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Volume 1

Bacillus subtilis

The Molecular Biology of the Bacilli

Volume I: *Bacillus subtilis*

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Preface

Since the discovery of DNA-mediated transformation in *Bacillus subtilis* by Spizizen in 1958, this organism has emerged as a second major prokaryotic subject for the study of molecular biology, providing a valuable gram-positive counterpoint to *Escherichia coli*. Although universal aspects of biology have properly received the lion's share of attention to date, particularistic studies that stress differences between organisms will probably become increasingly important. In this regard the study of *B. subtilis* will undoubtedly assume a major role as a representative of a second major class of prokaryotes. Some problems, like the control of sporulation and the uptake and integration of transforming DNA, can obviously be approached readily in *B. subtilis*; and their study will be facilitated by the considerable body of knowledge that exists concerning its genetics, biochemistry, and physiology.

Bacilli are also of major importance in the fermentation industry since they elaborate a variety of useful enzymes and antibiotics. As a consequence of this, industrial-scale techniques for the cultivation of bacilli are relatively well developed. The ability of several bacilli to excrete large amounts of certain proteins into the extracellular space is a particularly attractive feature for commercial exploitation. Unfortunately, little is known about the molecular basis of protein excretion in the bacilli and the coupling, if any, of excretion and synthesis. It is likely that this and the intriguing problems associated with growth stage related regulation (see Chapter 11) will yield to investigation within the next few years. This optimism is partly based on the advent of molecular cloning in *B. subtilis*.

The present treatise is directed toward two overlapping groups of scientists: those who are concerned with the use of the *B. subtilis* system as a tool for the study of molecular biology and those who wish to increase the medical, veterinary, and industrial usefulness of this and related organisms. In selecting the subject matter of this volume, I have given primary attention to those areas of research that have been traditionally investigated in this organism (sporulation, defective bacterio-

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phages, transformation), to those topics in which outstanding progress has been made in recent years, and to those subjects that are of interest to the two groups of scientists described above. There has been no attempt to be comprehensive. It is hoped that subsequent volumes, when warranted by progress in the field, will make up for any omissions.

I would like to thank Eugenie Dubnau and Issar Smith for many discussions during the course of this work and for help with some of the editing. To Annabel Howard, my special appreciation for much of the secretarial work associated with preparing this volume.

David A. Dubnau

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1

The Genetic Map of *Bacillus subtilis*

DENNIS J. HENNER AND JAMES A. HOCH

Introduction

Studies have focused on many different aspects of *Bacillus subtilis*, including biochemical and morphological changes during sporulation, germination, and outgrowth; mechanisms of genetic exchange in transformation and transduction; cell wall synthesis, cell growth, and division; enzymatic characterization of anabolic and catabolic pathways; and a host of others. The isolation of mutants defective in these processes with subsequent genetic analysis has resulted in our contemporary genetic map. As the most thoroughly characterized gram-positive microorganism, *B. subtilis* allows an evolutionary comparison of chromosome organization with that of other, distantly related, microorganisms (*10*). In addition, a genetic analysis of the primitive differentiation cycle this mi-

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croorganism undergoes, i.e., sporulation–germination, permits an assessment of unique aspects of chromosome structure and gene organization that may be attributed to developmental gene expression.

The advent of molecular cloning with its precise definitions of physical distances between mutations within genes or between genes themselves dictates that some relationship be formulated between observed genetic distance (i.e., cotransfer frequency) in transformation or transduction and actual physical distance between mutations. An initial attempt to define this relationship is presented below. Although imprecise at this time, the analysis allows an estimation of the physical distance from the genetic distance. A more detailed rationale for some of the assumptions used can be found in an earlier work (*66*).

A. Conversion of Genetic Cotransfer Frequency to Physical Distance between Markers

The frequency of genetic cotransfer between various linked markers is characteristic of the system used for genetic analysis. In general, the larger the piece of DNA transferred in the system, the higher the probability that two linked markers will be cotransferred. In *B. subtilis*, we have two useful systems of genetic analysis that differ greatly in the size of the piece of DNA transferred: PBS1 transduction and transformation. PBS1 transduction may allow fragments as large as 10% of the entire chromosome to be transferred, whereas transformation with naked DNA probably occurs with fragments $\frac{1}{10}$–$\frac{1}{20}$ the size of the transducing fragment (*66*). In order to compute the physical distance between markers on the transforming or transducing DNA fragment from genetic cotransfer frequencies obtained in each system, one must derive an expression that relates genetic values to physical distance.

The relationship between the frequency of cotransduction of markers and the physical distance between the markers on a transducing piece of DNA has been the subject of several mathematical models (*91,198*). The basic features of these models include the possibility that factors other than recombinational events will affect the apparent frequency of recombination between any two markers. In order to be cotransduced the two markers must be contiguous on a single fragment of transducing DNA and, as the distance between them increases, their chance of being on the same transducing fragment decreases. Furthermore, as the markers become closer to the end of the transducing fragment, the size of the pieces of DNA outside the markers in which crossing-over might occur becomes limited, reducing the number of cotransductants. The models also make certain assumptions about the transduction process.

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They assume that recombination is linearly proportional to the length of DNA in which the recombination can occur, and that transducing fragments are of uniform length and have random end points. None of these assumptions has been verified in either the PBS1 transduction system or the transformation system, and there is evidence in some transduction systems for violation of certain of these assumptions, i.e., marker-specific effects, nonreciprocal crosses, preferred end points (*91*). However, the theoretical predictions of the models have shown good agreement with observed distances in P22 transduction in *Salmonella typhimurium* (*32,91*).

Kemper's formula (*91*), $C = 1 - t + t(\ln t)$, where C is the frequency of cotransduction of two markers and t is the fractional length of the transducing fragment separating the markers, has been used to convert cotransduction frequencies to physical distances along a transducing or transforming DNA fragment. A plot of this equation is shown in Fig. 1. An inspection of this plot shows that the cotransduction frequency falls quickly as the physical distance between two markers increases. For example, two markers, A and B, separated by half the length of a transducing fragment ($0.5t$), will show only 15% cotransduction, not 50%. This model predicts that recombination values will not be additive. If a marker, C, is halfway between the markers A and B above, it will be $0.25t$ from each and show 60% recombination from each, but the two outside markers, A and B, will show only 85% recombination, not 120%. Thus, in composing the final genetic map of *B. subtilis* the genetic distances from PBS1 transduction were converted to physical distances by

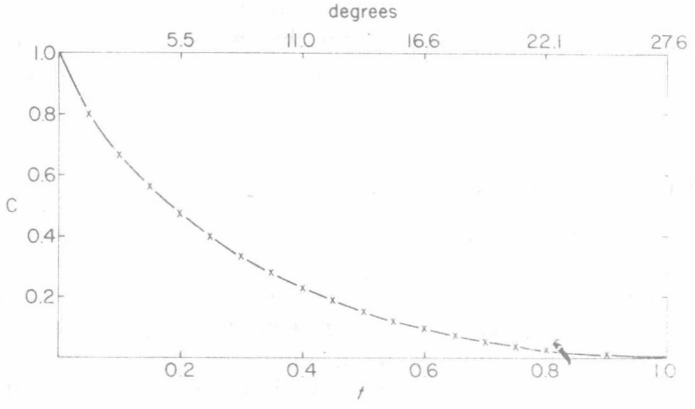

Fig. 1. Cotransfer frequency (C) in genetic analysis as a function of physical distance between markers (t) on donor DNA molecule plotted according to the equation $C = (1 - t) + t(\ln t)$.

the curve in Fig. 1. The validity of using this formula to correct PBS1 transduction values has been verified with a number of markers (*66*).

B. Relationship between the Computed Physical Distance and the Amount of DNA between Markers

Application of the Kemper formula to transduction or transformation data allows one to reduce these data to a value t that gives the fractional length of the transducing or transforming DNA fragment between any two markers. If the average length of these fragments were known, in terms of base pairs, an estimate of the number of base pairs between markers could be obtained. This relationship is simply $D = t(T)$, where D is the distance between markers in base pairs, t is the fractional length of the transducing or transforming DNA fragment, and T is the average size of this fragment in base pairs. In PBS1 transduction we have calculated that T is approximately 150×10^6 daltons or 250 kbp of DNA (*66*). Thus any set of genetic data for cotransfer between two markers may be converted to kilobase pairs of DNA between the markers by application of these equations. Such information is useful in many contexts, such as cloning experiments where decisions on what the chances for success are of overlapping hybridization experiments in moving from one marker to another if only the genetic distance between the markers is known. As more segments of the *B. subtilis* genome become available as cloned fragments, we shall have the opportunity to determine more precisely DNA distances between markers and their relationship to genetic cotransfer values.

Using a T value for PBS1 transduction of 150×10^6 daltons, we have estimated that the *B. subtilis* genome has a size of approximately 2×10^9 daltons (*66*), a value in agreement with physical determinations (*11,53*).

C. Detailed *Bacillus subtilis* Map

The genetic map of *B. subtilis* is circular and uninterrupted. We have chosen to divide this circle into 360° and to locate individual markers by a degree designation. Unfortunately, some regions of the chromosome are too crowded to permit a detailed genetic map in a circular format. Thus, a circular map with landmark loci (Fig. 2) may be used for orientation with the detailed map presented in Fig. 3. The circular map has been divided into 10 segments of 36° each. We have chosen to use the *guaA* locus as 0° because it is close to the origin of replication and is

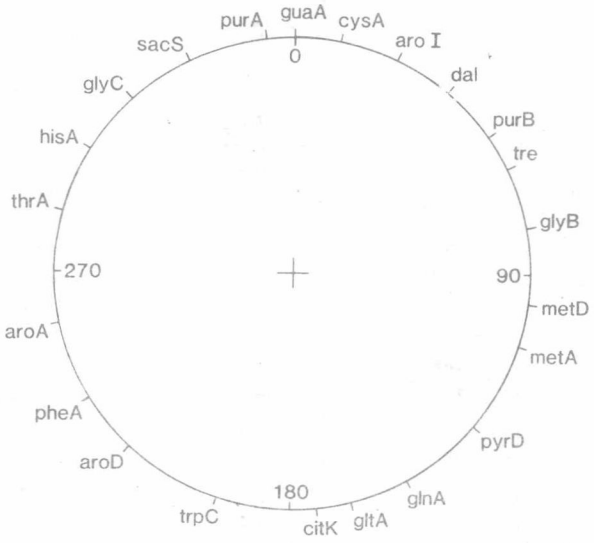

Fig. 2. Abbreviated genetic map of *B. subtilis* showing the landmark genetic markers.

an easily selectable marker in this region that has been used for a large number of genetic crosses.

A variety of new markers have been placed on this *B. subtilis* map that were not present on the previous version (*66*). These have been localized by their cotransduction with known genetic markers and positioned accordingly. No attempt was made to recalibrate the entire map using the new cotransduction data. One region, comprising the *metD–recA* interval, has been reanalyzed and some major changes made in both the order of markers and the intervals between markers. The order of the *cysC* and *pyr* cluster has been reversed in orientation from the previous map. There are a variety of conflicting reports regarding the order of these loci (*203;* R. Buxton, personal communication; K. Yamane, personal communication), but some recent mapping data favor a reversal of the previously published order (Ordal and Hoch, in preparation). Several other loci ordered with respect to *cysC* and *pyr* have also been moved. Many intervals between loci in this region have been changed, with a significant reduction in the overall size of the region. It would not be too startling if there proved to be an inversion of the *cycC–pyr* region in some strains of *B. subtilis*.

Since *B. subtilis* 168 is the most widely used strain of *B. subtilis*, we had difficulty in deciding whether to place markers introduced from

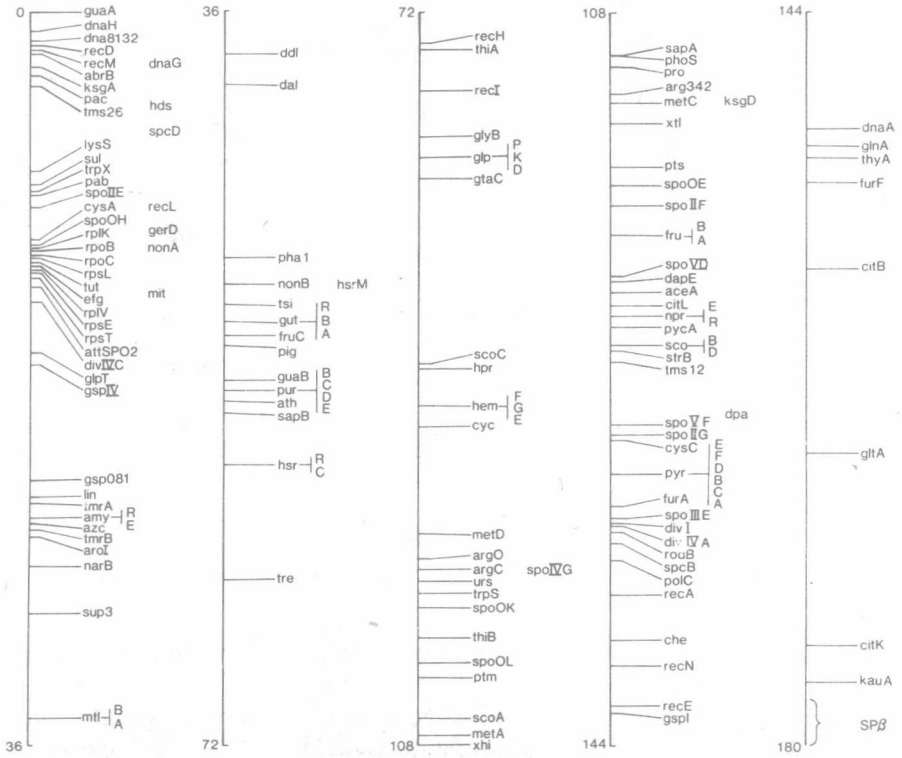

Fig. 3. Detailed genetic map of *B. subtilis*.

related strains on the genetic map. For example, the restriction–modification systems from various related strains may be introduced by transformation into the 168 strain and mapped by PBS1 transduction (*164*). We have placed these markers on the present map, since we feel that there must be a nearly homologous region in *B. subtilis* 168 in order for these markers to be introduced into the chromosome.

Many markers placed on this map have never been ordered with respect to their neighbors but mapped only with respect to landmark loci in the region. We hope that the publication of this genetic map will encourage workers to order new (and old) loci with respect to nearby markers on the map. Until such crosses are performed, the order and position of loci must be regarded as approximate and the original literature should be consulted.

The cloning of various genes of *B. subtilis* will certainly be useful in the near future in generating an accurate map of *B. subtilis*. Exact dis-

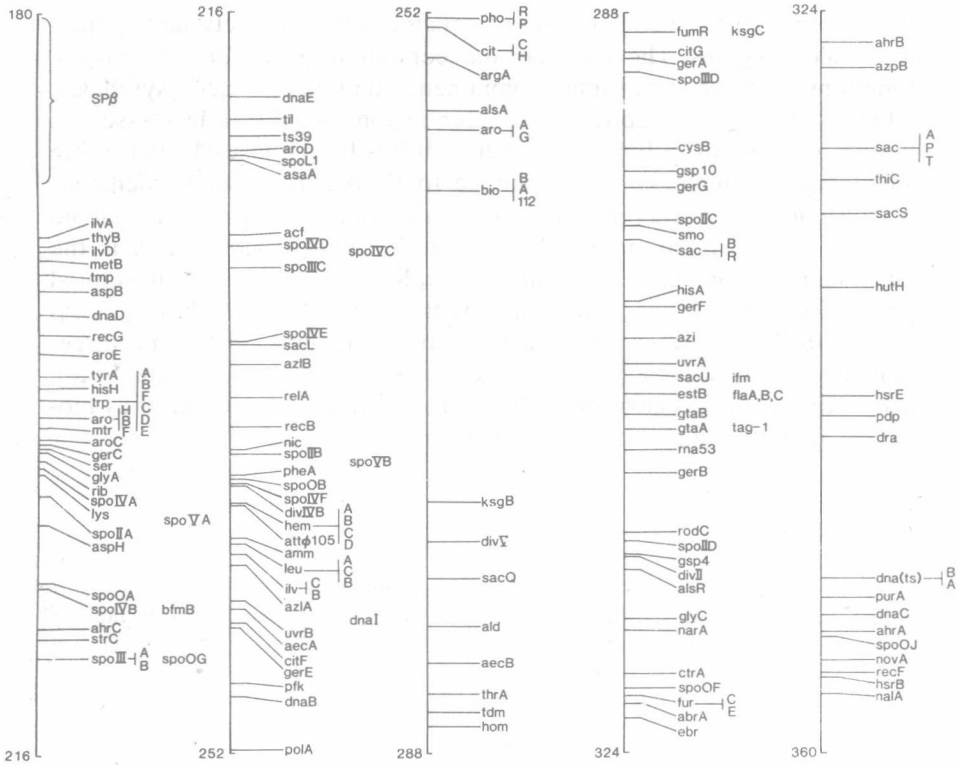

Fig. 3 Continued.

tances between loci will be determined, and the experimental relationship between physical distances and cotransformation and cotransduction determined. Conversely, the genetic map should prove to be of use in cloning experiments. For example, a marker that is not easily selectable could be cloned by selecting for a known nearby marker. Also, if the general chromosome organization of the various *B. subtilis* strains is similar, one might be able to screen for the desired gene from the related strain by hybridization with the similar cloned segment from the 168 strain.

D. Genetically Silent Regions

Several features of chromosome organization are common to both *B. subtilis* and *E. coli* that may indicate some fundamental structural constraints on gene distribution. In both organisms there is a tendency for

the known loci to cluster in groups separated by relatively large genetically silent regions. The major clusters contain the genes for maintenance functions such as ribosomal components and amino acid synthesis, whereas the loci that appear in sparser regions seem of a less essential nature, e.g., sugar utilization enzymes. It has been suggested that these silent regions might be less available to RNA-synthesizing machinery because of a structural role in the folded and condensed bacterial nucleoid (*10,66*). One striking similarity between *B. subtilis* and *E. coli* is the large silent region near the terminus of replication, although a substantial proportion of this region is occupied by the temperate bacteriophage SPβ in *B. subtilis*. Whether silent regions are an integral part of the three-dimensional structure of bacterial genomes or whether they simply are less active coding regions for other reasons should be answered as molecular cloning makes these regions available for study.

Acknowledgments

We are grateful to our colleagues for unpublished mapping data. This work was supported, in part, by grants GM19416 and GM25891 awarded by the National Institute of General Medical Sciences, PHS/DNHS.

Appendix: *Bacillus subtilis* Genetic Loci

Gene symbol	Mnemonic	Map position[a]	Phenotype, enzyme deficiency, or other characteristics	References[b]
abrA	Antibiotic resistance	325	Partial suppressor of stage-0 mutant phenotypes	A
abrB	Antibiotic resistance	5	Partial suppressor of stage-0 mutant phenotypes	*184*
absA	Antibiotic sensitivity	—	Partial suppressor of stage-0 mutant phenotypes; see *abrB*	*83, 84*
absB	Antibiotic sensitivity	—	Partial suppressor of stage-0 mutant phenotypes; see *abrB*	*83, 84*
ace	Acetate	122	Pyruvate dehydrogenase defect, linked to *pycA*	C
acf	Acriflavine	235	Acriflavine resistance	*13, 82*

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