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Short Communication

REEXAMINATION OF CHROMOSOMAL LOCI OF HUMAN MUSCLE ACTIN GENES BY FLUORESCENCE *IN SITU* HYBRIDIZATION

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Summary Human genes for cardiac (*ACTC*), skeletal (*ACTA*), and vascular type (aortic type or α-) smooth (*ACTSA*) muscle actins have been localized to chromosomes 15q14, 1q42.1, and 10q23.3, respectively, by fluorescence *in situ* hybridization.

Key Words human, muscle actin gene, chromosomal loci, fluorescence *in situ* hybridization

Actin is one of the major components in muscle cells. In higher vertebrates, six different actins have been identified (Vandekerckhove and Weber, 1979); they are four muscle actins, *i.e.*, skeletal, cardiac, and two smooth muscle (enteric, gizzard or γ-type and vascular, aortic or α-type) actins, and two (β and γ) non-muscle cytoplasmic actins. In this study, we reexamined chromosome localizations of their genes by fluorescence *in situ* hybridization (FISH).

Chromosome slides were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes by excess-thymidine synchronization and bromodeoxyuridine release technique (Inazawa *et al.*, 1993). Plasmid DNA or phage DNA was labeled with biotin-16-dUTP (Boehringer, Mannheim, Germany) by nick-translation. The labeled probe was prehybridized with Cot-1 DNA (Life Technologies, Gaithers-

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berg, USA) and then used for hybridization (Inazawa *et al.*, 1993) as a probe. After washing finally with 1 × SSC (0.15 M NaCl and 0.015 M Na-citrate), the slides were incubated with fluorescein isothiocyanate-conjugated avidin (Boehringer) and counterstained with propidium iodide for G-banding, which was delineated through a UV-2A filter (Nikon, Tokyo).

The probe for the human cardiac muscle actin gene (*ACTC*) was the 13 kb *Eco*RI genomic DNA fragment cloned into pBR322 (pHRL83-R1, Gunning *et al.*, 1984). Figure 1A shows that *ACTC* is localized to chromosome 15 at the region q14. The gene has been located by Gunning *et al.* (1984) using human-mouse somatic cell hybrids to 15q11-qter. Kramer *et al.* (1992) suggested from linkage analysis that the gene is likely to exist between two genetic markers, D15S24 (15q13) and D15S1 (15q14-q21). Our result above is consistent with these. Thierfelder *et al.* (1993) mapped the familial hypertrophic cardiomyopathy locus to chromosome 15q2 and demonstrated that the cardiac actin gene is not there, with which our result is also in a good agreement.

The probe for the human skeletal actin gene (*ACTA*) was an 11 kb *Hin*dIII genomic DNA fragment cloned into PBR322 (pHask1, Taylor *et al.*, 1988). *ACTA* was localized to human chromosome 1 at the region q42.1 (Fig. 1C). Akkari *et al.* (1994) assigned the gene to 1q42 by FISH, which is more finely mapped here.

The human vascular type smooth muscle actin gene (*ACTSA*) was previously localized by us to chromosome 10 at the region q22-q24 by *in situ* hybridization using a tritium-labeled 2.7 kb probe (Ueyama *et al.*, 1990). We reexamined the *ACTSA* locus by FISH using longer probes. Clones for *ACTSA* were isolated from a human gene library (EMBL4, Takiguchi *et al.*, 1988), and two overlapping clones, λHACTSA-1 and λHACTSA-6, were used as the probe. The former contained exons 1–4 (out of 9 exons) and the latter contained the upstream region (about 11 kb) and exon 1. Figure 1E shows that the gene is localized to chromosome 10 at the region q23.3, which confirms our previous data and is more precise.

The other muscle actin gene, the enteric type smooth muscle actin gene (*ACTA3*), was located to human chromosome 2 using rodent-human somatic cell hybrids (Miwa *et al.*, 1991), which was confirmed by FISH (at p13.1, Ueyama *et al.*, in press).

Fig. 1. Refined mapping of human muscle actin genes by FISH. About 70 (pro)metaphase chromosome preparations were analyzed, and twin-spot signals on both chromatids of homologous chromosomes in more than 20% of them were detected as follows: the cardiac muscle actin gene (*ACTC*), the skeletal muscle actin gene (*ACTA*), and the vascular type smooth muscle actin gene (*ACTSA*) at 15q14 (A and B), 1q42.1 (C and D), and 10q23.3 (E and F), respectively. A, C, and E, FISH; B, D, and F, G-banding patterns.

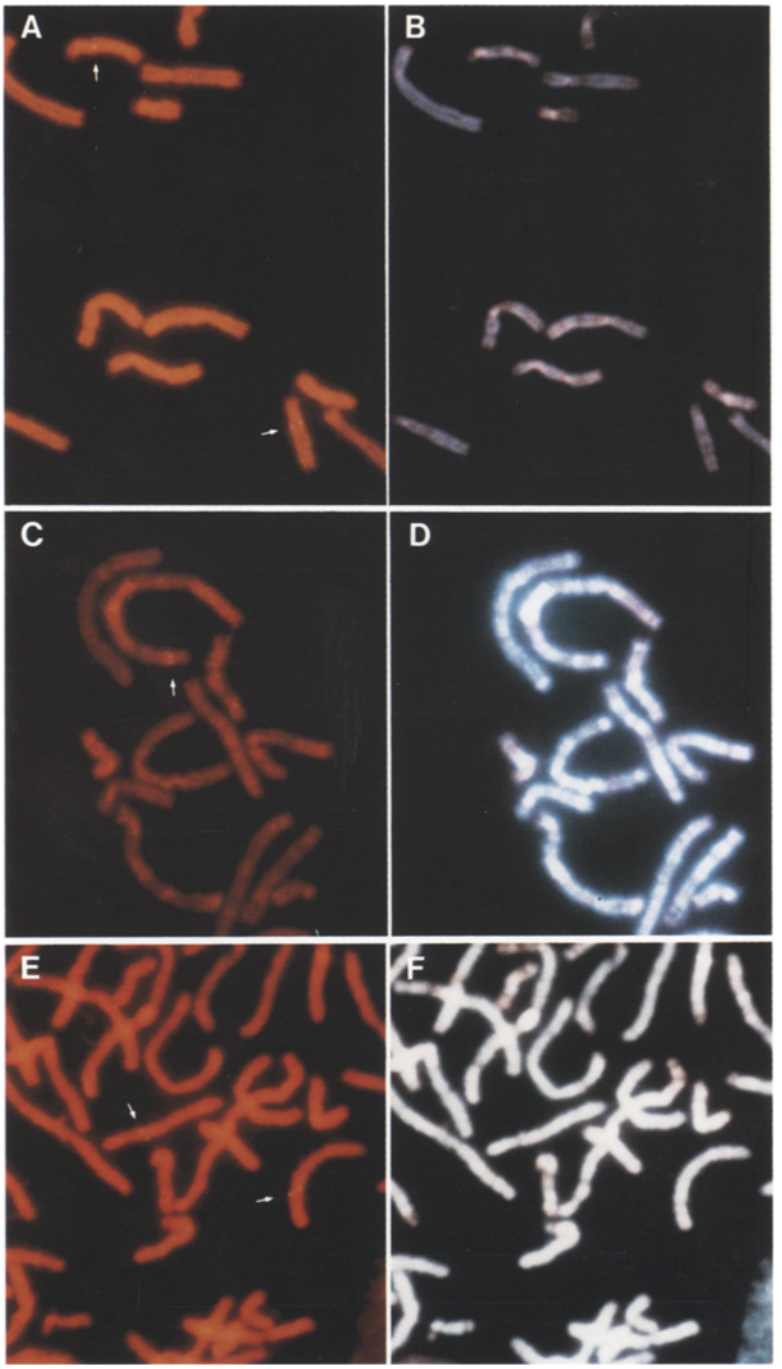

Fig. 1

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