ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1994, p. 1205–1208
0066-4804/94/$04.00+0

Vol. 38, No. 5

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

J. E. HODGSON,[1]* S. P. CURNOCK,[2] K. G. H. DYKE,[2] R. MORRIS,[3] D. R. SYLVESTER, AND M. S. GROSS[3]

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

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 (ΔG = 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.

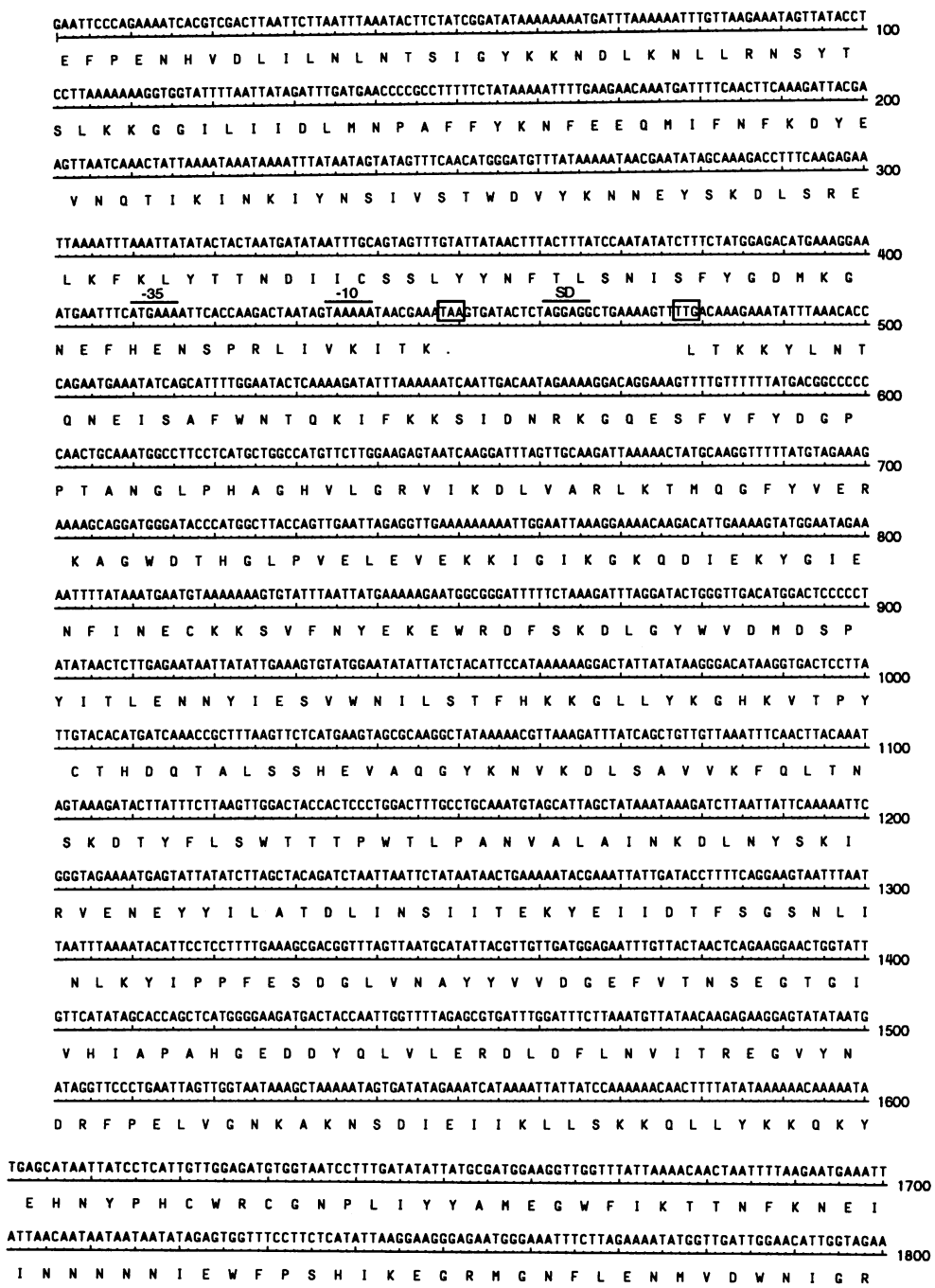

FIG. 1. Nucleotide sequence encompassing the mupirocin resistance gene and its flanking regions. The predicted amino acid sequences of the gene products are also presented. Mupirocin resistance gene (ORF A), nucleotides 477 to 3549. ORF B, nucleotides 1 to 457. ORF C, nucleotides 3621 to 4013. Translational 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

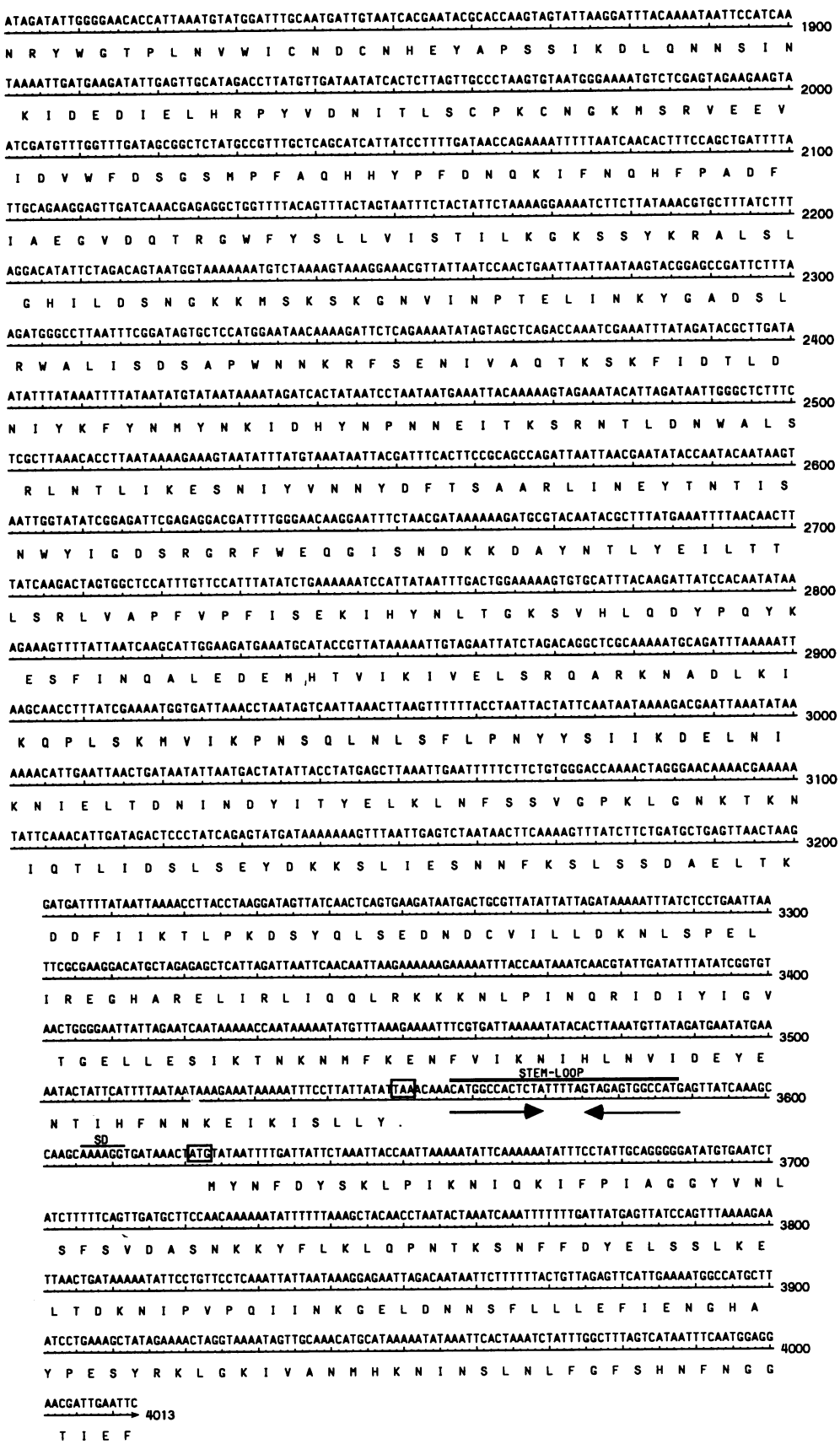

FIG. 1—*Continued.*

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[a]:				
	S. aureus	*E. coli*	*Methanobacterium thermoautotrophicum* (12)	*Saccharomyces cerevisiae* (14)	*Tetrahymena thermophila* (6)
Mupr	52/30	50/27	57/35	58/36	56/34
S. aureus		63/42	56/34	51/28	53/29
E. coli			52/31	50/28	50/25
Methanobacterium thermoautotrophicum				57/36	55/31
Saccharomyces cerevisiae					68/48
Tetrahymena thermophila					

[a] 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.

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