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Revision of the Linkage Map of *Bacillus subtilis* 168: Indications for Circularity of the Chromosome

J. LEPESANT-KEJZLAROVÀ,* J.-A. LEPESANT, J. WALLE, A. BILLAULT, AND R. DEDONDER

Unité de Biochimie Cellulaire, Institut de Biologie Moléculaire, Centre National de la Recherche Scientifique, Université PARIS VII, 2 place Jussieu, 75.221 Paris Cedex 05, France

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A revision of the linkage map of the *Bacillus subtilis* 168 chromosome has been undertaken with the use of the generalized transducing phage PBS1. The mapping of four new markers (*narB1*, *mtlB1*, *aroI906*, and *tre-12*) has allowed a determination of the relative orientation of the *purB-dal* segment and its linkage with the *lin* markers. The chromosomal segment comprised between the *sacQ36* and *gtaA12* markers has been linked with the *narA1*, *ctrA1*, and *sacA321* markers. The *recA1* marker has been mapped relative to the *thyA* and *citB17* markers. Indications of linkage have been found between the *tre-12* and *catA* markers and the *aroG932* and *sacQ36* markers. According to these results, a circular genetic map of the chromosome of *B. subtilis* 168 is presented. Taken together, the transduction data and the order of marker replication determined by Harford in the accompanying paper support strongly the hypothesis of a symmetrical and fully bidirectional mode of replication for the *B. subtilis* 168 chromosome.

The elaboration of the chromosomal map of *Bacillus subtilis* 168 as it appears in recent compilations (5, 16, 34, 35, and see Fig. 1) is the result of the combination of two different approaches to genetic mapping.

First, transformation by exogenous deoxyribonucleic acid and transduction with the generalized transducing bacteriophage PBS1 have been extensively used in locating an increasing number of genetic markers on what we shall call linkage groups, or mapping segments. The difficulty in finding appropriate selective markers for linking these segments to one another has imposed a limitation to the complete resolution of the chromosomal map by these methods.

Second, three methods, marker frequency (31), density transfer (32), and selective excretion of deoxyribonucleic acid (3), have been used to assess the relative order of replication of markers on the genetic map, and the results (3, 7, 13, 18, 20, 26, 31, 32) have been interpreted on the basis of two assumptions: (i) the genome of *Bacillus subtilis* is composed of only one replication unit, and (ii) the replication process is initiated at an origin located close to the *purA* locus and proceeds unidirectionally towards a terminus located near the *metB* locus. This has resulted in the genetic structure which is schematized in Fig. 1. It should be pointed out that these methods have been applied to a relatively restricted number of markers assigned to several independent linkage groups as defined by co-transduction data, and conflicting results have been obtained in assessing the replication position of at least two markers, *hisA1* and *ura-1* (7, 18, 26, 32).

Two lines of evidence appeared later to invalidate the unidirectional hypothesis and thus led to a reevaluation of the conclusions derived from replication data. On the basis of autoradiographic studies by Wake, direct evidence has been obtained for physical circularity of the *B. subtilis* chromosome and symmetrical bidirectional replication over its total length (10, 28, 29). A bidirectional replication process had been proposed independently (22) to reconcile conflicting results obtained by transduction and density transfer analysis in assessing the position of the *sacA* locus relative to *purA16*. Three markers mapped by transformation in the *sacA* locus *sacA1* (22) and *sacA21* and *sacA24* (J. Lepesant-Kejzlarovà, unpublished data) were shown to replicate after *purA16* and before *purB6* (20, 26). Conversely, transduction mapping placed the *sacA* locus to the left of the *purA16* marker (22). Additional genetic evidence in favor of bidirectionality over a limited region close to the origin has been reported by Hara and Yoshikawa (11).

In view of these facts, a revision of the linkage map was initiated by means of transduction analysis in order to confirm and to connect the

known linkage groups to one another by the mapping of new markers made available from different sources. A part of this work has been reported in a preliminary communication (23).

In parallel, a revision of the replication order of numerous markers by the density transfer method has been undertaken by Harford (12) who has also independently confirmed some of the PBS1 linkage data presented here.

MATERIALS AND METHODS

Bacterial strains. The strains of *B. subtilis* used in this study are listed in Table 1.

Phages. The generalized transducing phage PBS1 (27) was used in all transduction crosses, and phage SPO1 (22) was used for the detection of phage-resistant and phage-sensitive recombinant phenotypes.

Media. MM, C, and SP media were as previously described (22). SPBSA is SP medium supplemented with 1.5% heat-denatured bovine serum albumin (BSA fraction V, Nutritional Biochemicals Corp.). SPBSAG is SPBSA medium supplemented with 2% glucose.

Transduction procedure. A slight modification in the preparation of transducing PBS1 lysates was introduced during the course of this study. Bacteria were grown in antibiotic medium number 3 (Bacto-Penassay broth, Difco). When the optical density (Beckman D. U. spectrophotometer, 1-cm light pathway, 650 nm) reached 1 (around 5×10^8 cells per ml) bacteria were diluted to an optical density of 0.2 in fresh antibiotic medium number 3 supplemented with 2.0 mM $MgSO_4$ and infected with phage at a low multiplicity of infection. Otherwise, the previous procedure was followed for the preparation of lysates and transduction of recipient strains (22).

Selection of recombinants and scoring of recombinant classes. After selection on appropriate medium, recombinants were picked as patches onto the same selective medium and further examined for unselected markers by replica plating.

In the case of a weak co-transfer (below 10%) or in the case of clones arising from quadruple crossovers, the relevant recombinants were purified through single-colony isolation on SP medium, and three different colonies from each isolate were patched on SP plates and further checked for unselected markers by replica plating. Strains bearing the markers involved in the crosses were included as controls in all master plates for replica plating analysis. In all transduction crosses, a reversion control of the recipient was performed. Revertants were also examined for their phenotypic characteristics as a further control. Phenotypes and selection of recombinants for new markers introduced in this study are given in Table 2.

Recombinants for auxotrophic markers were selected on MM medium supplemented with 0.1% glycerol as a carbon source and 20 μg of the appropriate auxotrophic requirements per ml, except L-arginine, which was added to a final concentration of 500 μg/ml.

The selection procedure for lincomycin-resistant (Lin^r) recombinants (13) was modified according to Harford (personal communication): 0.1 ml of an appropriate dilution of the transduction mixture was diluted in 5 ml of MM medium supplemented with 0.1% glycerol, 10 μg of auxotrophic requirement per ml, 250 μg of Casamino Acids per ml (Difco), and 1% Bacto-agar (Difco). This mixture was plated on a 10-ml base layer of the same medium. After an incubation of 6 to 7 h at 37 C for expression of the Lin^r recombinants, the plates were overlayered with 5 ml of semisolid tryptose blood agar base (22 g/liter [Difco]) containing 220 μg of lincomycin per ml (Upjohn). After a 48-h incubation at 37 C, the recombinants were picked as patches onto tryptose blood agar base (33 g/liter) plates supplemented with 55 μg of lincomycin per ml and further tested for unselected markers by replica plating.

The streptomycin-resistant phenotype (Str^r) was checked by replica plating on TBAB plates supplemented with 1500 μg of streptomycin per ml (Specia) (13).

Dal^+ recombinants were selected according to Dul and Young (8), and Suc^+ recombinants were selected as previously described (22).

The Rec^- phenotype (*recA1* marker) was examined on MM plus 0.1% glucose medium plates supplemented with 0.05 μg of mitomycin C per ml (Sigma) (15).

Uvr^r (*uvr-1* marker) (15), Pha^r (*gtaA12* [33] and *pha-1* markers confering resistance to *B. subtilis* phage SPO1) and Suc^c (*sacT30* marker, constitutive synthesis of sucrase [22]) phenotypes were tested as previously described (22).

The hyperproduction of extracellular levansucrase (Lvs^h) and extracellular proteases (Prt^h) phenotypes confered by the pleiotropic *sacQ* and *sacU* mutations (F. Kunst, J. Lepesant-Kejzlarovà, J.-A. Lepesant, M. Pascal, A. Billault, and R. Dedonder, Biochimie, in press; 22) were checked in parallel; Lvs^h phenotype was checked by using a color test as previously reported (22) and Prt^h phenotype was checked by replica plating on SPBSA medium by the method of Michel et al. (24). Clones were scored as Prt^h when surrounded by a clear halo of hydrolysis after incubation for 16 h at 37 C.

The Prt^h phenotype confered by the *catA* marker (19) was examined by replica plating on SPBSAG and by scoring the presence of the halo after 36 to 40 h of incubation at 37 C.

Expression of results. Mapping results are expressed as percentage of recombination by the following convention: percentage of recombination = 1 − (co-transfer) (2).

For the sake of simplicity in the presentation of the results, all the co-transfer data determined in the course of this study have been summarized (see Table 3).

RESULTS

Position of the sacQ36 to gtaA12 segment. The position of this segment on the map shown in Fig. 1 has been deduced from the relative order of replication of the *thr-5*, *hisA* and *argC* loci obtained by Dubnau et al. with strain W23

TABLE 1. *List of B. subtilis strains*

Strain	Genotype	Origin
Reference		
PG594	*trpC2 metC3 mtlB1*	H. Chalumeau
BD71	*hisA1 argC4 ura-1*	D. Dubnau
VUB12	*trpC2 thyA thyB nov-1*	N. Harford
VUB23	*trpC2 thyA thyB lin-2 spcB2*	N. Harford
VUB29	*lin-2*	N. Harford
VUB30	*lin-4*	N. Harford
VUB34	*thyA thyB pac-3 strA1 spcB2 lin-2*	N. Harford
GSY111	*trpC2 ilvA1*	C. Anagnostopoulos
GSY 250	*trpC2 argA2*	C. Anagnostopoulos
GSY1025	*trpC2 metB4 recA1*	C. Anagnostopoulos
GSY1057	*hisA1 ura-1 uvr-1*	C. Anagnostopoulos
BD92	*cysB3 hisA1 trpC2*	C. Anagnostopoulos
PG642	*purB6 sorR1*	P. Gay
GSY292	*trpC2 gltA1*	J. A. Hoch
JH417	*trpC2 citB17*	J. A. Hoch
WB906[a]	*aroI906*	J. A. Hoch
WB932[a]	*aroG932*	J. A. Hoch
CU479	*trpC2 ctrA1*	S. A. Zahler-P. Winter
CU495	*trpC2 narA1*	S. A. Zahler-P. Winter
CU636	*trpC2 narB1*	S. A. Zahler-P. Winter
60229	*trpC2 ald*	E. Freese
60935	*trpC2 metC dal*	E. Freese
SCR1026	*lys phe arg catA*	J. Ito
SB5	*ura-1 hisA1 trpC2*	J. Lederberg
168TT	*thyA thyB trpC2*	F. Rothman
Mu8 u5 u16 u12	*leu8 metB5 purA16 tre-12*	N. Sueoka
BD34	*thr-5 leu-8 metB5*	N. Sueoka
BD53	*purB6 metB5 leu8*	N. Sueoka
BC369	*hisA1 argC4 metD1 pha-1*	F. E. Young
Constructed[b]		
QB1	*sacA321 trpC2*	Reference 22
QB7	*sacA321 purB6*	BD53 $\xrightarrow{tf}$ QB1
QB14	*sacA321 his A1*	BD71 $\xrightarrow{tf}$ QB7
QB15	*sacA78*	Reference 22
QB16	*sacA78 ilvA1*	QB $\xrightarrow{tf}$ GSY111
QB39	*sacT30 trpC2*	Reference 22
QB95	*sacS3 trpC2*	Reference 22
QB103	*sacA321 trpC2 hisA1*	Reference 22
QB123	*sacA321 ctrA1 trpC2*	CU479 $\xrightarrow{tf}$ QB103
QB300	*sacQ36 trpC2*	Reference 22
QB316	*sacU25 trpC2*	Reference 22
QB320	*sacU37 trpC2*	Reference 22
QB322	*sacU200 trpC2*	Reference 22
QB511	*gtaA12 hisA1*	Reference 22
QB518	*gtaA12 sacU25*	QB316 $\xrightarrow{td}$ QB511
QB526	*trpC2 hisA1 thr-5*	BD34 $\longrightarrow$ BD92
QB537	*trpC2 cysB3*	BD34 $\longrightarrow$ BD92
QB539	*trpC2 cysB3 thr-5*	QB537 $\xrightarrow{td}$ QB536
QB637	*sacQ36 trpC2 thr-5*	QB300 $\xrightarrow{td}$ QB539
QB668	*sacA321 leu8 metB5 hisA1*	BD34 $\xrightarrow{tf}$ QB103
QB683	*sacU25 metD1 argC4 pha-1*	QB316 $\xrightarrow{td}$ BC369
QB684	*sacU37 metD1 argC4 pha-1*	QB320 $\xrightarrow{td}$ BC369
QB685	*sacU200 metD1 argC4 pha-1*	QB322 $\xrightarrow{td}$ BC369
QB687	*sacA321 trpC2 tre-12*	MU8U5U16U12 $\xrightarrow{tf}$ QB103
QB691	*hisA1 narA1*	QB14 $\xrightarrow{tf}$ CU495
QB692	*sacA321 narA1*	QB14 $\xrightarrow{tf}$ CU495
QB693	*purB6 tre-12*	Mu8u5u16u12 $\xrightarrow{tf}$ QB7
QB698	*trpC2 sacQ36 ald*	60229 $\xrightarrow{td}$ QB637
QB782	*thyA ilvA1*	QB16 $\xrightarrow{td}$ 168TT

TABLE 1—*Continued*

Strain	Genotype	Origin
QB788	*thyB trpC2*	BD34 $\xrightarrow{tf}$ 168TT
QB790	*thyA thyB citB17 trpC2 recA1*	*citB17* from JH417 and *recA1* from GSY1025 introduced by transformation in several steps in 168TT
QB800	*hisA1 pha-1*	SCR1026 $\xrightarrow{td}$ BC369
QB803	*hisA1 metD1 argC4 sacA321*	BC369 $\xrightarrow{tf}$ QB103
QB804	*hisA1 pha-1 catA*	SCR1026 $\xrightarrow{td}$ BC369
QB805	*trpC2 metC purB6 dal sorR1*	PG642 $\xrightarrow{td}$ 60935
QB811	*leu-8 metB5 sacA321 dal*	60935 $\xrightarrow{tf}$ QB668
QB815	*trpC2 narB1 lin-2*	VUB23 $\xrightarrow{td}$ CU636
QB817	*metB5 sacA321 dal narB1*	CU636 $\xrightarrow{tf}$ QB811
QB818	*leu-8 metB5 sacA321 argA2*	GSY250 $\xrightarrow{tf}$ QB668
QB819	*sacA321 narB1 mtlB1*	PG594 $\xrightarrow{td}$ QB817
QB820	*metB5 dal sacA321 aroI906*	WB906 $\xrightarrow{tf}$ QB811

[a] The strain carries also a *str*^{r} marker.

[b] Strains were constructed in this laboratory by congression of markers at saturating concentrations of deoxyribonucleic acid ($\xrightarrow{tf}$) or by PBS1 transduction ($\xrightarrow{td}$). Arrows point from the donor to the recipient.

TABLE 2. *Description of the new genetic markers introduced in this study*

Genetic marker	Phenotype	Selection of recombinants
ald	Ald$^-$: Lack of L-alanine dehydrogenase. Absence of growth on L-alanine as a carbon source (Trowsdale et al., submitted for publication).	MM medium + 0.1% L-alanine (Merck) + 0.0015% Casamino Acids.
aroI906	Aro$^-$: Lack of shikimate kinase (17). Auxotrophic requirement for tryptophan, phenylalanine, and tyrosine.	MM medium + 0.1% glycerol.
catA	Prt^h: Hyperproduction of exocellular proteases even in the presence of glucose (Ito and Spizizen [19] and personal communication).	Nonselective marker.
citB17	Cit$^-$: Lack of aconitase. Auxotrophic requirement for glutamate (16).	MM medium + 0.1% glucose.
ctrA1	Ctr$^-$: Absolute requirement for cytidine in the absence of ammonium (Winter and Zahler, personal communication; [35]).	MM medium in which $(NH_4)_2SO_4$ is replaced by 0.2% KNO_3 + 0.1% glucose.
gltA1	Glt$^-$: Lack of glutamine 2-ketoglutarate aminotransferase (16). Auxotrophic requirement for either glutamate or asparate (Hoch, personal communication).	MM medium + 0.1% glucose.
mtlB1	Mtl$^-$: Lack of mannitol-1-phosphate dehydrogenase. Inability to use mannitol as a carbon source and inhibition of growth by mannitol (Chalumeau, personal communication).	C medium + 0.1% mannitol (Merck).
narA1 *narB1*	Nar$^-$: Lack of nitrate reductase. No growth with nitrate as sole nitrogen source (Winter and Zahler personal communication; [35]).	The same as Ctr$^+$. Addition of cations [10^{-5} M $MnSO_4$, 10^{-3} M $Ca(NO_3)_2$, 5 μg of ferric ammonium citrate per ml] improves growth of Nar$^+$ recombinants.
pha-1	Pha^r: Resistance to SPO1; resistance to other *B. subtilis* phages has not yet been tested.	Nonselective marker in PBS1 transduction crosses.
sacU25 *sacQ36*	Lvs^h, Prt^h: Pleiotropic phenotype; hyperproduction of exocellular levansucrase and proteases (Kunst et al., in press; [22]).	Nonselective markers.
tre-12	Tre$^-$: Inability to use α-α trehalose as a carbon source (20).	MM medium + 0.1% α-α trehalose (Merck + 0.0015% Casamino Acids (Difco).

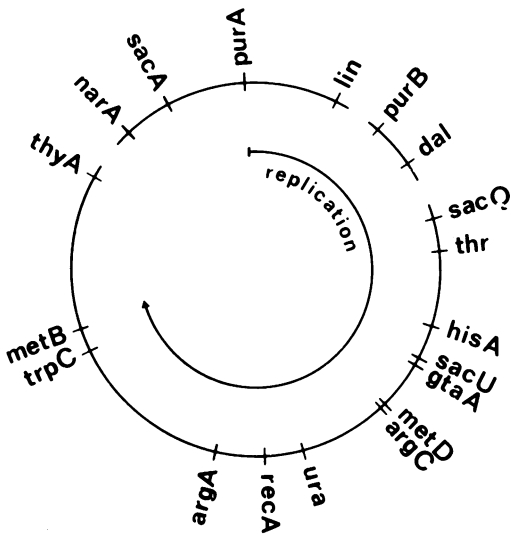


FIG. 1. *Simplified linkage map of the B. subtilis 168 chromosome based on recent compilations (5, 16, 34, 35).*

(7) and Borenstein and Ephrati-Elizur with strain 168TT (3).

Genetic continuity between the *hisA1* and *argC4* markers was first proposed by Dubnau et al., who found a co-transfer between those two markers (7). Later, Grant and Simon (9), Young et al. (33), and Boylan et al. (4) questioned the validity of this demonstration, since they failed to obtain a significant co-transfer of *hisA1* or several markers linked to it with the *argC4* or *metD1* markers, except in specific strains such as BC369 (33).

Similarly, in the course of mapping the *sacU* locus, several attempts to link either the *hisA1* or *sacU* markers in two-factor crosses with both *metD1* or *argC4* were negative even in the strain BC369 (22) (Table 3). It should be noted that in these crosses the phage-resistant marker harbored by the strain BC369, which could be assessed to be *gtaC51* (see Table 10 of reference 33), behaved as a non-co-transducible marker with either *metD1*, *argC4*, or *hisA1* (Table 3). Since this marker appears to be enzymatically and genetically distinct from any known phage-resistant marker (see Discussion), it will be referred provisorily to as *pha-1*. An extension of the segment including *argC4* was recently obtained by mapping of the *catA* locus (see Table 9; [19]). However, this marker again did not appear to be co-transducible with *hisA1* (Table 3).

These negative results have been ascribed to abnormalities of the recombination process in the *argC* to *hisA* region (4, 33). It also appeared possible that the *sacQ* to *gtaA* segment was misplaced. Arguments in favor of the latter assumption are provided by the replication data of Harford (12) which confirm the previous results of O'Sullivan and Sueoka (26) and Huang et al. (18), who found the *hisA1* marker replicating ahead of *thr-5*.

Taking these facts into account and assuming that replication is bidirectional, we investigated a new position for the *sacQ* to *gtaA* segment by testing the linkage of *sacQ36* or *gtaA12* with markers at the ends of other segments (Table 3). A definite co-transfer was detected between the *gtaA12* marker and the *narA1* marker mapped by Winter and Zahler (personal communication; [34]) beyond the *sacA* locus.

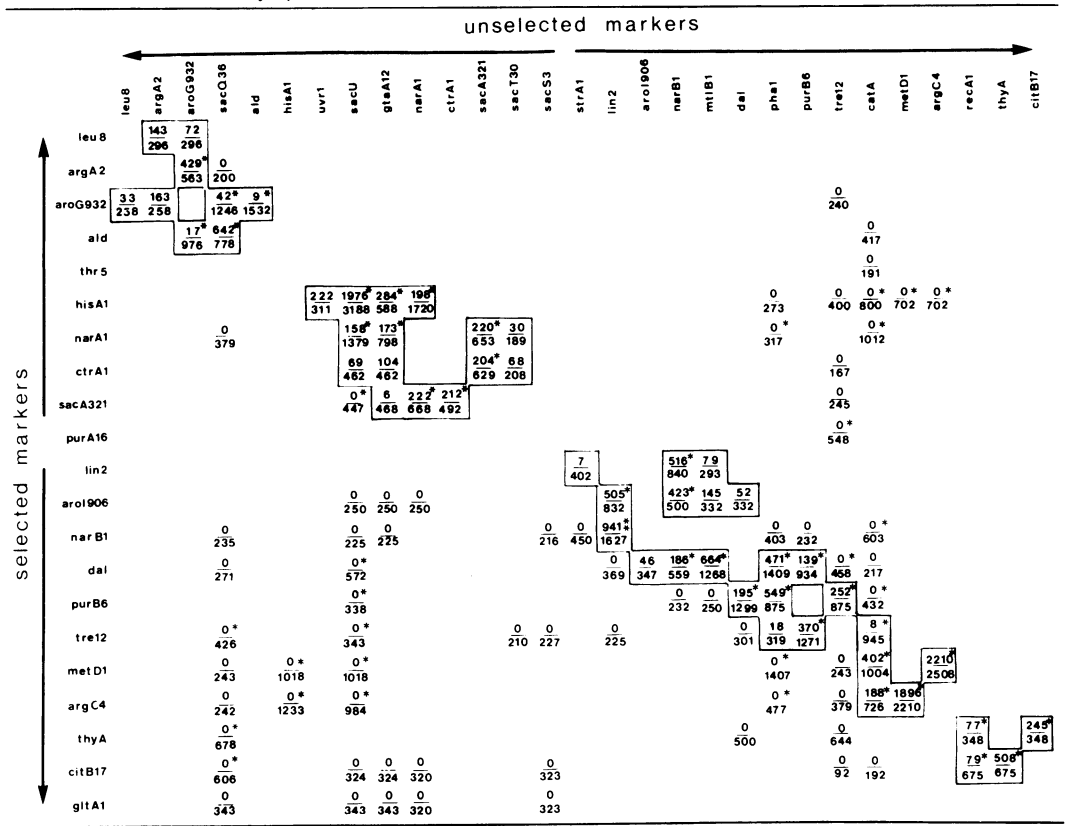
A series of three- or four-factor crosses involving the *hisA1*, *uvr-1*, *sacU25*, *ctrA1*, and *sacA-321* markers located previously on both sides of the *gtaA12* to *narA1* region were performed (Table 4; [23]). The results of these crosses were all compatible with the order represented on the map of Fig. 2. Similar results were obtained with two additional *sacU* markers, *sacU-37* and *sacU200*.

Direct co-transduction between *narA1* and *hisA1* was observed by using three different strains: SB5, BC369, and QB103.

Extension of the segment including purB and demonstration of genetic continuity between the lin and dal markers. Until recently, a relatively small number of markers had been located in the vicinity of the *purB* locus (34). Dul and Young reported an extension of this region with the mapping of the *dal* marker, but no linkage with other known outside markers was found (8). Indications that the position of this region on the chromosome was correct were obtained by Anagnostopoulos (personal communication), who found a weak linkage between the *lin* locus and two suppressor mutations which were co-transducible with the *purB* locus. However, the orientation of this segment could be only arbitrarily fixed on the basis of these data (Fig. 1).

Several indications prompted us to examine the location of a set of new markers (*tre-12*, *aroI906*, *narB1*, and *mtlB1*) relative to *purB6* and *dal*. According to the results of Harford (12), *aroI906* and *narB1* were shown to replicate in front of *purB6*, while *tre-12* replicates after it as first reported by Kennett and Sueoka (20). The *mtlB1* marker was investigated, since a failure to link this marker to any known marker was encountered by Chalumeau (personal communication).

TABLE 3. *Summary of the co-transduction frequencies measured in the course of this study*[a]



[a] Markers have been placed according to their relative position on the two replication arms symbolized by the arrows. *, Pooled values from several independent determinations; ⁑, identical value has been obtained with *lin4*.

The co-transfer of *tre-12* with markers replicating approximately at the time of *purB6* was tested in two-factor crosses (Table 3), and a linkage was found with *purB6*. As *tre-12* is not co-transducible with *dal*, it was placed on the opposite side of the *purB* segment. The finding that the *pha-1* marker is localized in between the *purB* and *dal* loci (Table 5) enabled us to perform a series of three-factor crosses which confirmed the supposed sequence of markers in the segment (Table 5 and Fig. 3).

Conversely, the *narB1*, *aroI906* and *mtlB1* markers appeared in two-factor crosses to be co-transducible with the *dal* marker although *narB1* and *mtlB1* were unlinked with *purB6* (Table 3). Three-factor crosses involving either *dal*, *narB1*, and *mtlB1* or *dal*, *aroI906*, and *mtlB1* were performed (Table 6).The results of these crosses show clearly the position of *mtlB1* between *narB1* and *dal* or between *aroI906* and *dal*. The relative position of *aroI906* and *narB1* markers could not be established in the second cross of Table 6, since the QB820 recipient strain displays a Nar⁻ phenotype. It should be pointed out that the original strain WB906 and all strains in which *aroI906* has been introduced by congression at saturating concentrations of deoxyribonucleic acid show equally this Nar⁻ phenotype. The tentative order *aroI906*, *narB1*, *dal* (Fig. 3) is proposed on the basis of co-transfer values found in separate two-factor crosses (Table 3).

Since *aroI906*, *narB1*, and *mtlB1* appeared to be distant from *dal*, their linkage with the *lin* locus was tested. A definite linkage was obtained between *lin-2* and the *narB1*, *aroI906*, and *mtlB1* markers, but no indications of co-transfer between *dal* and *lin-2* could be found (Tables 3 and 6). Essentially, identical results have been obtained by Harford (12), and the two sets of data are compatible with the order shown on the map of Fig. 3.

Reassessment of the structure of the metD1 to thyA linkage group. The genetic continuity

inside the segment extending from *metD* to *thyA* appears to be firmly established on the basis of co-transduction data reported in the literature, except on one particular point concerning the linkage of *recA* with *argA*. (For reviews see references 5, 16, 34, and 35. The mapping of *thyA* by Neubauer and Zahler is as yet unpublished and is cited in reference 34.)

Hoch and Anagnostopoulos (14) first reported a weak co-transfer of *recA1* with *argA10*. In addition, Le Hegarat and Anagnostopoulos (21) and Le Hegarat (Thèse de doctorat d'Etat, Université Paris XI, Paris, France, 1973) found a weak linkage of *recA1* with both the *argA11* and *phoP* markers.

The replication data obtained by Harford (12) conflict with the structure of the *metD* to *thyA* linkage group suggested by these mapping results, since *argA2* appears to replicate at the same time as *argC4* in front of *leu-8*, *pheA1*, *metC3* and well before *ura-1*. These results were suggestive of another configuration for the *argA*

TABLE 4. *Four-factor transduction crosses involving markers located in the hisA1-sacA321 region*

Recipient genotype	Donor genotype	Recombinants			Implied order
		Selection	Classes	No.	
hisA1 narA1 (QB691)	*sacU25 gtaA12* (QB518)	His$^+$	His$^+$Lvs^{h}Pha^{r}Nar$^+$	33	*hisA1 sacU25*
			His$^+$Lvs^{h}Pha^{r}Nar$^-$	143	*gtaA12 narA1*
			His$^+$Lvs^{h}Pha^{s}Nar$^+$	0	
			His$^+$Lvs^{h}Pha^{s}Nar$^-$	79	
			His$^+$Lvs$^+$Pha^{r}Nar$^+$	0	
			His$^+$Lvs$^+$Pha^{r}Nar$^-$	1	
			His$^+$Lvs$^+$Pha^{s}Nar$^+$	1	
			His$^+$Lvs$^+$Pha^{s}Nar$^-$	78	
narA1 sacA321 (QB692)	*sacU25 gtaA12* (QB518)	Suc$^+$	Suc$^+$Nar$^+$Pha^{r}Lvs^h	0	*sacU25 gtaA12*
			Suc$^+$Nar$^+$Pha^{r}Lvs$^+$	5	*narA1 sacA321*
			Suc$^+$Nar$^+$Pha^{s}Lvs^h	0	
			Suc$^+$Nar$^+$Pha^{s}Lvs$^+$	129	
			Suc$^+$Nar$^-$Pha^{r}Lvs^h	0	
			Suc$^+$Nar$^-$Pha^{r}Lvs$^+$	1	
			Suc$^+$Nar$^-$Pha^{s}Lvs^h	0	
			Suc$^+$Nar$^-$Pha^{s}Lvs$^+$	333	
ctrA1 sacA321 trpC2 (QB123)	*sacU25 gtaA12* (QB518)	Ctr$^+$	Ctr$^+$Suc$^+$Pha^{r}Lvs^h	0	*sacU25 gtaA12*
			Ctr$^+$Suc$^+$Pha^{r}Lvs$^+$	8	*ctrA1 sacA321*
			Ctr$^+$Suc$^+$Pha^{s}Lvs^h	0	
			Ctr$^+$Suc$^+$Pha^{s}Lvs$^+$	139	
			Ctr$^+$Suc$^-$Pha^{r}Lvs^h	68	
			Ctr$^+$Suc$^-$Pha^{r}Lvs$^+$	28	
			Ctr$^+$Suc$^-$Pha^{s}Lvs^h	1	
			Ctr$^+$Suc$^-$Pha^{s}Lvs$^+$	218	

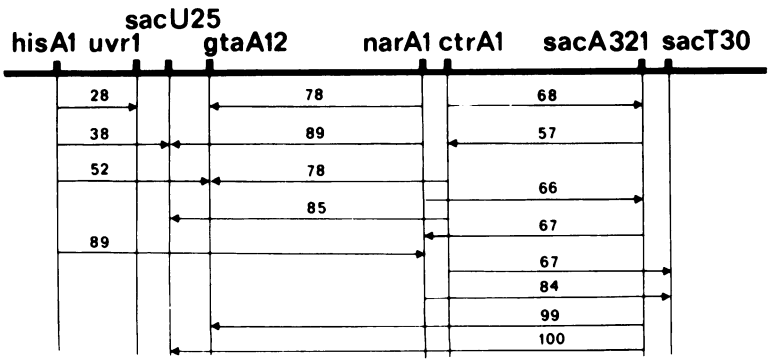


FIG. 2. *Genetic map of the hisA to sacT region. Distances are expressed as percentage of recombination in PBS1 transduction; values are taken from Table 3. The arrows point from the selected to the unselected markers.*

TABLE 5. *Mapping of tre-12 and pha-1 markers relative to purB6 by three-factor crosses*

Recipient genotype	Donor genotype	Recombinants			Implied order
		Selection	Classes	No.	
purB6 dal trpC2 metB5 sorR (QB805)	*pha-1 hisA1 catA* (QB804)	Ade$^+$	Ade$^+$Pha^{r}dal$^+$	77	*dal pha-1 purB6*
			Ade$^+$Pha^{r}dal$^-$	90	
			Ade$^+$Pha^{s}dal$^+$	1	
			Ade$^+$Pha^{s}dal$^-$	125	
purB6 tre-12 (QB693)		Tre$^+$	Tre$^+$Ade$^+$Pha^r	18	*pha-1 purB6 tre-12*
			Tre$^+$Ade$^+$Pha^s	78	
			Tre$^+$Ade$^-$Pha^r	0	
			Tre$^+$Ade$^-$Pha^s	223	

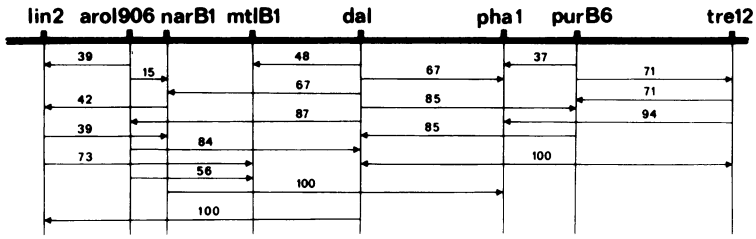


FIG. 3. *Genetic map of the lin to tre region. Distances are expressed as percentage of recombination in PBS1 transduction; values are taken from Table 3. The arrows point from selected to the unselected markers.*

TABLE 6. *Mapping of aroI906, narB1, and mtlB1 markers relative to dal by three-factor transduction crosses and linkage of narB1 with lin-2*

Recipient genotype	Donor genotype	Recombinants			Implied order
		Selection	Classes	No.	
narB1 dal metB5 sacA321 (QB817)	*trpC2 metC3 mtlB1* (PG594)	Dal$^+$	Dal$^+$Mtl$^+$Nar$^+$	3	*narB1 mtlB1 dal*
			Dal$^+$Mtl$^+$Nar$^-$	76	
			Dal$^+$Mtl$^-$Nar$^+$	131	
			Dal$^+$Mtl$^-$Nar$^-$	87	
aroI906 dal (QB820)	*mtlB1 narB1 sacA321* (QB819)	Dal$^+$	Dal$^+$Mtl$^+$Aro$^+$	2	*aroI906 mtlB1 dal*[a]
			Dal$^+$Mtl$^+$Aro$^-$	161	
			Dal$^+$Mtl$^-$Aro$^+$	44	
			Dal$^+$Mtl$^-$Aro$^-$	122	
narB1 trpC2 (CU636)	*lin-2 strA1 pac-3 spcB2 thyAB* (VUB34)	Nar$^+$	Nar$^+$Lin^{r}Str^r	0	*strA1 lin-2 narB1*
			Nar$^+$Lin^{r}Str^s	229	
			Nar$^+$Lin^{s}Str^r	0	
			Nar$^+$Lin^{s}Str^s	221	
		Lin^r	Lin^{r}Nar$^+$Str^r	0	
			Lin^{r}Nar$^+$Str^s	218	
			Lin^{r}Nar$^-$Str^r	7	
			Lin^{r}Nar$^-$Str^s	177	

[a] The position of *narB1* was not deduced from this cross (see text).

to *thyA* segment, placing *argC* and *argA* on opposite replication arms of the chromosomes and creating a gap delimited by the *thyA* and *recA1* markers.

In order to confirm this assumption the linkage of *recA1* with *thyA* was tested in three-factor crosses involving *recA1*, *thyA*, and *citB17* in strain QB790 (*recA1*, *thyA*, *thyB*, *citB17*, *trpC2*). It is a well-established fact that in *B. subtilis* the Thy$^+$ phenotype is conferred by the simultaneous presence of two mutations affecting the unlinked *thyA* and *thyB* loci, the *thyA*$^+$ *thyB*$^-$ and *thyA*$^-$ *thyB*$^+$ strains being phenotypically Thy$^+$ (1, 30). As a consequence, the

QB788 strain (*thyB trpC2*) has been used as a donor in order to avoid the appearance of *thyA*$^-$ *thyB*$^+$ recombinants when selection was made for Thy$^+$ recombinants. The *thyA*$^+$ *thyB*$^-$ constitution of strain QB788 was checked by transduction into strain QB82 (*ilvA1 thyA*) with selection for Ilv$^+$. As expected from the linkage of *thyB* with *ilvA1* (1, 30) *thyA*$^-$ *thyB*$^-$ (thymine-requiring) colonies were recovered in this cross (Table 7).

The results of the cross of QB790 with QB788 demonstrate a linkage of *recA1* with *thyA* (80% recombination) and are in agreement with the order *citB17*, *thyA*, *recA1* (Table 7). Sensitivity to aminopterin (1, 30) of donor, recipient, and recombinant strains was not examined in this study. Independent results of Harford (12) tend to confirm the *recA1-thyA* linkage.

The linkage of *citB* with *gltA* has been found by Hoch and Zahler but is as yet unpublished (personal communications, [16, 34]). Otherwise, the linkage of *recA1* with *ura-1* has been reported by three different authors (6, 12, 14).

Indications for circularity of the genetic map. The new data reported here, together with co-transduction results reported by other authors (34), establish the continuity of two large linkage groups. One extends from *tre* to *sacQ* and the other extends from *metD* to *argA*.

Positive indications were obtained for linking these two groups to one another. In repeated crosses (Table 8) *aroG932* was found to be weakly linked with both *sacQ36* and *ald* (a marker recently mapped between the *thr-5* and *sacQ36* markers and very close to the latter marker [J. Trowsdale, D. A. Smith, G. W. Gould, submitted for publication]).

Since the mapping of *aroG* relative to *argA*

TABLE 7. *Three-factor transduction crosses involving recA1, thyA, and citB17; verification of the ThyA*$^+$ *ThyB*$^-$ *constitution of strain QB788*

Recipient genotype	Donor genotype	Recombinants			Implied order
		Selection	Classes	No.	
recA1 thyA thyB citB17 trpC2 (QB790)	*thyB trpC2* (QB788)	Thy$^+$	Thy$^+$Cit$^+$Rec$^-$	196	*recA1 thyA citB17*
			Thy$^+$Cit$^+$Rec$^+$	49	
			Thy$^+$Cit$^-$Rec$^-$	75	
			Thy$^+$Cit$^-$Rec$^+$	28	
		Cit$^+$	Cit$^+$Thy$^-$Rec$^-$	165	
			Cit$^+$Thy$^-$Rec$^+$	2	
			Cit$^+$Thy$^+$Rec$^-$	431	
			Cit$^+$Thy$^+$Rec$^+$	77	
thyA ilvA1 (QB782)	*thyB trpC2* (QB788)	Ilva$^+$	Ilva$^+$Thy$^-$Trp$^+$	30	*ilvA1 thyB1 trpC2*
			Ilva$^+$Thy$^-$Trp$^-$	52	
			Ilva$^+$Thy$^+$Trp$^+$	13	
			Ilva$^+$Thy$^+$Trp$^-$	1	

TABLE 8. *Linkage of aroG932 with ald and sacQ36 and mapping of aroG932 relative to argA2 and leu-8 by three-factor transduction crosses*

Recipient genotype	Donor genotype	Recombinants			Implied order
		Selection	Classes	No.	
aroG932 (WB932)	*sacQ36 ald trpC2* (QB698)	Aro$^+$	Aro$^+$Lvs^{h}Ald$^-$	4	*aroG932 sacQ36 ald*
			Aro$^+$Lvs^{h}Ald$^+$	12	
			Aro$^+$Lvs$^+$Ald$^-$	0	
			Aro$^+$Lvs$^+$Ald$^+$	453	
sacQ36 ald trpC2 (QB698)	*aroG932* (WB932)	Ald$^+$	Ald$^+$Lvs^{h}Aro$^+$	62	
			Ald$^+$Lvs^{h}Aro$^-$	0	
			Ald$^+$Lvs$^+$Aro$^+$	329	
			Ald$^+$Lvs$^+$Aro$^-$	5	
aroG932 (QB932)	*leu-8 argA2 metB5 sacA321* (QB818)	Aro$^+$	Aro$^+$Arg$^-$Leu$^-$	32	*leu-8 argA2 aroG932*
			Aro$^+$Arg$^-$Leu$^+$	131	
			Aro$^+$Arg$^+$Leu$^-$	1	
			Aro$^+$Arg$^+$Leu$^+$	94	

TABLE 9. *Three-factor transduction crosses involving catA, metD1, argC4 markers*

Recipient genotype	Donor genotype	Recombinants			Implied order
		Selection	Classes	No.	
hisA1 argC4 metD1 pha-1 (BC369)	*catA lys phe arg* (SCR1026)	Arg$^+$	Arg$^+$Met$^+$Prt^h	54	*catA metD1 argC4*
			Arg$^+$Met$^+$Prt$^+$	215	
			Arg$^+$Met$^-$Prt^h	0	
			Arg$^+$Met$^-$Prt$^+$	58	

has not been reported in detail (17), the position of *aroG932* was further examined in a three-factor cross involving *leu-8*, *argA2*, and *aroG932*. Results of this cross confirmed the order, *aroG932*, *argA2*, *leu-8* (Table 8). On the opposite chromosome arm a very weak linkage was found between the *tre-12* and *catA* markers in repeated two-factor crosses (Table 3).

The mapping of *catA* has not been reported in full detail by Ito and Spizizen (19) and its position relative to *metD1* and *argC4* was ascertained by three-factor transduction crosses of the type summarized in Table 9. Results of these crosses were in agreement with the order *catA*, *metD1*, and *argC4*. It should be noted that no co-transfer of *catA* was found with other markers except *tre-12*, *metD1*, and *argC4* (Table 3).

DISCUSSION

The transductional mapping data reported above lead to important revisions and extensions of the linkage map of the *B. subtilis* 168 chromosome as compared to former representations.

The segment comprised of the region between the *sacQ36* and *gtaA12* markers has been inverted and transposed into a new position. Genetic continuity between this segment and the segment commencing with *narA1* and including *purA* appears to be firmly established on the basis of the co-transfers found between markers extending from *hisA1* to *sacA321*.

The segment comprising the *purB* locus has been extended by the mapping of *tre-12* at one end and three new markers, *mtlB1*, *aroI906*, and *narB1*, at the other. The relative orientation of this segment and continuity with the *purA* segment is clearly established on the basis of the co-transfer of *mtlB1*, *aroI906*, and *narB1* with the *lin-2* marker. This has been confirmed by Harford (12).

In this study, the *pha-1* marker has been located between the *dal* and *purB6* markers. No deficiency in phosphoglucomatase activity was observed in all strains bearing *pha-1* (unpublished results). Thus *pha-1* appears to affect a gene regulating phage resistance which is different from the *gtaA*, *gtaB*, and *gtaC* loci previously characterized and mapped by Young et al. (33). Two other markers, *sorR1* (obtained from Chalumeau; regulatory gene of the sorbitol operon) and *furB* (obtained from Zahler; resistance to 5-fluorouracil), have been also located between the *dal* and *purB6* markers. A detailed report of these data will be published elsewhere.

These new results together with results reported by other authors (see reference 35 for a compilation of transduction data) define a large linkage group extending from *tre-12* to *sacQ36*. Co-transfer values between markers inside this segment are high with the exception of the *sacS* to *purA* distance which is over 90% recombination (22).

The finding of a linkage between *recA1* and *thyA* leads to the inversion of the *thyA-aroG* segment as it is shown in Fig. 4 and the definition of a second large linkage group extending from *catA* to *aroG*. It is possible that the measurement of the *recA1* to *thyA1* distance could have been influenced by the conditions under which the selection of Thy$^+$ recombinants was made. Wilson et al. noted that up to one-third of Thy$^+$ recombinants die if placed directly onto selective medium (30). However, it can be noted that in this series of crosses the *citB* to *thyA* distance appears to be approximately the same when the primary selection is made either for Cit$^+$ or Thy$^+$ recombinants (75 and 70% co-transfer, respectively). Neubauer and Zahler obtained a somewhat smaller value of 45% co-transfer (cited in reference 34 and personal communication). The linkage of *recA1* with *citB17* or *thyA* has not been tested in the reverse direction since no primary selection for Rec$^+$ recombinants has yet been made.

In this context, further testing of markers located close to *recA1* like *aspA* (16) for linkage with *thyA* and *citB17* would help in confirming these preliminary results.

Otherwise the genetic continuity over the *catA* to *aroG* segment appears to be clearly established on the basis of several sets of independent results. (For a review see reference 34. The unpublished map position of the *citB* and *gltA* loci has been personally communi-

cated to us by Hoch and Zahler.) We are currently examining the linkage relationship between the *citK*, *gltA*, *citB*, *thyA*, and *recA* loci.

The weak linkages found between *aroG932* and *sacQ36* and between *tre-12* and *catA* indicate that the two major linkage groups are joined together with the circular configuration shown in Fig. 4. However, these indications of genetic circularity should not be regarded as definitive and the mapping of additional markers in the areas of weak co-transfer is crucially needed in order to substantiate these results.

An argument in favor of the validity of the *tre-12–catA* linkage can be taken from a recent report of Naumov et al. (25). These authors mapped by phage AR9 transduction an adenosine triphosphate-dependent nuclease marker (*rec342*) and a thiamine marker *thi78* between *purB6* and a glycine marker, *gly133*. They established the sequence *purB6*, *rec342*, *thi78*, *gly133*, and observed also a weak co-transfer (4%) between *gly133* and *argC4*. The mapping of *rec342*, *thi78*, and *gly133* relative to *tre*, *catA*, *metD*, and *argC4* is currently under investigation with the use of PBS1 transduction.

This assumption of circularity of the genetic map on the basis of the present data is strongly supported by the autoradiographic evidence of Wake which demonstrates physical circularity of the chromosome (10, 28, 29). The density transfer results of Harford (12) suggest that since *argA2* and *argC4* replicate relatively soon after *tre-12* and *thr-5*, the genetic distances separating these markers are probably not more than 100 to 200 transduction map units in extent.

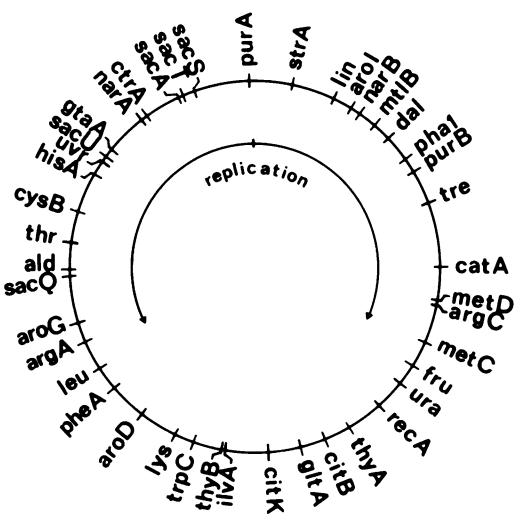


FIG. 4. *Revised genetic map of the B. subtilis 168 chromosome. For simplicity, only markers used in this study and a few other markers are presented.*

The new linkage assignments on the revised map of Fig. 4 and the replication orders determined by Harford in the accompanying paper (12) strongly support the hypothesis of a circular chromosome which replicates bidirectionally over two arms of approximately equal length from an origin located close to the *purA16* marker. These findings corroborate the conclusions of Wake deduced from the autoradiographic visualization of the replication of the *B. subtilis* 168 chromosome (29).

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