Characterization and cloning of the *gerC* locus of *Bacillus subtilis* 168

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A *Bacillus subtilis* *gerC* spore germination mutant demonstrating a temperature-sensitive response to L-alanine as germinant has been characterized in detail. The *gerC58* mutation is 50% cotransformed with *aroB* in the gene order *gerC*–*aroB*–*trpC*. The mutation is responsible for a severe growth defect which is manifest at all growth temperatures and is most extreme on rich media. A second, unlinked, mutation in the original strain suppressed this growth defect, but spores of the suppressed strain failed to germinate in alanine at 42 °C. As this germination defect is dependent on the presence of the *gerC58* allele, it is likely to be the direct result of a mutant *gerC* protein. The *gerC* gene therefore appears to have a role in both spore germination and vegetative cell growth. A gene library of *Bcl*I-digested *B. subtilis* chromosomal DNA was constructed in phage vector ϕ 105J27. A derivative containing the *gerC* region was obtained by complementation of the growth defect of an unsuppressed *gerC58* strain. This phage contained a 6.3 kb insert of bacterial DNA, which is above the reported packaging limit of the phage. It failed to form visible plaques, although it could be handled as a prophage and sufficient phage particles be isolated to allow characterization of the insert. A deletion derivative generated *in vitro* and carrying only 2.9 kb of insert DNA also complemented the *gerC* defect. This *gerC* locus is the second locus to be implicated in alanine-stimulated germination. The first, *gerA*, is a developmentally controlled operon whose gene products are present only in the spore. This study of *gerC*, in contrast, reveals a role in spore germination for a normally essential vegetative protein.

Introduction

A number of genetic loci in *Bacillus subtilis* whose products are important in spore germination have been identified by mutant analysis (Piggot *et al.*, 1981). Of the loci concerned, two have been identified that are only defective in germination in alanine; these are *gerA* and *gerC*. Both were described in the first analysis of *ger* mutants, by Trowsdale & Smith (1975). The *gerA* locus has been studied in detail, Sammons *et al.* (1981) suggesting that the properties of *gerA* mutants were consistent with the hypothesis that the locus could encode an alanine-sensitive receptor in the spore. The region was cloned (Moir, 1983) and has been sequenced (Feavers *et al.*, 1985; Zuberi *et al.*, 1987). The *gerA* operon comprises three genes whose products are likely to be membrane associated; expression of these genes is confined to the forespore compartment during sporulation (Feavers *et al.*, 1990).

The *gerC* locus has been studied in much less detail; the original report described two mutations, *gerC58* and

gerC88, both of which conferred temperature-sensitive spore germination in alanine.

Attempts to carry out precise mapping of the *gerC* mutations (Moir *et al.*, 1979) were frustrated by the presence of a number of mutations in the strains, all cotransducible with the *ger* marker. The first complication in the strains was the presence (within the starting strain from which the mutants were derived) of an allele blocking the spore germination response at an intermediate stage. This locus was called *tzm* by Trowsdale & Smith (1975) and later designated *gerJ* and characterized as a member of a class of cortex-defective mutants (Warburg & Moir, 1981; Warburg *et al.*, 1986). It leads to a decreased loss of optical density during germination and a pink colouration in the germination plate test involving reduction of a tetrazolium salt.

The original *gerC* mutant isolates grew abnormally on rich agar; at 42 °C the colonies were extremely small, whereas at 30 °C they were of normal size (the *Wrd*[–] phenotype). On minimal glucose medium these colonies were normal at all temperatures tested. As both growth in rich agar and germination were temperature sensitive, it was originally thought that both might result from a single mutation. Moir *et al.* (1979) carried out PBS1

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transduction crosses, using *lys*⁺ as the selected marker, which succeeded in separating both *gerJ* and *wrd* mutations from *gerC*. Transductants that were *Wrd*⁻ but *Ger*⁺, for example, were obtained in the cross. The *gerC* defect was only found in transductants that had also acquired the donor *trpC* marker, placing the mutation very close to or on the *lys*-distal side of *trp*. *Lys*⁺ transductants that were *GerC*⁻ had also acquired the donor *wrd* and *gerJ* mutations, and thus this cross, while establishing the gene order *lys-wrd-gerJ-(gerC, trp)* did not yield any *gerC* mutants that were free from the linked mutations.

This paper contains a reassessment of the *gerC* locus and describes the successful separation of a *gerC* mutation from the linked but independent mutations. The unexpected discovery of a severe growth defect associated directly with *gerC* allowed the selection of clones carrying the wild-type complementing gene.

Methods

Bacterial strains used are listed in Table 1. Routine subculture of strains was done in Difco Penassay broth (PAB) or on Oxoid nutrient agar no. 2 (NA) plates, unless otherwise stated in the text. Glucose minimal medium (MA) for the selection of transformants was that of Anagnostopoulos & Spizizen (1961) supplemented with amino acids as required. Media for the culture of phage 105 derivatives were described by Errington & Jones (1987). Brain heart infusion broth (BHIB; Oxoid) was used for the preparation of phage 105 lysates. BBL trypticase (Moir & Brammar, 1976) was used for *gerC*⁺ selection.

Transformation of competent cells. This was done as described by Warburg & Moir (1981). Recombinants selected were purified by single-colony isolation on selective medium before testing of unselected

markers. The tetrazolium reaction used to score *Ger*⁺ and *Ger*⁻ colonies as Tzm-red or -white, respectively, was as described previously (Zuberi *et al.*, 1985) except that lysogens of ϕ 105J27, MY1 and MY2 were incubated for 4 d at 30 °C to obtain sporulation in colonies.

PBS1 transduction. This was according to Jamet & Anagnostopoulos (1969).

Spore germination studies. Spores were prepared on NA plates and washed as described by Moir *et al.* (1979). Germination was monitored as loss of OD₅₈₀ after addition of germinants, by the procedure described in Moir *et al.* (1979), except that Tris/HCl was 10 mM, pH 8.4 and KCl was 2.25 mg ml⁻¹.

Construction of a gene library, protoplast transfection and induction of lysogens. These procedures were according to Errington & Jones (1987). Lysogeny was tested by cross-streaking against a ϕ 105 clear mutant.

Complementation of the 'tiny' (Tny) phenotype is explained in the text; for spot complementation tests, 10 µl aliquots of small-scale induced lysates or of an appropriate dilution of a purified phage preparation were spotted onto a lawn of Tny⁻ cells spread on NA, along with a separate vector control. Results were scored after 24–48 h incubation at 30 °C.

Results and Discussion

Previous attempts to separate *gerC* from its linked mutations had utilized PBS1 transduction, selecting *Lys*⁺. A PBS1 cross selecting *MetB*⁺ was attempted in the hope of obtaining *gerC* mutants without linked secondary mutations by selecting a marker on the side of the region opposite to *lys*. The results were still difficult to interpret in terms of gene order for both *gerC58* and *gerC88* donor strains; as transductants carrying *gerC58* showed less residual germination at the non-permissive temperature, this marker was chosen for further study.

Table 1. Strains of *Bacillus subtilis* 168

Strain	Genotype	Origin or reference
1604	<i>trpC2</i>	Moir <i>et al.</i> (1979)
BD111	<i>cysB3 thrA5 trpC2</i>	D. Dubnau
CU267	<i>ilvB2 leuB16 trpC2</i>	S. Zahler
CU1064	<i>metB5 attSPβ</i>	S. Zahler
1558	<i>gerC58 wrd tzm(gerJ51) trpC2</i>	Trowsdale & Smith (1975)
AM677	<i>thrA5 trpC2 gerC58</i> (suppressed)	This work
QB99	<i>hisH2 leuA8</i>	1A89 of BGSC*
1A563	<i>trpB7</i>	BGSC
SB70	<i>tyrA1 hisH2†</i>	1A173 of BGSC*
SB137	<i>aroB2 hisH2†</i>	Nester <i>et al.</i> (1963)
AM726	<i>tyrA1 hisH2 ger</i> ⁺	PBS1 [SB70] → CU1064
AM727	<i>aroB2 hisH2 ger</i> ⁺	PBS1 [SB137] → CU1064
BG4004	<i>argC4 mtr-264 amyE::[P_{trp} trpE'-lacZ' Cm^R]</i>	Shimotsu & Henner (1986)
NG17.23	<i>spoIVA67 trpC2</i>	Piggot & Coote (1976)
FB75	<i>ecp-1</i>	Raugei <i>et al.</i> (1981)
AM355	<i>gerA570 trpC2</i>	Zuberi (1985)

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† These strains already carry a spontaneous germination mutation that maps in *gerA* (A. Moir, unpublished results).

Fig. 1. Germination of AM677. Spores of AM677 (*gerC58*) (●), 1604 (*ger*⁺) (■) and AM355 (*gerA570*) (▲) prepared at the same time under the same conditions were heat-activated at 80 °C for 20 min in distilled water and germinated in: (a) alanine and KCl at 42 °C, (b) asparagine, glucose, fructose and KCl at 42 °C, and (c) alanine and KCl at 25, 37 and 42 °C.

An attempt was made to transfer the *gerC58* mutation into an unmutagenized background by congression. The original strain 1558 was used as source of donor DNA (final concentration 20 µg ml⁻¹) in a transformation cross with strain BD111 as recipient; Cys⁺ transformants were selected on minimal agar containing tryptophan and threonine, purified and tested for germination phenotype by the tetrazolium plate test. Only three out of 643 transformants were Tzm-white and grew to a normal extent on rich agar. About 3% of the transformants had a severe growth defect on NA and did not sporulate; these were not studied further at the time.

The three Tzm-white transformants were characterized and shown to possess a temperature-sensitive defect in alanine-stimulated germination like the parental strain (Fig. 1). One of these, strain AM677, was used as a starting point for more detailed mapping by transformation crosses.

The results of transformation crosses between AM677 and strains carrying auxotrophic markers in the *trp* region revealed an unexpected asymmetry of behaviour. If AM677 was the recipient strain, Tzm-white recombinants were obtained amongst the transformants. If, in contrast, this strain was used as donor, no such transformants were obtained, and instead a significant proportion of the recombinants grew as visible but very small colonies on the minimal selective agar (dubbed the 'tiny' or Tny⁻ phenotype; colony diameter was about one-fifth that of Tny⁺). The results of several crosses are shown in Table 2.

Cross 1, using strain AM677 as recipient, demonstrates that the likely gene order is *gerC-trpC-hisH*. Cross 2, using this strain as donor, generates the gene order *tny-trpC-hisH*, where the *tny* mutant allele is responsible for the growth defect of recombinants and shows similar transformation linkage to that in cross 1 for *gerC*. Cross 3 is a control, showing that strain 1604 does not carry a *tny* mutant allele linked to *trpC*. Cross 4 confirms and extends the gene order *gerC(tny)-trpC-hisH-tyrA*. The overall deduced map location of *gerC*, based on these and other crosses with strains AM727 and 1A563 (Yazdi, 1989), is summarized in Fig. 2.

gerC and *tny* markers map close together, and have not been separated in any of our transformation crosses. In crosses 2 and 4 of Table 2, and in other unpublished crosses, all Tny⁺ recombinants tested (at least 300) were Ger⁺. In order to test whether Ger⁻ recombinants from cross 1 carried the *tny* mutant allele, chromosomal DNA was isolated from four Ger⁻ His⁺ and four Ger⁺ His⁺ recombinants from cross 1 in Table 2. These DNAs were used to transform strain QB99 (*leuA8 hisH2*) to His⁺. At least 200 transformants from each of the eight crosses were tested for unselected markers. Whenever a Ger⁺ donor was used, none of the recombinants were Tny⁻. If a *gerC* mutant donor was used, however, about 20% of the transformants had the characteristic Tny⁻ growth defect. Thus the *gerC* mutant allele and the *tny* mutant allele had segregated together in all of the eight transformants tested from cross 1. The genetic data suggest, but do not unequivocally prove, that *gerC58* and

Table 2. Mapping of *gerC58* by transformation

Cross	Donor	Recipient	Selection	Transformant class	No. of crossovers required for predicted gene order*	No. of transformants
1	QB99 (<i>leuA8 hisH2</i>)	AM677 (<i>gerC58 thrA5 trpC2</i>)	Trp ⁺	His ⁺ Ger ⁻	2	23
				His ⁺ Ger ⁺	2	15
				His ⁻ Ger ⁻	2	32
				His ⁻ Ger ⁺	2	30
2	AM677	QB99	His ⁺	Trp ⁺ Tny ⁺ (Ger ⁺)	2	31
				Trp ⁺ Tny ⁻	4	1
				Trp ⁻ Tny ⁺ (Ger ⁺)	2	50
				Trp ⁻ Tny ⁻	2	18
3	1604 (<i>trpC2</i>)	QB99	His ⁺	Trp ⁺ Tny ⁺ (Ger ⁺)	NA	28
				Trp ⁺ Tny ⁻		0
				Trp ⁻ Tny ⁺ (Ger ⁺)		72
				Trp ⁻ Tny ⁻		0
4	AM677	AM726 (<i>tyrA1 hisH2</i>)	Tyr ⁺	His ⁺ Trp ⁺ Tny ⁺ (Ger ⁺)	2	22
				His ⁺ Trp ⁺ Tny ⁻	4	0
				His ⁺ Trp ⁻ Tny ⁺ (Ger ⁺)	2	55
				His ⁺ Trp ⁻ Tny ⁻	2	9
				His ⁻ Trp ⁺ Tny ⁺ (Ger ⁺)	2	15
				His ⁻ Trp ⁺ Tny ⁻	4	1
				His ⁻ Trp ⁻ Tny ⁺ (Ger ⁺)	4	2
				His ⁻ Trp ⁻ Tny ⁻	4	1

NA, Not applicable – *tny* marker not segregating in cross.

* Predicted gene order is: cross 1, *gerC*–*trpC*–*hisH*; cross 2, *tny*–*trpC*–*hisH*; cross 4, *gerC* (*tny*)–*trpC*–*hisH*–*tyrA*.

tny represent the same mutational site; if they do not, they must map extremely close to each other. The analysis is made more difficult by the failure of the unsuppressed *tny* mutant to sporulate – so that the Ger phenotype can only be scored in Tny⁺ recombinants or in suppressed backgrounds. The cotransfer of the *gerC* marker with *tny*⁺ in all of over 300 recombinants does suggest that the mutations are very close, if not identical. Our failure to separate them makes recombination index analysis impossible, and reversion analysis of Tny⁻ is complicated by the observed suppression. The simplest interpretation consistent with all our observations is that the two phenotypes result from the same mutation.

Considerable effort had been expended in attempting to separate *gerC58* from the growth defect; it was finally realized that the likely interpretation of our data was that strain AM677 and the original *gerC* mutant strain 1558 both harbour some second compensating mutation, not linked to *trp* in transformation crosses. This suppressed the growth defect resulting from the *tny* (*gerC*) mutation and left the strain spore-forming but germination-defective. In retrospect, the frequent class of congressants with an abnormal growth phenotype generated in early attempts to obtain a singly mutant *gerC58* strain were probably single mutants, whereas AM677 either acquired both suppressor and *gerC*, by a double congression event or because of linkage of the suppressor to the selected marker, or was a *gerC* transformant

carrying a spontaneously generated suppressing mutation that arose at an early stage of colony development.

Strains carrying the unsuppressed *gerC58* mutation proved very difficult to handle; any attempt to subculture them in rich medium, either in liquid or on plates, led to the rapid accumulation of suppressed mutants with improved growth rate. Cultures of the unsuppressed strain were routinely generated by inoculating together six colonies from an MA plate into 3 ml of minimal glucose medium, and incubating at 37 °C for about 8 h to generate a mid-exponential-phase culture. Liquid cultures were stable only if aerated efficiently; after 30 min at room temperature any unaerated culture would lyse spontaneously. Attempts to make glycerol stocks of these strains failed, as only suppressed derivatives could be recovered even after short periods of storage. Colonies of the unsuppressed strain had to be generated directly when required by transformation of strain QB99 with DNA from strain AM677, selecting His⁺. Transformants that were Tny⁻, recognized by their small colony size on MA, were purified twice on MA and used fresh in the procedure described above.

Cloning of the *gerC* region

A library of *Bcl*I-generated fragments of chromosomal DNA isolated from strain 1604 was generated by cloning into the *Bam*HI site of the cloning vector ϕ 105J27 (Jones

Fig. 2. The genetic map of the *gerC*-*trp* region; not drawn to scale. The numbers represent map distance (= 100 - percentage cotransformation). The arrow points to the selected marker in each case. Where several identical crosses had been carried out, the average map distance is shown.

& Errington, 1987). Because an unsuppressed *tny* (*gerC58*) strain grows very poorly on rich agar it was possible to detect complementation by a *gerC*⁺ hybrid phage on the basis of improved growth of lysogens in plaques on NA. *Tny*⁻ cells (100 µl aliquots), grown in minimal glucose medium to OD₆₀₀ = 0.25 and concentrated twofold, were infected with 2×10^6 p.f.u. from the recombinant phage pool (or 2×10^6 p.f.u. of $\phi 105J27$ as control), and plated in a 3 ml BBL trypticase overlay on NA plates. After overnight incubation at 30 °C, the plates infected with the recombinant phage pool contained 2–15 faster-growing clusters of cells on a very thin lawn of *Tny*⁻ cells; in contrast, the control infections with vector alone gave no such colonies. Any further incubation beyond the 24 h period permitted the growth of revertants and/or suppressed derivatives on both experimental and control plates.

Lysogens from eight of the clusters that appeared early were purified to single colonies on NA, and then heat-induced. Filter-sterilized lysates were spotted onto a lawn of *Tny*⁻ cells on NA and incubated for 24–48 h at 30 °C. Lysates derived from seven out of the eight cultures were positive, complementing the *Tny*⁻ growth defect as demonstrated in Fig. 3(a). The transducing phages were purified to homogeneity through successive cycles of lysogenization of a *Tny*⁻ strain, purification of individual lysogens, and induction.

Attempts to purify the transducing phages by single-plaque isolation were unsuccessful. Separate plate lysates were made from several hundred individual phage plaques obtained by plating the induced lysates on CU267; none ever showed transducing activity. The

transducing phage fails to make plaques on *Tny*⁺ lawns, although it was possible to see the centres of infection as heavily growing spots on *Tny*⁻ lawns (Fig. 3b). Estimation of phage DNA purified from the lysate in Fig. 3(b) suggests that this plate was infected with about 10^5 transducing particles; the efficiency of detection of *Tny*⁺ lysogenic centres is therefore very low. The visible plaques in lawns represent an even smaller proportion of the total titre of the lysate and represent deletion mutants. All the transducing isolates obtained showed similar complementation behaviour and lacked plaque-forming ability. One, named $\phi 105MY1$, was retained for more detailed analysis.

Since the cloning had exploited repair of the *Tny*⁻ growth defect, it was necessary to test whether the germination defect was simultaneously complemented on lysogenization of the *Tny*⁻ strain by $\phi 105MY1$. The germination behaviour of 20 *Tny*⁺ complemented lysogenic transductants of an unsuppressed *Tny*⁻ strain was examined in a tetrazolium overlay test. All twenty were Tzm-red and hence *Ger*⁺; the cloned DNA therefore complemented the *gerC* (*tny*) locus, repairing both growth and germination defects.

Two litres of a complemented lysogen carrying $\phi 105MY1$ as prophage was grown in BHIB, heat-induced and the phage particles harvested by PEG precipitation, purified by centrifugation on a CsCl step gradient and phenol-extracted. The yield of phage DNA, 50 µg from 2 litres of lysate, was only one-fortieth of that routinely obtained by induction of a vector-containing lysogen, but was sufficient for initial characterization of the inserted DNA.

The 2.9 kb *Sst*I fragment and the 3.4 kb *Xba*I fragment from phage MY1 have been subcloned separately into pUC119 (M. Yazdi & S. Greenhalgh, unpublished experiments). Additional sites defined in the cloned DNA (Fig. 4) were derived by restriction mapping of plasmid subclones.

The lack of plaque-forming ability and poor yield of phage on induction can be explained by the size of the DNA insert in ϕ 105MY1. The 6.3 kb of cloned DNA would mean that the total size of the hybrid genome, at 41.2 kb, is 1 kb larger than the published upper size limit for efficient packaging of ϕ 105DNA in the phage head (Errington & Pughe, 1987).

ϕ 105MY1 contains only two *Xba*I sites, flanking a 3.4 kb segment of cloned DNA at the right-hand end of the *gerC*-containing 6 kb *Bcl*I fragment (Fig. 4). As it was likely that the failure of ϕ 105MY1 to make visible plaques is related to the large insert size, it was presumed that deletion of 3.4 kb from the insert might restore plaque-forming ability.

Five hundred nanograms of ϕ 105MY1 DNA was cut with *Xba*I in 20 μ l; the digest was diluted to 50 μ l and ligated. The ligation mix (1.0 μ l) was used in protoplast transfection of strain CU267 and 100 visible plaques were obtained. Three phage isolates were purified, used to lysogenize strain CU267, and their DNA isolated from small-scale induced lysates. Their restriction maps were identical; all had lost the 3.4 kb *Xba*I fragment, and retained the expected 2.9 kb *Xba*I–*Sma*I fragment (Fig. 4). One of these phages was named ϕ 105MY2.

Strain AM677 was lysogenized with ϕ 105MY2, and with another two identical isolates. Five lysogens were tested for each lysate; all were Tzm-red (*Ger*⁺). The DNA remaining in these hybrids was therefore sufficient to complement the *gerC58* defect of strain AM677. The *gerC* gene is therefore contained in the 2.9 kb *Xba*I–*Sma*I fragment of ϕ 105MY2, which can be easily isolated in quantity.

The cloned DNA which complements *gerC* is derived from the region defined genetically as the *gerC* locus, since the clone could recombine with (although not complement) the *aroB* mutation of strain SB137, as determined by a significant but low frequency of transduction by ϕ 105MY1 in spot transductions on selective minimal agar (Yazdi, 1989). Data from Southern blotting of the *aro*–*trp* region (D. Henner, personal communication) suggests that a 6 kb *Bcl*I fragment extends to the *trp*-distal side of *aro*, in the region that would be predicted from Fig. 2 to contain *gerC*; this is similar to the estimated size of the cloned fragment. Recent preliminary sequence data confirm that the *trp*-proximal end of the cloned DNA lies within the *aroB* gene (S. Greenhalgh, unpublished).

The *gerC* locus as defined by mapping is located close

Fig. 3. Complementation of the *Tny*[–] growth defect by phage 105 MY1. (a) Ten microlitres of induced lysates were spotted on a lawn of *Tny*[–] cells on NA, and the plates incubated at 30 °C for 48 h. On this plate 14 lysates were positive. The negative, vector, control is present in an area of the plate to the right of centre but the spot cannot be clearly seen. *Tny*⁺ revertants or pseudo-revertants can be seen scattered throughout the surface of the plate. (b) Complementation at the single-plaque level. One hundred microlitres of a 10³-fold dilution of a CsCl-purified preparation of phage MY1 was plated with *Tny*[–] cells in an overlay on NA and incubated overnight at 30 °C. The development of more densely-growing lysogens in invisible 'plaques' can be seen, as well as plaques of more normal appearance, generated by deletions in the transducing phage genome.

to several other markers; *spoIVA* (33% cotransformed with *trpC2*; Piggot & Coote, 1976), *mtr* (40–55% cotransformed with *trpE*; Hoch *et al.*, 1971) and *ecp* (46% cotransformed with *aroB*, calculated from data in Raugei *et al.*, 1981) are also reported as mapping in this region. Attempts were made to complement strains NG17.23, BG4004 and FB75. An area in a lawn of each strain on

Fig. 4. Summarized restriction map of $\phi 105\text{MY1}$ and its deletion derivative MY2. Vector DNA immediately flanking the 6.3 kb of cloned DNA is shown as a stippled region; restriction sites remaining in the multiple cloning site on either side of the insert are indicated. The actual fusion points of vector and cloned DNAs in phage MY1 are hybrid sites formed by fusion of *Bam*HI and *Bcl*I cohesive ends. Phage MY2 was generated from MY1 by deletion of the internal 3.4 kb *Xba*I fragment. E, *Eco*RI, H, *Hind*III, P, *Pst*I, V, *Eco*RV, Sa, *Sa*I, Sm, *Sma*I, Sp, *Sph*I, Ss, *Sst*I, St, *Stu*I, X, *Xba*I.

NA was infected with a 10 μl drop of $\phi 105\text{MY1}$, estimated from DNA content to contain about 10^7 phages ml^{-1} . After overnight incubation at 30 °C, single colonies were isolated by streaking from the area of infection. After confirmation of their lysogenic status, the phenotype of 50 was tested in each case. None of the three markers was complemented by the cloned DNA. In this procedure repair by recombination would not be detected; in fact, the *mtr-264* mutation can be repaired by recombination with the cloned DNA (D. Henner, personal communication). The *spoIVA* gene has now been cloned, and the restriction map of the *gerC* region does not overlap with that of the *spoIVA* clone (J. Errington, personal communication).

The complexity of the original *gerC* strains and the difficulty in studying them because of linked mutations has led to the intensive analysis of only one of the two available; the second mutation, *gerC88*, generates anomalous mapping data (Moir *et al.*, 1979; M. Yazdi, unpublished results), and the phenotype of a strain carrying a single mutation at this locus is not known. The strategy of first cloning the wild-type gene corresponding to *gerC58* means that it should now be possible to establish whether the two mutant alleles map in the same place. Lysogens generated by infection of $\phi 105\text{MY2}$ into a slow-growing *ger-88* strain grow at the same rate as the wild-type. This suggests that *ger-88* is also complemented by the cloned DNA. As both mutants came from the same mutagenesis (Trowsdale & Smith, 1975) it is quite possible that they are differently suppressed derivatives of the same original mutation. The failure to obtain many *gerC* mutants in past mutagenesis regimes is not surprising, given the major growth defect that may result.

The nature of the *gerC* defect and how it is suppressed by an unlinked mutation remains obscure. Since the *gerC* defect causes a temperature-sensitive germination phenotype, it is more likely to be a missense than a nonsense mutation. The defect in germination is reversi-

ble, as germination is initiated if the temperature is shifted back to the permissive from the non-permissive temperature (Trowsdale, 1973). If the *gerC58* mutant gene encodes a protein with some degree of function in germination at low temperature it is surprising that the defect in growth is exhibited at all temperatures – although the level of residual function required might be higher for growth. Even on minimal medium, the unsuppressed *gerC* mutant does not grow faster at 30 °C than at higher temperatures. The suppression could be tRNA-mediated or could involve activation of an alternative function that would substitute for *gerC* in growth, but not in germination; alternatively some compensating mutation could have arisen in a gene whose product interacts with the *gerC* product. It is not clear at this stage whether a variety of different mutations can suppress *gerC58*, as no attempt has yet been made to analyse the suppression phenomenon.

The evidence suggests that the defects in growth and germination both result from the same *gerC58* mutation. The *gerC* gene would therefore be unlike all other *ger* loci so far identified, as its product is likely to have a role in both growth and alanine-stimulated germination. The putative *gerC* product would not, however, be involved in the alternative germination route requiring both an amino acid and sugars. The only other locus in which mutations have been found to affect alanine germination specifically is *gerA*, which is a developmentally regulated operon encoding three proteins that are probably membrane-associated (Zuberi *et al.*, 1987); the model currently proposed is that the germinant L-alanine binds to a complex of these proteins and that the signal is somehow then transduced. The *gerC* gene product is implicated in this process, and may be involved in assisting the interaction with germinant or in transducing the initial stimulus. The likelihood that it represents a vegetative protein required for normal growth adds further to its interest and importance for our understanding of spore germination mechanisms.

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