Nucleotide sequence of a cDNA encoding an alternative form of LEF-1

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Proteins derived from alternatively spliced forms of transcription factor genes have been discovered and shown to be involved in the regulation of their target gene expression (for review; 1). Since alternative forms usually lack one or two functional domains that mediate transcriptional activation, the suppressive effect on the function of their normal counterpart is explained by competitive joining to the transcriptional machinery. As for the lymphoid enhancer-binding factor LEF-1, two major transcripts (4.2 and 2.7 kb), which we propose to call LEF-1L and LEF-1S, respectively, have been detected in lymphoid organs as well as pre-B, pre-T, and T cell lines. So far, only the cDNA for LEF-1L has been cloned and analysed (2, 3).

A mouse thymic cDNA library in which insert sizes are restricted between 1.5 and 3.0 kb was screened with a PCR amplified LEF-1L probe (nucleotides 1030–1481 of cDNA clone GN8, Figure 1b in (2)). One clone we obtained carried a novel 5′ part whereas the 3′ hybridizing part included the DNA-binding HMG box (4) encoding region. An EcoRI 0.3kb fragment within the novel 5′ part detected exclusively the 2.7 kb transcript by Northern blot analysis (data not shown). Sequence analysis revealed that this clone was 2740bp long (DDBJ, EMBL and GenBank accession number D16503) and that nucleotides 1673–2740 were identical with the 3′ part of LEF-1L, which corresponds to the third and following exons (unpublished data). Open reading frame search unveiled a 5′ untranslated region of 1804 bp, the same reading frame usage as LEF-1L and a coding region of 852 bp. Although nucleotides 1673–1804 are noncoding in LEF-1S, they are coding for amino acids in LEF-1L (2). *In vitro* translation of the RNA obtained by *in vitro* transcription of the LEF-1S cDNA clone produced a single polypeptide with a molecular weight of 38 kD (Figure 1), which is compatible with the value predicted from the newly found open reading frame (31,830 Dalton). Because this alternative form does not contain the acidic amino acid region (5) and a part of the proline rich region (6) of the transcription factor, it is expected that LEF-1S is capable of binding to DNA but unlikely to directly activate the expression of its target genes. From these results, we speculate that LEF-1S serves as a transcriptional repressor that binds to the same sites as LEF-1L binds.

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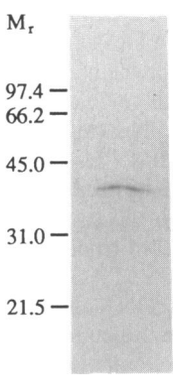

Figure 1. *In vitro* translated LEF-1S polypeptide. The LEF-1S polypeptide was translated in a reticulocyte lysate from the LEF-1S cDNA clone derived transcript and size-fractionated by SDS–PAGE, The sizes of molecular mass markers are indicated in kD.