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NOTES

The Leucine-Responsive Regulatory Protein, Lrp, Modulates Microcin J25 Intrinsic Resistance in *Escherichia coli* by Regulating Expression of the YojI Microcin Exporter▽

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Many *Escherichia coli* K-12 strains display an intrinsic resistance to the peptide antibiotic microcin J25. In this study, we present results showing that the leucine-responsive regulatory protein, Lrp, is involved in this phenotype by acting as a positive regulator of YojI, a chromosomally encoded efflux pump which expels microcin out of cells. Exogenous leucine antagonizes the effect of Lrp, leading to a diminished expression of the pump and an increased susceptibility to microcin J25.

Microcin J25 (MccJ25) is a plasmid-encoded, 21-amino-acid antibacterial peptide produced by *Escherichia coli* (3, 30). It is active against *E. coli*, *Salmonella*, and *Shigella* strains. MccJ25 intake is mediated by the outer membrane receptor FhuA and the inner membrane proteins TonB, ExbB, ExbD, and SbmA (31, 32). *E. coli* RNA polymerase is the target of antibiotic action (12, 37). The binding site for MccJ25 is located in the secondary channel of the enzyme (1, 21), which provides a route by which the nucleotide substrates reach the catalytic site. Thus, MccJ25 inhibits transcription by clogging the channel and blocking the access of substrates to the active center (1, 21).

Four genes (*mcjA*, *mcjB*, *mcjC*, and *mcjD*) are required for MccJ25 synthesis, export, and immunity (34, 35). The *mcjA* gene encodes the primary structure of MccJ25 as a 58-amino-acid precursor, from which a 37-amino-acid N-terminal leader is removed. The 21-residue mature peptide has a compact, extraordinary structure, which consists of an eight-residue lariat ring and a C-terminal tail which folds on itself and passes through the ring, where it is sterically trapped (2, 29, 36). The *mcjB* and *mcjC* gene products are involved in this assembly process (14). The *mcjD* product has a dual role. It works as a dedicated ATP-binding cassette exporter of MccJ25 and, at the same time, provides immunity against both the endogenous MccJ25 synthesized in producer cells and the exogenous microcin that gains entry (11, 35).

We have observed that in twofold serial dilution assays on LB medium or M9 minimal medium plates with several *E. coli* K-12 strains as indicators (including the widely used strain MC4100), MccJ25 gives rise to turbid zones of growth inhibition. These observations suggested that *E. coli* strains have an intrinsic partial resistance to MccJ25. However, the polyauxotrophic strain AB1133 gives completely clear halos and is routinely used in our laboratory as an indicator strain. We reasoned that a study of this unusual sensitivity could give us a clue to understanding the origin of the natural resistance of other *E. coli* strains. In the present work, we demonstrate that the increased sensitivity of AB1133 depends on the leucine used to supplement the AB1133 auxotrophy for this amino acid. The leucine effect seems to be mediated by the global regulator Lrp, which acts as a positive regulator of an endogenous chromosome-encoded MccJ25 efflux pump, YojI. Exogenous leucine would antagonize the effect of Lrp, leading to a diminished expression of the pump and to the accumulation of microcin in AB1133 cells.

Sensitivity to MccJ25 was tested by a spot-on-lawn assay, as follows. Twofold serial dilutions of a purified MccJ25 preparation (1 mg/ml) were spotted (10 μl) onto M9 medium plates and dried. Aliquots (50 μl) of cultures to be tested for sensitivity in stationary phase were mixed with 3 ml of top agar (0.7% agar) and overlaid onto the plates. After overnight incubation, the plates were examined for different degrees of inhibition.

Our first working hypothesis to explain the higher susceptibility of strain AB1133 to MccJ25 was that the partially resistant strains possess a genetic marker which is absent or mutated in the fully susceptible AB1133 strain. To investigate this possibility, mating experiments were done using the partially resistant strain AB259 (HfrH, prototrophic) as the donor and the multiply marked AB1133 [*thr-1 leuB6* Δ(*gpt-proA*)*62 hisG4 argE3*] strain as the recipient. This allowed us to map the resistance marker to the 98- to 5-min region of the *E. coli* K-12 map. A bacterial cross was then performed between AB259 and AB1133 by using the gradient-of-transmission method of De Haan et al. (10) and selecting for Thr^+. Recombinants

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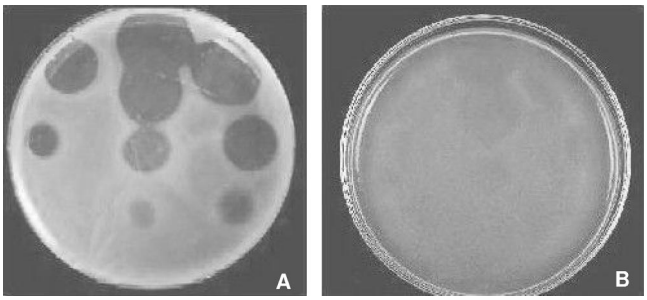

FIG. 1. Comparison of the susceptibilities of strains AB1133 (A, control) and AB1133 transduced to *leuB*$^+$ (B) to MccJ25. Twofold serial dilutions of MccJ25 (1/2 to 1/1,024) were spotted onto LB medium plates. After the spots dried, the plates were overlaid with a suspension of AB1133 (left) or the transductional cells (right). Note the very turbid (almost invisible) spots given by the transductional strain.

were scored for inheritance of Leu$^+$, ProA$^+$, and MccJ25 resistance. This experiment indicated that the locus responsible for resistance was located at approximately 2 min on the *E. coli* K-12 genetic map. Its location was then more precisely determined by P1 transduction. The bacteriophage P1*vir* propagated on AB259 was used to transduce AB1133 to Leu$^+$. One hundred twenty transductants were tested for MccJ25 resistance by a cross-streaking assay. All of them had become partially resistant to the antibiotic. When some selected transductants were subjected to a serial dilution test, all of them showed very turbid halos, in contrast to the parental strain AB1133 (Fig. 1). The same results were obtained when the transduction experiment was repeated with strain MC4100 as the donor and AB1133 as the recipient: resistance to MccJ25 was 100% cotransduced with *leuB*. These results indicated that *leuB* inactivation in AB1133 was related to the increased susceptibility of this strain to MccJ25.

Susceptibility to MccJ25 is affected by the concentration of leucine in the culture medium. For growth of AB1133, M9 medium plates were supplemented with the appropriate amino acids at 50 μg/ml each. We hypothesized that the greater sensitivity of AB1133 to MccJ25 was not due to the *leu* mutation per se but to the leucine added to the medium. If so, one might expect that the addition of leucine should also increase the sensitivity of intrinsically resistant strains to MccJ25. To test this possibility, the response of strain AB259 to MccJ25 was examined by a spot-on-lawn assay with M9 minimal medium plates supplemented with 12.5 and 25 μg/ml of leucine. With the leucine-supplemented medium, the susceptibility to MccJ25 of this strain was similar to that of AB1133 and varied with the external abundance of the amino acid, increasing with increased leucine concentration (Fig. 2).

The leucine effect on MccJ25 susceptibility is mediated by the leucine-responsive regulatory protein, Lrp. It is well known that exogenous leucine affects the expression of some genes regulated by Lrp, a global regulator of metabolism in *E. coli* (4, 22, 23). This suggested that the effect of leucine on microcin susceptibility could be exerted through Lrp. Altogether, six different patterns of regulation by Lrp have been recognized, depending upon whether Lrp acts negatively or positively and upon the way in which leucine affects expression. For those cases in which Lrp acts positively as an activator, leucine sometimes overcomes the effect of Lrp (thus causing reduced expression), sometimes intensifies the effect of Lrp, and sometimes has no effect on Lrp-mediated activation. Similarly, for cases in which Lrp acts negatively, there are examples in which leucine overcomes the effect, is required for the effect, or has no effect upon Lrp-mediated repression. To test the idea that the leucine effect on MccJ25 susceptibility could be mediated by Lrp, we compared the responses of the isogenic strains MC4100 and RO64 (MC4100 *lrp*::Tn*10*) to the antibiotic in M9 minimal medium and in the same medium containing either 12.5 or 25 μg/ml of leucine by using a twofold serial dilution assay. Two important results were obtained from this experiment (Fig. 3). First, in M9 medium without leucine, the mutant RO64 was more susceptible to MccJ25 than the parent MC4100 and showed clear halos, since it was even more sensitive than AB1133. This indicated that Lrp is implicated in the resistance to MccJ25. Second, in contrast to MC4100, addition of leucine to the medium did not increase the sensitivity of

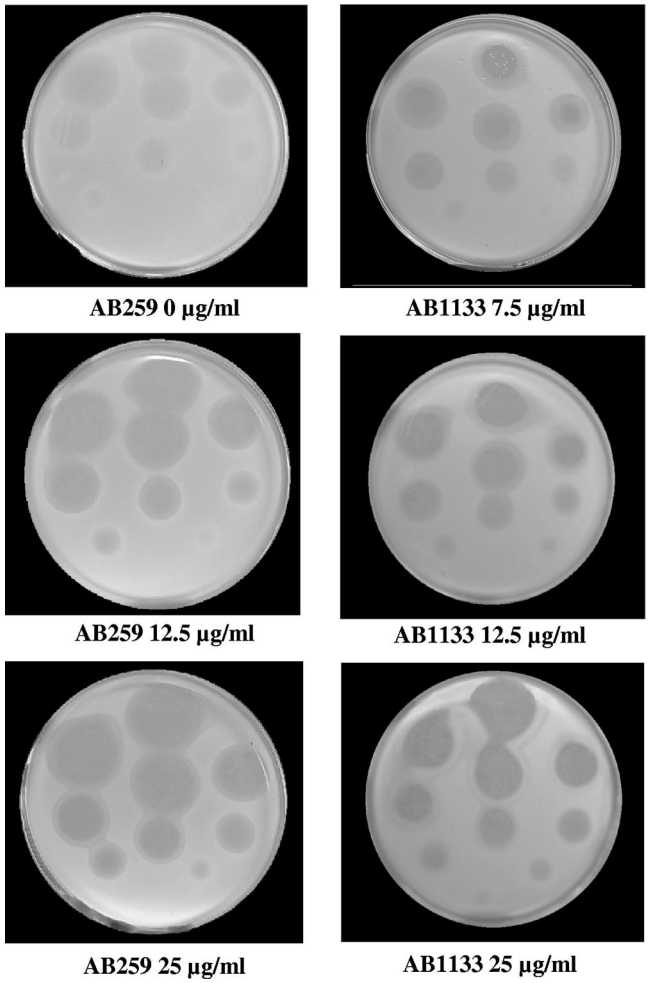

FIG. 2. Comparison of the susceptibilities of strains AB259 (intrinsically resistant to microcin) and AB1133 to MccJ25 in the presence of different leucine concentrations. For the auxotrophic strain AB1133, 7.5 μg/ml was the minimal concentration of the amino acid necessary for growth. Note that for both strains, the inhibition halos become clearer and larger with increasing concentrations of leucine.

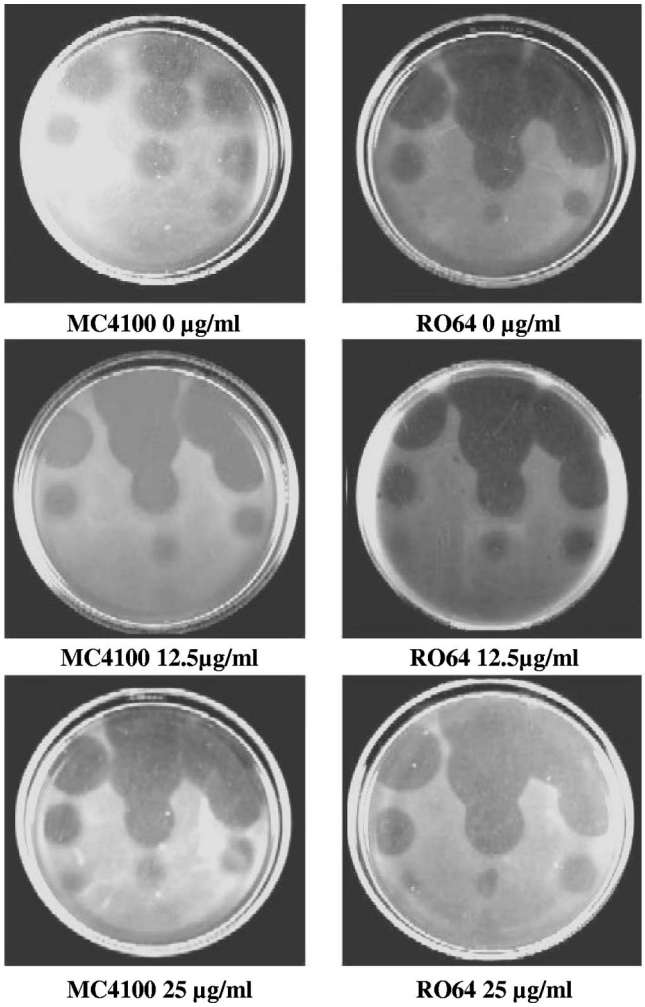

FIG. 3. Comparison of the susceptibilities of strains MC4100 (parent) and RO64 (*lrp*-null mutant derivative) to MccJ25 in the presence of increasing concentrations of leucine. Note that, in contrast to MC4100, the addition of leucine to the medium did not increase the sensitivity of RO64 to the antibiotic.

RO64 to the antibiotic. This is just what is to be expected if Lrp is a mediator of the leucine effect.

To more precisely assess the effect of leucine on sensitivity to MccJ25, we determined the MIC of MccJ25 for strains MC4100 and RO64 in M9 medium and in the same medium supplemented with 50 μg/ml of leucine (Table 1). This experiment confirmed that strain MC4100 is more resistant than RO64 to MccJ25, showing in unsupplemented medium a MIC of MccJ25 that was eightfold higher than that for RO64, whereas in the presence of leucine, the MIC for MC4100 was twofold higher. As expected, the MIC for RO64 remained unchanged when the strain was grown in the presence of the amino acid.

It should be noted that the effect of leucine was enhanced when proline and threonine (25 μg/ml each) were also added to the culture medium (results not shown). This is in keeping with the finding that the *lrp* gene is repressed in cells grown in glucose minimal medium with either the α-ketoglutarate or the oxalacetate family of amino acids (23). The net result of these processes (the leucine-sensitizing effect and *lrp* repression) would be a susceptibility level to microcin that is higher than that observed with leucine alone.

Lrp positively regulates YojI, an inner-membrane MccJ25 efflux pump. The above-described results suggested that Lrp was somehow involved in intrinsic resistance to MccJ25. Previously, we determined that *E. coli yojI*, an open reading frame of unknown function which encodes a putative membrane protein belonging to the ATP-binding cassette family of exporters (25), is capable of using MccJ25 as a substrate (13). In that study, we constructed the plasmid pCLO7, a pUC18 derivative with a 4.2-kb SmaI-PstI chromosomal fragment containing *yojI* as the only intact open reading frame. Overexpression of *yojI* from pCLO7 renders *E. coli* strains (such as AB1133 and MC4100) completely resistant to MccJ25. Therefore, one possible explanation for the above-described results is that Lrp could be acting as a positive regulator of *yojI* transcription, which would lead to an increase in YojI levels in the membrane. The enhanced efflux of exogenous microcin that enters cells would result in an increased resistance to the antibiotic. To confirm this hypothesis, we first examined the DNA region upstream of the *yojI* gene. Sequences resembling those of the *E. coli* σ^{70} promoter consensus −10 and −35 elements were found (17), with a spacer region of 15 bp (the optimal is 16 to 18 bp) (Fig. 4). *yojI* lacks an obvious ribosome-binding site (the consensus sequence is TAAGGAGG (33). These data suggest that the *yojI* gene of *E. coli* is poorly expressed. We detected several putative Lrp-binding sites in the *yojI* upstream region, which strongly suggests that Lrp functions as a transcriptional regulator of *yojI*. One of these sites (Fig. 4, site VI) shows a perfect fit to the 12-bp Lrp consensus binding sequence (TTT ATTCtNaAT) proposed by Rex et al. (28). Except for sites V and VI (Fig. 4), the rest of the potential Lrp binding sites identified are located far upstream from the putative promoter. The functional importance of the presumptive Lrp binding sites was assessed by PCR amplification of four regions containing the putative promoter and progressively greater amounts of DNA from the region upstream of the promoter, using pCLO7 plasmid DNA as a template (Fig. 5). Oligonucleotide primers were engineered to contain EcoRI and SalI restriction sites. PCR products were digested with these enzymes and cloned into the EcoRI and SalI sites of plasmid pAH125 (16) upstream of the promoterless *lacZ* reporter gene carried by this vector, resulting in the plasmids pLrp1 to pLrp4 (Fig. 5A). They were then introduced into the *pir*$^+$ host strain DH5α λpir to allow replication of the vector, which contains the conditional origin of replication from the plasmid R6K. Expression of the promoter fusions was measured using the method of Miller (20) with cultures grown overnight in LB medium. As shown in Fig. 5B, the plasmid pLrp4, bearing the DNA fragment containing the *yojI* promoter and all the po-

TABLE 1. Influence of leucine on the MIC of MccJ25

Strain	MIC (μg/ml) for medium	
	M9	M9 plus Leu (50 μg/ml)
MC4100	0.97	0.24
RO64	0.12	0.12

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ATCTCTATTCGCCCATTGATCCGCAAACAAATAAACCGTGGCCCGCCATGCCACAGagTtTTCaTAAT
TAGAGATAAGCGGGTAACTAGGCGTTTGTTTATTTGGCACCGGGCGGTACGGTGTCTCAAAAGTATTA
                                                                  I
TTATGTCAACGTGCGGCTACGGCGGCGGGCTATCCAGATTTCCAGCCAGATGCTTGTCTTATCAACCG
AATACAGTTGCACGCCGATGCCGCCGCCCGATAGGTCTAAAGGTCGGTCTACGAACAGAATAGTTGGC

CTACGCTCCTGGCGCGAAACTGTCGCTGCATCAGGATAAAGACGAACCGGATCTGCGCGCGCCAATTG
GATGCGAGGACCGCGCTTTGACAGCGACgtAGTCcTATTTCTGCTTGGCCTAGACGCGCGCGGTTAAC
                           II
TTTCTGTTTCTCTGGGCTTACCCGcgatTTtTCAATTTGGCGGCCTGAAACGAAATGATCCGCTCAAA
AAAGACAAAGAGACCCGAATGGGCGCTAAAAAGTTAAACCGCCGGACTTTGCTTTACTAGGCGAGTTT
                           III
CGTTTgTTgTTggaACATGGCGATGTGGTGGTATGGGGCGGTGAATCGCGGCTGTTTTATCACGGTAT
GCAAACAACAACCTTGTACCGCTACACCACCATACCCCGCCACTTAGCGCCGACAAAATAGTGCCATA
    IV
TCAACCGTTGAAAGCGGGGTTTCATCCACTCACCATCGACTGCCGCTACAACCTGACATTCCGTCAGG
AGTTGGCAACTTTCGCCCCAAAGTAGGTGAGTGGTAGCTGACGGCGATGTTGGACTGTAAGGCAGTCC

CAGGTAAAAAAGAATAAAAATAAGAATTATTATTGCTGTGCGCGCGAAGATGTTTAAACTGCGGGCTG
GTCCATTttTTCTTATTTTTTATTCTTAATAATAACGACACGCGCGCTTCTACAAATTTGACGCCCGAC
      V      VI          -35          -10
TAATTCATTGTCCGGGTTTTCTGCATGGAACTTCTTGTACTTGTCTGGCGGCAGTATCGCTGGC
ATTAAGTAACAGGCCCAAAAGACGTACCTTGAAGAACATGAACAGACCGCCGTCATAGCGACCG
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FIG. 4. Nucleotide sequence of the regulatory region of the *yojI* gene. Coding and noncoding strands are shown. The presumptive −35 and −10 RNA polymerase binding sites have been shaded. The start codon (ATG) for *yojI* translation is in boldface. Regions corresponding to possible Lrp recognition sequences, labeled I to VI, are in boldface type and underlined. Lowercase letters represent nucleotides which are different from the canonical ones (the consensus is TTTATTCtNaAT). Sequences V and VI are in opposite orientations (as indicated by the arrows) and overlap by one base pair, which has been shaded.

tential Lrp binding sites, showed, as expected, the highest β-galactosidase values. Fusions in the plasmids pLrp1 (containing only Lrp site VI) and pLrp2 (which also carried Lrp sites IV and V) had only 10% of the basal activity of fusion in pLrp4, suggesting that these sites by themselves may not have a significant role in *yojI* activation. In contrast, when a DNA fragment incorporating Lrp site III (Fig. 4) was used to drive *lacZ* in plasmid pLrp3, β-galactosidase activity increased to values that paralleled those seen in pLrp4, indicating that sequence III is important for full *yojI* activation. However, we cannot exclude from this analysis the possibility that this sequence is necessary but not sufficient for promoter activation and that an interaction between Lrps bound at this site and at downstream sites might be necessary for promoter expression. This may require the intervening DNA to be bent by the Lrp protein (24).

Further evidence that Lrp regulates YojI expression came from experiments in which we transformed the strains MC4100

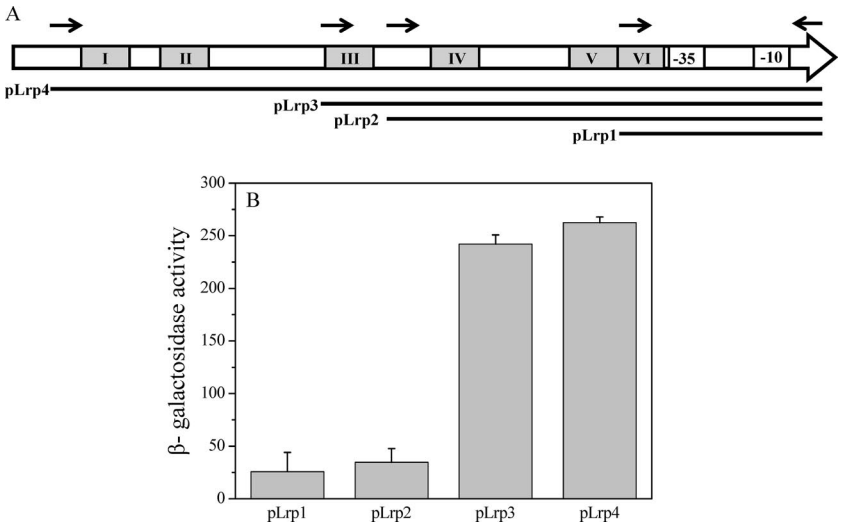

FIG. 5. Construction and activities of the *lacZ* transcriptional fusions under the control of the *yojI* promoter region. (A) The box at the top is a schematic representation of the *yojI* upstream sequences shown in Fig. 4. The arrowhead at the right end indicates the direction of transcription. The presumptive −10 and −35 promoter sequences (open boxes) and the locations of the six putative Lrp binding sites (shaded boxes) are shown. Solid lines represent *yojI* upstream regions fused to the *lacZ* gene in the promoter-probe plasmid pAH125, and the names of the resulting constructs are given at the left. Oligonucleotide primers used to amplify the different regions are shown by arrows. Predicted sizes of the PCR fragments subcloned into pLrp1 through pLrp4 are 100, 255, 297, and 498 bp, respectively. The scheme is not drawn to scale. (B) β-Galactosidase activities for cells carrying corresponding fusion plasmids. Strains were grown overnight in LB medium, and activities of β-galactosidase (expressed in Miller units) were assayed. Background expression values of the *lacZ* gene on the vector were subtracted. Values represent the averages of three independent assays, each performed in duplicate.

TABLE 2. β-Galactosidase activities of chromosomal versus plasmid-borne *yojI-lacZ* fusions

Strain	β-Galactosidase activity ± SD (Miller units)[a]
MC4100 (*yojI′-lacZ*)	80.24 ± 1.9
RO64 (*yojI′-lacZ*)	23.53 ± 2.3
MC4100(pCLO7 *yojI-lacZ*)	116.40 ± 1.27
RO64(pCLO7 *yojI-lacZ*)	16.49 ± 0.84

[a] Mean values ± standard deviations (SD) were obtained from five independent experiments.

and RO64 (MC4100 *lrp*::Tn*10*) with plasmid pCLO7 to determine the effect of Lrp depletion on the expression of the plasmid-borne *yojI* gene. Transformants were selected on LB medium-ampicillin (50 μg/ml) and tested for sensitivity to MccJ25 by a serial dilution assay. As expected, MC4100(pCLO7) became fully resistant to MccJ25, whereas no increase in resistance was seen with RO64(pCLO7), which was as sensitive as the parent strain RO64. At first sight, these results could be taken to mean that very little if any YojI is synthesized from pCLO7 in the Lrp-deficient host. However, as we shall see below, the expression of a chromosomal *yojI-lacZ* fusion was significantly reduced but not eliminated in an *lrp*-null background (Table 2). Therefore, it would be reasonable to assume that in the absence of Lrp, the residual transcription of *yojI* in pCLO7 should lead to some degree of microcin resistance. One likely explanation for the apparent all-or-none effect seen with the plasmid pCLO7 may be provided by the observation that an *lrp*-null mutant is significantly more sensitive to MccJ25 than a Δ*yojI* mutant (results not shown), suggesting that, besides YojI, an additional MccJ25 resistance factor is also positively controlled by Lrp. It is therefore possible that on Lrp deprivation, the amount of YojI synthesized from pCLO7 is not sufficient to overcome the MccJ25 sensitivity produced by the reduced level of this putative factor. Be that as it may, the results described in this section are clearly consistent with the role of Lrp in the regulation of *yojI*.

Effect of Lrp on the expression of chromosomal and plasmid-borne *yojI-lacZ* fusions. The above-described findings strongly suggested that Lrp influences transcription activation at *yojI*. To further test this, we measured the expression of *yojI* in the presence and in the absence of Lrp by using a chromosomal *yojI-lacZ* transcriptional fusion constructed as follows. We started with a derivative of strain DH5α in which the *yojI* gene had been replaced by a chloramphenicol resistance cassette via a λ Red recombinase-mediated gene replacement (9). The Δ*yojI* mutation from this strain was transduced into MC4100, and the cassette was subsequently removed by using the FLP recombinase produced by a conditionally replicating plasmid (9), thus creating an unmarked *yojI* deletion. The single-copy *lacZ* transcriptional fusion to *yojI* was generated in MC4100 Δ*yojI* by the procedure developed by Ellermeier et al. (15). The fusion was then transduced into strain RO64. Both fusion strains were grown in LB medium to stationary phase, and β-galactosidase activity was measured using the method described by Miller (20). As shown in Table 2, expression of the fusion was almost four times lower in the absence of Lrp. Similar results were obtained with a *yojI-lacZ* translational fusion contained in plasmid pCLO7. This fusion was obtained by insertion of Tn*5lacZ* (19) into the *yojI* gene. The fusion plasmid was introduced into the mutant RO64 and the parent MC4100, and β-galactosidase activities were measured as described above. As seen in Table 2, in the Lrp-deficient background, there was a sevenfold reduction in β-galactosidase activity compared with that in the control strain MC4100. These results confirmed that Lrp upregulates *yojI* expression.

Ectopic expression of *yojI* restores MccJ25 resistance in an *E. coli lrp* mutant. In the plasmid pCLO7, *yojI* is under the control of its native promoter. If *yojI* is indeed regulated by Lrp, it is to be expected that cloning of *yojI* devoid of its regulatory sequences and under the control of another promoter should lead to Lrp-independent expression of the gene. To test this prediction, strains MC4100 and RO64 were transformed with the plasmid pTrcHyojI, a pTrc6His derivative (25). This plasmid contains *yojI* under the control of the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible *trc* promoter and expresses a C-terminal His-tagged YojI protein. The His tag does not interfere with the ability of YojI to confer microcin resistance (13). Note that the *yojI* gene in this plasmid was derived by amplification of just the coding portion of the wild-type gene and, thus, lacks its regulatory region (25). We then examined the susceptibility of the transformants to MccJ25 (IPTG was not added to agar plates, since we have previously seen that basal expression from the *trc* promoter is enough to confer full resistance). Both the MC4100 and the RO64 cells carrying pTrcHyojI showed complete resistance to MccJ25 (no inhibition halos were detected in a spot-on-lawn test). These results show that ectopic expression of the *yojI* gene from plasmid pTrcHyojI made the expression of *yojI* independent of Lrp, providing strong evidence that *yojI* is the primary point at which the Lrp protein interacts.

In summary, this study demonstrates that the presence of Lrp is required for expression of YojI and that addition of leucine to the culture medium reduces this activation, presumably by antagonizing the effect of Lrp. The attenuation of the effect of Lrp by exogenous leucine has also been shown for some operons regulated by Lrp (5, 26). The mechanism by which Lrp acts is now thought to involve different associations of Lrp monomers. Lrp self-associates to hexadecamers instead of the dimer conformation previously suggested (6, 7, 8). Growth in minimal medium containing leucine leads to a relatively high intracellular concentration of the amino acid, inducing a transition of the hexadecamer into leucine-bound octamers, which are presumed to have little or no affinity for binding sites (8).

Finally, we have recently reported that there is a direct correlation between guanosine 3′,5′-bispyrophosphate (ppGpp) accumulation in stationary-phase strains and MccJ25 resistance. Furthermore, our results indicated that the alarmone is required to induce production of YojI (27). On the other hand, Landgraf et al. (18) demonstrated that ppGpp functions as an activator of *lrp* gene expression. In light of these findings, an interpretation of the present data is that *yojI* is induced indirectly by ppGpp via the increase in cellular Lrp levels. Lrp thus appears as a major determinant of the natural resistance of many *E. coli* strains to MccJ25.

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REFERENCES

1. **Adelman, K., J. Yuzenkova, A. LaPorta, N. Zenkin, J. Lee, J. T. Lis, S. Borukhov, M. D. Wang, and K. Severinov.** 2004. Molecular mechanism of transcription inhibition by peptide antibiotic microcin J25. Mol. Cell **14:**753–762.
2. **Bayro, M. J., J. Mukhopadhyay, G. V. T. Swapna, J. Y. Huang, L.-C. Ma, E. Sineva, P. E. Dawson, G. T. Montelione, and R. H. Ebright.** 2003. Structure of antibacterial peptide microcin J25: a 21-residue lariat protoknot. J. Am. Chem. Soc. **125:**12382–12383.
3. **Blond, A., J. Péduzzi, C. Goulard, M. J. Chiuchiolo, M. Barthélémy, Y. Prigent, R. A. Salomón, R. N. Farías, F. Moreno, and S. Rebuffat.** 1999. The cyclic structure of microcin J25, a 21-residue peptide antibiotic from *Escherichia coli*. Eur. J. Biochem. **259:**747–755.
4. **Calvo, J. M., and R. G. Matthews.** 1994. The leucine-responsive regulatory protein, a global regulator of metabolism in *Escherichia coli*. Microbiol. Rev. **58:**466–490.
5. **Chen, C., and E. B. Newman.** 1998. Comparison of the sensitivities of two *Escherichia coli* genes to in vivo variation of Lrp concentration. J. Bacteriol. **180:**655–659.
6. **Chen, S., M. H. Rosner, and J. M. Calvo.** 2001. Leucine-regulated self association of leucine-responsive regulatory protein (Lrp) from *Escherichia coli*. J. Mol. Biol. **312:**625–635.
7. **Chen, S., Z. Hao, E. Bieniek, and J. M. Calvo.** 2001. Modulation of Lrp action in *Escherichia coli* by leucine: effects on non-specific binding of Lrp to DNA. J. Mol. Biol. **314:**1067–1075.
8. **Chen, S., and J. M. Calvo.** 2002. Leucine-induced dissociation of *Escherichia coli* Lrp hexadecamers to octamers. J. Mol. Biol. **318:**1031–1042.
9. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
10. **De Haan, P. G., W. P. M. Hoekstra, C. Verhoef, and H. S. Felix.** 1969. Recombination in *Escherichia coli*. III. Mapping by the gradient of transmission. Mutat. Res. **8:**505–512.
11. **Delgado, M. A., J. O. Solbiati, M. J. Chiuchiolo, R. N. Farías, and R. A. Salomón.** 1999. *Escherichia coli* outer membrane protein TolC is involved in the production of the peptide antibiotic microcin J25. J. Bacteriol. **181:**1968–1970.
12. **Delgado, M. A., M. R. Rintoul, R. N. Farías, and R. A. Salomón.** 2001. *Escherichia coli* RNA polymerase is the target of the cyclopeptide antibiotic microcin J25. J. Bacteriol. **183:**4543–4550.
13. **Delgado, M. A., P. A. Vincent, R. N. Farías, and R. A. Salomón.** 2005. YojI of *Escherichia coli* functions as a microcin J25 efflux pump. J. Bacteriol. **187:**3465–3470.
14. **Duquesne, S., D. Destoumieux-Garzón, S. Zirah, C. Goulard, J. Péduzzi, and S. Rebuffat.** 2007. Two enzymes catalyze the maturation of a lasso peptide in *Escherichia coli*. Chem. Biol. **14:**793–803.
15. **Ellermeier, C. D., A. Janakiraman, and J. M. Slauch.** 2002. Construction of targeted single copy *lac* fusions using λ Red and FLP-mediated site-specific recombination in bacteria. Gene **290:**153–161.
16. **Haldimann, A., and B. L. Wanner.** 2001. Conditional-replication, integration, excision, and retrieval plasmid-host systems for gene structure-function studies in bacteria. J. Bacteriol. **183:**6384–6393.
17. **Hawley, D. K., and W. R. McClure.** 1983. Compilation and analysis of *Escherichia coli* promoter DNA sequences. Nucleic Acids Res. **11:**2237–2255.
18. **Landgraf, J. R., J. Wu, and J. M. Calvo.** 1996. Effects of nutrition and growth rate on Lrp levels in *Escherichia coli*. J. Bacteriol. **178:**6930–6936.
19. **Manoil, C.** 1990. Analysis of protein localization by use of gene fusions with complementary properties. J. Bacteriol. **172:**1035–1042.
20. **Miller, J. H.** 1992. A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
21. **Mukhopadhyay, J., E. Sineva, J. Knight, R. M. Levy, and R. H. Ebright.** 2004. Antibacterial peptide microcin J25 inhibits transcription by binding within and obstructing the RNA polymerase secondary channel. Mol. Cell **14:**739–751.
22. **Newman, E. B., and R. Lin.** 1995. Leucine-responsive regulatory protein: a global regulator of gene expression in *E. coli*. Annu. Rev. Microbiol. **49:**747–775.
23. **Newman, E. B., R. T. Lin, and R. D'Ari.** 1996. The leucine/Lrp regulon, p. 1513–1525. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. ASM Press, Washington, DC.
24. **Nickerson, C. A., and E. C. Achberger.** 1995. Role of curved DNA in binding of *Escherichia coli* RNA polymerase to promoters. J. Bacteriol. **177:**5756–5761.
25. **Nishino, K., and A. Yamaguchi.** 2001. Analysis of a complete library of putative drug transporter genes in *Escherichia coli*. J. Bacteriol. **183:**5803–5812.
26. **Platko, J. V., and J. M. Calvo.** 1993. Mutations affecting the ability of *Escherichia coli* Lrp to bind DNA, activate transcription, or respond to leucine. J. Bacteriol. **175:**1110–1117.
27. **Pomares, M. F., P. A. Vincent, R. N. Farías, and R. A. Salomón.** 2008. Protective action of ppGpp in microcin J25-sensitive strains. J. Bacteriol. **190:**4328–4334.
28. **Rex, J. H., B. D. Aronson, and R. L. Somerville.** 1991. The *tdh* and *serA* operons of *Escherichia coli*: mutational analysis of the regulatory elements of leucine-responsive genes. J. Bacteriol. **173:**5944–5953.
29. **Rosengren, K. J., R. J. Clark, N. L. Daly, U. Goransson, A. Jones, and D. J. Craik.** 2003. Microcin J25 has a threaded side chain-to-backbone ring structure and not a head-to-tail cyclized backbone. J. Am. Chem. Soc. **125:**12464–12474.
30. **Salomón, R. A., and R. N. Farías.** 1992. Microcin 25, a novel antimicrobial peptide produced by *Escherichia coli*. J. Bacteriol. **174:**7428–7435.
31. **Salomón, R. A., and R. N. Farías.** 1993. The FhuA protein is involved in microcin 25 uptake J. Bacteriol. **175:**7741–7742.
32. **Salomón, R. A., and R. N. Farías.** 1995. The peptide antibiotic microcin 25 is imported through the TonB pathway and the SbmA protein. J. Bacteriol. **177:**3323–3325.
33. **Shine, J., and L. Dalgarno.** 1974. The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA **71:**1342–1346.
34. **Solbiati, J. O., M. Ciaccio, R. N. Farías, and R. A. Salomón.** 1996. Genetic analysis of plasmid determinants for microcin 25 production and immunity. J. Bacteriol. **178:**3661–3663.
35. **Solbiati, J. O., M. Ciaccio, R. N. Farías, J. E. González-Pastor, F. Moreno, and R. A. Salomón.** 1999. Sequence analysis of the four plasmid genes required to produce the circular peptide antibiotic microcin J25. J. Bacteriol. **181:**2659–2662.
36. **Wilson, K. A., M. Kalkum, J. Ottesen, J. Yuzenkova, B. T. Chait, R. Landick, T. Muir, K. Severinov, and S. A. Darst.** 2003. Structure of microcin J25, a peptide inhibitor of bacterial RNA polymerase, is a lassoed tail. J. Am. Chem. Soc. **125:**12475–12483.
37. **Yuzenkova, J., M. Delgado, S. Nechaev, D. Savalia, V. Epshtein, I. Artsimovitch, R. A. Mooney, R. Landick, R. N. Farias, R. Salomon, and K. Severinov.** 2002. Mutations of bacterial RNA polymerase leading to resistance to microcin J25. J. Biol. Chem. **277:**50867–50875.