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Assignment of the human CRABP-II gene to chromosome 1q21 by nonisotopic in situ hybridization

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Summary. Two highly conserved forms of cellular retinoic acid binding protein (CRABP-I and CRABP-II) have been described, and one, CRABP-II, is highly expressed in human skin. We have utilized a 10-kb fragment containing the human CRABP-II (hCRABP-II) gene (isolated from a human genomic library) to localize hCRABP-II to human chromosome 1 band q21 by fluorescence in situ hybridization. Localization to 1q was confirmed by hybridization of a hCRABP-II cDNA clone against a human-mouse hybrid cell line containing a t(1;6)(q21;q13)) translocation chromosome. The hCRABP-II gene is therefore localized to a band known to contain several other genes that are expressed in the context of epidermal differentiation, including profilaggrin, loricrin, involucrin, and calcyclin.

Introduction

Retinoic acid (RA) is an important signaling molecule in embryonic development (Eichele 1989) as well as in the growth and differentiation of human skin (Sporn and Roberts 1983). In the skin, RA inhibits terminal differentiation (Fuchs and Green 1981; Yaar et al. 1981) and accelerates proliferation (Connor and Lowe 1983; Fisher et al. 1991). Many of the pleiotypic actions of RA and other retinoids are likely to be mediated by nuclear retinoic acid receptors (RAR; Petkovich et al. 1987; Giguere et al. 1988) acting as ligand-dependent transcription factors. However, other cellular retinoid binding proteins have been described, including retinol binding protein (CRBP) and cellular retinoic acid binding protein (CRABP; Ong and Chytil 1975; Siegenthaler and Saurat 1987). While the cellular functions of these proteins remain unknown, CRBP is likely to be important in regulating the transport, storage, or metabolism of vitamin A (retinol), whereas CRABP is likely to be involved in nuclear transport or cytoplasmic sequestration of RA (Wang and Gudas 1984; Takase et al. 1986).

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Two forms of CRABP have been identified through the use of biochemical and molecular cloning techniques, and termed CRABP-I and CRABP-II (Shubeita et al. 1987; Giguere et al. 1990). We have recently isolated cDNAs encoding both molecules from human skin and fibroblast libraries and demonstrated that CRABP-II mRNA is much more highly expressed under basal conditions, and is selectively upregulated by RA in human skin and in cultured human dermal fibroblasts (Astrom et al. 1991). CRABP-II transcripts are also selectively upregulated by agents that promote keratinocyte differentiation in vitro (Astrom et al. 1991), suggesting that CRABP-II may be an important mediator of the effects of RA on keratinocyte growth and differentiation.

Recently, the human gene encoding hCRABP-I has been localized to chromosome 15 by DNA blot hybridization using a panel of human-rodent hybrid cell lines (van Kessel et al. 1991). However, the chromosomal localization of hCRABP-II has not been reported. Here, we make use of fluorescence in situ hybridization (FISH) to determine the chromosomal localization of hCRABP-II, and confirm this localization by blot hybridization analysis of a human-mouse hybrid cell line. The results indicate that hCRABP-II is localized to a region that includes several other genes that are expressed in a coordinate fashion during the process of keratinocyte differentiation.

Materials and methods

DNA probes

An approximately 10-kb *Xho*I-*Hind*III fragment of the genomic DNA clone lambda 2.1 containing the entire human CRABP-II gene was subcloned into the phagemid Bluescript SK- (Astrom et al., in preparation). The plasmid was digested with *Eco*RI and used for preparation as a fluorescent probe by nick translation as described previously (Meese et al. 1992).

For screening of genomic blots, the 436-bp hCRABP-I PCR product and the hCRABP-II cDNA lambda f1.1 insert were subcloned (Astrom et al. 1991), digested with *Eco*RI, isolated using

1.2% LMP agarose gels (BRL), and labeled with ^{32}P dCTP by random priming (Feinberg and Vogelstein 1983).

Fluorescence in situ hybridization (FISH)

Slide preparation and hybridization conditions utilized were performed using the procedure of Pinkel and colleagues (Tkachuk et al. 1990), modified to include COT-1 blocking DNA (BRL) at 1 μg/hybridization reaction.

DNA blot hybridization

Genomic DNA was isolated from suspension cells by SDS-proteinase K lysis and phenol-chloroform extraction as previously described (Gross-Bellard et al. 1973). Human and mouse DNA was used as a control and for comparison to hybrid DNA. The source of the DNA was the human-mouse hybrid clone HAL26-12, containing a t(1;6) translocation chromosome [der(6)t(1;6)(6pter →6q13::1q21→1qter)] as well as human chromosomes 3, 15, 17, and 21 (Meese et al. 1992). DNAs were restricted with *Bam*HI and *Sac*I, separated on 1% agarose gels (Southern 1975), and transferred to derivitized nylon membranes (Zeta-Probe, Bio-Rad) under alkaline conditions as described by the manufacturer. Prehybridization was performed for at least 2 h at 42°C in 50% formamide, 5 × SSC, 50 m*M* sodium phosphate, pH 7.0, 1 × Denhardt's solution (Denhardt 1966), and 100 μg/ml each yeast tRNA and sonicated denatured salmon sperm DNA. Hybridizations were performed at 42°C for 16–24 h in the same buffer containing 10% dextran sulfate (Pharmacia), using 1–2 × 10^6 cpm probe (1 × 10^9–3 × 10^9 cpm/μg) per ml hybridization solution.

Results

A genomic clone containing an insert of approximately 10 kb encompassing the hCRABP-II gene was used to probe peripheral blood lymphocyte metaphase spreads by fluorescence in situ hybridization (Fig. 1). A total of 24 cells were completely analyzed, with 95% (60/66) of the total hybridization signals localized to chromosome 1. A total of 19/24 (80%) of cells showed signal on chromatids of chromosome 1 with 14/19 (74%) of cells displaying hybridization to both homologues. The hybridization signals observed were localized by first G-banding cells and then performing FISH on the identical cells to localize accurately the hybridization signal. As depicted in the ideogram in Fig. 1, the hybridization signal localizes hCRABP-II to 1q21.2.

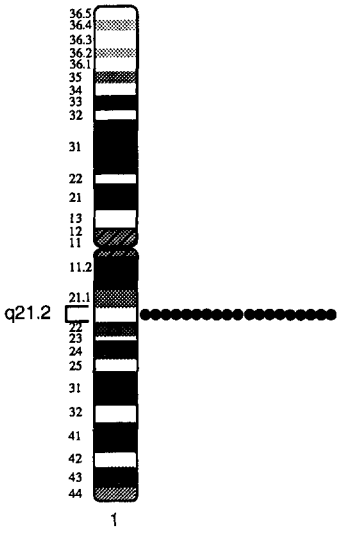

Fig. 1. Ideogram of human chromosome 1 and the fluorescence in situ hybridization pattern for normal peripheral blood lymphocyte metaphases probed with the hCRABP-II gene. The *circles* represent hybridization signals along chromosome 1 observed in 19 cells (see text)

To confirm the FISH results localizing hCRABP-II to the long arm of chromosome 1, and to rule out the possibility of crosshybridization between hCRABP-I and hCRABP-II, a mouse-human hybrid (HAL26-12) containing a t(1;6) translocation chromosome [t(1;6)(q21;q13)] was analyzed by DNA blot hybridization against the hCRABP-I and hCRABP-II cDNA probes. As shown in Fig. 2, hybrid DNA digested with *Bam*HI or *Sac*I displayed the expected human, as well as murine bands, when probed with the hCRABP-II probe. Consistent with the presence of chromosome 15 in a fraction of the hybrid cells (Meese et al. 1992), a faint band of about 24 kb was detected by the CRABP-I probe in *Sac*-I-digested hybrid DNA.

Discussion

Nonisotopic (fluorescence) in situ hybridization is a powerful technique, which can be used to localize genes rapidly and accurately to chromosomal subregions on metaphase chromosomes, as well as to visualize them in interphase nuclei (Tkachuk et al., 1990; for review see Lichter and Ward 1990). In this study, we have used this technique to determine the chromosomal localization of the human CRABP-II gene. Metaphase chromosome preparations probed with a recently isolated genomic DNA fragment containing the entire hCRABP-II gene (Åström et al., in preparation) clearly map hCRABP-II to 1q21, and suggest further sublocalization to 1q21.2 (Fig. 1).

We have confirmed the localization of hCRABP-II to 1q by DNA blot hybridization, making use of a mouse-human hybrid containing a human t(1;6)(q21;q13) translocation chromosome (Fig. 2). This translocation chromosome retains the distal long arm of chromosome 1 (:1q21→qter). For this application, the hCRABP-II probe used was a cDNA rather than a genomic clone in order to minimize hybridization to *Alu* family sequences and other repeats. The observed hybridization of the human probe to mouse DNA was not unexpected, as the human and mouse cDNA sequences display 93.5% homology (Astrom et al. 1991). hCRABP-I and -II cDNA probes displayed clearly different patterns of hybridization despite the 77.4% amino acid homology between the two cDNAs (Astrom et al. 1991). hCRABP-I has recently been localized to human chromosome 15 (van Kessel et al. 1991), portions of which are present in approximately 25% cells from the HAL26-12 hybrid line (Meese et al. 1992). Consistent with this observation, a faint 24-kb band was visible in *Sac*-I-digested hybrid DNA after hybridization against the CRABP-I probe. Nevertheless, the bands observed after probing HAL26-

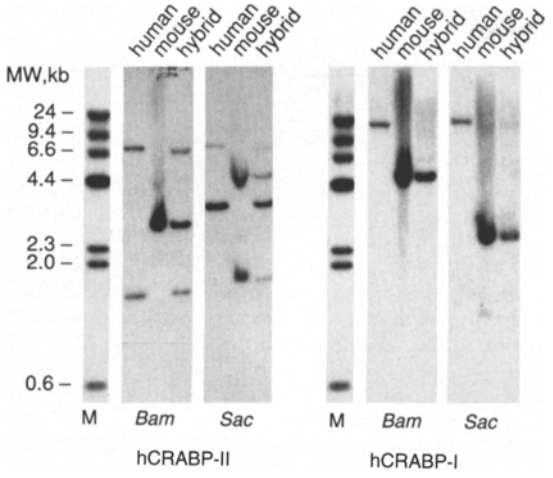

Fig. 2. Hybridization of hCRABP-II *(left)* and hCRABP-I *(right)* cDNA probes against *Bam*HI *(Bam)* and *Sac*I *(Sac)*-digested human, mouse and HAL26-12 hybrid DNA. *M HindIII*-digested λ DNA size markers

12 DNA with hCRABP-II cDNA must reflect specific hybridization to the hCRABP-II gene on human chromosome 1q, rather than the hCRABP-I gene on chromosome 15, because the human-derived bands that hybridize to the hCRABP-I probe differ in size from those hybridizing to CRABP-II. Taken together, the FISH and blot hybridization results clearly demonstrate that hCRABP-II is distal to the 1q21 breakpoint involved in this translocation, and strongly suggest that the location of this gene is 1q21.2.

Recently, several genes that are specifically expressed in the context of keratinocyte differentiation have been localized to 1q21. These include loricrin and involucrin, two major components of the cornified cellular envelope (Yoneda et al. 1991; Simon et al. 1989), and profilaggrin, which is involved in the aggregation of intermediate filaments during keratinocyte differentiation (McKinley-Grant et al. 1989). In addition, calcyclin, a calcium-binding protein that is regulated during the hair cycle and is expressed only in postmitotic cells (Wood et al. 1991) also maps to 1q21 (Ferrari et al. 1987). Given the prominent regulation of hCRABP-II in the context of epidermal differentiation and in response to RA treatment (Åström et al. 1991), it is tempting to speculate that the colocalization of these genes to 1q21 reflects a common mode of regulation. There are precedents for colocalization of genes known or suspected to be functionally related, for example, the HLA complex on chromosome 6p, the familial andenomatous polyposis complex located on chromosome 5q21 (Kinzler et al. 1991; Nishido et al. 1991), the type II keratin genes located on chromosome 12 (Rosenberg et al. 1991), and retinoic acid receptor-homeobox gene clusters located on chromosomes 7, 10, and 15 (Mattei et al. 1991). Additional high resolution mapping studies will be required to determine whether the functionally related genes that map to 1q21 are in fact closely physically linked, and to begin to explore the molecular basis for co-expression of these genes in human skin.

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