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Regional Mapping of Two Subunits of Transcription Factor E4TF1 to Human Chromosome

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Genes of human transcription factor E4TF1 subunits, E4TF1a and E4TF1b, were mapped to region q21 of human chromosome 21 and to region q11.21 of human chromosome 7, respectively, by using a fluorescence *in situ* hybridization method.

Key words: Transcription factor — E4TF1 — Fluorescence *in situ* hybridization — Human chromosome 7 — Human chromosome 21

E4TF1 was originally identified as one of the factors responsible for transcription of the adenovirus early region 4 (E4) gene.[1)] E4TF1 is composed of at least two types of subunits,[2)] E4TF1a and E4TF1b, with molecular masses of 60 and 53 kDa, respectively. E4TF1a alone can bind to a specific DNA sequence but does not activate transcription *in vitro*. E4TF1b alone neither binds to DNA nor stimulates transcription. However, E4TF1b can interact with E4TF1a. The heterodimers composed of the two subunits further dimerize, resulting in the formation of a tetrameric complex which stimulated transcription *in vitro*.[3)] We have also isolated their cDNAs from a HeLa cell cDNA library.[4)] E4TF1a has a DNA binding motif of the *ets* oncogene family. E4TF1b contains four tandemly repeated Notch-ankyrin motifs essential to the interaction of E4TF1b with E4TF1a.[3)] The sequence data revealed that E4TF1 subunits were highly homologous to GA binding protein (GABP) subunits. GABP was purified as a factor that recognized the *cis*-acting element of the herpes simplex virus immediate early gene from rat liver cells.[5)] They immunologically cross-react with each other. Recently, several transcriptional factors, EF-1A,[6)] NRF-2,[7)] β-factor,[8)] and RBF-1[9)] have been found to be immunologically related to GABP or E4TF1. They are responsible for transcription of the adenovirus early region 1a, the rat cytochrome *c* oxidase subunit IV gene, the mouse ribosomal protein L32 gene, and the human retinoblastoma gene, respectively. We report here the result of the regional mapping of E4TF1 subunit genes by *in situ* hybridization.

Fluorescence *in situ* hybridization (FISH) was performed as described previously.[10)] In brief, each biotinylated cDNA probe of 1.7 kbp E4TF1a and 1.2 kbp E4TF1b was hybridized to human metaphase chromosomes which were denatured at 75°C for 3 min in 70% formamide/2 ×SSC (0.3 *M* NaCl, 0.03 *M* sodium citrate). Hybridization signals were detected with 3-hydroxy-N-2′-biphenyl-2-naphthalenecarboxyamide (HNPP; Aisin Cosmos) staining after treatment with anti-biotin Fab′ alkaline phosphatase conjugate (1:100; Boehringer) for 45 min at 37°C. Chromosomes were counterstained with Hoechst-quinacrine for simultaneous detection of FISH signals and Q-bands. Microscopic visualization and photographs were performed with a standard Nikon Microphoto microscope, using a BV-2A filter (excitation wavelength, 400–440 nm) and Fuji ASA 400 color film.

Figs. 1A and 2A show clear and strong fluorescent signals on the long arm of chromosome 21 at band q21 when the E4TF1a probe was used and on the long arm of chromosome 7 at band q11.21 in the case of the E4TF1b probe, respectively. These results indicate that the E4TF1a and E4TF1b genes are located on region q21 of human chromosome 21 and region q11.21 of human chromosome 7, respectively (Figs. 1B and 2B).

These chromosomal localizations are of particular interest, since structural abnormalities were frequently identified at chromosome region 7q11 in tumor cell lines derived from non-small-cell lung cancer,[11,12)] and at chromosomal band 21q21-q22 in lipomas.[13)] These findings would suggest that E4TF1 genes are involved in these cancers. Furthermore, the DNA-binding activity of E4TF1, including E4TF1a and E4TF1b, was increased to a maximum in the late G1 phase of the cell cycle (Sawada and Handa, unpublished data), suggesting that E4TF1 is

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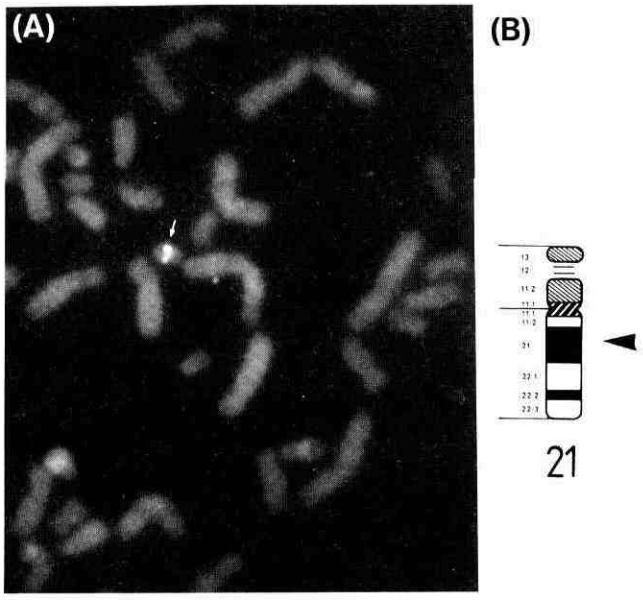

Fig. 1. *In situ* hybridization of E4TF1a probe to human metaphase chromosomes. (A) Partial metaphase showing the site of hybridization to human chromosome 21. Hybridization signals were detected with HNPP staining. The arrow indicates hybridized fluorescent spots. (B) Ideogram of human chromosome 21 showing the location of the E4TF1a gene on q21 (arrowhead), based on analysis of simultaneous detection of FISH signals and Q-bands.

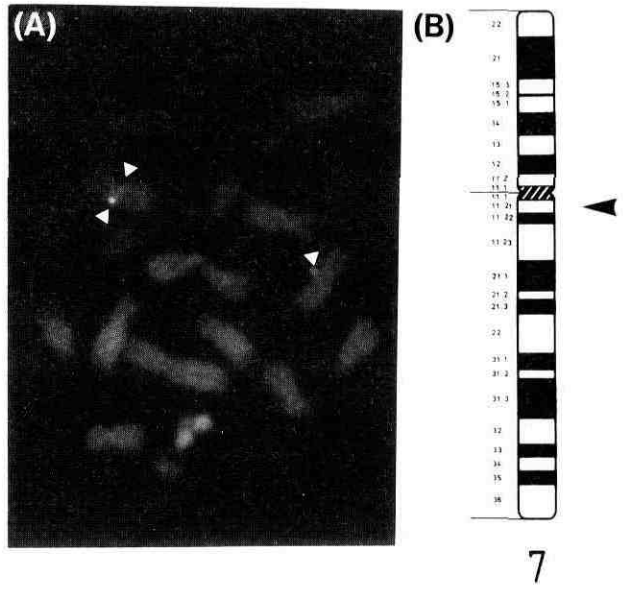

Fig. 2. *In situ* hybridization of E4TF1b probe to human metaphase chromosomes. (A) Metaphase showing the site of hybridization to human chromosome 7. Hybridization signals were detected with HNPP staining. Arrows indicate hybridized fluorescent spots. (B) Ideogram of human chromosome 7 showing the location of the E4TF1b gene on q11.21 (arrowhead), based on analysis of simultaneous detection of FISH signals and Q-bands.

involved in cell growth. Precise analysis of each E4TF1 subunit gene to examine the possible influence of their alteration on carcinogenesis or transformation is in progress.

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