Genome-Wide Comparative Chromosome Map between Human and the Forrest's Pika (*Ochotona forresti*) Established by Cross-Species Chromosome Painting: Further Support for the Glires Hypothesis

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Key Words

Chromosome painting • Evolution • Forrest's pika • Lagomorpha • Mapping • Rabbit

genome; and (3) Robertsonian translocations have contributed to the karyotype differences between *O. forresti* and *O. cuniculus*.

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Abstract

Comparative genomic data for ochotonids (pikas) are important for understanding the karyotype evolution of lagomorphs. Here, we have established the first genome-wide chromosomal homologies between human and the Forrest's pika (*Ochotona forresti*, 2n = 54) by cross-species chromosome painting. Integration of our map with the published comparative chromosome map between human and rabbit has enabled the establishment of an indirect homology map between Forrest's pika and domestic rabbit (*Oryctolagus cuniculus*, 2n = 44), representing the 2 Lagomorpha families: Leporidae and Ochotonidae. Our results demonstrate that (1) most of the proposed ancestral eutherian synteny or syntenic associations have been retained in the *O. forresti* genome; (2) the HSA1/10p association, one of the 2 signature rearrangements that were proposed to support the grouping of the orders Lagomorpha and Rodentia into a monophyletic clade called Glires, is also present in the *O. forresti*

The order Lagomorpha consists of 2 families: Leporidae (hares and rabbits), and Ochotonidae (pikas), about 91 species [Hoffmann and Smith, 2007], of which the domestic rabbit (*Oryctolagus cuniculus*, 2n = 44) is the most well-studied species mainly due to its importance as a model for biomedical research [Korstanje et al., 1999].

The phylogenetic placement of Lagomorpha on the eutherian tree remains a topic of debate. Nevertheless, the grouping of Lagomorpha and Rodentia into a monophyletic clade called Glires represents the current consensus view that is supported overwhelmingly by both morphological and molecular evidence [Luckett and Hartenberger, 1993; Murphy et al., 2001a; Reyes et al., 2004; Asher et al., 2005; Kriegs et al., 2007; Arnason et al., 2008; Schneider and Cannarozzi, 2009]. Furthermore, the Glires concept appears to be supported by molecular cytogenetic evidence. The existence of conserved association(s) of hu-

man (HSA) homologous chromosomal segments in domestic rabbit [Korstanje et al., 1999] and squirrels [Richard et al., 2003; Stanyon et al., 2003; Li et al., 2004, 2006] has led to the suggestion that the HSA1/10p association and, to a lesser extent, the HSA9/11 association may represent the cytogenetic signature characterizing the clade Glires [Stanyon et al., 2003; Li et al., 2004]. Nevertheless, the presence of such signature rearrangements in Ochotonidae remains to be established.

Comparative cytogenetic studies have made a great contribution to our understanding of karyotype evolution of species within the families Leporidae and Ochotonidae, respectively [Hsu and Benirschke, 1971; Stock, 1976; Capanna et al., 1991; Ivanitskaya, 1991; Robinson et al., 2002; Robinson, 2006 and literature cited therein]. However, earlier cross-family comparison based on G-banding has only revealed a few conserved chromosomal segments between representative species from Leporidae and Ochotonidae, leading to the suggestion that tandem fusion could be responsible for the family-level karyotype divergence in Lagomorpha [Stock, 1976].

Cross-species chromosome painting is the most robust method for establishing genome-wide comparative chromosome maps in mammals. Such comparative chromosome maps, usually using human chromosomes as the reference, have enabled the reconstruction of ancestral eutherian genome organization and landscape chromosomal rearrangements accompanying the radiation of major phylogenetic lineages [see Ferguson-Smith and Trifonov, 2007 for review]. Up to now, the comparative chromosome maps between human and representative species of the extant 18 eutherian orders (including one Lagomorpha species, *Oryctolagus cuniculus*) [Korstanje et al., 1999] have been reported [see Ferguson-Smith and Trifonov, 2007 for review; Nie et al., 2008]. Nevertheless, genome-wide comparative chromosome maps between human and representative species of Ochotonidae, as well as between representative species of Leporidae and Ochotonidae have not been reported hitherto.

In this study, we have established the first genome-wide chromosomal homology map between human and Forrest's pika (*Ochotona forresti*, OFO, $2n = 54$) by cross-species chromosome painting with human chromosome-specific painting probes. Such a map sheds further insight into the ancestral lagomorph karyotype and chromosomal rearrangements underlying the karyotype divergence between Leporidae and Ochotonidae and provides further support for the Glires hypothesis.

Materials and Methods

Metaphase Preparations and DAPI-Banding

The *O. forresti* fibroblast cell line was derived from skin biopsies of a male Forrest's pika collected from Gaoligong Mountain, Yunnan, China. Metaphase preparations were made following conventional methods as previously described [Yang et al., 2003a]. The *O. forresti* chromosomes were karyotyped based on inverted DAPI-banding patterns that are similar to the G-banding patterns.

Fluorescence in situ Hybridization

Human chromosome-specific painting probes were made from flow-sorted chromosomes by degenerate oligonucleotide primed-PCR (DOP-PCR) [Telenius et al., 1992]. Cross-species chromosome painting between *O. forresti* and human was carried out as previously described [Yang et al., 1999]. After post-hybridization washes, biotin-labeled probes were visualized using Cy3-avidin (final concentration 2 $\mu\text{g/ml}$, Amersham). After detection, slides were then mounted in Vectashield medium with DAPI (4,6-diamidino-2-phenylindole, Vector Laboratories) and covered with $22 \times 32 \text{ mm}^2$ coverslips.

Microscopy

Fluorescence in situ hybridization (FISH) images were captured using the Genus system (Applied Imaging Corp.) as previously described [Yang et al., 2004]. Hybridization signals were assigned to specific chromosomes or chromosomal regions as defined by enhanced DAPI-banding patterns.

Results

Painting O. forresti Chromosomes with Human Probes

The male *O. forresti* has a $2n = 54$ karyotype (fig. 1). The X and Y chromosomes are a large submetacentric chromosome and a small bi-armed chromosome, respectively. To establish the chromosomal homology map between human and *O. forresti*, 22 human autosomal paints and the X chromosome paint were hybridized onto the metaphases of *O. forresti*. Representative FISH examples are shown in figure 2. The cross-species hybridization results are summarized on a DAPI-banded karyotype of *O. forresti* (fig. 1). In the genome of *O. forresti*, 9 human chromosome painting probes (HSA6, 9, 11, 13, 17, 18, 20, 21, and X) each hybridized onto one *O. forresti* chromosomal segment or chromosome; 9 human probes (HSA1, 2, 8, 10, 14–16, 19, and 22) each detected 2 chromosomal regions; 5 human probes (HSA3–5, 7, and 12) each delineated 3 homologous segments. In total, 22 human autosomal painting probes defined 41 conserved segments in the *O. forresti* genome. All previously proposed ancestral human homologous segment associations such as HSA3/21, 4/8, 7/16, 12/22 (twice), 14/15, and 16/19 [Yang et al., 2003b and references cited therein] were also found

Fig. 1. DAPI-banded karyotype of *O. forresti*. The segments homologous to regions of human (HSA) and *O. cuniculus* (OCU) are indicated to the right of each *O. forresti* chromosome. The assignment of *O. cuniculus* homologies to each *O. forresti* chromosome is based on the published data of Korstanje et al. [1999] and Chantry-Darmon et al. [2005]. Note that OCU10 and OCU11 are interchanged in Korstanje et al.'s study. In addition, the association

HSA 12pq/22qt has not been detected in the rabbit genome in an earlier study [Korstanje et al., 1999], but the presence of HSA 12pq/22qt association on *O. cuniculus* chromosome 4q has been confirmed by a later study [Chantry-Darmon et al., 2005]. Arrows indicate the positions of centromeres of some chromosomes, which are otherwise ambiguous.

in the *O. forresti* genome. Besides these postulated ancestral eutherian synteny, 6 adjacent segment associations (HSA1/10, 3/12, 9/15, 10/11, 12/14, and 18/20) were present in the *O. forresti* genome, with 5 of these associations (i.e. HSA3/12, 9/15, 10/11, 12/14, and 18/20) most likely resulting from Robertsonian translocations as suggested by the position of centromeres.

Discussion

Cross-species chromosome painting with human chromosome-specific painting probes has allowed us to construct the first genome-wide comparative chromosome map between human and *O. forresti*, a representative species of the pika family. An integrated analysis of

our comparative chromosome map and the previously published maps [Korstanje et al., 1999; Robinson et al., 2002] provides new insight into the comparative genome organization of the Lagomorpha.

Karyotype Relationship between O. cuniculus and O. forresti

Comparative maps between human and rabbit (*Oryctolagus cuniculus*) have been established by using various methods, including reciprocal chromosome painting with both human and *O. cuniculus* chromosome-specific probes, and comparative gene mapping [Korstanje et al., 1999; Hayes et al., 2002; Chantry-Darmon et al., 2003, 2005]. The comparison of our human – *O. forresti* comparative map with the published human – *O. cuniculus* comparative map has enabled us to deduce the subchro-

Fig. 2. Examples of cross-species chromosome painting. **a** HSA chromosome 9 probe hybridized onto the short arm of *O. forresti* chromosome 3. **b** DAPI-banding of the same *O. forresti* metaphase. **c** HSA chromosome 15 probe hybridized onto the long arm of *O. forresti* chromosome 3. **d** DAPI-banding of the same *O. forresti* metaphase.

mosomal correspondence between *O. forresti* and *O. cuniculus* as well as human, based on the genome-wide homologies delimited by comparative chromosome painting and banding comparison (fig. 1). The comparative results demonstrate that these 2 lagomorph species, representing the 2 lagomorph families, share at least 29 conserved segments equivalent to human chromosomal segments or segment associations 1a, 1b/10p, 2pq, 2q, 3a/21, 3b, 4a/8p, 4b, 5a, 6, 7a, 7b/16p, 7c, 8q, 9, 10q, 11, 12p, 12q/22qt, 12qt/22q, 13, 14a, 15/14b/15, 16q/19q, 17, 18, 19p, 20 and X. Ignoring the unpainted heterochromatic regions, 8 *O. forresti* chromosomes (OFO4, 5, 15, 16, 18, 24, 25, and X) show complete chromosomal conservation with their *O. cuniculus* counterparts; thirteen *O. forresti* chromosomes (OFO6, 9–13, 17, 19–23, and 26) each correspond to one *O. cuniculus* chromosomal segment; 6 *O. forresti* chromosomes (OFO1–3, 7, 8, and 14) each correspond to 2 *O. cuniculus* chromosomal segments or chromosomes. Most homologous segments/chromosomes between *O. forresti* and *O. cuniculus* defined by human painting probes also display a high degree of conservation in banding patterns, with the exception of *O. for-*

resti chromosomes 1p, 3q, 4, 6 and 11 and their corresponding homologues in *O. cuniculus*. Intrachromosomal rearrangements such as inversion or centromere repositioning may have accounted for the banding variations within OFO1p–OCU16, OFO3q–OCU17, OFO4–OCU12, OFO6–OCU7q, and OFO11–OCU14q.

In addition to these intrachromosomal changes, karyotype differences between *O. forresti* and *O. cuniculus* appear to be mainly due to Robertsonian translocations. For example, *O. forresti* chromosomes 1 (homologous to HSA1/10/11) and 3 (homologous to HSA9/15/14/15) can be derived through a few Robertsonian translocations from *O. cuniculus* chromosomes 1 (homologous to HSA9/11), 16 (homologous to HSA1/10) and 17 (homologous to HSA15/14/15). The same situation is also observed in *O. forresti* chromosomes 2 and 13, 6 and 17, 10 and 14, 20 and 26, and 12 and 22 versus *O. cuniculus* chromosomes 3 and 15, 7, 8 and 20, 10, and 11, respectively (fig. 1). In contrast, previous G-banding comparison has suggested that tandem fusions have played a major role in the evolution of lagomorph karyotypes [Stock, 1976]. There is indeed one obvious example of chromosomal

evolution via tandem fusion: the evolution of *O. cuniculus* chromosome 2p apparently could be explained by a tandem fusion of *O. forresti* chromosomes 21 (= HSA4) and 23 (= HSA4/8p). However, it is most probably that *O. forresti* chromosomes 21 and 23 have evolved from *O. cuniculus* chromosome 2p via a fission event as the HSA4/8p association represents a conserved ancestral synteny. Therefore, together our results suggest that Robertsonian translocations, rather than tandem fusions, are the dominant type of interchromosomal rearrangements (fig. 1). However, further comparative chromosome painting data from more pika species are required to determine the direction of karyotype evolution of lagomorphs.

Further Cytogenetic Evidence for the Superorder Glires

Previous comparative analyses have indicated that HSA1/10p and HSA9/11 (to a lesser extent) associations are considered to be the cytogenetic signatures characterizing the clade Glires [Stanyon et al., 2003; Li et al., 2004]. Our results show that the HSA1/10p association appears to be present in the *O. forresti* chromosome 1. Thus, the existence of HSA1/10p association in the pika genome provides further cytogenetic evidence for the Glires hypothesis. However, the HSA1/10p association is absent in the genomes of *Pedetes capensis* (Pedetidae), *Sicista betulina* (Dipodidae), and *Castor fiber* (Castoridae) [Graphodatsky et al., 2008], it seems likely that this association has been disrupted in their common ancestor. Interestingly, HSA1/10 is also present in all Perissodactyla species for which the Zoo-FISH data are available [Yang et al., 2003a, 2004; Trifonov et al., 2008] and in the Malayan flying lemur (*Galeopterus variegatus*) [Nie et al., 2008]. However, reciprocal chromosome painting data show that the HSA1/10 association detected in both Perissodactyla species and the Malayan flying lemur came from the segments homologous to HSA1 and 10q [Yang et al., 2003a, 2004; Nie et al., 2008; Trifonov et al., 2008]. Therefore, the HSA1/10p association is indeed a cytogenetic signature that supports the grouping of orders Lagomorpha and Rodentia into the superorder Glires.

Although HSA9/11 association is found in domestic rabbit [Korstanje et al., 1999; Chantry-Darmon et al., 2005], it is absent in the genomes of other lagomorphs so far investigated. The karyotypic relationships within the leporids have been examined by chromosome painting using *O. cuniculus* chromosome-specific probes [Robinson et al., 2002]. The results showed that *O. cuniculus* chromosome 1 (homologous to HSA9/11) has broken up in the leporid lineage (i.e. homologous to HSA9 and 11,

respectively) [Robinson et al., 2002]. Moreover, HSA9/11 association is also absent in the genome of *O. forresti* in our study. Nevertheless, comparative chromosome maps between human and representative species of the Rodentia [Richard et al., 2003; Stanyon et al., 2003; Li et al., 2004, 2006; Graphodatsky et al., 2008] have suggested that the HSA9/11 association could be regarded as a component of the ancestral karyotype of the Rodentia [Graphodatsky et al., 2008]. Considering that the family Ochotonidae consists of about 30 species, comparative chromosome painting data from more representative pika species, in particular those with lower diploid numbers, are needed to further validate if the HSA9/11 association could represent the shared derived cytogenetic character of the superorder Glires.

Conservation of Ancestral Eutherian Syntenies in O. forresti

The comparative chromosome maps between human and representative species of the extant 18 eutherian orders have been used to infer the composition of the ancestral eutherian karyotype (AEK) [Chowdhary et al., 1998; Murphy et al., 2001b; Richard et al., 2003; Yang et al., 2003b; Svartman et al., 2006; Ferguson-Smith and Trifonov, 2007]. Our results show that the ancestral synteny equivalent to human chromosomes 2pq, 2q, 6, 8q, 9, 10q, 11, 13, 17, 18, 19p, 20, and X have been conserved in the genome of *O. forresti*, while the ancestral synteny of HSA1, 5, and 7a each have been fragmented by additional fission events in the *O. forresti* genome. In addition, our results demonstrate that the postulated ancestral eutherian syntenic associations (i.e. HSA3/21, 4/8p, 7/16p, 14/15, 12pq/22qt, 12qt/22q, 16q/19q) are all present in the *O. forresti* genome. Therefore, most of the proposed AEK have been retained in the *O. forresti* genome. However, *O. forresti* clearly has a derived karyotype. By comparing our result with the putative AEK as suggested by Ferguson-Smith and Trifonov [2007], we can speculate that at least 11 chromosome fissions, 7 chromosome fusions, 1 inversion, and 3 centromere repositionings were needed to convert the karyotype of $2n = 46$ AEK to that of $2n = 54$ *O. forresti*. Therefore, the *O. forresti*, like the *O. cuniculus*, has a relatively conserved genome organization.

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