Characterization of a Novel Type of Serine/Threonine Kinase That Specifically Phosphorylates the Human Goodpasture Antigen* (Received for publication, December 4, 1998, and in revised form, January 28, 1999)

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Goodpasture disease is an autoimmune disorder that occurs naturally only in humans. Also exclusive to humans is the phosphorylation process that targets the unique N-terminal region of the Goodpasture antigen. Here we report the molecular cloning of GPBP (Goodpasture antigen-binding protein), a previously unknown 624-residue polypeptide. Although the predicted sequence does not meet the conventional structural requirements for a protein kinase, its recombinant counterpart specifically binds to and phosphorylates the exclusive N-terminal region of the human Goodpasture antigen in vitro. This novel kinase is widely expressed in human tissues but shows preferential expression in the histological structures that are targets of common autoimmune responses. The work presented in this report highlights a novel gene to be explored in human autoimmunity.

Goodpasture (GP)3 disease is an autoimmune disorder described only in humans. In GP patients autoantibodies against the non-collagenous C-terminal domain (NC1) of the α3 chain of collagen IV cause a rapidly progressive glomerulonephritis and often lung hemorrhage, the two cardinal clinical manifestations of the GP syndrome (see Ref. 1 for review). Since the NC1 domain is a highly conserved domain among species and between the different collagen IV α chains (α1–α6) (2), the exclusive involvement of the human α3(IV)NC1 in a natural autoimmune response suggests that this domain has structural and/or biological peculiarities of pathogenic relevance. Consistent with this, the N terminus of the human antigen is divergent, and it contains a unique 5-residue motif, KRGDS9, that conforms to a functional phosphorylation site for type A protein kinases (3, 4). Furthermore, the corresponding human gene, but not the other human related or homologous genes from other species, generates multiple transcripts by an exclusive alternative splicing phenomenon (5–7). Recent studies indicate that the phosphorylation of the N terminus of the GP antigen by cAMP-dependent protein kinase is up-regulated by the presence of the alternative products.3 Thus, specific serine/threonine phosphorylation appears to be a major biological difference between the human antigen, antigen from other species, and the homologous domains from other human α(IV) chains and therefore might be important in pathogenesis (1, 4).

Here we report the cloning and characterization of a novel type of serine/threonine kinase that specifically binds to and phosphorylates the unique N-terminal region of the human GP antigen.

MATERIALS AND METHODS

Synthetic Polymers

Peptides—GP pep1, KKKGKDSGSPATWTTRGFVFT, representing residues 3–23 of the human GP antigen, and GP pep1α, KGKRGDAGSPATWTTRGFVFT, a mutant Ser9 to Ala9, were synthesized by MedProbe and CHIRON. FLAG peptide, DYDDDDK, was from Sigma.

Oligonucleotides—The following as well as several other GPBP-specific oligonucleotides were synthesized by Genosys and Life Technologies, Inc.: ON-GPBP-54m, TCGAATTCACCATGGCCCCACTAGCCGCACTCAAAGGACGACGATGACAAG and ON-GPBP-55c, CCGAGGCCGACGAGTTCAGCTCTCTCTCTATTTCTGATTTGT; ON-HNC-B-N-14m, CGGGATCCCGCTAGCTAAGCCAGGCAAGGATGG; ON-HNC-B-N-16c, CCGGATCCATGCTAAGCCAGGCAAGGATGG; ON-HNC-B-N-16c, CCGGATCCATGCTAAGCCAGGCAAGGATGG.

Isolation and Characterization of cDNA Clones

Encoding Human GPBP

Several human λ-gt11 cDNA expression libraries (eye, fetal and adult lung, kidney, and HeLa S3, from CLONTECH) were probed for cDNAs encoding proteins interacting with GP pep1. Nitrocellulose filters (Millipore) prepared following standard immunoscreening procedures were blocked and incubated with 1–10 nmol per ml of GP pep1 at 37 °C. Specifically bound GP pep1 was detected using M3/1A monoclonal antibodies (7). A single clone was identified in the HeLa-derived library (HeLa1). Specificity of fusion protein binding was confirmed by similarly binding recombinant eukaryotic human GP antigen. The EcoRI cDNA insert of HeLa1 (0.5 kb) was used to screen further the GenBank3‡‡† EBI Data Bank with accession number AF136450. § Supported by a postdoctoral fellowship sponsored by Fundación Valenciana de Investigaciones Biomédicas, by Exploraciones Radiológicas Especiales, S.A., and by Bancaixa. ¶¶ Recipient of a fellowship from the Conselleria de Educacio i Cienc i de la Comunitat Valenciana.

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The abbreviations used are: GP, Goodpasture; bp, base pair; GPBP and rGPBP, native and recombinant Goodpasture antigen-binding protein; GST, glutathione S-transferase; HLA, human lymphocyte antigen; kb, kilobase pairs; NC1, non-collagenous domain; PH, pleckstrin homology; PAGE, polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; ORF, open reading frame.

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1 The nucleotide sequence(s) reported in this paper has been submitted to the GenBank3‡‡† EBI Data Bank with accession number(s) AF136450.

2 Several human λ-gt11 cDNA expression libraries (eye, fetal and adult lung, kidney, and HeLa S3, from CLONTECH) were probed for cDNAs encoding proteins interacting with GP pep1. Nitrocellulose filters (Millipore) prepared following standard immunoscreening procedures were blocked and incubated with 1–10 nmol per ml of GP pep1 at 37 °C. Specifically bound GP pep1 was detected using M3/1A monoclonal antibodies (7). A single clone was identified in the HeLa-derived library (HeLa1). Specificity of fusion protein binding was confirmed by similarly binding recombinant eukaryotic human GP antigen. The EcoRI cDNA insert of HeLa1 (0.5 kb) was used to screen further the same library and to isolate overlapping cDNAs. The largest cDNA (2.4 kb) containing the entire cDNA of HeLa1 (n4) was fully sequenced.

Northern and Southern Blots

Pre-made Northern and Southern blots (CLONTECH) were probed with HeLa1 cDNA following the manufacturers' instructions.

Plasmid Construction, Expression, and Purification of Recombinant Proteins

GPBP-derived Material—The original λ-gt11 HeLa1 clone was expressed as a lysogen in Escherichia coli Y1059 (8). The corresponding β-galactosidase-derived fusion protein containing the N-terminal 150 residues of GPBP was purified from the cell lysate using an AP TGagarose column (Roche Molecular Biochemicals). The EcoRI 2.4-kb fragment of n4 was subcloned in Bluescribe M13+ vector (Stratagene).

2 J. Saus, manuscript in preparation.
Additional reading on some of the topics discussed in the text:

- Goodpasture Syndrome
- Antibody Production
- In Vivo Dephosphorylation of rGPBP
- Nucleotide Sequence Analysis
- Physical Methods and Immunological Techniques
- Computer Analysis
- RESULTS

For a comprehensive understanding, please refer to the full text for detailed information.
predicted polypeptide has a large number of phosphorylatable (17.9%) and acidic (16%) residues with an unequal distribution along the sequence. Serine, which is the most abundant residue (9.3%), shows preference for two short regions of the protein where it comprises nearly 40% of the amino acids compared with an average of less than 7% throughout the rest of the polypeptide chain. It is also noteworthy that the more N-terminal serine-rich region consists mainly of a Ser-Xaa-Yaa repeat. Acidic residues are preferentially located at the three N-terminal quarters of the polypeptide with nearly 18% of the residues being acidic. These residues represent only 9% in the most C-terminal quarter of the polypeptide, resulting in a polypeptide chain with two electrically opposite areas. At the N terminus, the polypeptide contains a pleckstrin homology (PH) domain that has been implicated in the recruitment of many signaling proteins to the cell membrane where they exert their biological activities (17). Finally, a bipartite nuclear targeting sequence (18) exists as an integral part of a heptad repeat region that meets all the structural requirements to form a coiled-coil (16).

Protein data bank searches revealed homologies almost exclusively within the approximately 100 residues at the N-terminal region harboring the PH domain. The PH domain of the oyster-binding protein, which displays an overall identity of 33.5% and a similarity of 65.2% with our cloned protein, is the most similar. In addition, the Caenorhabditis elegans cosmid F25H2 (GenBank™ accession number Q93569) contains a hypothetical ORF that displays an overall identity of 26.5% and a similarity of 61% throughout the entire protein sequence indicating that similar proteins are present in lower invertebrates. Several tagged human expressed sequences (GenBank™ accession numbers AA287878, AA287561, AA307431, AA331618, AA040134, AA158618, AA040807, AA122226, AA158617, AA121104, AA412432, AA412433, AA282679, and N27578) demonstrated a high degree of nucleotide identity (above 96%) with the corresponding stretches of the GPBP cDNA, suggesting that they represent human GPBP. Interestingly, the tagged sequence AA287878 shows a gap of 67 nucleotides within the sequence corresponding to the GPBP 5′-untranslated region, suggesting that the GPBP pre-mRNA is alternatively spliced in human tissues (not shown).

The distribution and expression of the GPBP gene in human tissues was first assessed by Northern blot analysis (Fig. 2A). The gene is expressed as two major mRNA species between 4.4- and 7.5-kb in length and other minor species of shorter lengths. The structural relationship between these multiple mRNA species is not known, and their relative expression varies between tissues. Striated muscle (skeletal and heart) is the tissue with highest expression, whereas lung and liver show the least.

Southern blot studies using genomic DNA from different species indicate that homologous genes exist throughout phylogeny (Fig. 2B). Consistent with the human origin of the probe, the hybridization intensities decrease in a progressive fashion as the origin of the genomic DNA moves away from humans in evolution.

**Experimental Determination of the Translation Start Site**—To confirm experimentally the predicted ORF, eukaryotic expression vectors containing either the 2.4-kb of cDNA of n4′ or only the predicted ORF tagged with a FLAG sequence (Fig. 1).
Expression and Characterization of Yeast rGPBP—Yeast expression and FLAG-based affinity purification were combined to produce rGPBP (Fig. 4A). A major polypeptide of ~89 kDa along with multiple related products displaying lower M<sub>r</sub> were obtained. The recombinant material was recognized by both anti-FLAG and specific antibodies guaranteeing the fidelity of the expression system. Again, however, the M<sub>r</sub> displayed by the major product was notably higher than predicted and even higher than the M<sub>r</sub> of the 293 cell-derived recombinant material, supporting the idea that GPBP undergoes post-translational modifications.

Fig. 2. Distribution of GPBP in human tissues (Northern blot) and in eukaryotic species (Southern blot). A random priming 32P-labeled HeLa1 cDNA probe was used to identify homologous messages in a Northern blot of poly(A<sup>+</sup>) RNA from the indicated human tissues (A) or in a Southern blot of genomic DNA from the indicated eukaryotic species (B). Northern hybridization was performed under highly stringent conditions to detect perfect matching messages and at low stringency in the Southern blot to allow the detection of messages with mismatches. No appreciable differences in the quality and amount of each individual poly(A<sup>+</sup>) RNA were observed by denaturing gel electrophoresis and when probing with human β-actin cDNA a representative blot from the same lot. The numbers denote the position and the sizes in kb of the RNA or DNA markers used.

Fig. 3. Experimental determination of the translation start site. A, the two cDNAs present in pc-n4<sup>+</sup> and pc-FLAG-n4<sup>+</sup> plasmids used for transient expression are represented as black lines. The relative position of the corresponding predicted (n4<sup>+</sup>) or engineered (FLAG-n4<sup>+</sup>) translation start site is indicated (Met). B, the extracts from control (+), pc-n4<sup>+</sup> (n4<sup>+</sup>), or pc-FLAG-n4<sup>+</sup> (FLAG-n4<sup>+</sup>) transfected 293 cells were subjected to SDS-PAGE under reducing conditions in 10% gels, and the separated proteins were transferred to a polyvinylidene difluoride membrane (Millipore) and blotted with the indicated antibodies. The numbers and bars indicate the molecular mass in kDa and the relative positions of the molecular weight markers, respectively.

Although the purification system provides high quality material, the presence of contaminants with a protein kinase activity could not be ruled out. The existence of contaminants was also suggested by the presence of a FLAG-containing 40-kDa polypeptide displaying no reactivity with specific antibodies nor incorporation of 32P in the phosphorylation assays (Figs. 4A and 5A). To identify precisely the polypeptide harboring the protein kinase activity, we performed in vitro kinase renaturation assays after SDS-PAGE and Western blot (Fig. 6). We successfully combined the use of specific antibodies (lane 1) and autoradiographic detection of in situ 32P incorporation (lane 2), and we identified the 89-kDa rGPBP material as the primary (lanes 2) or transient (lanes 3) modes were cultured in the presence of H<sub>3</sub>32PO<sub>4</sub>. The recombinant material specifically immunoprecipitated contains 32P indicating that the phosphorylation of GPBP occurs in vivo and is likely to be a physiological process.

The rGPBP Is a Serine/Threonine Kinase That Phosphorylates the N-Terminal Region of the Human GP Antigen—Although GPBP does not contain the 12 conserved structural regions required to define the classic catalytic domain for a protein kinase, the recent identification and characterization of novel non-conventional protein kinases (19–27) encouraged the investigation of its phosphorylating activity. Addition of [γ-32P]ATP to rGPBP either from yeast or 293 cells (not shown) in the presence of Mn<sup>2+</sup> and Mg<sup>2+</sup> resulted in the incorporation of 32P as Ser(P) and Thr(P) in the major and related products that were recognized by both anti-FLAG and specific antibodies (Fig. 5, A and B), indicating that the affinity purified material contains a Ser/Thr protein kinase. To characterize this activity further, GPpep1, GPpep1Ala<sup>9</sup> (a GPpep1 mutant with Ser<sup>9</sup> replaced by Ala), native and recombinant human antigens, and native bovine antigen were assayed (Fig. 5C). Affinity purified rGPBP phosphorylates all human-derived material to a different extent; however, in similar conditions no appreciable 32P incorporation was observed in the bovine-derived substrate. The lower 32P incorporation displayed by GPpep1Ala<sup>9</sup> when compared with GPpep1 and the lack of phosphorylation of the bovine antigen indicates that the kinase present in rGPBP discriminates between human and bovine antigens and that Ser<sup>9</sup> is a target for the kinase.

Although the purification system provides high quality material, the presence of contaminants with a protein kinase activity could not be ruled out. The existence of contaminants was also suggested by the presence of a FLAG-containing 40-kDa polypeptide displaying no reactivity with specific antibodies nor incorporation of 32P in the phosphorylation assays (Figs. 4A and 5A). To identify precisely the polypeptide harboring the protein kinase activity, we performed in vitro kinase renaturation assays after SDS-PAGE and Western blot (Fig. 6). We successfully combined the use of specific antibodies (lane 1) and autoradiographic detection of in situ 32P incorporation (lane 2), and we identified the 89-kDa rGPBP material as the primary (lanes 2) or transient (lanes 3) modes were cultured in the presence of H<sub>3</sub>32PO<sub>4</sub>. The recombinant material specifically immunoprecipitated contains 32P indicating that the phosphorylation of GPBP occurs in vivo and is likely to be a physiological process.
polypeptide harboring the Ser/Thr kinase activity. The lack of $^{32}$P incorporation in the rGPBP-derived products as well as in the 40-kDa contaminant further supports the specificity of the renaturation assays and locates the kinase activity to the 89-kDa polypeptide. Recently, it has been shown that traces of protein kinases intimately associated to a polypeptide can be released from the blot membrane, bind to, and phosphorylate the polypeptide during the labeling step (28). To assess this possibility in our system, we performed the renaturation studies using a small piece of membrane containing the 89-kDa polypeptide either alone or together with membrane pieces representing the different regions of the blot lane. We observed similar $^{32}$P incorporation at the 89-kDa polypeptide regardless of the co-incubated pieces (not shown), indicating that if there are co-purified protein kinases in our sample they are not phosphorylating the 89-kDa polypeptide in the renaturation assays unless they co-migrate. Co-migration does not, however, appear to be a concern since rGPBP deletion mutants displaying different mobilities also have kinase activities and could similarly be in blot renatured (not shown).

**Immunohistochemical Localization of the Novel Kinase—**To investigate GPBP expression in human tissues, we performed immunohistochemical studies using specific polyclonal (Fig. 7) or monoclonal antibodies (not shown). Although GPBP is widely expressed in human tissues, it shows tissue and cell specificity. In the kidney, the major expression is found at the epithelial cells of the tubules and at the mesangial cells and podocytes of the glomerulus. At the lung alveolus, the antibodies display a linear pattern suggestive of a basement membrane localization along with staining of pneumocytes. Liver shows low expression in the parenchyma but high expression in biliary ducts. The expression at the central nervous system is observed in the white matter and not in the neurons of the brain. In testis, a high expression in the spermatogonium contrasts with the lack of expression in the Sertoli cells. The adrenal gland shows a higher level of expression at the cortical cells versus the medullar. In the pancreas, GPBP is preferentially expressed in Langerhans islets versus the exocrine moiety, and in prostate, GPBP is expressed in the epithelial cells but not in the stroma (Fig. 7). Other locations with high expression of GPBP are striated muscle, epithelial cells of intestinal tract, and Purkinje cells of the cerebellum (not shown). In general, in the tissues where GPBP is highly expressed the staining pattern is mainly diffuse cytosolic. However in certain
locations there is, in addition, an important staining reinforce-
ment at the nucleus (spermatogonium), at the plasma mem-
brane (pneumocyte, hepatocyte, prostate epithelial cells, white
matter), or at the extracellular matrix (alveolus) (Fig. 7).

DISCUSSION

Our data show that GPBP is a novel non-conventional serine/
threonine kinase and present evidence that indicate that GPBP
discriminates between human and bovine GP antigens and
targets the exclusive human phosphorylatable region in vitro.
Although the presence of additional protein kinases in the
affinity-purified rGPBP cannot completely be ruled out, several
lines of evidence indicate that the 89-kDa polypeptide is the
only kinase therein. First, we found no differences in auto-
or trans-phosphorylation among rGPBP samples purified either
in the presence of 150 mM or 0.5, 1, or 2 M salt (not shown)
suggesting that rGPBP does not carry kinases intimately
bound. Second, the presence of a FLAG-containing yeast-de-
derived kinase in our samples is not a concern since material
purified using GPBP-specific antibodies show no differences in
phosphorylation (not shown). Third, a deletion mutant of GPBP
displays reduced auto- and trans-phosphorylation activities,3
suggesting that is the only material in the rGPBP with the
ability to carry out phosphate transfer.

Although GPBP is not homologous to other non-conventional
kinases, they share some structural features including a N-
terminal α-helix coiled-coil (26, 27), serine-rich motifs (24),
high phosphoamino acids content (27), bipartite nuclear local-
arization signal (27), and the absence of a typical nucleotide or

3 A. Raya and J. Saus, unpublished observations.
ATP-binding motif (24, 27).

Immunohistochemistry studies show that GPBP is a cytosolic polypeptide also found in the nucleus, associated with the plasma membrane and likely at the extracellular matrix associated with basement membrane, indicating that it contains the structural requirements to reach all these destinations. The nuclear localization signal and the PH domain confer to it the potential to reach the nucleus and the cell membrane, respectively (17, 29, 30). Although GPBP does not contain the structural requirements to be exported, at the 5’-end untranslated region of its mRNA exists an upstream ORF of 130 residues with an in-frame stop codon at the beginning (Fig. 1). An mRNA editing process inserting a single base pair (U) would generate an operative in-frame start site and an ORF of 754 residues containing an export signal immediately downstream of the edited Met (not shown). Polyclonal antibodies against a synthetic peptide representing part of this hypothetical extra sequence display a linear vascular reactivity in human tissues suggestive of an extracellular basement membrane localization.3 Alternatively, a splicing phenomenon could generate transcripts with additional unidentified exon(s) that would provide the structural requirements for exportation. The multiple cellular localization, the high content in Tyr(P), and would provide the structural requirements for exportation. The approach. These studies revealed that specific serine phosphorylation in vitro suggest that GPBP in addition is the target of specific tyrosine kinase activity and therefore likely involved in specific signaling cascade(s).

The idea that common pathogenic events exist at least for some autoimmune disorders is suggested by the significant number of patients displaying more than one autoimmune disease, and also by the strong and common linkage that some of these diseases show to specific major histocompatibility complex haplotypes (31, 32). The experimental observation that the autoantigen is the leading moiety in autoimmunity and that a limited number of self-components are autoantigenic (31) suggest that these components share biological features with important consequences in self/non-self recognition by the immune system. One possibility is that triggering events by altering different but specific self-components would result in abnormal antigen processing. In certain individuals expressing a particular major histocompatibility complex specificity, the abnormal peptides could be recognized by non-tolerized T cells and trigger an immune response (1). We explored the GP antigen to identify biological features of relevance in autoimmune pathogenesis. Since the human antigen is a natural autoantigen but not the homologous counterparts from other superior mammals, and only α3 is involved in autoimmunity but not the remaining five α chains, comparative studies among NC1 domains were a useful initial approach. These studies revealed that specific serine phosphorylation as well as pre-mRNA alternative splicing are biological hallmarks of the human versus the other species GP antigens (4, 5). These two features are also associated with the biology of other autoantigens including acetylcholine receptor and myelin basic protein (4). The latter is suspected to be the major antigen in multiple sclerosis, another exclusively human autoimmune disease in which the immune system targets the white matter of the central nervous system. GP disease and multiple sclerosis are human disorders that display a strong association with the same HLA class II haplotype (HLA DRB1*1501) (32, 33). This along with the recent report of death by GP disease of a multiple sclerosis patient carrying this HLA specificity (34) support the existence of common pathogenic events in these human disorders.

Phosphorylation of specific serines has been shown to change intracellular proteolysis (35–40). Conceivably alterations in protein phosphorylation can affect processing and peptide presentation and thus mediate autoimmunity. GP antigen-derived peptide presentation by the HLA-DR15 depends more on processing than on preferences of relatively indiscriminate DR15 molecules (41), suggesting that if processing is influenced by abnormal phosphorylation, the resulting peptides would likely be presented by this HLA. Our more recent data indicate that in both the GP and myelin basic protein systems, the production of alternative splicing products serves to regulate the phosphorylation of specific and structurally homologous cAMP-dependent protein kinase sites,2 suggesting that this or a closely related kinase is the in vivo phosphorylating enzyme. Alterations in the degree of antigen phosphorylation, caused either by an imbalance in alternative products or by the action of an intruding kinase that deregulates phosphorylation of the same motifs, could lead to an autoimmune response in predisposed individuals. Accordingly, we found that in kidney, GP patients express relatively more alternative products than control individuals (5) and that GPBP phosphorylates the human GP antigen at a major cAMP-dependent protein kinase phosphorylation site in an apparently unregulated fashion since alternative products do not affect antigen phosphorylation.3

Although GPBP is ubiquitously expressed, in certain organs and tissues it shows a preference for cells and tissue structures that are a target of common autoimmune responses as follows: the Langerhans cells (type I diabetes); the white matter of the central nervous system (multiple sclerosis); the biliary ducts (primary biliary cirrhosis); the cortical cells of the adrenal gland (Addison disease); striated muscle cells (myasthenia gravis); spermatogonium (male infertility); Purkinje cells of the cerebellum (paraneoplastic cerebellar degeneration syndrome); and epithelial intestinal cells (pernicious anemia, autoimmune gastritis and enteritis). Although it is premature to draw definitive conclusions on the pathogenic involvement of GPBP, all the above observations point to this novel kinase as an attractive candidate to be considered when envisioning a model for human autoimmune disease.

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REFERENCES
1. Saus, J. (1998) in Goodpasture’s Syndrome: Encyclopedia of Immunology (Delves, P. J., and Roitt, I. M., eds) 2nd Ed., pp. 1005–1011, Academic Press Ltd., London.
2. Leinonen, A., Mariyama, M., Mochizuki, T., Tryggvason, K., and Reeder, S. T. (1994) J. Biol. Chem. 269, 26172–26177.
3. Quinones, S., Bernal, D., Garcia-Sogo, M., Elena, S. F., and Saus, J. (1992) J. Biol. Chem. 267, 19780–19784.
4. Revert, F., Penades, J. R., Plana, M., Bernal, D., Johansson, C., Itarte, E., Cervera, J., Wieslander, J., Quinones, S., and Saus, J. (1993) J. Biol. Chem. 270, 13254–13261.
5. Bernal, D., Quinones, S., and Saus, J. (1993) J. Biol. Chem. 268, 12090–12094.
6. Pouzol, I., Xia, Y., and Wilson, C. B. (1994) J. Biol. Chem. 269, 2342–2348.
7. Penades, J. R., Bernal, D., Revert, F., Johansson, C., Fresquet, V. J., Cervera, J., Wieslander, J., Quinones, S., and Saus, J. (1995) Eur. J. Biochem. 229, 754–760.
8. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
9. Coligan, J. E., Dunn, B. N., Ploegh, H. L., Speicher, D. W., and Winfield, P. T. (1996) Current Protocols in Protein Science, John Wiley & Sons, Inc., New York.
10. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1998) Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York.
11. Ferrel, J. E., and Martin, G. S. (1991) Methods Enzymol. 200, 430–435.
12. Doyle, W. J., van der Geer, P., and Hunter, T. (1991) Methods Enzymol. 201, 110–149.
13. Hsu, S. M., Raine, L., and Fanger, H. (1981) J. Histochem. Cytochem. 29, 577–580.
Goodpasture Syndrome

14. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 3389–3402
15. Bairoch, A., Bucher, P., and Hofmann, K. (1997) Nucleic Acids Res. 25, 217–221
16. Lupas, A. (1996) Trends Biochem. Sci. 21, 375–382
17. Lemmon, M. A., Falasca, M., Ferguson, K. M., and Schlessinger, J. (1997) Trends Cell Biol. 7, 237–242
18. Boulikas, T. (1993) Crit. Rev. Eukaryotic Gene Expr. 3, 193–227
19. Csermely, P., and Kahn, C. R. (1991) J. Biol. Chem. 266, 4943–4950
20. Maru, Y., and Witte, O. N. (1991) Cell 67, 459–468
21. Beeler, J. F., LaRochelle, W. J., Chedid, M., Tronick, S. R., and Aaronson, S. A. (1994) Mol. Cell. Biol. 14, 982–988
22. Csermely, P., Miyata, Y., Schneider, T., and Yahara, I. (1995) J. Biol. Chem. 270, 6381–6388
23. Dikstein, R., Ruppert, S., and Tjian, R. (1996) Cell 84, 781–790
24. Eichinger, L., Bommies, L., Vandekerckhove, J., Schleicher, M., and Gettermans, J. (1996) EMBO J. 15, 5547–5556
25. Côté, G. P., Luo, X., Murphy, M. B., and Egelhoff, T. T. (1997) J. Biol. Chem. 272, 6846–6849
26. Ryan, A. G., Ward, M. D., Mendola, C. E., Pavur, K. S., Dorovkov, M. V., Wiedmann, M., Erdjument-Bromage, H., Tempst, P., Parmar, T. G., Prostko, C. R., Germino, F. J., and Hait, W. N. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4884–4889
27. Fraser, R. A., Heard, D. J., Adam, S., Lavigne, A. C., Le Douarin, B., Tora, L., Losson, R., Rochette-Egly, C., and Chambon, P. (1998) J. Biol. Chem. 273, 16199–16204
28. Langelier, Y., Champoux, L., Hamel, M., Guilbault, C., Lamarche, N., Gaudreau, P., and Massie, B. (1998) J. Biol. Chem. 273, 1435–1443
29. Lemmon, M. A., and Ferguson, K. M. (1998) Curr. Top. Microbiol. Immunol. 228, 39–74
30. Rebecchi, M. J., and Scarlata, S. (1998) Annu. Rev. Biophys. Biomol. Struct. 27, 503–528
31. Roit, I. (1994) in Autoimmune Diseases: Essential Immunology, 8th Ed., pp. 383–439, Blackwell Scientific, Oxford
32. Erlich, H., and Apple, R. (1998) in MHC Disease Associations: Encyclopedia of Immunology (Delves, P. J., and Roitt, I. M., eds) 2nd Ed., pp. 1690–1709, Academic Press Ltd., London
33. Phelps, R. G., Turner, A. N., and Rees, A. J. (1996) J. Biol. Chem. 271, 18549–18553
34. Henderson, R. D., Saltissi, D., and Pender, M. P. (1998) Acta Neurol. Scand. 98, 134–135
35. Litsky, J. M., and Johnson, G. V. W. (1992) J. Biol. Chem. 267, 1563–1568
36. Brown, K., Gerstberger, S., Carlson, L., Franceso, G., and Siebenlist, U. (1995) Science 267, 1485–1488
37. Chen, Z. J., Parent, L., and Maniatis, T. (1996) Cell 84, 585–586
38. Aberle, H., Bauer, A., Stappert, J., Kispert, A., and Kemler, R. (1997) EMBO J. 16, 3797–3804
39. Regnier, C. H., Song, H. Y., Gao, X., Goeddel, D. V., Cao, Z., and Rothe, M. (1997) Cell 90, 373–383
40. Vlach, J., Hennecke, S., and Amati, B. (1997) EMBO J. 16, 5334–5344
41. Phelps, R. G., Jones, V. L., Coughlan, M., Turner, A. N., and Rees, A. J. (1998) J. Biol. Chem. 273, 11440–11447