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Nucleotide sequences of B1 SINE and 4.5S$_I$ RNA support a close relationship of zokors to blind mole rats (Spalacinae) and bamboo rats (Rhizomyinae)

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ABSTRACT

Until recently, zokors (Myospalacinae) were assigned to the Cricetidae family. However, analysis of mitochondrial and nuclear genes suggests a sister relationship between zokors and subterranean rodents of the Spalacidae family, namely blind mole rats (Spalacinae) and bamboo rats (Rhizomyinae). Here, we cloned and sequenced copies of the B1 short interspersed element (SINE) from the genome of zokor *Myospalax psilurus*. The consensus nucleotide sequence of zokor B1 was very similar to spalacids and rhizomyids, but not cricetids. Similar to spalacids (*Spalax microphthalmus*) and rhizomyids (*Tachyoryctes splendens*), zokor contained two variants of the 4.5S$_I$ small nuclear RNA. The longer variant (L-variant, 104 nucleotides) was found only in zokor, spalacids and rhizomyids. The short, or S-variant (98 nucleotides), had a wider distribution; however, analysis of the nucleotide sequences of S-variants of 4.5S$_I$ RNA confirmed that zokors are closely related to spalacids and rhizomyids, but not to cricetids. The evolution of the 4.5S$_I$ RNA genes and pseudogenes is discussed.

1. Introduction

Zokors are Asiatic burrowing rodents that comprise two genera, *Myospalax* and *Eospalax*. Their taxonomic position is controversial. Tullberg (1899) first suggested a relationship between zokors and two families of subterranean muroid rodents, Spalacidae and Rhizomyidae. Later, other morphologists also assigned zokors to Spalacidae (Miller and Gidley, 1918; Chaline et al., 1977). Gradually, however, an alternative classification that assigned zokors (subfamily Myospalacinae) to Cricetidae became accepted (Simpson, 1945; Chaline et al., 1977; Gromov and Polyakov, 1977; Carleton and Musser, 1984; Carrol, 1988; Pavlinov, 2003). Zokors could also be regarded as a separate branch within arvicolines rather than an independent derivative of the ancestral cricetid stock. Thus, Kretzoi (1955) amended arvicolines to the family level, placed myospalacines as a subfamily, and assigned the same rank to true lemmings (Lemminae) and mole voles (Ellobiinae). This taxonomic affiliation of zokors was recently supported by Agadjanian (2009). Molecular studies initially confirmed the relationship between zokors and cricetids (Michaux and Catzeflis, 2000; Michaux et al., 2001). However, the results of Norris et al. (2004) as well as Jansa and Weksler (2004) suggested that *Myospalax* is a sister group to the clade containing the Spalacinae and Rhizomyinae subfamilies.[2] These discrepancies have been attributed to the misidentification of a single sample of zokor in the study by Michaux and Catzeflis.

To date, phylogenetic data on zokor has been obtained primarily through analysis of mitochondrial and nuclear genes. The possibility remains that genetic similarities among zokors, mole rats and bamboo rats could be the result of convergence caused by a similar (subterranean) mode of life. Additionally, phylogenetic analysis of gene nucleotide sequences requires complex computer programs, and the use of different algorithms can result in different trees. Ideally, gene-based phylogenetic trees should be confirmed by analysis of other kinds of molecular markers (Shedlock and Okada, 2000; Murphy et al., 2004; Kriegs et al., 2007; Kramerov and Vasetskii, 2009). We have developed several markers in our laboratory that can potentially shed light on the phylogeny of zokors. One of these markers, the B1 *s*hort *i*nterspersed *e*lement (SINE) is likely not under selection pressure (Veniaminova et al., 2007b). A second marker, the 4.5S$_I$ small nuclear RNA, has yet to be functionally defined (Gogolevskaya and Kramerov, 2002). Both sequences often exhibit distinct features among rodent families. Here, we investigated the feasibility of using these markers to determine whether zokors are a sister group to hamsters (Cricetidae), or to mole rats (Spalacinae) and bamboo rats (Rhizomyinae).

Abbreviations: SINE, short interspersed element; LINE, long interspersed element; Pol III, RNA polymerase III; PAGE, polyacrylamide gel electrophoresis.

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[2] Recently, the rank of spalacids and rhizomyids has been lowered to subfamilies.

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Fig. 1. Alignment of nucleotide sequences of cDNA clones from golden hamster (*Mesocricetus auratus*), brown rat (*R. norvegicus*) and Russian mole rat (*S. microphthalmus*) (Gogolevskaya and Kramerov, 2002). Four variants of mole rat 4.5S$_I$ RNA are shown: S variant, major L1 variant, and minor L2 and L3 variants. Only nucleotides that differ from the hamster RNA sequence are indicated. Dashes indicate gaps. The nucleotide sequences of the PCR primers are shown above the alignment.

SINEs or short retroposons are repetitive 80 to 400 basepair (bp) sequences that are interspersed over the eukaryotic genome and are amplified through reverse transcription (Kramerov and Vassetzky, 2005; Ohshima and Okada, 2005). The mammalian genome contains two to four families of SINEs that vary in number from 10^4 to 10^6 copies per genome. Individual nucleotide sequences are 65–90% similar. Most SINE families are derived from a cellular tRNA; however, some SINE families also descended from 7SL RNA or 5 S rRNA. SINEs are transcribed by RNA polymerase III (Pol III) from an internal promoter in their 5′ region. The classical SINE promoter consists of two boxes (A and B) that are spaced 30–40 bp apart. SINEs are considered nonautonomous mobile elements, since they do not encode proteins and utilize the reverse transcriptase of *l*ong *in*terspersed *e*lements (LINEs) for amplification. SINEs of placental mammals usually proliferate with the help of LINE-1.

The first two SINE families discovered, B1 of mice, rats, and hamsters (Kramerov et al., 1979; Krayev et al., 1980; Haynes et al., 1981) and Alu of primates (Deininger et al., 1981), originated from 7SL RNA, a component of the signal recognition particle (SRP). SRP is a cytoplasmic ribonucleoprotein present in all eukaryotes that is involved in the translation of secreted proteins (Ullu and Tschudi, 1984). SINEs derived from 7SL RNA have subsequently been identified in tree shrews (Scandentia), but not other placentals (Nishihara et al., 2002; Vassetzky et al., 2003; Kriegs et al., 2007). B1 SINEs are present in the genomes of all rodents analyzed to date, and B1 SINE sequence variants, typically single-nucleotide substitutions and small indels, have been identified that are specific to some rodent families (Veniaminova et al., 2007b). Analysis of these sequence variations (Veniaminova et al., 2007a,b; Churakov et al., 2010) supports one of prevailing rodent trees. Here, we analyzed B1 SINE nucleotide sequences to confirm the relationship between zokors, mole rats and bamboo rats.

The 4.5S$_I$ small nuclear RNA was discovered in the 1970s (Ro-Choi et al., 1972), but its function remains unknown. Synthesis of 4.5S$_I$ RNA is carried out by Pol III, and it exhibits some sequence similarity to the 5′-region of the B2 SINE (Serdobova and Kramerov, 1998). In *Mus musculus* and *Rattus norvegicus*, a single genomic locus harbors three 4.5S$_I$ RNA genes that occupy 80 kb on chromosome 6 and 44 kb on chromosome 4, respectively (Gogolevskaya and Kramerov, 2010). In addition, hundreds of retropseudogenes of 4.5S$_I$ RNA are interspersed throughout the genome. 4.5S$_I$ RNA is specific to only four related rodent families: Muridae, Cricetidae, Spalacidae and Rhizomyidae (Gogolevskaya and Kramerov, 2002).[3] The 4.5S$_I$ RNA of golden hamster is very similar to rat and mouse, differing only by a single nucleotide (Fig. 1). On the other hand, the differences between mole rat and rat/mouse 4.5S$_I$ RNA are more considerable (eight substitutions and two single-nucleotide insertions).

A long 4.5S$_I$ RNA species (4.5S$_I$ RNA-L) has been uncovered in mole rat that differs from the common short species (4.5S$_I$-S) by a number of nucleotide substitutions and a 7-nucleotide insertion (Fig. 1). Here, we showed that the long and short 4.5S$_I$ RNA variants of mole rat are also present in bamboo rats and zokors, but not in other rodents, including hamsters. These data indicate a sister relationship between myospalacids, spalacids and rhizomyids, but not cricetids.

2. Materials and methods

2.1. DNA and RNA isolation and electrophoresis

Genomic DNA, ethanol-preserved tissue and live animals were kindly provided by the researchers listed in Supplementary Table 1. DNA was isolated from liver, kidney or muscle by incubation of the tissue with proteinase K followed by phenol/chloroform extraction. Total RNA was isolated from the liver by the guanidine isothiocyanate method (Chomczynski and Sacchi, 1987).

2.2. Cloning of B1 SINE

Zokor genomic DNA (5.0 μg) was digested with *Eco*RI and *Hin*dIII and then separated by 1% agarose gel electrophoresis. DNA fragments of 0.5–1.2 kb were collected by reverse electrophoresis on a DEAE membrane. DNA was eluted from the membrane and precipitated by ethanol using 10 μg of glycogen as a carrier. The isolated genomic fragments (0.5 μg) were ligated into 0.3 μg of pGEM3Z digested with *Eco*RI and *Hin*dIII and used to transform XL-1 Blue *Escherichia coli* cells. Colony hybridization was carried out at 60 °C in 4× SSC containing 0.5% SDS, 5× Denhardt's solution, 0.1 mg/ml boiled herring sperm DNA and a ^{32}P-labeled murine B1 probe (Vassetzky et al., 2003). Nitrocellulose filters were washed in 0.1× SSC containing 1% SDS at 42 °C and positive colonies were identified by autoradiography. B1-containing *E. coli* clones were purified by two additional rounds of colony hybridization.

2.3. Northern hybridization

RNA (30 μg) was separated by electrophoresis on 6% polyacrylamide gels containing 7 M urea. RNA was transferred onto a Hybond XL membrane using a semi-dry electroblotting apparatus (TE 77 PWR, Amersham Bioscience). Hybridization was carried out at 42 °C as described for cloning of B1 SINE, with the addition of 50% formamide to the hybridization solution. Preparation of the ^{32}P-labeled 4.5S$_I$ RNA-

[3] In the cited article, we followed the traditional classification considering mole rats and bamboo rats as two different families, Spalacidae and Rhizomyidae, respectively. 4.5S$_I$ RNA was not studied in Rhizomyidae, but its genes/pseudogenes were found in the *Rhizomys pruinosus* genome.