JOURNAL OF BACTERIOLOGY, Nov. 1991, p. 6790–6797
0021-9193/91/216790-08$02.00/0

Vol. 173, No. 21

Targeted Disruption of the *Myxococcus xanthus* Orotidine 5′-Monophosphate Decarboxylase Gene: Effects on Growth and Fruiting-Body Development

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Received 3 June 1991/Accepted 5 July 1991

The *Myxococcus xanthus* gene coding for orotidine 5′-monophosphate (OMP) decarboxylase (EC 4.1.1.23) was cloned. The *M. xanthus uraA* gene efficiently complemented an *Escherichia coli* OMP decarboxylase mutant, permitting it to grow in the absence of uracil. Electroporation of *M. xanthus* with a circular plasmid carrying a selectable *uraA*::*kan* gene disruption resulted in homologous recombination at the chromosomal *uraA* locus. Chromosomal integration of the gene disruption plasmid created heterozygous (*uraA*$^+$/*uraA*::*kan*) tandem duplications. These tandem duplications were unstable and segregated auxotrophic *uraA*::*kan* daughters at frequencies of 2×10^{-4} to 8×10^{-4} per viable cell. Rare *uraA*::*kan* segregants were easily obtained by selecting for resistance to the toxic analog 5-fluoroorotic acid. Our experiments suggest that the cloned *uraA* gene could facilitate the use of gene duplications in the genetic analysis of *M. xanthus* development. The *uraA* mutants could utilize uracil, uridine, or uridine 5′-phosphate for growth, indicating that *M. xanthus* has pyrimidine salvage pathways. During multicellular development, *uraA*::*kan* gene disruption mutants sporulated to wild-type levels but formed smaller and more numerous aggregates than did their *uraA*$^+$ parent, regardless of whether uracil was added to the medium. Pyrimidine deprivation of *uraA* mutants, under conditions that otherwise supported vegetative growth, failed to induce fruiting-body development or sporulation.

Myxococcus xanthus undergoes a developmental fruiting cycle involving multicellular aggregation and subsequent sporulation of many of the aggregated cells. Fruiting-body development is a response to nutrient starvation, but the molecular nature of the initiating event is not well understood (15).

M. xanthus grows primarily on peptides and amino acids (8, 13). Amino acids provide carbon skeletons for essential biosynthetic pathways, and their oxidative degradation yields energy. When cells growing on a synthetic medium are specifically starved for an essential amino acid (valine, leucine, isoleucine, or methionine), or if an amino acid auxotroph is starved for its required amino acid, fruiting development occurs (9, 19). Starvation for carbon or phosphate will also induce fruiting development (19). These development-inducing conditions could be explained by a single mechanism whereby development is triggered by a lowering of intracellular levels of charged tRNAs. Consistent with this idea, amino acid analogs that inhibit tRNA charging induce fruiting development (19). It is unclear, however, whether the regulatory signals initiating development also occur in response to limitation for essential nutrients less closely related to protein synthesis. To better understand the scope of starvation sensing, we examined the role of pyrimidine biosynthesis during fruiting development. Pyrimidine nucleotides, originating with UMP, serve as precursors for RNA and DNA synthesis. They also function catalytically in cell wall biosynthesis.

We have isolated the *M. xanthus uraA* gene coding for orotidine 5′-monophosphate (OMP) decarboxylase, the enzyme that catalyzes the final step in de novo UMP biosynthesis (18). Using *M. xanthus* mutants that lack a functional *uraA* gene, we found that pyrimidine starvation hindered growth but did not initiate development. Furthermore, de novo UMP biosynthesis was not even required for developmental sporulation.

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MATERIALS AND METHODS

Chemicals. Orotate, OMP, uracil, uridine, 5′-UMP, 2′,3′-UMP, and kanamycin sulfate were obtained from Sigma. The analog 5-fluoroorotic acid (FOA) was a gift from R. Davis.

Bacteria and media. Bacterial strains used in this work are described in Table 1. CTT broth, a rich growth medium, and A1 medium, a defined medium, were made as previously described (5). For plates, media were solidified with 1.5% agar (Difco). CTT soft agar contained 0.7% agar. CTT agar was supplemented with FOA (100 μg/ml) after the autoclaved media had cooled to 60°C. Complete dissolution of FOA required stirring with a magnetic stirrer for 20 min. *M. xanthus* strains were grown in CTT broth (5) with aeration at 32°C. Cell density was measured in a Klett-Summerson colorimeter with a red filter. For fruiting development, growing cultures were sedimented at 10,000 × *g* and resuspended to a Klett reading of 1,000 in TPM buffer (10 mM Tris, 1 mM KPO_4, 10 mM $MgCl_2$, pH 7.6). Twenty-microliter drops were placed on TPM starvation agar, allowed to dry, and incubated at 32°C for 2 to 3 days.

M. xanthus pyrimidine growth requirements were assayed by densely streaking bacteria onto the chemically defined M1 agar media (25). M1 agar plates were unsupplemented or supplemented with 1 mM uracil, uridine, 5′-UMP, 2′,3′-UMP, orotate, or OMP. Growth was scored after incubation for 1 week at 32°C in a humid chamber.

DNA manipulations. Routine DNA manipulations were performed essentially as described previously (22). *M. xanthus* chromosomal DNA was isolated as previously described previously (2). DNA blot hybridizations, using Ny-

TABLE 1. Strains

Strain	Genotype	Derivation	Source
M. xanthus			
DK101	*sglA1*		D. Kaiser
DK1622	Wild type		D. Morandi
DK7636	Tn*5lac*Ω*7636*	P1*clr100*Cm::Tn*5lac* × DK1622 (Km^r)	H. Kimsey
DK7817	*sglA1 uraA*$^+$/*uraA*::*kan*	pHK611[*Sac*I::*kan*] × DK101 (Km^r)	This work
DK7818	*sglA1 uraA*$^+$/*uraA*::*kan*	pHK611[*Sac*I::*kan*] × DK101 (Km^r)	This work
DK7819	*sglA1 uraA*::*kan*	pHK611[*Sac*I::*kan*] × DK101 (Km^r)	This work
DK7820	*sglA1 uraA*$^+$/*uraA*::*kan*	pHK611[*Sac*I::*kan*] × DK101 (Km^r)	This work
DK7821	*sglA1 uraA*$^+$/*uraA*::*kan*	pHK611[*Sac*I::*kan*] × DK101 (Km^r)	This work
DK7837	*sglA1 uraA*::*kan*	DK7817 (FOA^r)	This work
DK7838	*sglA1 uraA*::*kan*	DK7818 (FOA^r)	This work
DK7840	*sglA1 uraA*::*kan*	DK7819 (FOA^r)	This work
DK7841	*sglA1 uraA*::*kan*	DK7820 (FOA^r)	This work
DK7842	*sglA1 uraA*::*kan*	DK7821 (FOA^r)	This work
E. coli			
DH5α	ϕ80d*lacZ*Δ*M15* Δ*lacU169 recA1 endA1 hsdR17 supE44 thi-1 gyrA relA1*		
BNN46	*trp lac hsdR pyrF*	MC1000	R. Davis

tran membranes (Schleicher & Schuell), were performed in 50% formamide according to the method of Davis et al. (6). DNA probes for hybridization were radiolabelled with [α-^{32}P]dATP (Amersham), using the random hexamer primer method (11). Unincorporated nucleotide was removed by gel filtration through Bio-Gel P-30 resin (Bio-Rad).

Plasmid pHK611[*Sac*I::*kan*] was constructed as follows. One microgram of pHK611 DNA was digested with *Sac*I and blunt ended by exonuclease treatment with T4 DNA polymerase. The treated plasmid DNA was ligated to 1 μg of purified *Hin*cII fragment carrying the *kan* gene of Tn*903* (21) in a 25-μl reaction. Kanamycin-resistant (Km^r) transformants obtained in *Escherichia coli* DH5α were analyzed by restriction mapping.

Electroporation. Electroporation conditions for *M. xanthus* were originally defined by Rodriguez (21a). *M. xanthus* DK101 was grown to exponential phase in CTT broth. Cells were collected by centrifugation at 10,000 × *g* for 5 min and washed two to four times in electroporation buffer (270 mM sucrose, 5 mM KPO_4 [pH 7.6], 15% glycerol). Portions (0.2 ml) of chilled prepared cells were mixed with 1 to 5 μg of supercoiled plasmid DNA and immediately subjected to an electric field pulse of 5,000 V/cm at a 25-μF capacitor setting, using a Bio-Rad GenePulser apparatus (7). Time constants in the range of 2 to 10 ms were sought. The contents of the cuvette were added to 3 ml of molten CTT soft agar and plated on CTT agar containing 40 μg of kanamycin sulfate per ml. Km^r transformants were toothpicked from the agar overlay after 5 to 7 days incubation at 32°C.

***M. xanthus* genomic library.** DNA fragments generated by partial *Sau*3A1 digestion of *M. xanthus* DK7636 chromosomal DNA were ligated to approximately equal amounts of *Bam*HI-digested, dephosphorylated pUC18 plasmid DNA (24). Total DNA concentration in the ligation was kept at 50 μg/ml to favor intermolecular ligation. Ligation products were transformed into *E. coli* DH5α (12). Restriction analysis of plasmid DNA from 20 randomly chosen transformants indicated that approximately one-half of all clones contained inserts greater than 2 kbp in size. Plasmid DNA was prepared from independent pools, each composed of 1,000 to 5,000 transformants. Portions of the pooled transformants were stored at −80°C in 10% glycerol.

RESULTS

Cloning. An *M. xanthus* gene coding for OMP decarboxylase activity was isolated from a recombinant plasmid bank by screening for clones that genetically complement the pyrimidine growth requirement (Ura$^-$) of *E. coli pyrF* strain BNN46. Plasmid pHK600 was isolated from one Ura$^+$ candidate and shown to contain a 10.5-kbp insert fragment (Fig. 1). To verify that this cloned insert derives from the *M. xanthus* chromosome, DK1622 DNA was digested with *Eco*RI, *Bam*HI, and *Pst*I and analyzed by DNA blot hybridization. The number and sizes of fragments hybridizing to a pHK600 DNA probe indicated that the 10.5-kbp insert of pHK600 is a unique fragment derived from the *M. xanthus* chromosome (data not shown).

pyrF-complementing activity is retained on a 1.8-kbp *Xba*I-*Pst*I restriction fragment (Fig. 1) derived from the center of the cloned insert in pHK600. Complementing

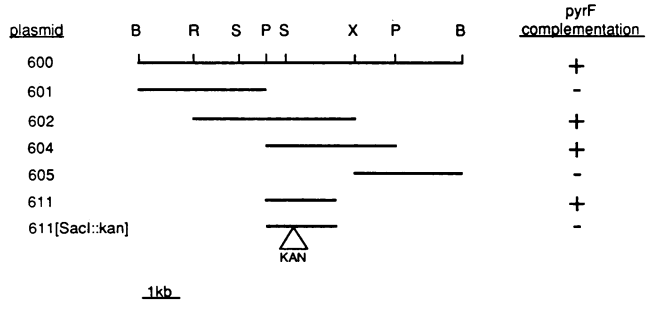

FIG. 1. Localization of the *pyrF*-complementing region of *M. xanthus* DNA. Subcloned regions, indicated by lines, were constructed in pUC18. To test *pyrF* complementation, plasmid transformants of *E. coli* BNN46 were streaked onto minimal M9 agar without uracil and growth at 32°C was assayed. +, growth; −, no growth. B, *Bam*HI; P, *Pst*I; R, *Eco*RI; X, *Xba*I; S, *Sac*I.

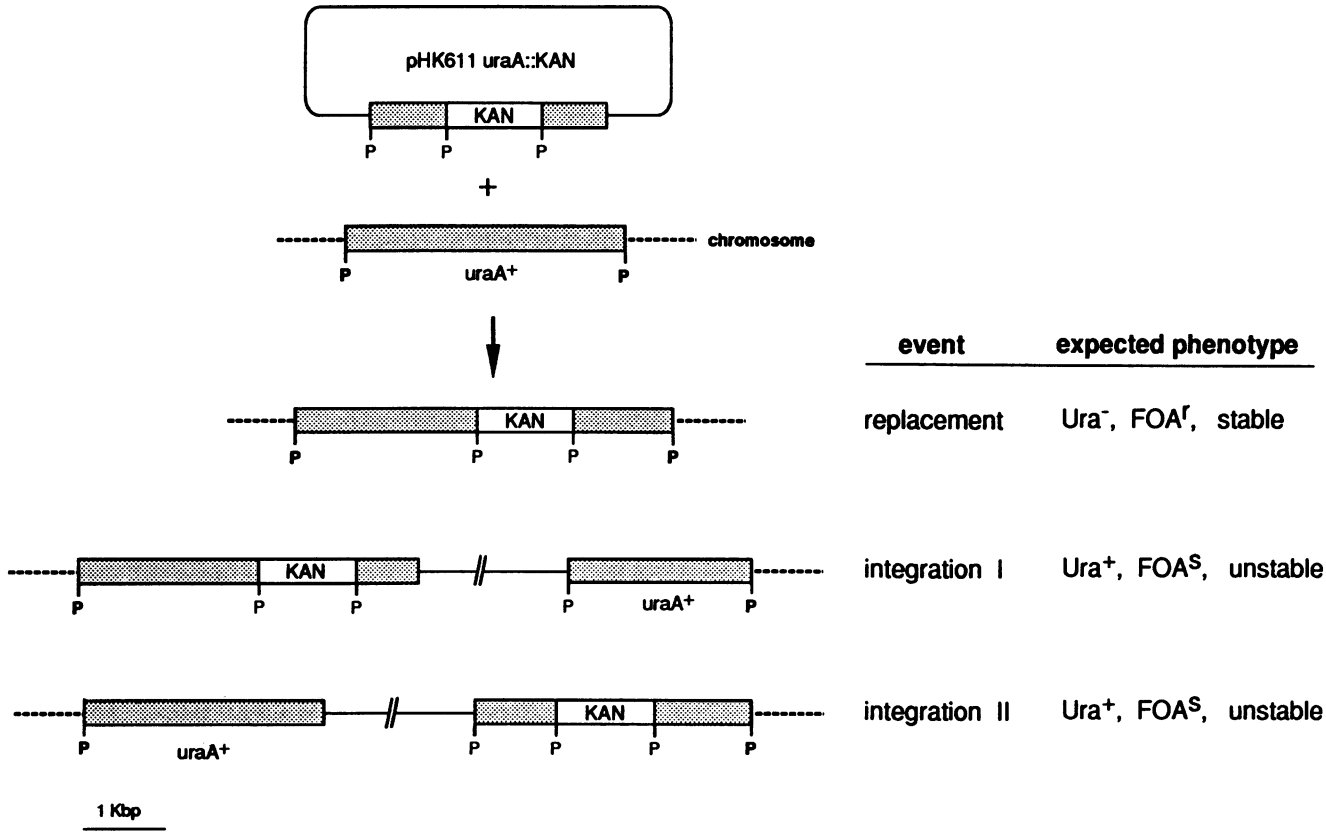

FIG. 2. Possible products of recombination between electroporated plasmid pHK611[*uraA*::*kan*] and the *M. xanthus* chromosome. The chromosomal *uraA* gene resides on a 3.5-kbp *Pst*I restriction fragment. *Pst*I restriction sites are denoted by P. Those in boldface type indicate *Pst*I restriction sites on the recipient chromosome. The *Pst*I restriction sites diagrammed on the integrating plasmid were all generated in the construction of pHK611[*Sac*I::*kan*]. Integration event I occurs to the left of the *kan* gene, and integration event II occurs to the right.

activity was destroyed when the neomycin phosphotransferase I gene (*kan*) fragment, which confers resistance to kanamycin, was inserted at a unique *Sac*I restriction site, demonstrating that the *Sac*I site lies in the gene or genes responsible for the OMP decarboxylase activity detected by the growth assay.

Genetic test of function. To determine whether the cloned *M. xanthus* DNA of pHK600 encodes a unique and functional OMP decarboxylase, the chromosomal segment corresponding to pHK611 was replaced by the gene disruption carried on pHK611[*Sac*I::*kan*]. We reasoned that disruption of a unique and biosynthetically functional OMP decarboxylase gene would generate an auxotrophic strain requiring an external pyrimidine source for growth, whereas disruption of a gene expressing a fortuitous or duplicate OMP decarboxylase activity would not result in such a requirement.

Gene replacement in *M. xanthus* was accomplished by electroporating circular pHK611[*Sac*I::*kan*] plasmid DNA into strain DK101 and selecting for Km^r transformants. Five stably Km^r electroporants were recovered from three independent experiments. The transformation efficiency was approximately 1 μg^{-1}. Our experience with electroporating *M. xanthus* strains suggests that low transformation efficiencies correlate with high cell cohesiveness.

The genetic fate of electroporated plasmid DNA in the five Km^r transformants was determined by physical and genetic methods. We expected plasmid DNA to recombine with the chromosome by homologous recombination, since pUC18 cannot replicate in *M. xanthus* and homologous recombination is known to occur when similar plasmids are introduced into *M. xanthus* by P1 transduction (20). Homologous recombination between a circular plasmid and the chromosome could lead to integration of the entire plasmid or replacement of chromosomal sequences by homologous sequences on the plasmid. Physical maps diagramming the double-crossover and two single-crossover products are shown in Fig. 2. The targeted gene resides on a 3.5-kbp *Pst*I fragment. Since the 1.5-kbp *kan* insertion is precisely flanked by *Pst*I sites, gene replacement should give rise to two new *Pst*I fragments of sizes 2.2 and 1.2 kbp. Plasmid integration, however, could give rise to one of two structures, depending on the point of recombination. Type I integrants should have three *Pst*I fragments with predicted sizes of 3.5 kbp and a doublet of 2.2 kbp; type II integrants should display three fragments of sizes 5.8, 1.2, and 1.0 kbp.

If the mutated gene on pHK611[*Sac*I::*kan*] bears the sole, functional *M. xanthus* OMP decarboxylase gene, then plasmid integration can be distinguished from gene replacement by the phenotype of the resulting strains. Gene replacement will yield simple gene disruptions (*uraA*::*kan*) that are stably Ura^- Km^r. Plasmid integration, however, will generate heterozygous gene duplications (*uraA*$^+$/*uraA*::*kan*) that are Ura^+ due to the presence of an intact *uraA* gene. Tandem duplications are not stably maintained in *M. xanthus* (2) because intramolecular homologous recombination between the duplicated region can result in excision of the integrated

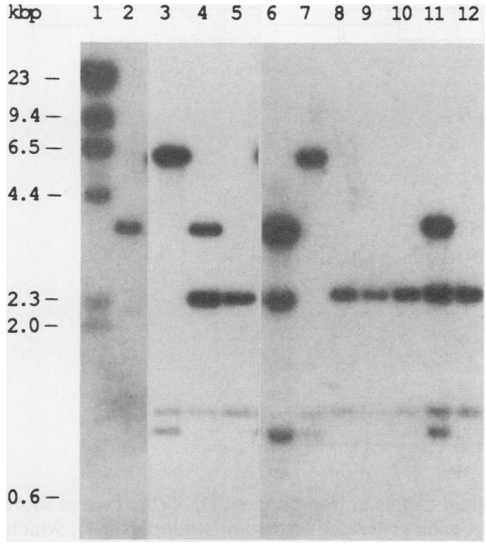

FIG. 3. Autoradiogram from DNA blot hybridization analysis of pHK611[*uraA*::*kan*] electroporants in *M. xanthus*. Total DNA was digested with *Pst*I and electrophoresed through a 0.7% agarose gel. Gel contents were transferred to a Nytran membrane and hybridized as described in Materials and Methods. The 3.5-kbp *Pst*I fragment served as a probe. Lanes 3 to 7 contain DNA from the five primary Km^r transformants; lanes 8 to 12 contain DNA from one Ura^- FOA^r daughter from each electroporant, loaded in the same order as lanes 3 to 7. Lanes: 1, *Hin*dIII-digested λ DNA; 2, DK101; 3, DK7817; 4, DK7818; 5, DK7819; 6, DK7820; 7, DK7821; 8, DK7837; 9, DK 7838; 10, DK7840; 11, DK7841; 12, DK7842.

plasmid. Therefore, Ura^+ plasmid integration strains should produce Ura^- Km^r daughters at moderately high frequency. This frequency can be measured since rare Ura^- excision recombinants can be selected by plating for resistance to the analog FOA. FOA is toxic to cells if it is converted to 5-fluoro-UMP by the sequential action of orotate PRPP transferase and OMP decarboxylase. Cells lacking OMP decarboxylase cannot carry out this conversion and are FOA resistant (3).

To determine whether the five Km^r strains that arose by transformation have the expected chromosomal structures, DNA was prepared from each strain, digested with *Pst*I, and analyzed by DNA blot hybridization. In parallel, the same strains were tested for a pyrimidine nutritional requirement by observing growth on synthetic M1 agar. Lastly, to measure FOA^r and FOA^s CFU, cultures were plated on both CTT agar and CTT agar containing FOA.

Only transformant DK7819 (Fig. 3, lane 5) exhibits the 2.2- and 1.2-kbp *Pst*I fragments predicted for gene replacement. As expected, DK7819 requires exogenous uracil for growth on chemically defined M1 agar (Table 2). Growth was restored by millimolar concentrations of uracil, uridine, 5′-UMP, or 2′,3′-UMP but not by orotate or OMP, indicating that the pyrimidine requirement arose from the loss of an essential OMP decarboxylase activity. Consistent with this view, DK7819 is FOA^r (Table 2), as are OMP decarboxylase-deficient strains of *E. coli* and *Saccharomyces cerevisiae* (3). Vegetatively growing DK7819 is otherwise indistinguishable from DK101, its wild-type parent. DK7819 has maintained its Ura^- Km^r phenotype stably for more than 25 generations.

DNA from transformants DK7817 (Fig. 3, lane 3) and DK7821 (lane 7) both display the 5.8-, 1.2-, and 1.0-kbp *Pst*I fragments expected for a type II plasmid integration at the

TABLE 2. Genetic and structural features of pHK611[*uraA*::*kan*] transformants

Strain	Uracil growth Requirement[a]	Frequency of FOA resistance[b]	Physical structure
DK101	Ura^+	1.5×10^{-7}	
DK7817	Ura^+	7.8×10^{-4}	Integrant II
DK7818	Ura^+	3.6×10^{-1}	Integrant I
DK7819	Ura^-	1.6	Gene replacement
DK7820	Ura^+	2.3×10^{-3}	Multiple integrant
DK7821	Ura^+	2.0×10^{-4}	Integrant II

[a] Cultures were spotted onto M1 agar to determine their pyrimidine growth requirement.

[b] Growing cultures were plated for viable count determinations on CTT agar and CTT agar plus FOA.

uraA locus. Both are Ura^+, demonstrating that a functional *uraA* gene is still present in these strains. Both strains also give rise to Km^r Ura^- FOA^r progeny at a frequency of 2×10^{-4} to 8×10^{-4} per viable cell (Table 2), suggesting that the instability of the *uraA* gene in these two strains is due to excision of the integrated plasmid by homologous recombination.

To confirm this interpretation, DNA from two Ura^- Km^r segregants, DK7837 (DK7817 parent) and DK7842 (DK7821 parent), was digested with *Pst*I and analyzed by DNA blot hybridization. Both have the 2.2- and 1.2-kbp fragments expected for a simple *uraA*::*kan* gene disruption (Fig. 3, lanes 8 and 12). The instability of the *uraA* gene in DK7817 and DK7821 must therefore be due to the recombinational loss of a gene duplication created by plasmid integration. The frequencies of duplication loss measured in these experiments are similar to the frequencies reported for loss of tandem duplications in *Salmonella typhimurium* and *E. coli* (1). An alternative path to generate Ura^- Km^r segregants is gene conversion without loss of the integrated plasmid. We have not yet seen this event with *uraA* plasmid integrant strains; however, Stephens and Kaiser (23) reported evidence for gene conversion of point mutations at the *mglA* motility locus. The tandem duplications present in DK7817 and DK7821 create partial diploids and therefore afford the opportunity to assess complementation between two *uraA* alleles; as expected, the *uraA*::*kan* gene disruption is recessive to the $uraA^+$ gene.

The chromosome structure of transformant DK7818 (Fig. 3, lane 4) is more complex; it has the 3.4- and doublet 2.2-kbp fragments consistent with a type I plasmid integration event but also displays an unexpected 1.2-kbp fragment of lower intensity. This pattern could be interpreted as the superposition of fragment patterns of a type I plasmid integration and a gene replacement (3.4, doublet 2.2; 2.2 and 1.2 kbp). The test culture of DK7818 was Ura^+ but yielded Ura^- FOA^r daughters at a frequency much higher than that found for duplication strains DK7817 or DK7821 (Table 2). DNA from one Ura^- FOA^r daughter was Km^r and had only the 2.2- and 1.2-kbp *Pst*I fragments expected for a gene replacement (Fig. 3, lane 9). DK7818 may have originated by type I plasmid integration but then segregated the gene duplication early in the growth of the culture. It should be noted that the gene duplications described here would not be stably maintained by kanamycin selection because the *kan* gene is not at the novel joint of the duplication but rather is within the duplicated region itself (1) (Fig. 2).

The pattern of *Pst*I fragments in transformant DK7820 (Fig. 3, lane 6) appears anomalous, but the 3.4-, 2.2-, and

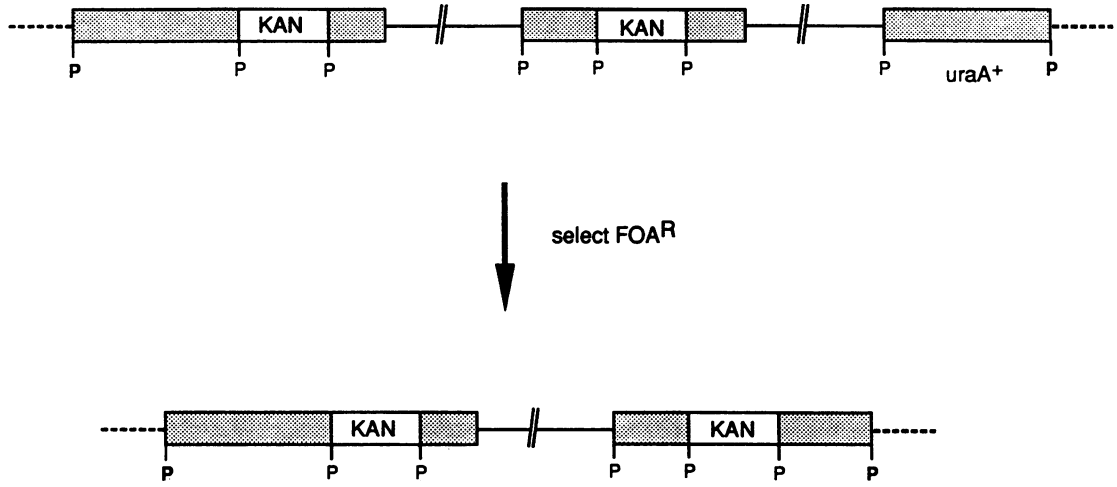

FIG. 4. Chromosome structure of multiply integrated pHK611[*uraA*::*kan*] plasmid copies in transformant DK7820. Two or more plasmid molecules integrate to give the structure shown at top. Loss of the functional *uraA* gene generated segregant strain DK7841, which has four *Pst*I fragments (Fig. 3, lane 11). The structure shown at bottom is consistent with this *Pst*I fragment pattern.

1.0-kbp fragments are readily explained by the integration of multiple plasmid molecules or the integration of a preexisting multimeric plasmid molecule. The higher intensity of the 3.4-kbp *Pst*I band is consistent with this interpretation since this fragment would arise multiple times in a chromosome with this structure. DK7820 is Ura^+, demonstrating that a functional *uraA* gene is still present in the strain, but gives rise to Ura^- FOA^r daughters at a rate similar to that of duplication strains DK7817 and DK7821 (Table 1). DNA from one such Ura^- FOA^r daughter was analyzed by blot hybridization and found to contain four *Pst*I fragments of sizes 3.4, 2.2, 1.2, and 1.0 kbp (Fig. 3, lane 11). This fragment pattern fits the prediction if recombinational loss of only the single $uraA^+$ gene was selected by FOA resistance. Two copies of the *ura*::*kan* gene disruption would remain in the segregant, as shown in Fig. 4.

Fruiting development of *uraA*::*kan* strains. All of the *uraA*::*kan* mutant strains listed in Table 1 exhibit similar altered patterns of developmental aggregation. The most obvious defect is a five- to sixfold increase in the density of fruiting structures within a developing spot: 72 fruiting bodies per cm^2 for wild-type DK101 versus 408 fruiting bodies per cm^2 for *uraA*::*kan* strains (Fig. 5). The fruiting bodies produced by *uraA*::*kan* mutants are noticeably smaller than those produced by wild-type DK101. Occasional ringlike aggregates can also be found at the perimeter of a developing spot of mutant cells (Fig. 5), similar to the aberrant aggregates associated with *frz* mutants (26) but more variable in size. The $uraA^+$ tandem duplication strains DK7817 and DK7821 formed normal fruiting bodies containing normal spores, demonstrating that the aggregation defects observed in *uraA*::*kan* mutants are due solely to the *uraA* gene disruption and not to a second, unrelated mutation. Attempts to restore normal aggregation to the *uraA*::*kan* mutants by adding uracil or uridine to the agar medium failed, suggesting that the aggregation defect is not primarily due to lowered levels of pyrimidine nucleotide precursors.

Although their aggregation behavior is altered, *uraA*::*kan* mutant strains give rise to wild-type levels of heat- and sonication-resistant myxospores during fruiting development on an agar surface (Fig. 5). To examine the possibility that a second OMP decarboxylase functions during fruiting-body development, fruiting development was carried out on starvation agar in the presence of the toxic analog FOA. We observed that the number of heat- and sonication-resistant spores produced by developing *uraA*::*kan* cells was not reduced by FOA treatment, whereas the titer of resistant spores produced by the DK101 parent strain was reduced approximately 100-fold (2.1×10^6/ml versus 2.0×10^4/ml). The maintenance of FOA resistance of *uraA* mutants during fruiting development argues against the presence of a second development-specific enzyme. It appears that the de novo pyrimidine biosynthetic pathway is not required for fruiting-body sporulation even though many genes are expressed specifically during fruiting development (16).

Induction of development by starvation. The effect of pyrimidine starvation upon vegetatively growing *uraA*::*kan* mutant cells was examined. Previous work had shown that development could be triggered by transferring growing cells to a buffer containing calcium and magnesium as its only nutrients (17). More specifically, development could also be induced by amino acid starvation (9, 13) or by phosphorus or carbon starvation (19). The aim was to test whether specific starvation for pyrimidines could also initiate multicellular development.

Cultures of *uraA*::*kan* mutants were spotted at high cell density on synthetic A1 agar lacking uracil or on A1 agar containing 40 μM or 1.0 mM uracil. Forty micromolar uracil was observed to be a growth-limiting concentration. Fruiting-body development, as judged by the appearance of darkened aggregate structures or refractile myxospores, did not occur in either *uraA*::*kan* mutant tested or in the parent strain DK101 under conditions of uracil limitation or starvation (not shown). Multicellular aggregation or sporulation also failed to occur when wild-type cells were incubated at high cell density on vegetative CTT agar containing FOA, a toxic pyrimidine precursor (not shown). Apparently, specific starvation for pyrimidine metabolites is not sufficient to initiate the developmental program.

DISCUSSION

An *M. xanthus* gene coding for OMP decarboxylase activity was isolated. Three pieces of evidence together demonstrate that this gene is the sole, functional *M. xanthus*

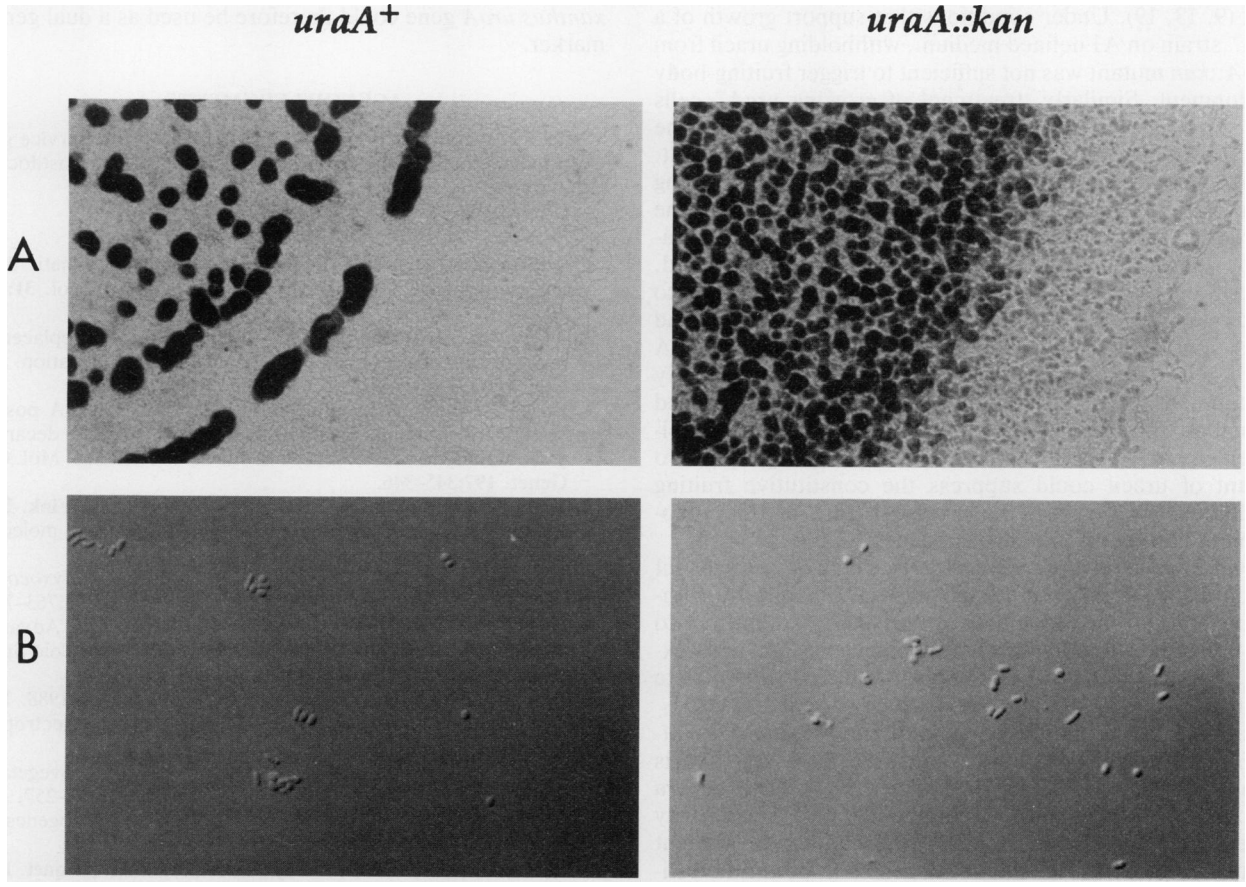

FIG. 5. Developmental defects of *M. xanthus uraA*::*kan* mutants. (A) Cultures were allowed to develop at high cell density on TPM starvation agar for 3 days. *uraA*::*kan* mutants sporulate to wild-type levels but aggregate into smaller, more numerous fruiting structures. Swarming cells at the perimeter occasionally form doughnutlike structures. Photographs were obtained by using a Wilde-Heerbrugg dissecting microscope. (B) Wet mounts of myxospores from mature fruiting bodies. *uraA*::*kan* spores are environmentally resistant and morphologically similar to wild-type myxospores. Photographs were obtained by using a Zeiss Axiophot optical microscope with Nomarski optics.

OMP decarboxylase gene. (i) A recombinant plasmid carrying the *M. xanthus* gene complements the pyrimidine requirement of an *E. coli pyrF* mutant. (ii) An insertion mutation of the cloned *M. xanthus* gene, when reintroduced into the chromosome so that it replaces the normal allele, causes a pyrimidine growth requirement. Growth of the *uraA* gene disruption strain is restored by exogenous uracil, uridine, or UMP but not by orotate, the pyrimidine substrate for the final OMP decarboxylation step in UMP biosynthesis. (iii) The derived amino acid sequence of the cloned *M. xanthus* gene shows regions of similarity to OMP decarboxylases from many diverse organisms (15a). Although we have not looked directly in developing cells for a second OMP decarboxylase activity, two arguments render it unlikely that a second, development-specific enzyme exists. First, *uraA* mutant strains remain resistant to the toxic analog FOA during development as well as vegetative growth, whereas wild-type *M. xanthus* is FOA sensitive during growth and development. Second, DNA blot hybridization reveals no other sequences highly homologous to *uraA* that would suggest the existence of another, related gene.

The *uraA*::*kan* mutant strains described here permitted an examination of the role of de novo UMP biosynthesis in fruiting-body development. It was observed that *uraA*::*kan* mutant strains sporulated normally in the absence of significant levels of pyrimidines in the media. This finding suggests that the de novo pathway is not required for sporulation during fruiting-body development. Many genes are expressed during the sporulation phase of development (16); nevertheless, development takes place on an agarose medium lacking any external source of carbon or nitrogen. The sporulation proficiency of *uraA* null mutants suggests that precursors for spore-specific RNA synthesis may derive from an intracellular salvage pathway that degrades existing vegetative RNAs, such as rRNA.

Although sporulation of *uraA*::*kan* mutants is normal, multicellular aggregation is not; *uraA* mutant cells aggregate into small numerous fruiting structures. The pattern of cell movement at the edge of a developing spot (formerly the edge of the dried inoculum spot) is also altered. These altered motility patterns are not rescued by addition to the agar medium of levels of uracil or uridine that efficiently rescue vegetative growth. This indicates that the altered aggregation of *uraA* mutants is not due to a decrease in the intracellular pools of pyrimidine nucleotides but more likely is due to accumulation of another metabolite before the block in UMP biosynthesis.

A second issue raised by these data is how starvation initiates fruiting-body development. Previously, it was shown that growth limitation for a diverse set of essential and nonessential amino acids could initiate fruiting develop-

ment (9, 13, 19). Under conditions that support growth of a *uraA*$^+$ strain on A1 defined medium, withholding uracil from a *uraA*::*kan* mutant was not sufficient to trigger fruiting-body development. Similarly, treatment of growing *uraA*$^+$ cells with sublethal concentrations of FOA, a toxic pyrimidine analog, was also not able to trigger fruiting development. Our results suggest that whatever the development-inducing signal may be, it is not generated in response to pyrimidine starvation. These results could mean that productive starvation is sensed by the lack of charged tRNA. Amino acid, carbon, or phosphorus starvation might be expected to induce development by limiting the availability of charged tRNA. Serine hydroxamate, an inhibitor of seryl-tRNA synthase in *E. coli*, has been shown to induce fruiting-body development (19). Hemphill and Zahler previously reported a uracil auxotrophic mutant that carried out fruiting development even when no amino acid was limiting (13). No amount of uracil could suppress the constitutive fruiting development. However, the precise nature of this auxotrophic mutation was not determined.

Given an efficient intracellular salvage pathway, it may not be possible to greatly limit pyrimidine availability by withholding uracil from a uracil auxotroph or by adding FOA to wild-type cells. Such a salvage mechanism might also explain the ability of *uraA* auxotrophic mutants to complete fruiting-body sporulation in the absence of added uracil.

Transcription of the *pyr5-6* gene of *Dictyostelium discoideum*, which codes for OMP decarboxylase activity, is strongly regulated during development (10). The genera *Myxococcus* and *Dictyostelium*, although not evolutionarily related, both initiate development in response to nutrient deprivation (14). The cloned *uraA* gene will permit investigations of *uraA* gene regulation during fruiting-body development in *M. xanthus*.

Genetic tools. Previously, only P1 transduction could be used to introduce recombinant plasmids into *M. xanthus*. These plasmids are large because they must carry a portion of the P1 chromosome. Here we report that direct electroporation of circular plasmid DNA yields stable transformants in which the plasmid integrates into the *M. xanthus* chromosome by homologous recombination. Although the number of transformants reported here is small, plasmid integration occurred more frequently (four of five instances) than gene replacement.

Integrating plasmids are used to create genetic duplications of small portions of the *M. xanthus* chromosome. These duplications are useful for performing genetic complementation tests as well as for replacing a chromosomal allele with an in vitro gene construct. Often it is necessary to allow the duplication to segregate. For example, segregation from the tandem duplication used in a complementation test can prove that both alleles were originally present in the duplication. Previously, finding segregants was a laborious procedure since tandem duplications are only moderately unstable (2). One solution to this problem utilizes a genetic marker for which the presence of the marker gene can first be selected, and then later its absence can also be selected. The *URA3* gene, which encodes the biosynthetic enzyme OMP decarboxylase in yeast cells, functions in this way (4). The *URA3* gene is directly selectable by using uracil prototrophy (Ura$^+$); the mutant form of the gene can be selected directly by using the analog FOA. In *M. xanthus*, direct selection for FOA resistance allowed rapid segregation of tandem duplications. Defined growth media exist for *M. xanthus*, so Ura$^+$ selections are possible. The cloned *M. xanthus uraA* gene could therefore be used as a dual genetic marker.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant GM 23441 to D.K. and by an American Cancer Society postdoctoral fellowship to H.H.K.

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