Proc. Natl. Acad. Sci. USA
Vol. 78, No. 5, pp. 2707–2711, May 1981
Biochemistry

Genetic assignment of resonances in the NMR spectrum of a protein: *lac* repressor

(gene regulation/^{19}F/3-fluorotyrosine/nonsense suppression)

MARY ANN C. JAREMA*, PONZY LU*†, AND JEFFREY H. MILLER‡

*Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104; and ‡Département de Biologie Moléculaire, Université de Genève, Geneva, Switzerland

Communicated by Howard K. Schachman, January 15, 1981

ABSTRACT **By using a systematic genetic approach, the resonances in the ^{19}F NMR spectrum of 3-fluorotyrosine-substituted *lac* repressor protein have been assigned. The NMR data indicate that each monomer of the repressor consists of two distinct and independent domains. One domain, the NH_2-terminal sixth of the primary sequence, which has been shown to be very important for DNA binding, is very mobile. The remaining COOH-terminal sequence is more rigid. Ligands of the repressor, which affect its DNA binding capability, lead to conformational changes in the COOH-terminal domain. The approach to the assignment of spectral features taken here can be extended to other systems.**

The *lac* repressor of *Escherichia coli* is the ideal system for an examination of the molecular mechanism of gene regulation (1–3). The entire DNA sequence for the *lacI* gene, which codes for the repressor, and the amino acid sequence of this protein have been independently determined (4, 5). It is a tetrameric protein, total molecular weight 154,520, with 360 amino acids in the subunit without either bound metal ions or cofactors (2). In our genetic analysis of this protein, we have been able to generate 90 nonsense mutations and in most cases determine their position in the 360 codons in the *lacI* gene (6–8). Because there are a number of nonsense suppressors that can insert different amino acids in response to the chain-terminating nonsense codons (UAG, UAA, or UGA) (9), we have been able to use this collection to look at the *in vivo* properties of over 300 different repressors each with a single amino acid substitution (7). Among these 90 mutations we have characterized nonsense mutations at the positions corresponding to all of the eight tyrosines and both tryptophans in the subunit.

With the ability to substitute other amino acids for tyrosine or tryptophan at specific positions in the sequence through nonsense suppression, it is possible to introduce specific alterations in the corresponding fluorescence (10) or NMR (11) spectra of altered *lac* repressors. In the latter case we simply note which resonance or resonances are missing in the NMR spectra of *lac* repressors isolated from strains containing suppressed nonsense mutations at each tyrosine position. We use this approach here for the assignment of the resonances from the eight tyrosines in the ^{19}F NMR spectrum of the substituted *lac* repressor.

We chose 3-fluorotyrosine to introduce ^{19}F as a nuclear spin probe and to simplify the NMR spectrum. The ^{19}F NMR spectrum shows only a single resonance for each of the eight tyrosines. This substituted *lac* repressor binds to inducer and operator DNA and releases the operator DNA upon binding the *lac* operon inducer (12) in a manner almost identical to repressor with normal tyrosines (13). The advantages of this amino acid analogue and ^{19}F NMR have been described and utilized in several systems (14–21).

Table 1. Effect of amino acid substitution at tyrosine positions of *lac* repressor (from ref. 7)

Wild-type amino acid	Amino acid substitution*			
	Su-1 (Ser)	*Su-2* (Gln)	*Su-3* (Tyr)	*Su-6* (Leu)
Tyrosine-7	∓	∓	+	∓
Tyrosine-12	+	+	+	+
Tyrosine-17	−	−	+	∓
Tyrosine-47	−	−	+	−
Tyrosine-126	+	+	+	+
Tyrosine-204†	+	+	+	+
Tyrosine-273†	i^s	$i^{s/r}$	+	+
Tyrosine-282†	−	−	+	+

* Effects are: +, normal repressor activity; −, β-galactosidase constitutive, ∓, β-galactosidase partially inducible; i^s, β-galactosidase not inducible; $i^{s/r}$, isopropyl β-D-thiogalactoside required for repression.
† These are the actual positions of these three tyrosine residues (4, 5). Residues 204, 273, and 282 were previously, incorrectly, labeled 193, 260, and 269, respectively. All references to earlier work have included this correction.

The peak assignment strategy that we use here is not limited to tyrosine or to the fluorine nucleus. It also allows the insertion of a nuclear spin label, using suppression, at any of the 90 amino acids positions where we have nonsense mutations in the *lacI* gene (11). Furthermore, any gene product existing or inserted into *E. coli* is amenable to the approach that we have taken with *lac* repressor.

The properties of *lac* repressors with substitutions at the eight tyrosines *in vivo* in several nonsense suppressor strain backgrounds are summarized in Table 1. A variety of properties are represented. Some positions tolerate any substitution and some, none. Glutamine substitution at 273 leads to a reversal of the inducer function; at 282, it leads to loss of quaternary structure (22). The altered repressors used here are isolated from *E. coli* strains carrying the respective altered *lacI* gene and an amber suppressor, *Su-6*, which inserts a leucine in response to the UAG triplet. This particular substitution was chosen because it gave the largest number of functional repressors *in vivo* (Table 1). For all of the spectra shown here, *lac* repressor was isolated from the appropriate strain grown and manipulated to incorporate 3-fluorotyrosine (12).

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

Abbreviations: i^+, i^-, etc., see Table 1; iPrSGal (= IPTG), isopropyl β-D-thiogalactoside; NphFuc (= ONPF), *o*-nitrophenyl D-fucoside.

† To whom reprint requests should be addressed.

MATERIALS AND METHODS

The suppressed derivatives of the strains containing amber mutations at each tyrosine were constructed as follows: the mutations (6, 7) were crossed into the *lac* region of a heat-inducible λ prophage by genetic recombination, and the strains were converted to suppressor-positive by transducing to arginine independence and scoring for the presence of a suppressor by the conversion of the i^- (constitutive) to i^+ (wild type) phenotype. The strains carried an *argE* amber mutation, and were transduced with lysates prepared on an $argE^-$, $Su\text{-}6^+$ strain.

DL-3-Fluorotyrosine was synthesized from 3-fluoro-*p*-anisaldehyde (Aldrich), and characterized as in ref. 12.

The repressors were isolated as described in refs. 10 and 12. Tetrameric cores were isolated according to the procedure described in ref. 22, except the digestion buffer was 1 M Tris·HCl/30% (vol/vol) glycerol/0.01 M 2-mercaptoethanol, pH 7.6. The NMR spectra were obtained on a Nicolet NT 150 spectrometer operating at 141.1765 MHz at 25°C, using a 90° pulse and a 20-mm-diameter sample tube. The resonances were not significantly narrowed when the protons were decoupled, thus all of the spectra here are undecoupled. The Fourier transforms were made on 15,000–25,000 transients with 8000 data points and a 5000-Hz sweepwidth with an acquisition time of 0.4 sec. The chemical shifts were measured relative to an external standard of 80 mM trifluoroacetic acid in 25% (vol/vol) ^2H_2O. Protein concentrations in all cases were between 10 and 40 μM in tetramers (1.3–5.3 mg/ml) in a solution of 0.25 M Tris·HCl/0.2 M KCl/3 mM dithiothreitol/0.1 mM EDTA in 25% ^2H_2O (pH is that of the reading from the meter at 25°C). The recycle time was 0.5 sec except where indicated. Although the longest longitudinal relaxation time was 0.4 sec, no significant differences in relative intensities were observed with recycle times as long as 3 sec.

RESULTS

Resonances of NH_2-Terminal and Core Tyrosines. Geisler and Weber (23) demonstrated that *lac* repressor can be selectively cleaved between amino acids 50 and 60, depending on the protease used. The digestion yields, per tetramer: (*i*) four NH_2-terminal headpieces with DNA binding activity, including tyrosines 7, 12, 17, and 47; and (*ii*) a tetrameric core with the inducer binding site, including tyrosines 126, 204, 273, and 282 (4, 5, 23, 24). The same occurs in the case of the 3-fluorotyrosine-substituted form of the *lac* repressor.

In Fig. 1 we have superimposed the ^{19}F NMR spectrum of the core on the spectrum of the intact repressor in the absence (Fig. 1*A*) and presence (Fig. 1*B*) of the gratuitous inducer (1) isopropyl β-D-thiogalactoside (iPrSGal), respectively. Consistent with the biochemical observation that the iPrSGal binding site is in the tetrameric core (23), the ^{19}F NMR spectrum of the core shows the major change upon binding of iPrSGal: the loss of intensity at 60.2 ppm and the appearance of a resonance at 58.2 ppm. The core spectra are superimposable on the broad resonances of the intact repressor in both cases, an observation that makes the description of the peak assignments in the subsequent discussion simpler but is not a requirement in the approach taken here. The identification of the resonances with particular tyrosine residues in Fig. 1 is derived from the data shown in Fig. 2.

Resonances from Tyrosines 12 and 126. The altered repressors having tyrosine residues 12 or 126 replaced by leucine give the spectra (thick lines) shown in Fig. 2 *C* and *F*, respectively. It can be seen, from the comparison with the wild-type spectrum, which resonance is removed by the substitution involved (indicated by the arrow). The conditions, pH or presence of ligands, were chosen to provide the clearest difference in the spectral regions of interest. iPrSGal was used for the set of spectra shown in Fig. 2*F* for the assignment of tyrosine-126 because it allows a better display of the very broad resonances due to the core tyrosines.

Resonances from Tyrosines 7, 17, and 47. The altered repressor with a leucine for tyrosine substitution at position 7 gives the spectrum (thick line) shown in Fig. 2*B*. The arrow in the figure indicates the position of missing intensity at 61.0 ppm. A comparison of the spectrum from the altered repressor with tyrosine-17 substituted with the spectrum from the repressor with tyrosine-7 substituted is shown in Fig. 2*D*. These

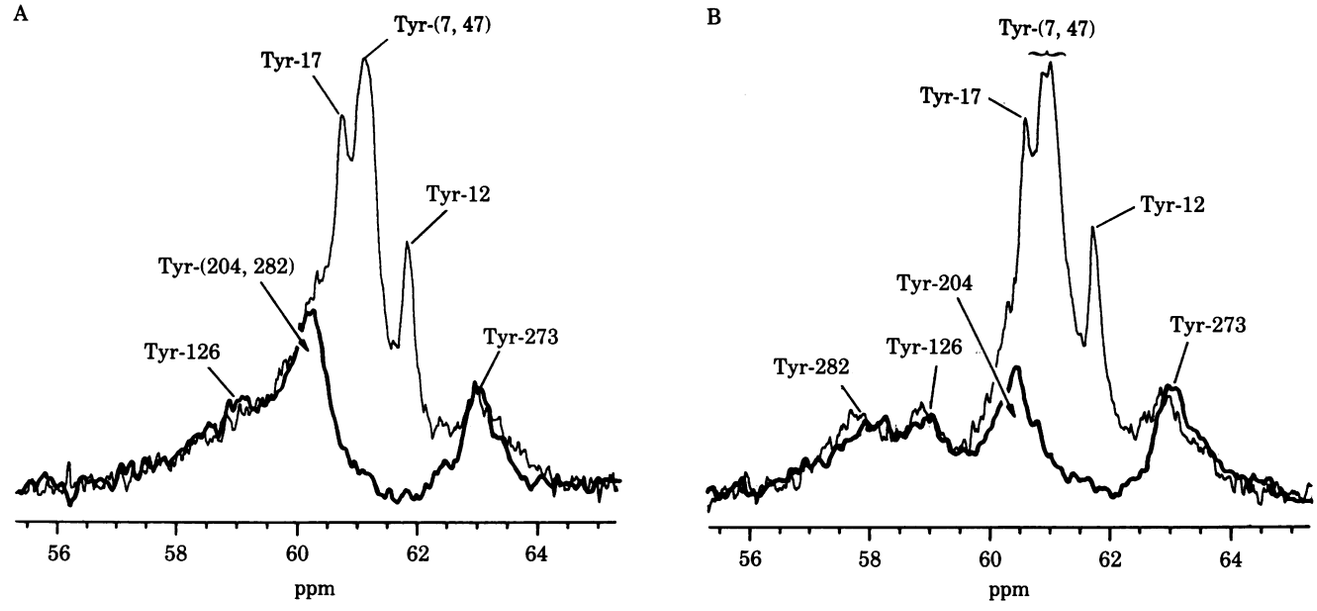

FIG. 1. ^{19}F NMR spectra of the wild-type *lac* repressor (thin lines) compared with the spectra of the tetrameric core derived by trypsin digestion (thick lines), both at pH 7.4. (*A*) No iPrSGal (IPTG); (*B*) 0.2 mM iPrSGal present with both core and intact repressor. The peak assignments are described in the text.

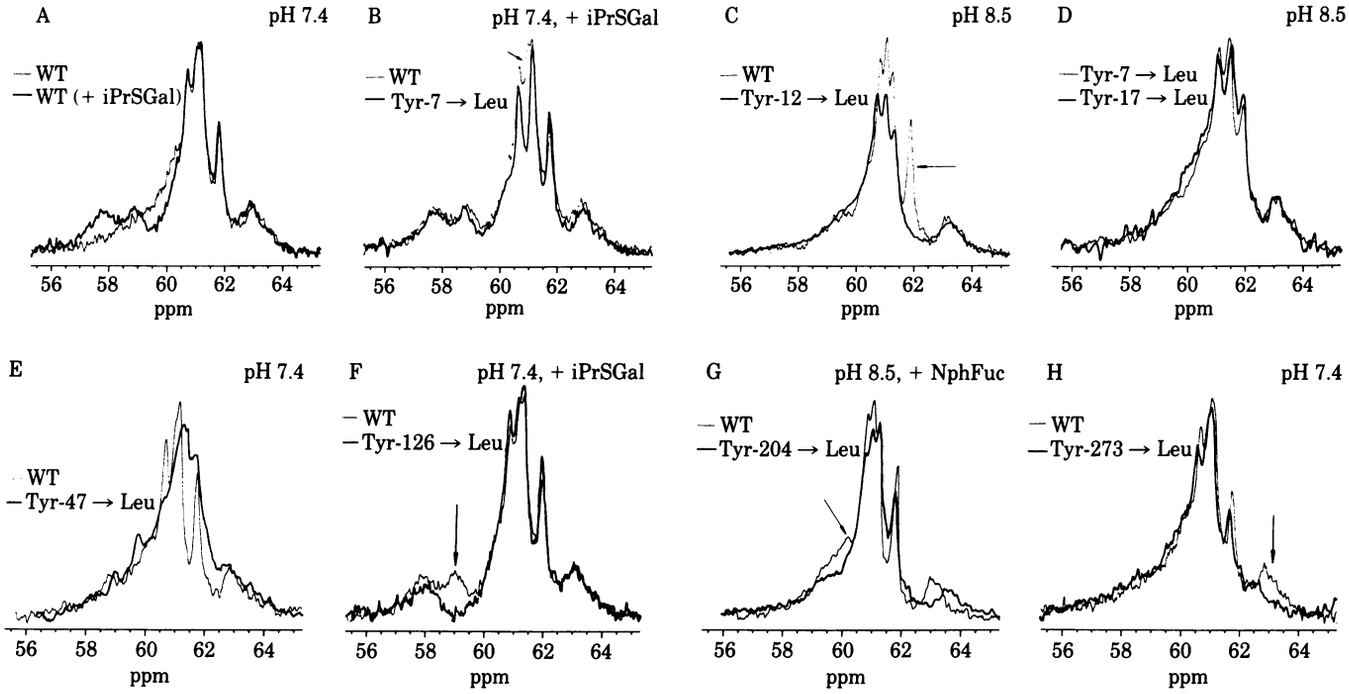

FIG. 2. Comparison of the spectra from the collection of altered repressors with the spectrum from wild-type (WT) repressor. The arrows indicate the positions where resonances are lost due to leucine substitution for tyrosine. The conditions for both spectra in each panel along with the species of altered repressor are indicated. Spectral parameters are those given in the text except for *C*, *D*, *E*, and *G*, for which a 3-sec recycle time was used. The repressors were isolated by the procedures of refs. 10 and 12 with the addition of a DEAE-Sephadex A-50 column as the last step. For *G* the *o*-nitrophenyl D-fucoside (NphFuc; = ONPF) concentration was 1 mM.

two spectra are quite similar, with the missing intensity at 60.7 ppm. Because this feature is present in Fig. 2*B*, we assign it to tyrosine-17. This leaves the resonance from tyrosine-47 to the center at 61.0 ppm. The importance of this residue to the structure of the protein is seen in Fig. 2*E*, in which denatured material is evident. The tyrosine-17 resonance at 60.7 ppm is visible as a shoulder. This assignment of the four NH_2-terminal tyrosine resonances has been confirmed by looking at the spectra of the four altered NH_2-terminal headpieces.

A general observation is that the altered *lac* repressors that give wild-type properties *in vivo* also result in repressor proteins that seem more stable under the conditions used in these experiments. Leucine substitutions at tyrosines 17 and 47 result in loss of operator binding *in vivo*, with tyrosine-7 showing a less pronounced difference (Table 1). The spectra for the repressors altered at tyrosines 17 and 47 show broader resonances along with detectable chemical shifts in the center of the spectrum. Substitution at tyrosine-7, which gives a partially wild-type phenotype *in vivo*, results in a spectrum that is superimposable on the wild-type spectrum with the exception of the missing resonance (Fig. 2*B*).

Resonance from Tyrosine-204. When the spectrum from the altered repressor with a substitution at position 204 is compared with that of the wild type, one detects a loss of intensity at the foot, 60.3 ppm, of the resonance that we assign to tyrosine-47 (see Fig. 1). This difference can be enhanced by comparing the wild-type spectrum with this altered repressor spectrum in the presence of *o*-nitrophenyl D-fucoside (NphFuc), an anti-inducer (25) (Fig. 2*G*). We also note loss of relative intensity in the NH_2-terminal resonances as well as a difference at 63.0 ppm, as one would expect if a broad resonance were removed in the case of the repressor altered at tyrosine-204. The loss of intensity at 63.0 ppm is compensated by the broad resonance at 63.5 ppm. We are seeing a change in the environment of the tyrosine corresponding to the 63.0 ppm peak due to the leucine substitution for tyrosine at position 204. This assignment is also confirmed by changes in the spectrum of the core alone, upon the addition of NphFuc.

Resonances from Tyrosines 273 and 282. A comparison of the spectrum of the altered repressor with a substitution at position 273 with the wild type is shown in Fig. 2*H*. The only difference is the broad resonance centered at 63.0 ppm. The fact that this resonance was shifted upfield by substitution at position 204 also assures us that it is indeed due to the native protein and not an artifact of aggregated or denatured protein.

At this point the remaining resonance at 57.7 ppm in the presence of iPrSGal and at 60.0 ppm in the absence of iPrSGal must be tyrosine-282. The resonance at 57.7 ppm occurs in the repressors with substitutions at positions 126, 204, or 273 when iPrSGal is present. In the absence of iPrSGal it is at the same position as the resonance due to tyrosine-204, because there is greater intensity in this region. Furthermore, we see the simultaneous loss of intensity at 60.0 ppm with a gain in intensity at 57.7 ppm when the tryptic core is titrated with iPrSGal.

DISCUSSION

Implications of the Data. The most striking feature of the spectra, as illustrated by Fig. 1, is the fact that the tetrameric core spectra are superimposable on the spectra of the corresponding intact repressor. The converse of this result has been observed in the proton NMR spectrum of the NH_2-terminal headpiece by others (26, 27)—i.e., the aromatic region of the spectrum contains most of the narrow lines in the ^1H NMR spectrum of the intact repressor. The corresponding ^{19}F NMR spectrum of the NH_2-terminal headpiece is superimposable on the narrow resonances in the center of the ^{19}F NMR spectrum of the intact repressor, a result anticipated from the ^1H NMR observations. These observations suggest that very little change occurs in the tyrosine environments or relative mobilities upon

the removal of the NH_2-terminal 59 amino acids by proteolytic digestion of the *lac* repressor.

Proteins substituted with 3-fluorotyrosine are expected to exhibit a nuclear Overhauser enhancement of almost -1 in the fluorine resonances upon broad-band irradiation of the protons, because the overall tumbling motion of the macromolecule is too slow for the irradiation energy to be dissipated into the solvent during the time scale of the observation (14, 16). We have published a dynamic NMR analysis of the fluorine resonances upon proton irradiation in 3-fluorotyrosine-substituted *lac* repressor (28). The narrow resonances, which we now know correspond to the NH_2-terminal tyrosines, all showed nuclear Overhauser enhancements of -0.5 to -0.7, whereas the broad resonances, those from the core tyrosines, gave the expected values of close to -1. Note that even in a protein as small as the gene 5 product of fd phage, which has a single polypeptide chain of only 87 amino acids, the fluorines in the substituted form exhibit nuclear Overhauser enhancements of nearly -1 (29). Thus, the NH_2-terminal tyrosines in the *lac* repressor are in a very mobile portion of the molecule. The *lac* repressor is a protein with two domains: one part, the tetrameric core, is rigid, and the four headpieces are very flexible.

The denaturation of the *lac* repressor results in a single resonance at 61.5 ppm (12), the same chemical shift as free 3-fluorotyrosine. This corresponds to a position just between the peaks assigned to tyrosines 7/47 and 12. Thus, a consistent picture is formed that the tyrosines having the most mobility also have chemical shifts nearest the completely solvated free fluorotyrosine resonance position. This explains why both iodination (30) and nitration (31) of tyrosines result mainly in modification of the NH_2-terminal headpiece tyrosines, and perhaps why one can cleave away the NH_2-terminus. A comparison of the spectra from repressors missing tyrosine at positions 7 or 12 with those of repressors missing tyrosine at positions 17 or 47 suggests that the removal of one of the latter pair of tyrosines disrupts the *lac* repressor structure much more than the first two. This does not seem to be completely consistent with a proposed model for the NH_2-terminal fragment in which tyrosines 7, 12, and 17 must be a stacked array (32).

The broad resonance due to tyrosine-273 at 63.0 ppm is at the same position as the resonance from free 3-fluorotyrosine at high pH, at which the phenolic proton dissociates (12). This suggests that tyrosine-273 either is ionized in the protein or is participating in a hydrogen bond and is broadened due to immobilization. This is the residue that makes the repressor i^s (inducer insensitive) or i^r (inducer enhances binding to operator DNA) when it is replaced by serine or glutamine (Table 1). It is also tyrosine-273 that shifts further upfield in response to a leucine substitution at tyrosine-204, a residue also involved in inducer binding.

From nitration experiments with the core of wild-type *lac* repressor, Alexander *et al.* (31) have suggested that iPrSGal protects tyrosine-204 from nitration whereas NphFuc does not. In our data we see a slight downfield shift of tyrosine-204's resonance due to NphFuc but not due to iPrSGal. Because iPrSGal and NphFuc compete in their binding for *lac* repressor (33), it is likely that tyrosine-204 is affected by both ligands, but not identically, and thus the nitration difference. Binding of inducer (iPrSGal) results also in a large downfield shift of the tyrosine-282 resonance. Differences in the effect of inducer and anti-inducer on *lac* repressor conformation have been detected in a variety of spectroscopic ways by others (10, 34–38), and have been localized to the tetrameric core of the repressor (37, 39). The observations here establish that tyrosines 204 and 282 are involved.

Our spectra with oligo[d(A-T)] double helical fragments and *lac* operator DNA show significant broadening of the NH_2-terminal resonances. Because the binding of inducer reduces the affinity of the repressor for operator DNA, and the NH_2 terminus is implicated in the DNA binding function of the protein (24, 34), one might expect to see large changes in the NH_2-terminal tyrosine environments upon iPrSGal binding. The only change that we see reproducibly is the very slight resolution of the tyrosine-7/47 resonance into two (see Fig. 1*B*). NphFuc does not give this effect. This is consistent with Ogata and Gilbert's conclusion that the addition of inducer results in small changes in many points of the repressor molecule rather than a few major changes in the NH_2 terminus (40, 41).

Applications of the Peak Assignment Strategy to Other Systems. The difficulty of correlating NMR spectral features with specific atoms in the primary structure of a protein or a polynucleotide has limited the application of the full potential of NMR spectroscopy to molecules that are relatively small, such as lysozyme (monomer of 129 residues), (42–44) or bovine trypsin inhibitor (monomer of 67 residues) (45–47). In all cases a diverse repertoire of chemical modification methods along with the known x-ray structure were employed to make educated guesses as to the correct assignments (48–51). Even in propitious cases, considerable difficulties have been encountered in making peak assignments in the NMR spectrum where, for example, only four histidines (52–56) or six tryptophans were involved (57). Using the *lac* repressor, we show that peak assignment, in cases in which relatively large numbers of identical amino acid residues are involved, can be approached genetically.

The use of variants of a protein for the purpose of peak assignment has been made in several cases by taking advantage of natural variations. This has been done for hemoglobin (58–60), staphylococcal nuclease (61), pancreatic ribonuclease (62), and cytochromes (63–68). What we have done here is to show that it is possible to seek out and use variants in a systematic and complete way to make specific changes at the molecular level. The strategy taken here is quite applicable to other systems. One is not limited to the fluorine nucleus, to tyrosine, or to prokaryotic macromolecules. Once one can resolve the resonances, if necessary via isotopic enrichment or negative labeling, all one needs is a scheme to generate and order specific mutations in the gene for the translational or transcriptional product of interest. We have taken a classical approach here, utilizing specific mutagenesis and an extensive deletion mapping system (20). However, given the possibility of site-directed mutagenesis on specific DNA restriction endonuclease fragments of an isolated gene (69), coupled with the flexibility of modern cloning vehicles and associated technology (70), any gene product of interest is amenable to the peak assignment scheme demonstrated here.

We thank Betty Jarema for tireless technical assistance, Hans-Richard Rackwitz for suggestions on fluorotyrosine synthesis, and Stan Opella for helpful comments on the manuscript. This work was supported by grants from the American Cancer Society and National Institutes of Health, including a Career Award, to P.L. and by a grant from the Swiss National Fund to J.H.M.

1. Jacob, F. & Monod, J. (1961) *J. Mol. Biol.* **3,** 318–356.
2. Bourgeois, S. & Pfahl, M. (1976) *Adv. Protein Chem.* **30,** 1–99.
3. Miller, J. H. & Reznikoff, W. (1978) *The Operon* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
4. Farabaugh, P. J. (1978) *Nature (London)* **274,** 765–769.
5. Beyreuther, K. (1978) *Nature (London)* **274,** 767.
6. Miller, J. H., Ganem, D., Lu, P. & Schmitz, A. (1977) *J. Mol. Biol.* **109,** 275–301.
7. Miller, J. H., Coulondre, C., Hofer, M., Schmeissner, U., Sommer, H. Schmitz, A. & Lu, P. (1979) *J. Mol. Biol.* **131,** 191–222.
8. Miller, J. H., Coulondre, C. & Farabaugh, P. J. (1978) *Nature (London)* **274,** 770–775.

9. Gorini, L. (1970) *Annu. Rev. Genet.* **4,** 104–134.
10. Sommer, H., Miller, J. H. & Lu, P. (1976) *J. Biol. Chem.* **251,** 3774–3779.
11. Lu, P., Jarema, M. C., Rackwitz, H.-R., Friedman, R. L. & Miller, J. H. (1979) in *NMR and Biochemistry*, eds. Opella, S. J. & Lu, P. (Dekker, New York), pp. 59–66.
12. Lu, P., Jarema, M. C., Mosser, K. & Daniel, W. E. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3471–3475.
13. Gilbert, W. & Müller-Hill, B. (1967) *Proc. Natl. Acad. Sci. USA* **58,** 2415–2421.
14. Gerig, J. T. (1978) in *Biological Magnetic Resonance*, eds. Berliner, L. J. & Reuben, J. (Plenum, New York), Vol. 1, pp. 139–204.
15. Sykes, B. D. & Hull, W. E. (1978) *Methods Enzymol.* **49,** 270–295.
16. Sykes, B. D., Weingarten, H. J. & Schlesinger, M. J. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 469–473.
17. Anderson, R. A., Nakashima, Y. & Coleman, J. E. (1975) *Biochemistry* **14,** 907–917.
18. Hagen, D. S., Weiner, J. H. & Sykes, B. D. (1978) *Biochemistry* **17,** 3860–3866.
19. Hagen, D. S., Weiner, J. H. & Sykes, B. D. (1979) *Biochemistry* **18,** 2007–2012.
20. Kimber, B. J., Griffiths, D. V., Birdsall, B., King, R. W., Scudder, P., Feeney, J., Roberts, G. C. K. & Burgen, A. S. V. (1977) *Biochemistry* **16,** 3492–3500.
21. Robertson, D. E., Kroon, P. A. & Ho, C. (1977) *Biochemistry* **16,** 1443–1451.
22. Schmitz, A., Schmeissner, U., Miller, J. H. & Lu, P. (1976) *J. Biol. Chem.* **251,** 3359–3366.
23. Geisler, N. & Weber, K. (1977) *Biochemistry* **16,** 938–943.
24. Jovin, T., Geisler, N. & Weber, K. (1977) *Nature (London)* **269,** 668–672.
25. Jayaraman, K., Müller-Hill, B. & Rickenberg, H. V. (1966) *J. Mol. Biol.* **18,** 339–343.
26. Buck, F., Rüterjans, H. & Beyreuther, K. (1978) *FEBS Lett.* **96,** 335–338.
27. Wade-Jardetzky, N., Bray, R. P., Conover, W. W., Jardetzky, O., Geisler, N. & Weber, K. (1979) *J. Mol. Biol.* **128,** 259–264.
28. Opella, S. J., Friedman, R. A., Jarema, M. C. & Lu, P. (1979) *J. Magn. Reson.* **36,** 81–88.
29. Coleman, J. E. & Armitage, J. M. (1978) *Biochemistry* **17,** 5038–5045.
30. Fanning, T. C. (1975) *Biochemistry* **14,** 2512–2520.
31. Alexander, M. E., Burgum, R. A., Shaw, M. D. & Matthews, K. S. (1977) *Biochim. Biophys. Acta* **493,** 367–379.
32. Chandrasekaran, R., Jardetzky, T. S. & Jardetzky, O. (1979) *FEBS Lett.* **101,** 11–14.
33. Gilbert, W. & Müller-Hill (1966) *Proc. Natl. Acad. Sci. USA* **56,** 1891–1898.
34. Laiken, S. L., Gross, C. A. & von Hippel, P. H. (1972) *J. Mol. Biol.* **66,** 143–155.
35. Ohshima, Y., Matsura, M. & Horiuchi, T. (1972) *Biochem. Biophys. Res. Commun.* **47,** 1444–1450.
36. Matthews, K. S., Matthews, H. R., Thielmann, H. W. & Jardetzky, O. (1973) *Biochim. Biophys. Acta* **295,** 159–165.
37. Maurizot, J.-C. & Charlier, M. (1977) *Eur. J. Biochem.* **79,** 395–399.
38. Wu, F. Y.-H., Bandyopadhyay, P. & Wu, C.-W. (1976) *J. Mol. Biol.* **100,** 459–472.
39. Matthews, K. S. (1974) *Biochim. Biophys. Acta* **359,** 334–340.
40. Ogata, R. T. & Gilbert, W. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5851–5854.
41. Ogata, R. T. & Gilbert, W. (1979) *J. Mol. Biol.* **132,** 709–728.
42. Dobson, C. M., Ferguson, S. J., Poulsen, F. M. & Williams, R. J. P. (1978) *Eur. J. Biochem.* **92,** 99–103.
43. Cassels, R., Dobson, C. M., Poulsen, F. M. & Williams, R. J. P. (1978) *Eur. J. Biochem.* **92,** 81–97.
44. Poulsen, F. M., Hoch, J. C. & Dobson, C. M. (1980) *Biochemistry* **19,** 2597–2607.
45. Wüthrich, K. & Wagner, G. (1979) *J. Mol. Biol.* **130,** 1–18.
46. Wüthrich, K. & Wagner, G. (1979) *J. Mol. Biol.* **130,** 31–37.
47. Richarz, R., Sehr, P., Wagner, G. & Wüthrich, K. (1979) *J. Mol. Biol.* **130,** 19–30.
48. Campbell, I. D. (1977) in *NMR and Biology*, eds. Dwek, R. A., Campbell, I. D., Richards, R. E. & Williams, R. J. P. (Academic, New York), pp. 33–50.
49. Wüthrich, K., Wagner, G., Richarz, R. & DeMarco, A. (1977) in *NMR and Biology*, eds. Dwek, R. A., Campbell, I. D., Richards, R. E. & Williams, R. J. P. (Academic, New York), pp. 51–62.
50. Dobson, C. M. (1977) in *NMR and Biology*, eds. Dwek, R. A., Campbell, I. D., Richards, R. E. & Williams, R. J. P. (Academic, New York), pp. 63–94.
51. Campbell, I. D., Dobson, C. M. & Williams, R. J. P. (1975) *Proc. R. Soc. London Ser. A.* **345,** 23–40.
52. Markley, J. L. (1975) *Biochemistry* **14,** 3546–3554.
53. Markley, J. L. (1975) *Biochemistry* **14,** 3554–3561.
54. Markley, J. L. & Finkenstadt, W. R. (1975) *Biochemistry* **14,** 3562–3566.
55. Patel, D. J., Cannel, L. I. & Bovey, F. A. (1975) *Biopolymers* **14,** 987–997.
56. Shindo, H., Hayes, M. B. & Cohen, J. S. (1976) *J. Biol. Chem.* **251,** 2644–2647.
57. Lenkinski, R. E., Dallas, J. L. & Glickson, J. D. (1979) *J. Am. Chem. Soc.* **101,** 3071–3077.
58. Fung, L. W.-M. & Ho, C. (1976) *Biochemistry* **14,** 2526–2535.
59. Wiechelman, K. J., Fairbank, V. F. & Ho, C. (1976) *Biochemistry* **15,** 1414–1420.
60. Viggiano, G., Wiechelman, K. J., Chervenick, P. A. & Ho, C. (1978) *Biochemistry* **17,** 795–799.
61. Roberts, G. C. K. & Jardetzky, O. (1970) *Adv. Protein Chem.* **24,** 447–545.
62. Leijenaar-Van denBerg, G., Migchelsen, C. & Beintema, J. J. (1974) *FEBS Lett.* **48,** 218–221.
63. Cohen, J. S. & Hayes, M. B. (1974) *J. Biol. Chem.* **249,** 5472–5477.
64. Dobson, C. M., Moore, G. R. & Williams, R. J. P. (1975) *FEBS Lett.* **51,** 60–65.
65. Oldfield, E., Norton, R. S. & Allerhand, A. (1975) *J. Biol. Chem.* **250,** 6381–6402.
66. Keller, R. M., Piettigreis, G. W. & Wüthrich, K. (1973) *FEBS Lett.* **36,** 151–156.
67. Moore, G. R. & Williams, R. J. P. (1980) *Eur. J. Biochem.* **103,** 533–541.
68. Moore, G. R. & Williams, R. J. P. (1980) *Eur. J. Biochem.* **103,** 543–550.
69. Taniguchi, T. & Weissmann, C. (1978) *J. Mol. Biol.* **118,** 533–565.
70. Wu, R., ed. (1979) *Methods Enzymol.* **68.**