Oncostatin M is a polypeptide cytokine having unique structure and diverse biological activities, including the ability to inhibit growth of certain cultured tumor cells. Here we have determined the disulfide bonding pattern of recombinant oncostatin M and have used site-directed mutagenesis to identify regions of this molecule necessary for receptor binding and growth inhibitory activities. Two intramolecular disulfide bonds, C6-C127 and C49-C167, were identified in recombinant oncostatin M. Analysis of mutations at each of the five cysteines in oncostatin M indicated that mutants C49S and C167S were inactive (<1/10 wild type activity) in growth inhibitory assays and radioreceptor assays. Carboxy-terminal deletion mutations terminating at S185 and beyond were active, but further shortening abolished activity in both assays. Two deletion mutants proximal to C49 (∆22-36 and ∆44-47) and insertion mutant GAG77 also were inactive. One deletion mutant, ∆87-90, had significantly (~3fold) increased activities in both growth inhibitory assays and radioreceptor assays. A potential amphiphilic domain was identified beginning at C167 and extending toward the carboxy terminus. Two mutants having altered hydrophobic residues within this domain (F176G and F184G) were inactive, suggesting that these residues are required for proper conformation of the receptor binding site. Taken together, these results indicate that biological activity of oncostatin M requires discontinuous regions of the molecule, including residues near the essential disulfide bond, C49-C167, and within a putative amphiphilic helix at the carboxy terminus. Oncostatin M thus belongs to a growing family of cytokines whose interactions with their respective receptors are mediated in part by known or predicted carboxy-terminal amphiphilic helices.

Oncostatin M is a polypeptide cytokine produced by lymphoid cells, which was originally identified by its ability to inhibit the growth of certain tumor cell lines but not normal fibroblasts. Further studies have shown that oncostatin M, like other cytokines, is multifunctional and may regulate cellular growth and gene expression of many cell types. Cellular receptors for oncostatin M were identified on a wide variety of cells (2-4). Although oncostatin M shares functional properties with other cytokines, its structure is unique. Sequence analysis of an oncostatin M cDNA clone (6) revealed a 252-amino acid precursor, with a 25-amino acid signal peptide. Subsequent studies (7) showed that approximately 31 amino acids were cleaved from the carboxyl terminus of recombinant oncostatin M and native oncostatin M from U937 cells. Carboxy-terminal processing of pro-oncostatin M increased its growth inhibitory activity on A375 melanoma cells, but not its ability to bind its receptor(s) (7). Oncostatin M shares no sequence homology with other known cytokines.

Regions of oncostatin M important for receptor binding and growth regulation are unknown. To address this question, we have identified cysteine residues involved in intramolecular disulfide linkages. We also have constructed plasmids containing site-directed mutations of oncostatin M and have expressed mutant proteins in COS cells. In this paper, we report on the functional characterization of a collection of these mutants. The results suggest that multiple regions of oncostatin M are functionally important and that one such region lies near a critical disulfide linkage.

MATERIALS AND METHODS

Cell Culture—A375 melanoma cells, COS cells, and 12981 lung carcinoma cells (1, 5, 6) were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 100 µg/ml of streptomycin, and 100 units/ml of penicillin.

Assessment of Disulfide Bonds—Purified recombinant oncostatin M from Chinese hamster ovary cells (25 µg) was dried in vacuo in a 1.5-ml polypropylene microfuge tube, and dissolved in 136 µl of 0.125 M ammonium bicarbonate, pH 8.1. Tryptic digestions were carried out in 25 µl of 0.125 M ammonium bicarbonate for 1 h at 37°C. The sample then was divided equally into two 1.5-ml microfuge tubes, 2-mercaptoethanol (4 µl) was added to one sample, and the tubes were flushed with nitrogen and incubated for 2 h at 25°C. The reaction mixtures were concentration to pH 2.0 with 0.1% trifluoroacetic acid.

Peptides were separated by HPLC on a reversed-phase Partisil C8 column (4.6 x 100 mm, Whatman). The column was equilibrated with 0.1% trifluoroacetic acid and acidic samples were applied at a flow rate of 1 ml/min. The column was then washed with 25 ml of 0.1% trifluoroacetic acid, and peptides were eluted with a linear gradient of 0-60% acetonitrile in 0.1% trifluoroacetic acid over a period of 150 min at a flow rate of 0.4 ml/min. Individual peptide peaks were collected and sequenced using an Applied Biosystems model 475A gas-phase sequencer.

Antiserum and Monoclonal Antibodies—Preparation and characterization of site-specific antisera recognizing a peptide corresponding to amino acids 6-19 of oncostatin M were reported previously (6, 7). (Numbers are from the amino-terminal alanine of natural and recombinant oncostatin M.) Anti-50-64 serum was prepared and characterized as described (7); binding of this antiserum was blocked by the

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed. Tel.: 206-728-0786. Fax: 206-728-1663.

1 M. Shoyab, unpublished work.
2 The abbreviations used are: HPLC, high performance liquid chromatography; DMEM, Dulbecco's modified Eagle's medium; GIA, growth inhibitory assay; RRA, radioreceptor assay; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; mAb, monoclonal antibody.
cognate peptide. Culture medium containing monoclonal antibody OM4 was provided by Dr. Susan Radka, Oncogen.

Construktion and Expression of Oncostatin M Mutations—The oncostatin M cDNA expression plasmid, pSPOM, has been described previously (6, 7). Mutant A22-36 was constructed by endonuclease digestion of pSPOM at unique BglII and BamHI restriction sites and by religation of digestion products, creating a deletion of two amino acids 22-36. Deletion mutant AS179 was constructed by cleaving pSPOM at a unique DraIII site, blunting overhanging nucleotides with the Klenow fragment of DNA polymerase I, and religating the digested plasmid. Internal deletion mutations (A44-47, A57-90, A118-121, A125-155, and A178-181); carboxy-terminal deletion mutations (A196-227, A190-227, A189-227, A186-227, A184-227, and A182-227); and base change mutations resulting in transversions (C6S, C49S, C80S, C127S, C167S, F169G, F167G, F184G, H171G, H174G, and H176G) were constructed by a combination of oligonucleotide-directed mutagenesis and polymerase chain reaction amplification techniques as described (7). Three codon insertions (GAG6, GAG77, GAG104, and GAG140) were constructed by inserting the oligonucleotide sequence GGCGCCGCGG, which encodes the amino acids glycine-alanine-glycine, preceding the indicated amino acid by oligonucleotide-directed mutagenesis and polymerase chain reaction amplification. Internal deletion mutations A195-226 was constructed by exonuclease digestion as described (8). Mutants A191-227 and A183-227 were described in a previous report (7), in which they were denoted A190 and A182, respectively. All constructs were cloned into HindIII/XhoI-cleaved plasmid pH3MPY (9). Each mutation was screened by restriction analysis and verified by dideoxy sequencing of the plasmid (10). Sequence analysis of mutant H171G revealed that random secondary point mutations had occurred during construction, resulting in additional mutations (K11OR and M113V).

COS cells were transfected with pSPOM or plasmids encoding mutant forms of oncostatin M as described previously (6). Twenty-four hours after transfection, serum-free conditioned medium was added, and the cells were cultured for an additional 48 h. Media were then collected and assayed for growth inhibitory activity and radioreceptor activity as described (6, 7). A portion of each collection was dialyzed against 0.2 M acetic acid to allow for sample concentration necessary for SDS-PAGE and immunoblotting analysis.

Biological Assays—Biological assays for oncostatin M activity have been described in detail elsewhere (2, 5-7). One GIA unit was equivalent to ~0.1 ng in an assay volume of one ml. Concentrations of oncostatin M were estimated generally as linear over concentrations corresponding to ~0.12 to ~0.5 μg/ml oncostatin M. Oncostatin M concentrations were determined generally as means (coefficients of variation of 30%) of determinations from three serial 2-fold dilutions of culture. Dose-response curves determined using this method were linear over concentrations corresponding to -0.12 to -0.5 μg/ml oncostatin M. Oncostatin M mutants described in this paper were expressed at concentrations ranging from 0.14 to 2 μg/ml.

Computer-assisted Sequence Analysis—Secondary structure analysis of oncostatin M was performed on an IBM PS/2 Model 80 computer using Genepep (Riverside Scientific, Seattle, WA) and PC/Gene (Intelligenetics, Mountain View, CA) software.

RESULTS

Assignment of Disulfide Bonds—As a first step in determining important functional regions of oncostatin M, we determined disulfide bonding patterns of this molecule. Mature oncostatin M contains 5 cysteine residues, providing for two possible intramolecular disulfide linkages (6). To map disulfide bond(s), purified recombinant oncostatin M from Chinese hamster ovary cells (5, 7) was digested with trypsin, and peptide digests were analyzed by reversed-phase HPLC with and without reduction with 2-mercaptoethanol (Fig. 1). Peptide peaks present at lower levels in the reduced sample were isolated and subjected to amino acid sequence analysis. The peak denoted by a single asterisk gave the following sequences (3127. The fifth cysteine, corresponding to C6 and C127. These data permit assignment of two disulfide bonds in oncostatin M containing wild-type or mutant oncostatin M was compared with maximal binding in the absence of unlabeled oncostatin M. The amount of oncostatin M required to inhibit binding of 125I-labeled oncostatin M (at ~20 ng/ml) by 50% was determined and defined to be one RRA unit (equivalent to ~3 ng of purified recombinant oncostatin M in an assay volume of 0.1 ml). GIA and RRA activities (units/ml) were converted to specific activity values (units/ng) by normalizing for concentrations of oncostatin M determined by quantitative immunoblotting (see below and Ref. 7). Coefficients of variation for GIA and RRA activities determined in the same experiment ranged from 15 to 30%.

To compare results between experiments more easily, specific activities were converted to relative specific activities by dividing by specific activity values for wild type oncostatin M determined in the same experiment. Coefficients of variation for relative specific activity values ranged from 22 to 45%. Values presented are from representative experiments. In many cases, specific activity determinations were repeated several times, with determinations varying by 50%. Mutant forms of oncostatin M having relative specific activity values in either GIA or RRA of 1/10 of wild type were considered inactive. Mutant forms of oncostatin M having relative specific activities in either GIA or RRA of >2 were considered significantly more active than wild type.

Detection and Quantitation of Oncostatin M—SDS-PAGE (11); and immunoblotting analysis were performed as described (6, 7). Concentrations of wild type or mutant oncostatin M in serum-free culture medium were measured by quantitation as described in detail elsewhere (7). Serum-free culture media were diluted in DMEM dithiothreitol was added to a concentration of 10 mM, and proteins were denatured by heating to 95°C for 5 min. Samples were then applied to an nitrilotriacetic acid cross-linked gel. The gel was scanned by densitometer (Hoefer Scientific Instruments, San Francisco, CA) using transmitted light. Amounts of oncostatin M (in arbitrary units) were calculated from peak heights; they were converted to concentrations by comparison with peak heights from a standard curve obtained by serial dilution of a known amount of purified oncostatin M in serum-free medium from mock-transfected COS cells. Oncostatin M concentrations were estimated generally as means (coefficients of variation of 30%) of determinations from three serial 2-fold dilutions of culture. Dose-response curves determined using this method were linear over concentrations corresponding to ~0.12 to ~0.5 μg/ml oncostatin M. Oncostatin M mutants described in this paper were expressed at concentrations ranging from 0.14 to 2 μg/ml.

Identification of Essential Cysteine Residues—Having established the disulfide bonding pattern of oncostatin M, we next investigated functionally important regions of the molecule by site-directed mutagenesis. Site-directed mutations were introduced into a cDNA encoding oncostatin M, and mutant proteins were expressed in COS cells (5-7). Serum-free media from transfected COS cells containing wild-type and mutant forms of oncostatin M were collected and analyzed by biological activity by GIA and RRA as described under "Materials and Methods." Samples of conditioned medium were sub-

1 S. Radka, J. C. Kallestad, P. S. Linsley, and M. Shoyab, submitted for publication.
Site Directed Mutants of Oncostatin M

Injected to SDS-PAGE and immunoblotting analysis to confirm production of oncostatin M; the sole exception to this was mutant Δ87–90 which was analyzed only by quantitative immunoblotting. Concentrations of oncostatin M in serum-free culture medium were then determined by quantitative immunoblotting (7). Concentrations determined in this manner corresponded roughly to the levels of oncostatin M seen following SDS-PAGE and immunoblotting analysis. Specific GIA and RRA activities were then calculated as described under "Materials and Methods" and Table I.

The first set of mutants was designed to test which of the 5 cysteine residues are essential for biological activity. In preliminary experiments, reduction and S-pyridylethylation (with 4-vinylpyridine) of oncostatin M reduced GIA activity by >90%, whereas S-pyridylethylation alone had little effect. This suggested that one or more of the disulfide linkages in oncostatin M were essential for biological activity. To confirm these observations, cysteine residues involved in disulfide bond formation were changed individually to serine by oligonucleotide-directed mutagenesis. Plasmids containing mutant constructs were transfected into COS cells, and relative specific activities of mutant oncostatin M released into serum-free conditioned medium were determined (Table I). The results indicated that cysteine to serine mutations at C6 and C127 had little effect on GIA and RRA activities, whereas mutations at C49 and C167 resulted in >90% loss of both GIA and RRA activities. Simultaneous mutations at C6 and C167 also resulted in inactive molecules. Changing the free cysteine at position 80 to serine resulted in a slight increase in both GIA and RRA activity, but this was not seen in other experiments. From these data, we conclude that the C49–C167 disulfide linkage is essential, but the C6–C127 linkage is not.

**TABLE I**

| Mutant    | Relative specific activity |
|-----------|---------------------------|
|           | GIA  | RRA  |
| C6S       | 0.19 | 0.78 |
| C49S      | <0.01| 0.06 |
| C80S      | 2.0  | 1.4  |
| C127S     | 1.3  | 0.60 |
| C167S     | 0.07 | 0.06 |
| C6S/C167S | <0.01| <0.01|

Sequential Deletion of Carboxyl-terminal Amino Acids—We previously showed (7) that mutant Δ183–227 was inactive in GIA and RRA, whereas mutant Δ191–227 was active, indicating that residues between position 183 and 191 were crucial for functional activity. To determine more precisely the extent of functionally important residues at the carboxyl terminus, we constructed and expressed a series of constructs having sequential deletions at the carboxyl terminus (Table II). Mutants terminating at position 185 and beyond were active, but further truncations abolished GIA and RRA activities. These data indicate that carboxyl-terminal residues up to and including S185 are essential for biological activity. Most carboxyl-terminal deletion mutants had 2–5-fold greater relative specific activity in GIA than in RRA. This increase in specific activity of oncostatin M in GIA, but not in RRA, was observed previously following removal of carboxyl-terminal residues distal to position 196 (7).

Scanning Deletion and Insertion Mutants—We next constructed a series of scanning deletion and insertion mutations by oligonucleotide-directed mutagenesis. Plasmids were constructed containing four separate deletion mutations spaced approximately 100 bases apart. These included three separate 4-codon deletions and one 15-codon deletion. Four plasmids with 3-codon insertions encoding the amino acids, -GAG-, were also constructed. Plasmids containing mutant constructs were transfected into COS cells, and serum-free media were

**TABLE II**

| Mutant    | Relative specific activity |
|-----------|---------------------------|
|           | GIA  | RRA  |
| Δ196-227  | 1.7  | 0.73 |
| Δ191-227  | 0.90 | 0.26 |
| Δ190-227  | 0.99 | 0.68 |
| Δ189-227  | 2.0  | 0.42 |
| Δ188-227  | 1.4  | 0.54 |
| Δ187-227  | 0.66 | 0.21 |
| Δ186-227  | 0.60 | 0.20 |
| Δ185-227  | 0.17 | 0.02 |
| Δ184-227  | 0.02 | <0.01|
| Δ183-227  | <0.03| <0.01|
| Δ182-227  | <0.03| <0.01|
tested for functional activities (Table III). Deletion mutants \(\Delta 22-36\) and \(\Delta 44-47\) were inactive in both GIA and RRA, indicating that a region proximal to the amino terminus from C49 was essential for activity. Mutant GAG77 was also inactive in GIA, although it had a low level of RRA activity. This mutant was also expressed at low levels (0.14 \(\mu\)g/ml). This insertion disrupts the putative N-linked glycosylation recognition sequence beginning at N75. The low levels of expression of this protein and its inactivity in GIA suggest that glycosylation at N75 may be important for functional expression and GIA activity. One deletion mutant, \(\Delta 87-90\), gave significantly higher GIA and RRA activities. We conclude that regions disrupted by two deletion mutations (\(\Delta 87-90, \Delta 152\)) and three insertion mutations (GAG6, GAG104, and GAG140) were not essential for activity.

Plasmids containing deletion mutations \(\Delta S179, \Delta 118-121,\) and \(\Delta 178-181\) were also constructed and transfected into COS cells, but produced no detectable oncostatin M (data not shown).

**Point Mutations in a Putative Carboxyl-terminal Amphiphilic Domain Reduce Biological Activity**—Results from cysteine and carboxyl-terminal deletion mutants indicated that C167 and inclusion of the carboxyl terminus through S185 were essential for biological activity, suggesting that a region between these residues might have important structural features. Examination of this region by Edmundson wheel analysis (12) revealed a potential amphiphilic helix extending from C167 to the tryptic-like cleavage site at R196. A portion of this helix, extending from R168 to S185 is shown in Fig. 2. To determine if residues from either the hydrophilic or the hydrophobic faces of this putative helix are functionally important, the histidine residues at positions 171, 174, and 178 and phenylalanine residues at positions 169, 176 and 184 were mutated to glycine residues by oligonucleotide-directed mutagenesis. As shown in Table IV, mutants F176G and F184G were inactive, whereas F169G, H171G, H174G, and H178G were active. Thus, mutations of these histidine residues were tolerated, but mutations at 2 of 3 phenylalanine residues inactivated oncostatin M.

**DISCUSSION**

A summary of the results described in this paper is depicted graphically in Fig. 3. Our results show that a number of regions in oncostatin M are not required for functional activity. They also show that one region of oncostatin M which is required includes the C49-C167 disulfide bond and certain residues proximal to this bond. Finally, we have shown that regions of oncostatin M discontinuous with the essential disulfide bond are also required for functional activity (e.g., mutant, GAG77). Thus, tertiary structural features of oncostatin M are required for functional activity.

In most cases, GIA and RRA activities of mutant proteins were affected in parallel. All mutants inactive in GIA also had reduced activity in RRA. Likewise, deletion mutant \(\Delta 87-90\) had significant increases in both activities. In some cases, however, relative GIA and RRA activities of mutant forms of oncostatin M were not affected equally. Several mutants had greater relative specific GIA activities than RRA activities (e.g., carboxyl-terminal deletion mutants shown in Table II, and mutant H178G, shown in Table IV). The basis for the greater relative GIA activities of these mutants is unclear, but it may be significant that all of these mutants had alterations at the carboxyl terminus.

In a previous study (7), we observed that cleavage of a hydrophilic domain from the carboxyl terminus of pro-oncostatin M likewise increased GIA, but not RRA activity of oncostatin M. This finding, together with the results of the present study, indicates that GIA activity of oncostatin M may be increased without affecting receptor binding by carboxyl-terminal modifications. Increased GIA activities possibly could result from altered stabilities of mutant forms during the relatively long term (3-day) GIA. Alternatively, carboxyl-terminal modifications may alter the signaling capacities of mutant forms subsequent to their receptor binding.
assays for oncostatin M (1-5) may help distinguish between these possibilities.

Residues proximal to C49 are also essential for functional activity of oncostatin M because deletion mutants A22-36 and A44-47 were inactive. This region of oncostatin M lies just proximal to the amino terminus from the essential C49-C167 disulfide bond (see Fig. 3). The importance of residues proximal to C49 is also indicated by monoclonal antibody (mAb) binding studies to be published elsewhere.4 These studies show that binding of mAb OM2 (which neutralizes GIA and RRA activity of oncostatin M) to mutants A22-36 and A44-47 was reduced greatly compared with binding of non-neutralizing mAb, OM1. Binding of both mAbs was affected by mutations in the putative carboxyl-terminal amphipathic domain. Results of these mAb binding studies, together with those of the present study, indicate that the tertiary structure of residues on both sides of disulfide bond C49-C167 is essential for functional activity of oncostatin M.

A predicted amphiphilic helix was identified, extending from C167 to the site of proteolytic cleavage (7) of the highly charged carboxyl-terminal domain from pro-oncostatin M (see Fig. 2). Eisenberg et al. (13) have proposed the hydrophobic moment, <μH>, of a protein sequence as a measure of the tendency of a sequence to form amphipathic helices. <μH> calculated for the putative carboxyl-terminal helix from oncostatin M (Fig. 2) was among the highest for known protein sequences, including many helices known from x-ray structure (data not shown). This region is important for functional activity, because several mutants from this region were inactive, including carboxyl-terminal deletion mutants terminating proximal to residue 180 and two point mutations, F176G and F184G. A second significant helical moment (<μH>, Ref. 13) is found in oncostatin M, centered at T27. Mutants Δ22-36 and Δ44-47, which were inactive, have alterations in this region.

Mutants with alterations in the hydrophilic face of the predicted carboxyl-terminal amphipathic helix (H171G, H174, and H178G) were active, whereas two of three mutants with alterations in the hydrophobic face (F176G and F184G) were inactive. The projected amphiphilic helix, should it exist in a soluble protein such as oncostatin M, is likely to be oriented so that the hydrophobic face is oriented toward the hydrophobic interior of the protein. The crucial phenylalanine residues therefore may not make direct contacts with the oncostatin M receptor, but rather may be required for maintaining proper position of the actual contact residues. It is noteworthy that other mutations in this region (A178-181 and ΔS179) were not detected by COS cells, possibly indicating a requirement for this region in proper folding, intracellular transport, and/or secretion of oncostatin M.

Carboxyl-terminal amphipathic helices are involved in binding of many cytokines to their respective receptors. Cunningham and Wells (14) showed that several residues which affected receptor binding were localized within the carboxy-terminal amphipathic helix of human growth hormone using alanine-scanning mutagenesis. Bazan (15) has discussed evidence that several cytokines having receptors with structural similarities to the human growth hormone receptor (16), including prolactin, interleukin-6, granulocyte-stimulating factor, and erythropoietin, also have carboxyl-terminal helices implicated in receptor binding. An amphipathic carboxyl-terminal helix has been implicated in receptor binding of interleukin-2 (17). Finally, St. Charles et al. (18) demonstrated that residues believed to be involved in heparin binding by platelet factor four were contained in a carboxyl-terminal amphipathic helix.

Although these other cytokines share little primary sequence homology, with the exception of platelet factor four, they all have, or are predicted to have, tertiary structures consisting of four helix bundles (15, 19). It is not known whether oncostatin M also shares this structural motif, although analysis of secondary structure by the method of Garnier (20) predicts that oncostatin M has a high helical content (39.2% helical), similar to predicted values for human growth hormone (42.3%) and interleukin-2 (58.1%). Likewise, as discussed above, several regions of oncostatin M have high <μH>, possibly indicating that amphipathic helices contribute significantly to the tertiary structure of oncostatin M.

Regardless of tertiary structure considerations, none of the above cytokines has a disulfide bonding pattern like that of oncostatin M. One cytokine family which does have a disulfide bonding pattern similar to oncostatin M is the interferon family. This family has the same disulfide linkage motif (Cl-Cz, C6-C127) and nonessential regions are indicated by dashed lines. Active mutants are enclosed in unshaded boxes, and inactive mutants are enclosed in shaded boxes.

Acknowledgments—We thank Scott Austin for production of oligomers; Michael Neubauer and Joseph Cook for help in DNA sequence analysis; Dr. David Eisenberg for useful discussion of amphipathic helices; Drs. Timothy Rose and Susan Radka for their helpful discussions; Drs. O. Haffar, P. Fell, and S. Arufo for comments on the manuscript; and L. Linsley for assistance with the manuscript.

REFERENCES

1. Zarling, J. M., Shoyab, M., Marquardt, H., Hanson, M. B., Lioubin, M. N., and Todaro, G. J. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 9739-9743.
2. Horn, D., Fitzpatrick, W. C., Gompper, P. T., Ochs, V., Bolton-Hanson, M., Zarling, J. M., Malik, N., Todaro, G. J., and
Site Directed Mutants of Oncostatin M

Linsley, P. S. (1990) Growth Factors 2, 157–165

3. Brown, T. J., Rowe, J. M., Shoyab, M., and Gladstone, P. (1990) in Molecular Biology of the Cardiovascular System (Roberts, R., and Schneider, M. D. eds) pp. 195–206, Wiley-Liss, Inc. New York

4. Brown, T. J., Lioubin, M. N., and Marquardt, H. (1987) J. Immunol. 139, 2977–2983

5. Linsley, P. S., Bolton-Hanson, M., Horn, D., Malik, N., Kallestad, J. C., Ochs, V., Zarling, J. M., and Shoyab M. (1989) J. Biol. Chem. 264, 4282–4289

6. Malik, N., Kallestad, J. C., Gunderson, N. L., Austin, S. D., Neubauer, M. G., Ochs, V., Marquardt, H., Zarling, J. M., Shoyab, M., Wei, C., Linsley, P. S., and Rose, T. M. (1989) Mol. Cell. Biol. 9, 2847–2853

7. Linsley, P. S., Kallestad, J. C., Ochs, V., and Neubauer, M. (1990) Mol. Cell. Biol. 10, 1882–1890

8. Henikoff, S. (1984) Gene(Amst.) 28, 351–359

9. Stamenkovic, I., Clark, E. A., and Seed, B. (1989) EMBO J. 8, 1403–1410

10. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467

11. Laemmli, U. K. (1970) Nature 227, 680–685

12. Schiffer, M., and Edmundson, A. B. (1967) Biophys. J. 7, 121–135

13. Eisenberg, D., Weiss, R. M., and Terwilliger, T. C. (1982) Nature 299, 371–374

14. Cunningham, B. C., and Wells, J. A. (1989) Science 244, 1081–1085

15. Bazan, J. F. (1990) Immunol. Today 11, 350–354

16. Bazan, J. F. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 6934–6938

17. Landgraf, B., Cohen, F. E., Smith, K. A., Gadski, R., and Ciardelli, T. L. (1989) J. Biol. Chem. 264, 816–822

18. St. Charles, R., Waiz, D. A., and Edwards, B. F. P. (1989) J. Biol. Chem. 264, 2092–2099

19. Brandhuber, B. J., Boone, T., Kenney, W. C., and McKay, D. B. (1987) Science 238, 1707–1709

20. Garnier, J. P., Osguthorpe, J. and Robson, B. (1978) J. Mol. Biol. 120, 97–105

21. Wetzel, R. (1981) Nature 289, 606–607

22. Wetzel, R., Johnston, P. D., and Czarnecki, C. W. (1988) in The Biology of the Interferon System (De Maeyer, E., and Schellekens, H., eds) pp. 101–112, Elsevier Science Publishers, Amsterdam

23. De Maeyer, E., and De-Maeyer Guignard, J. (eds.) (1988) Interferons and Other Regulatory Cytokines p.14, Wiley Interscience, New York

24. Williams, R. W. (1985) J. Biol. Chem. 260, 3937–3940

25. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132