Vb SUMMARY

In recent years, three structurally related gene families which are important for the formation of a functional epidermis were localized to the long arm of human chromosome 1. They encode structural as well as regulatory proteins which cooperate during epidermal differentiation. Coordinated expression and the close linkage of the genes in chromosomal region 1q21 lead to the definition of a new gene complex, the epidermal differentiation complex (EDC).

Two resources essential for the characterization of the EDC were generated within this study. To examine the genomic organization of the EDC, a contig of 24 yeast artificial chromosomes (YACs) covering 6 Mb of region 1q21 was assembled. A total of 43 genes, 20 newly generated hybridization markers, and seven polymorphic sequence-tagged site (STS)-markers were mapped within the contig. Except for two smaller DNA segments all of these loci were at least doubly linked. To achieve a higher resolution, a SalI restriction map of the YAC contig was generated and, to verify the mapping results, a genomic restriction map covering approximately 4,5 Mb of region 1q21. Using two YACs flanking the EDC as probes for dual colour fluorescence *in situ* hybridization (FISH) the chromosomal orientation of the gene complex was determined. The positioning of seven STS-markers yielded an integrated map of region 1q21 as a valuable tool for linkage analyses.

For the transcriptional analysis of genes involved in epidermal differentiation a gridded cDNA library synthesized from differentiating keratinocytes was constructed. This library was unique in terms of its size (10 filters carrying 18432 doubly spotted clones each) and the included transcripts which even covered late stages of differentiation and enabled rapid and systematic investigations of the whole keratinocyte transcriptome. In addition, a database for the management of the cDNA library was developed.

Furthermore, a gene identification method using YACs as probes to screen a cDNA library was improved. The subtractive hybridization of a gridded cDNA library with non overlapping YACs resolved nearly all problems with non specific hybridization previously described; approximately 90% of the subtractively selected cDNA clones could be mapped within the EDC.

The cDNA sequences represented 40 different transcripts originating from 33 genes. Of these 21 genes were newly assigned to the EDC: *ADAR1*, *ANXA9*, *HAX1*, *LAMRL6*, *PIAS3*, *PIP5K1A*, *PSMB4*, *PSMD4*, *PSMD8L*, *RBM8*, and *TPM3*, as well as ten previously uncharacterized genes (*NICE1* to *NICE10*), one of which was homologous to *NOTCH2*. Four additional genes, *CHRNB2*, *RPTN*, *S100A11*, and *TDRKH*, were localized within the EDC independently of the subtractive hybridization. The established gene families of the EDC were extended by two new members, *RPTN* and *S100A11*, and a polymorphic transcript of the *SPRR3* gene was identified. Expression and sequence analyses of

NICE1 mapping as well within the core region of the EDC suggested the existence of a new, differentiation-specifically expressed gene family.

The recognition of the corresponding genes in the mouse genome verified an orthologous linkage group on mouse chromosome 3. Likewise, paralogous regions on human chromosomes 1, 6, 9, and 19 were confirmed. Moreover, four of the newly assigned EDC genes have homologues on the long arm of chromosome 15, indicating a fifth paralogous region; a further step in the elucidation of the evolution of chromosomes.

As a result of this study the EDC has been extended from 2 to 6 Mb, now including 52 genes which are almost completely expressed in keratinocytes and therefore potentially contribute to the formation of the epidermis. Because the transcriptional analysis was not performed exhaustively, the number of genes should still increase. Moreover, the extension of the EDC on chromosome 1 is still unknown, making further investigations of this remarkable region of the human genome desirable.