Assignment of Psoriasin to Human Chromosomal Band 1q21: Coordinate Overexpression of Clustered Genes in Psoriasis

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Psoriasin is an abundant low molecular weight protein in keratinocytes from psoriatic lesions. Because of similarities in gene structure and expression to other genes on human chromosomal band 1q21, we hypothesized that psoriasin might also map to this region. To test this hypothesis, we identified and used a genomic λ clone (λ9.2) as a probe for fluorescent *in situ* hybridization. λ9.2 detected the 1q21 region in 81% of 52 chromosomes 1 examined, although it also hybridized to acrocentric chromosomes. λ9.2 DNA yielded polymerase chain reaction amplification of a 121-bp sequence colinear with psoriasin cDNA, as did genomic DNA from hybrid cell lines containing all or part of chromosome 1. The psoriasin gene was present on a 380-kb yeast artificial chromosome clone that was previously mapped to 1q21 and shown to contain calcyclin; here it is also shown to contain MRP8 and CaN19. Psoriasin and several other tightly linked 1q21 genes were markedly overexpressed in psoriatic lesions, suggesting a role for these clustered genes in the regulation of epidermal proliferation.

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The process of epithelial differentiation is evolutionarily ancient and well-conserved (Bereiter-Hahn *et al*, 1984). Therefore, the mechanism by which genes central to this process are regulated is likely to be ancient and conserved as well. One such mechanism might involve the clustering of functionally related genes, a recurrent phenomenon in eukaryotic as well as prokaryotic genomic organization (Ohta, 1991). Evidence for the importance of gene clustering for appropriate coordinate gene expression is provided by several homeobox gene clusters (Gehring, 1992), the human β-like globin gene cluster (Townes and Behringer, 1990), and the variable regions of the T-cell receptor γ gene (Lefranc and Rabbitts, 1991; Allison, 1993). Gene clustering might simply be a result of evolutionary duplication; however, it is difficult to explain the clustering of structurally unrelated but functionally related genes, such as occurs in the histocompatibility locus antigen locus (Trowsdale *et al*, 1991), by this mechanism.

To date, at least twenty genes expressed during epithelial differentiation have been localized to the proximal long arm of human chromosome 1. Many of these genes belong to the small proline-rich protein (SPRR) and S100 gene families[1] (Volz *et al*, 1993). The SPRR genes are tightly clustered (Gibbs *et al*, 1993), and several members of the family are expressed at high levels during terminal differentiation of epidermal keratinocytes (Gibbs *et al*, 1990). Although their functions are currently unknown, SPRR gene products are major components of the cornified envelope of the epidermal cornified layer (Marvin *et al*, 1992). The S100 genes all contain two copies of the helix-turn-helix EF-hand structural motif found in numerous calcium-binding proteins and are thought to play an important role in exerting the effects of calcium on cell growth and differentiation (Heizmann and Hunziker, 1991). With the exception of S100β (21q22) and calbindin-D9k (X), all known S100 genes map to the 1q21 region (Heizmann and Hunziker, 1991; Engelkamp *et al*, 1993). Several additional skin-expressed genes also map to the 1q21 region, including loricrin, involucrin, filaggrin, trichohyalin, cellular retinoic acid-binding protein II (CRABP II), dermatopontin, and β-glucocerebrosidase (Kingsmore *et al*, 1990; Backendorf and Hohl, 1992; Elder *et al*, 1992; Superti-Furga *et al*, 1993). Of these, loricrin and involucrin appear to have evolved from the SPRR family and filaggrin and trichohyalin from the S100 family, by a process of duplication and divergence (Backendorf and Hohl, 1992).

Psoriasis is a chronic skin disorder characterized by marked hyperproliferation and altered differentiation of keratinocytes in the context of activated cell-mediated immunity and inflammation (Barker, 1991). Celis *et al* (1990) have used 2-dimensional gel

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[1] Listed below are the HGM gene symbols and GenBank/EMBL accession numbers for the 1q21 S100 genes discussed in this paper. For purposes of familiarity, the former names are used throughout this manuscript: S100α: HGM symbol S100A1, GenBank/EMBL accession number X58079. CaN19: HGM symbol S100A2, GenBank/EMBL accession number M87068. CACY (calcyclin): HGM symbol S100A6, GenBank/EMBL accession number J02763. Psoriasin: HGM symbol S100A7, GenBank/EMBL accession numbers M86757. MRP8 (calgranulin A): HGM symbol S100A8, GenBank/EMBL accession number M21005. MRP14 (calgranulin B): HGM symbol S100A9, GenBank/EMBL accession number M21064.

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Abbreviations: LCL, lymphoblastoid cell line; PCR, polymerase chain reaction; RT-PCR: reverse transcription-polymerase chain reaction; SPRR, small proline rich region; FISH, fluorescence *in situ* hybridization; YAC, yeast artificial chromosome.

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Table I. PCR Primers

Gene	Direction	Primer Sequence	Product Size (bp)	Reference
Psoriasin (mRNA)	Forward (Primer 1)	AGCAAAGATGAGCAACAC	288	(Madsen *et al*, 1991)
	Reverse (Primer 2)	GCGCTGCTCCATGGCTCT		
Psoriasin (genomic)	Forward (Primer 3)	CTGGTGGGAGAAGACATT	120	(Madsen *et al*, 1991)
	Reverse (Primer 4)	AGCCACAGACTACCACAA		
Calcyclin	Forward	GGATCGTGAAATTGCAAGGCTGATGGAAGA		(Ferrari *et al*, 1987)
	Reverse	TTCGGAAGCCAAGACGCAAGGGTAAATTTG	234	
MRP14	Forward	GGAGAATAAGAATGAAAAGGTC		(Lagasse and Clerc, 1988)
	Reverse	AAATAAAGTCTCTTCCTCCAA	376	
MRP8 (Genomic)	Forward	TTGGGAACAGGGTGGTAGTAT		(Lagasse and Clerc, 1988)
	Reverse	GAGTGGAGAGGGAGGAAAAAT	267	
MRP8 (mRNA)	Forward	TGCAGACGTCTGGTTCAAAG		(Lagasse and Clerc, 1988)
	Reverse	CTCTGGGCCCAGTAACTCAG	153	
SPRR2-1	Forward	GTCAAAGTATCCACCCAAGAGC		(Gibbs *et al*, 1993)
	Reverse	AGAAAGAAGCTCCCTGTGTATCC	146	
CaN19	Forward	GGCACTCATCACTGTCAT		(Lee *et al*, 1992)
	Reverse	AAAACTCAAAGGCATCAA	123	

electrophoresis to identify proteins that display altered expression in psoriatic lesions as compared to normal skin. Two of these proteins had been identified previously as members of the S100 family: MRP8/calgranulin A and MRP14/calgranulin B. An additional protein identified by this strategy as strongly upregulated in psoriatic lesions proved to be novel and was named psoriasin (Madsen *et al*, 1991). The amino acid sequence of psoriasin revealed a single potential calcium-binding EF-hand structure (Madsen *et al*, 1991). Given that psoriasin, MRP8, and MRP14 displayed similarities in gene structure and regulation, we hypothesized that these genes might share a common origin and, as a result, a common genomic location. To test this hypothesis, we have mapped the psoriasin gene using a combination of fluorescence *in situ* hybridization (FISH), somatic cell hybrid, and yeast artificial chromosome (YAC) methodologies. In addition, we have confirmed the coordinate overexpression of psoriasin and several closely linked genes in psoriatic lesions.

MATERIALS AND METHODS

Polymerase Chain Reaction (PCR) Amplification Total RNA was extracted from keratome biopsies of normal and lesional psoriatic skin as described earlier (Elder *et al*, 1990) and amplified by reverse transcription-polymerase chain reaction (RT-PCR) using 1 μg of total RNA (Kawasaki, 1990). Human placental DNA was prepared by the SDS-proteinase K technique (Gross-Bellard *et al*, 1973) and amplified as described (Saiki *et al*, 1985). The sequences of all primers used in this study are given in **Table I**. Amplifications contained 100 ng of DNA, a 200 μM concentration of each dNTP, 50 pmol of each primer, 14 mM Tris-HCl (pH 8.3), 1.0–1.5 mM magnesium chloride, 50 mM potassium chloride, and 1.25 units of *Taq* DNA polymerase (Promega, Madison, WI) in a total volume of 100 μl. Amplification was performed with 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1–3 min, with initial denaturation at 94°C for 3 min and final extension at 72°C for 7 min. PCR products were size-fractionated by electrophoresis in 1.5% Sea-Plaque™ agarose gels (FMC, Rockland, ME) or 2.0% agarose gels and visualized by ethidium bromide staining.

Cloning and Sequencing of Psoriasin PCR Products PCR products were eluted from agarose gels using the Geneclean kit (Bio 101, La Jolla, CA) and cloned into the pCR vector (TA Cloning Kit, Version 1.3, Invitrogen, San Diego CA) as described by the manufacturer. Positive clones were subjected to DNA sequencing on both strands using T7 and SP6 primers or M13 forward and reverse primers, making use of an automated DNA sequencer (Applied Biosystems, Model 373A, Version 1.2.0, Foster City, CA) available through the DNA Sequencing Core Facility, University of Michigan.

Cloning of Psoriasin Genomic DNA Approximately 2×10^6 plaques from a human placental genomic λ FIX II library (Stratagene, La Jolla, CA) were screened using the psoriasin RT-PCR product as probe (Ausubel *et al*, 1988). One of the clones, λ9.2, was further characterized by PCR amplification and DNA sequencing as described for genomic DNA above, except that 50 ng of phage DNA was used for amplification.

Isolation and Analysis of YAC Clones YAC clones were isolated from a human YAC library (Burke *et al*, 1987) by PCR screening, as described (Hardas *et al*, 1994). Intact yeast chromosomes were size-fractionated using YAC DNA from 2×10^5 cells prepared in low melting point agarose plugs (GibcoBRL, Gaithersburg, MD) as described (Chandrasekharappa *et al*, 1992). Contour-clamped homogeneous electric field electrophoresis was performed as described (Chu *et al*, 1986) using a commercially available apparatus (CHEF-DR, Bio-Rad, Richmond, CA) with minor modifications: electrophoresis was performed at 200 V, 20- to 50-s ramp, at 14°C for 32 h in circulating 0.5× TBE buffer (Sambrook *et al*, 1989). After electrophoresis, the DNA was alkali-denatured and transferred to a nylon membrane (Zeta Probe, Bio-Rad) as described by the manufacturer. Multimeric λ ladders (FMC Bioproducts) served as size markers and were detected by addition of 0.5 ng of random-primed λ DNA to the hybridization solution.

Fluorescence *In Situ* Hybridization (FISH) Procedures for preparation of normal human metaphase chromosome spreads and hybridization conditions used for FISH have been described (Tkachuk *et al*, 1990), except that heat-denatured Cot 1 repetitive DNA (Gibco-BRL) was used to block repetitive sequences (Zhang *et al*, 1993). The probe DNAs were labeled by nick translation using biotin-11-dUTP (Koch *et al*, 1986). Hybridization was detected by sequential incubations with avidin-conjugated fluorescein isothiocyanate, biotinylated rabbit anti-avidin IgG, and avidin-conjugated fluorescein isothiocyanate as described (Zhang *et al*, 1993).

Genomic DNAs The human-Chinese hamster ovary hybrid cell lines GM07299 and GM06318B were obtained from the Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ. In line GM07299, 35% of cells contain human chromosome 1 and 96% contain human X, whereas 100% of line GM06318B cells contain X as the sole human chromosome. The human-mouse hybrid clone HAL26–12 contains a t (1;6) translocation chromosome [der(6) t (1;6) (6pter-6q13::1q21–1qter)] as well as portions of human chromosomes 3, 15, 17, and 21 (Meese *et al*, 1992). The human lymphoblastoid cell lines (LCL) 18 and 37 were generously provided by Dr. Rajan Nair. Genomic DNA was isolated from these cell lines as well as from Chinese hamster ovary cells and normal mouse fibroblasts by SDS-proteinase-K lysis (Gross-Bellard *et al*, 1973) and then subjected to PCR amplification and size fractionation as described earlier for human genomic DNA.

RNA Preparation and Northern Blot Hybridization Forty-μg samples of total RNA derived from keratome biopsies of normal or lesional psoriatic skin were size-fractionated by electrophoresis over 1% denaturing formaldehyde-agarose gels, then blotted and hybridized as described above for genomic DNA. The blots were sequentially hybridized against random-primed (Feinberg and Vogelstein, 1983) probes prepared from gel-purified psoriasin, calcyclin, MRP 14, MRP8, and SPRR2–1 PCR products (Saiki *et al*, 1985) using the primer sequences shown in **Table I**. The same blots were also probed for CaN19 (Lee *et al*, 1992) expression using a full-length CaN19 cDNA insert. This cDNA was isolated from a human keratinocyte library by oligonucleotide screening, and its identity was confirmed by double-stranded DNA sequencing (data not shown). As a control for RNA

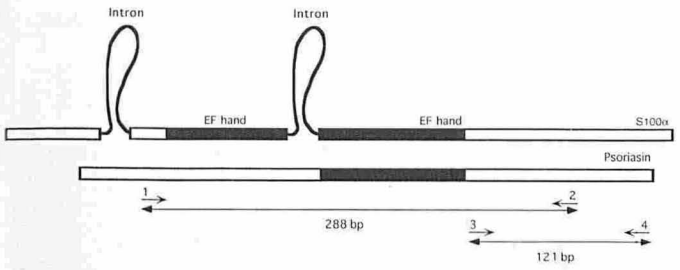

Figure 1. Schematic comparison of the S100α gene and psoriasin mRNA. *Solid bars*, position of EF hands on the respective sequences. *Small arrows*, positions of primers 1 through 4 within the psoriasin cDNA sequence (see text). *Large arrows*, sizes of the amplified fragments obtained from psoriasin cDNA.

loading and intactness, the blots were also probed with p36B4, which encodes the ribosomal phoshoprotein PO (Laborda, 1991).

RESULTS

A 288-bp fragment encompassing most of the psoriasin transcript was amplified by reverse transcription-polymerase chain reaction (RT-PCR) using as template polyadenylated RNA from 8 pooled keratome biopsies of lesional psoriatic skin, and the primers designated 1 and 2 in **Fig 1** and **Table I**. The 288-bp RT-PCR product was then gel-purified and used to screen a human placental genomic library. After secondary screening, two clones, called λ 9.1 and λ 9.2, were selected for further analysis.

As the structure of the psoriasin gene is presently unknown, two additional primers (designated 3 and 4 in **Fig 1** and **Table I**) were empirically designed to amplify a 121-bp product from the 3′ end of the psoriasin transcription unit. This region was predicted to lack introns, based on the known exon-intron structures of other S100 genes **(Fig 1)**. This strategy yielded PCR products of equal size from reverse-transcribed mRNA and from genomic DNA, confirming the lack of introns in this region of the psoriasin gene (data not shown). Amplification using primers 3 and 4 yielded the expected 121-bp product from human placental DNA **(Fig 2A)**, and hybridized to a cloned and sequenced 121-bp psoriasin probe (**Fig 2B**, and see below). An additional 240-bp PCR product was amplified from genomic DNA **(Fig 2A)**, but failed to hybridize to the 121-bp probe **(Fig 2B)**. This band was cloned and sequenced, and found to belong to the human LINE 1 interspersed repetitive sequence family (data not shown). Primers 3 and 4 were then used to amplify λ 9.2 and λ 9.1. The expected 121-bp product was obtained from genomic DNA and λ 9.2, but not from λ 9.1. Neither phage yielded the 240-bp LINE-1 product **(Fig 2)**.

λ 9.2 was then used to probe metaphase spreads from normal human peripheral blood lymphocytes by FISH. In a total of 26 metaphases analyzed, containing 52 evaluable chromosomes 1 by G-banding, hybridization to 1q21–22 was observed on 42 of 52 chromosomes examined (81%). Of the 42 positive chromosomes, 26 (62%) displayed hybridization to both chromatids. In addition to the 1q21 signal, faint hybridization to the short arms of one or more of the acrocentric chromosomes 13, 14, 15, 21, and 22 was also detected in 18 of 26 (69%) of the metaphases analyzed. To further confirm, therefore, that psoriasin resides within the 1q21 region and not on one of the acrocentric chromosomes, three rodent-human somatic cell hybrid cell lines and nine contiguous YACs known to be derived from the 1q21 region were screened for psoriasin by PCR. The expected 121-bp PCR product was amplified from and detectable by hybridization in cell line GM07299, which contains human chromosomes 1 and X, but not from cell line GM06318B, which contains X as its only human chromosome **(Fig 2)**. The 121-bp band was also observed in HAL26–12, a mouse-human hybrid line that retains the distal long arm of human chromosome 1 (1q21–1qter) as well as portions of human chromosomes 3, 15, 17, and 21 (Meese *et al*, 1992). Of nine YACs tested,

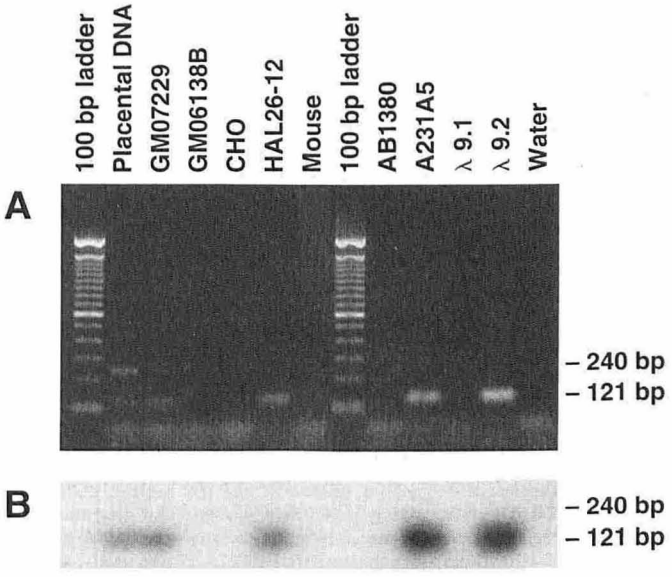

Figure 2. Psoriasin sequences are present on proximal human chromosome 1q, YAC A231A5, and bacteriophage clone λ9.2. Genomic DNA from human placenta, various rodent-human somatic hybrid cell lines, and rodent controls (100 ng/reaction), from YAC A231A5 and parental strain AB1380 (100 ng/reaction), and from bacteriophage clones λ9.1 and λ9.2 (50 ng/reaction) were amplified by PCR using the primers 3 and 4 **(Fig 1)**. *A*, ethidium bromide-stained gel, with the mobility of the observed 121- and 240-bp PCR products indicated to the right. *B*, the gel was blotted and probed with the cloned 121-bp psoriasin PCR amplification product from human genomic DNA, whose sequence is shown in **Fig 3**. Template DNAs used are indicated above the figure.

spanning approximately 1.2 megabases of the 1q21 region (Zhao and Elder, manuscript in preparation), only YAC A231A5 (formerly referred to as A2315A in Hardas *et al*, 1994) yielded the expected 121-bp PCR product **(Fig 2**, and data not shown).

To verify further that the 121-bp PCR product derives from the psoriasin gene and not a pseudogene, the 121-bp fragment was cloned and sequenced from reverse-transcribed lesional psoriasis RNA, genomic DNA, λ 9.2, and A231A5 **(Fig 3)**. To exclude

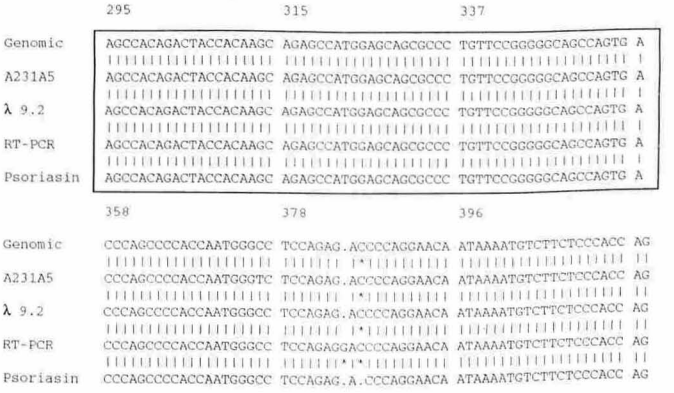

Figure 3. The psoriasin 3′ untranslated region contains a possible hypermutable sequence. DNA sequences of psoriasin PCR products amplified from genomic DNA, YAC A231A5, λ9.2, and lesional psoriatic skin cDNA are aligned with the published (Madsen *et al*, 1991) psoriasin sequence. *Vertical lines*, identity; *asterisks*, mismatches; and *periods*, gaps inserted to maximize homology. Nucleotide position numbers are those used in the published sequence (Madsen *et al*, 1991). *Boxed* information indicates translated sequences.

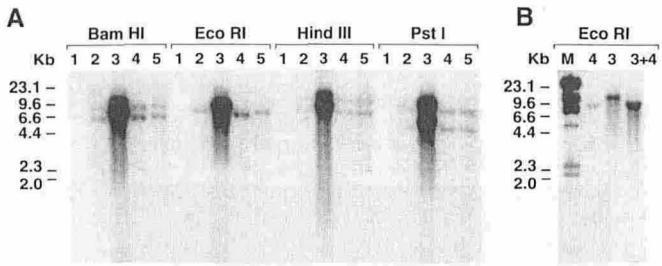

Figure 4. Psoriasin hybridizes to human genomic DNA and YAC A231A5 DNA in a single-copy pattern. Southern blot hybridization analysis of human, yeast, and YAC A231A5 to psoriasin is shown, revealing identical hybridization patterns typical of single copy genes in both human and YAC A231A5. Blots were hybridized to the 121-bp psoriasin from human genomic DNA described in the legend for **Fig 2**. *A*, blot hybridization of DNA samples digested with the four different restriction enzymes indicated above the figure. DNAs used include: *1*, yeast strain AB1380 (1 μg); *2*, human placental (5 μg); *3*, YAC A231A5 (0.1 μg); *4*, LCL 18 (5 μg); *5*, LCL 37 (5 μg). *B*, mixing experiment showing that genomic DNA and YAC A231A5 generate identical hybridization patterns. DNA samples are numbered as in *A*, except that 0.05 μg of A231A5 was used.

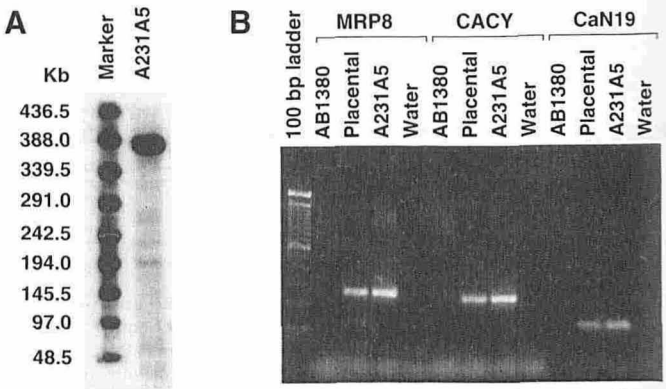

Figure 5. Psoriasin resides within 0.4 megabase of calcyclin, MRP8, and CaN19. *A*, YAC size estimation by PFGE and Southern hybridization. Total YAC A231A5 DNA was fractionated by PFGE, blotted to nylon, and hybridized to Cot1 DNA plus tracer amounts of λ phage DNA (to detect the marker DNA). M indicates lambda ladders. Marker sizes (in kb) are indicated to the left of the figure. *B*, PCR screening of human placental, YAC A231A5, and yeast strain AB1380 with primers designed to detect MRP8, calcyclin (CACY), and CaN19 genomic sequences (see **Table I**). DNA samples and primers are indicated above the figure.

possible variation during PCR amplification, three independent clones were sequenced from human placental DNA and YAC A231A5. Throughout the 31 nucleotides of translated region sequence analyzed, all eight clones analyzed were identical to the published cDNA sequence (Madsen *et al*, 1991). In all eight clones, however, an additional C residue was present following residue 385 in the 3′ untranslated region (**Fig 3**, and data not shown). In addition, the RT-PCR product also displayed a reiterated G residue following position 384 that was not present in any other PCR product or in the published psoriasin sequence (Madsen *et al*, 1991).

Because these nucleotide differences left open the question whether the 121-bp 1q21 psoriasin-like sequence might represent a pseudogene, we used the 121-bp cloned psoriasin fragment from genomic DNA to probe a genomic blot containing restriction endonuclease-digested genomic DNA from three individuals, and A231A5 **(Fig 4)**. All three human genomic DNA samples produced the same, single-copy hybridization pattern on each of the four restriction enzyme digests; however, YAC A231A5 consistently yielded more slowly migrating bands than did genomic DNA **(Fig 4*A*)**. Because the mobility shift was consistent across restriction digests, we hypothesized that they might represent an artifact due to differences in preparation and/or loading of human *versus* yeast DNA. Mixing experiments confirmed that this was the case **(Fig 4*B*)**.

We have previously reported the isolation and FISH mapping of YAC A231A5, identified by PCR screening using calcyclin gene primers (Hardas *et al*, 1994). This YAC contains approximately 380 kb of human genomic DNA, as estimated by pulsed field gel electrophoresis (PFGE) and Southern blot analysis **(Fig 5*A*)**. To further localize the psoriasin gene with respect to calcyclin and other 1q21 genes, we screened A231A5 using primers derived from other known 1q21 genes (Volz *et al*, 1993). As shown in **Fig 5*B***, A231A5 contains at least three other 1q21 genes in addition to psoriasin: MRP 8, calcyclin, and CaN 19.

To assess the expression of psoriasin and other nearby 1q21 genes in psoriasis lesions, a Northern blot containing total RNA isolated from keratome skin biopsies of normal and lesional psoriatic skin was prepared and probed with psoriasin, MRP14, MRP8, SPRR2–1, calcyclin, and CaN19, all of which have been mapped to 1q21 (Dorin *et al*, 1990; Elder *et al*, 1992; Gibbs *et al*, 1993; and this work). As shown in **Fig 6**, psoriasin, MRP8, MRP14, SPRR2–1, and CaN19 were all markedly overexpressed in psoriatic lesions, with calcyclin being overexpressed to a lesser degree. In contrast, no difference in expression was observed for the control gene, 36B4.

DISCUSSION

Taken together, our results demonstrate that psoriasin maps to the q21 region of human chromosome 1 and further localizes this gene to within 380 kb of calcyclin, CaN19, and MRP8. This region is known to contain at least eight members of the S100 gene family (Engelkamp *et al*, 1993; Volz *et al*, 1993; Hardas *et al*, 1994). Thus it is of interest that psoriasin displays weak amino acid homology to members of the S100 family **(Table II)**, including identity or conservative substitution at 13 of 16 consensus residues within the 29-amino acid EF-hand motif found in all S100 proteins (Madsen *et al*, 1991). Therefore, we suggest that psoriasin and the S100 genes may have arisen from a common ancestor by a process of local gene duplication and divergence.

Although the psoriasin genomic clone λ 9.2 hybridized to several acrocentric chromosomes, it did not hybridize to 21q or X, where other known members of this gene family have been localized (Heizmann and Hunziker, 1991; Englekamp *et al*, 1993). We cannot formally rule out the possibility that pseudogenes or other S100 family members may exist in the human genome; however, the non-1q21 chromosomal bands detected by λ 9.2 would seem better explained by non-S100, low copy number interspersed repeats presumably present in this clone. In support of this argument, and in agreement with previous reports (Lee *et al*, 1992), the 121-bp psoriasin probe detected a single copy band pattern in genomic DNA **(Fig 4)**. Moreover, identical bands were detected in YAC A231A5 when YAC and genomic DNA were mixed in order to overcome a previously-recognized (Brownstein *et al*, 1989) artifact of electrophoretic mobility **(Fig 4*B*)**. These results would not be expected if the non-1q21 chromosomal bands detected by FISH were due to the psoriasin sequences themselves.

We detected an additional C residue at position 385 in eight of eight cloned psoriasin PCR products sequenced, deriving from six independent PCR reactions (**Fig 3**, and data not shown). Thus, this residue is unlikely to be a PCR artifact. As this residue resides in the 3′ untranslated region of the psoriasin transcript, its absence would have no effect on the amino acid sequence of the translation product (Madsen *et al*, 1991). As we appear to have generated a reiterated G residue following nucleotide 384 during amplification and/or cloning of the RT-PCR product **(Fig 3)**, it is possible that this region of the gene may be hypermutable. Even if pseudogenes and/or reiterated psoriasin-like genes are responsible for these results, they must reside on 1q21, as the hybridization patterns

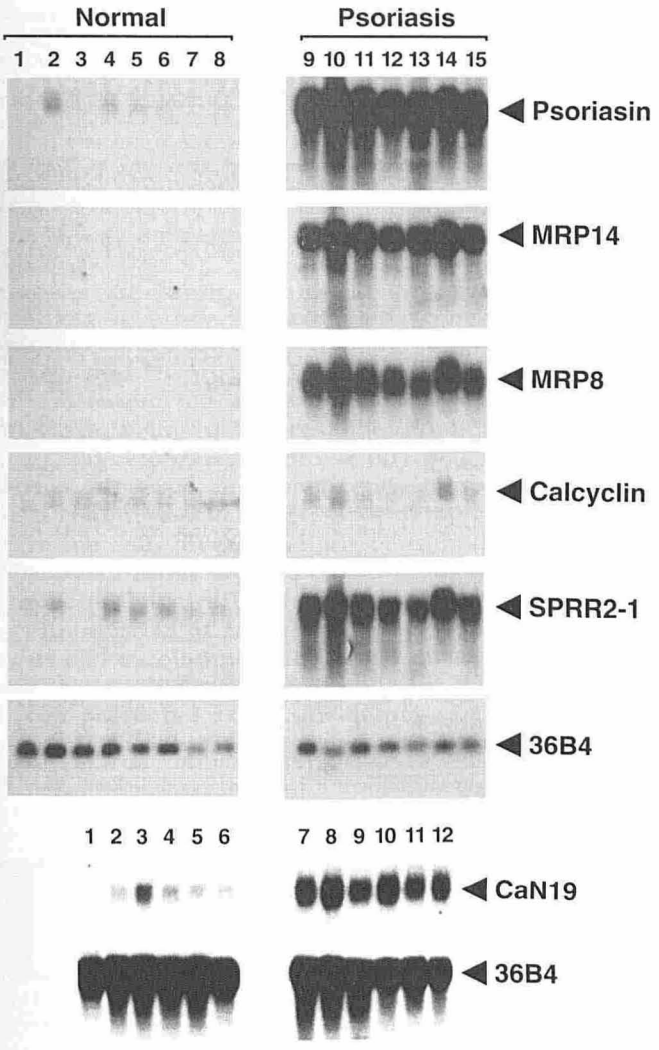

Figure 6. Multiple 1q21 genes are markedly overexpressed in psoriatic skin. Autoradiographs of sequential hybridizations of duplicate Northern blots are shown. RNAs were extracted from keratome biopsies of normal skin or lesional psoriatic skin. Based on comparison of probe specific activities and autoradiographic exposure times (not shown), the relative abundance of the transcripts shown are estimated as follows (from high to low): MRP14, MRP8, psoriasin, SPRR2–1, CaN19, and calcyclin.

produced by genomic DNA and YAC A231A5 are single-copy and identical **(Fig 4)**.

Our Northern blotting observations **(Fig 6)** confirm those of Celis and co-workers regarding overexpression of MRP8, MRP14, and psoriasin in psoriasis (Madsen *et al*, 1991) and extend them to another member of the S100 family: CaN19, a putative tumor suppressor gene (Lee *et al*, 1992). Calcyclin was also overexpressed in psoriasis lesions, albeit less markedly so; this may reflect the facts that calcyclin is known to be selectively expressed in postmitotic hair follicle keratinocytes (Wood *et al*, 1991) situated too deeply to be well sampled by keratome biopsy. We also observed marked overexpression of SPRR2–1 in psoriasis lesions **(Fig 6)**. There is no apparent homology between members of the S100 and SPRR gene families **(Table II)**. Thus, like the histocompatibility locus antigen locus, the 1q21 region appears to represent an example of clustering of structurally unrelated but coordinately expressed and functionally related genes.

Locus control regions are DNA sequences capable of high-density binding of diverse transcription factors, conferring high-level expression of nearby genes in a tissue-specific manner (Townes and Behringer, 1990). The overexpression of tightly clustered genes reported here in the context of psoriasis suggests the hypothesis that one or more locus control regions-like sequences may be present in the 1q21 region. Alternatively, duplication of gene-regulatory sequences could have occurred during the course of evolution. Further studies will be required to address the molecular basis of the apparent coordinate regulation of these genes, and to determine their role in the control of epidermal cell proliferation and differentiation.

Table II. Protein Sequence Homologies Between Psoriasin and Other S100 Family Members[a]

Gene	Length of Protein Sequence	Length of Psoriasin Sequence Compared	Percent Similarity to Psoriasin	Percent Identity to Psoriasin
MRP14	114 aa[b]	101 aa	49.4	25.8
MRP8	93 aa	96 aa	58.8	32.9
Calcyclin	90 aa	91 aa	50.0	28.4
CaN19	98 aa	100 aa	50.5	22.1
S100A	94 aa	89 aa	53.6	22.6
SPRR2-1	72 aa	76 aa	25.0	9.7

[a] Comparison is made by using the PILEUP program of the GCG software package.
[b] aa, amino acids.

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