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Chromosomal Locations of Three *Bacillus subtilis din* Genes

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Previously isolated DNA damage-inducible (*din*) genes of *Bacillus subtilis* have been mapped on the bacterial chromosome by bacteriophage PBS1-mediated transduction. The *din* genes have been localized to three positions on the *B. subtilis* map. *dinA* cotransduction with the *hisA* locus was 80%, while *dinC* cotransduction with this marker was about 56%. *dinB* is unlinked to *hisA*, but its cotransduction with the *dal-1* and *purB* loci was 84 and 22%, respectively.

All organisms have developed systems for the surveillance, repair, and maintenance of the primary structure of DNA. Some of these repair mechanisms are under complex regulatory networks that are induced or derepressed after cellular DNA damage. The most extensively studied of these is the SOS regulon of *Escherichia coli* (8, 17, 18). Briefly, this inducible system consists of at least 17 unlinked chromosomal genes that are transcriptionally repressed by the product of the *lexA*$^+$ gene. Once the SOS system is activated, the *recA*$^+$ gene product is altered such that it directly causes or initiates the inactivation of this cellular repressor in addition to certain prophage repressors (14–16). The genes that are under the control of the cellular repressor (LexA protein) have been termed *din* (DNA damage inducible). Many of these genes were identified after the generation of gene fusions with the Mu d(*lac*) vector (5). Several of the *din* genes have been characterized with regard to the proteins encoded and their functions (1, 2, 6, 17). The isolation and mapping of these *din* genes greatly facilitated the dissection of the molecular control mechanisms for the SOS regulon.

An SOS-like system (SOB) has been identified in the gram-positive sporeforming bacterium *Bacillus subtilis* (9–11, 19, 21). The SOB regulon is of particular interest because it has been shown to be activated when the bacteria differentiate into their competent states (10, 11, 20). Therefore, the complete characterization of this inducible repair system should yield valuable information on the regulation of differentiation and development in *B. subtilis*. Previously, *din* genes were isolated in *B. subtilis* by using the insertional mutagenesis system Tn*917*:*lacZ* developed by Youngman and his colleagues (13, 22, 23). Approximately 15 *din* fusions were identified after more than 30,000 colonies were screened. These colonies were initially identified by their increased production of β-galactosidase in the presence of mitomycin C compared with β-galactosidase activity when the bacteria were grown in the absence of any DNA-damaging agent (9). Although these 15 fusions were isolated, it was not known how many *din* genes were actually identified. The initial data indicated that the kinetics of β-galactosidase induction differed among the isolates and that two of the isolates demonstrated increased sensitivity to DNA-damaging agents. Thus, it appeared that among the 15 *din* fusions, there were insertions in different loci on the chromosome. Therefore, to ascertain the positions of the *din* insertions, the fusions were mapped to the *B. subtilis* chromosome by bacteriophage PBS1-mediated transduction (4; Table 1).

Transductants were selected first for erythromycin resistance and then for the indicated auxotrophic marker.

PBS1-mediated cotransduction frequencies were determined by a protocol obtained from K. Sandman which has been described previously (4). Donor strains were grown until motile in Difco antibiotic medium no. 3 supplemented with 40 μg of tryptophan per ml, 0.5% glucose, 0.1% yeast extract, and 0.05% casein hydrolysate. The cells were infected with a stock of PBS1 lysate at a multiplicity of infection of 0.1, incubated 30 min at 37°C with gentle mixing, diluted 1/10 in the same medium, and incubated for 30 min before the addition of 4 μg of chloramphenicol per ml. After an additional 2 h of incubation, the culture was placed at 37°C without aeration overnight. Lysates were treated with 50 μg of DNase A per ml, centrifuged to remove bacterial debris, and filtered through a 0.45-μm-pore-size filter.

Recipient strains were grown until motile in M broth (1% tryptone, 0.5% yeast extract, 1% NaCl, 5 mM $MgCl_2$, 5 mM $CaCl_2$, and 0.5 mM $MnCl_2$), infected at a multiplicity of infection of 1.0, and incubated for 15 min at 37°C with aeration. Cells were centrifuged and suspended in M broth, and erythromycin-resistant colonies were selected for as previously described (9) (the antibiotic resistance determi-

TABLE 1. Mapping of *din* loci

Donor	Recipient[a]	No. linked/ total no. tested	Secondary marker	Cotransduction frequency (%)
din-3	KS115	181/348	*hisA*	52
din-4	KS115	66/100	*hisA*	65
din-5	KS115	73/160	*hisA*	46
din-7	QB928	231/245	*dal-1*	94
din-7	QB928	49/245	*purB*	20
din-8	KS115	110/200	*hisA*	55
din-10	KS115	60/100	*hisA*	60
din-12	KS115	108/200	*hisA*	54
din-16	KS115	169/283	*hisA*	60
din-17	KS115	224/400	*hisA*	56
din-18	KS115	224/400	*hisA*	56
din-21	KS115	236/400	*hisA*	59
din-22	KS115	246/400	*hisA*	60
din-23	KS115	126/200	*hisA*	63
din-76	KS115	105/126	*hisA*	83
din-77	KS115	73/100	*hisA*	73

[a] Strain KS115 (*cysA14 metC3 trpC2 leuA8 hisA1*) was obtained from K. Sandman. Strain QB928 (*dal-1 purB33 aroI906 trpC2*) was obtained from the *Bacillus* Genetic Stock Center.

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TABLE 2. Gene order determination[a]

Donor	Recipient	Selected marker	Colony genotype[b]					Total[c]	Gene order
			Ery	*hisA*	*gtaA*	*dal-1*	*purB*		
YB1324	KS115	Ery	1	0	0			18	
			1	1	0			2	
			1	0	1			304	
			1	1	1			83	
		hisA	0	1	0			196	
			1	1	0			1	
			0	1	1			12	
			1	1	1			62	
									hisA–gtaA–din-17
YB1325	KS115	Ery	1	0	0			20	
			1	1	0			0	
			1	0	1			220	
			1	1	1			260	
		hisA	0	1	0			189	
			1	1	0			3	
			0	1	1			10	
			1	1	1			148	
									hisA–gtaA–din-18
YB1326	KS115	Ery	1	0	0			7	
			1	1	0			0	
			1	0	1			210	
			1	1	1			278	
		hisA	0	1	0			191	
			1	1	0			2	
			0	1	1			17	
			1	1	1			175	
									hisA–gtaA–din-21
YB1327	KS115	Ery	1	0	0			27	
			1	1	0			1	
			1	0	1			195	
			1	1	1			221	
		hisA	0	1	0			204	
			1	1	0			0	
			0	1	1			20	
			1	1	1			126	
									hisA–gtaA–din-1
YB1328	KS115	Ery	1	0	0			7	
			1	1	0			4	
			1	0	1			194	
			1	1	1			289	
		hisA	0	1	0			108	
			1	1	0			1	
			0	1	1			10	
			1	1	1			158	
									hisA–gtaA–din-4
YB1329	KS115	Ery	1	0	0			7	
			1	1	0			2	
			1	0	1			217	
			1	1	1			275	
		hisA	0	1	0			183	
			1	1	0			0	
			0	1	1			22	
			1	1	1			145	
									hisA–gtaA–din-8
YB1330	QB928	Ery	1			0	0	19	
			1			1	0	167	
			1			0	1	8	
			1			1	1	32	
		dal-1	0			1	0	250	
			1			1	0	122	
			0			1	1	3	
			1			1	1	45	
									dal-1–din-7–purB

[a] Strains YB1324, YB1325, YB1326, YB1327, YB1328, and YB1329 were constructed by transforming strain YB1323 (*metB5 trpC2 gtaA100 xin-1* Spβ[s]) with DNA isolated from the appropriate *din* fusion strains. Strain YB1330 is the original isolate of the *din-7* fusion strain (9). Strains KS115 and QR928 are defined in footnote *a* to Table 1.

[b] Donor markers are designated by a 1, and recipient markers are designated by a 0.

[c] Number of transductants having a particular genotype.

nant is part of transposon Tn*917*). After 48 h of incubation at 37°C, the resistant colonies (1 μg of erythromycin and 25 μg of lincomycin per ml) were picked to fresh media and examined for the Din phenotype (9, 23) and for auxotrophic markers.

For the two fusions, *din-76* and *din-77* (9), that resulted in increased sensitivity to the DNA-damaging agents UV and mitomycin C, cotransduction with the *hisA* locus was approximately 80%. This is the same cotransduction frequency as that associated with the *uvrA* locus (3, 7) and is in agreement with published data which indicate that bacteria carrying the *din-76* fusion are deficient in excision repair (9). Because of these data, the *dinA* designation was chosen for fusions *din-76* and *din-77*. One particular fusion, *din-7*, did not demonstrate any cotransduction with the *hisA* locus. Instead, this fusion, now designated *dinB7*, was shown to have a cotransduction of 84% with the *dal-1* locus and 22% with the *purB* locus (Table 1), and the order of the genes, as established by three factor crosses, was found to be *dal-1–dinB–purB* (Table 2). The remaining *din* fusions all mapped in the region of the chromosome near the *hisA* locus. The results demonstrated a cotransduction of between 46 and 71% for these *din* fusions (Table 1) and the *hisA* locus. Three factor crosses were performed on seven of these *din* fusions, and the order established indicated that all of these *din* fusions (*din-1*, *din-4*, *din-8*, *din-17*, *din-18*, *din-21*, and *din-22*) mapped proximal to the *gtaA* locus and distal to the *hisA* locus such that the order was *din-gtaA-hisA* (Table 2). Although all of these remaining *din* fusions are clustered in one region of the chromosome, it still is not known whether these fusions represent transposon insertions within one or more genes. However, the DNA sequences adjacent to three of the fusions, *din-17*, *din-18*, and *din-21*, have been cloned and sequenced (data not shown), and the results indicate that these three fusions represent insertions within the same gene but at different locations. The gene represented by these three fusions has been designated *dinC*. We have not yet determined whether the remaining *din* fusions are in the *dinC* gene or whether these insertions represent other closely linked genes that are under the control of the SOB regulon. The clustering of genes controlling similar phenomena has been previously demonstrated with *B. subtilis* (15). Therefore, it would not be surprising to discover that more than one SOB-regulated gene is in the region of the chromosome that has a cotransduction of approximately 50% with the *hisA* locus. In fact, phenotypic evidence suggests that more than one SOB-regulated gene does exist in this region of the chromosome. Specifically, strains carrying the *din-23* fusion show a decreased ability to be transformed with chromosomal DNA (9), while in strains carrying the *din-22* fusion, the SOB system cannot be induced with nalidixic acid (P. Love, personal communication).

Love and Yasbin (11) demonstrated that the *recE*$^+$ gene is DNA damage inducible and is under the regulation of the SOB regulon. In addition, O'Kane et al. have identified two additional *din* genes that are of resident prophage origin (12). Collectively, the data indicate that there are at least six loci scattered over the *Bacillus* chromosome that are under the control of the SOB regulon.

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