed using the appropriate secondary antibody (antibiotin fluorescein, antidig Texas Red) (Vector Labs and Molecular Probes). Images were captured on an Olympus AX70 using a CCD cooled camera and Applied Imaging Cytovision software.

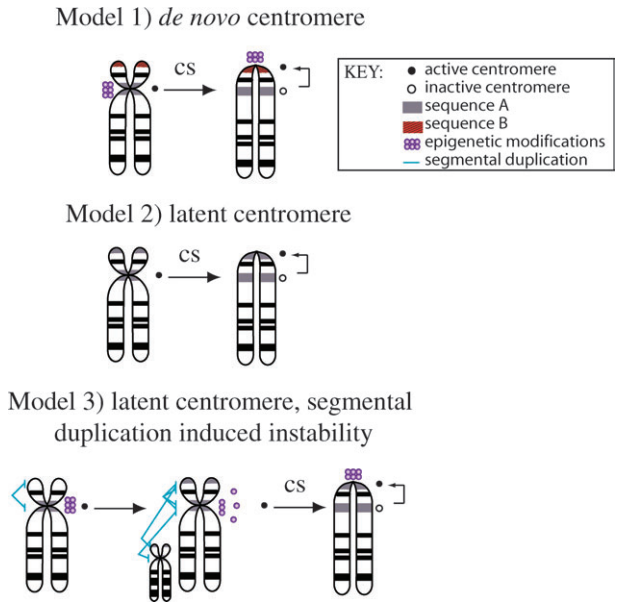

Figure 1. Schematic diagram of the models proposed to explain the derivation of a centric shift/centromere repositioning. The key is shown to the right.

Southern Analysis

Genomic DNA (10 μg) from the representative species was digested with the named enzyme in the presence of buffer at 37°C (New England Biolabs). KERV was labeled with P32-dCTP by random priming and hybridized to DNA (transferred to a Nylon N+ membrane) at 55°C in 500 mM Na_2HPO_4 (pH 7.0), 7% SDS, and 1 mM EDTA. Posthybridization washes were performed in 2× SSC, 0.1% SDS, 1× SSC, 0.1% SDS, or 0.5× SSC, 0.1% SDS at either 65°C or 55°C. Species analyzed include: *Aepyprymnus rufescens, Bettongia penicillata, Dendrolagus goodfellowi, D. lumholtzi, Dorcopsis luctuosa, Hypsiprymnodon moschatus, Isoodon macrourus, I. obesulus, Lagorchestes conspicillatus, Macropus agilis, M. antilopinus, M. dorsalis, M. eugenii, M. giganteus, M. parma, M. parryi, M. robustus, M. rufogriseus banksianus, M. rufogriseus rufogriseus, M. rufus, Monodelphis domestica, Notoryctes typhlops, Onychogalea fraenata, O. unguifera, Petrogale mareeba, P. penicillata, P. persephone, P. purpureicollis, P. xanthopus, Potorous tridactylus, Pseudochirulus herbertensis, Pseudochirus peregrinus, Sarcophilus harrisii, Setonix brachyurus, Thylogale thetis, Vombatus ursinus, Wallabia bicolor.*

Neocentromeres and Centromere Repositioning

Three theories describing the possible mechanism for neocentromere emergence in the context of centromere repositioning have been posited (Figure 1). The first proposes an epigenetic mechanism for repatterning of a segment of chromatin to perform as a competent site of kinetochore attachment and assembly. A lack of identifiable, shared satellite sequence features among cases of dicentric chromosomes and neocentromeres (Alonso et al. 2003; Barry et al. 1999; Lo et al. 2001a; Lo et al. 2001b; Sullivan and Willard 1998) suggests that this mechanism is likely independent of DNA sequence. Another theory, not necessarily exclusive of the first, proposes that a latent centromere may act as a primer for centromere emergence (Choo 1997; du Sart et al. 1997). Under its initial description, the latent centromere hypothesis relies on the presence of a centromere-specific sequence at the site of imminent centromere formation. Recently, this hypothesis has been modified to suggest that there may be latent chromatin and/or genomic structures that act as a mark for centromere formation (Ventura et al. 2004). The third combines elements of the first two, proposing that an increase in instability in a locus may induce repair mechanisms that ultimately can trigger chromatin repatterning (Ventura et al. 2003).

"Satellite DNA," a phrase that originally applied to satellite bands observed in ultracentrifuge density gradients, is commonly used to describe any tandemly repetitive sequence (John 1988).While satellite DNA families can be species or chromosome specific (Singer 1982), their seemingly ubiquitous presence at or near centromeric domains across a wide variety of organisms suggests that they play a role in centromere function (Eichler 1999; Henikoff et al. 2001; Willard 1990). Earlier studies in simian cell lines transfected with human alpha satellite sequences showed that these repetitive regions could form de novo centromeres on existing chromosomes (Haaf et al. 1992). However, later studies characterizing neocentromeric DNA in dicentric chromosomes showed that classical human alpha satellites are not the essential element dictating centromere location in humans (Barry et al. 1999; du Sart et al. 1997; Sullivan and Willard 1998). Concordantly, Williams et al. (1998) have shown that neocentromeres in *Drosophila* do not contain classical repeated DNA sequences. Thus, it is clear that while satellite DNA may be sufficient for centromere function, it is not required (Csink and Henikoff 1998; Willard 1990). Such a disparity between sequence features among active and inactive centromeres argues against a latent centromere hypothesis for neocentromerization. However, this position becomes more difficult to support when considering centromere repositioning in karyotypic evolution. In this context, cryptic repeats may be lost due to increased recombination at recently inactivated centromere locations (Jackson 2003; Ventura et al. 2003), as well as through genetic drift.

Using fluorescence in situ hybridization of BAC probes spanning several chromosomes and *in silico* analyses, Ventura et al. (2003) identified an ancestral centromere in HSA15q25. This centromere was inactivated at the time of the fission event that resulted in HSA14, HSA15, and the emergence of two new centromeres. This ancestral location coincides with neocentromere formation in 15q24–26 in at least two human cases, supporting the latent centromere hypothesis. The geographic coincidence of these neocentromeres, however, does not extend to the sequence level as one neocentromere maps >8 Mb from the ancestral centromere (Ventura et al. 2003).

Neocentromeres have also been identified at HSA3q26-qter (Muller et al. 2000; Ventura et al. 2004), HSA13q21–32 (Ventura et al. 2003), and HSAXq13 (Ventura et al. 2001). In the case of chromosomes 3 and 13, neocentromeres are found at higher frequencies than expected under random models of chromosome aberration (Amor and Choo 2002), implicating the latent centromere hypothesis in defining centromere competent locations. These observations are not taken from sequence analyses or mapping data alone but are considered in light of the evolution of active and ancestral centromere locations across primates. Each hot spot for neocentromere emergence in humans colocalizes to an ancestral centromere that has been inactivated during primate evolution. Like the case of the ancestral centromere in HSA15q25, there is little sequence homology between the inactive and active centromere sites. The reuse of centromere locations, often referred to as centromere seeding, implies that a latent centromeric site may function as both euchromatin and as a neocentromere.

Thus, while the DNA obviously is not a strict dictator of centromere competence, features conserved at inactive centromere locations may retain the capacity to become active centromeres. These features may exist in the form of as yet unidentified epigenetic marks or architectural features, such as segmental duplications, of the surrounding genomic landscape.

Ventura et al. (2003) have proposed a link between persistent recombinogenic segmental duplications and neocentromere potential. In this model, a locus characterized by a high frequency of segmental duplications, such as that found in the region surrounding HSA15q24–26 and its homologous segments in other primate species, may develop sites for neocentromerization through repair processes following chromosome rearrangement induced by these duplications. A coincidental association between an increased frequency of segmental duplications and chromosome rearrangement at loci near ancestral centromeres is supported by evidence of duplication events at 15q25 in human samples (Gratacos et al. 2001). A similar observation has been found for the ancestral, inactive centromere located in HSA6p22 (Eder et al. 2003). This region is also defined as rich in segmental duplications, and instability associated with these duplicons has been linked to 21-hydroxylase deficiency (Tusie-Luna and White 1995). In contrast to these findings, there is a paucity of segmental duplications in the ancestral centromere-neocentromere association identified in HSA3q26 (Ventura et al. 2004).

When examining centromere repositioning and centromere seeding across active and ancestral centromere locations within a karyotype, several factors influencing the sequence structure and content of these regions must be considered. Duplicons that are the result of pericentric segmental duplications likely undergo heterochromatic reformation to convert to euchromatin and become subject to an increased recombination rate once they are no longer restricted to centric domains (Jackson 2003). Timing the duplication event is difficult as it may be the result of pericentric duplications from the centromere prior to its inactivation rather than from the newly active centromere into an euchromatic site. Likewise, instability at the ancestral centromeric regions may be the result of the decay in pericentric heterochromatin following epigenetic repatterning once centromere repositioning has taken place (Jackson 2003).

The obvious shuffling of chromosomal segments in an evolutionary context, typified by breaks of synteny (BOS), implies that centromere repositioning may be a much more common occurrence than previously appreciated. Intriguingly, recent genomic analyses have shown that segmental duplications are strongly correlated with BOS between human and mouse (Armengol et al. 2003). Therefore, the contribution of segmental duplications to genomic diversity must be discussed in the context of centromere emergence.

Segmental Duplications

The assembly of sequences from large contiguous chromosomal regions from human, mouse, and a few other mammalian models has made possible high-resolution comparative mapping of regions of synteny on both large and small scales. What has become clear is that the organization of the genomes of these organisms has been heavily influenced by duplication, followed by tandem insertion or transposition of regions encompassing single genes or large DNA segments. Furthermore, these high-resolution comparative maps reveal that the distribution of these segmental duplications frequently coincide with unstable genomic regions and disease loci.

Comparisons of the assembled mouse and human genome sequences have led to some insights into the gross karyotypic changes between these two distantly related mammals. Remarkable power to trace the evolutionary trajectories of duplicated segments (duplicons), however, has come from concerted sequencing and mapping efforts in several primate models. It is estimated that approximately 5.2% of the human genome exists as duplicons, many derived in the last 35 million years (Bailey et al. 2002; Bailey et al. 2001; Consortium 2001). Interestingly, these duplicated segments tend to be concentrated in pericentromeric and subtelomeric regions, showing tenfold enrichment in these regions compared to euchromatin (Bailey et al. 2001). Furthermore, duplicons localizing to pericentromeric and subtelomeric regions are more likely to originate interchromosomally, while euchromatic duplicons tend to be intrachromosomal in origin (Bailey et al. 2001; Eichler 2001). Duplicated segments in euchromatin frequently comprise low copy repeats (LCRs) that likely arise from and are prone to nonhomologous recombination. Nonhomologous recombination serves not only to expand LCRs but can also lead to micro-deletions and inversions within or encompassing these segments. Such instability imbued by intrachromosomal segmental duplications can have dramatic effects on gene expression and chromosomal integrity. Analyses of intrachromosomal segmental duplications in humans have shown them to be associated with 169 known regions of instability and 24 human genomic disorders (Bailey et al. 2002; Stankiewicz and Lupski 2002).

Tracing the trajectory of both intrachromosomal duplicons, as highlighted in studies of instability, and interchromosomal duplicons in an evolutionary context can uncover events responsible for species-specific genomic alterations. Interchromosomal duplications have been found in phylogenetic mapping studies of paralogous regions between species of great apes. A complex series of rearrangements was described in analyses of a pericentromeric DNA fragment found on the long-arm of human chromosome 21 (HSA21). This sequence was found to be the product of intrachromosomal duplication (Potier et al. 1998), followed by interchromosomal transposition (to HSA2q, HSA13, and HSA18) in great apes after the divergence of orangutans (Golfier et al. 2003).

Another example of the complex history of sequences that may have contributed to the formation of rearrangements during primate karyotypic evolution can be found in the elegant studies of the evolution of the ancestral fusion site in HSA2q13–2q14.1 (Fan et al. 2002a; Fan et al. 2002b; Martin et al. 2002; Mefford and Trask 2002). Chromosome 2 in humans derives from the fusion of two chromosomes that have remained separate in other primate species. Fan et al. (2002a) traced the history of the sequences present at this fusion site and found that this region has undergone a complex series of rearrangements and duplicative exchanges, including several rounds of intrachromosomal and interchromosomal duplications, an inversion and subsequently a fusion between duplicated segments to form HSA2.

Thus, genome architecture, as defined by nongenic regions of the genome, can act as a catalyst for chromosome rearrangements through the action of segmental duplication and nonallelic homologous recombination. The propensity toward intrachromosomal rearrangements in human chromosome evolution (Pevzner and Tesler 2003a; Pevzner and Tesler 2003b; Postlethwait et al. 2000) has made delineating the complex history of human chromosomal segments across other mammalian lineages challenging. Comparisons of mouse and human genome sequences have shown that 53% of evolutionary breakpoint rearrangements defined at BOS between these two species associate with segmental duplications (Armengol et al. 2003). Additionally, Thomas et al. (2003) have identified recent (~5–7 mya) pericentromeric segmental duplications on mouse chromosomes 5 and 6 that have been implicated in a chromosome fission event, resulting in the derivation of two new centromeres, as well as chimeric transcript formation. While defining the trajectory of some of these rearrangements and determining whether the duplications are associative or causative has not been accomplished, an intriguing correlation between karyotypic evolution and segmental duplications is emerging.

Marsupial Karyotypic Evolution

Descriptions of chromosome homologies and karyotypes within Marsupialia are extensive, with over 70% of known marsupial species karyotyped (Hayman 1977; Hayman 1990). Chromosome evolution within Macropodidae (kangaroos, wallabies, and rat kangaroos), a group that radiated into ~77 species over 22 mya (Kirsch and Lapointe 1995; Rens et al. 2003), has been comprehensively studied in terms of G-banding, chromosome rearrangements, and homologies. This group of marsupials is characterized by an extensive diversification of karyotypes, with diploid numbers ranging from 2n=10, 11 in *W. bicolor* to 2n=32 in *A. rufescens*, all derived from an ancestral 2n=22 karyotype (Hayman 1990; Rens et al. 2003; Rofe 1979).

Central to the description of this karyotypic diversity is the involvement of the centromere, either through its location on the chromosome or its involvement in fissions, translocations, inversions, fusions, and shifts. The involvement of the centromere in the chromosomal rearrangements giving rise to the karyotypic diversification within Macropodidae has been elegantly highlighted in a recent study tracing the phylogenetic distribution of 19 conserved syntenic segments across several marsupial families (Rens et al. 2003).

Further evidence for the correlation between centromere dynamics and karyotypic diversity in marsupials has been described in detailed analyses of genome rearrangement and instability found in dysgenic interspecific hybrids within the *Macropus* genus. Several hybrids display karyotypic aberrations almost exclusively associated with centromeric abnormalities, including translocations and amplifications (O'Neill et al. 1998; O'Neill et al. 2001). Detailed analyses of five hybrids from two different species crosses have shown instabilities linked to kangaroo endogenous retrovirus (KERV) (see below), attributed to a significant copy-number increase of this sequence in the centromere (unpublished data; O'Neill et al. 1998). Recent research has shown that this centromeric amplification also associates with fusion and fission events, as well as knob-formation, a potentially meiotically driven element (Rhoades and Dempsey 1966). Thus, it appears that the centromere, or at least centromere-associated sequences, may have played a pivotal role in chromosome restructuring and centromere repositioning in macropodines.

Retroviruses and Centromere-specific Sequences

The link between segmental duplications, BOS, and centromere repositioning becomes more intriguing in light of new data from the marsupial model species *M. eugenii*. A previously identified retroviral element, KERV, has been localized to all of the active centromeres of this species (Ferreri et al. 2004). Previously characterized as a centromeric element within another macropodine (kangaroos and wallabies) species, this sequence is preserved as a centromeric repeat as well as a functional retroviral element. BACs containing this sequence have been mapped onto metaphase chromosomes of *M. eugenii* (Ferreri et al. 2004; for a current map, see Figure 2). Significantly, 72% of these clones (35 total) map to BOS between conserved chromosome segments as determined through cross-species reciprocal chromosome painting (Ferreri et al. 2004; Rens et al. 2003). When the rearrangements these blocks have experienced

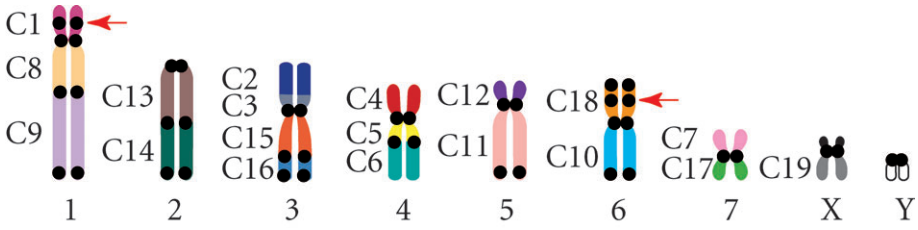

Figure 2. Ideogram indicating the 19 conserved chromosome segments (Rens et al. 2003) and the location of KERV/satellite sequences as determined through primed in situ hybridization (Ferreri et al. 2004) and BAC mapping (black dots). Arrows indicate the location of non-BOS associated BACs at sites that correspond to centromere locations in other marsupial species.

within other marsupials are considered, 100% of these clones map to latent or active centromere locations within this species.

Active centromere sites within *M. eugenii* also contain another centromeric satellite that experiments show possesses CENP-B binding capability (K. Bulazel et al., unpublished data). The colocalization of signals for this satellite and KERV at all active centromeres within this species suggests that these elements are tightly associated. Each of the KERV-positive BACs, therefore, was screened by dot blot hybridization at high stringency with the complete satellite sequence. Surprisingly, 100% of these BACs showed positive hybridization with this probe. Therefore, neither sequence is restricted to centromeric domains within this species. Furthermore, 11 interstitial, latent centromere locations thus far mapped harbor sequences that may indicate centromere competence.

The appearance of these interstitial sequences is likely the result of segmental duplications—although replicative transposition cannot be ruled out, given the direct involvement of a retroviral sequence. Evidence from hybrid studies indicates that this element may undergo bursts of activity (O'Neill et al. 1998), enabling mobilization to other locations within the genome through autonomous replication machinery. However, the location of interstitial sequences is not randomly distributed, as might be expected under a mobilization scenario. While it is evident that KERV has retained activity (Ferreri et al. 2004; O'Neill et al. 1998), the location of active KERV sequences has not been defined. The nonrandom positive correlation between the location of KERV and centromeres (as well as BOS) indicates that these paralogous sequences may in fact be the product of segmental duplication events. Detailed studies of chromosome rearrangements within humans and homologies between mice and humans have shown an increased concentration of repeated DNA, retroelements, and segmental duplications at BOS (Armengol et al. 2003; Bailey et al. 2004; Bailey et al. 2003; Dehal et al. 2001), suggesting a mechanistic association between chromosome rearrangement and these genomic elements (Armengol et al. 2003; Bailey et al. 2004; Bailey et al. 2003).

The colocalization of the BOS with active centromere-associated sequences is significant, as each of these sites is involved in chromosome rearrangements that karyotypically identify divergent marsupial species. Each of these sites is also the location of active centromeres in other marsupial species. The appearance of these sequences may be the result of past duplication events from active centromere locations; however, phylogenetic inference from karyotypic studies (see O'Neill et al. 2004 for a review) indicates that the appearance of at least some of these sequences predates the centromere repositioning event. This strongly implies that there are many latent centromere locations within the marsupial karyotype that are centromere competent.

Although the order of events over the course of *M. eugenii* chromosome evolution in relation to KERV, the functional satellite, and BOS remains to be determined, examining the structure and relationship of these sequences as functionally disparate portions of the genome could lead to major insights into centromere seeding, competence, and emergence.

Conservation of Breaks of Synteny and Centromere Sequences

Preliminary analyses of other macropodine species indicate that the location of at least three KERV and satellite-positive BACs to BOS is conserved in two other species, *M. rufogriseus* (red-necked wallaby) and *W. bicolor* (swamp wallaby) (Ferreri et al. 2004). The BAC that maps to the BOS and active centromere on *M. eugenii* chromosome 7 was used as a probe on metaphase chromosomes of *P. assimilis* (allied rock-wallaby), a genus that diverged early in the macropodine radiation (Eldridge and Close 1993; O'Neill et al. 2004). Within this genus, examples of centromere repositioning are abundant. *P. assimilis* contains an acrocentric chromosome 7, the likely result of a centric shift or other unknown mechanism (Eldridge and Close 1993).This BAC maps to the interstitial, inactive centromere location, not the derived acrocentric location (Figure 3). This indicates that centromere repositioning has occurred on this chromosome and further reinforces our results from *M. eugenii* (which indicate that BOS retain centromere sequences).

Conservation of KERV has been defined in other marsupial groups in an effort to extend our analyses beyond the family Macropodidae. KERV sequences have been identified through Southern analyses in all marsupial species

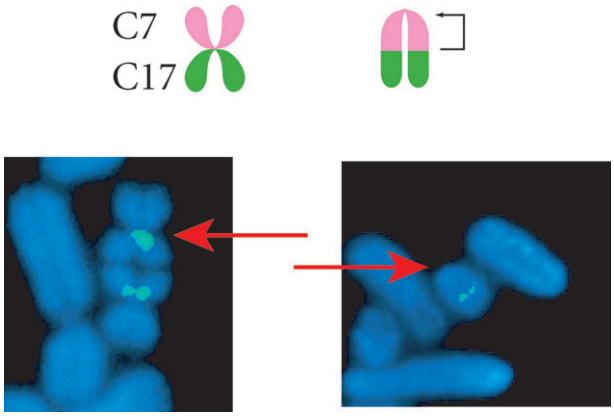

Figure 3. Centromere shift on chromosome 7 between *M. eugenii* and *P. assimilis*. (A) A schematic representation of the shift in correlation to the conserved chromosome segments, indicated on the left. (B) Fluorescence in situ hybridization of the same BAC (green) to metaphase chromosome 7 (blue) of *M. eugenii* (left) and *P. assimilis* (right). The centromere for each chromosome is indicated with a red arrow.

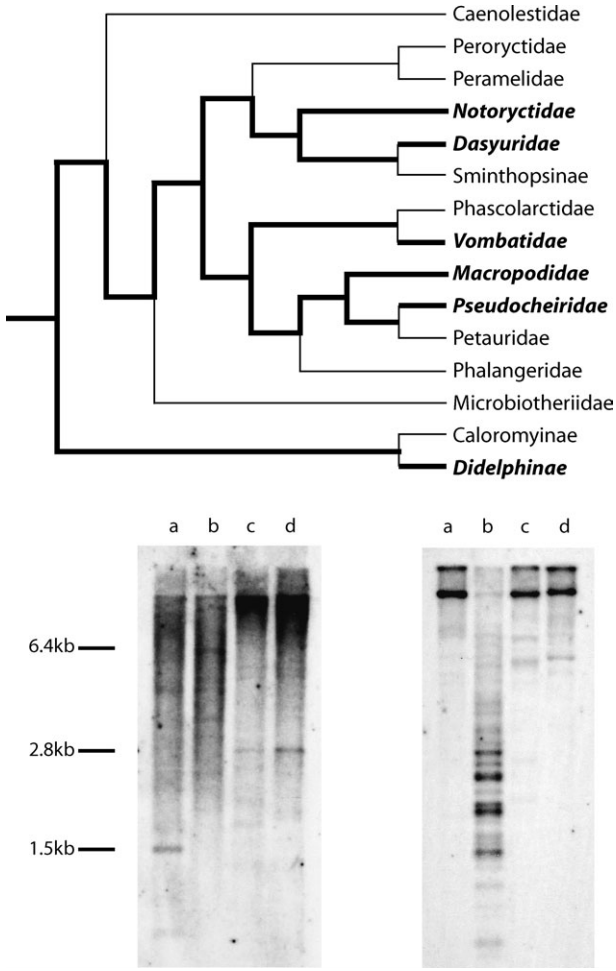

Figure 4. (A) Phylogeny (Amrine-Madsen et al. 2003) of marsupial families and subfamilies. Superimposed onto the branches are the terminal lineages in which KERV sequences have been identified (bold) and the inferred ancestral conservation of KERV (bold). Lineages in which analyses were performed are indicated in italics. Note that only these six families have been studied; an absence of KERV cannot be inferred in any lineage from this date set. (B) Southern analyses of *M. eugenii* KERV (left panel) and the functional centromeric satellite (right) in *M. domestica*. A size reference is shown to the left. Genomic DNA was digested with (a) *BglII*, (b) *EcoRI*, (c) *MspI,* and (d) *PstI*.

examined thus far, a data set that includes 36 species and six families (Figure 4A). Comparative analyses for another organism for which a genome sequence will be available afford opportunities to examine the potential involvement of segmental duplications in defining BOS and latent centromere retention. To this end, a portion of the KERV sequence containing portions of the *gag-pro-pol* genes was used to screen genomic DNA from the South American marsupial *Monodelphis domestica*. Southern analyses under low stringency conditions show several distinct hybridizing fragments within the genome of this species (Figure 4B). The data has been confirmed by preliminary BLAST sequence alignment between the *M. rufogriseus* KERV sequences and the initial release of BAC end sequence for *M. domestica (*data not shown). Likewise, Southern analyses indicate that the functional satellite associated with KERV is also conserved in this species (Figure 4B). However, the retention of functional domains (i.e., CENPB DNA binding domains) has not been determined. Conservation of this sequence across ~180 million years of divergence is remarkable given the apparent lack of conservation of satellites within other eukaryotic systems. The extent of sequence conservation, retention of functional retroviral genes, and correlation with centromeres or BOS must be determined.

It is evident from studies of neocentromere hot spots in the human karyotype and ancestral centromere locations in primates that the latent centromere hypothesis, either defined by sequence or the involvement of segmental duplications, may explain centromere emergence in primate lineages. The data presented herein, in contrast to that from primates, clearly indicates that latent centromere sequences are stringently conserved within marsupials. Our data, showing an involvement of segmental duplications with BOS within macropodines and retention of latent centromeres following centromere repositioning, supports the latent centromere hypothesis in defining centromere locations within this group of mammals. The remarkable retention of latent centromeres throughout marsupials, a large and karyotypically diverse group of mammals, may have facilitated the chromosomal radiations that several marsupial families have experienced.

The retention of latent centromeres, either as a sequence-specific or functional domain, within the karyotypes of a group of mammals may provide a means of introducing new karyotypic variability or of restricting gene flow. This

retention does not necessarily constitute a selective advantage per se, but it implicates latent centromeres in the process of karyotypic diversification. This may be achieved randomly through the acquisition of a fertility barrier in the form of a heterozygote for centromere locations on homologous chromosomes. Such a mechanism may foster sympatric or parapatric speciation. While heterozygotes may present as the result of a random event, as has been observed in neocentromere cases in humans, they may be found in the form of intra- and interspecific hybrids. As is evident from our own analyses of centromere rearrangements in hybrids, this portion of the genome may be destabilized and rapidly altered. Such alterations, perhaps through the use of latent centromeres, may result in the rescue of otherwise deleterious heterozygosity and/or may allow for introgression of novel genomic domains. Furthermore, fixation of new karyotypes within a population may proceed rapidly due to other forces, such as meiotic drive.

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