Supplemental Material

Importance of the tmRNA system for cell survival when transcription is blocked by DNA-protein crosslinks

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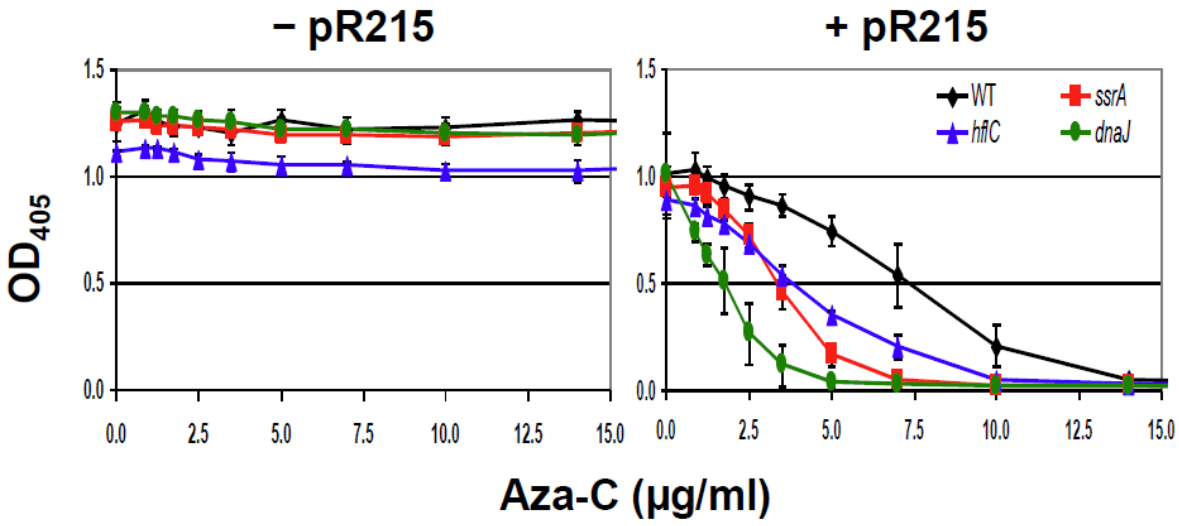
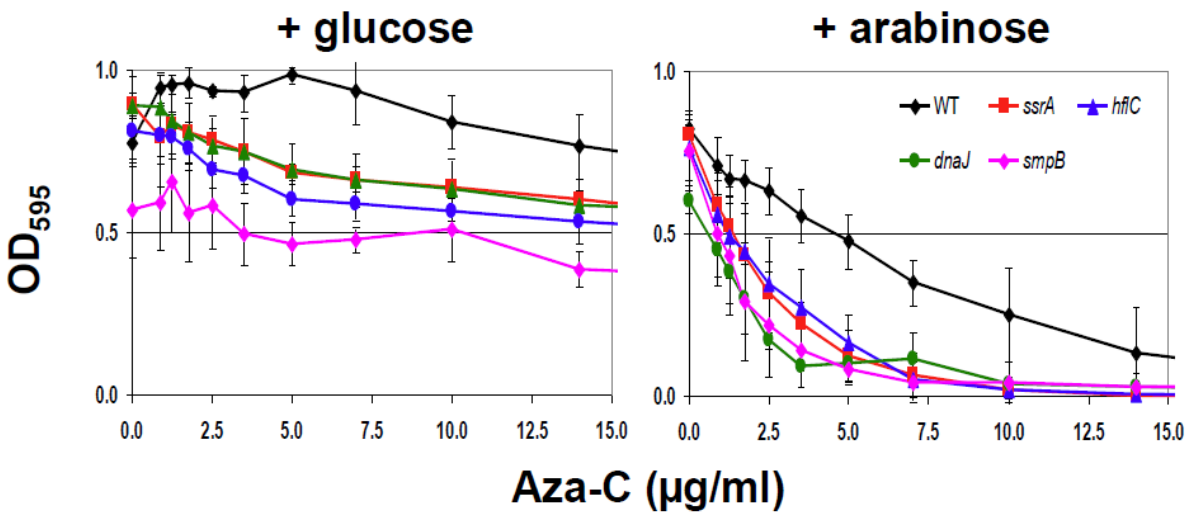
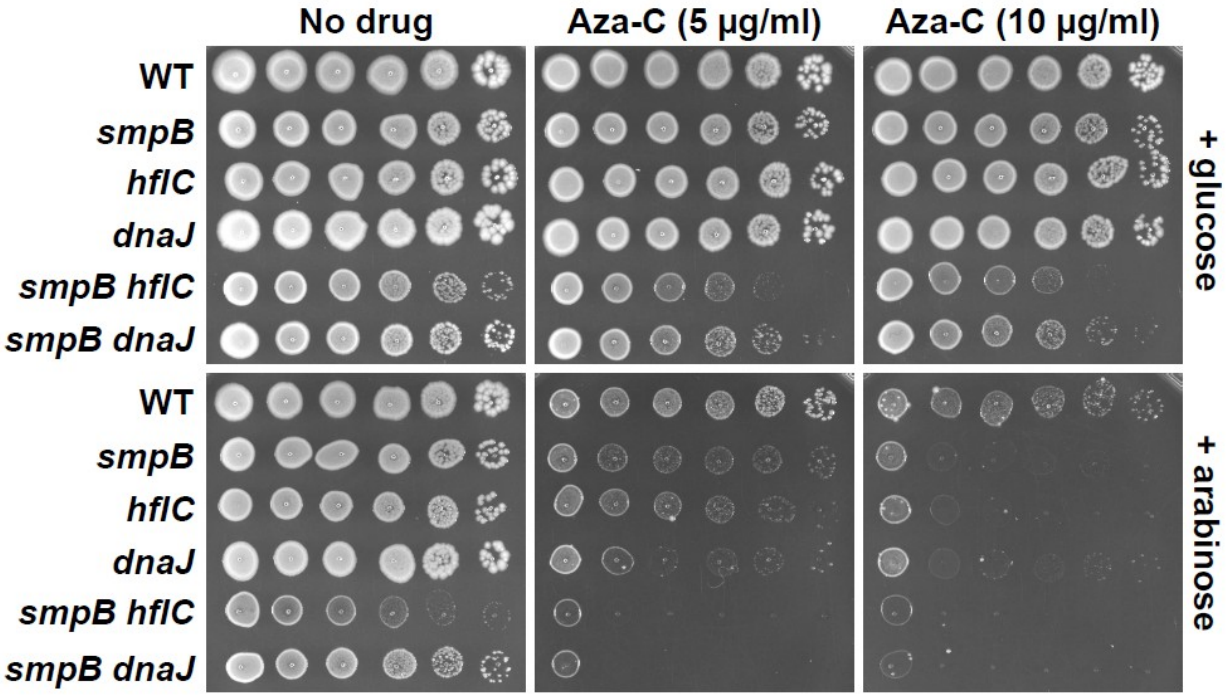


Supplemental Figure 1. Hypersensitivity of mutants in MIC assay depends on M.EcoRII-expressing plasmid. This graph presents the average of 3 independent minimum inhibitory concentration (MIC) assays. Overnight cultures of HK21 (WT) or HK21 derivatives, with or without the M.EcoRII-expressing plasmid pR215, were pelleted, resuspended in LB, and diluted to 2 x 10^5 cells/ml. Appropriate antibiotics and tetrazolium dye (Biolog Inc.) were added to each of the cell suspensions, which were then delivered into wells of a microtiter plate (75 µl/well). Aza-C (Sigma-Aldrich) stocks were prepared at twice the desired concentrations, and 75 µl of each was delivered into the appropriate wells to give a final volume of 150 µl. The background optical density of each well was read at 405 nm (Anthos Plate Reader 2001) immediately, and the plate was then incubated at 37°C for 20 hours with shaking before the final optical density reading. Standard deviations are shown as error bars (N = 3).



Supplemental Figure 2. Hypersensitivity of mutants in MIC assay depends on arabinose induction of M.EcoRII. This experiment was done as described above for Supplemental Figure 1 except that the cells contained the arabinose-inducible M.EcoRII plasmid pBAD-MEcoRII and the wells of the microtiter plates contained LB with either glucose (0.2%) or arabinose (0.05%). In this experiment, turbidity was measured directly by optical density at 595 nm (and tetrazolium dye was not included). Standard deviations shown as error bars (N = 3).



Supplemental Figure 3. Aza-C hypersensitivity of *smpB* and double mutants with arabinose-inducible M.EcoRII. HK21 cells (WT) or the indicated mutant derivatives, all carrying plasmid pBAD-MEcoRII, were diluted from overnight cultures to approximately 4 x 10^8 cells/ml. Ten-fold serial dilutions were generated across a 96-well plate and 5 µl of each dilution was spotted onto LB plates with no drug or the indicated concentration of aza-C. The upper panels are plates that contained glucose (0.2%), while the lower panels are plates that contained arabinose (0.05%). Plates were photographed after overnight incubation at 37°C.

Bcm (µg/mL)

Aza-C (µg/mL)

	0	2.5	5	7.5	10	12.5	15	20	22.5	25	27.5	30
0	0.72	0.803	0.766	0.702	0.721	0.642	0.618	0.396	0.234	0.081	0.01	0.002
1	0.361	0.368	0.386	0.374	0.345	0.323	0.242	0.033	0.006	0.003	0.004	0.007
2.5	0.152	0.138	0.129	0.098	0.088	0.064	0.021	0.005	0.004	0.004	0.006	0.006
5	0.026	0.022	0.015	0.007	0.003	0.001	0	0	0	0	0.002	0.001
7.5	0.011	0.012	0.011	0.007	0.005	0.005	0.006	0.005	0.005	0.004	0.005	0.005
10	0.007	0.007	0.005	0.005	0.005	0.004	0.005	0.004	0.004	0.004	0.003	0.004
12.5	0.007	0.006	0.005	0.005	0.004	0.004	0.005	0.005	0.005	0.004	0.005	0.036
15	0.008	0.007	0.006	0.007	0.006	0.006	0.008	0.006	0.006	0.007	0.007	0.004

Supplemental Table 1. Sample growth inhibition data for azaC / bicyclomycin double drug experiment. This and the subsequent Supplemental Tables and Figure illustrate the analysis of double-drug titrations. In this experiment, HK22 pBAD-MEcoRII cells from an overnight culture were diluted to an OD_{560} of 0.5, diluted an additional 2000-fold, and then mixed in equal volume with a solution containing twice the indicated concentrations of aza-C and bicyclomycin (Bcm). The drug titrations were set up in a 96-well microtiter plate in checkerboard fashion by adding increasing concentrations of Bcm in the left to right direction and increasing concentrations of aza-C in the top to bottom direction. The final Bcm concentrations ranged from 0 to 30 µg/mL (increasing in 2.5 µg/mL increments) and the final aza-C concentrations included 0, 1 µg/mL, and from 2.5 to 15 µg/mL (increasing in 2.5 µg/mL increments) as shown in the Table. Plates were incubated at 37°C with shaking in a BioTek EL_x808 microplate reader for 12 hours, with cell turbidity measured every 15 minutes. The end-point used to calculate inhibition was set to a time near the end of exponential growth in the absence of drugs, namely the time at which the growth rate had dropped to 10% of the maximum growth rate (equal to 10.5 hours for the experiment shown in this Table). The OD_{630} values measured in the microplate reader at that time are shown in the Table above.

The experiment measuring growth inhibition by streptolydigin and bicyclomycin was done in an identical fashion except for the following: (1) the bacterial strain EW1B was used to allow permeability to streptolydigin; (2) the cells were diluted 200-fold instead of 2000-fold; (3) the final Bcm concentrations (in addition to 0 µg/mL) ranged from 0.36 to 36 µg/mL (increasing in 66.7% serial increments) and the final streptolydigin concentrations (in addition to 0 µg/mL) ranged from 0.21 to 4.5 µg/mL (increasing in 66.7% serial increments).

95% Growth Inhibition

Experiment	Aza-C	Bcm	FIC
1	7.5	0	
1	0	25	
1	1	20	**0.93**
1	2.5	12.5	**0.83**
1	7.5	2.5	**1.10**
1	5	7.5	**0.97**
2	7.5	0	
2	0	22.5	
2	7.5	2.5	**1.11**
2	5	5	**0.89**
2	2.5	15	**1**
2	1	20	**1.02**
3	5	0	
3	0	27.5	
3	2.5	15	**1.05**
3	1	20	**0.93**
4	7.5	0	
4	0	25	
4	5	2.5	**0.77**
4	2.5	12.5	**0.83**
4	1	20	**0.93**
		Average:	**0.95**
		St. Dev:	0.11

75% Growth Inhibition

Experiment	Aza-C	Bcm	FIC
1	5	0	
1	0	20	
1	2.5	7.5	**0.88**
1	1	15	**0.95**
2	5	0	
2	0	20	
2	2.5	10	**1.00**
2	1	20	**1.20**
3	2.5	0	
3	0	25	
3	1	20	**1.20**
4	2.5	0	
4	0	22.5	
4	1	10	**0.84**
		Average:	**1.01**
		St. Dev:	0.16

50% Growth Inhibition

Experiment	Aza-C	Bcm	FIC
1	2.5	0	
1	0	20	
1	1	7.5	**0.78**
2	2.5	0	
2	0	20	
2	1	10	**0.90**
3	1	0	
3	0	22.5	
4	2.5	0	
4	0	22.5	
4	1	10	**0.84**
		Average:	**0.84**
		St. Dev:	0.06

Supplemental Table 2. FIC values from the aza-C / bicyclomycin titrations. From the OD_{630} measurements at the set end point (the data in Supplemental Table 1 above), the fractional inhibitory concentrations (FIC) were calculated. For each level of aza-C, the FIC was calculated for the first concentration of bicyclomycin (Bcm) that gave the indicated level of inhibition (95%, 75% or 50%), and for each concentration of Bcm, the FIC was calculated for the first concentration of aza-C that gave 95%, 75% or 50% inhibition. FIC values were calculated using the following formula:

$$FIC = \frac{MIC, aza\text{-}C\ in\ combination}{MIC, aza\text{-}C\ alone} + \frac{MIC, Bcm\ in\ combination}{MIC, Bcm\ alone}$$

As an example, the wells with the lowest drug concentrations that gave at least 95% inhibition are highlighted in Supplemental Table 1. For the combination of aza-C at 2.5 µg/mL and Bcm at 15 µg/mL, the FIC value was calculated as:

$$FIC = \frac{2.5\ \mu g/mL}{5\ \mu g/mL} + \frac{15\ \mu g/mL}{27.5\ \mu g/mL} = 1.05$$

This Table summarizes these FIC values from four different experiments for the three different levels of growth inhibition. The MIC values for each drug alone in each experiment are also shown (with no FIC value). The average and standard deviations from all FIC values are shown at the bottom.

95% Growth Inhibition

Experiment	Stl	Bcm	FIC
1	2.70	0	
1	0	36	
1	1.62	1.68	**0.65**
1	0.97	21.6	**0.96**
2	2.70	0	
2	0	36	
2	1.62	7.78	**0.82**
2	0.97	21.6	**0.96**
3	2.70	0	
3	0	36	
3	1.62	7.78	**0.82**
3	0.97	21.6	**0.96**
		Average	**0.86**
		St. Dev:	0.13

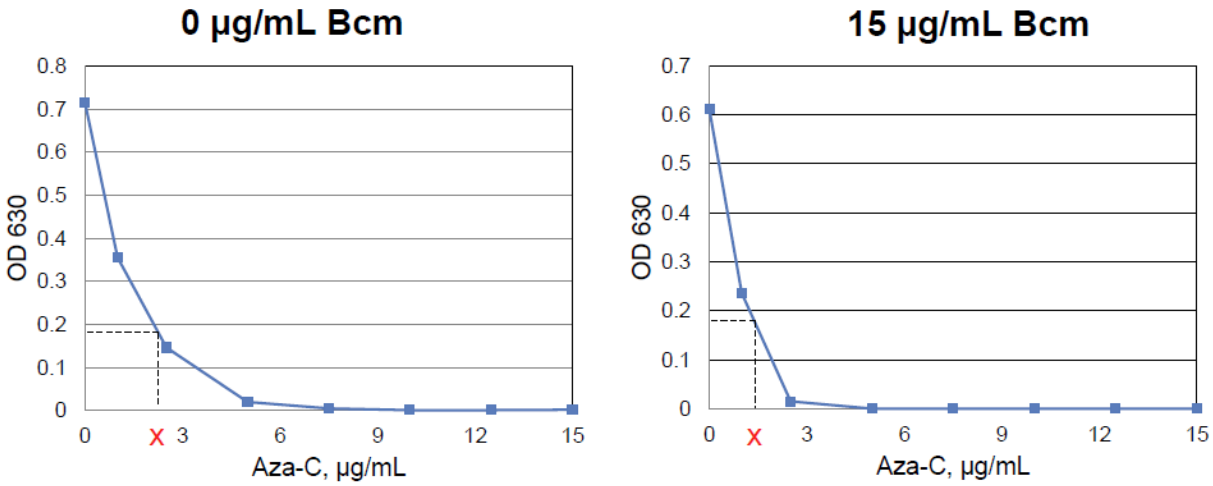
75% Growth Inhibition

Experiment	Stl	Bcm	FIC
1	1.62	0	
1	0	36	
1	0.97	12.96	**0.96**
1	0.58	21.6	**0.96**
2	1.82	0	
2	0	36	
2	0.97	12.96	**0.89**
2	0.58	21.6	**0.92**
3	1.62	0	
3	0	36	
3	0.97	12.96	**0.96**
3	0.58	21.6	**0.96**
		Average	**0.94**
		St. Dev:	0.03

50% Growth Inhibition

Experiment	Stl	Bcm	FIC
1	1.62	0	
1	0	36	
1	0.97	4.67	**0.73**
1	0.58	21.6	**0.96**
2	1.62	0	
2	0	21.6	
2	0.97	0.36	**0.62**
3	0.97	0	
3	0	36	
3	1.67	0.6	**1.74**
3	0.35	21.6	**0.96**
		Average	**1.00**
		St. Dev:	0.44

Supplemental Table 3. FIC values from the streptolydigin / bicyclomycin titrations. This Table summarizes the FIC values for the streptolydigin / Bcm titrations, calculated and presented exactly as above (legend to Supplemental Table 2).



Supplemental Figure 4. Developing the isobolic graphs for drug interaction. Based on the OD_{630} measurements from the end points described above (e.g. the data in Supplemental Table 1), we estimated the concentration of either aza-C or streptolydigin necessary to achieve a certain growth inhibition (95%, 75% or 50%) at each defined concentration of Bcm. These estimations were made by plotting the endpoint OD_{630} as a function of aza-C or streptolydigin concentration at that particular Bcm concentration; two examples are shown in this Figure (0 and 15 mg/mL Bcm, left and right respectively). The OD_{630} values that represent 95%, 75% and 50% inhibition are calculated (based on the end point OD_{630} of the well that has neither drug), and the estimated aza-C or streptolydigin concentration that would achieve this inhibition is then determined from the graph (in essence, interpolating between the two adjoining data points). The determination for 75% inhibition (OD_{630} = 0.72 X 0.25 = 0.18) is shown in this Figure, represented by dotted lines. For growth with either drug alone, we estimated the drug concentration required to give 95%, 75% or 50% inhibition exactly as shown in the left panel of this Figure for aza-C.