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LINE-1 element in the vole *Microtus subarvalis*

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Highly repetitive DNA sequences of the LINE-1 (L1) family, which are interspersed in the genomes of various organisms, are among the many transposable elements deserving attention. L1 elements are widely distributed throughout the mammalian genomes (Burton et al. 1986). Their occurrence was first demonstrated in man and other mammals. Subsequently, L1-like elements were found to occur in insects and higher plants (DiNosera and Sakaki 1990).

No definite role has yet been assigned to L1 elements. Their presence in virtually all organisms suggests that they might be important for the structural organization and function of the genome. The sequence features of L1 include two open reading frames (ORFs), the short 5′ proximal ORF1 and the about four times longer 3′ proximal ORF2. L1 element contains a poly(A) tail at the 3′ end and is flanked by short direct repeats (Fanning and Singer 1987). The sequence similarity between L1 and retroviruses and related transposable elements suggests that the predicted ORF2 encoded protein is involved in the transposition of L1 element. The support for the involvement of L1-encoded proteins in L1 transposition has come from in vitro studies (Leibold et al. 1990). More recently it was shown that human L1 element encodes reverse transcriptase activity (Dombroski et al. 1991; Mathias et al. 1991).

We describe here genomic L1 element detected in voles of the Microtus genus. Genomic DNA was prepared from vole liver according to standard procedures (Henry et al. 1990); complete digestion was performed with endonuclease *Eco*RI. Comparative restriction analysis of genomic DNAs from five vole species is shown in Fig. 1. Clearly, the number and distribution of bands corresponding to DNA repeats are specific to each of the species. The intense band is common to the four species, and it represents the same

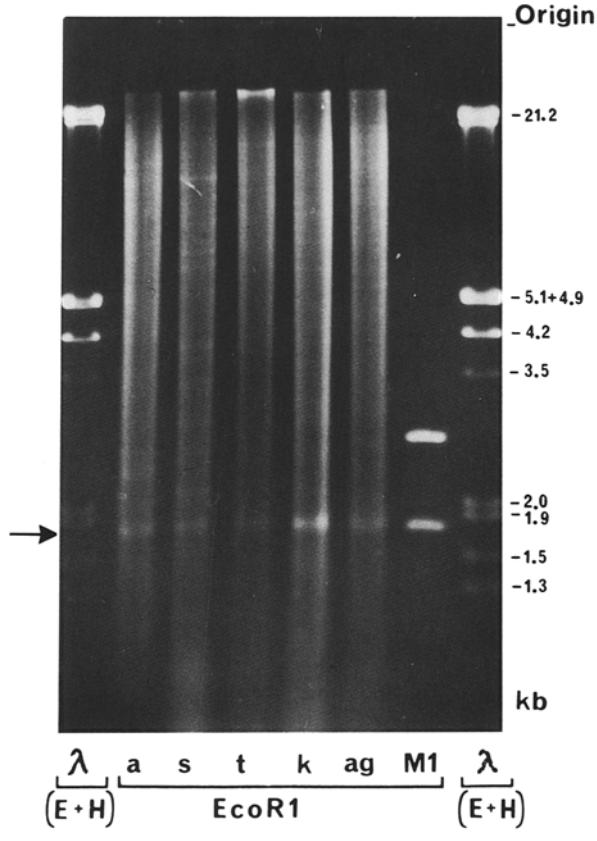

Fig. 1. Restriction digests of genomic DNAs from five vole species of the genus *Microtus: M. arvalis* (a), *M. subarvalis* (s), *M. transcaspicus* (t), *M. kirgizorum* (k), *M. agrestis* (ag). The vole species share a 1.8-kb fragment **(arrow).** Lane λ contains *Eco*RI-*Hin*dIII digest of λ DNA. Lane M1 contains pMs1 digested with *Eco*RI.

The nucleotide sequence data reported in this paper have been submitted to EMBL Data Library and have been assigned the accession number X59853 M.SUBARVALIS, LINE 1 DNA.

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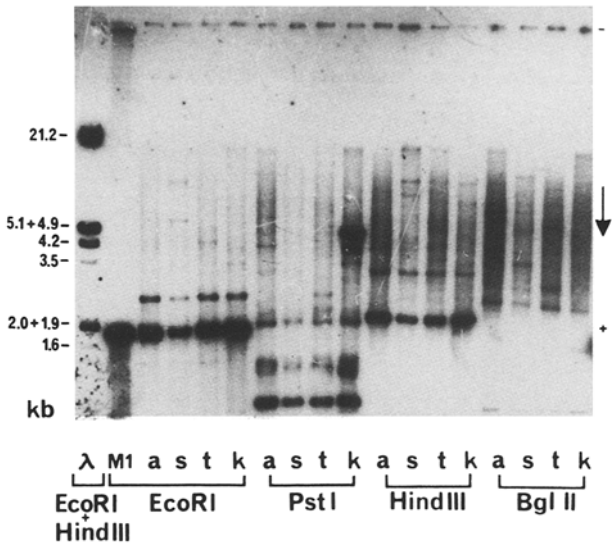


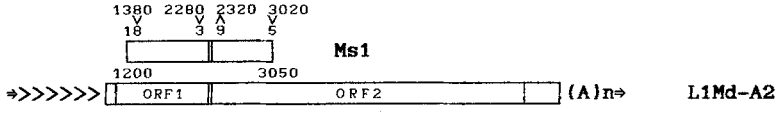
Fig. 2. Southern blot analysis of DNAs from four vole species: *M. arvalis* (a), *M. subarvalis* (s), *M. transcaspicus* (t), *M. kirgizorum* (k). Vole genomic DNAs were digested with restriction endonucleases *Eco*RI, *Pst*I, *Hin*dIII, and *Bgl*II, separated by 1.0% agarose gel electrophoresis, transferred onto capron filters, and hybridized with ^{32}P-labeled pMS1. Lane M1 contains fragment pMs1. Lane λ contains ^{32}P-labeled λDNA fragments produced by digestion with *Eco*RI-*Hin*dIII.

copy number for each. The arrow indicates the 1.8-kb fragment present in the patterns of all the vole species. Henceforth it will be referred to as Ms1. The region of the agarose gel containing Ms1 was cut from it, and the DNA was recovered by electroelution. Ms1 was ligated into the *Eco*RI site of plasmid pUC19 (Sambrook et al. 1989).

Distribution of Ms1 in the five vole species and various organisms was analyzed by Southern blotting (Fig. 2). The results of Southern blot analysis assured us that Ms1 is present in the genomes of all the vole species. It is noteworthy that, along with a common major band, the restriction patterns showed a set of minor bands specific to each species (Fig. 2).

Copy number for Ms1, as determined by dot hybridization at 65°C (Kafatos et al. 1979), was 10^5 per genome in the five vole species. For a more complete characterization of Ms1, we performed sequence analysis by the method of Maxam-Gilbert with acetone precipitation (Baram and Grachev 1985). We carried out a search for Ms1 in the EMBL Data library, and we determined that there is approximately a 70% homology between Ms1 and mouse L1Md-A2 (Loeb et al. 1986). Figure 3 gives the alignment for Ms1 and mouse L1Md-A2. The region showing homology spans from ORF1 and the start of ORF2. Further experi-

A



B

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SQ   SEQUENCE  1834 BP;   360 A;   303 C;   364 G;   807 T.
     GAATTCCAGG TTTAGTCCGT GGTGATAATA CACAGGTGGT TATGGATATT TTTTTGTAAC
     TGTGGAAGTT TGCTTTGTTA ACGAGGATGT GGTCAATTTT CGAGAAGGTT CCATGAGCTG
     CAGAGAAGAA GGTATATTCT TTTCTATTTG GGTGGAATGT TCTATAGACG TCTGTTAAGT
     CCATTTGATT CAATACGTCC ATTAATTCTC TTATATCTCT GTTAGGTTTC TGTCTGATTG
     ACCTGTCCAT TGGTGAAAGA GGCATGTTGA GATCTCCTAC TATTAGTGTG TGCGGTTTGA
     TGGCAGCCTT GCGTTTTAGT ATTGTTTCTT TTATATATGT GGGTGCTTTT ATATTAGGGG
     CATAAATATT CAGGATTGAG ACCTCATCCT GATCAATTGT TCCTGTTATG AGTATAAAAT
     ATCCCTCTCC ATCTCTTCTG ATTGATTTAA GTTTGAAGTC AACTTTGTTA GAAATTAATA
     TGGCCACACC TGCTTGTTTC TTAGATCCAT TTGCTTGATA AACCTTTTCC CATCCCTTTA
     CCCTAAGTAG GTGCCTGTCT TTGTGGTTGA GGTGTGTTTC TTGTAAACAG CAGAACGTTG
     GATCCTGTCT GCGCATCCAA TCTCTTAGCC TGTGCCTTTT TATAGGTGAG TTGAGTCCAT
     TGACATTAAG GGATATTAAT GACCAGTGGT TGCTAACTCC GGTCACTTTT TTAGTCATAT
     AGTTTGTGTG TTTGCTTTCT TTTAGTTGCG CTGGTAAAGG GTGTCTAGAA GTTTGAGTAA
     TTGTGGTCAT TGTTGGTCTC CTTGGTTTGT GATTTTCCTT CTATTACTTT CTGTAAGGCT
     GGATTTGTGG CTACGTATTG TTTAAATTTG TTTTTATCCT GAAAATTTTG TTTTCTGTAT
     TTATAGTGAA CGAAAGCTTG GCCGGGCATA GTAGTCTGGG CTTGCATCCA TGTTCTCTTA
     GCTTTTGCAG TATATCTATC CAAGACCTTC TGGCTTTCAT GGTTTCTATA GAGTAGTCAG
     GCGTAAGTCT GATAGGTTTA CCTTTATATG TAATTTGACC TTTTTCCTTT GCAGCTCTTA
     ATATTTTTTC TTTATTCTGT ATGTTTTTGTG TTCTGATTAT TATATGGCGA GGAGATGTTT
     TTTTTTGGTC CAGTCTATTC GGTGTTCTGT ATGCTTCTTG AACCTTCAAA GGAATATCTT
     TCTTTAGGCT GGGGAAGTTT TCTTCTATAA TTTCATTAAA TATATTTTCT GAACCATTGA
     GCTGTATTTC TTCTGCTTCT TCTATCTTAA TTATTCTTAG GTTTGGTCTT TTTATTGTGT
     CCCATATTTG TGAATGTTTT GTGATGAGAA TTTGTTGGTT TTGCTGTTTT CTTTGATCAG
     TGTGTTAATT TTCTCTATGG TATCTTCAGT GTCTGACATT CTTTCTTCTA ACTCTTGTAA
     TCTGTTGGTA GTACTTGTCT CTATAGTTCC TGTTCGTTTA CCCAGATTTT CCATCTCCAA
     CCTTCGCTCG GTTTGTGTTT TGTTCATTAC TTCCATTTCA TTCTTCAGGT CTTGAACCAT
     TTCCCTTACC TGTTTGATTG CATTTTCTTG TTTCTCTTGA TTTTCTTGGG TATCTTTGAG
     CGATTTATTA ATTTCCTCTA CCTTTTTGTT TTTAATCTCT AATTGTTTAT GGCAGTTTTT
     CACCTCCTGT TTAAGGTCTT CTATTATTTT CAAAAAATTC ACTTTTGAGT CGATTTCTTC
     TAATTCTTCT GGAGTAGGGT GTACAATTCT TCTTATTTCG GGATCCTTGG GTTCTGGTGA
     TGTCATGTTG CCTTTCAGGT TGTTTGAGGA ATTC
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Fig. 3. Structure of Ms1 sequence. **(A)** Scheme of sequence features of Ms1 in comparison with mouse L1Md-A2 element (Loeb et al. 1986). Insertions and deletions in Ms1 in accordance with L1Md-A2 are designated as ⋁ and ⋀, respectively. The upper number indicates its location; the lower one, size in bp. ⇒, 13 bp terminal repeats. >>>, 5′terminal repeating region. ORF1, ORF2, open reading frames. (A)n, A-rich 3′ tail. 1200, 3050, the start and the end of homology region. **(B)** Nucleotide sequence of Ms1.

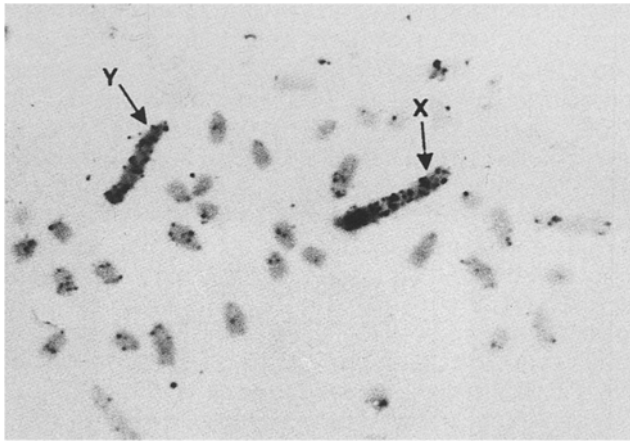

Fig. 4. In situ hybridization of Ms1 fragment to *Microtus subarvalis* metaphase chromosomes. **Arrows** indicate sex vole chromosomes.

ments are needed to determine whether Ms1 fragment is full-length L1 element or truncated like its counterpart in the genomes of all the other species.

We determined the chromosomal localization of Ms1, using in situ hybridization on vole metaphase chromosomes. Tritium-labeled pMs1 fragment was used as a probe. As Fig. 4 shows, Ms1 is scattered throughout the vole genome with a preferential localization on the X and Y chromosomes. Our data did not allow us to make inferences concerning the possible relation of Ms1 and particular functional regions of the genome. Nevertheless, Ms1 distribution on vole chromosomes is of interest when taking into account the data showing high dispersion of L1 elements in the mammalian genomes (Singer 1989). It is unclear why Ms1 has such localization on the vole chromosomes, and additional investigations are required to understand this phenomenon.

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References

Baram, G.I., Grachev, S.A. (1985). Use of lithium perchlorate for isolation and analysis of oligo- and polynucleotides. Bioorganicheskaya khimia 11, 1420–1422. (in Russian).

Burton, F.H., Loeb, D.D., Voliva, C.F., Martin, S.L., Edgell, M.H., Hutchison, C.A. (1986). Conservation throughout Mammalia and extensive protein-encoded capacity of the highly repeated DNA long interspersed sequence one. J. Mol. Biol. 187, 291–304.

DiNosera, P.P., Sakaki, Y. (1990). LINEs: a superfamily of retrotransposable ubiquitous DNA elements. Trends Genet. 6, 29–30.

Dombroski, B.A., Mathias, S.L., Nanthakumar, E., Scott, A.F., Kazazian, H.H., Jr. (1991). Isolation of an active human transposable element. Science 254, 1805–1808.

Fanning, T.G., Singer, M.F. (1987). LINE-1: a mammalian transposable element. Biochem. Biophys. Acta 910, 203–212.

Henry, M.J., Raina, A.K., Ridgway, R.L. (1990). Isolation of high-molecular-weight DNA from insects. Anal. Biochem. 185, 147–150.

Kafatos, F.S., Jones, C.W., Efstratiadis, A. (1979). Determination of nucleic acids sequence homologies and relative concentrations by dot hybridization procedures. Nucleic Acids Res. 7, 1541–1542.

Leibold, D.M., Swergold, G.D., Singer, M.F., Thayer, R.E., Dombroski, B.A., Fanning, T.G. (1990). Translation of LINE-1 DNA elements in vitro and in human cells. Proc. Natl. Acad. Sci. USA 87, 6990–6994.

Loeb, D.D., Padgett, R.W., Hardies, S.C., Shehee, W. Ron, Comber, M.B., Edgell, M.H., Hutchison, C.A. (1986). The sequence of a large L1Md element reveals a tandemly repeated 5′ end and several features found in retrotransposons. Mol. Cell. Biol. 6, 168–182.

Mathias, S.L., Scott, A.F., Kazazian, H.H., Jr. (1991). Reverse transcriptase encoded by a human transposable element. Science 254, 1808–1810.

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual,* 2nd ed. (Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press).

Singer, M.F. (1989). LINE1 sequences: human transposable elements. In *Evolutionary Tinkering in Gene Expression,* M. Grunberg-Monago, B.E.C. Clark, H.G. Zachau, eds. (New York: Plenum Publishing Corp.), pp. 155–162.