

and provocative speculation is that the elimination of  $V_{\beta}11^{+}$  T cells by I-E may require engagement of their TCR with a self peptide in association with self I-E (15), and the absence of deletion may reflect an absence of the putative self peptide during the neonatal week. Thus, the absence of a self peptide in the thymus either permanently or for a limited time would result in the occurrence of autoreactive T cells in peripheral organs. Regardless of why  $V_{\beta}11^{+}$  T cells are not deleted in I-E<sup>+</sup> neonatal mice, the finding of maturation of these autoreactive T cells and their emigration to the spleen provides a plausible explanation for the paradox of clonal deletion and the existence of autoreactive T cells in the normal adult peripheral lymphoid organs.

If T cells that are normally deleted in the thymus can leave the thymus during the neonatal period, why do they not cause autoimmune diseases in the normal mice? Although we have suggested that these cells persist in the normal adults, we have not yet ruled out the possibility that they are subsequently deleted, particularly as they recirculate through the medulla of a more mature thymus. Alternatively, these nondeleted T cells may persist, but tolerance mechanisms other than deletion (so-called peripheral tolerance) normally prevent them from being activated by self antigens (16). This is suggested by the finding that the sizes of the  $V_{\beta}11^{+}$  T cells in the D3TX mice were in the range of nonactivated small lymphocytes (Fig. 1H). That autoimmune diseases elicited by D3TX can be prevented by normal spleen T cells also supports this possibility (10).

The representation of large numbers of  $V_{\beta}11^{+}$  T cells among the  $\alpha\beta$  T cells in the spleens of 3-day-old mice was unexpected. The finding is important in the consideration of autoimmunity that results from D3TX. Since thymectomy eliminates the source of T cells, D3TX should fix the T cell repertoire of the mice to one enriched in  $V_{\beta}11^{+}$  (and  $V_{\beta}3^{+}$ ) T cells. This is indeed the case since the  $V_{\beta}11^{+}$  T cells in the lymph node and spleen of adult D3TX mice are significantly enriched in relation to those T cells that can normally mature in the thymus. In our preliminary study,  $V_{\beta}3^{+}$  T cells also represented a high percentage of the  $\alpha\beta$  T cells in B6AF1 mice after D3TX (13). In addition, D3TX mice had 40% of the normal number of  $\alpha\beta$  T cells (Fig. 1, A and B). D3TX, therefore, markedly skews the T cell repertoire of adult mice to one enriched in those T cells that are normally deleted in the adult thymus.

The finding that D3TX mice develop a high incidence of organ-specific autoimmune diseases supports the notion that T

cells with specific  $V_{\beta}$  segments have a direct role in organ-specific autoimmunity. In this regard, mice treated with cyclosporine A also have mature, autoreactive T cells in the thymus (3, 17), and they develop autoimmune diseases similar to those of D3TX mice (18). Furthermore, in mice of the H-2<sup>u</sup> haplotype, experimental autoimmune encephalomyelitis induced by immunization with the peptide 1-11 of myelin basic protein is associated with the function of  $V_{\beta}8$ -specific T cells (19). Further studies of mice with autoimmune disease should help to determine whether clonal deletion is the mechanism of tolerance to organ-specific autoantigens and to further clarify the role of the undeleted autoreactive clones in autoimmune disease pathogenesis.

*Note added in proof:* Schneider *et al.* (23) also observed that  $V_{\beta}6^{+}$  cells were not deleted in the neonatal Mls<sup>a+</sup> mice. Their findings therefore supported our results, and were an inspiration to this study.

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## Human KGF Is FGF-Related with Properties of a Paracrine Effector of Epithelial Cell Growth

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Keratinocyte growth factor (KGF) is a human mitogen that is specific for epithelial cells. The complementary DNA sequence of KGF demonstrates that it is a member of the fibroblast growth factor family. The KGF transcript was present in stromal cells derived from epithelial tissues. By comparison with the expression of other epithelial cell mitogens, only KGF, among known human growth factors, has the properties of a stromal mediator of epithelial cell proliferation.

GROWTH FACTORS THAT ARE SECRETED by certain cells and act on nearby responsive cells function in the development of multicellular organisms (1). Such paracrine-acting growth factors also appear to participate in the renewal of normal hematopoietic cell populations (2). Epithelial cells that line the skin and gastrointestinal (GI) tract also turnover rapidly. Although there are several growth factors that include epithelial cells among their known targets, none has yet been established as important in proliferation of normal epithelial tissues. We recently purified a

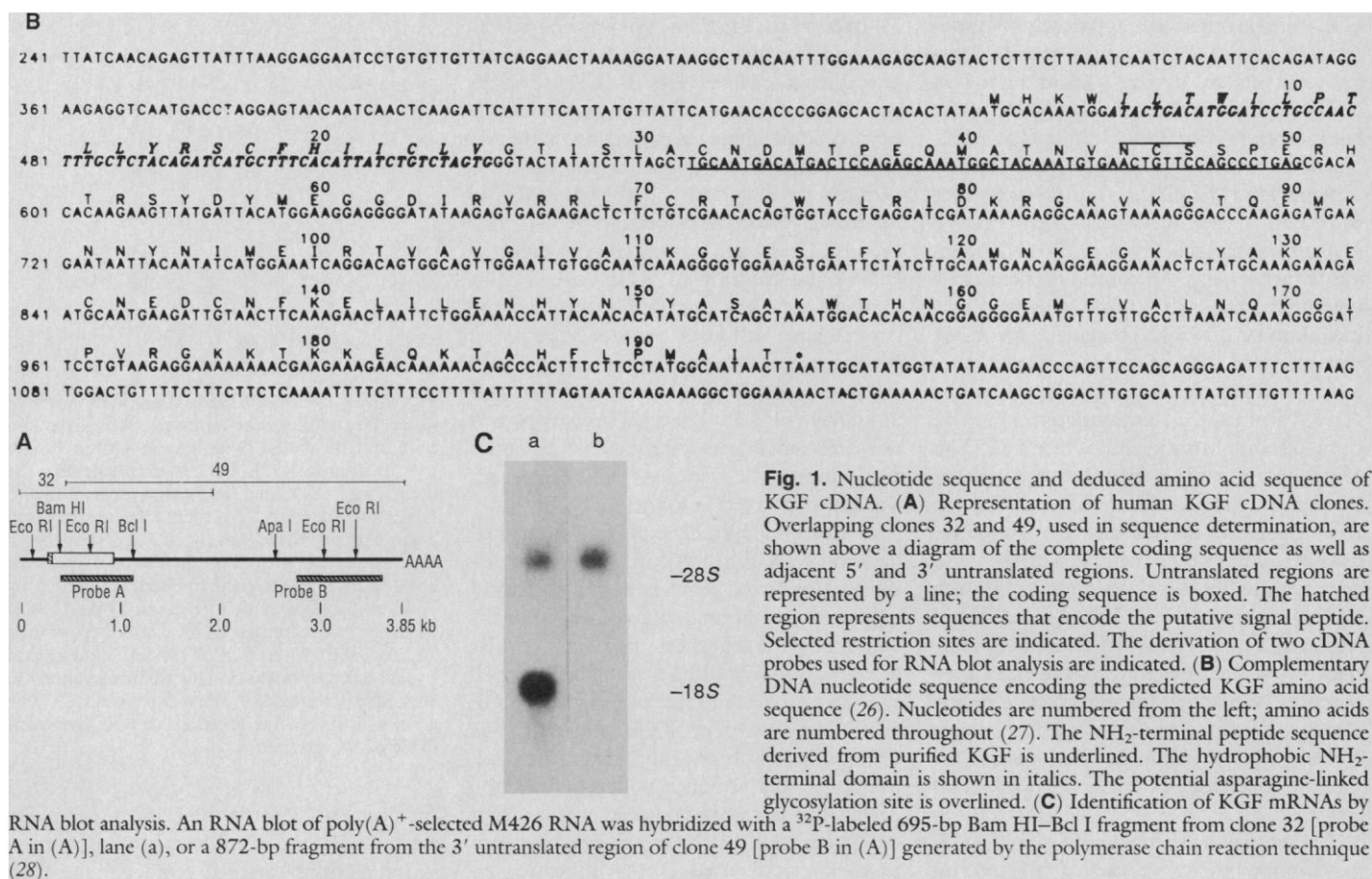
growth factor, keratinocyte growth factor (KGF), that is active on keratinocytes and appears to be specific for epithelial cells (3). We now describe the isolation of the cDNA for KGF and its possible role in epithelial cell growth and development.

Oligonucleotide probes were generated

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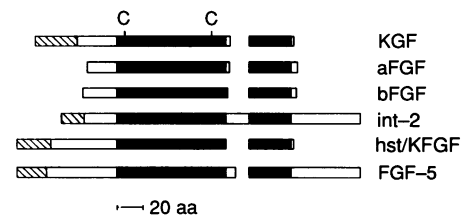
on the basis of the experimentally determined amino acid sequence of KGF (3) and then used to screen an oligo(dT)-primed cDNA library prepared from M426 human embryonic lung fibroblasts, the initial source of the factor (4). Of ten plaque-purified clones analyzed, one, designated clone 49, had an insert of 3.5 kb, whereas the rest had inserts ranging from 1.8 to 2.1 kb. Analysis of the smaller clones revealed several common restriction sites. Sequence analysis (5) of a representative clone, designated clone 32, along with clone 49, demonstrated that they were overlapping cDNAs (Fig. 1A), which when aligned established a continuous 3.85-kb sequence that contained the complete KGF coding sequence (Fig. 1B).

A likely ATG initiation codon was located at nucleotide position 446, establishing a 582-bp open reading frame, which ended at a TAA termination codon at nucleotide position 1030. This open reading frame encodes a putative 194-amino acid polypeptide with a calculated molecular size of 22,512 daltons. The sequence flanking the ATG codon did not conform to the proposed GCC(G/A)CCATGG consensus sequence for optimal initiation by eukaryotic ribosomes (6), although there was an A residue three nucleotides upstream of the

ATG codon. An A residue at this position is the most conserved nucleotide in the consensus sequence. This ATG codon was preceded 85 nucleotides upstream by a TGA stop codon in the same reading frame. A 19-amino acid sequence, which was consistent with the experimentally determined NH<sub>2</sub>-terminal sequence of purified human KGF, began 32 amino acids downstream of the proposed initiation codon. The predicted KGF amino acid sequence contained one potential N-linked glycosylation site (Asn-X-Ser) from residues 45 through 47.

To search for homology between KGF and any known protein, we analyzed the National Biomedical Research Foundation data base with the FASTP program of Lipman and Pearson (7). The predicted primary structure of KGF was related to those of acidic (aFGF) and basic fibroblast growth factor (bFGF), as well as *int-2*, *hst/KFGF*, and *FGF-5*-encoded proteins. The FGFs are heparin-binding mitogens with broad target cell specificities (8). *FGF-5* and *hst/KFGF* are transforming genes, originally detected by DNA-mediated gene transfer (9), whereas *int-2* was identified as an oncogene by proviral integration of mouse mammary tumor virus (10).

The primary KGF translation product, like those of *hst/KFGF* and *FGF-5*, contains



**Fig. 2.** Topological comparison of the FGF family of related molecules (29). Alignment of the six proteins revealed two major regions of homology, spanning amino acids 65 to 156 and 162 to 189 in the predicted KGF sequence, which were separated by a short, nonhomologous series of amino acids. In the case of the *int-2* product, the length of this sequence was 17 residues; in the *hst* product, the two homologous regions were contiguous. In the aligned regions, KGF was 44% identical to the *int-2* product (mouse), 41% identical to *FGF-5* (human), 39% identical to bFGF (human), 37% identical to aFGF (human), and 33% identical to the *hst* product (human); all six proteins were identical at 19% of the residues and, allowing for conservative substitutions, they showed 28% similarity. The two protein domains that share most similarity are shown by shaded boxes. Hatched boxes indicate putative signal peptide sequences. The position of two conserved cysteine residues are shown.

a hydrophobic NH<sub>2</sub>-terminal region, which is probably a signal sequence (11) (Fig. 2). The finding that this NH<sub>2</sub>-terminal domain is not present in the mature KGF molecule (Fig. 1B) supports this conclusion. The

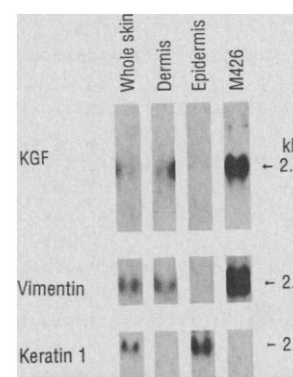
FGFs are synthesized apparently without signal peptides (12). The *int-2*-encoded protein contains an atypically short region of NH<sub>2</sub>-terminal hydrophobic residues (13), but it is not known if the protein is secreted. The *int-2*- and *FGF-5*-encoded proteins also contain long COOH-terminal extensions compared to the other family members.

A probe spanning most of the KGF coding sequence (Fig. 1A, probe A) detected a predominant 2.4-kb transcript as well as a less abundant, ~5-kb transcript by RNA blot analysis (14) of polyadenylated [poly(A)<sup>+</sup>] M426 RNA (Fig. 1C). A probe derived from the 3' untranslated region of clone 49, distal to the end of clone 32 (Fig. 1A, probe B) only hybridized to the larger message (Fig. 1C). Thus, it appears that the KGF gene is transcribed as two alternative mRNAs. Two other members of the FGF gene family, bFGF (12) and *int-2* (15), also express multiple RNAs. The 3' untranslated region of the KGF cDNA contained many ATTTA sequences, which have been proposed to be markers for the selective degradation of transiently expressed, unstable RNAs (16) and might in part account for the low abundance of the larger KGF transcript.

To investigate the functional role of KGF, we examined the expression of its transcript in a variety of human cell lines and tissues. The predominant 2.4-kb KGF transcript was detected in each of several stromal fibroblast lines derived from epithelial tissues of embryonic, neonatal, and adult sources (Fig.

3). In contrast, the transcript was not detected in normal glial cells (17), nor in a variety of epithelial cell lines (Fig. 3). The transcript was also evident in RNA extracted from normal adult kidney and organs of the GI tract, but not from lung or brain. For comparison, we also analyzed the same RNAs for transcripts of transforming growth factor- $\alpha$  (TGF- $\alpha$ ) and epidermal growth factor (EGF). In contrast to KGF, the TGF- $\alpha$  message was not detected in any of the stromal fibroblast lines, but was expressed in varying amounts in the different epithelial cell lines; a small amount was also detected in kidney (Fig. 3). The EGF transcript was not detected in any of the same cell lines and, among the various tissues analyzed, was only observed in kidney (Fig. 3).

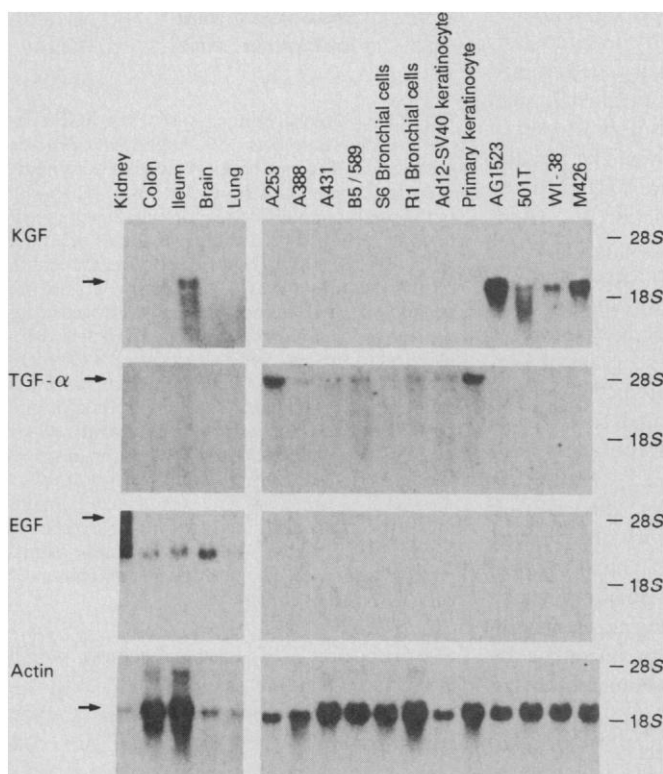
In order to further explore the stromal pattern of KGF expression, whole skin tissue was dissected from newborn mice and separated into dermal and epidermal layers by mild tryptic digestion (18). Total cellular RNA was extracted from each layer, as well as from whole skin, and screened for KGF expression (14). The KGF transcript was observed in whole skin; moreover, the KGF transcript was specifically detected in the dermis but not in the epidermal layer (Fig. 4). As controls for the enrichment for each tissue layer, we used DNA probes for vimentin and keratin 1, which are specific for mesenchymal and epithelial cells, respectively (19, 20). The striking specificity of KGF RNA expression in stromal cells from epithelial tissues supports the concept that this



**Fig. 4.** Expression of KGF in the skin of newborn mice. Whole skin was removed from 1-day-old mice and incubated overnight at 4°C in 0.25% trypsin solution (18). On the following day the dermal and epidermal layers were separated and RNA was extracted from these two layers as well as from intact mouse skin (4). RNA (20  $\mu$ g) from each specimen, including the human fibroblast line M426, was screened for KGF transcript by RNA blot analysis with a <sup>32</sup>P-labeled Pvu II-Ssp I fragment of the human KGF cDNA (nucleotides 162 to 1380). Detection of vimentin and keratin 1 (K1) transcripts was done with human vimentin and mouse K1 cDNA derived probes (30). The arrows indicate the location of the transcript detected by each probe.

factor is important in the normal mesenchymal stimulation of epithelial cell growth.

Interactions between epithelial and mesenchymal tissues are important during normal development (21). However, the specific factors responsible for such interactions have not been well established. Although EGF and TGF- $\alpha$  are structurally related growth factors with epithelial as well as fibroblast specificity, our results and those of previous studies indicate that neither shows the specific patterns of expression in stromal cells from epithelial tissues displayed by KGF (22). The insulin-like growth factors are synthesized predominantly in cells of mesenchymal origin in human fetal tissues (23) but exert paracrine effects on multiple cell types. Whereas aFGF and bFGF possess activity for epithelial cells in addition to their known endothelial and fibroblast targets (24) and their transcripts are present in some stromal cell lines (25), neither are apparently synthesized with signal peptides (12). Thus, while they may have a role in epithelial cell renewal, their mechanisms of release are not known and may only be associated with cell damage (8). In contrast, we demonstrate that the structurally related KGF has evolved as a secreted, epithelial cell-specific growth factor, the transcript of which is widely expressed in epithelial tissues and is specifically observed in stromal cell types of epithelial tissue origin. Thus, among the few known growth factors with epithelial cell activity, only the newly identified KGF has properties consistent



**Fig. 3.** Comparison of KGF mRNA expression in normal human tissues and cell lines with that of TGF- $\alpha$  and EGF. Total RNAs were prepared and analyzed by RNA blotting (14). The following cell lines were used: squamous cell carcinomas A253, A388, and A431; mammary epithelial cells B5/589; immortalized bronchial epithelial cells S6 and R1; Ad12-SV40-immortalized keratinocytes and primary keratinocytes; neonatal foreskin fibroblast AG1523; adult skin fibroblast 501T; and embryonic lung fibroblasts WI-38 and M426.

with that of a major paracrine effector of normal epithelial cell proliferation.

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26. The KGF cDNA sequence may be requested from GenBank by accession number M25295.
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28. R. K. Saiki *et al.*, *Science* **230**, 1350 (1985); probe B was derived from KGF cDNA (nucleotides 2781 to 3653). Amplification was performed with two 18-base oligonucleotide primers, and the amplified fragment was resolved on an agarose gel and recovered by electroelution.
29. Recently another member of the FGF gene family, designated *FGF-6*, was identified on the basis of homology to a human *hst* probe [I. Marics *et al.*, *Oncogene* **4**, 335 (1989)]; hence, KGF is the seventh member of the FGF family.
30. Hybridization with the human KGF and vimentin probes was performed in 40% formamide, 5 $\times$  SSC, 2.5 $\times$  Denhardt's solution, 10% Dextran sulphate, 25 mM sodium phosphate buffer (pH 6.5), and yeast transfer RNA (100  $\mu$ g/ml) at 42°C. Filters were washed at 55°C with 1 $\times$  SSC and 0.1% SDS for 30-min intervals. Hybridization with the mouse K1 probe was carried out under more stringent conditions in 50% formamide and the filters were washed at 60°C with 0.1 $\times$  SSC and 0.1% SDS.
31. We thank J. Wong for assistance in RNA blot analysis, W. Weinberg and S. Yuspa for providing the mouse keratin probe, R. Baserga for providing the human vimentin cDNA, J. Lechner for providing the immortalized bronchial epithelial lines S6 and R1, M. Stampfer for providing the immortalized mammary epithelial line B5/589, M. Kraus and S. Rudikoff for helpful discussions, and S. R. Tronick for assistance in running DNA sequence analysis computer programs. Supported in part by a Japanese Overseas Cancer Fellowship from the Foundation for Promotion of Cancer Research to T.M.

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## Light Adaptation in Cat Retinal Rods

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It has long been an open question whether individual rod receptors in the mammalian retina show any light adaptation. The prevailing evidence so far has suggested that these cells, unlike those in lower vertebrates, adapt little if at all. The experiments on cat rods reported here, however, indicate that this is not really true. Since the cone system in the cat retina has a fairly high light threshold, the rods also need to adapt so that they do not saturate with light before the cones fully take over vision at higher light intensities. In similar experiments, adaptation was found in rods of other mammalian species, including primates.

**L**IGHT ADAPTATION ALLOWS THE visual system to maintain its ability to detect contrast despite large changes in the light level. Although this phenomenon has been well characterized in many ways, it is still not clear how much of light adaptation is at the "network" level (that is, resulting from synaptic interactions in the retina) and how much of it resides in the photoreceptors themselves (1). For rods, the receptors for dim light, there is overwhelming evidence in cold-blooded vertebrates that these cells can adapt to light (2–6). In mammals, however, the cumulative evidence has been against the existence of any rod adaptation (1, 7, 8). The most recent and direct evidence, on the basis of electrical recordings from single rods of the macaque monkey, has also indicated that these cells show negligible adaptation to light (9). Thus, there may be a fundamental difference in rod behavior between mammals and lower vertebrates and, perhaps more generally, between warm-blooded and cold-blooded animals (10). To further examine this question, we have studied single rods from the cat retina. Surprisingly, these cells showed

clear evidence of light adaptation.

An eye was removed from a cat under pentobarbital anesthesia in dim red light. In infrared light and under physiological saline solution (11), the eye was coronally hemisected, and several small pieces of retina were removed from the posterior eyecup. These samples were stored at 5°C in Dulbecco's modified Eagle's medium (Gibco) for use over a period of ~10 hours. When needed, a piece of retina was transferred into physiological saline solution and finely chopped on a layer of cured Sylgard (Dow Corning). We then recorded membrane current from a single rod outer segment projecting from a retinal fragment, after sucking the outer segment into a glass pipette filled with the same saline and connected to a current-recording amplifier (12). This outer segment was stimulated transversely with diffuse, unpolarized light at 500 nm (12). The temperature in the immediate vicinity of the recorded cell was maintained at 38° to

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