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Determination of Hypersensitivity to Genotoxic Agents Among *Escherichia coli* Single Gene Knockout Mutants

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Summary

We have tested the KEIO collection of 3985 different viable single gene knockouts in *Escherichia coli* to identify genes whose loss increases sensitivity to one or more of six different chemotherapeutic agents and mutagens: Bleomycin (BLM), Cisplatin (CPT), ICR-191 (ICR), 5-azacytidine (5AZ), Zebularine (ZEB), and 5-bromo-2'-deoxyuridine (5BdU). We discovered a set of 156 strains that display a significant increase in sensitivity to at least one of the agents tested. Each genotoxic agent generates a distinct "sensitivity profile" that is characteristic of the agent. Comparison with an independent study of sensitivity profiles for an extensive set of antibiotics pinpoints those effects that are relatively specific for each agent. In some cases engineered double mutants have greatly increased effects. These results provide insight into the mechanism of action of each agent, and define targets for the design of co-drugs that can potentiate these agents. An example is the finding that mutants lacking one of several genes in the folate biosynthetic pathway are hypersensitive to ZEB, leading to a demonstration of synergy between trimethoprim and ZEB.

Introduction

Bacteria have often served as model systems for the interaction of genotoxic agents with DNA, and for analyzing the effects of mismatch repair (MMR), nucleotide excision repair (NER), base excision repair (BER), and recombinational repair systems on DNA lesions, as exemplified by studies with cisplatin [1–9], leading to similar studies of DNA repair of such lesions in higher cells [7,10–14]). In this study we sought mutants in *Escherichia coli* that have increased sensitivity to one of six or more genotoxic agents [bleomycin (BLM), cisplatin (CPT), ICR-191 (ICR), 5-azacytidine (5AZ), zebularine (ZEB), and 5-bromo-2'-deoxyuridine (5BdU)], to define the cell's intrinsic resistance to them, the intrinsic resistome, and to uncover unexpected pathways involved in sensitizing cells to one or more of these agents. Some of these pathways might have counterparts in higher cells and thus prove useful in sensitizing human cells to lower doses of these agents. This would reduce the toxic side effects associated with drug treatment [10,15], as well as the secondary tumors resulting from the mutagenic effects of the agents themselves. Moreover, the increased potency of the drug could retard the development of resistance that often limits treatment [16].

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The six genotoxic agents, BLM, CPT, ICR-191, 5AZ, ZEB, and 5BdU (see Figure 4) that we used to screen the KEIO collection of gene knockouts in *E. coli* for hypersensitivity are both cytotoxic and mutagenic in *E. coli*. However, they are also used in various cancer chemotherapeutic programs, and in some cases are linked to human disease. BLM exerts its effects by chelating iron and other metal ions, ultimately generating superoxide and hydroxide free radicals that cause double-strand breaks in DNA. These are cytotoxic if unrepaired [17,18]. BLM is part of effective regimens, often with CPT, for the treatment of testicular cancer [19], and also for Hodgkins lymphoma [20]. CPT acts by making inter- and intra- strand cross links in DNA [2–4], thus interfering with DNA replication, and is used to treat a variety of cancers, being particularly effective against testicular tumors [21–26]. ICR (ICR191 [27]) is an effective frameshift mutagen in bacterial and eukaryotic cells [28–31]. It causes increased killing of repair deficient HCT116 cells compared with repair proficient cells, and has been suggested as a chemotherapeutic compound on that basis [32]. 5AZ and ZEB [33] are cytidine analogs that are incorporated into DNA and RNA during replication and transcription. This leads to mutations in bacteria and higher cells [e.g. 34–37]. The anticancer effects of 5AZ [38,39] and ZEB [33,44] occur by interfering with the methylation of DNA, thereby reversing the effects of methylation-induced silencing of tumor suppressor genes [38–41]. 5AZ [38] and ZEB [41,52] inhibit DNA methyltransferase, and ZEB is also a competitive inhibitor of cytidine deaminase [45], an enzyme that reduces the potency of cytidine analogs. Therefore ZEB is used in combination with 5AZ to enhance the antitumor activity of 5AZ [e.g. 46]. 5BdU was found to increase the cytotoxicity induced by 1,3-bis-(2-chloroethyl)-1-nitrosourea and cisplatin in human glioma cells [47], and was previously used to sensitize cells to radiation therapy [48]). It is worth noting that phagocytes produce 5BdU via myeloperoxidase in human inflammatory tissue, and this may contribute to the link between chronic inflammation and cancer [49].

In the work reported here, we screened the KEIO collection of 3,985 mutants [50], each with a different gene knockout, for strains that were more sensitive to one or more drugs, and then quantified the effects by defining the minimum inhibitory concentration, MIC [51]. In some cases engineered double mutants have pronounced effects. We detected a number of mutants with altered DNA recombination and repair that have been reported in previous studies [e.g. 1–6,8–9; see Discussion for details], but also found numerous additional mutants affected in functions related not only to DNA, but also cell wall and cell membrane, different metabolic reactions, and gene regulation. We thus have generated a “sensitivity profile” for each agent that represents an additional dimension in agent characterization, namely the response of a comprehensive set of mutants to each chemical.

Materials and Methods

E. coli strains

The KEIO collection is, as described in Baba *et al.* [50], from the starting wild-type strain BW25113 [52]. This strain (*lacI^q rrnB_{T14} ΔlacZ_{WJ16} hsdR514 ΔaraBAD_{AH533} ΔrhaBAD_{L_{D78}}*) is the wild-type strain used in the experiments reported here, unless otherwise stated. Briefly, each of the 3,985 strains in the KEIO collection carries a complete deletion of a different gene, with a *kan* insert replacing each gene. We used the following strains for donors of the indicated markers by P1 transduction. CGSC6911 – *tolC6::miniTn10* – (*E. coli* genetic stock center); CGSC7553 – *recG265::cat* – (*E. coli* Genetic stock Center); DY330 [53] was converted to *recC::cat* [54] and used as a donor for P1 transduction. N3055 (*uvrA::Tn10*) was a gift of Graham Walker.

***E. coli* genetic methods**

Unless otherwise stated, all genetic methods are as described by Miller [55]. P1 transduction was used to generate double mutants in the BW25113 background.

Plates with Agents

We prepared LB plates with different concentrations of each agent by making a fresh stock solution (1 mg/ml CPT, BLM, ICR, ZEB, & 5dBU in sterile distilled water, and 24 mg/ml 5AZ in DMSO), dissolving for at least one hour, and adding to the agar just prior to pouring. All plates were used within several hours of preparation.

Screening with the Deutz cryoreplicator

The Deutz cryoreplicator [56] contains 96 prongs on individual springs, allowing its frequent application to frozen glycerol cultures. The KEIO collection [50] is maintained on two sets of 45 96-well micro titer plates and stored in glycerol at -80°C . Each set of 45 plates contains one copy of the entire collection. We used the odd numbered plate set (plates 1,3,5,7...etc). A Deutz cryoreplicator was used to transfer cells from the frozen plates to 0.5ml media in 96-well plates, which were incubated overnight and then the replicator was used to transfer a microdrop to microtiter wells with fresh LB medium containing 50 $\mu\text{g/ml}$ kanamycin, to prevent the growth of contaminants. After 3 hours of growth these plates were printed onto LB plates with different concentrations of each agent. For the initial screening, kanamycin was present in the plates, but in final retests and MIC determinations kanamycin was not present. Initially, 200 $\mu\text{g/ml}$ of CPT, 0.3 $\mu\text{g/ml}$ BLM, and 20 $\mu\text{g/ml}$ ICR was used to screen all 45 plates in the collection. (Figure 1 shows an example). The MIC (minimum inhibitory concentration) for the starting strain (BW25113) is 250 $\mu\text{g/ml}$ for CPT, 1.25 $\mu\text{g/ml}$ for BLM, and >30 $\mu\text{g/ml}$ for ICR. Strains appearing more sensitive to one of these agents were retested at 50, 100, 150, 200, and 250 $\mu\text{g/ml}$ CPT, 0.125, 0.313 and 0.5 $\mu\text{g/ml}$ BLM, and 10, 15, and 20 $\mu\text{g/ml}$ ICR. We created a condensed library consisting of all the sensitive strains as well as 283 strains found to be sensitive to one of 22 antibiotics (57). We tested 5AZ and ZEB against the condensed library of strains at different concentrations (5AZ 60, 80 and 100 $\mu\text{g/ml}$ and ZEB 60 and 100 $\mu\text{g/ml}$). The MIC of the starting strain was determined to be >200 $\mu\text{g/ml}$ 5AZ and 150 $\mu\text{g/ml}$ ZEB. Then we tested the condensed library from the first three screens against an array of 5BdU concentrations (50, 100, 200, and 300 $\mu\text{g/ml}$), and the only mutant found to be hypersensitive to 5BU was *dam*. While the starting strain was found to have a MIC of >1000 $\mu\text{g/ml}$ 5BU, the MIC of the *dam* mutant was 50 $\mu\text{g/ml}$.

Determination of MIC

Minimum inhibitory concentrations [18] were determined either by applying 10^4 – 10^5 colony forming units to an LB plate with the appropriate concentration of the desired antibiotic and examining the plate after 18 hr incubation at 37°C , or by inoculating liquid cultures with 10^5 cells and observing growth after 18 hours [18]. Most of the mutants in the KEIO collection grow indistinguishably from the wild-type on LB plates under the conditions tested.

Combination experiments with zebularine and trimethoprim

The starting strain was grown over night in minimal medium (Medium A [55]), then subcultured for 6 hrs, also in minimal medium until a density of $\sim 10^8$ cells. The subculture was then diluted 10^{-2} , and 50 μl of the dilution was added to 5ml of minimal media with or without each agent. Cultures were incubated at 37°C for 18 hrs, and then the optical density of each culture was taken at 600 nm. First, concentrations of ZEB and trimethoprim (TMP) to be used were determined ($>70\%$ growth for each drug alone as compared to the control). A concentration of 0.03 $\mu\text{g/ml}$ TMP and two concentrations of 0.04 $\mu\text{g/ml}$ and 0.05 $\mu\text{g/ml}$

ZEB were determined. For the experiments, a control (no drug), each drug alone, and TMP in combination with both ZEB concentrations were all tested in triplicates.

Colony Density Analysis

96-well colony grids were photographed using a Canon 8.1 megapixel camera. The images were then cropped before analysis. Density Analysis of the colonies was performed using software ImageJ (<http://rsbweb.nih.gov/ij/>). First, background removal was performed uniformly across every image, and then the contrast for each was increased uniformly by 2%. The raw values obtained from this program were then input into excel, in which the raw values of the two control strains, *ebgA* and *bglB*, were subtracted from the values of all other colonies. The absolute values resulting from this indicate the level of sensitivity, higher values being most sensitive and vice versa. These adjusted values were then combined into a single chart, which was then condensed by setting a cutoff value of 7,000 for each genotoxic agent tested. However, these values only account for one concentration of each agent, and thus do not give the same rank-order information as is given by visual inspection, though still providing accurate quantitative sensitivities. Additionally, mutants that only show growth of revertant colonies yield lower sensitivity values due to a strong signal from their dense growth. This phenomenon, as well as the high cut-off values chosen for these values in an attempt to minimize noise, is responsible for lower number of hits in this table when compared to Figure 2 (described in Figure S3), although all the hits from this method were found via visual inspection.

Validation

A collection of 3,985 strains will contain some errors, and some impure strains. Mori and coworkers (58) have published a validation of the entire collection, extensively testing each strain, and identifying those that are incorrect or are suspected to be incorrect. We have removed these strains from our charts and tables. The impurity problem can be minimized by repurifying and retesting, as was done here. We also analyzed 22 mutants that were of interest by PCR analysis, and/or sequencing, using two primer pairs for each, and verified in two PCR experiments for each that they all carried a *kan* insert in place of the correct gene, and that there was no presence of the original gene sequence elsewhere (via a duplication). We reported these validations for mutants deleted for *argO*, *degP*, *dacA*, *glpD*, *trxB*, *xseA*, *sapC*, *rplI*, *tufA*, *ycdZ*, *dinB*, *recA*, *oxyR*, *rpsf*, *flgB*, *ppiD* (57), and carried out the same analysis for *uvrA*, *uvrC*, *udk*, *umuC*, and *umuD* in the current study. In the case of *rpsF*, we carried out both PCR and sequencing for validation. An additional two mutants can be identified by their phenotypes (*dam*, mutator; *lon*, mucoid formation). We had reason to suspect the *recF* deletion mutant, and a similar analysis revealed that the *recF* deletion strain in the first copy of the KEIO strains is incorrect, although the *recF* strain in the second copy is correct [57], and this has been used in the work reported here.

Chemicals

Cisplatin, ICR-191, 5-azacytidine, 5-bromo-2'-deoxyuridine, trimethoprim, and kanamycin were purchased from Sigma (St. Louis, MO). Bleomycin was purchased from CalBiochem (San Diego, CA). Zebularine was a gift from Victor E. Marquez.

Results

Screening for increased sensitivity to Genotoxic Agents

The entire KEIO collection of 3,985 single gene knockout strains of *E. coli* was screened for mutants with increased susceptibility to CPT, BLM, and ICR (see Materials and Methods). The mutant strains that appeared to have greater sensitivity than the wild-type were purified

from single colonies and retested against a set of concentrations for each of the three agents used in the screen, generating a condensed mutant library. This library was added to a condensed library of 283 strains detected by screening for increased sensitivity to any of 22 different antibiotics [57]. The condensed library was then tested against 5AZ, ZEB, and 5BdU at varying concentrations. Additionally, 5AZ and ZEB were screened against the half of the KEIO collection that contains most of the assigned genes. Figure 1B shows a set of cropped images of mutants grown on different concentrations of CPT to illustrate the varying sensitivities between the strains.

The results from all of the tests are summarized in Figure 2, which lists the strains with the highest sensitivity to all six agents using a three-tiered color designation for each data point, the darkest color depicting greatest sensitivity (See Figure S1 for the specific MIC values). The sensitivities of the strains listed in this table were scored by visual inspection, taking their growths across various concentrations into account. Figure 2 also compares the data for the six agents with a compilation of the results from our studies of 22 antibiotics tested against the same KEIO collection [57]. (Although 283 gene knockout mutants show increased sensitivity to one of the antibiotics tested, we have only considered those mutants that are more sensitive to at least one of the six genotoxic agents tested). This comparison allows us to distinguish mutants that show sensitivity to multiple antibiotics and genotoxic agents from mutants that show more specific effects, and both types of mutants are evident. The MDS column in Figure 2 indicates the degree of multidrug sensitivity for each knockout mutant, with the darkest shade showing the highest degree of MDS, and the empty (white) boxes indicating no increased sensitivity to any of the antibiotics tested. We consider these results in more detail in the Discussion.

Quantifying sensitivity values

Digital photographs of the retested strains were analyzed using colony density analysis software (described in Materials and Methods). To minimize background, sensitivity values were adjusted according to two control strains, and a cut-off value was chosen. The adjusted values above this cutoff were taken and compiled into a list of strains shown in Figure S2. All of these strains had been found by visual inspection as described above for Figure 2.

Testing double knockout mutants for greater sensitivity

We constructed a number of double knockout strains (see Materials and Methods) to test for further increases in sensitivity to CPT. The results are shown in Table 1. It can be seen that in a number of cases the double mutants are considerably more sensitive than either single mutant. For example, *uvrA recC* and *uvrA recG* double mutants display a MIC of 25 µg/ml with CPT, whereas each parent single mutant displays a MIC of 100 µg/ml CPT.

Combination treatments

One of the objects of this work is to detect pathways or proteins that when inhibited might increase the sensitivity to a particular agent. There are four mutants, *ygfA*, *folB*, *folP*, and *nudB*, defective in different reactions in the folate biosynthetic pathway that are more sensitive to one or more of the agents tested. The hypersensitivity of three of these to ZEB led us to examine whether a combination of trimethoprim (TMP), an inhibitor of dihydrofolate reductase, and ZEB would show synergistic effects. Figure 3 displays the results using ZEB and TMP in combination against the starting strain, BW25113, which clearly show that concentrations of TMP and ZEB that give little growth inhibition by themselves result in virtually a total inhibition of growth when used in combination.

Discussion

We chose to look at mutants that are more sensitive to at least one of six genotoxic agents, CPT, BLM, ICR, 5AZ, ZEB, and 5BdU, in order to uncover pathways of damage avoidance and repair not yet described for these agents, and to define the total “intrinsic resistome” for each compound. We screened the KEIO collection [50] of 3,985 single gene knockout *E. coli* strains and after retesting defined a set of 156 mutants with increased sensitivity to one or more of the agents. Figure 2 lists these mutants according to the function affected. Previous work on mutants sensitive to these agents has focused on those deficient in DNA recombination and repair. CPT, BLM, and 5AZ have been well studied, ICR and 5BdU less so in this regard, and ZEB virtually not at all. Marinus, Essigmann, and coworkers [5,6,9], among others [1,2], have shown that mutants deficient in the *recA,B,C* pathway, *recF*, and nucleotide excision repair deficient mutants such as *uvrA*, are more sensitive to CPT than wild-type, as are *dam* mutants [3]. Some of these sensitivities emanate from CPT-induced DNA double-stranded breaks [59]. Additional work from Marinus and Essigmann [60] showed that *recA,B,C,D*, and also *recN*, *recG*, and *ruvC*, but not *recF*, are more sensitive to BLM than wild-type. The results in Figure 2 are in good agreement with these findings, particularly considering that different types of measurements have been used. Ikeda and coworkers [61] have reported moderately increased sensitivity of *hns* mutants to BLM at 30°C, but we have not detected sensitivity in our experiments at 37°C. An extensive study of 5AZ sensitivity [62] examined 25 repair and recombination mutants, and detected *recA,B,C,G*, *ruvA,C*, *uvrD* mutants as being significantly more sensitive than wild-type. Again, the results in Figure 2 are in agreement with these findings. They also detected sensitivities with *priA*, *priB*, and *polA* mutants. Valid *priB* and *polA* mutants are absent from the KEIO collection [58]. Strains lacking adenine methylase, *dam*, have been shown to be sensitive to base analogs such as 2-aminopurine, and 5-bromouracil, and attributed to the generation of MMR-dependent double strand breaks [63]. Finally, Exo VII has been shown to be involved in mutation avoidance by acridines [64].

With regard to DNA repair, new results shown in Figure 2 include the involvement of Exo VII in protecting the cell from CPT and ZEB, as strains lacking either subunit (*xseA,B*) are more sensitive to CPT and those lacking at least the large subunit (*xseA*) are more sensitive to ZEB. Also, Exo III (*xthA*) protects against the toxicity of both 5AZ and ZEB. The novel finding that *rnhA* mutants are more sensitive to BLM, CPT, 5AZ, and ZEB raises the question of how an enzyme that degrades RNA of DNA-RNA hybrids protects the cell from these agents. The involvement of *recA,B,C,G,N*, *ruvA*, *uvrC,D*, and *xerC,D* in protecting the cell from the toxic effects of ZEB are also revealed in Figure 2. New roles for detoxifying enzymes are exemplified by the finding that ICR has greatly increased cytotoxicity in *ybdB* mutants lacking a detoxifying protein for methylglyoxal.

Figure 2 shows that each of the six genotoxic agents has a distinct “sensitivity profile”. Some of the mutants are also sensitive to multiple antibiotics [57]. We indicate these in the MDS (multiple drug sensitivity) column. These mutants have defects in one of a number of different functional categories, including recombinational repair (e.g. *recABC*), efflux (*acrAB*, *tolC*), membrane and cell wall synthesis and maintenance (*rfaCDEG*, *envC*, *lpxLM*), and control of gene expression (*nusB*, *rseA*, *xapR*, *yciT*). The more specific sensitivities are of greater interest here, and they are also distributed among each functional category. Twenty-five of the mutants are not sensitive to any of the antibiotics tested [57] but are sensitive to one of the six genotoxic agents studied here.

Although 5AZ, ZEB, and 5BdU are base analogs, their sensitivity profile fingerprints are completely different. Only one mutant, among those tested, namely the *dam* knockout, is more sensitive to 5BdU, with a MIC of 50 µg/ml. Even at 1,000 µg/ml, no other mutants

appear to be sensitive. 5BdU is in the deoxyribonucleotide form, whereas 5AZ and ZEB are in the ribonucleotide form. Although this might explain the lack of sensitivity of some of the other strains to 5BdU, it does not explain the marked difference between 5AZ and ZEB in these experiments (*e.g.* see the different responses of *xerCD*, *tdk* and *fis* to these two agents).

Among the relatively specific mutants, several are noteworthy. *yebR* mutants lack free methionine-(R)-sulfoxide reductase, and are strongly sensitive only to ZEB (Figure 2), and not to any of the 22 antibiotics tested. The same is true for *ydfP* and *yhcB*. Several mutants defective in the folate biosynthesis pathway are sensitive to BLM, CPT, and particularly ZEB. Actually, there are 13 genes involved in the multi-step branched pathway for folate synthesis in *E. coli*. Seven of these are deleted in viable mutants in the KEIO collection, and four of these *ygfA*, *folB*, *folP*, and *nudB* show sensitivities to genotoxic agents. We have grouped these in Figure 2. *upp* (uracil phosphoribosyltransferase) mutants are sensitive to only 5AZ and ZEB. *ybcN* mutants, lacking a prophage encoded base flipping protein, are sensitive to only BLM among the agents tested here, and only rifampicin among the 22 antibiotics tested [57]

Kishony and coworkers looked at effects of combinations of two different antibiotics for a series of antibiotics [65]. They found that TMP showed synergistic effects with aminoglycosides and with sulfamonomethoxine, but not with 13 other classes of drugs. The synergistic effects we detected between ZEB and trimethoprim, an inhibitor of bacterial dihydrofolate reductase, shown in Figure 3, underscore one of the objects of this study, which is to pinpoint ways to potentiate currently used drugs by identifying pathways of sensitivity. These results suggest possible synergy between ZEB and methotrexate, a related inhibitor of human dihydrofolate reductase that is used in chemotherapy [66].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Examples of *E. coli* mutants that possess hypersensitivity to cisplatin, as seen in the KEIO collection screen

A) Shown are two 96-well colony grids. On the left are colonies printed onto media containing Luria Broth and Agar only (no drug), and on the right onto media containing a sub-lethal concentration of cisplatin (200µg/ml). Two mutants, *recR* and *uvrA*, do not grow at this concentration, and upon retesting were determined to have minimum inhibitory concentrations of 200 and 100 µg/ml CPT, respectively. B) The growth profiles of *recR* and *uvrA* that show significant sensitivity to cisplatin with respect to the starting strain (BW25113) are illustrated in order of increasing sensitivity. The figure is a composite of printed colonies (see Materials and Methods) of each strain on media with different concentrations of CPT.

Figure 2. Top Sensitive *E. coli* Strains to genotoxic agents

156 single gene knockout mutants were found to be hypersensitive to one or more genotoxic agents. Here strains are organized first by gene category [1(red) - DNA replication, recombination and repair; 1A(red) - functions indirectly affecting category 1; 2(green) - transport, efflux, cell wall and cell membrane synthesis; 2A(teal) - chaperones and functions related to 2; 3(orange) - protein synthesis; 3A(orange) - RNA processing; 4(blue) - central metabolic reactions; 5(purple) – regulation; 6(yellow) - prophage encoded genes, cell adhesion; 7(black) - unassigned genes], then alphabetically, though some strains involved in the same pathway are grouped together. Strains with stronger susceptibilities are shown in the darkest shade, medium susceptibilities in medium shade, and weak susceptibilities in lightest shade. Column 5 (MDS) indicates the degree of multi-drug sensitivity for each knockout mutant, which we determined in a separate study (25), with the darkest shade showing the highest degree of MDS (sites sensitive to 8 or more agents), the medium shade 4–7, the light shade 1–3, and the empty (white) boxes indicating no increased sensitivity to any of the antibiotics tested.

Figure 3. Combination experiments between zebularine and trimethoprim

The hypersensitivity of folate biosynthesis mutants to ZEB led us to examine whether a combination of trimethoprim (TMP), an inhibitor of dihydrofolate reductase, and ZEB would show synergistic effects, using the starting strain (BW25113). This figure shows that concentrations of TMP 0.03µg/ml and ZEB 0.04 and 0.05µg/ml that give little growth inhibition by themselves result in virtually a total inhibition of growth when used in combination.

Figure 4. Structures of genotoxic agents studied

The “R” group on the base analogs represents the ribose ring, which is in the deoxyribonucleotide form for 5BdU, and in the ribonucleotide form for 5AZ and ZEB (interchangeable for cytidine, used here as a structure reference).

Table 1
Sensitivities of double knockout mutants to CPT

We constructed a number of double knockout strains to test for further increases in sensitivity to CPT. In a number of cases, the double mutants are considerably more sensitive than either single mutant. Here, we list the minimum inhibitory concentrations of single and double knockout mutants against CPT.

Minimum Inhibitory Concentrations (MICs) in Single and Double *E. coli* Knockout Strains

Strain	MIC (CPT, µg/ml)	Double Mutant	MIC (CPT, µg/ml)
<i>BW25113</i>	200–250		
<i>uvrA</i>	100		
<i>recG</i>	100	<i>uvrArecG</i>	25
<i>recC</i>	100	<i>uvrArecC</i>	25
<i>recO</i>	150	<i>uvrArecO</i>	50
<i>tolC</i>	150	<i>uvrAtolC</i>	100
<i>gcvA</i>	150	<i>gcvArecC</i>	50