Zoological Studies 34, Supplement I (1995)

Zoological Studies

Applications of the Confocal Fluorescence Microscopy in the Study of the Centrosomal Proteins

Tang K. Tang and Chieh-Ju C. Tang

Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan, R.O.C.

During past several years, we have identified and characterized two proteins: NuMA (Nuclear Mitotic Apparatus protein) and protein 4.1 (P4.1). NuMA, originally described as a predominantly nuclear protein, is present in the interphase nucleus and concentrated in the spindle pole of mitotic cells, while P4.1 is an erythrocyte membrane skeletal protein whose function is believed to strengthen red cell morphology. We and others have recently isolated the cDNAs that encoded NuMA and P4.1, respectively. Our results showed that both NuMA (Tang et al. 1993, 1994) and P4.1 (Tang et al. 1990a,b) constitute multiple isoforms heterogeneous in size. The heterogeneity of NuMA (Tang et al. 1993) and P4.1 (Tang et al. 1991, Huang et al. 1993) appears to be arisen by alternative RNA splicing from a single gene.

Confocal fluorescence microscopes have an improved resolution over conventional fluorescence microscopes by producing images in which out-of-focus blur is essentially absent. We have applied the confocal fluorescence microscopy to finely localize the subcellular distribution of NuMA and P4.1. In order to elucidate the localization of various NuMA isoforms, we have developed a transient expression system (Tang et al. 1994). Expression of NuMA-large shows cell cycle-dependent distribution indistinguishable from that of human NuMA; it is present in the interphase nuclei and segregates to the spindle pole during mitosis. However, expression of the two other isoforms (NuMA-medium and -small) shows distinct subcellular localization. NuMA-medium and NuMA-small are distributed in the cytosol and appear to be clustered mainly at the centrosomal region. When cells enter into mitosis, NuMA-medium and -small move into the mitotic spindle pole. In addition, we have mapped the NuMA gene to human chromosome 11q12->q13.5 by a fluorescence in situ hybridization technique. The NuMA DNA was biotin labeled and used as a probe to hybridize the metaphase chromosomes. The chromosome DNA was stained with propidium iodide and the biotinylated probe was detected by FITC-conjugated avidine under a confocal laser-scanning microscope (BioRad MRC 600).

For localization of the large P4.1 isoforms (135 kDa) within the cells, an antibody (anti-5') that specifically recognized the N-terminal end of this large P4.1 isoform was generated. The mouse 3T3 fibroblasts at various cell cycle stages were processed for immunofluorescence with anti-5' and anti-tubulin antibodies. Our results showed that the large P4.1 was mainly detected at the interphase centrosome and formed several large aggregates within interphase nuclei. In prophase stage, the large P4.1 was clearly detected at the separated centrosomes. However, some P4.1 spread within the cytosol. When cells entered metaphase, the large P4.1 was found at the spindle poles and the mitotic microtubules. Finally, in telophase cells, the large P4.1 was located at the midbodies as well as the centrosomes. Interestingly, when we processed the mouse sperms for immunostaining with our anti-5' antibody, the large P4.1 was mainly detected at the basal body complex (centrosomal region) at the base of the sperm tail.

The differential subcellular localization of various NuMA and P4.1 isoforms suggest that some uncharacterized functions of these proteins have not been identified yet.

REFERENCES

Huang JP, C-J Tang, KH Kou, VT Marchesi, EJ Benz, TK Tang. 1993. Genomic structure of the locus encoding protein 4.1:Structural basis for complex combinational patterns of

tissue-specific alternative RNA splicing. J. Biol. Chem. **268:** 3758-3766.

Tang C-J, TK Tang. 1991. Rapid localization of membrane skeletal protein 4.1 gene (EL-1) to human chromosome 1p33->1p34.2 by non-radioactive in situ hybridization. Cytogenet. Cell genet. 57:119.

Tang TK, Z Qin, TL Leto, VT Marchesi, EJ Benz. 1990a. Heterogeneity of mRNA and protein products arising from the protein 4.1 gene in erythroid and non-erythroid tissues. J. Cell Biol. **110:** 617-624.

Tang TK, CE Mazzucco, EJ Benz, VT Marchesi. 1990b. Molecular cloning and nuclear localization of lymphoid membrane skeletal protein 4.1. In Biochemical and Structural Dynamics of the Cell Nucleus, eds. E Wang, JL Wang, S Chien, CW Wu, WY Cheung. San Diego:Academic Press, pp. 43-57.

Tang TK, C-J Tang, Y-L Chen, C-W Wu, 1993. Nuclear proteins of the bovine esophageal epithelium. (II).The NuMA gene gives rise to multiple mRNAs and gene products reactive with monoclonal antibody W1. J. Cell Sci. **104:** 249-260.

Tang TK, C-J Tang, Y-J Chao, C-W Wu. 1994. Nuclear Mitotic Apparatus Protein (NuMA): Spindle association, nuclear targeting and differential subcellular localization of various NuMA isoforms. J. Cell Sci. **107:** 1389-1402.