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Genetic Evidence that GTP Is Required for Transposition of IS*903* and Tn*552* in *Escherichia coli*

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Surprisingly little is known about the role of host factors in regulating transposition, despite the potentially deleterious rearrangements caused by the movement of transposons. An extensive mutant screen was therefore conducted to identify *Escherichia coli* host factors that regulate transposition. An *E. coli* mutant library was screened using a papillation assay that allows detection of IS*903* transposition events by the formation of blue papillae on a colony. Several host mutants were identified that exhibited a unique papillation pattern: a predominant ring of papillae just inside the edge of the colony, implying that transposition was triggered within these cells based on their spatial location within the colony. These mutants were found to be in *pur* genes, whose products are involved in the purine biosynthetic pathway. The transposition ring phenotype was also observed with Tn*552*, but not Tn*10*, establishing that this was not unique to IS*903* and that it was not an artifact of the assay. Further genetic analyses of purine biosynthetic mutants indicated that the ring of transposition was consistent with a GTP requirement for IS*903* and Tn*552* transposition. Together, our observations suggest that transposition occurs during late stages of colony growth and that transposition occurs inside the colony edge in response to both a gradient of exogenous purines across the colony and the developmental stage of the cells.

Transposons are defined as distinct regions of DNA that have the ability to move from one genomic location to another, a process mediated by element-encoded transposases (8, 9). While the main focus of transposition research has been in determining the detailed mechanisms of transposition, relatively little attention has been paid to how transposition is regulated in vivo or the in vivo requirements for the process (35). Transposon movement can result in gene inactivation (by insertional inactivation or deletion) or activation (by creation or introduction of promoters upstream of genes). In addition, intramolecular transposition can result in extensive DNA rearrangements, including deletions, inversions, and duplications of chromosome segments; these events are thought to play an important role in facilitating genome evolution (7–9). As the consequences of transposition can be either dire or favorable for the host organism, it is intuitive that the host has the capability to regulate this process. There are multiple examples of how transposons regulate their own movement, but very little work has focused on the role played by the host in regulating transposition. To address this, we have generated an insertion mutant library of *Escherichia coli*, which we have used to screen for host genes involved in regulation of IS903 transposition (E. Twiss, A. Coros, N. Tavakoli, and K. M. Derbyshire, unpublished data).

The first comprehensive genetic screen for host mutants affecting transposition was carried out in 1985; it revealed a role for *dam* methylation in decreasing the rate of IS*10*, IS*903*, and IS*50* transposition and the level of IS*10* transposase expression (39). Transposon ends containing fully methylated *dam* sites were shown to be less active than were hemi-methylated ends, suggesting that transposition would be elevated following passage of a replication fork through the element. Thus, the timing of transposition of certain elements is at least partially correlated with the cell replication cycle. Other biochemical and genetic studies have identified host factors, such as integration host factor, histone-like nucleoid structuring protein, and Hu, as playing an important—but not essential—role in transposition of many elements (30, 41, 51, 52, 55). Indeed, the observation that these nucleoid-associated proteins can influence both transposition and site-specific recombination reinforces the proposal that these proteins communicate cellular status to the recombination machinery (6, 53). A satisfying aspect of this hypothesis is that, since the abundance of nucleoid-associated proteins varies during cell growth, their relative availability would accordingly serve as a read-out of the cell's environment (4). The involvement of other host proteins in the transposition process (for example, proteases, chaperones, DnaA, ribosome-associated proteins) (13, 29, 31, 49, 57) suggests that this form of "cellular input" plays a much greater role than has currently been defined. Our unpublished results and the findings described here support this idea, by defining additional host factors that modulate transposition.

To monitor the effect of host mutants on IS*903* transposition, we have employed a papillation assay in which independent transposition events are observed as blue papillae on a bacterial colony (Fig. 1); mutants that affected transposition were thus detected as changes in the number of blue papillae within a colony (12, 52). One intriguing group of host mutants identified in our screen resulted in a distinct ring of papillae near the periphery of the colony, as if transposition were coordinated with colony development. We show here that these "ring mutants" are all defective in purine biosynthesis and that the "rings of transposition" reflect a requirement for GTP.

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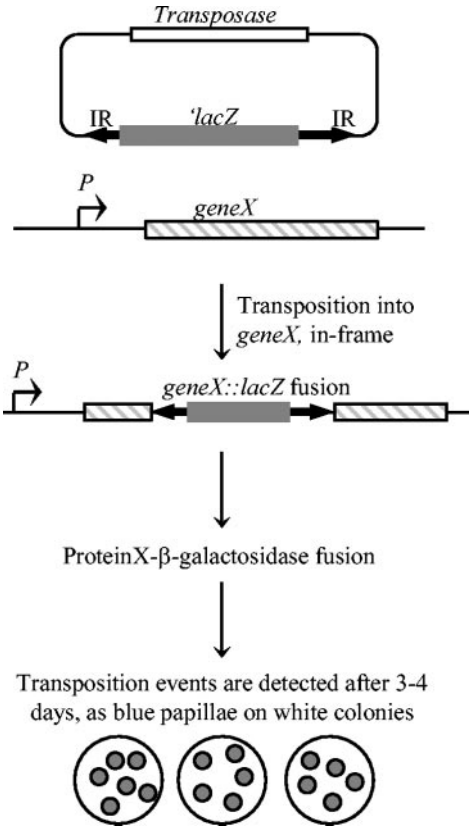

FIG. 1. Papillation assay used to identify the effect of *pur* mutants on transposition of IS*903*, Tn*10*, and Tn*552*. A *lacZ* mutant strain was transformed with a plasmid carrying the transposase gene and its cognate transposon. The transposon carries a *lacZ* gene, which lacks transcriptional and translational start signals and is therefore not expressed (*'lacZ*). Transposition of this element (Tn*'lacZ*) into an expressed gene (*geneX*) in the correct orientation and reading frame will generate a *geneX*::*lacZ* fusion, which will express a β-galactosidase fusion protein. Cells within a colony containing such transposition events can be detected as blue papillae on a white colony, after prolonged incubation on rich medium containing lactose and the indicator X-Gal. Inverted repeats (IR) define the ends of the transposon and are recognized by the transposase.

MATERIALS AND METHODS

Reagents and chemicals. Restriction enzymes and DNA-modifying enzymes were purchased from New England Biolabs and were used according to the manufacturer's instructions. The Fail Safe enzyme mix used in the PCRs and the EZ::TN <KAN-2> Tnp transpososome kit were purchased from Epicenter. Adenine was purchased from Sigma Chemical Company. Oligonucleotides used for PCR primers, λ Red mutagenesis, and DNA sequencing were purchased from Integrated DNA Technologies (Table 1). DNA sequencing was used to confirm and map mutations and was performed by the Molecular Genetics Core at the Wadsworth Center.

Bacterial strains. All *E. coli* strains used in the papillation assays are derived from DH10B, and their relevant genotypes are given in Table 2. Relevant mutations, including those received from the *E. coli* Genetic Stock Center (Yale University), or created by λ Red mutagenesis (58), were transduced into DH10B (*recA* mutant). Since transduction requires RecA, the *E. coli recA* gene was expressed in *trans* from a temperature-sensitive, Ap^r plasmid, pET001. Following successful transduction, pET001 was cured by nonselective growth at 42°C.

Source of mutants. The *purD*, *purF*, *purH*, and *purL* mutants were identified as Km^r colonies that formed rings of papillae during the screen of the insertion library (Twiss et al., unpublished). The *purK*, *guaB*, and *spoT*205::Km mutations were transduced into the DH10B strain using lysates derived from the strains CAG18566 *purK3200*::Tn*10*Km (37, 50), CAG18469 *gua-26*::Tn*10* (17, 50), and CF6650 *spoT205*::Km (56), respectively.

The *purA*, *rpoS*, *ndk*, *spoT*, and *relA* deletion mutations were made using the λ Red mutagenesis system (58). Described briefly, a 1.5-kb PCR fragment was created with two 62-bp-long primers that each contained a 5′ region homologous to the flanks of the targeted gene and a 3′ end homologous to the Km^r replacement cassette (Table 1). Here, the Km^r template was carried on pKD4 (11) and is flanked by FRT sites (Flp recombination target), which can be used to excise the Km^r gene via site-specific recombination (see below). The PCR product was created using "hot start" and "touch-down" PCR for high specificity (10, 15), and the fragment was purified using DNA Clean & Concentrator-5 from Zymo Research, digested with DpnI enzyme to destroy the template plasmid, and repurified. Ten to 100 ng of the PCR product was transformed into the λ Red strain DY329 (58), and transformants were selected on media containing kanamycin (25 μg/ml). The deletion was confirmed by two PCRs, one using a pair of primers flanking the region of interest and a second reaction using a primer that anneals within the integrated segment (oKD293) and a flanking primer. P1 lysates were made from Km^r transformants and were used to transduce the mutations into DH10B containing pET001.

The *spoT relA* double mutant strains were created by removal of the Km^r cassette in one gene replacement, via site-specific recombination between the two FRT sites, before transduction of the second Km^r-marked gene. This was achieved by transformation of the Δ*relA*::Km strain with pCP20, which is an Ap^r, temperature-sensitive plasmid encoding the Flp recombinase (11). Once established at the permissive temperature, Ap^s Km^s segregants were enriched by liquid growth without selection at 42°C, before colony purification. Ap^s Km^s clones, containing the unmarked deletion, were identified by patching onto selective medium. The resulting mutant was then transduced with a lysate made from the Δ*spoT*::Km mutant, thus creating a Δ*relA* Δ*spoT*::Km double mutant. The process was also used to create a Δ*spoT* Δ*rel*A::Km mutant.

In the course of these strain constructions, we discovered that DH10B contains a *relA1* allele; the *relA* gene contains an IS*2* insertion. This allele has been described previously, but it has never been assigned to DH10B. It has residual RelA function, since the IS*2* insertion splits the gene into two functional peptides (33). To confirm that the ring phenotype was not due to this *relA*::IS2 mutation, we transduced the wild-type *relA* gene into a *guaB* mutant background. The ring phenotype occurred independently of the *relA1* allele (data not shown).

Papillation assay. A mutant library was generated in DH10B by transpososome mutagenesis using the Km^r EZ::TN <KAN-2> Tnp transposome kit (Twiss et al., unpublished). Host mutants affecting transposition were identified using a papillation assay (12). Briefly, pNT105 (52), which carries the IS*903* transposase and an IS*903* transposon containing a cryptic *lacZ* gene (Tn*'lacZ*), was transformed into competent DH10B (Fig. 1). After transformation, cells were recovered in 250 μl LB medium for 1 to 2 h at 37°C and were plated on papillation medium, which contains Luria-Bertani agar, 20 μg/ml chloramphenicol, 0.05% lactose, and 40 μg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). Transformants were incubated at 30°C in a humid container for 3 to 4 days. Similar constructs carrying a cryptic *lacZ* gene were generated for Tn*10* and Tn*552* (52) and used as above to monitor transposition. Briefly, the Tn*552* genes *tnpAB* were cloned into a pBR322 derivative carrying a Tn*552'lacZ* transposon, to generate pET003. A Tn*10'lacZ* transposon (26) was constructed in pACYC184 carrying the Tn*10* transposase expressed from the P*taq* promoter (23), to generate pET005.

Adenine gradient plates were employed to study the effect of supplemental adenine on the papillation phenotype of *pur* mutants. These plates were created by pouring 15 ml of papillation medium supplemented with 80 μg/ml adenine into petri dishes and allowing the medium to set while the plate rested at an angle. The petri dishes were then transferred to a flat surface, and 15 ml of papillation medium (without adenine) was poured over the solidified adenine base to create a constant thickness of medium and a gradient of supplemental adenine across the plate.

Mating-out assay. Mating-out assays were performed as described previously (52). The target conjugative plasmid was pUBF101, which contains a region preferred by IS*903* for insertion (25), and the recipient was DH1 (Nal^r). pKD498 was used as the transposon delivery plasmid. Mating assays were performed on *purK* and *purF* mutants.

GTP-binding assay. A modified protocol of an ATP-binding assay (16) was used to determine whether GTP is a substrate of the Tn*552* transposase. Approximately 0.65 μg of purified transposase was incubated with 2.25 μCi [α-^{32}P]GTP (3,000 mCi/mmol; Perkin Elmer Life Sciences) in a buffer containing 25 mM HEPES, pH 7.5, 2 mM dithiothreitol, 200 mM NaCl, 1 mM $MgCl_2$, and 8% sucrose for 10 min in a microtiter dish at room temperature. After the incubation, the sample was UV-irradiated in a Spectrolinker (Spectronics Cor-

TABLE 1. Oligonucleotides used in this study

Gene target	Primer	Sequence
λ Red knockouts[a]		
purA	OKD499	GTAACAACGTCGTCGTACTGGGCACCCAATGGGGTGACGAAGgtgtaggctggagctgcttc
	OKD500	TACGCGTCGAACGGGTCGCGCAGAATCATGGTTTCAGTACGAatgggaattagccatggtcc
	OKD521	ggtaacaacgtcgtcgtactg
	OKD522	tacgcgtcgaacgggtcgcgc
rpoS	OKD536	TTTTGCTTGAATGTTCCGTCAAGGGATCACGGGTAGGAGCCAgtgtaggctggagctgcttc
	OKD537	CAGCCTCGCTTGAGACTGGCCTTTCTGACAGATGCTTACTTAatgggaattagccatggtcc
	OKD544	gttgtcggtagcagacggtct
	OKD545	gaaccagttcaacacgcttgc
ndk	OKD540	ACGCTGGTACAGACAACAACAGAACAATTTACAGAGGTAAAAgtgtaggctggagctgcttc
	OKD541	CGGATGCCACGTTTGCACGCGGCATTTACGAAATTATTAACGatgggaattagccatggtcc
	OKD542	gacatatgctattccggcctc
	OKD543	caatagtcaacggccctgttg
relA	OKD557	ATTTGCCGATTTCGGCAGGTCTGGTCCCTAAAGGAGAGGACGgtgtaggctggagctgcttc
	OKD558	TCTACATTGTAGATACGAGCAAATTTCGGCCTAACTCCCGTGcatatgaatatcctccttag
	OKD553	gcattaacgtagccgggatcc
	OKD554	ctggatatgttcccacacacg
spoT	OKD559	GCTATTGCTGAAGGTCGTCGTTAATCACAAAGCGGGTCGCCCgtgtaggctggagctgcttc
	OKD560	CCTGGCGAGCATTTCGCAGATGCGTGCATAACGTGTTGGGTTcatatgaatatcctccttag
	OKD555	caggaacagcaagagcaggaa
	OKD556	cagatgcgtgcataacgtgtt
Km[r]	OKD293	ccaagctcttcagcaatatcac
Complementation[b]		
purD	OKD429	at**ggatcc**actgactgctgcattccc
purH	OKD430	ga**gtcgac**gtcagttagggatcacg
purF	OKD433	aa**cggccg**ctggcgcgtcttatcagg
	OKD434	aa**cggccg**actttgcaggatttcgg

[a] The 62-base-long oligonucleotides used for λ Red recombineering were designed with two domains: the segment homologous to the gene targeted for knockout is capitalized, and the segment homologous to the Km[r] gene of pKD4 is in lowercase (11). The oligonucleotides for *relA* and *spoT* knockouts differ because they include the FRT sites. Oligonucleotides OKD521–22, 542–5, and 553–6 were used for PCR confirmation of the λ Red knockout, and they anneal to sequences up- and downstream of the target gene. OKD293 anneals within the Km[r] gene and was used as an internal primer, together with a flanking primer, to confirm replacements.

[b] Oligonucleotides used for complementation contain EagI, BamHI or SalI restriction sites (boldface), to facilitate cloning of the amplified gene into pBR322, for complementation studies.

poration) at 0.12 J/cm^2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis loading buffer was added to the samples, which were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The gel was visualized by autoradiography.

RESULTS AND DISCUSSION

Insertions in genes encoding the purine biosynthetic pathway cause a ring-of-transposition phenotype. We have begun a comprehensive screen for host mutants that affect transposition. To generate a library of host mutants, we have used a Tn*5* derivative encoding kanamycin resistance (Km[r]) and delivered the transposon by electroporation (18). In the screen, 30 colonies exhibited a distinctive phenotype: Lac$^+$ papillae, indicative of independent transposition events within a colony, occurred predominantly in a ring just within the periphery of the colony (Fig. 2, compare panel A with C and E). We note that although papillae are not entirely absent from the center of the colony, the number is clearly reduced; transposition appears to be coordinated with colony development, since papillae develop from cells inside the periphery of the colony. The ring phenotype was confirmed in each case by restreaking onto fresh medium. Finally, for each mutant, the Km[r]-marked mutation was transduced into a clean genetic background. The ring phenotype and Km[r] cotransduced with 100% efficiency. Cloning and sequence analysis identified each ring mutant as having an insertion in a *pur* gene, whose protein products are enzymes involved in the purine biosynthetic pathway in *E. coli* (Fig. 3). Insertions in *purF*, *purD*, *purL*, and *purH* were identified from the screen (Fig. 2C and E, and data not shown). An independently isolated Tn*10* insertion in *purK* was also tested in the papillation assay. This mutation was transduced into DH10B and was shown to cause a ring phenotype (Fig. 2G).

The *purD* and *purL* insertion mutants were complemented with their wild-type genes. This confirmed that the effect of the mutations was due to disruption of the purine biosynthetic pathway and not due to a polar effect on genes found in the vicinity of the *pur* genes (Fig. 2CC and DD).

***pur* mutants cause a ring phenotype for IS*903* and Tn*552* but not Tn*10*.** We assayed two other transposons to determine whether the ring phenotype was specific to IS*903*. We selected two DDE transposons that are not closely related at the amino acid level and that transpose by different mechanisms. Tn*10* moves via a cut-and-paste mechanism, while Tn*552* transposes by a replicative pathway; therefore, the two are expected to have differing host requirements (20, 22). No rings of papillae were observed for Tn*10* (data not shown), but all *pur* mutants caused a ring phenotype for Tn*552* (Fig. 2). This latter result indicates that disruption of the purine biosynthetic pathway is not specific to IS*903*. However, the absence of rings observed for Tn*10* indicates that this phenotype is not a general effect on transposition imposed by the assay conditions. Equally important, we can rule out more trivial explanations as being responsible for the formation of transposition rings. For example, the phenotype is unlikely to be due to transcriptional effects, as

TABLE 2. *E. coli* strains and plasmids used in this work

Strain or plasmid	Relevant genotype	Reference or source
Strain		
DH10B	F$^-$ *araD139* Δ(*ara leu*)7697 Δ*lacX74 galU galK rpsL deoR* ϕ80*dlacZ*Δ*M15 endA1 nupG recA1 mcrA* Δ(*mrr hsdRMS mcrBC*)	19
DH10B derivatives		
purA	DH10B, *purA*::Km	λ Red system (this work)
purD	DH10B, *purD*::Km	Host mutant screen (this work)
purF	DH10B, *purF*::Km	Host mutant screen (this work)
purH	DH10B, *purH*::Km	Host mutant screen (this work)
purK	DH10B, *purK3200*::Tn*10*Km	Transduction with lysate from CAG18566 (50)
purL	DH10B, *purL*::Km	Host mutant screen (this work)
guaB	DH10B, *guaB*::*tet*	Transduction with lysate from CAG18469 (50)
ndk	DH10B, *ndk*::Km	λ Red system (this work)
rpoS	DH10B, *rpoS*::Km	λ Red system (this work)
spoT205	DH10B, *spoT205*::Km	Transduction with lysate from CF6650 (56)
spoT205 guaB	DH10B, *spoT205*::Km, *guaB*::*tet*	Transduction of *spoT205*::Km from CF6650 into DH10B, *guaB*::*tet*
Δ*spoT*	DH10B, Δ*spoT*	λ Red system (this work)
Δ*relA*	DH10B, Δ*relA*	λ Red system (this work)
Δ*spoT relA*::Km	DH10B, Δ*spoT relA*::Km	λ Red system (this work)
Δ*relA spoT*::Km	DH10B, Δ*relA spoT*::Km	λ Red system (this work)
DY329	Lam$^-$, IN(*rrnD-rrnE*)1, *rph-1* Δ*lacU169 nadA*::Tn*10 gal490* λ*c1857* Δ(*cro-bioA*)	58
DH1	*recA1 gyrA96* Nal^r recipient strain for mating-out assay	21
Plasmid		
pKD4	*oriR6K*γ, Ap^r, Km^r, *rgnB*(Ter)	11
pCP20	Carries yeast Flp recombinase gene (FLP); Cm^r, Ap^r; temperature-sensitive replication	11
pNT167	The *purH* and *purD* genes from DH10B cloned between the BamHI and SalI sites of pBR322	This work
pAC1	*purL* gene with upstream sequences cloned between BamHI and SalI sites of pBR322	This work
pET001	The *E. coli recA* gene cloned into the SmaI site of pRR10-ts97	This work
pRR10-ts97	Ap^r, temperature sensitive	40
pUBF101	RP1 derivative (Tet^r), conjugative plasmid, containing a preferred insertion site for IS*903*	25
pKD498	pKD100 derivative carrying an IS*903* transposon and transposase; Ap^r, Cm^r, Km^r	52
pNT105	Cm^r pACYC184 derivative expressing IS*903* transposase and carrying a cryptic *′lacZ* transposon with IS*903* ends	52
pET003	pBR322 derivative expressing Tn*552* transposase and TnpB and carrying a cryptic *′lacZ* transposon with Tn*552* ends; Ap^r	This work
pET005	pACYC184 derivative expressing Tn*10* transposase and carrying a cryptic *′lacZ* transposon with Tn*10* ends; Cm^r	This work

both transposases are expressed from different promoters (*lacUV5* for IS*903* and the native promoter for Tn*552*), or plasmid effects, as the two transposons are delivered from different plasmids (pACYC184 and pBR322, respectively).

Supplemental adenine in the medium can suppress the ring papillation phenotype of *pur* mutants. In *E. coli*, purines are acquired by two routes: either they are synthesized de novo via the purine biosynthetic pathway, or else they are scavenged from the surrounding medium via the purine salvage pathway (59) (Fig. 3). Since *pur* gene mutations disrupted the former process, we hypothesized that the transposition phenotype resulted from a combination of purine deficiency at the center of the colony, plus an ability to scavenge purines at the edge of the colony: hence the ring of transposition. To address this, we poured transposition assay plates such that a concentration gradient of adenine would be present in the medium. These gradient plates gave rise to wild-type papillating colonies in areas of high adenine concentration, while colonies growing in regions of low adenine concentration retained their ring phenotype (Fig. 2S to X). These findings support the idea that the ring phenotype is related to a purine deficiency during colony growth.

An inability to synthesize guanine is responsible for the ring phenotype. The *pur* mutants described above catalyze reactions upstream of the IMP branch point in the purine biosynthetic pathway. The protein products of *purA* and *guaB* catalyze reactions downstream of the IMP branch point and result in synthesis of adenine and guanine, respectively (Fig. 3). A *guaB*::Tn*10* insertion mutation and a *purA* deletion, created using the λ Red system, were transduced into DH10B and then

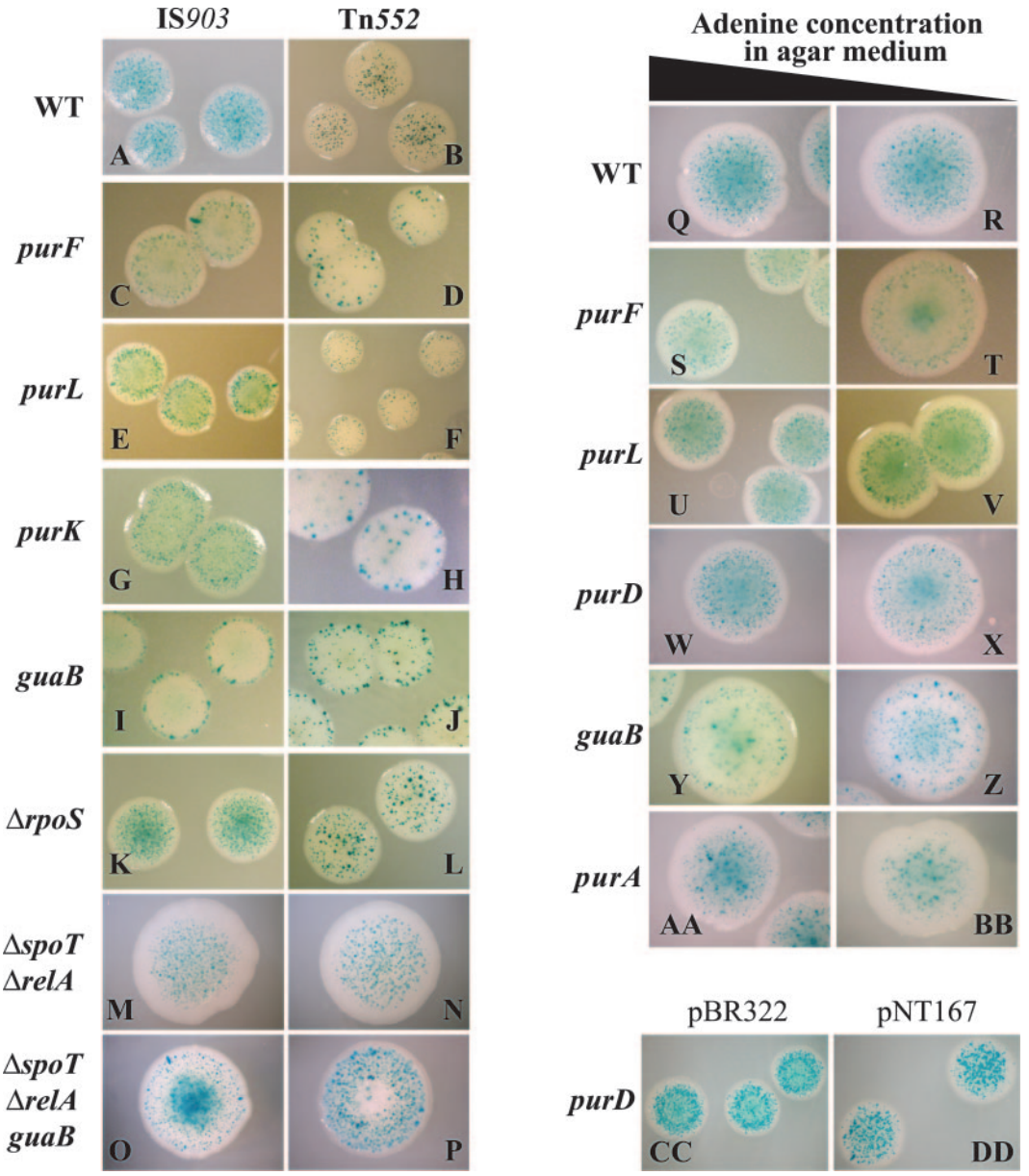

FIG. 2. Papillation phenotype of wild-type and mutant colonies containing either IS*903* or Tn*552*. The left-hand panel (A-P) shows examples of rings of papillae for IS*903* and Tn*552* in a variety of mutant backgrounds that affect purine metabolism. No rings are observed with deletions of *rpoS*, *relA*, or *spoT* (K-N). Note that a ring phenotype is restored in the *rpoS relA guaB* triple mutant but that the rings are either faint (O, IS*903*) or more diffuse (P, Tn*552*) than with the *guaB* mutant alone (I, J). The right-hand panel (Q-BB) shows that excess exogenous adenine can rescue the wild-type papillation phenotype. Papillation of IS*903* is shown for the same purine mutants as above but on adenine gradient plates. Colonies growing at low concentrations of adenine (right column) exhibit a ring phenotype. By contrast, high concentrations of adenine rescue the phenotype, except for *guaB*, which retains the ring phenotype (Y). The bottom right panels (CC-DD) demonstrate that the wild-type *purD* gene encoded by pNT167 complements the *purD* insertion, in contrast to the empty vector, pBR322.

tested in the papillation assay. The *purA* mutant was defective for transposition, as the overall level of papillation was reduced, but no rings were observed (Fig. 2BB). By contrast, in the *guaB* mutant, both IS*903* and Tn*552* generated rings of papillae, indicating that a defect in GMP biosynthesis (or a defect further along that pathway) is what causes the ring phenotype (Fig. 2I and J). We note that the *guaB*-mediated rings differed in two respects from those of the upstream mutations. First, the *guaB*-mediated ring was located closer to the periphery of the colony. Second, the appearance of the *guaB* rings occurred from days 8 to 10 in the transposition assay, whereas the appearance of the rings of other *pur* gene mutants occurred on day 4 of the transposition assay. We currently have no explanation for these differences, but it is possible that the notably slower growth of the *guaB* mutant causes a reduced colony size and slower development of the ring. Another observation that correlates guanine availability with *pur* gene mutant effects on transposition was the fact that *guaB* colonies

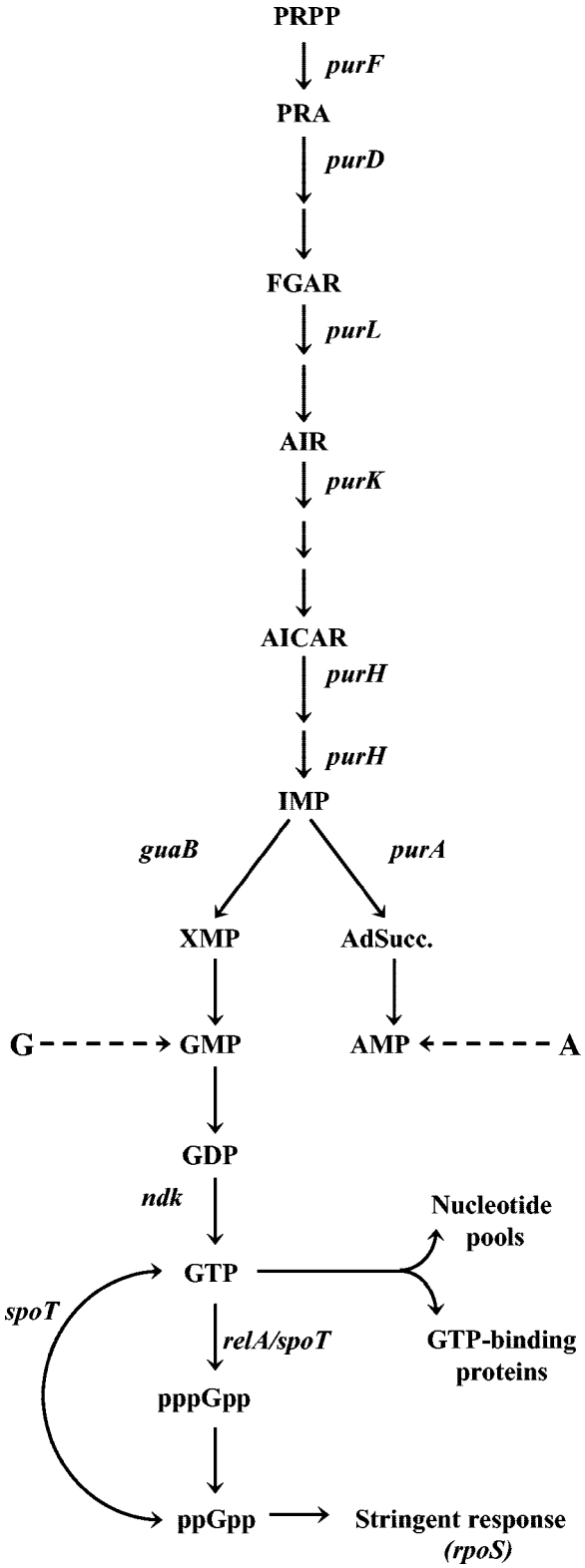

FIG. 3. The purine biosynthetic pathway is shown in a simplified form (modified from reference 59), highlighting the mutated genes that were tested in the papillation assay (PRPP, 5-phospho-α-D-ribosyl 1-pyrophosphate; PRA, 5-phospho-β-D-ribosylamine; FGAR, 5′-phosphoribosyl-*N*-formylglycinamide; AIR, 5′-phosphoribosyl-5-aminoimidazole; AICAR, 5′-phosphoribosyl-4-carboxamide-5-aminoimidazole; AdSucc., adenylosuccinate; ppGpp, guanosine-tetraphosphate). In addition to synthesis of purines, cells can scavenge purines (A and G) from the medium (dashed arrows), which is the primary source until exogenous supplies are depleted. The transposition phenotype of *pur* mutants with either IS*903* or Tn*552* transposon derivatives is shown in Fig. 2.

growing in close proximity to one another developed rings 2 to 3 days earlier than did those colonies that were more dispersed (data not shown). Presumably, growth in areas of high colony density is subject to greater competition for scavenged nutrients, so the effects of disruption of the purine biosynthetic pathway are manifested sooner. Finally, we note that the *guaB* mutant was not suppressed by addition of exogenous adenine to the medium (Fig. 2Y to Z). This confirms that transposition is responding to a guanine deficiency rather than an indirect effect of a *guaB* mutation on adenine availability.

Together, these data suggest that transposition of IS*903* and Tn*552*, but not Tn*10*, requires guanine (either directly or indirectly). This is further supported by the absence of rings in other biosynthetic mutants, including *purA* and pyrimidine biosynthetic mutants (*carB* and *pyrC*; data not shown) (5, 50); such an absence indicates that the phenotype is not indicative of a general titration of available metabolites. The purine biosynthetic pathway is normally repressed by PurR, until exogenous purine sources are exhausted from the medium in later stages of colony growth. Thus, our data are consistent with the idea that transposition occurs late in colony growth—hence, the reduction in papillae at the center of the colony, where purines are depleted from the medium and cannot be synthesized by the *pur* mutants. However, the cells inside the growing edge of the colony can scavenge purine precursors from the medium, with the result that transposition occurs in these cells and that a ring of papillae is formed.

The effect of *pur* mutations on transposition is colony specific. A mating-out assay was performed to determine whether the *pur* mutations also had an effect on transposition in cells growing in a liquid culture. The results from the mating assay showed that *pur* mutants had wild-type transposition frequencies (data not shown), thus indicating that the effect of *pur* mutants on transposition is specific to the colony environment. This finding was expected, given that these cells would not be subject to purine deprivation by the same developmental and spatial restriction as cells grown as a biofilm on solid medium.

The link between purine deprivation and transposition requires GTP and is independent of ppGpp metabolism. We considered that the two most likely requirements of guanine in transposition would be for processes requiring GTP or ppGpp (Fig. 3). GTP could be required directly in the transposition reaction, or GTP-binding proteins could be required for modulation of transposition. GTP is also a precursor of ppGpp, which is required for triggering the stringent response (Fig. 3). An inability of the cells to mediate the stringent response might prevent transposition, except at the edges of the colony, where purines could still be scavenged. A precedent for limitation of transposition to stationary-phase events has been established for a *Pseudomonas putida* transposon, Tn*4652* (27), whose expression is dependent on the stationary-phase sigma factor (RpoS), which responds to cellular ppGpp levels.

In *E. coli* the two genes whose protein products synthesize

ppGpp are *relA* and *spoT* (56). Individual, and combined, deletion derivatives of each of these two genes were constructed using λ Red mutagenesis. All combinations of *relA* and *spoT* deletion mutations (and acquired and independently isolated *relA* and *spoT* mutants; data not shown) exhibited a wild-type papillation phenotype and did not generate rings, indicating that the ring phenotype must be caused by a defect upstream of ppGpp (Fig. 2M to N, and data not shown). Moreover, when these two mutations were combined with the *guaB* mutation, the ring phenotype was restored, confirming that the limited availability of GMP or GTP causes the ring phenotype (Fig. 2O and P). In addition, an *rpoS* deletion mutant had a wild-type papillation phenotype, demonstrating that the rings seen with *pur* mutants are not caused by a disruption of the stringent response mediated by this stationary-phase sigma factor (Fig. 2K and L).

The lack of rings observed with *spoT* and *relA* mutants is consistent with the hypothesis that the ring phenotype reflects a requirement for GTP during transposition and that in *pur* mutants, guanine is scavenged from the medium. To prevent conversion of scavenged guanine into GTP, we generated a mutation in the nucleoside diphosphokinase gene, *ndk* (Fig. 3). No papillae (indicative of transposition) were observed with either IS*903* or Tn*552* (data not shown). Taken together, the results of these genetic experiments suggest that the ring phenotype reflects the ability of cells at the edge of a growing colony to scavenge guanine, for synthesis of GTP, which is required for transposition. The absence of rings with other mutants including *purA*, *pyr*, *relA*, *spoT*, and *rpoS* indicates that this is a GTP-specific effect unique to IS*903* and Tn*552*.

The transposition requirement for GTP could be indirect. Two, of many possible, scenarios are the involvement of a host GTP-binding protein in the transposition reaction per se or that a GTP-dependent pathway might regulate transposition or a host factor required for transposition. An alternative, and more parsimonious, explanation is that the IS*903* and Tn*552* transposases are GTP-binding proteins, although neither contain the canonical GTP-binding consensus sequence (14, 43). The availability of active Tn*552* transposase (gift of N. Grindley, Yale University) allowed us to perform a preliminary GTP-binding assay (16). The results were negative (data not shown). Direct evidence for, or against, the involvement of GTP in the transposition reaction will require reconstitution of the complete transposition reaction, including the Tn*552* accessory protein, TnpB. This protein is present in our papillation assay but has not yet been purified.

Transposition occurs in later stages of colony growth. Several lines of evidence support the idea that transposition occurs in nutrient-starved cells, during late stages of colony development. In wild-type cells, papillae are only detected after 3 to 4 days of growth, and they are absent from the growing margin, suggesting that transposition occurs in older cells in the middle of the colony, which are more likely to be nutrient starved. In *pur* mutants, transposition was observed just inside the periphery of the colony and not in the center, consistent with the idea that transposition does not occur (at least, not with high frequency) until later stages of colony development, and then predominantly in peripheral cells that are becoming subject to nutrient deprivation. If transposition occurred at early growth stages, *pur* mutant cells in the center of the colony would still have access to exogenous purines and would thereby have the ability to undergo transposition. Evidence supporting a preference for transposition in late growth has also been reported for IS*1*, Mu, and Tn*4652* (discussed above), indicating this may be a common theme and further suggesting that transposition occurs in response to the cellular environment. IS*1* insertions into the *bgl* locus occur preferentially in stationary-phase cells (32, 38), and the formation of *araB-lacZ* fusions by a Mu prophage is triggered by prolonged carbon starvation (45, 47). Under nutrient-restricted conditions, there may be a link between transposition and the stringent response. However, it is clear from our analysis that the ring phenotype is not dependent on the ability to synthesize ppGpp (Fig. 2 M and N).

GTP and transposition. Our results provide the first evidence of a role for GTP in bacterial transposition, although two eukaryotic transposons have been shown to respond very differently to GTP in in vitro reactions. The transposase encoded by the P element from *Drosophila* binds GTP and contains a canonical GTP-binding-site motif (28, 34). More recently, GTP has been shown to act as a conformational effector that promotes assembly of the active transposase-DNA complex in vitro (D. Rio, personal communication). By contrast, in VDJ recombination, which is mediated by the RAG1/2 recombinase and mechanistically is identical to transposition (3, 24), GTP has been shown to inhibit transposition by blocking capture of the target DNA (54). Although neither the IS*903* nor the Tn*552* transposase contains a detectable GTP-binding motif, we note that such a motif is present in the Tn*552* accessory protein, TnpB (42). TnpB is related to two characterized transposon-encoded proteins: MuB, from bacteriophage Mu, and the TnsC protein of Tn*7*. Both MuB and TnsC are ATP-binding proteins and are important regulators of transposition (1, 2, 16). Tn*552* TnpB is required for in vivo transposition, and it could potentially be responding to available GTP. A more rigorous determination of whether TnpB is an ATP- or a GTP-binding protein must await this protein's purification.

The ring of transposition phenotype is striking. Remarkably, such rings have been described previously when monitoring formation of *araB-lacZ* fusions carried by the bacteriophage Mu (46). Although these rings formed under quite different conditions and were continuous rings of β-galactosidase expression, rather than individual papilla, they are a result of Mu transposition, requiring transposase activity and Mu ends (48). The rings observed with Mu were not dependent on purine auxotrophy, but were consistent with colony pattern formation and differentiation triggering transposition. These and other Mu-mediated fusions only occurred after prolonged incubation and nutrient starvation (summarized in reference 44). Thus, there are tantalizing similarities between transposition of the two elements in response to the cellular environment. Newman and Shapiro have also elegantly demonstrated that chemical gradients in the medium can result in altered expression of genes, and this expression depends on the spatial location of cells within a colony (36). Here, we observe a similar effect on transposition, in which a purine gradient combined with the spatial differentiation of cells generates a transposition ring. The fact that no papillae are detected at the edge of the colony, where purines are presumably abundant, argues that a second component is suppressing transposition. The ring-like organization of the papillae suggests that this second component is

dependent on the location of the cells with respect to the growing colony edge. We anticipate that the isolation of additional host mutants will shed greater light on the regulation of transposition both within a cell, and within a cellular community in a colony biofilm, which more closely resembles the natural bacterial habitat.

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