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Genetics

Developmental cell interactions in *Myxococcus xanthus* and the *spoC* locus

(tandem duplications/sporulation/plasmid integration/genetic complementation/gene conversion)

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ABSTRACT **The product(s) of the *Myxococcus xanthus spoC* locus is required for two multicellular activities in fruiting body development, rippling and sporulation. Ripples, which are formed early in development, are spatially separated ridges of cells that move synchronously. Myxospores are heat-resistant resting cells that are formed near the end of the developmental process. To investigate the function of *spoC*, it was cloned in an *Escherichia coli* plasmid, then transferred to *M. xanthus* by specialized transduction with coliphage P1. The plasmid, which cannot replicate in *M. xanthus*, integrated into the *M. xanthus* chromosome, producing two copies of the *spoC* locus in tandem. Cells containing one copy of a mutant allele and one copy of the wild-type allele displayed the wild-type phenotype. Cells containing two different mutant alleles failed to ripple or sporulate, implying that all four independent *spoC* mutations are in the same gene or unit of transcription. Homozygous mutant duplications arose from constructions in which DNA from a *spo*$^+$ donor was transduced into a *spoC* recipient, or vice versa, at an average frequency of 14%, indicating that gene conversion was a frequent event.**

Myxobacteria, such as *Myxococcus xanthus*, are prokaryotes with a developmental cycle that involves interactions between cells (1). When *M. xanthus* is plated at a high cell density on a low-nutrient agar, many thousands of cells move to a central location, where they form an aggregate having a species-characteristic shape (2, 3). A mature fruiting body is produced when the rod-shaped cells within the aggregate become spherical, dormant myxospores.

Some mutants of *M. xanthus* that exhibit normal vegetative growth are apparently defective in cell–cell interactions essential for development (4, 5). When these mutants are placed under conditions that would induce the wild type to form fruiting bodies, they start to develop, but their development stops before myxospore formation. These mutants do form myxospores when they are mixed with developing wild-type cells, as if they fail to produce, but remain responsive to, essential intercellular stimuli. Pairwise testing of 57 mutants divided them into four groups designated SpoA, -B, -C, and -D. Sporulation occurred in mixtures of two mutants belonging to different groups but not in mixtures of two mutants of the same group.

One of these groups, SpoC, is also defective in rippling. During fruiting body development, *M. xanthus* cells accumulate in parallel ridges, which move in concert like ripples on the surface of water (6, 7). The spatial coordination of this motion would seem to require interactions between cells. Among the four groups of Spo mutants, only the members of group C fail to form ripples (7). All the SpoC mutants are deficient in both fruiting body development and rippling, but they form a single group in the mixed sporulation test described above and are genetically linked to each other (5). To determine how many genes are at the *spoC* locus we have cloned the locus in an *Escherichia coli* plasmid. The plasmid was transduced into *M. xanthus*, where the cloned segment recombined with its homolog in the *M. xanthus* chromosome to form a tandem duplication of the cloned segment. In this way heterozygotes were constructed and dominance and genetic complementation tests were performed. The data argue for a single transcription unit at the locus, and some of the implications of this finding are explored.

MATERIALS AND METHODS

Rippling and Fruiting. Vegetative cultures were grown in CTT broth (8). To induce fruiting body development and to test rippling, colonies from CTT agar plates were picked with a toothpick and transferred to CF agar (4). After 1 day at 32°C, 1 μl of purified *Sarcina lutea* murein at 20 mg/ml was placed on a section of the colony and allowed to dry (7). Plates were incubated 24 hr at 32°C and observed for ripples under a stereomicroscope. Plates were incubated for 2 additional days at 32°C (total of 4), then examined for heat-resistant spores (5).

Cloning of *Myxococcus* Genes. Because the Tn5 insertion Ω1519 is 75% cotransduced with *spoC* mutations (5), the locus may occupy the same *Bam*HI fragment with the kanamycin phosphotransferase from Tn5. Restriction endonuclease *Bam*HI cuts once in Tn5 next to the kanamycin phosphotransferase (9) but cuts *M. xanthus* sequences infrequently. Accordingly, *Bam*HI restriction fragments from *spoC*$^+$ and *spoC*$^-$ strains containing Tn5 Ω1519 were ligated to pREG411, described below, and *E. coli* Q358 (10) was transformed for kanamycin resistance by following the cloning procedures described by Davis *et al.* (11). To join restriction fragments, 5.6 μg of *Bam*HI-digested vector was mixed with 20 μg of *Bam*HI-digested *M. xanthus* DNA in a total volume of 400 μl. Plasmid clones were obtained from four different strains: pLJS9 from DK1531 (SpoC$^+$), as well as three *spoC*$^-$ alleles, pLJS10 with *spo-741*, pLJS11 with *spo-653*, and pLJS12 with *spo-731*. All four plasmids contain a 21-kilobase (kb) insert extending from the center of Tn5 through the kanamycin phosphotransferase one direction into the *Myxococcus* chromosome (Fig. 1). The recombinant DNA experiments described here follow the National Institutes of Health guidelines.

Transfer of Plasmid Clones from *Escherichia coli* to *Myxococcus*. The coliphage P1 will inject its DNA into *M. xanthus* but it fails to multiply or to establish lysogeny (12, 13). P1 specialized transducing particles may be used to transfer plasmids to *M. xanthus* (unpublished results). The cloning vector, pREG411, is a pBR322-derived plasmid with a 6.7-kb *Eco*RI restriction

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Abbreviations: kb, kilobase(s); Km^r and Km^s, kanamycin-resistant and kanamycin-sensitive; Tc^r, tetracycline-resistant; Spo, capacity to sporulate; Rpl, capacity to ripple.

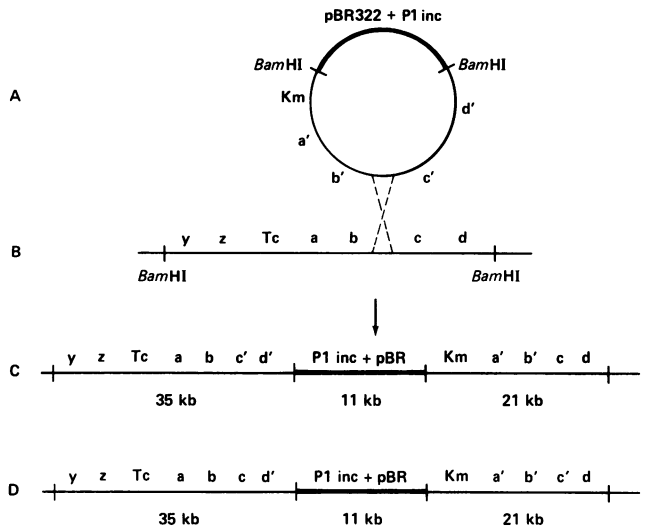

FIG. 1. The circular plasmid (*A*) contains two *Bam*HI fragments, the one represented by the thicker line being the vector, pREG411, and the one represented by the thinner line being the cloned *M. xanthus* fragment that contains a gene encoding kanamycin resistance (*Km*). The recipient chromosome (*B*) contains an insertion of Tn*5*-132 (*Tc*) (which includes a gene encoding tetracycline resistance) that is located in the same position (relative to *M. xanthus* sequences) as the wild-type Tn*5* (*Km*) in the plasmid. Plasmid and recipient can be distinguished from each other by the size of their *Bam*HI fragments, their antibiotic resistance, and their *M. xanthus* genotype. A crossover in homologous sequences between *b'* and *c'* leads to the integration of the plasmid to generate a kanamycin-resistant tetracycline-resistant (Km^r Tc^r) strain that is a partial diploid for those genes contained on the plasmid (*C*). (*D*) Consequences of an alternative recombination between *c'* and *d'*.

fragment encoding P1-specific incompatibility (14). When an infecting P1 establishes lysogeny in an *E. coli* strain carrying pREG411, a cointegrate between P1 and pREG411 is formed by recombination in their homologous P1-incompatibility DNA sequences. Upon induction to lytic growth, P1 begins packaging within the P1 segment of the cointegrate and many of the resulting phage particles contain the entire pREG411 plasmid. In practice Q358 containing a plasmid was infected with P1 *cam clr-100* (15) in L broth (15) at a multiplicity of 0.1 for 15 min at room temperature. Infected cells were plated on L agar containing kanamycin at 50 μg/ml, to select for maintenance of the plasmid pREG411 containing a segment of *M. xanthus* DNA, and chloramphenicol at 12.5 μg/ml, to select for maintenance of P1. After incubation at 30°C for 24 hr, drug-resistant colonies were picked and streaked. For thermal induction of the cointegrate lysogens, the culture was incubated in L broth with 10 mM $MgSO_4$ at 30°C until the cell density reached about 2×10^8 cells per ml, then shifted to 40°C for 35 min and 37°C for an additional 60–80 min. Chloroform was added; the suspension was sedimented at 10,000 × *g* for 10 min; and the supernatant was retained as the P1 stock.

To transfer the plasmid to *M. xanthus*, 10 μl of the P1 stock was mixed with 100 μl of exponentially growing *Myxococcus* in CTT broth for 15 min at room temperature. The mixture was plated on CTT agar containing kanamycin at 40 μg/ml and incubated at 32°C for 4–5 days. During this infection, homologous recombination between P1 incompatibility sequences can regenerate the original circular plasmid. Southern blots and nick-translations were performed according to the procedure of Davis *et al.* (11). Procedures for analysis of segregation from tandem duplications were developed by L. Avery (personal communication).

RESULTS

To determine if the loss of ability to sporulate and the loss of ability to ripple in *spoC* mutants result from the same mutation, mutant *spoC* alleles were transduced into wild-type *M. xanthus*, using an insertion of transposon Tn*5*, Ω1519, that is 75% cotransduced with *spoC* by myxophage Mx8 (5). It was found that all the Km^r transductants that had lost the ability to sporulate also had lost the ability to ripple, and all that had retained the ability to sporulate retained the ability to ripple. Because four independently isolated *spoC* mutants were tested, and because more than 575 transductants have been examined in this and subsequent experiments without separating the two properties, the *spoC* locus must be required for both rippling and sporulation.

Construction of Partial Diploids for *spoC*. Plasmid pREG411 containing a *M. xanthus* DNA insert can integrate into the *M. xanthus* chromosome by a single homologous recombination event anywhere within its inserted *M. xanthus* DNA as diagrammed in Fig. 1. This is the only event that can lead to a Km^r transductant because pREG411 cannot replicate in *M. xanthus* and because the *M. xanthus* insert terminates within Tn*5*. Consequently a second homologous recombination event that would replace chromosomal tetracycline resistance and adjacent chromosomal DNA with kanamycin resistance and adjacent cloned DNA from the plasmid is impossible. The entire plasmid integrates into the chromosome, duplicating in tandem those sequences common to the cloned DNA and chromosome.

Restriction analysis supports this mechanism. Fig. 2 shows a Southern blot of DNA from transductants that have acquired kanamycin resistance from the plasmid clone. All contain restriction fragments characteristic of both the donor and recipient, as expected of a duplication. Lane 1 shows plasmid pLJS9, which was also used as the probe for the other lanes. pLJS9 yields two *Bam*HI restriction fragments, one that is 11 kb and corresponds to the vector, pREG411, and one 21-kb fragment that corresponds to the cloned *M. xanthus* DNA. Digests of three more independent plasmid clones, pLJS10, pLJS11, and pLJS12, contain the same two fragments. Lane 2 shows *Bam*HI-digested DNA of the spo^+ Tn*5* strain (DK1531) from which the cloned segment was derived, and it contains the same 21-kb fragment present in pLJS9. Lane 3 is *Bam*HI-digested DNA from DK3332, a strain that contains Tn*5*-132, a modified Tn*5* in which the center fragment encoding kanamycin resistance has been replaced with a fragment encoding tetracycline resistance from Tn*10* (9). Although Tn*5*-132 occupies the same position (Ω1519) in DK3332 that wild-type Tn*5* does in DK1531, the fragment hybridizing with the probe is much larger in DK3332 because Tn*5*-132 lacks

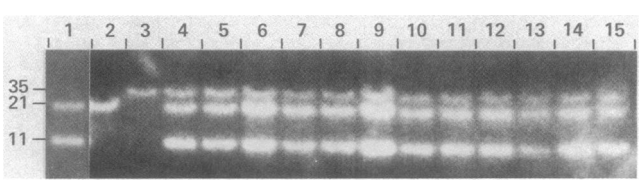

FIG. 2. Southern blot of *Bam*HI restriction fragments of duplications; pLJS9 was used as the probe. Lane 1, pLJS9 (clone); lane 2, DK1531 (parent of clone); lane 3, DK3332 (recipient); lanes 4–15 are duplications made by inserting pLJS9 into either $spoC^+$ or $spoC^-$ recipients otherwise isogeneic with DK3332. Strains with duplications having the ability to sporulate and to ripple (Spo^+ Rpl^+ phenotype): lane 4, DK2652 ΩpLJS9; lanes 5–7, three independent isolates of DK3332 ΩpLJS9; lane 8, DK2653 ΩpLJS9; lane 9, DK2655 ΩpLJS9; and lane 10, DK2657 ΩpLJS9. Strains with duplications having the Spo^- Rpl^- phenotype: lanes 11 and 12, independent isolates of DK3332 ΩpLJS9; lane 13, DK2653 ΩpLJS9; lane 14, DK2655 ΩpLJS9; lane 15, DK2657 ΩpLJS9. Numbers on the left give length in kb.

Table 1. Phenotypes of partial diploids for *spoC*

Parents		Phenotype		
Donor	Recipient	Spo$^+$ Rpl$^+$	Spo$^-$ Rpl$^-$	% +
pLJS9 (*spo*$^+$)	DK2652 (*spo*$^+$)	118	0	100
pLJS9 (*spo*$^+$)	DK2653 (*spo-741*)	14	7	67
pLJS10 (*spo-741*)	DK2652 (*spo*$^+$)	67	5	93
pLJS9 (*spo*$^+$)	DK2655 (*spo-653*)	15	3	86
pLJS11 (*spo-653*)	DK2652 (*spo*$^+$)	85	11	89
pLJS9 (*spo*$^+$)	DK2657 (*spo-731*)	16	7	70
pLJS12 (*spo-731*)	DK2652 (*spo*$^+$)	23	5	82
pLJS9 (*spo*$^+$)	DK3332 (*spo-3330*)	18	2	90

The donor strains were *E. coli* carrying the Tn*5* Km^r plasmids designated in the first column. Phage P1 grown on the donor was used to transduce the *M. xanthus* strain in the second column (the recipient) and carrying Tn*5* tetracycline resistance. Both Tn*5*s are located at position Ω1519. The Km^r Tc^r transductants carry a duplication of Ω1519 and adjacent sequences, including *spoC*. Transductants were scored for their capacity to sporulate (Spo$^+$ or Spo$^-$) and for their capacity to ripple (Rpl$^+$ or Rpl$^-$) as described in the text.

a *Bam*HI site. A *spoC*$^+$ and four different *spoC*$^-$ strains, otherwise isogeneic with DK3332, have been constructed for use as recipients in the construction of tandem duplications. All these strains carry Tn5-132 at Ω1519 and are distinguished from strains carrying wild-type Tn5 in the same position by their restriction pattern (compare lanes 2 and 3) as well as by their antibiotic resistances. Lanes 4 through 15 are restriction digests of transductants formed by introduction of pLJS9 into each of the five different isogeneic recipients just described. These Km^r transductants yield both *Bam*HI fragments of the donor (11 and 21 kb) in addition to the unique 35-kb fragment found in the recipient. They carry kanamycin resistance from the donor and the allelic tetracycline resistance of the recipient. Therefore they behave like strains containing a tandem duplication of a segment of the *M. xanthus* chromosome adjacent to Ω1519.

To test whether the cloned 21-kb *M. xanthus* fragment carries *spoC*$^+$, duplications constructed by introducing pLJS9 into *spoC*$^-$ recipients were tested for their ability to sporulate (Spo) and to ripple (Rpl). As shown in Table 1, 67–100% of the Km^r transductants were phenotypically Spo$^+$ and Rpl$^+$, showing that pLJS9 carries an active *spoC*$^+$ locus. Reciprocal crosses, in which a plasmid containing the homologous 21-kb *M. xanthus* segment from a *spoC*$^-$ strain was transduced into a *spoC*$^+$ recipient, DK2652, also produced a majority of Spo$^+$ Rpl$^+$ transductants (Table 1).

Segregation From Spo$^+$ Rpl$^+$ Partial Diploids. Tandem duplications in *M. xanthus* are unstable, as expected (16), and characterization of the segregants helps to elucidate their structure. When partial diploids for the 21-kb *spoC* segment are grown in the absence of antibiotic selection, kanamycin resistance (contributed by the donor plasmid) is lost at an average rate of 0.2% per generation, but tetracycline resistance (from the recipient chromosome) is stably maintained. *Bam*HI restriction fragments from the kanamycin-sensitive (Km^s) segregants were examined by Southern blots using pLJS9 as the probe (Fig. 3). Lanes 1 and 13 represent the original partial diploids and lanes 2–12 represent segregants from 11 of the independent constructions shown in Fig. 2. In each case the Km^s segregant has lost two fragments, identified by their size as those contributed by the donor plasmid. The segregants always retain one fragment identified by its size as having originated from the *M. xanthus* chromosome. Thus, segregation begins with recombination between duplicated *M. xanthus* sequences and always results in loss of the pREG411 sequence and the kanamycin phosphotransferase gene.

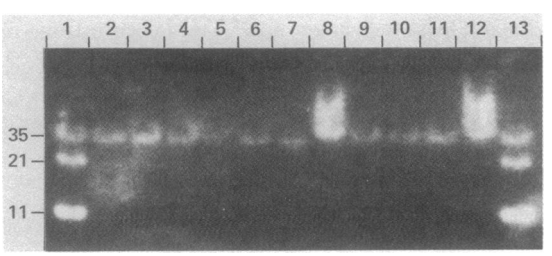

FIG. 3. Southern blot of *Bam*HI restriction fragments, with pLJS9 used as the probe. Lanes 1 and 13 are Km^r Tc^r duplications. Lanes 2–12 are Km^s Tc^r segregants of the duplications shown in Fig. 2. Km^s Tc^r segregants from Spo$^+$ Rpl$^+$ partial diploids: lane 2, a segregant from DK2652 ΩpLJS9 (shown in lane 1); lanes 3 and 4, segregants from independent isolates of DK3332 ΩpLJS9; lane 5, segregant from DK2653 ΩpLJS9; lane 6, segregant from DK2655 ΩpLJS9. Km^s Tc^r segregants from Spo$^-$ Rpl$^-$ partial diploids: lane 7, segregant from DK2657 ΩpLJS9; lanes 8 and 9, segregants from independent isolates of DK3332 ΩpLJS9 (one of the isolates is shown in lane 13); lane 10, segregant from DK2653 ΩpLJS9; lane 11, segregant from DK2655 ΩpLJS9; lane 12, segregant from DK2657 ΩpLJS9.

The genotypes of segregants from *spoC*$^+$/*spoC*$^-$ partial diploids are shown in Table 2. Sporulation and rippling always segregated together. Both *spoC*$^+$ and *spoC*$^-$ segregants arose from all five strains, clearly demonstrating the presence of two different copies of the *spoC* locus in the partial diploid. Two types of partial diploid strains are evident in Table 2: those that segregated *spoC*$^+$ at a high frequency (62–80%) and those that segregated *spoC*$^+$ at a low frequency (6–12%). Two types of diploids are expected: one from integration of the incoming *spo*$^+$ plasmid to the left of *spoC* in the recipient and the other from integration to the right, as illustrated in Fig. 1 *C* and *D*. Both high- and low-frequency types are found whether SpoC$^+$ was the donor or the recipient in the original construction. The fre-

Table 2. Segregation from *spoC*$^+$/*spoC*$^-$ partial diploids

Parents		Genotype of haploid segregants		
Donor	Recipient	*spoC*$^-$	*spoC*$^+$	% +
pLJS9 (Spo$^+$)	DK3332 (*spo-3330*)	12	37	76
pLJS9 (Spo$^+$)	DK3332 (*spo-3330*)	13	21	62
pLJS12 (*spo-731*)	DK2652 (Spo$^+$)	1	4	80
pLJS9 (Spo$^+$)	DK3332 (*spo-3330*)	22	3	12
pLJS11 (*spo-653*)	DK2652 (Spo$^+$)	16	1	6

spoC$^-$/*spoC*$^+$ partial diploids constructed with the donors and recipients listed in columns 1 and 2 were grown without antibiotics for 10 generations. Individual Km^s segregants were examined for Spo and Rpl to determine whether they were *spoC*$^+$ or *spoC*$^-$. Spo and Rpl always cosegregated. Lines 1 and 2 show two partial diploids generated independently from the same donor and recipient strains.

quency of SpoC$^+$ segregants should be high when *spoC* is closer to the junction between the two copies of the duplicated sequence than *spoC*$^+$. The vector and the kanamycin resistance gene are located at the junction (Fig. 1) and are lost from every segregant.

Genetic Complementation Tests. Given the dominance of *spoC*$^+$ over *spoC*$^-$ as demonstrated in Table 1, *trans* tests can be performed between pairs of different *spoC* mutations to see whether they belong to the same genetic complementation group. Each of the *spoC*$^-$ plasmids pLJS10, -11, and -12 was transduced into the four isogeneic *spoC*$^-$ recipients listed in Table 1 and the transductants were tested for their ability to sporulate and to ripple. The homoallelic duplications *spo-741/spo-741*, *spo-653/spo-653*, and *spo-731/spo-731* were uniformly Spo$^-$ Rpl$^-$. In these tests (data not shown), all the nine different heteroallelic double heterozygotes had the mutant (Spo$^-$ Rpl$^-$) phenotype. Thus, all four of the *spoC* loci belong to the same genetic complementation group.

Homozygous Partial Diploids. A fraction (10–33%) of the Km^r transductants from a *spo*$^+$ donor into a *spoC*$^-$ recipient, have a Spo$^-$ Rpl$^-$ phenotype (Table 1). The same phenomenon also occurs with a *spoC*$^-$ donor and a *spo*$^+$ recipient, and at about the same frequency (7–18%). These strains behave like partial diploids, because they have restriction fragments characteristic of both the donor and the recipient (Fig. 2, lanes 11–15). Moreover, Km^s segregants from the Spo$^-$ Rpl$^-$ transductants were examined by Southern blot hybridization (Fig. 3) and found to have lost the same two fragments (shown in lanes 8–12) as the segregants from Spo$^+$ Rpl$^+$ duplications (shown in lanes 2–7).

If these Spo$^-$ Rpl$^-$ transductants are partial diploids harboring an unexpressed *spo*$^+$ allele, then they should segregate some Spo$^+$ haploid colonies. However, all 337 Km^s segregants from 11 such partial diploids were found to be *spo*$^-$. Another possibility is that the initial transductant is a *spoC*$^-$/*spoC*$^+$ heteroduplex that segregates *spoC*$^-$ and *spoC*$^+$ homoduplexes at the first cell division. To test this possibility, Km^r colonies were picked directly from transduction plates and streaked out on kanamycin medium, and the resulting sibling colonies were tested for sporulation to examine the homogeneity of their phenotype. From 9 independent partial diploids classified as SpoC, all 118 sibling colonies had the Spo$^-$ phenotype. (From 18 independent partial diploids classified as Spo$^+$, all 151 sibling colonies were Spo$^+$.) Because the initial Spo$^-$ Km^r partial diploids have a uniform Spo$^-$ phenotype and because their Km^s segregants are all Spo$^-$, these strains must contain two *spo*$^-$ alleles. To test the possibility that mutations generating *spo*$^-$ might have occurred in pLJS9 during its transfer or incorporation into *M. xanthus*, partial diploids were constructed using pLJS9 (*spo*$^+$) as the donor and the *spo*$^+$ DK2652 as the recipient. All the resultant duplications were Spo$^+$ Rpl$^+$ (Table 1) and 140 segregants from 31 different constructions were all Spo$^+$ Rpl$^+$. If the second *spoC*$^-$ allele does not arise by mutation of *spo*$^+$, then it might arise by a mismatch repair that utilizes the *spoC*$^-$ allele as repair template. This possibility is examined in *Discussion*.

DISCUSSION

Three lines of evidence indicate a role for the *spoC* locus in developmental interactions between cells. First, *spoC* mutants fail to form myxospores in fruiting bodies unless they are allowed to develop mixed with *spoC*$^+$ cells. Dominance of *spoC*$^+$ over *spoC*$^-$ in heterozygous partial diploids rules out production of an antagonist by the mutant allele. Second, all four *spoC* mutants fail to form the traveling cell-accumulations known as ripples. Because *spoC* mutants exhibit normal cellular motility (7), their failure to ripple most likely reflects a defect in a long-range coordination of cell movement necessary for rippling. The sporulation and rippling defects go together. All of the more than 575 mutant, transductant, and segregant strains examined were either *spoC*$^-$ and phenotypically Spo$^-$ Rpl$^-$ or *spoC*$^+$ and phenotypically Spo$^+$ Rpl$^+$. By genetic complementation all four *spoC* mutants, which arose by independent mutations, lie in a single gene or transcription unit. Third, rippling can be induced by addition of murein or four components of *M. xanthus* murein (7). The same set of four components induces the *spoC* mutants to sporulate (17). Taken together, the data imply that the product(s) of one locus, *spoC*, is required for an intercellular stimulus that initiates sporulation, that coordinates cell movement in ripples, and that is coupled to the metabolism of murein.

Some homozygous *spoC*/*spoC* partial diploids arise in crosses involving a *spo*$^+$ donor and a *spoC* recipient. How do homozygotes arise from heteroallelic parents? A mechanism that would involve integration, excision of a circular element containing a *spoC* allele, and a second integration of the circle can be ruled out because the observed rate of excision from a duplication of the cloned segment studied here is 0.2% per generation, but the frequency of homozygotes is much higher (7–33% of Km^r transductants). Because the frequency of homozygotes is the same whether the *spoC* allele comes from donor or recipient, the two partners must make similar contributions. Generation of homozygotes via a heteroduplex and mismatch repair would fit the observations. A scheme that is consistent with results from bacteriophage λ (18, 19) and with data on repair in *M. xanthus* (20) is illustrated in Fig. 4. A cross strand exchange between corresponding strands of the donor plasmid, represented as a circle, and the chromosome produces a recombination intermediate of the type proposed by Holliday (21) (Fig. 4*A*). The crossover bridge moves laterally along the DNA, extending the pair of heteroduplexes between donor and recipient (*B*). Cleav-

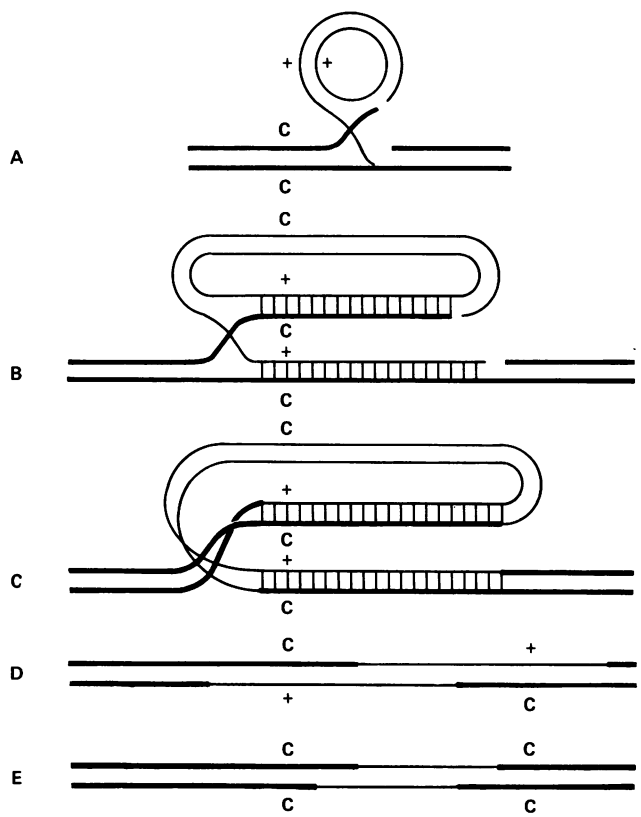

FIG. 4. Mechanism suggested for the formation of homozygous partial diploids. Light lines represent the donor plasmid; heavy lines, the recipient chromosome.

age and exchange of the second pair of corresponding strands resolves the intermediate (*C*). If the initial exchange had occurred to the right of *spoC* and if the crossover bridge moved leftward past the second copy of *spoC*, then both copies of the *spoC* locus would lie in heteroduplex regions (*D*). Fox *et al.* (18) have observed that a DNA branch, such as a crossover bridge, can migrate through point mutations, but is arrested by long regions of nonhomology. Therefore migration would be expected to stop at a Tn*5* insertion in the donor or recipient unmatched in the other. Finally, repair of mismatched base pairs should generate *spoC*/*spoC* homozygotes (*E*), spo^+/spo^+ homozygotes, which have also been observed from analogous clones of *spoD* (unpublished observations), and $spoC^-/spo^+$ heterozygotes.

The method of transfer of cloned sequences from *E. coli* to *M. xanthus* described here is quite general and could be used in any of the wide range of bacteria into which bacteriophage P1 can inject DNA.

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