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GENOMICS

Genomics 82 (2003) 245–249

www.elsevier.com/locate/ygeno

Short Communication

Reciprocal chromosome painting shows that squirrels, unlike murid rodents, have a highly conserved genome organization

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Received 20 November 2002; accepted 4 April 2003

Abstract

We present the first report of reciprocal chromosome painting between humans and a rodent. Gene mapping and sequencing data lead to the generalization that rodent genomes are highly rearranged. In contrast, our results show a surprising conservation of genome structure between humans and squirrels. The synteny of 12 human chromosomes was entirely conserved (5, 6, 9, 11, 13–15, 17, 18, 20, 21, and X). Of the 12 syntenic associations of human chromosomes present in the squirrel, six are well-known ancestral eutherian associations (3/21, 4/8, 7/16, 12/22, 14/15, 16/19). Apparently, few derived translocations characterize the evolutionary origin of the rodents. One association (10p/1qter) may be a cladistic marker for the cohort Glires, linking rodents and lagomorphs.

Keywords: Chromosomal homology; Zoo-FISH; Synteny; Phylogeny; Sciurids

Over the past decade chromosomal homology between literally dozens of species in a wide range of mammalian orders was assigned by Zoo-FISH [1,2]. Even though reciprocal hybridizations provide more detailed information on homology, most of these reports used human chromosome probes in unidirectional painting [3–7].

Rodents are one placental mammalian order in which Zoo-FISH using human paints has met with almost no success [8,9]. Despite their importance in biomedical research and the wealth of gene mapping data available for mouse and rat, there is as yet no chromosome painting map to connect a rodent genome with humans or any other nonrodent species.

This report is the first study of chromosomal homology between humans and a rodent established by chromosome painting. Unlike the human/mouse gene map comparisons, the results show a surprising conservation of genome structure between humans and squirrels.

We employed fluorescence-activated chromosome sorting and degenerate oligonucleotide primed PCR (DOP-PCR) to construct chromosome paints for both humans and the common Eastern gray squirrel. PCR primers, amplification, and labeling conditions were as previously described [4,9,10]. The chromosomal homology between squirrels and humans was mapped by reciprocal fluorescence in situ hybridization (FISH). Hybridization and detection procedures were as previously described [5]. Metaphase chromosome preparations of two male Eastern gray squirrels, *Sciurus carolinensis*, were prepared from primary fibroblast cell cultures according to standard procedures. To facilitate chromosome identification, most chromosomes were banded prior to in situ hybridization by G-banding in addition to the DAPI banding concurrent with FISH [11].

The bivariate flow karyotype of the Eastern gray squirrel ($2n = 40$) was resolved into 20 peaks and DOP-PCR provided chromosome paints from each peak. These paints were then hybridized to squirrel metaphases to identify the chromosome content of each peak of the flow karyotype. Chromosomes 10/11, 11/13, 12/13/14, and 16/17 often sorted together. Nevertheless, the FISH signals of these chromosomes in both the squirrel and the human genome could be assigned unequivocally, since the two homologues of these chromosomes appeared in varying combinations with other chromosomes in the flow karyotype.

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0888-7543/03/$ – see front matter
doi:10.1016/S0888-7543(03)00109-5

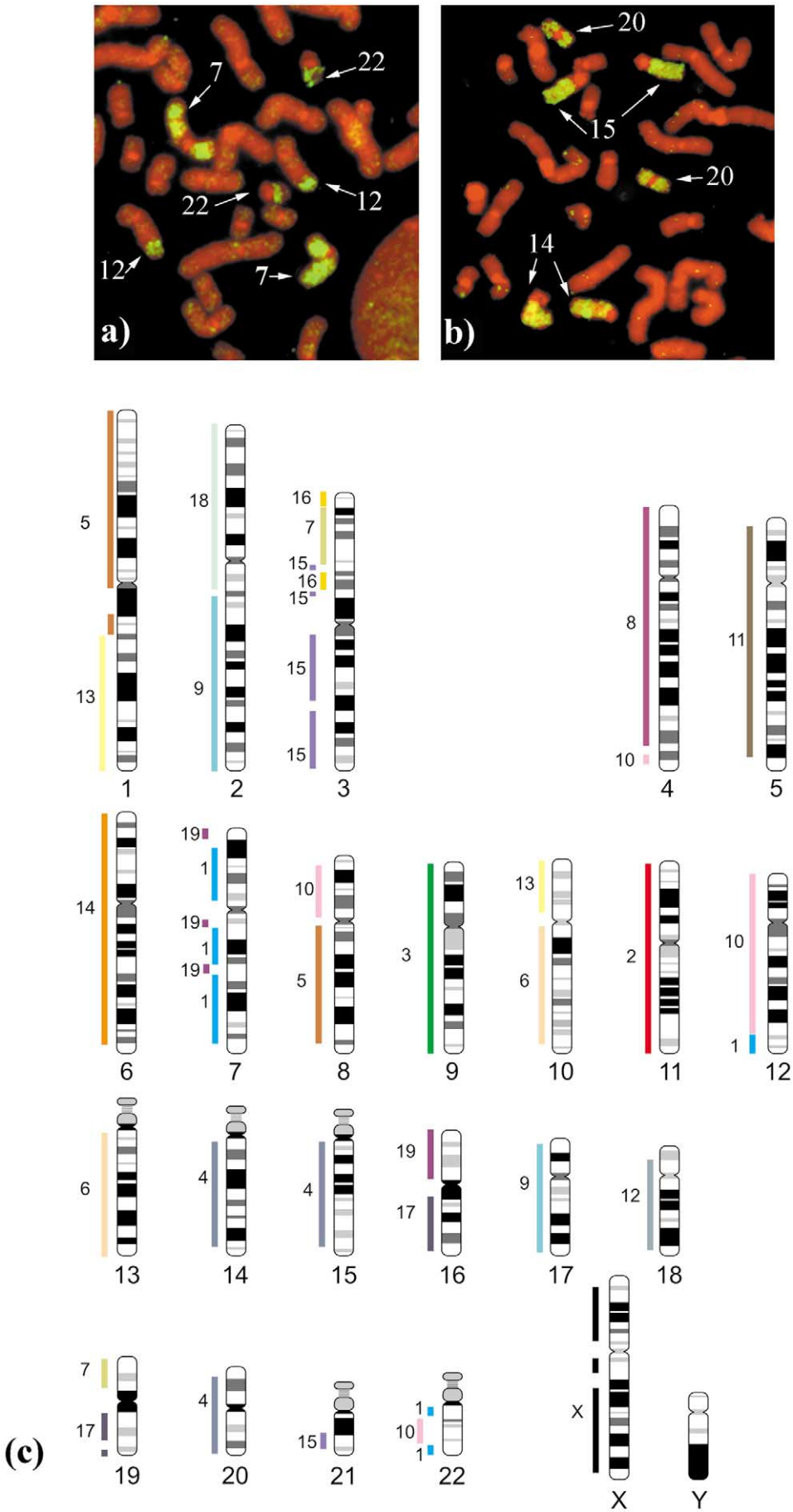

Fig. 1. (a) Squirrel Chr. 1 hybridizes to human 7, 12, and 22. (b) Squirrel Chr. 4 hybridizes to human 14, 15, and 20. (c) Idiogram of human karyotype with hybridizations of squirrel chromosomes numbered to the left. Some small segments remained unhybridized on chromosomes 3, 4, 7, and 19.

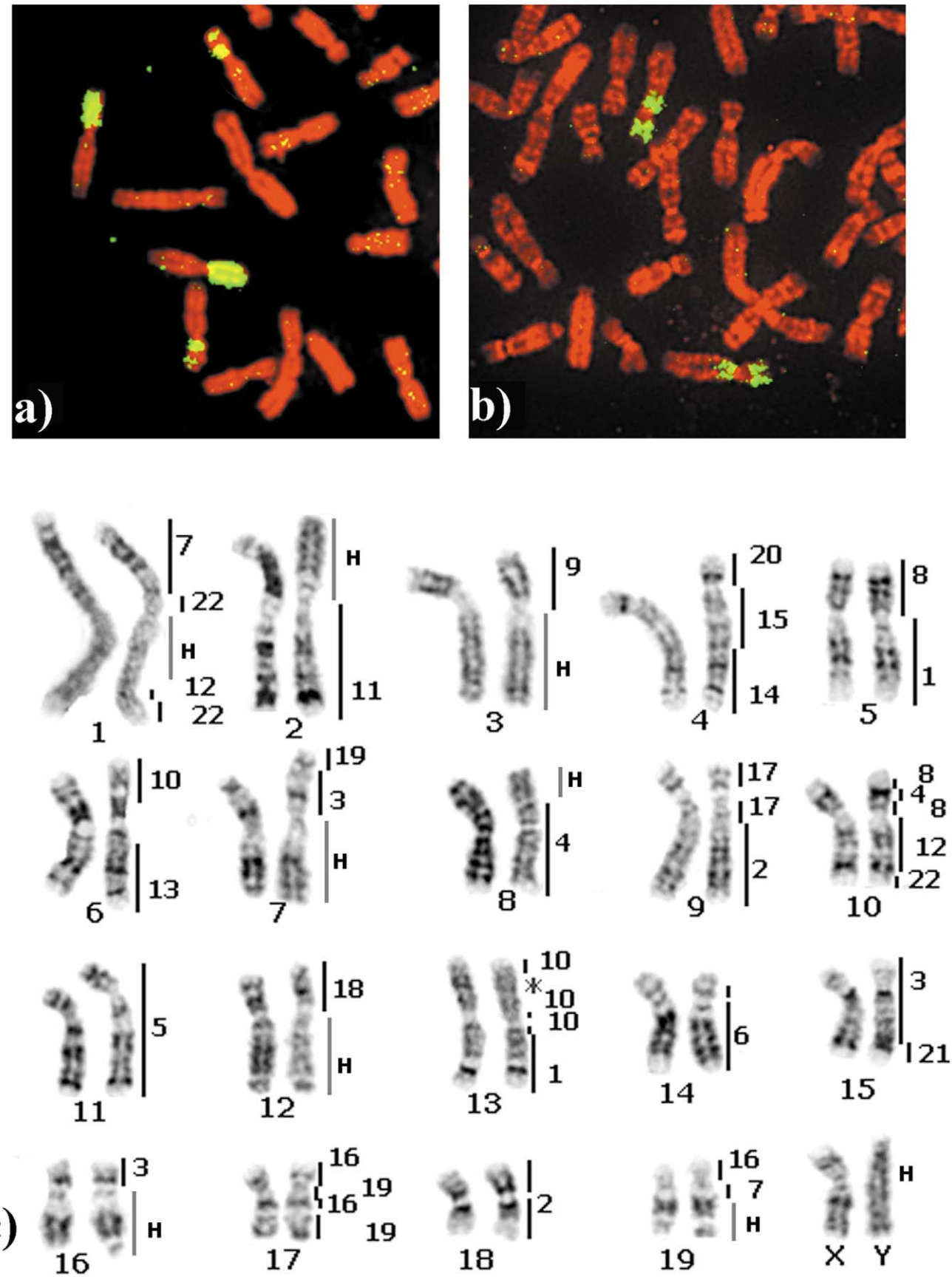

Fig. 2. (a) Human chromosome 8 paint lights up squirrel Chr. 5p and two signals on squirrel 10p. (b) Human chromosome 17 hybridizes to the short arm and pericentromeric region of squirrel 9. (c) G-banded karyotype of Eastern gray squirrel with chromosomes numbered below and human chromosome hybridizations assigned to the right. The lateral lines marked with an H show the presence of heterochromatin blocks that remained unhybridized.

Paint probes of the Eastern gray squirrel chromosomes were hybridized to human metaphases and 43 signals were detected on the human karyotype (Fig. 1). Eight Eastern gray squirrel chromosome probes (2, 3, 8, 11, 12, 14, 18, and X) provided a single hybridization signal on human chromosomes. The Y-chromosome paint produced no signal. Eleven human autosomes (5, 6, 9, 11, 13–15, 17, 18, 20, and 21) were painted by single squirrel chromosome paints.

Paints specific to each human chromosome except the Y were used to hybridize squirrel metaphases (Fig. 2). The 23 human paints (autosomes plus X) produced 39 clear signals on the squirrel karyotype. Nine squirrel chromosomes (2, 3, 8, 11, 12, 14, 16, 18, and X) were hybridized only by single human chromosome probes. The complete synteny of 12 human chromosomes (5, 6, 9, 11, 13, 14, 15, 17, 18, 20, 21, and X) was maintained in the squirrel karyotype. Fourteen associations between human chromosomes are present in the squirrel karyotype: 1/8, 1/10, 2/17, 3/19, 3/21, 4/8, 7/16, 7/22, 8/12, 10/13, 12/22 (three times), 14/15, 15/20, and 16/19 (two times). Associations that are separated only by unhybridized areas (heterochromatic regions or centromeres) were also counted.

Gene mapping and sequencing data amply illustrate that the most extensively analyzed rodent genomes, the mouse and rat genomes, are highly rearranged compared to humans and other mammals [12–14]. It has become a consensus that rodents have more highly fragmented genomes than other mammalian orders. Contrary to expectations, this report shows extensive conservation of chromosomal syntenies between a rodent, the Eastern gray squirrel, and humans. Our data show that the squirrel has a conserved genome not far removed from the architecture found in many other mammalian orders. Indeed, the squirrel karyotype is phenetically closer to humans and cats than it is to either rats or mice. The karyotype of the gray squirrel displays a high similarity to reconstructions of ancestral placental mammal karyotypes [1,2,15]. According to our chromosome painting data, only eight translocations, one inversion, and one chromosome break would be necessary to derive the squirrel karyotype from the reconstructed ancestral placental karyotype. We can conclude that few derived translocations characterized the evolutionary origin of the rodents.

Research on a wide range of mammalian species shows that about half (6 of 14) of the chromosome associations in the squirrel karyotype are probably ancestral associations: 3/21, 4/8, 7/16, 12/22, 14/15, 16/19 [1,2,15]. The other associations (1/8, 1/10, 2/17, 3/19, 7/22, 15/20, 8/12, 10/13) appear to be derived. Squirrel, rat, and mouse have the associations 1/10 and 8/12 [13]. These associations could have been present in the last common ancestor of squirrels and murids but the high number of rearrangements in murid genomes makes convergence more likely and weakens this hypothesis. The apparent absence of the other associations in both these species, however, more strongly suggests that the remaining associations did not constitute genomic elements present in the last common ancestor of all rodents.

The cytogenetic data are suggestive of a phylogenetic link between lagomorphs and rodents. Of the apparently derived associations present in the squirrel karyotype the association 1/10 is also found in the rabbit. Further, the reciprocal painting of rabbit probes onto human suggests that rabbit 16 and squirrel 13 are probably homologous because both chromosome paints go to human 10p and most of human 1qter [16]. The painting data would then support the classification of rodents and lagomorphs into the superorder Glires, a relationship recently supported by nuclear DNA sequence analysis [17].

As noted above, the high evolutionary rates found in the mouse genome suggest that comparisons of the mouse syntenic associations for reconstructing the ancestral genome of mammals must be conducted with extreme care. If we use the chromosome associations found in the squirrel as a minimum for rodents, then the mouse has at least 76 additional associations that appeared one or more times [14]. High evolutionary rates also mean that the chance of convergence giving rise to "red herrings" is great. The conclusions of Postlethwait et al. [18] illustrate this danger. They concluded that "because individual chromosomes in zebrafish and mouse contain putative orthologs of Hsa11, Hsa15 and Hsa19q . . . these chromosomes were syntenic in the last common ancestor of zebrafish and tetrapods. . . ." Based on the absence of syntenic associations of homologs to human 15, 19q, and 11 in the squirrel, primates, and various species from other mammalian orders, it now appears improbable that these associations are ancestral for placental mammals. An ancestral mammalian chromosome containing 17/16p/7b/22 seems equally unlikely.

Acknowledgments

The authors thank Lisa Adams for her assistance in tissue culture and other laboratory procedures and William Murphy and Marta Svartman for their comments.

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