GENOMICS 21, 359–363 (1994)

Direct Evidence for Homologous Sequences on the Paracentric Regions of Human Chromosome 1

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Received September 13, 1993; revised January 25, 1994

Calcyclin is a member of the S100 family of proteins, many of which are encoded by genes that have been localized to the proximal long arm of human chromosome 1 (bands q21–q22). A 450-kb yeast artificial chromosome clone containing the human calcyclin gene was identified by PCR screening and used as a probe for fluorescence *in situ* hybridization (FISH). Along with the expected hybridization to 1q21, simultaneous, specific hybridization to the centromeric region of the short arm of chromosome 1 was also observed. An identical pattern of hybridization was observed when microdissected 1q21 DNA sequences were used as a probe for FISH, confirming the presence of homologous sequences flanking both sides of the centromere of human chromosome 1. These results are consistent with a model in which human chromosome 1 arose by insertion of the centromere and heterochromatin into an ancestral chromosome containing chromosome-specific repetitive sequences. © 1994 Academic Press, Inc.

INTRODUCTION

Recently, over 20 genes expressed in the context of epithelial differentiation have been mapped to the band q21 region of human chromosome 1, suggesting that gene clustering might reflect the evolution of skin and other epithelia (Backendorf and Hohl, 1992; Engelkamp *et al.*, 1993; Hardas *et al.*, 1993; Volz *et al.*, 1993). Calcium is known to be an important mediator of epithelial differentiation (Yuspa *et al.*, 1989); thus, it is of interest that at least nine members of the S100 family of calcium-binding proteins have been mapped to the 1q21 region. One of these proteins, calcyclin, was originally described as a cell-cycle-regulated gene associated with proliferating cells (Ferrari *et al.*, 1987). However, in the skin, it is most highly expressed on postmitotic, terminally differentiated cells of the hair follicle (Wood *et al.*, 1991). In addition to calcyclin, other members of the S100 family that have been mapped to 1q21 include placental calcium binding protein, lipocortin II small subunit (p11), calgranulins A (MRP8) and B (MRP14), and S100α, β, D, E, and L (also known as CaN 19) (Dorin *et al.*, 1990; Engelkamp *et al.*, 1993; Lee *et al.*, 1992). The genes encoding the small proline-rich proteins SPRR1, SPRR2, and SPRR3 are also localized to 1q21–q22 (Gibbs *et al.*, 1993); however, they display no obvious homology to the S100 proteins and appear to compose a separate gene family. Profilaggrin, trichohyalin, loricrin, and involucrin are also major proteins expressed during the terminal differentiation of skin and hair (Gibbs *et al.*, 1993; Juhlin *et al.*, 1992; Lee *et al.*, 1993a); notably, these genes also map to the 1q21–q22 region (Fietz *et al.*, 1992; Lee *et al.*, 1993b). Recently, profilaggrin and trichohyalin have been shown to contain N-terminal domains that bind calcium and are related to the S100 family (Lee *et al.*, 1993a; Markova *et al.*, 1993; Presland *et al.*, 1992), whereas loricrin and involucrin contain N- and C-terminal domains that are homologous to the SPRR family (Backendorf and Hohl, 1992). Interestingly, members of all these families are markedly overexpressed in skin diseases characterized by altered proliferation and differentiation (Celis *et al.*, 1990; Hardas *et al.*, 1993; Saintigny *et al.*, 1992). However, at present, it is unclear whether the proximity of these genes is important for their coordinate expression or simply reflects their evolutionary history.

To identify additional, novel genes relevant to epidermal differentiation that map to this region, we have been identifying yeast artificial chromosome (YAC) clones that map to the 1q21 region. In the course of ruling out chimerism in these clones by fluorescence *in situ* hybridization (FISH), we have identified chromosome-specific repeated sequences in the paracentric regions of chromosome 1 and confirmed their existence using chromosome microdissection. These results support a model for the evolution of human chromosome 1 suggested by earlier studies of mouse–human homologies in this region (Dorin *et al.*, 1990; Moseley and Seldin, 1989).

MATERIALS AND METHODS

YAC A2315A was isolated from the Washington University library (Burke *et al.*, 1987) by PCR screening of a gridded array of YACs

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0888-7543/94 $6.00

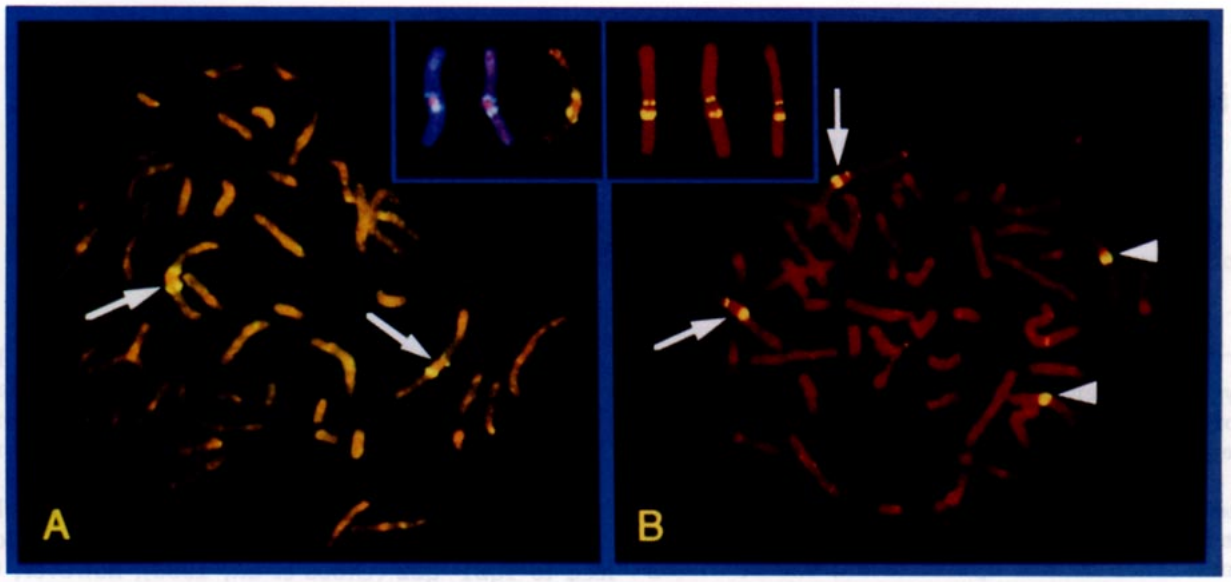

FIG. 1. Regional mapping of YAC A2315A by FISH. (**A**) Representative metaphase preparation, counterstained with propidium iodide, demonstrating hybridization of YAC A2315A to proximal 1p, bands 1q21–q22. Note that specific hybridization to 1p12 is also seen. (**B**) Representative metaphase preparation demonstrating hybridization of PCR-amplified Micro-FISH probe derived from the HAL 26-12 translocation breakpoint, demonstrating the hybridization of the probe to proximal 1q, bands q21–q22 and to proximal 1p. Note that specific hybridization to 6q13 is also seen. Insets, magnified images of positive individual chromosomes. (**Left**) A2315A probe. (**Right**) HAL 26-12 Micro-FISH probe.

using the primers 5′-GGATGCTGAAATTGCAAGGCTGATGGA-AGA-3′ (forward) and 5′-TTCGGAAGCCAAGACGCAAGGGTAAAT-TTG-3′ (reverse), derived from positions 2543–2572 and 2758–2787 of the published calcyclin genomic DNA sequence (Ferrari *et al.*, 1987), respectively. Total yeast DNA from this YAC clone was biotinylated by nick-translation using the Bio-Nick kit (Gibco BRL) and used as a probe for *in situ* hybridization on normal human metaphase spreads as previously described (Elder *et al.*, 1992). The signal was visualized using a two-layer system of avidin-conjugated fluorescein isothiocyanate (AvFITC) followed by biotinylated rabbit antiavidin IgG and AvFITC as previously described (Volz *et al.*, 1993; Zhang *et al.*, 1993). Epifluorescence microscopy was performed using a Zeiss Axiophot microscope equipped with a dual bandpass flourescein–rhodamine filter. The preparation and use of microdissected probes for FISH (Micro-FISH) have been described (Meese *et al.*, 1992; Trent *et al.*, 1989; Willard *et al.*, 1987).

RESULTS

As shown in Fig. 1, YAC A2315A produced strong hybridization signals to the band q21–q22 region of chromosome 1, as expected. Positive hybridization signals were observed on both homologues in all 17 metaphases studied, with signals observed on both chromatids in 27 of 34 chromosomes examined (79%). Unexpectedly, hybridization to the centromeric region of the short arm of chromosome 1 was also observed in all metaphases that showed positive hybridization to 1q21–q22, with obvious sparing of the centromere and heterochromatin and no specific hybridization to any other chromosome (arrows, Fig. 1; inset, Fig. 1A).

Because the hybridization to the proximal p region was quite strong and specific, we hypothesized that this 450-kb YAC might contain sequences homologous to both areas of chromosome 1. To test this hypothesis and to rule out the possibility of YAC chimerism, we hybridized biotin-labeled microdissected DNA [a procedure we termed Micro-FISH (Meese *et al.*, 1992; Trent *et al.*, 1989; Willard *et al.*, 1987)] to normal metaphase chromosomes. The dissected sequences originated from a translocation breakpoint within a mouse–human hybrid cell line (HAL 26-12) that contained a t(1;6) translocation chromosome [der(6)t(1;6)(6pter–6q12::1q21–qter)] (Meese *et al.*, 1992). Using a biotinylated probe prepared from these sequences by nick translation, we also observed a strikingly similar pattern of hybridization to both proximal 1p and proximal 1q (arrows, Fig. 1; inset, Fig. 1B). As expected, hybridization to chromosome 6q was also observed (arrowheads, Fig. 1B), consistent with the origin of the microdissected probe spanning the t(1;6) translocation breakpoint.

To investigate further the localization of these repetitive sequences, two additional YACs mapping to the 1q21–q22 region were obtained by PCR screening the YAC library with primers derived from the genes encoding MRP14 [5′-GGAGAATAAGAATGAAAAGGTC-3′ (forward), 5′-TTGGAGGAAGAGATCTTATTTG-3′ (reverse), positions 3451–3472 and 3806–3825, respectively (Lagasse and Clerc, 1988)] and involucrin [5′-TCAGGAGTCCCTCAAGACTGTT-3′ (forward) and 5′-CATTTCCAGTTGCTCATCTCTC-3′ (reverse), positions 77–98 and 449–470, respectively (Eckert and Green, 1986)], each of which has been localized to this region (Dorin *et al.*, 1987; Yoneda *et al.*, 1991). In contrast to YAC A2315A, each of these YACs yielded specific hybridization to only the 1q21–q22 region (data not shown). Taken together, our results argue strongly against chimerism of YAC A2315A and instead indicate the presence of specific sequences located within

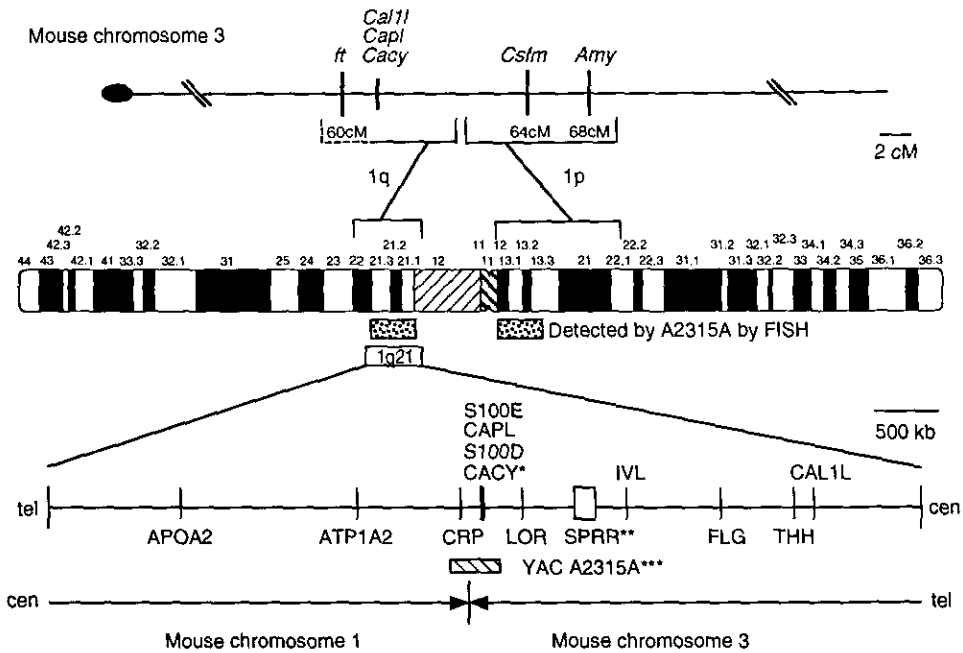

FIG. 2. *Genomic organization of the 1q21 region in human and mouse. A genetic map of mouse chromosome 3, a cytogenetic map of* human chromosome 1, and a tentative physical map of the 1q21 region are shown. The proposed (Moseley and Seldin, 1989) location of centromere insertion into an ancestral chromosome locally colinear with mouse chromosome 3 is shown above the idiogram of human chromosome 1, and the proposed breakpoint of that ancestral chromosome locally colinear with band 1q21 but now represented on mouse chromosomes 1 and 3 is shown below the physical map. Dashed lines indicate that the telomeric extent of mouse chromosome 3 sequences represented in human band 1q21 is unknown. (*) indicates that S100E, CAPL, and S100D reside within 15 kb of CACY (Engelkamp *et al.*, 1993). (**) indicates that two SPRR1, seven SPRR2, and one SPRR3 gene reside within 300 kb of each other in the approximate region indicated by the open box (Gibbs *et al.*, 1993). (***) indicates that YAC A2315A is approximately 450 kb in length and contains CRP, CACY, S100D, S100E, S100L (CaN19), and MRP8 by PCR screening (B.H. and J.T.E., in preparation).

this YAC that are homologous to sequences on centromeric 1q.

DISCUSSION

Comparative mapping of the human and mouse genomes has revealed preservation of gene order on a 45.1-cM segment of DNA extending from bands p22 to q32 on human chromosome 1, which in the mouse is found in two conserved linkage groups located on chromosomes 1 and 3 (Moseley and Seldin, 1989). These data support a model in which a translocation of an ancestral chromosome took place during the genesis of mouse chromosomes 1 and 3, whereas insertion of the centromere and heterochromatin into that ancestral chromosome occurred during the genesis of human chromosome 1 (Moseley and Seldin, 1989). The relationships between the mouse and the human chromosomes predicted by this model are depicted in Fig. 2. Our results would be consistent with that model, assuming that the centromere and heterochromatin became inserted in a region of the ancestral chromosome between chromosome-specific repeated sequences.

A physical map of the 1q21 region has recently been published (Volz *et al.*, 1993), and map information from the literature (Collins *et al.*, 1992; Dorin *et al.*, 1990; Engelkamp *et al.*, 1993; Fietz *et al.*, 1992; Gibbs *et al.*, 1993; Hardas *et al.*, 1993; Lee *et al.*, 1993b; Moseley and Seldin, 1989; Oakey *et al.*, 1992; Volz *et al.*, 1993) and from our laboratory has been used to orient the physical map with respect to the centromere (Fig. 2). The orientation shown is based on the reported locations of *Cal1l* on mouse chromosome 3 (Saris *et al.*, 1987) and CRP on human and mouse chromosomes 1 (Yunis and Whitehead, 1990; Collins *et al.*, 1992), the location of CAL1L with respect to calcyclin on the physical map of the 1q21 region (Volz *et al.*, 1993), and the presence of both CACY and CRP on YAC A2315A but not on any other contiguous YACs (B.H. and J.T.E., in preparation). Further work is required to confirm this orientation, which should be regarded as tentative. Given the orientation shown, however, it is evident that the proposed centromeric insertion event must have occurred distal to CAL1L, outside the region containing all known S100-like and SPRR genes, loricrin, involucrin, profilaggrin, and trichohyalin. By this interpretation, it is unlikely that the repetitive sequences arose as a consequence of the insertion event itself.

The lack of specific hybridization of either the YAC A2315A or Micro-FISH probes to sites elsewhere in the genome argues that these sequences are not simply interspersed repetitive DNA [such as the *Alu* (Slagel *et al.*, 1987; Willard *et al.*, 1987) or LINE (Skowronski and Singer, 1986) families], but rather are specific for the paracentric region of chromosome 1. These sequences are unlikely to be derived from the paracentric

heterochromatin of chromosome 1, because no hybridization to these regions is observed with either probe, and because the HAL 26-12 t(1;6) translocation breakpoint excludes these sequences (Meese *et al.*, 1992). There are other reports of chromosome-specific repetitive sequences detected using YACs or cosmids as probes for FISH (Baldini *et al.*, 1993; Dauwerse *et al.*, 1992; Stallings *et al.*, 1992), and at least one of these involves telomeric 1p and telomeric and subtelomeric 1q (Baldini *et al.*, 1993). However, the sequences observed here are likely to be different, because (i) the pattern of hybridization is different and (ii) loss of hybridization signal was reported on 1q but not telomeric 1p after washing at 42°C, whereas the signals observed here were stable to extended washing at this temperature. Sequence analysis of a chromosome-16-specific, low-abundance repetitive element revealed similarities to a minisatellite, in that the repeat unit was only 40 bp in length (Stallings *et al.*, 1992). It remains to be determined whether the repeated sequences we have identified represent members of a cluster of homologous genes, diverged repeats such as the chromosome-specific alphoid satellite sequences (Haaf and Willard, 1992) or other low-abundance repeats such as those present on both arms of chromosome 16 (Stallings *et al.*, 1992).

The clustering of structurally related genes is being reported with increasing frequency as the mapping of the human genome progresses (Ohta, 1991). Although it may be possible to explain clustering on the basis of evolutionary duplication and divergence alone, it is difficult to explain the colocalization of highly divergent genes, such as the SPRR and S100 families, by this mechanism. It is possible that the divergence between these two families is simply so ancient that no similarities can be detected. Alternatively, the presence of at least two distinct groups of genes in the 1q21 region that appear to be functionally interrelated and coordinately expressed suggests that mechanisms beyond the presence of gene-specific response elements and transcription factors may be involved. One possible mechanism would be the opening of the chromatin in this region over a very long range in a tissue-specific fashion, as appears to be accomplished by the locus control region in the human β-like globin gene cluster (Townes and Behringer, 1990). The presence of a centromere and heterochromatin would be expected to disrupt this process; therefore, we would predict that few, if any, S100-like or SPRR-like genes will be identified on centromeric 1p. It will be of interest to test this hypothesis by identifying the reduplicated sequences, determining whether they represent expressed genes, assessing their structural relationships to other 1q21 genes, and measuring their expression patterns during epidermal differentiation.

ACKNOWLEDGMENTS

This work was supported by an award (R01 AR42248) from the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, USPHS (J.T.E., B.H.), by the Department of Veterans Affairs, Veterans Administration Hospital, Ann Arbor, MI (J.T.E.), and by the Babcock Memorial Trust. We are grateful to Dr. S. Chandrashekharappa for assistance and advice with YAC cloning and analysis and to Ann Burgess for excellent technical assistance.

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