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Characterization of a member of the immunoglobulin gene superfamily that possibly represents an additional class of growth factor receptor

(protein-tyrosine kinase)

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ABSTRACT **We have screened cDNA libraries prepared from embryonic chicken tissues to isolate additional genes encoding growth factor receptors. Nucleotide sequencing of a cDNA encoding a gene, which we have termed *klg*, revealed it to represent an additional member of the immunoglobulin gene superfamily, which also possesses extensive sequence similarity to the protein-tyrosine kinase growth factor receptor genes. The *klg* gene was shown to encode a 140-kDa glycoprotein. However, the sequence of the tyrosine kinase domain is unusual in that the aspartate residue located within the highly conserved Asp-Phe-Gly triplet is replaced by an alanine residue. The presence of this aspartate has previously been found to be essential for tyrosine kinase activity. Consistent with the replacement of this aspartate, we were unable to detect any evidence of an associated kinase activity with the *klg*-encoded protein. These observations raise the possibility that the *klg* gene product represents a newly discovered class of receptor that plays a role in signal attenuation rather than signal propagation.**

Cell growth and differentiation is controlled by a complex network of cell–cell and cell–growth factor interactions. Polypeptide growth factors activate target cells by binding to cell-surface receptors, after which the signal is transduced across the cell membrane. Although the biochemical details of these receptor-regulated pathways vary among different signaling systems, the cell-surface receptors themselves can be divided into families of structurally related molecules. The receptor protein-tyrosine kinases (PTKs) represent the largest and best characterized of these cell-surface receptor gene families (1). This family of growth factor receptors comprises an extracellular ligand-binding domain that is linked through a transmembrane domain to a cytoplasmic catalytic domain, which functions as a ligand-stimulated tyrosine kinase.

The PTK catalytic domains of these enzymes are very highly conserved and can be divided into 11 structurally conserved subdomains (2). Mutagenesis studies have shown that within these domains certain invariant amino acid residues are essential for the kinase activity of these enzymes. For example, the lysine residue that is involved in binding of ATP is essential for kinase activity, as are the two aspartate residues found within either the His-Arg-Asp (HRD) or Asp-Phe-Gly (DFG) triplets (3). Biochemical studies have addressed the mechanisms by which the tyrosine kinase activity of these receptors is activated. The formation of dimers on ligand binding has been implicated as playing a critical role (1, 4). Thus, experimentally, when dimers are formed between an inactive and an active kinase, the subsequent signal to the cell is impaired, implying that intermolecular interactions are important for signal transmission. Recently, receptors have been described that could function as the inactive partner in such interactions: for example, the forms of the trkB protein that contain only the extracellular domain (5) or the kinase-inactive versions of the c-kit receptor (6). These molecules represent candidates for proteins with the potential for playing roles in signal attentuation.

We have isolated a gene with a structure reminiscent of that of a growth factor receptor but with an unusual structural change in the kinase domain. The DFG triplet is replaced by the sequence Ala-Leu-Ser (ALS). The other 10 kinase subdomains are conserved. In addition to this unusual change within the "kinase" domain, this protein is a member of the immunoglobulin gene superfamily having seven immunoglobulin-like loops in its extracellular domain. We have given this gene the designation *klg* (*k*inase-*l*ike *g*ene) until a more functional designation can be made. In this report, we describe the isolation and characterization of this gene.*

MATERIALS AND METHODS

Isolation of *klg* cDNA Clones. A 10-day-old chicken embryonic brain λgt10 cDNA library was kindly provided by J. Levy (7) and a λgt11 cDNA library prepared from 10-day-old chicken embryos was kindly provided by B. Vennstrom (8). The 1.0-kilobase (kb) *Pst* I/*Pvu* II DNA fragment of the v-*sea* oncogene (9) was used as a probe.

Nucleotide Sequencing. DNA sequence was determined by dideoxynucleotide chain termination methods with modified T7 DNA polymerase (10). Most cDNAs were sequenced from both single-stranded M13 phage DNA and double-stranded plasmid DNA.

RNA Analysis. Total cellular RNA was isolated from embryonic or 14-day-old chicken tissues and cell lines by the guanidine thiocyanate and $CsCl_2$ procedure (11). Poly(A)-containing mRNA was electrophoresed on agarose/formaldehyde gels, transferred to GeneScreen nylon membranes (DuPont), and hybridized with a *klg*-specific probe.

Antisera. klg restriction enzyme fragments were subcloned into trpE expression vectors and overexpressed as trpE fusion peptides in the C600 strain of bacteria as described (12). Polyclonal rabbit antisera were then raised against these trpE fusion peptides.

Metabolic Labeling, Immune Precipitation, and Western Blotting. Metabolic labeling and immune precipitation were as described (13). For determination of the glycosylation state of the klg proteins, cells were labeled in the presence of tunicamycin (5 μg/ml; Sigma). Western blotting of immunoprecipitates was as described (14).

***In Vitro* Kinase Assays.** Cells were extracted and immunoprecipitated as described above and the immune complexes

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Abbreviation: PTK, protein-tyrosine kinase.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M63437).