

## Molecular Characterization of the Gene Encoding High-Level Mupirocin Resistance in *Staphylococcus aureus* J2870

J. E. HODGSON,<sup>1\*</sup> S. P. CURNOCK,<sup>2</sup> K. G. H. DYKE,<sup>2</sup> R. MORRIS,<sup>3</sup> D. R. SYLVESTER, AND M. S. GROSS<sup>3</sup>

*Department of Biotechnology, SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth Surrey, RH3 7AJ,<sup>1</sup> and Microbiology Unit, Department of Biochemistry, University of Oxford, Oxford,<sup>2</sup> United Kingdom, and SmithKline Beecham Pharmaceuticals, Biopharmaceuticals R&D, King of Prussia, Pennsylvania, 19406<sup>3</sup>*

Received 22 October 1993/Returned for modification 16 December 1993/Accepted 25 February 1994

**The nucleotide sequence of the *ileS* gene conferring high-level resistance to mupirocin in *Staphylococcus aureus* J2870 has been determined. The gene sequence is substantially different from that of the native *ileS* gene of *S. aureus*, indicating that high-level resistance to mupirocin results from the acquisition of a novel *ileS* gene.**

The antibiotic mupirocin (pseudomonic acid A) is produced by *Pseudomonas fluorescens* (8). Mupirocin is an analog of isoleucine which competitively inhibits amino acid activation by isoleucyl tRNA synthetase and thus inhibits protein synthesis (10, 11). Antibacterial activity is largely restricted to gram-positive organisms (13, 22), many gram-negative bacteria being mupirocin resistant due to poor penetration through the outer membrane (24). As a result of its intrinsic antibacterial activity against gram-positive species, mupirocin is used as a topical agent for the treatment of staphylococcal and streptococcal infections.

High-level resistance (>1,000 µg/ml) to mupirocin has been reported in coagulase-positive and coagulase-negative staphylococci together with evidence that the resistance was present within the staphylococcal population before mupirocin was used therapeutically (17). In many instances high-level mupirocin resistance in staphylococci is mediated by plasmids which are transferrable to susceptible recipient strains (17, 18). Similarly, transfer of mupirocin resistance from *Staphylococcus aureus* J2870 by conjugal mating resulted in a strain (*S. aureus* J2947) which was highly resistant to mupirocin and contained a 25-kb plasmid (7).

The mupirocin resistance gene has been cloned from *S. aureus* J2947, and the nucleotide sequence of a small section of the gene has been determined (7). The deduced amino acid sequence shows homology to the isoleucyl tRNA synthetase of *Escherichia coli* (25), indicating that mupirocin resistance might be the result of a modified isoleucyl tRNA synthetase.

The 4-kb fragment conferring mupirocin resistance was subcloned from pOX301 into pGEM 5Zf (Promega Corp., Madison, Wis.) for nucleotide sequencing. The nucleotide sequence was determined for both strands of the gene by the dideoxynucleotide chain termination method (21) by using the Sequenase kit, version 2.0. (US Biochemical, Cleveland, Ohio). Sequencing was carried out directly from denatured plasmid DNA using either vector primers or primers complementary to appropriate regions of the known sequence.

The complete nucleotide sequence of the 4-kb insert from pOX301, which confers resistance to mupirocin in *S. aureus* J2870, has been determined. Computer analysis indicates the presence of a complete open reading frame (ORF A) flanked on either side by putative incomplete ORFs (ORF B and ORF C), which extend beyond the limits of the cloned DNA (Fig. 1).

ORF A starts at the initiation codon TTG at position 477

and terminates at the stop codon TAA, located at position 3549. Thus ORF A contains 3,072 bp, encoding a 1,024-amino-acid polypeptide with an  $M_r$  of 118,857, with a predicted pI of 7.4. ORF A is preceded by a ribosome binding site with an intervening AT-rich spacer (seven of ten) which is essential for expression in gram-positive bacteria (1). The initiation codon UUG was first reported for the *S. aureus* β-lactamase (15) and has since been described as the initiation codon for a range of gram-positive and gram-negative genes. Although in *E. coli* the efficiency of initiation from a single protein start site is AUG>GUG>UUG (19), it is not known if there is any functional significance in the use of UUG as the initiation codon for the mupirocin-resistant isoleucyl tRNA synthetase. N-terminal protein sequencing has confirmed the UUG initiation codon (data not shown). ORF B and ORF C failed to show significant homology to data base protein sequences; consequently their function remains unknown.

We have identified a putative consensus sequence for a promoter 66 bases upstream of the initiation codon (Fig. 1). The -35 region is a four-of-six match with the consensus sequence TTGACA. An 18-bp spacer is followed by a -10 region that matches four of six bases with the consensus sequence TATAAT. It is considered unlikely that the promoter is not within the cloned DNA fragment, since mupirocin resistance is expressed in *S. aureus* when the DNA fragment is cloned in either orientation (7). Immediately downstream of the ORF is a stem-loop structure typical of rho-independent terminators, which possesses a good probability of formation according to free energy calculations ( $\Delta G = 26.2$  kcal [ca. -109.6 kJ]) (23). If transcription does terminate at this stem-loop structure, the promoter for the putative ORF C is not obvious.

The primary protein structure of ORF A shows significant homology to isoleucyl tRNA synthetases from other species. Within the protein are the HXGH and KMSKS motifs characteristic of class I tRNA synthetases (16, 25), which are required for ATP hydrolysis (20) and the GWD motif, which is conserved among all isoleucyl tRNA synthetases (5) and has a major role in amino acid activation. Dyke et al. (7) showed that deletions extending into ORF A abolished mupirocin resistance. The combined evidence supports the assumption that high-level resistance to mupirocin is due to the presence of an additional plasmid-borne isoleucyl tRNA synthetase gene (*ileS*). Further evidence supporting this assumption is provided by biochemical evidence (9), which showed that two distinct isoleucyl tRNA synthetase activity peaks were present in *S. aureus* strains highly resistant to mupirocin.

\* Corresponding author. Phone: 0737 364400. Fax: 0737 364440.

```

GAATCCAGAAAAACACGTCGACTTAATCTCTTAATTAATACTTCTATCGGATATAAAAAAATGATTTAAAAAATTTGTTAAGAAATAGTTATACCT 100
E F P E N H V D L I L N L N T S I G Y K K N D L K N L L R N S Y T
CCTTAAAAAAGGTGGTATTTAATTATAGATTTGATGAACCCCGCCTTTTCTATAAAAAATTTGAAGAACAATGATTTCAACTTCAAAGATTACGA 200
S L K K G G I L I I D L M N P A F F Y K N F E E Q M I F N F K D Y E
AGTTAATCAAACCTATTAATAATAAAATTTATAAGTATAGTTTCAACATGGGATGTTTATAAAAAAATACGAATATAGCAAAGACCTTTCAAGAGAA 300
V N Q T I K I N K I Y N S I V S T W D V Y K N N E Y S K D L S R E
TTAAAAATTAATATATACTACTAATGATATAATTTGCAGTAGTTGTATTATAACTTTACTTTATCCAATATATCTTCTATGGAGACATGAAAAGAA 400
L K F K L Y T T N D I I C S S L Y Y N F T L S N I S F Y G D M K G
ATGAATTCATGAAAATTCACCAAGACTAATGTAATAAACGAAATTAAGTATGATCTCTAGGAGCTGAAAAGTTTTGCAAGAATAATTTAAACACC 500
N E F H E N S P R L I V K I T K . L T K K Y L N T
CAGAATGAAATATCAGCATTGTGAATACTCAAAGATATTTAAAAATCAATTGACAATAGAAAAGGACAGGAAAGTTTTGTTTTTATGACGGCCCC 600
Q N E I S A F W N T Q K I F K K S I D N R K G Q E S F V F Y D G P
CAACTGCAATGGCCTTCTCTGCTGGCCATGTTCTTGAAGAGTAAATCAAGGATTAGTTGCAAGATAAAAACTATGCAAGGTTTTTATGAGAAA 700
P T A N G L P H A G H V L G R V I K D L V A R L K T M Q G F Y V E R
AAAAGCAGGATGGGATACCCATGCTTACCAGTTGAATTAGAGGTTGAAAAAAAATGGAATTAAGGAAAAACAAGCATTGAAAAGTATGGAATAGAA 800
K A G W D T H G L P V E L E V E K K I G I K G K Q D I E K Y G I E
AATTTTATAATGAATGTAATAAAGTGTATTTAATATGAAAAAGAATGGCGGATTTTTCTAAGATTAGGATACGSGTTGACATGGACTCCCCCT 900
N F I N E C K K S V F N Y E K E W R D F S K D L G Y W V D H D S P
ATATAACTCTTGAGAATAATATATGAAAGTGTGAATATATTCTACATTCATAAAAAAGGACTATTATATAAGGACATAAGGTGACTCCTTA 1000
Y I T L E N N Y I E S V W N I L S T F H K K G L L Y K G H K V T P Y
TTGTACACATGATCAAAACCGCTTAAGTCTCATGAAGTAGCGCAAGGCTATAAAAAAGTAAAGATTATCAGCTGTTGTTAAATTTCAACTTACAAAT 1100
C T H D Q T A L S S H E V A Q G Y K N V K D L S A V V K F Q L T N
AGTAAAGATACTTATTTCTAAGTTGGACTACCACTCCCTGGACTTTGCCGCAATGTAGCATTAGCTATAAATAAAGATCTTAATTTCAAATAATTC 1200
S K D T Y F L S W T T T P W T L P A N V A L A I N K D L N Y S K I
GGGTAGAAAATGAGTATTATCTTAGCTACAGACTAATTAATCTATAAATACTGAAAAATACGAAATATTGATACCTTTTCAGGAAGTAAATTAAT 1300
R V E N E Y Y I L A T D L I N S I I T E K Y E I I D T F S G S N L I
TAATTTAAAATACATTCCTCTTTGAAAGCAGCGTTAGTAAATGATATTACGTTGTTGATGAGAAATTTGTTACTAACTCAGAAGGAACGGTATT 1400
N L K Y I P P F E S D G L V N A Y Y V V D G E F V T N S E G T G I
GTTTCATATAGCACCAGCTCATGGGAAGAGTACTACCAATGGTTTTAGACCGTGATTGGATTCTTAAATGTTATAACAAGAGAAGGAGTATAATG 1500
V H I A P A H G E D D Y Q L V L E R D L D F L N V I T R E G V Y N
ATAGGTTCCCTGAATGTTGGTAATAAAGCTAAAAATAGTGATATAGAAATCATAAAATATTATCCAAAAACAACCTTTTATATAAAAAACAAAAATA 1600
D R F P E L V G N K A K N S D I E I I K L L S K K Q L L Y K K Q K Y
TGAGCATAATTATCCTCATGTTGGAGATGTGTAATCCTTTGATATATTATGCGATGGAAGGTTGGTTTTATAAACAACATAATTTAAGAATGAAAT 1700
E H N Y P H C W R C G N P L I Y Y A H E G W F I K T T N F K N E I
ATTAACAATAATAATAATAGAGTGGTTTCTTCTCATATTAAGGAAGGAGAATGGGAAATTTCTTAGAAAAATAGGTTGATTGGAACATTGCTAGAA 1800
I N N N N N I E W F P S H I K E G R M G N F L E N M V D W N I G R

```

FIG. 1. Nucleotide sequence encompassing the mupirocin resistance gene and its flanking regions. The predicted amino acid sequences of the gene products are also presented. Mupirocin resistance gene (ORF A), nucleotides 477 to 3549. ORF B, nucleotides 1 to 457. ORF C, nucleotides 3621 to 4013. Translational start and stop codons are boxed. -35 and -10 Pribnow boxes as well as the ribosome-binding site are indicated (overlines). The stem-loop structure corresponding to the proposed transcriptional termination signal of ORF A is defined by the boldface overline.

A comparison of the mupirocin-resistant isoleucyl tRNA synthetase and the native protein from *S. aureus* Oxford (4) reveals only 52% similarity and 30% identity (Table 1). It is clear, therefore, that high-level resistance to mupirocin has not arisen by mutations in the native *S. aureus* *ileS* gene which have been selected by mupirocin therapy. (In fact the respective genes have only 57% DNA identity.) This supports the finding

of Rahman et al. (17), who identified strains of *S. aureus*, highly resistant to mupirocin, which were originally isolated 20 years before the commercial introduction of mupirocin. The comparison further indicates that high-level resistance to mupirocin has arisen by a route different from that for the acquisition of moderate-level resistance (<512 µg/ml), which can be selected in vitro following serial subculture in the presence of

ATAGATATTGGGAACACCCATTAATGTATGGATTGCAATGATTGTAATCACGAATACGCCAAGTAGTATTAAGGATTTACAAAATAATTCATCAA 1900  
 N R Y W G T P L N V W I C N D C N H E Y A P S S I K D L Q N N S I N  
 TAAAATTGATGAAGATATTGAGTTGCATAGACCTTATGTTGATAATACACTCTTAGTTGCCCTAAGTGAATGGGAAAATGCTCCGAGTACAAGAAGTA 2000  
 K I D E D I E L H R P Y V D N I T L S C P K C N G K M S R V E E V  
 ATCGATGTTGGTTTGATAGCGGCTCTATGCCGTTTCTCAGCATCATTATCCTTTTGATAACCCAGAAAATTTTAAATCAACACTTTCCAGCTGATTTTA 2100  
 I D V W F D S G S H P F A Q H H Y P F D N Q K I F N Q H F P A D F  
 TTGCAGAAGGAGTTGATCAAACGAGAGGCTGGTTTTACAGTTTACTAGTAATTTCTACTATTCTAAAAGGAAAATCTTCTATAAACGCTGTTTATCTTT 2200  
 I A E G V D Q T R G W F Y S L L V I S T I L K G K S S Y K R A L S L  
 AGGCATATTTAGACAGTAATGGTAAAAAATGTCTAAAAGTAAAGGAACGTTTAAATCCAACGAATTAATAAAGTACGGACCCGATCTTTTA 2300  
 G H I L D S N G K K M S K S K G N V I N P T E L I N K Y G A D S L  
 AGATGGGCTTAATTTCCGATAGTCTCCATGGAATAACAAAAGATTCTCAGAAAATAGTAGCTCAGACCAAAATCGAAAATTTATAGATACGCTTGATA 2400  
 R W A L I S D S A P W N N K R F S E N I V A Q T K S K F I D T L D  
 ATATTTATAAATTTATAATATGTATAATAAATAGATCACTATAATCTAATAATGAAATTACAAAAGTAGAAAATACATTAGATAATGGGCTCTTTC 2500  
 N I Y K F Y N M Y N K I D H Y N P N N E I T K S R N T L D N W A L S  
 TCGCTTAAACACCTTAATAAAAAGAAAGTAATATTTATGTAATAATACGATTTCACTTCCGACCCAGATTAATTAACGAATATACCAATACAATAAGT 2600  
 R L N T L I K E S N I Y V N N Y D F T S A A R L I N E Y T N T I S  
 AATTGGTATATCGGAGATTGAGAGGACGATTTGGGAACAAGGAATTTCTAACGATAAAAAGATCGCTACAATACGCTTTATGAAATTTAAACACTT 2700  
 N W Y I G D S R G R F W E Q G I S N D K K D A Y N T L Y E I L T T  
 TATCAAGACTAGTGGCTCCATTTGTTCCATTTATATCTGAAAAATCCATTATAATTTGACTGGAAAAGTGTGCAATTTACAAGATTATCCACAATATA 2800  
 L S R L V A P F V P F I S E K I H Y N L T G K S V H L Q D Y P Q Y K  
 AGAAAGTTTTATTAATCAAGCATTGGAAGATGAAATGCATACCGTTATAAAAATTTGTAATAATCTAGACAGGCTCGCAAAAATGCAGATTTAAAAAT 2900  
 E S F I N Q A L E D E M H T V I K I V E L S R Q A R K N A D L K I  
 AAGCAACCTTTATCGAAAATGGTATTAACCTAATAGTCAATAACTTAAGTTTTTACCTAATTAATCAATAATAAAGACGAATTAATATATA 3000  
 K Q P L S K M V I K P N S Q L N L S F L P N Y Y S I I K D E L N I  
 AAAACATGAATTAAGTATAATTAATGACTATATACCTATGAGCTTAAATTTGAATTTTTCTTCTGTGGACCAAACTAGGGAACAAAACGAAAA 3100  
 K N I E L T D N I N D Y I T Y E L K L N F S S V G P K L G N K T K N  
 TATTCAAACATTGATAGACTCCCTATCAGAGTATGATAAAAAAGTTAATTTAGTCTAATAAATCTCAAAGTTTATCTTCTGATGCTGAGTTAACTAAG 3200  
 I Q T L I D S L S E Y D K K S L I E S N N F K S L S S D A E L T K  
 GATGATTTATAATTAACCTTACCTAAGGATAGTTATCAACTCAGTGAAGATAAGTACTGCGTTATATTATAGATAAAAATTTATCTCCTGAATTA 3300  
 D D F I I K T L P K D S Y Q L S E D N D C V I L L D K N L S P E L  
 TTCGCGAAGGACATGCTAGAGAGCTCATTAGATTAATCAACAATTAAGAAAAAGAAAAATTTACCAATAAATCAACGATTGATATTATATCGGTGT 3400  
 I R E G H A R E L I R L I Q Q L R K K K N L P I N Q R I D I Y I G V  
 AACTGGGAATATTAGAATCAATAAAAACCAATAAAAAATGTTTAAAGAAAATTTCTGATTAATAATATACACTTAAATGTTATAGATGAATATGA 3500  
 T G E L L E S I K T N K N M F K E N F V I K N I H L N V I D E Y E  
 AATACTATTCATTTAATAATAAAGAAAATAAAAAATTCCTTATTATATAAACAACATGGCCACTCTATTTTACTAGAGTGGCCATGAGTTATCAAAGC 3600  
 N T I H F N N K E I K I S L L Y . ← → ←  
STEM-LOOP  
SD  
 CAAGCAAAGGTGATAAATATGATAAATTTGATTATCTAAATACCAATAAAAATATTCAAAAATTTTCTTATTCAGGGGGATATGTAATCT 3700  
 H Y N F D Y S K L P I K N I Q K I F P I A G G Y V N L  
 ATCTTTTTCAGTTGATGCTTCAACAAAAAATTTTTTAAAGTACAACCTAATACTAAATCAAAATTTTTTGATTAGAGTTTCCAGTTTAAAAGAA 3800  
 S F S V D A S N K K Y F L K L Q P N T K S N F F D Y E L S S L K E  
 TTAAGTATAAAAAATTTCTGTTCCCTCAAATTTAATAAAGGAGAATTAGACAATAATCTTTTTTACTGTTAGAGTTCATTGAAAATGGCCATGCTT 3900  
 L T D K N I P V P Q I I N K G E L D N N S F L L L E F I E N G H A  
 ATCTGAAAGCTATAGAAAATAGTAAAATAGTGAACATGCATAAAAAATATAAATCACTAAATCTTTGGCTTTAGTCATAATTTCAATGGAGG 4000  
 Y P E S Y R K L G K I V A N M H K N I N S L N L F G F S H N F N G G  
 AACGATTGAATC  
→ 4013  
 T I E F

FIG. 1—Continued.

TABLE 1. Homology of mupirocin resistance protein (Mupr) to isoleucyl tRNA synthetases

Mupr or tRNA synthetase	Homology (% similarity/% identity) of Mupr to tRNA synthetases from <sup>a</sup> :				
	<i>S. aureus</i>	<i>E. coli</i>	<i>Methanobacterium thermoautotrophicum</i> (12)	<i>Saccharomyces cerevisiae</i> (14)	<i>Tetrahymena thermophila</i> (6)
Mupr	52/30	50/27	57/35	58/36	56/34
<i>S. aureus</i>		63/42	56/34	51/28	53/29
<i>E. coli</i>			52/31	50/28	50/25
<i>Methanobacterium thermoautotrophicum</i>				57/36	55/31
<i>Saccharomyces cerevisiae</i>					68/48
<i>Tetrahymena thermophila</i>					

<sup>a</sup> Numbers in parentheses after organisms are references.

increasing concentrations of mupirocin (2, 3). It is possible that the resistant isoleucyl tRNA synthetase gene has been acquired from another organism. However, comparison of the mupirocin-resistant isoleucyl tRNA synthetase with known isoleucyl tRNA synthetases (Table 1) has failed to produce conclusive evidence for the source of the resistant isoleucyl tRNA synthetase.

**Nucleotide sequence accession number.** The nucleotide sequence shown in Fig. 1 has been deposited in GenBank under the accession number X75439.

#### REFERENCES

- Band, L., and D. J. Henner. 1984. *Bacillus subtilis* requires a stringent region for gene expression. *DNA* 3:17–21.
- Capobianco, J. O., C. C. Doran, and R. C. Goldman. 1989. Mechanism of mupirocin transport into sensitive and resistant bacteria. *Antimicrob. Agents Chemother.* 33:156–163.
- Casewell, M. W., and R. L. R. Hill. 1985. *In vitro* activity of mupirocin (pseudomonic acid) against clinical isolates of *Staphylococcus aureus*. *J. Antimicrob. Chemother.* 15:523–531.
- Chalker, A., J. M. Ward, A. P. Fosberry, and J. E. Hodgson. 1994. Analysis and toxic overexpression in *Escherichia coli* of a staphylococcal gene encoding isoleucyl-tRNA synthetase. *Gene* 141:97–103.
- Clarke, N. D., D. C. Lién, and P. Schimmel. 1988. Evidence from cassette mutagenesis for a structure-function motif in a protein of unknown structure. *Science* 240:521–523.
- Csank, C., and D. W. Martindale. 1992. Isoleucyl-tRNA synthetase from the ciliated protozoan *Tetrahymena thermophila*: DNA sequence, gene regulation and leucine zipper motifs. *J. Biol. Chem.* 267:4592–4599.
- Dyke, K. G. H., S. P. Curnock, P. Golding, and W. C. Noble. 1991. Cloning of the gene conferring resistance to mupirocin in *Staphylococcus aureus*. *FEMS Microbiol. Lett.* 77:195–198.
- Fuller, A. T., G. Mellows, M. Woodford, G. T. Banks, K. D. Barrow, and E. B. Chain. 1971. Pseudomonic acid: an antibiotic produced by *Pseudomonas fluorescens*. *Nature (London)* 234:416–417.
- Gilbart, J., C. R. Perry, and B. Slocombe. 1993. High-level mupirocin resistance in *Staphylococcus aureus*: evidence for two distinct isoleucyl tRNA synthetases. *Antimicrob. Agents Chemother.* 37:32–38.
- Hughes, J., and G. Mellows. 1978. Inhibition of isoleucyl-transfer ribonucleic acid synthetase in *Escherichia coli* by pseudomonic acid. *Biochem. J.* 176:305–318.
- Hughes, J., and G. Mellows. 1980. Interaction of pseudomonic acid A with *Escherichia coli* B isoleucyl tRNA synthetase. *Biochem. J.* 191:209–219.
- Jenal, U., T. Rechsteiner, P.-Y. Tan, E. Bühlmann, L. Meile, and T. Leisinger. 1991. Isoleucyl tRNA synthetase of *Methanobacterium thermoautotrophicum* Marburg. *J. Biol. Chem.* 266:10570–10577.
- Lamb, Y. J. 1991. Overview of the role of mupirocin. *J. Hosp. Infect.* 19(Suppl. B):27–30.
- Martindale, D. W., Z. M. Gu, and C. Csank. 1989. Isolation and complete sequence of the yeast isoleucyl tRNA synthetase gene (ILS1). *Curr. Genet.* 15:99–105.
- McLaughlin, J. R., C. L. Murray, and J. C. Rabinowitz. 1981. Unique features in the ribosome binding site sequence of the Gram-positive *Staphylococcus aureus*  $\beta$ -lactamase gene. *J. Biol. Chem.* 256:11283–11291.
- Myers, A. M., and A. Tzagoloff. 1985. MSW, a gene coding for mitochondrial tryptophanyl tRNA synthetase. *J. Biol. Chem.* 260:15371–15377.
- Rahman, M., S. Connolly, W. C. Noble, B. Cookson, and I. Phillips. 1990. Diversity of staphylococci exhibiting high-level resistance to mupirocin. *J. Med. Microbiol.* 33:97–100.
- Rahman, M., W. C. Noble, and B. Cookson. 1987. Mupirocin-resistant *Staphylococcus aureus*. *Lancet* 2:387.
- Reddy, P., A. Peterkofsky, and K. McKenny. 1985. Translational efficiency of the *Escherichia coli* adenylate cyclase gene: mutating the UUG initiation codon to GUG or AUG results in increased gene expression. *Proc. Natl. Acad. Sci. USA* 82:5656–5660.
- Rossman, M. G., A. Liljas, C.-I. Branden, and L. J. Banaszak. 1975. Evolutionary and structural relationships among dehydrogenases, p. 61–102. *In* P. D. Boyer (ed.), *The enzymes*, 3rd ed. vol. 11. Academic Press, Orlando, Fla.
- Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74:5463–5467.
- Sutherland, R., R. J. Boon, K. E. Griffin, P. J. Masters, B. Slocombe, and A. R. White. 1985. Antibacterial activity of mupirocin (pseudomonic acid), a new antibiotic for topical use. *Antimicrob. Agents Chemother.* 27:495–498.
- Tinoco, I., Jr., P. N. Borer, B. Dengler, M. D. Levine, O. C. Uhlenbeck, D. M. Crothes, and J. Gralla. 1973. Estimation of secondary structure in ribonucleic acids. *Nature (London) New Biol.* 246(150):40–41.
- Vaara, M. 1992. The outer membrane as the penetration barrier against mupirocin in Gram-negative enteric bacteria. *J. Antimicrob. Chemother.* 29:221–222.
- Webster, T., H. Tsai, M. Kula, G. A. Mackie, and P. Schimmel. 1984. Specific sequence homology and three-dimensional structure of an aminoacyl transfer RNA synthetase. *Science* 226:1315–1317.