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Mapping the essential structures of human ribosomal protein L7 for nuclear entry, ribosome assembly and function

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Abstract **Human large subunit protein L7 carries multiple nuclear localization signals (NLS) in its structure: there are three monobasic partite NLSs at the NH_2-region of the first 54 amino acid residues and a bipartite in the middle section at position of 156–167. The C-region of the last 50 amino acid residues displays membrane binding nature, and might involve in forming a nuclear microbody for pre-nucleolar ribosome assembly. The middle section covers 144 amino acid residues which are essential for the structure and function of ribosome. This is evident from findings that truncated L7 without the NH_2-region or the C-region, or missing both regions, is capable of reaching nucleolus and incorporating in ribosome, however, only ribosomes bearing truncated L7 without the NH_2-region is capable of engaging in polysome formation. Combining with the phylogenic findings from homologous sequence alignment, the NH_2-region of L7, besides being as a eukaryotic expansion segment, can be excluded from building a functional eukaryotic ribosome.**

Keywords: Nuclear localization signal; Eukaryotic ribosomal protein; Ribosome assembly; Ribosome expansion segment; Nuclear microbody

1. Introduction

An intriguing aspect of ribosome assembly in the eukaryotic cell is that it is another level of control for gene expression. Ribosomes are assembled from ribosomal proteins and ribosomal RNAs in the nucleolus, and are then exported to the cytoplasm to carry out translation. To accomplish such a mission, ribosomal proteins, after being synthesized, are imported to the nucleus thence to the nucleolus. The nuclear entry of ribosomal proteins, like other nuclear proteins, depends upon a nuclear localization signal (NLS) and uses cargo/carrier machinery to cross the nuclear pores [1]. An earlier survey of ribosomal proteins [2] indicated that most of eukaryotic ribosomal proteins carry either a basic cluster sequence or a basic bipartite sequence [3]. Conversely, there are exceptions as reported in yeast ribosomal proteins S22 and S25 [4], which use an alternated sequence to gain the nuclear entry. This suggests that ribosomal proteins may have a different NLS type. Additionally, most ribosomal proteins carry more than one NLS-like sequence and their real activity in respect to nuclear targeting have seldom been investigated. Human large ribosomal protein L7 represents a striking example, its sequence carries four basic cluster NLS-like type segments within the NH_2-region, and the number of these basic clusters in NH_2-region varies in homologous mammalian L7 proteins: there are six in the mouse and five in the rat [2,5]. So far, the function of these clusters in respect to nuclear targeting has never been experimentally determined. In addition, besides having a high RNA-binding nature [6], the repeated basic cluster sequence have been assumed to be the primary target of auto-antibodies [7], and to be frequently associated with nuclear-related auto-antibodies in auto-immune disease [8,9]. In this study, we have attempted to dissect the essential structure of human large ribosomal protein L7 in terms of nuclear targeting, the role in the functions of ribosome and that of ribosome assembly.

2. Materials and methods

2.1. Cloning and expressions of truncated L7 proteins

Plasmids that contain genes coded for full-length of human L7 protein were obtained from previous studies in this laboratory. The construction of the NH_2-truncated mutant L7 genes was carried out by the same PCR strategy using pGEM-T/L7 as the vector for a cytomegalovirus (CMV) promoter-driven plasmid.

2.2. Cellular localization of transiently expressed truncated L7 proteins

Plasmids that carried flag-tag L7 or mutant genes were transfected into HeLa cells that were seeded on a cover slide one day prior to the transfection. Cells were viewed at different time of post-transfection under a confocal fluorescence microscopy. Cellular localization of expressed flag-tagged L7 was determined using anti-flag antibody (M2, Eastman Kodak Co.) as the primary antibody. Detection of fluorescent was done by applying a second antibody of rhodamine-conjugated anti-mouse IgG antiserum, then exciting with a laser beam (568 nm wavelength).

2.3. Polysome assay

The polysome assay was carried out to detect the *in vivo* engagement of flag-tagged recombinant ribosomes in the protein synthesis. Briefly, embryonic kidney 293T cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum (Life Technologies, Inc.), 5 mM L-glutamine, 50 μg/ml streptomycin, and 50 U/ml penicillin. The cells were then transfected with CMV-driven plasmids that carried different flag-tagged L7 or truncated genes by a calcium phosphate procedure, and then the culture was continued in fresh medium. After 24 h post-transfection, cells (1×10^7 cells) were lysated and analyzed on a 12-ml 10–35% sucrose gradient containing buffer of 20 mM Tris–HCl, pH 7.6; 50 mM KCl and 3 mM $MgCl_2$. Gradients were centrifuged at 35,000 rpm for 165 min in Beckman SW41 rotor at 4 °C. Fractions (0.3 ml) were collected and monitored

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for absorbance at 254 nm wavelength to detect the standard polysome profile. Regions of subunits, monomers and polysome were subjected to the rRNA extraction and analyzed by 2% agarose gel electrophoresis.

For detection of recombinant protein in polyribosome, a modification of ribosomal proteins dot blotting was carried out. Polysome fractions containing ribosomal subunits, monomers and polysome, underwent total protein extraction with 67% acetic acid. The proteins were TCA-co-precipitated with 10 μg BSA, then spotted on nitrocellulose paper and analyzed for immuno cross-reactivity against anti-flag antibody.

3. Results

3.1. The structure within L7 participating in nuclear targeting

The long stretch of basic amino acid cluster at the NH_2-region of human ribosomal protein L7 has previously been suggested as potential basic cluster types of NLS [2,5,6]. To validate such a suggestion, genes coding mutant L7 proteins that have NH_2-terminal end deletion and other deletions were constructed with a flag tagged peptide adjacent to it (Fig. 1A).

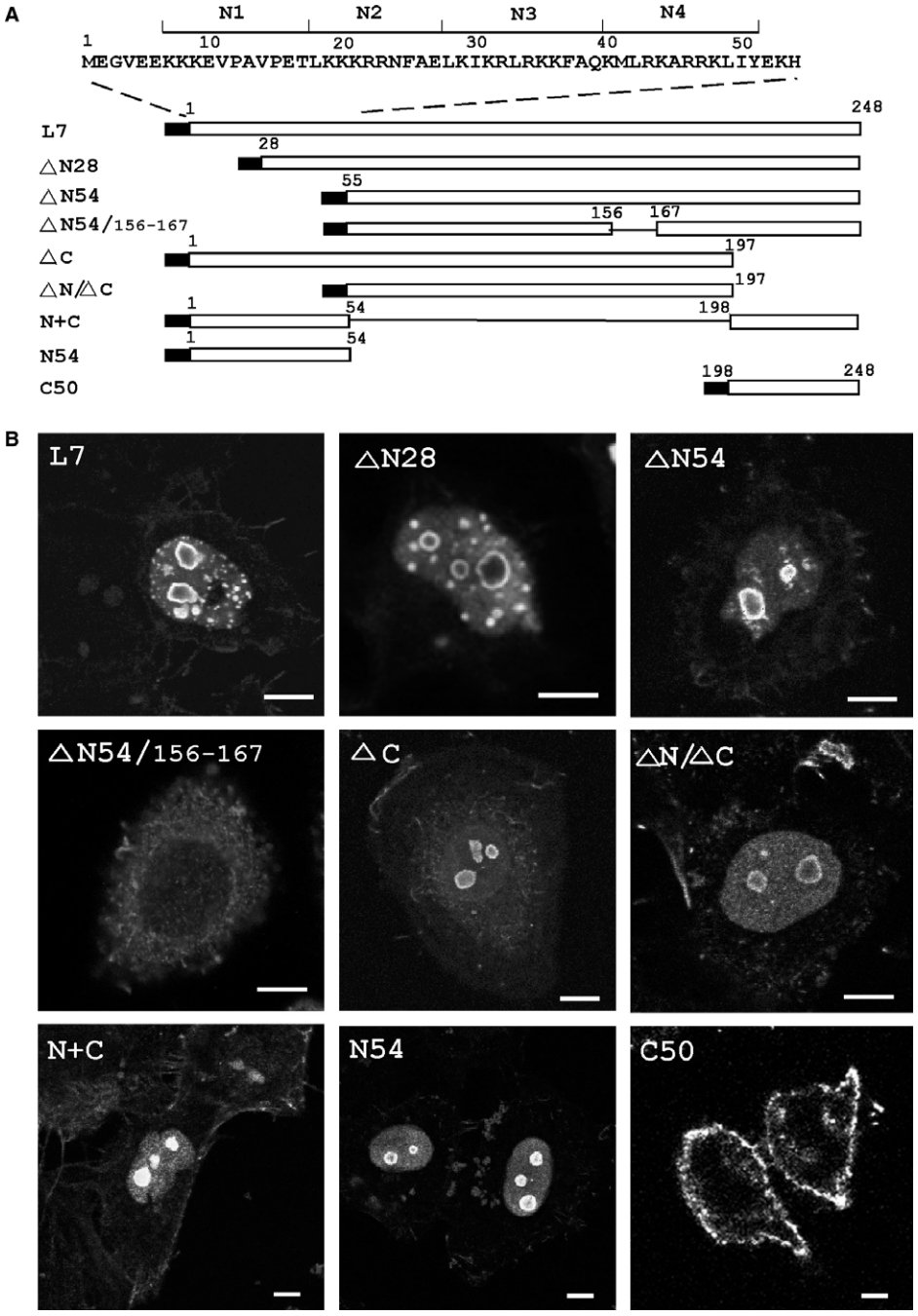

Fig. 1. Confocal analysis of sub-cellular localization of L7 protein and its mutant proteins. (A) The diagrams of mutant construction for this study. The first NH_2-terminal 54 amino acid residues are given and the possible basic cluster is showing in bold letters, N1, N2, N3, and N4 as suggested by Wool et al. (1995). The filled bar represents the flag-tagged peptide and the open bars are the remaining portions (as indicated by number) of L7. (B) The cellular distribution of flag-tagged L7 and mutant proteins at 24 h post-transfection viewed by immuo-fluorescent staining with a second FITC-conjugated antibody under a confocal microscopy. Anti flag-peptide antibody was used as the primary antibody. Bar = 5 μm.