Single-Gene Deletion Mutants of Escherichia coli with Altered Sensitivity to Bicyclomycin, an Inhibitor of Transcription Termination Factor Rho

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We have screened the entire KEIO collection of 3,985 single-gene knockouts in Escherichia coli for increased susceptibility or resistance to the antibiotic bicyclomycin (BCM), a potent inhibitor of the transcription termination factor Rho. We also compared the results to those of a recent study we conducted with a large set of antibiotics (A. Liu et al., Antimicrob. Agents Chemother. 54:1393-1403, 2010). We find that deletions of many different types of genes increase sensitivity to BCM. Some of these are involved in multidrug sensitivity/resistance, whereas others are specific for BCM. Mutations in a number of DNA recombination and repair genes increase BCM sensitivity, indicating that DNA damage leading to single- and double-strand breaks is a downstream effect of Rho inhibition. MDS42, which is deleted for all cryptic prophages and insertion elements (G. Posfai et al., Science 312:1044-1046, 2006), or W3102 deleted for the rac prophage-encoded kil gene, are partially resistant to BCM (C. J. Cardinale et al., Science 230:935-938, 2008). Deletion of cryptic prophages also overcomes the increased BCM sensitivity in some but not all mutants examined here. Deletion of the hns gene renders the cell more sensitive to BCM even in the Δkil or MDS42 background. This suggests that BCM activates additional modes of cell death independent of Kil and that these could provide a target to potentiate BCM killing.

In Escherichia coli, the ATP-dependent RNA-DNA helicase Rho (2, 31) terminates transcription at numerous sites (5, 10, 34). A recent study (4) revealed that critical Rho-dependent E. coli terminators are found in the cryptic prophages. Thus, Rho, aided by its cofactors NusA and NusG, is required to terminate transcription upstream of toxic foreign genes. Rho is the target for the antibiotic bicyclomycin (BCM) that binds specifically to Rho and inhibits its action (21, 39). BCM enhances transcription of horizontally transferred genes, including the various cryptic prophage genes that are otherwise silenced (4). One of these, the kil gene of the cryptic rac prophage, is lethal to the cell when expressed (18) and represents a major cause of cell death upon Rho inactivation. Cells lacking the kil gene are still sensitive to BCM but only at higher concentrations than that for the wild type (4).

What other pathways render cells sensitive to Rho inactivation? Previous work identified a number of recombinational repair functions that sensitized cells to rho mutations (17). We recently showed that Rho prevents replication fork collapse caused by transcription overrunning replication, resulting in DNA double-strand breaks (36). We wished to determine if other functions were involved in protecting the cell from BCM (intrinsically resistance). We therefore screened the KEIO collection (1) of 3,985 individual E. coli gene deletion mutants to identify mutants hypersensitive to BCM. All KEIO deletions were deliberately constructed so that the kan promoter is directed downstream and the kan gene is followed by no known terminator. This eliminates effects from transcriptional polarity, although translational polarity may, in some cases, still be possible. These mutants were compared with those in a similar study we carried out with 22 other antibiotics (23). We show that many different types of functions protect against Rho inactivation and that mutants lacking some of these functions sensitize the cell to BCM even in the absence of Kil function and other cryptic prophage genes.
containing glucose as a blank. Samples with an optical density greater than 0.500 were diluted 1:4 in minimal glucose. Percent growth was determined by setting respective controls to 100% growth and determining the percent growth of the average of each set of triplicates. An interaction is defined as additive when the percent growth of the antibiotic pair is equal to the product of the two percent growth values for each antibiotic alone (37), here defined as the predicted additive effect (FAE). When the antibiotic pair has a percent growth significantly lower than the predictive additive effect, this is defined as a synergistic interaction.

High-throughput screening method. For further details, see the report by Liu et al. (23). Briefly, cultures from the KEIO collection, maintained on 45 96-well microtiter plates, were taken out of frozen glycerol stored at \(-80^\circ\text{C}\) using the Deutz cryoreplicator (14), transferred to sterile microtiter wells containing 0.5 ml of LB broth (LB), and incubated at 37°C overnight. Approximately 3 to 5 ml of the resulting saturated culture was transferred to microtiter wells with fresh LB medium containing 50 \(\mu\text{g/ml}\) kanamycin to prevent the growth of contaminants (all strains taken from the KEIO collection are Kan\(^+\)). After 3 h of growth, the Deutz cryoreplicator was used to print microdrops of the subculture onto LB agar plates (with no kanamycin) containing various concentrations of BCM and incubated at 37°C overnight.

Construction of double mutants. The following strains (H. Mori and B. Wan- ner, unpublished data) were used as P1 donors to transduce mutants (e.g., the \(\text{kil}\) mutant) from the KEIO collection (1) or strain MDS42 (30) chloramphenicol resistance: the \(\text{trxB}:\text{cat}, \text{fabF}:\text{cat}, \text{hms}:\text{cat}, \text{ydfA}:\text{cat}, \text{ydhT}:\text{cat}, \text{recA}:\text{cat}, \text{argO}:\text{cat}, \text{and dam}:\text{cat}\) strains. Each strain contains a chloramphenicol-resistant marker that replaces the deleted gene region. The methodology used is as described by Miller (27).

Chemicals. Chloramphenicol, nitrofurantoin, neomycin, tobramycin, ciprofloxacin, and triclosan were purchased from Sigma (St. Louis, MO). Ciprofloxacin was purchased from ICN Biomedicals, Inc. (Aurora, OH).

Screening for increased sensitivity to BCM. The entire KEIO collection of almost 4,000 single-gene knockout mutants was screened using the high-throughput method described above against three subinhibitory concentrations of BCM: 20, 23, and 25 \(\mu\text{g/ml}\). The MIC of BCM for the starting strain (BW25113) (11) under these conditions is 30 \(\mu\text{g/ml}\). Approximately \(4 \times 10^7\) exponentially growing cells were spotted onto LB agar plates containing either 20, 23, or 25 \(\mu\text{g/ml}\) BCM and allowed to grow overnight. Mutants displaying inhibited growth at any of the subinhibitory concentrations tested were determined to have increased susceptibility to BCM. All hypersusceptible candidates were then repurified and retested using the same methodology.

Screening for increased resistance to BCM. The entire KEIO collection was also screened for mutants with increased resistance to BCM compared to that of the wild type. The KEIO collection was screened against three higher levels of BCM: 40, 45, and 50 \(\mu\text{g/ml}\). All resistant candidates were then purified from single colonies and retested using the same methodology.

RESULTS

Screening for BCM hypersensitivity. The entire KEIO collection of 3,985 strains (1) was screened for mutants that are more sensitive than the wild type to 25 \(\mu\text{g/ml}\) BCM, using a Deutz cryoreplicator (14) and 96-well plates (see Materials and Methods for details). Mutants showing increased susceptibility were then purified and retested against a series of BCM concentrations, yielding a set of 76 strains with significantly enhanced susceptibility to BCM. Figure 1 displays examples of the data. Figure 2 shows all of the mutants with increased BCM sensitivity. Colors and numbers indicate the different functions lacking in each mutant (see the legend to Fig. 2). The strongest sensitivities are indicated in darker colors. Quantitative values are provided in Table 1. Whereas Rho is the primary target of BCM (39), many different functions provide intrinsic resistance to BCM, including those concerned with DNA replication, recombination, and repair, as well as functions involved in cell wall and cell membrane synthesis, chaperoning, protein synthesis, and general metabolism. In addition, several genes encoding transcriptional regulators suppress BCM sensitivity. Figure 2 also summarizes the susceptibilities of the BCM-sensitive mutants to a set of other antibiotics (23) to identify those with reduced intrinsic resistance to multiple drugs (e.g., the \(\text{tolC}, \text{rimK}, \text{pgmB}\), and \(\text{yciT}\) mutants, among others) or those with a more specific pattern of resistance, such as \(\text{trxA}\) and \(\text{trxB}\), which are hypersensitive only to BCM and rifampin (RIF). Ten of the 83 mutants (18%), shown in bold type, display enhanced susceptibility only to BCM.

Effect of deleting cryptic prophage genes. BCM inhibition of Rho induces transcription read-through of many genes, including the \(\text{kil}\) gene of the cryptic \(\text{raco}\) prophage. Kil expression largely accounts for BCM sensitivity. Deletion of \(\text{kil}\) or of \(\text{kil}\) with all insertion elements and cryptic prophages (strain MDS42) yields significant resistance to BCM (4). We asked whether any of the BCM-sensitive mutants would be suppressed by deletions of cryptic prophages. Figure 3A confirms that deletion of \(\text{kil}\) increases resistance to BCM in otherwise wild-type \(E.\ coli\). It also reveals that MDS42 is more BCM resistant than a strain deleted of \(\text{kil}\) alone. Figure 3B through D detail three mutations that increase wild-type \(E.\ coli\) susceptibility to BCM. With \(\text{argO}\) (Fig. 3B), both \(\text{kil}\) and the multiple deletions in MDS42 suppress the increased sensitivity and restore resistance to wild-type levels. On the other hand, \(\text{hns}\) strains remain sensitive to BCM in a \(\text{kil}\) or an MDS42 background (Fig. 3C). Interestingly, the BCM sensitivity of a \(\text{recA}\) strain is partially suppressed in an MDS42 background but not in a strain bearing only a \(\text{kil}\) deletion (Fig. 3D). We found that the \(\text{recA}\) mutant showed sensitivity in MG1655 (data not shown), which is isogenic with MDS42. Our interpretation is that the cryptic prophages contribute to the increased BCM sensitivity in \(\text{recA}\) strains.
FIG. 2. Sensitivity profile for bicyclomycin (BCM). This sensitivity profile displays 76 single-gene knockout strains with increased sensitivity to BCM and their corresponding, collective sensitivity to 22 other antibiotics (MDS). These strains are organized by gene category, indicated by different colors:

- 1 (red): DNA replication, recombination, repair
- 1A (red): functions indirectly affecting category 1
- 2 (green): transport, efflux, cell wall and membrane synthesis
- 2A (teal): chaperones and functions related to category 2
- 3 (orange): protein synthesis
- 3A (orange): RNA processing
- 4 (blue): central metabolic reactions
- 5 (purple): regulation
- 6 (yellow): prophage-encoded functions, cell adhesion
- 7 (black): unassigned genes

Sensitivity to BCM is indicated with one of four levels of intensity: darker shades indicate stronger susceptibilities, lighter shades indicate medium susceptibilities, the lightest shades indicate medium-weak susceptibilities, and the lightest, halved shades indicate the weakest susceptibilities. The genes indicated in bold are uniquely sensitive to BCM.

MDS indicates the degree of multidrug sensitivity of each BCM-sensitive strain. Darker brown shades indicate sensitivity to 8 or more other antibiotics, medium brown shades indicate sensitivity to 4 to 7 other antibiotics, light brown shades indicate sensitivity to 1 to 4 other antibiotics, and no shading indicates unique sensitivity to BCM. dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; ACP, acyl carrier protein.

### Gene Product Description

<table>
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<tr>
<th>Gene</th>
<th>BCM</th>
<th>MDS</th>
<th>Categ</th>
<th>Gene Product Description</th>
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<td>DNA adenine methylase</td>
<td>1</td>
<td>DNA strand exchange and recombination protein</td>
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<tr>
<td><strong>recA</strong></td>
<td>DNA helicase, dsDNA/ssDNA exonuclease</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>recB</strong></td>
<td>DNA helicase, dsDNA/ssDNA exonuclease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>recC</strong></td>
<td>ssDNA and dsDNA binding, subunit of RecFOR complex</td>
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<tr>
<td><strong>recD</strong></td>
<td>DNA repair protein; subunit of RecFOR complex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>recE</strong></td>
<td>Recombination and repair protein, subunit of RecFOR complex</td>
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<td></td>
<td></td>
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<tr>
<td><strong>recF</strong></td>
<td>DNA helicase, resolution of Holliday junctions</td>
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<tr>
<td><strong>recG</strong></td>
<td>Single-stranded-DNA-specific exonuclease recG</td>
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<td><strong>recH</strong></td>
<td>Recombination and repair protein</td>
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<td></td>
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<tr>
<td><strong>recI</strong></td>
<td>E. coli ABC transporter, subunit C; UV damage repair</td>
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<tr>
<td><strong>wgrA</strong></td>
<td>DNA-dependent ATPase I and helicase II</td>
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<tr>
<td><strong>wseC</strong></td>
<td>Exodeoxyribonuclease VII large subunit</td>
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<td><strong>yebG</strong></td>
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<td>Replication fork repair protein</td>
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<td>Thiorodoxin reductase</td>
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<td><strong>yoeI</strong></td>
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<tr>
<td><strong>aceA</strong></td>
<td>AcrAB-TolC Multidrug Efflux transporter, periplasmic lipoprotein subunit</td>
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<tr>
<td><strong>vocC</strong></td>
<td>AcrAB-TolC Multidrug Efflux transporter, outer membrane subunit</td>
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<tr>
<td><strong>argQ</strong></td>
<td>Arginine outward transporter</td>
<td></td>
<td></td>
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<tr>
<td><strong>cncC</strong></td>
<td>Cytokinesis - murein hydrolase</td>
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<tr>
<td><strong>cpxD</strong></td>
<td>Putative curl production protein</td>
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<tr>
<td><strong>deh</strong></td>
<td>D-alanine-D-alanine ligase, B subunit</td>
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<tr>
<td><strong>deoD</strong></td>
<td>Putative lipoprotein - inner membrane</td>
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<td><strong>nhaA</strong></td>
<td>Sodium/proton transporter</td>
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<tr>
<td><strong>nhpC</strong></td>
<td>NhpC-putative lipoprotein hydrolase</td>
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<tr>
<td><strong>pgrA</strong></td>
<td>Peptidoglycan-associated lipoprotein precursor</td>
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<tr>
<td><strong>glnA</strong></td>
<td>2</td>
<td>Aminoacyl-tRNA synthetase</td>
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<tr>
<td><strong>glnB</strong></td>
<td>Phosphatidylglycerophosphatidylglycerol B</td>
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<tr>
<td><strong>lufB</strong></td>
<td>Sec-independent protein translocase</td>
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<td></td>
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<tr>
<td><strong>luxC</strong></td>
<td>Sec-independent protein translocase</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>yehO</strong></td>
<td>Predicted inner membrane protein</td>
<td></td>
<td></td>
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<tr>
<td><strong>yceF</strong></td>
<td>Probable intracellular seption protein</td>
<td></td>
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<tr>
<td><strong>ydeG</strong></td>
<td>Predicted inner membrane protein</td>
<td></td>
<td></td>
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<tr>
<td><strong>ydeH</strong></td>
<td>Predicted inner membrane protein</td>
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<td>Predicted inner membrane protein</td>
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<tr>
<td><strong>hscA</strong></td>
<td>Hap20; member of Hap70 protein family</td>
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<tr>
<td><strong>lon</strong></td>
<td>DNA-binding, ATP-dependent protease</td>
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<td><strong>yapA</strong></td>
<td>Peptidyl-prolyl cis-trans isomerase and chaperone</td>
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<td><strong>yedK</strong></td>
<td>Regulator of FtsI protein</td>
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<td><strong>rimI</strong></td>
<td>Ribosomal-protein-alanine acetyltransferase</td>
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<td><strong>rimK</strong></td>
<td>Ribosomal protein S6 modification protein</td>
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<td><strong>yedL</strong></td>
<td>SOS ribosomal protein L1</td>
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<td><strong>yedN</strong></td>
<td>Subunit of 50S ribosomal subunit</td>
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<td><strong>gcnB</strong></td>
<td>3A, Pol(A) polymerase (Plasmid copy number protein)</td>
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<td><strong>rlmE</strong></td>
<td>3A, SOS ribosomal protein L36</td>
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<td><strong>gapH</strong></td>
<td>3A, RNA pyrophosphohydrolase</td>
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<td><strong>catA</strong></td>
<td>4</td>
<td>Cardiolipin synthetase</td>
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<tr>
<td><strong>dadF</strong></td>
<td>4</td>
<td>Diaminopimelate epimerase</td>
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<td><strong>flgB</strong></td>
<td>4</td>
<td>E-lactococcal ACP synthase</td>
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<td><strong>yaeU</strong></td>
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<td>Iron-sulfur cluster scaffold protein</td>
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<td><strong>gpmM</strong></td>
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<td>Phosphoglycerate mutase, cofactor independent</td>
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<td><strong>ileS</strong></td>
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<td>Cystine desulfurase monomer</td>
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<td><strong>yjeC</strong></td>
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<td>F-prophage/acytoplasmic</td>
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<td>Predicted 4Fe-4S cluster-containing protein</td>
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<td>Glucose-6-phosphate 1-dehydrogenase</td>
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<td><strong>vedA</strong></td>
<td>5</td>
<td>Cell division modulator</td>
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<tr>
<td><strong>fur</strong></td>
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<td>Ferric uptake regulation protein,</td>
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<td>H-NS transcriptional dual regulator</td>
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<td>Transcriptional regulatory protein</td>
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<tr>
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<td>DNA-binding transcriptional regulator (deoT)</td>
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<td><strong>zgrQ</strong></td>
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<td>Qm prophage; predicted protein</td>
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<td>Qm prophage; predicted protein</td>
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<td>e1.4 prophage; predicted DNA transcriptional regulator</td>
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TABLE 1. Bicyclomycin sensitivity of single mutants listed by level of susceptibility

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<th>Strain</th>
<th>MIC (µg/ml)</th>
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<td>BW25113...</td>
<td>30</td>
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<tr>
<td>yafB' mutant</td>
<td>≤20</td>
</tr>
<tr>
<td>ycdC' mutant</td>
<td>≤20</td>
</tr>
<tr>
<td>hns mutant</td>
<td>≤20</td>
</tr>
<tr>
<td>ycdM mutant</td>
<td>≤20</td>
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<tr>
<td>ycdF' mutant</td>
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<tr>
<td>dnaK mutant</td>
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<td>ydfA mutant</td>
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<td>rimK mutant</td>
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<td>recD mutant</td>
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<tr>
<td>dapa' mutant</td>
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<td>&gt;25</td>
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<tr>
<td>ycdZ' mutant</td>
<td>&gt;25</td>
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* Gene knockouts are from the KEIO collection, using BW25113 as the starting strain. Strains listed in bold are uniquely sensitive to bicyclomycin.

Single-gene knockouts that increased resistance to BCM. We also screened the entire KEIO collection for mutants more resistant to BCM than the wild type. Table 2 lists mutants that are resistant to BCM concentrations of 40 µg/ml or higher. Again, mutants defective in many types of functions are involved. Of particular note are mutants deleting the e14 prophage and the hsf gene (see Discussion). The cutoff here of 40 µg/ml is too high to detect the kil deletion in this collection, as this mutant does not grow above 32 µg/ml BCM (see Fig. 3).

Pairwise interactions with other drugs. We also examined a number of pairwise interactions as described by Yeh et al. (37). Table 3 shows the results of testing subinhibitory concentrations of BCM in minimal medium with subinhibitory concentrations of nine different antibiotics. Only the two aminoglycosides tested (NEO, TOB) were synergistic with BCM.

DISCUSSION

We employed the antibiotic bicyclomycin (BCM), a potent, specific inhibitor of Rho (39), to identify E. coli mutants with changes in sensitivity to the drug. Cardinale et al. (4) used microarrays to show that BCM treatment preferentially increases the expression of genes derived from recent horizontal transfer, including cryptic prophage as well as noncoding intergenic regions. A major cause of death upon Rho inactivation is expression of the kil gene of the rae cryptic prophage, whose product, KilR, inhibits the essential cell division function FtsZ (7). Cells deleted of kil were relatively resistant to BCM, despite massive gene deregulation due to read-through of terminators at the ends of operons. Our screen of a set of E. coli deletion mutants with altered BCM resistance (Fig. 1) defines the genes involved in intrinsic resistance to BCM and also provides information on the type of downstream events that occur after inactivation of Rho by BCM. Some of the gene deletions identified in Fig. 1 that increase sensitivity to BCM are involved in general intrinsic resistance to multiple antibiotics, as indicated by the intensity of color in the MDS (multidrug sensitivity) column. These include genes involved in recombination and recombinational repair of double-strand breaks (recA, -B, -C), the main efflux pump in E. coli (acrA, tolC), genes involved in cell wall and cell membrane synthesis and integrity, and transporters and chaperones. This also includes several genes encoding ribosomal proteins (rplA, rmlI) or their modification (rimK), a variety of transcriptional regulators (fur, hns, yciT), and genes with uncharacterized functions.

Recent work identified a number of recombinational repair functions that support resistance to BCM. It is proposed that Rho prevents replication fork collapse and double-strand breaks caused by replisome/RNA polymerase (RNAP) collisions (36). The numerous DNA recombination and repair functions in Fig. 1 support these findings. The recA, -B, and -C system repairs double-strand breaks, and the recFORJ system is involved in the repair of single-strand breaks (6, 24, 33). Mutants lacking the latter system increase sensitivity to only a select group of antibiotics, namely, those that damage DNA. Similarly, the recovery of uvrC, uvrD, and uvrE in mutants (Fig. 1) further supports the idea that BCM treatment ultimately leads to DNA damage that is normally prevented by Rho. Interestingly, BCM does not induce the SOS system (36). We
have physically detected chromosomal breaks in cells treated with BCM. This underscores the complexities of antibiotic action (see, e.g., reference 19). Of particular interest are mutants that are specifically sensitive to BCM and not to any of the other antibiotics. Three of these appear to involve DNA repair proteins, the rdgC, yebG, and yfjY mutants. The ring-structured RdgC protein binds DNA and is associated with recombination and replication fork repair (3). Because it inhibits RecA-mediated DNA strand exchange reactions (15), it is considered to be a negative regulator of RecA action. Mahdi et al. (25) proposed that RecA drives the reversal of collapsed forks, rendering cells more dependent upon recombination to reestablish replication. In Fig. 1, we can see specific sensitivities within each category. For example, mutants lacking the thioredoxin/thioredoxin reductase system (the trxA and trxB mutants) (13) are specifically sensitive to BCM and RIF, and two mutants defective in septation, the minC and yciB mutants, are partially or completely specific for BCM. Mutants lacking the 50S ribosomal protein L36 (rpmI mutant) or the ribosomal protein alanine acetyltransferase (rimI mutant) are sensitive to only BCM among the antibiotics tested.

### TABLE 2. Bicyclomycin-resistant single mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (μg/ml)</th>
<th>Gene production description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW25113</td>
<td>30</td>
<td>RNA chaperone facilitating sRNA-mRNA pairing interactions</td>
</tr>
<tr>
<td>hff mutant</td>
<td>&gt;50</td>
<td>Acetyl-coenzyme A synthetase (AMP forming)</td>
</tr>
<tr>
<td>proC mutant</td>
<td>50</td>
<td>Proline ABC transporter</td>
</tr>
<tr>
<td>recB mutant</td>
<td>50</td>
<td>Anti-sigma factor</td>
</tr>
<tr>
<td>efeR mutant</td>
<td>50</td>
<td>Peroxidase; cryptic ferrous iron transporter</td>
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<tr>
<td>tmrU mutant</td>
<td>50</td>
<td>tRNA methyltransferase</td>
</tr>
<tr>
<td>fltG mutant</td>
<td>50</td>
<td>Flagellar motor switch protein</td>
</tr>
<tr>
<td>flgY mutant</td>
<td>50</td>
<td>Flagellar P-ring protein</td>
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<tr>
<td>hnsF mutant</td>
<td>50</td>
<td>Purine nucleoside phosphoromutase</td>
</tr>
<tr>
<td>ynaI mutant</td>
<td>50</td>
<td>Inner membrane protein</td>
</tr>
<tr>
<td>adhE mutant</td>
<td>45</td>
<td>Alcohol/acetaldehyde dehydrogenase</td>
</tr>
<tr>
<td>caiC mutant</td>
<td>45</td>
<td>Crotonobetainyl-coenzyme A reductase</td>
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<tr>
<td>rgyE mutant</td>
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<td>MFS transporter</td>
</tr>
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<td>hflE mutant</td>
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<td>ATP-dependent RNA helicase</td>
</tr>
<tr>
<td>gold mutant</td>
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<td>UDP-glucose 4-epimerase</td>
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<td>yciC mutant</td>
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<tr>
<td>gloB mutant</td>
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<tr>
<td>hypB mutant</td>
<td>40</td>
<td>Maturtion peptidease for hydrogenase 2</td>
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<td>yneJ mutant</td>
<td>40</td>
<td>Predicted DNA-binding transcriptional regulator, LysR type</td>
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<td>proA mutant</td>
<td>40</td>
<td>Transcription elongation factor</td>
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<tr>
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<td>Lysyl-tRNA synthetase</td>
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<td>e14 prophyte</td>
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<td>seq4 mutant</td>
<td>40</td>
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</tr>
<tr>
<td>proA mutant</td>
<td>40</td>
<td>Putative regulator of pyruvate oxidase</td>
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</table>

* Gene knockouts are from the KEIO collection, using BW25113 as the starting strain.
tease-deficient lon mutant is hypersensitive only to BCM and metronidazole. This is consistent with the finding that Lon protease degrades SulA (28), a potent inhibitor of FtsZ (8, 20), reducing SulA’s additive effect with BCM-stimulated expression of KilR. Highlighting the importance of FtsZ as a target of KilR is the finding that a deletion of hflq (22), a negative regulator of FtsZ (35, 38), yields cells that are more resistant to BCM (Table 2). For future applications, one might take advantage of these specific mutants in screening for antibiotics with properties similar to BCM.

Sozhamannan and Stitt (32) proposed a role for Rho in RNA degradation. Deletion of penB, encoding poly(A) polymerase 1 that polyadenylates RNAs, increased mRNA half-lives (29) and enhanced BCM sensitivity (Fig. 2). Moreover, deletion of rppH, which triggers mRNA degradation (12), also increased BCM sensitivity. Both of these results are consistent with the theory that Rho is involved in RNA turnover (32). It is possible that stabilization of the kilR transcript accounts for the hypersensitivity of these mutants, although an overall increase in RNA and RNA-DNA hybrids, toxic in Rho-deficient cells, is not ruled out.

We show that some of the increased susceptibilities to BCM could be overcome by deletion of kil alone or by deletion of all cryptic prophages and horizontally acquired genetic elements (strain MDS42). The deletions also increase BCM resistance in a wild-type background (4). Interestingly, in a BCM-sensitive mutant lacking the global regulator HNS, deletion of kil or placing the mutation in the MDS42 background did not enhance resistance. This raises the possibility of finding codrugs, or potentiators, of BCM by looking for inhibitors of, in this case, the HNS protein. Such an approach (9) might expand the use of BCM to other bacteria that lack the rnc prophage. We (36) have studied a set of mutants, several of which are not in the KEIO collection, that also enhance the sensitivity of MDS42 to BCM. Figure 3 also shows that MDS42 is more resistant to BCM, in some circumstances, than strains lacking kil alone, suggesting that other kil-like-encoded functions missing in MDS42 contribute to BCM toxicity. A candidate for one of these functions might be the ymfB gene of the e14 prophage (26), since deletion of this gene increases resistance to BCM (Table 2). This prophage carries a kil analogue, which Cardinale et al. (4) found to be upregulated approximately 10-fold by BCM.

We tested pairwise combinations of BCM with 9 different antibiotics (Table 3). Yeh et al. (37) have classified antibiotics into groups based on the patterns of their pairwise interactions. The data shown in Table 3, though not as extensive as that from Yeh et al., would at this point place BCM in the same grouping as CIP. This may not be surprising, given that BCM treatment results in expression of the Kil function that inhibits the cell division protein FtsZ and also inactivates Rho, leading to increased replisome/RNAP collisions and subsequent double-strand breaks (36), while CIP binds to DNA gyrase and blocks DNA synthesis, leading to double-strand breaks (16).

**ACKNOWLEDGMENTS**

We thank Barry Wanner for helpful advice. This work was supported by grant GM37219 from the National Institutes of Health to M.E.G. and by grant ES0110875 to J.H.M.

**REFERENCES**


**TABLE 3. Pairwise interactions**

<table>
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<tr>
<th>Antibiotic</th>
<th>% BCM-only growth</th>
<th>% antibiotic-only growth</th>
<th>% predicted additive growth (PAE)</th>
<th>% BCM + antibiotic growth</th>
<th>Interaction</th>
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<td>Cephradine (RAD)</td>
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<td>65.1</td>
<td>46.2</td>
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<td>82.6</td>
<td>61.8</td>
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<td>Nitrofurantoin (NIT)</td>
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<td>78.3</td>
<td>55.7</td>
<td>54.9</td>
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<td>Chloramphenicol (CHL)</td>
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<td>Erythromycin (ERY)</td>
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