gene loci mapped to date are dispersed on other human chromosomes (*27*).

That growth factors may play a role in the onset of neoplasia has been an attractive hypothesis for some time. The near identity of a gene sequence of *PDGF* and v-*sis* has added credence to this hypothesis (*21*). A characteristic translocation involving chromosome 4 (q21) and chromosome 11 has been observed in some cases of acute leukemia (*28*). However, recent phenotypic studies suggesting that the t(4;11)-associated acute leukemia represents a proliferation of myeloid progenitor cells (*29*) as well as the identification of the TCGF gene on q26–28 make it difficult to implicate TCGF in the etiology of this disease. Nonetheless, the occurrence of *TCGF* on chromosome 4 adds an important aspect to the continuing genetic analysis of oncogenes, growth factors, and retroviruses that have been implicated as regulators of normal growth and development as well as in the etiology of neoplasia (*27*).

LEONARD J. SEIGEL
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205
MARY E. HARPER*
Agouron Institute, 505 Coast Boulevard South, La Jolla, California 92037
FLOSSIE WONG-STAAL
ROBERT C. GALLO
Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda
WILLIAM G. NASH
STEPHEN J. O'BRIEN
Section of Genetics, National Cancer Institute, Frederick, Maryland 21701

References and Notes

1. D. A. Morgan, F. W. Ruscetti, R. C. Gallo, *Science* **193**, 1007 (1976); F. W. Ruscetti, D. A. Morgan, R. C. Gallo, *J. Immunol.* **119**, 131 (1977); S. Gillis, M. M. Ferm, W. Ou, K. A. Smith, *ibid.* **120**, 2027 (1978); F. W. Ruscetti, J. W. Mier, R. C. Gallo, *J. Supramol. Struct.* **13**, 229 (1980).
2. J. E. Gootenberg, F. W. Ruscetti, J. W. Mier, A. Gazdar, R. C. Gallo, *J. Exp. Med.* **154**, 1403 (1981).
3. S. Gillis and K. A. Smith, *Nature (London)* **268**, 154 (1977); S. Gillis, P. E. Baker, F. W. Ruscetti, K. A. Smith, *J. Exp. Med.* **148**, 1093 (1978); J. Watson, *ibid.* **150**, 1510 (1979).
4. B. J. Poiesz, F. W. Ruscetti, J. W. Mier, A. M. Woods, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6815 (1980); R. C. Gallo, B. J. Poiesz, F. W. Ruscetti, in *Modern Trends in Human Leukemia*, R. Neth, R. C. Gallo, T. Graft, K. Mannweiler, K. Winkler, Eds. (Springer-Verlag, Munich, 1981), vol. 5, pp. 502–514.
5. T. Waldmann *et al.*, *Clin. Res.* **31**, 547a (1983).
6. B. J. Poiesz, F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7415 (1980).
7. J. W. Mier and R. C. Gallo, *ibid.*, p. 6134.
8. T. Taniguchi, H. Matsui, T. Fujita, C. Takaoka, N. Kashima, R. Yoshimoto, J. Hamuro, *Nature (London)* **302**, 305 (1983).
9. S. Clark *et al.*, in preparation.
10. S. J. O'Brien, W. G. Nash, J. L. Goodwin, D. R. Lowry, E. H. Chang, *Nature (London)* **302**, 839 (1983); S. J. O'Brien, T. I. Bonner, M. Cohen, C. O'Connell, W. G. Nash, *ibid.* **303**, 74 (1983).
11. S. J. O'Brien and W. G. Nash, *Science* **216**, 257 (1982).
12. M. Bobro and J. Cross, *Nature (London)* **251**, 77 (1974).
13. S. J. O'Brien, J. M. Simonson, M. E. Eichelberger, in *Techniques in Somatic Cell Genetics*, J. Shay, Ed. (Plenum, New York, 1982); T. B. Shows and P. J. McAlpine, *Cytogenet. Cell Genet.* **32**, 221 (1982); H. Harris and P. A. Hopkinson, in *Handbook of Enzyme Electrophoresis in Human Genetics* (North-Holland, Amsterdam, 1976).
14. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
15. M. E. Harper, G. Franchini, J. Love, M. I. Simon, R. C. Gallo, F. Wong-Staal, *Nature (London)* **304**, 169 (1983); M. E. Harper, A. Ullrich, G. F. Saunders, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4458 (1981); M. E. Harper and G. F. Saunders, *Chromosoma* **83**, 431 (1981).
16. M. Popovic *et al.*, *Science* **219**, 856 (1983).
17. The human cells examined included: HeLa, BeWo, 143B, A673, JAR, WiDR, VA-2 [S. J. O'Brien *et al.*, *In Vitro* **16**, 119 (1980)], HUT-102 B2 p105 (*16*), and MJ (*16*). The restriction endonucleases used were: Eco RI, Pst I, Hind I, Xba I, Hind III, and Sst I. The patterns for all the cells with each restriction enzyme were identical.
18. W. G. Nash and S. J. O'Brien, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6631 (1982).
19. D. Owerbach *et al.*, *ibid.* **78**, 3123 (1981).
20. S. L. Naylor, A. Y. Sakaguchi, T. B. Shows, M. L. Law, D. Goeddel, P. W. Gray, *J. Exp. Med.* **157**, 1020 (1983).
21. R. F. Doolittle *et al.*, *Science* **221**, 275 (1983); M. D. Waterfield *et al.*, *Nature (London)* **304**, 35 (1983).
22. R. Dalla-Favera, R. C. Gallo, A. Giallongo, C. M. Croce, *Science* **218**, 686 (1982); D. C. Swan, O. W. McBride, K. C. Robbins, D. A. Keithley, E. P. Reddy, S. A. Aaronson, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4691 (1982).
23. U. R. Rapp *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4218 (1983).
24. T. I. Bonner, S. J. O'Brien, W. G. Nash, U. R. Rapp, C. C. Morton, P. Leder, *Science* **223**, 71 (1984).
25. G. Mark and U. R. Rapp, personal communication.
26. M. O. Dayhoff and W. C. Barker, *Atlas of Protein Sequence and Structure 1965* (National Biochemical Research Foundation, Washington, D.C., 1978).
27. J. D. Rowley, *Nature (London)* **301**, 290 (1983); see S. J. O'Brien, Ed., *Genetic Maps* [(Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 1984), vol. 3], for complete reference.
28. M. Oshimura, A. I. Freeman, A. A. Sanberg, *Cancer (Brussels)* **40**, 1161 (1977); E. L. Prigogina *et al.*, *Hum. Genet.* **53**, 5 (1979); H. Van Den Berghe *et al.*, *ibid.* **46**, 173 (1979).
29. J. L. Parkin *et al.*, *Blood* **60**, 1321 (1982); M. Nagasaka *et al.*, *ibid.* **61**, 1174 (1983).
30. We thank J. Simonson, R. Bauer, M. Eichelberger, N. White, and J. Love for excellent technical assistance and R. Reeves for discussion and technical advice. We thank G. Mark for communication of the unpublished sequence of c-*raf*-2 and for assistance in the sequence comparisons.
* Present address: Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda; Md. 20205.

14 September 1983; accepted 26 October 1983

Endogenous Regulation of Macrophage Proliferative Expansion by Colony-Stimulating Factor–Induced Interferon

Abstract. *Stimulation of cultures of murine bone-marrow cells with specific macrophage growth factor (colony-stimulating factor 1) resulted in the production of type 1 interferon. Neutralization of this endogenous interferon by antiserum directed against interferons α and β resulted in a significant enhancement of mononuclear phagocyte proliferation from committed marrow precursors. The effect of the antiserum was lost in cultures depleted of adherent cells, an indication that an adherent regulatory cell (or cells) in the marrow limits mononuclear phagocyte proliferation by producing antiproliferative interferon in response to high levels of specific growth factor.*

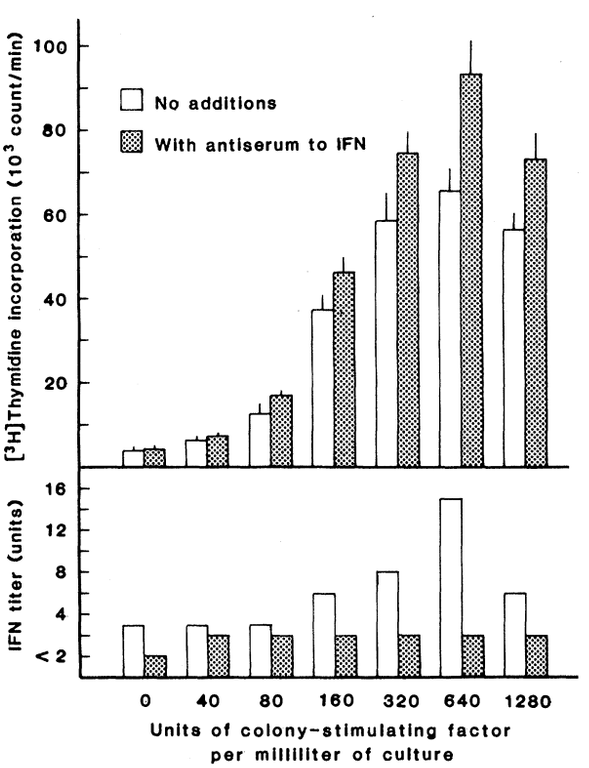
Maturation and differentiation of cells within the mononuclear phagocyte system results in a progressive loss of the capacity to proliferate in response to the specific growth factor known as colony-stimulating factor 1 (CSF-1) or macrophage growth factor (*1, 2*). The diminished proliferative capacity does not appear to be associated with a disappearance of specific receptors for CSF-1, since their expression is retained by more mature, differentiated macrophages (*2, 3*). Macrophages stimulated with CSF display various functional changes (*4*), including secretion of series E protaglandins (*5*), interleukin 1 (*6*), and interferon (IFN) (*4, 7*). Thus CSF-1 can act either as a proliferative signal for committed macrophage precursors (*2, 8*) and for immature mononuclear phagocytes (*1*) or as a stimulatory signal for more differentiated cells. Kurland *et al.* (*9, 10*) introduced the concept that mature macrophages in the marrow limit the CSF-induced expansion of monocytes and macrophages to appropriate levels by reacting to CSF within the local environment and producing series E prostaglandins; these prostaglandins act as potent inhibitors of CSF-induced mononuclear phagocyte proliferation from committed precursors (*11*). Thus CSF-1 may function in the bone marrow to regulate mononuclear phagocyte generation in both a positive and negative fashion. Series E prostaglandins, however, are not the only inhibitory molecules produced by CSF-stimulated macrophages. Interferon is also produced (*4, 7*) and may act as an antagonist to CSF-induced proliferation (*12–15*). Therefore mononuclear phagocyte proliferation might also be regulated by the balance of CSF and IFN in the environment of committed precursors. We obtained results that supported this hypothesis; the proliferative response of murine marrow preparations treated with purified CSF-1 was enhanced in the presence of antiserum that effectively neutralized murine IFN's

α and β (*15*). We have continued to investigate this finding and now report that IFN produced endogenously within CSF-stimulated marrow cultures limits the proliferation of committed mononuclear phagocyte precursors.

Both direct and indirect evidence for endogenous IFN production in marrow cultures is given in Fig. 1. Low-density murine femoral marrow cells (C3H/Anf; Cumberland View Farms, Clinton, Tennessee) were incubated in 96-well plates at a concentration of 1.25×10^5 cells per well in 0.2 ml of RPMI 1640 medium containing 10 percent fetal bovine serum (Microbiological Associates, Walkersville, Maryland), antibiotics, and dilutions of CSF-1. The CSF-1 (specific activity, 5×10^7 units per milligram of protein) was purified from serum-free supernatant medium of L929 cells as described by Waheed and Shadduck (*16*). Rabbit antiserum to mouse IFN's α and β (Enzo Biochem, New York) was added at a final dilution of 1:1000, a dilution that effectively neutralizes the antiproliferative effect of at least 200 units of IFN's α and β per milliliter (*15*). After 72 hours of incubation at 37°C in an atmosphere of 5 percent CO_2 in air, 0.1 ml of medium was removed from each well and assayed for IFN activity. The cultures remaining in the wells were then treated for an additional 6 hours with 1.0 μCi of [^{3}H]thymidine to determine their rate of proliferation. Detectable IFN increased to a maximum of 15 units in cultures stimulated with CSF at 640 units per milliliter. Cultures containing antiserum to IFN's α and β had 2 units or less of IFN activity. In this and in subsequent experiments, IFN production was correlated with proliferation rates as measured by incorporation of [^{3}H]thymidine. Optimal IFN levels were detected in cultures stimulated with doses of CSF that induced optimal levels of [^{3}H]thymidine incorporation. In addition, incorporation of antiserum to IFN's α and β resulted in an enhancement of proliferation in those cultures stimulated with CSF. This enhancement was significant ($P < 0.05$, Student's *t*-test) at CSF concentrations of 640 and 1280 unit/ml. As we reported earlier (*15*), this antiserum has no stimulating activity of its own and does not contain anti-CSF activity. These results implied that IFN produced as a result of CSF stimulation was suppressing the subsequent proliferation of committed precursors.

Augmentation of this enhancing effect of antiserum to IFN's α and β was accomplished by increasing the initial cell concentration in CSF-stimulated cultures (Fig. 2A). [^{3}H]Thymidine incorporation rates stimulated by CSF at 640 unit/ml were significantly enhanced by antiserum to IFN's α and β in cultures that contained at least 10^5 cells per well. Enhancement increased from approximately 30 percent at 10^5 cells per well to more than 70 percent in wells containing 1.5×10^5 to 2.0×10^5 cells per well ($P \leq 0.05$). In all cultures containing antiserum to IFN, IFN activity was detectable only at levels of ≤ 2 units, whereas cultures treated with CSF alone contained detectable IFN titers ranging from 4 units at 10^5 cells per well to 16 units at 1.5×10^5 to 2×10^5 cells per well.

Macrophages produce small amounts of IFN when stimulated with CSF-1 (*4*, *7*). Therefore, duplicate cultures of cells were treated as in Fig. 2A but transferred to empty wells after 2 hours of incuba-



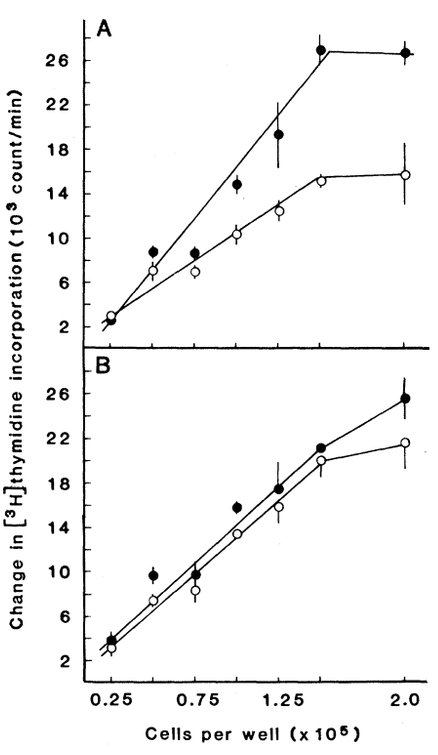


Fig. 1 (left). [^{3}H]Thymidine incorporation and IFN production by low-density murine femoral marrow cells stimulated with CSF-1. Marrow cells were centrifuged over mouse-density centrifugation medium (Lympholyte M; Cedarlane Laboratories), and low-density nucleated cells were collected from the interface, washed, and suspended in complete medium at 1.25×10^6 cells per milliliter. Cells were added to individual wells of 96-well plates in 0.1-ml volumes. CSF-1 and antiserum to IFN's α and β and their respective controls were each added to the wells in 0.05-ml volumes. Thus a total culture consisted of 0.2 ml containing 1.25×10^5 cells. After 72 hours of incubation at 37°C in a humid atmosphere of 5 percent CO_2 in air, 0.1 ml of culture medium was removed to assay for IFN; a semimicro vital stain assay was used with L929 cells challenged with vesicular stomatitis virus (*20*). In this assay, 1 unit of IFN is equal to approximately 1.4 international units. The remaining 0.1 ml of the marrow culture was treated for 6 hours with 1 μCi of [^{3}H]thymidine (specific activity, 2 Ci/mmole), harvested onto glass fiber filters with a cell harvester (Titertek, Flow Laboratories); [^{3}H]thymidine incorporation was determined by liquid scintillation spectrometry. IFN units represent the activity of pooled triplicate cultures, and [^{3}H]thymidine incorporation is given as the mean ± 1 standard error (S.E.) of the same triplicate cultures. Fig. 2 (right). Augmentation of the enhancing effect of antiserum to IFN's α and β accomplished by increasing the initial cell concentration in CSF-stimulated cultures. Low-density femoral marrow cells, prepared as described in the legend to Fig. 1, were incubated for 72 hours in 0.2-ml microwell cultures with an optimal dose of CSF-1 (640 unit/ml), with (●) or without (○) a 1:1000 dilution of antiserum to IFN's α and β. [^{3}H]Thymidine incorporation by these cultures was determined with a 6-hour treatment during the last 6 hours of culture. Data represent the results with CSF-stimulated cultures minus the results with their respective controls, which did not receive CSF. Values are given as means ± S.E. of triplicate cultures. In no case did control values exceed 1000 count/min. (A) Total cells; (B) nonadherent cells.

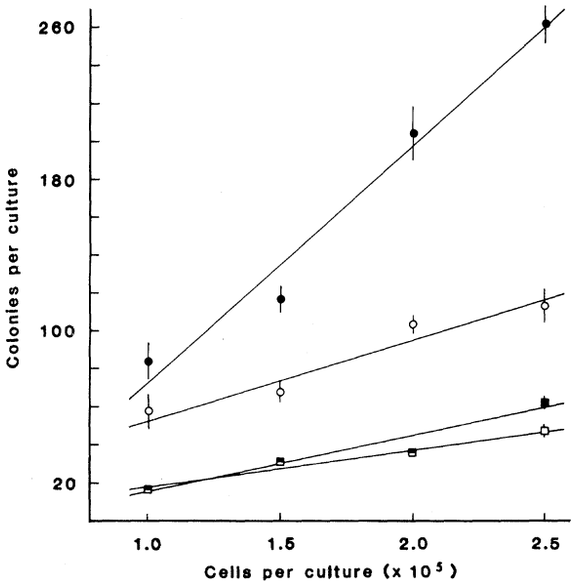


Fig. 3. Colony formation by CSF-1–stimulated femoral marrow cells incubated with (closed symbols) or without (open symbols) antiserum to IFN's α and β in 1:1000 dilutions. Values shown represent the mean ± 1 S.E. of triplicate cultures. Nucleated marrow cells were added to warm (41°C) RPMI 1640 medium containing 15 percent fetal bovine serum, antibiotics, and 0.3 percent agar (Bacto agar; Difco) and added to 35-mm plastic petri dishes containing 640 units of CSF-1 in complete medium. Antiserum to IFN's α and β was incorporated in the semisolid agar mixture prior to the addition of cells. The final volume per plate was 1.0 ml. After the cultures hardened, the plates were incubated for 7 days at 37°C in an atmosphere of 5 percent CO_2 in air. Colonies were enumerated microscopically at a magnification of ×37 as clearly discernible proliferative centers containing at least 25 cells. (○, ●) Total cells; (□, ■) nonadherent cells. Nonadherent cells were prepared by incubating marrow cells in 25-ml culture volumes at 1×10^7 cells per milliliter in RPMI 1640 medium containing 15 percent fetal bovine serum and antibiotics in 75-cm^2 tissue culture flasks. After 2 hours of incubation, nonadherent cells were harvested by vigorous washing of the flasks, and the cell concentrations were adjusted for addition to warm semisolid agar medium. Colony formation by nonadherent cell cultures was determined as described above.

tion. The transfer was performed by flushing the cultures repeatedly with a multichannel pipettor to dislodge nonadherent cells, including committed precursors; thus the transferred cells as new cultures were depleted of adherent cells (including macrophages and monocytes). After 72 hours of incubation these adherent cell–depleted cultures were assayed for IFN production and [^{3}H]thymidine incorporation. As shown in Fig. 2B, the presence of antiserum to IFN's α and β had no significant effect on [^{3}H]thymidine incorporation in these cultures, regardless of the cell concentration. In addition, detectable levels of IFN did not exceed 2 units in any of these cultures. On the basis of these experiments we conclude that CSF-1-induced proliferation of committed precursors, as measured by [^{3}H]thymidine incorporation, is limited by endogenous production of IFN, which requires the presence of adherent cells, presumably macrophages. In subsequent experiments, adherent marrow cells stimulated with CSF produced IFN in titers similar to those we have described, whereas IFN production has not been detected in any cultures depleted of adherent cells (data not shown).

Although we have previously shown that CSF-induced [^{3}H]thymidine incorporation is a reliable indicator of committed macrophage precursor proliferation under these conditions (*15*), the basic experiment was repeated with the classical assay for cloning of committed precursors in semisolid agar. Soft agar cultures (1 ml) containing increasing concentrations of nucleated marrow cells were incubated at 37°C for 7 days with CSF-1 (640 unit/ml), with or without a 1:1000 dilution of added antiserum to IFN's α and β. Colony formation was significantly enhanced ($P \leq 0.05$) by the addition of antiserum to IFN's α and β at all cell concentrations tested (Fig. 3). Again the enhancement afforded by the antiserum was dependent on cell concentration and ranged from approximately 30 percent at 10^5 cells to 160 percent at 2.5×10^5 cells per culture. The antiserum to IFN's α and β did not stimulate colony formation in the absence of CSF, and all unstimulated cultures contained fewer than ten colonies. Depletion of adherent cells by a 2-hour incubation of the marrow cells before initiating the cultures resulted in a diminution of the enhancing effect of antiserum to IFN's α and β. No enhancement of colony formation was observed at concentrations less than 2.5×10^5 cells per culture. A slight enhancement was observed only at the highest cell concentration.

Since the original proposal of Kurland and his co-workers (*5, 9–11*) that production of series E prostaglandins by CSF-induced macrophages has a negative regulatory role, evidence has been presented that macrophages within the marrow can act as suppressors of the myelopoietic response (*17–19*). Our results confirm the presence of an adherent regulatory cell that functions as a negative feedback inhibitor in a manner similar to that described by Kurland *et al.*; that is, exposure to a macrophage-active CSF results in the production of an inhibitor of CSF-induced proliferation of committed macrophage precursors. Proliferation of the committed precursors should then be determined by the balance of CSF and CSF-induced inhibitor (IFN) within the local environment of the committed precursors. Although the nature of this adherent cell remains unknown, circumstantial evidence other than its adherent nature suggests that it belongs to the mononuclear phagocyte system. First, macrophages exposed to CSF-1 produce small amounts of IFN (*4, 7*) in addition to series E prostaglandins, and second, the response is initiated after exposure to a macrophage-active CSF. The involvement of series E prostaglandins was not considered in our experiment; however, it will be important to determine whether both regulatory systems are operative and, if so, to determine if they act independently or in concert with each other and to determine the relative contributions of series E prostaglandins and IFN in regulation of CSF-induced macrophage proliferation.

ROBERT N. MOORE*
HAL S. LARSEN
DAVID W. HOROHOV
BARRY T. ROUSE

Department of Microbiology,
University of Tennessee,
Knoxville 37996

References and Notes

1. C. C. Stewart and H. S. Lin, *J. Reticuloendothel. Soc.* **23**, 269 (1978).
2. E. R. Stanley and L. J. Guilbert, *J. Immunol. Methods* **42**, 253 (1981).
3. L. J. Guilbert and E. R. Stanley, *J. Cell Biol.* **85**, 153 (1980).
4. R. N. Moore, J. T. Hoffeld, J. J. Farrar, S. E. Mergenhagen, J. J. Oppenheim, R. K. Shadduck, in *Lymphokines*, E. Pick, Ed. (Academic Press, New York, 1981), vol. 3, pp. 119–148.
5. J. I. Kurland and R. Bockman, *J. Exp. Med.* **147**, 952 (1978).
6. R. N. Moore, J. J. Oppenheim, J. J. Farrar, C. S. Carter, Jr., A. Waheed, R. K. Shadduck, *J. Immunol.* **125**, 1302 (1980).
7. R. N. Moore, P. S. Steeg, S. E. Mergenhagen, *Adv. Exp. Med. Biol.* **162**, 121 (1983).
8. A. W. Burgess, D. Metcalf, S. M. Watt, *J. Supramol. Struct.* **8**, 489 (1978).
9. J. I. Kurland, R. S. Bockman, H. E. Broxmeyer, M. A. S. Moore, *Science* **199**, 552 (1978).
10. J. I. Kurland, H. E. Broxmeyer, L. M. Pelus, R. S. Bockman, M. A. S. Moore, *Blood* **52**, 388 (1978).
11. L. M. Pelus, H. E. Broxmeyer, J. I. Kurland, M. A. S. Moore, *J. Exp. Med.* **150**, 277 (1979).
12. E. V. Hull, H. Schellekens, B. Lowenberg, M. J. DeVries, *Cancer Res.* **38**, 911 (1978).

13. G. R. Klimpel, W. R. Fleischman, K. D. Klimpel, *J. Immunol.* **129**, 176 (1982).
14. H. E. Broxmeyer, L. Lu, E. Platzer, C. Feit, L. Juliano, B. Y. Rubin, *ibid.* **131**, 1300 (1983).
15. R. N. Moore and B. T. Rouse, *ibid.*, p. 2374.
16. A. Waheed and R. K. Shadduck, *J. Lab. Clin. Med.* **94**, 180 (1979). One unit of CSF is that amount required for the production of one colony.
17. G. Spitzer, D. S. Verma, M. Beran, A. R. Zander, K. A. Dicke, K. B. McCredie, *Blood* **57**, 71 (1981).
18. G. Spitzer, D. S. Verma, M. Beran, A. R. Zander, K. B. McCredie, K. A. Dicke, *J. Natl. Cancer Inst.* **68**, 35 (1982).
19. H. E. Broxmeyer, J. Bognacki, P. Ralph, M. H. Dorner, L. Lu, H. Castro-Malaspina, *Blood* **60**, 595 (1982).
20. N. B. Finter, *J. Gen. Virol.* **5**, 419 (1969).
21. Supported by NIH grants AI-18960 and AI-14981.
* To whom correspondence should be addressed.

26 September 1983; accepted 17 October 1983

Spectrographic Representation of Globular Protein Breathing Motions

Abstract. *Two useful ways of describing the frequency composition of the breathing motions of globular proteins are the spectrogram and three-dimensional power spectrum, representations similar to those frequently used in speech analysis. In this report "low-frequency" vibrations of globular proteins, corresponding to the collective oscillations of atoms from many different residues, are considered. Radii of gyration fluctuations provide a sensitive way to characterize such concerted motions.*

A complete description of a globular protein requires not only a static three-dimensional x-ray structure but also an understanding of its flexibility and the role that structural fluctuations play in the protein's function. Globular proteins in solution exhibit a large variety of motions (*1, 2*). Evidence from a number of different experiments suggests that a globular protein is a fluid, dynamic structure involving rapid conformational fluctuations that allow relatively easy access of interior groups to various molecular probes (*3*). It has been hypothesized that the motions of globular proteins play an essential role in their function and may affect important processes such as binding of ligands, enzyme catalysis, hemoglobin cooperativity, immunoglobin action, electron transfer, and the assembly of supramolecular structures like viruses (*2, 4*).

This report addresses the "low-frequency" vibrations of globular proteins (*2, 5, 6*) that correspond to the collective oscillations of atoms from many different residues. In previous studies these breathing motions were investigated by representing the globular protein as a vibrating sphere of homogeneous elastic material (*5, 7*). In the research described here, protein breathing was investigated by determining the radius of gyration of 650 consecutive 0.147-psec atomic coordinate frames from the dynamic model of bovine pancreatic trypsin inhibitor (BPTI). The fluctuations in the equilibrium structure of BPTI have been examined in several molecular dynamics simulations (*4, 8, 9*). BPTI, a small globular protein (molecular weight, 6500), consists of one polypeptide chain with 58 amino acids and three disulfide bonds. The radius of gyration of BPTI, derived from atomic coordinates, was calculated from the relation

$$R_g = \sqrt{\frac{\Sigma Z_i a_i^2}{\Sigma Z_i}}$$

where Z_i is the atomic number for atom i increased by the number of attached hydrogens, the sums are over all atoms; R_g is the radius of gyration calculated about the electronic center of gravity for BPTI's dynamic structure; and a_i is the distance of atom i from the electronic center of gravity.

A useful way of describing the frequency composition of the R_g values through time is the three-dimensional power spectrum and spectrogram. In 1947 the search for an effective way to display and examine speech wave forms in natural speech led to the development of the sound spectrograph at Bell Laboratories. This spectrograph, which is in common use today, displays a trivariate representation of speech energy with abscissal time and ordinal frequency; relative intensity is indicated by darkness on the graph. In the present research a system for the computation of digital spectrograms and topographic spectral distribution functions of protein breathing motions was developed (*10*). In the topographic power spectrum (amplitude versus frequency versus time) shown in this report, hillocks are indicative of prominent periodicities in the concerted radial motions within the protein.

A three-dimensional power spectrum computed for the R_g fluctuations (Fig. 1) indicates most of the frequency power below 1 psec with a particularly prominent breathing mode centered at 3 psec. Higher frequencies (25 to 113 cm^{-1}) are evident to a lesser degree. The high ridge close to zero frequency may correspond to a slower radial oscillation or an "infrequent process" (*1*) that is not being observed long enough for adequate characterization in this conventional molecular dynamics simulation. Longer simulations would be required to determine the significance of such a slower oscillation.

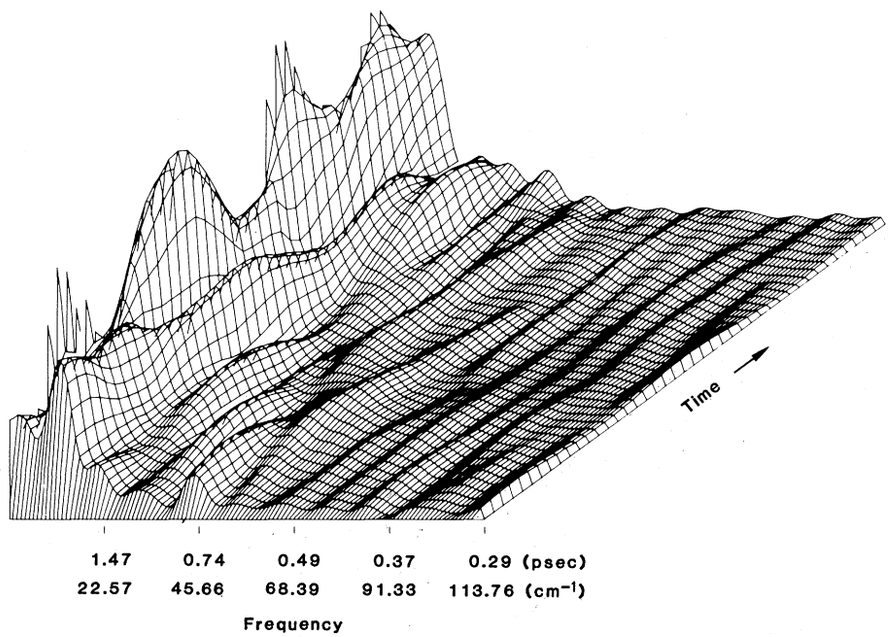


Fig. 1. Topographic spectral distribution function (power spectrum) calculated for the fluctuations in the radius of gyration of BPTI through time (96 psec of data are represented).