Reconstitution of Acetosyringone-Mediated Agrobacterium tumefaciens Virulence Gene Expression in the Heterologous Host Escherichia coli

SCOTT M. LOHRKE,† HONGJIANG YANG,‡ AND SHOUGUANG JIN*

Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, Florida 32610

Received 26 January 2001/Accepted 30 March 2001

The ability to utilize Escherichia coli as a heterologous system in which to study the regulation of Agrobacterium tumefaciens virulence genes and the mechanism of transfer DNA (T-DNA) transfer would provide an important tool to our understanding and manipulation of these processes. We have previously reported that the rpoA gene encoding the alpha subunit of RNA polymerase is required for the expression of lacZ gene under the control of virB promoter (virBp::lacZ) in E. coli containing a constitutively active virG gene [virG(Con)]. Here we show that an RpoA hybrid containing the N-terminal 247 residues from E. coli and the C-terminal 89 residues from A. tumefaciens was able to significantly express virBp::lacZ in E. coli in a VirG(Con)-dependent manner. Utilization of lac promoter-driven virA and virG in combination with the A. tumefaciens rpoA construct resulted in significant inducer-mediated expression of the virBp::lacZ fusion, and the level of virBp::lacZ expression was positively correlated to the copy number of the rpoA construct. This expression was dependent on VirA, VirG, temperature, and, to a lesser extent, pH, which is similar to what is observed in A. tumefaciens. Furthermore, the effect of sugars on vir gene expression was observed only in the presence of the chvE gene, suggesting that the glucose-binding protein of E. coli, a homologue of ChvE, does not interact with the VirA molecule. We also evaluated other phenolic compounds in induction assays and observed significant expression with syringaldehyde, a low level of expression with acetovanillone, and no expression with hydroxyacetophenone, similar to what occurs in A. tumefaciens strain A348 from which the virA clone was derived. These data support the notion that VirA directly senses the phenolic inducer. However, the overall level of expression of the vir genes in E. coli is less than what is observed in A. tumefaciens, suggesting that additional gene(s) from A. tumefaciens may be required for the full expression of virulence genes in E. coli.

Agrobacterium tumefaciens is a soil bacterium which infects plant wound sites and induces tumor formation. The bacterium harbors a large tumor inducing plasmid (Ti plasmid) encoding virulence genes and transfer DNA (T-DNA). The function of the virulence genes is the processing and transfer of the T-DNA from the Ti plasmid into susceptible plant cells, with subsequent integration into the host genome (for recent reviews, see references 27 and 56). Located on the T-DNA are genes that direct the biosynthesis of the plant growth regulators auxin and cytokinin in the infected cells (1, 49). The synthesis of these plant growth regulators results in a rapid, uncontrolled cell division leading to production of a characteristic tumor at the site of infection. In addition, the T-DNA contains genes for the synthesis of a unique class of compounds called opines, which Agrobacterium tumefaciens can utilize as a carbon and energy source (36).

In A. tumefaciens, the expression of virulence genes is under the control of a two-component regulatory system comprised of VirA and VirG (45, 52). VirA is an inner membrane histidine kinase (34, 54) which autophosphorylates in response to certain phenolic compounds released from wounded plants (44) with subsequent transfer of the phosphate moiety to the response regulator VirG (16, 22, 24). Once phosphorylated, VirG activates transcription from promoters containing a specific 12-bp sequence called the vir box, which is present in the promoters of all vir genes (25, 40). This expression is augmented by the presence of certain monosaccharides (5, 43) and an acidic pH (32), which is characteristic of plant wound sites. A periplasmic sugar-binding protein, ChvE, which is highly homologous to glucose-binding protein of Escherichia coli, interacts with the periplasmic portion of the VirA molecule in the presence of certain monosaccharides, including glucose and arabinose (2, 15). This interaction alone does not induce vir gene expression, but it sensitizes the VirA molecule to the phenolic inducers.

While A. tumefaciens is a potentially serious plant pathogen, the main interest in this organism is due primarily to its ability to transform plant cells. Researchers have developed delivery systems based on T-DNA transfer to engineer new traits into selected plant species (14). However, the exact mechanism of T-DNA transfer is still not well understood. The ability to use E. coli as a heterologous host in which to study the regulation of A. tumefaciens virulence genes and the mechanism of T-DNA transfer would constitute an excellent model system given the degree of characterization at both the biochemical and the genetic levels and the relative ease of genetic manipulation. However, all previous attempts to reconstitute inducer-mediated vir gene expression in E. coli have not been suc-
TABLE 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype or phenotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli DH5α</td>
<td>recA endA1 hsdR17 supE44 gyrA96 relA1 Δ(lacZYA-argF)U169 (d800lacZΔM15)</td>
<td>41</td>
</tr>
<tr>
<td>MC4100</td>
<td>F- araD139 Δ(argF-lac)U169 rpsL150 relA1 ilvD301 ptsF25 deoC1</td>
<td>7</td>
</tr>
<tr>
<td>M182</td>
<td>K-12 Δlac crp</td>
<td>8</td>
</tr>
<tr>
<td>M182Δcrp</td>
<td>K-12 Δlac crp</td>
<td>4</td>
</tr>
</tbody>
</table>

Plasmids

| pBI410           | virBp::lacZ IncP Te' | 5 |
| pPC401           | lac::VirF fusion in pTZ18R, Ap' | 25 |
| pSW191           | lac::VirF fusion in pTZ18R, Ap' | 54 |
| pSG092           | lac::VirB and lac::VirG in pTZ18R, Ap' | This work |
| pSY215           | virB::lacZ Plac::VirG (N54D) IncW Gm' | 31 |
| pSL107           | lac::VirB::VirG::VirBp::lacZ ColEI IncP Ap' Te' | 31 |
| pSL204           | lac::VirL::VirG::VirBp::lacZ IncP Te' | This work |
| pSI01010         | lac::VirB::VirBp::lacZ IncP Te' | This work |
| pSI0102          | lac::VirL::VirBp::lacZ IncP Te' | This work |
| pTZ18R/pTZ19R    | Cloning vectors, ColEI Ap' | U.S. Biochemicals |
| pTC110           | Cloning vector, IncW Km' Sp' | 10 |
| pPS1.3           | lac::poxA from A. tumefaciens A136 in pTZ19R, Ap' | 31 |
| pPS1.3R          | Non-lac::poxA from A. tumefaciens A136 in pTZ19R, Ap' | 31 |
| pHO98            | lac::poxA from A. tumefaciens A136 in pTC110, Km' IncW | 31 |
| pTC1.1           | lac::chvE from A. tumefaciens A136 in PCR21-TOPO, Ap' | This work |
| pTC2R2.4         | lac::chvE, lac::poxA from A. tumefaciens A136 in PCR21-TOPO, Ap' | This work |
| pQE30            | His expression vector, Ap' | Qiagen |
| pQE31            | A. tumefaciens His-RpoA, Ap' | 31 |
| pECH             | E. coli His-RpoA, Ap' | 31 |
| pAD8             | A. tumefaciens His-RpoA (deletion of C-terminal eight amino acids), Ap' | This work |
| pEA8             | E. coli His-RpoA (1–239 plus A. tumefaciens RpoA C-terminal eight amino acids), Ap' | This work |
| pADC             | A. tumefaciens His-RpoA (1–242 amino acids), Ap' | This work |
| pEN              | E. coli His-RpoA (1–247 amino acids), Ap' | This work |
| pENACN           | His-RpoA (E. coli 1–247 amino acids plus A. tumefaciens 248–336), Ap' | This work |
| pANEC            | His-RpoA (E. tumefaciens 1–242 amino acids plus E. coli 243–329) | This work |

cessful. Previously, we reported the identification of the rpoA gene, encoding the alpha subunit of RNA polymerase from A. tumefaciens, and that it was required for transcription of a virB promoter fused to lacZ (virBp::lacZ) in E. coli using a constitutively active VirG mutant [VirG(Con)] which can activate vir gene expression independent of VirA and inducers (31). In this study, we report the successful reconstitution of inducer-dependent expression of a virBp::lacZ fusion in E. coli utilizing lac promoter-driven virA and virG. Effects of various environmental conditions on virulence gene activation in E. coli are also described. Furthermore, by the gene fusion approach, the C-terminal domain of RpoA from A. tumefaciens is shown to be required for interaction with the transcriptional activator VirG.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** The bacterial strains used in this study are listed in Table 1. Bacterial strains were grown in either Luria-Bertani (LB) medium, mannitol glucose-Luria salts (MG/L) medium (50), or induction medium containing either 55 mM glucose, mannitol, or glycerol (53). When appropriate, the medium was supplemented with ampicillin (100 μg/mL), kanamycin (50 μg/mL), gentamicin (5 μg/mL), and tetracycline (20 μg/mL). For the growth of DH5α in induction medium, Casamino Acids (Difco) and thiamine were added to final concentrations of 0.1% (wt/vol) and 1 μg/mL, respectively. For M182 and M182Δcrp grown in induction medium, arginine and thiamine were added to final concentrations of 0.1% (wt/vol) and 200 μM, respectively. The virulence gene inducers acetosyringone, acetovanillone, hydroxyacetoephone, and syringalddehyde (Sigma) were added where indicated at a final concentration of 200 μM.

**Plasmid constructs and DNA manipulations.** The plasmids used in this study are listed in Table 1. Plasmids pZL2 and pECH are wild-type rpoA constructs from A. tumefaciens A136 (rpoAEc) and E. coli MC4100 (rpoAEc), respectively (31). Plasmid pAD8 encodes N-terminal His-tagged RpoAEc with the eight C-terminal amino acids deleted and was constructed by PCR amplification from pPS1.3 using the primers 5′-GGA AGG ATC CAT GAT TCA GAA GA-3′ and 5′-CCT GGA TCC TGC AGA TGA CTT ATC TG-3′ were used as...
RpoA of *A. tumefaciens*. To determine which domain of the *A. tumefaciens* RpoA is required for interaction with the VirG protein, a series of protein fusion constructs were generated, and the abilities of the hybrid *rpoA* constructs to activate transcription of *virBp: lacZ* in a VirG(Con)-dependent manner in *E. coli* MC4100 and pSY215 were evaluated (Fig. 1A). When introduced into DH5α, all of the fusion constructs clearly over-produced protein bands with predicted sizes (data not shown), but only pENACN was able to provide significant expression of the *virBp: lacZ* fusion (Fig. 1B). The wild-type His-tagged *A. tumefaciens* RpoA construct pZL2 exhibited reduced expression of *virBp: lacZ* relative to both pPS1.3 (59% reduction) and pENACN (48% reduction). Deletion of 8 and 94 amino acids from the C terminus of *A. tumefaciens* RpoA (pAD8 and pADC, respectively) resulted in a complete loss of *virBp: lacZ* expression. Additionally, addition of the eight amino acids from the *A. tumefaciens* RpoA C terminus to the C terminus of *E. coli* RpoA (pE8) also failed to activate transcription of *virBp: lacZ*. These results indicate that the C-terminal domain of the *A. tumefaciens* RpoA is required to interact with the VirG protein.

**Influence of rpoA copy number on expression of virBp: lacZ in *E. coli***. We have constructed pSL204, which contains lac promoter-driven virA and virG, as well as a virBp: lacZ reporter gene fusion. This construct is compatible with the *rpoA54* clones pHO98 and pPS1.3 (31). Transformation of DH5α containing pSL204 with pHO98 or pPS1.3 resulted in a blue color on MG/L and induction medium plates containing X-Gal and acetylsyringone. No blue color was observed on identical medium in the absence of acetylsyringone, indicating inducer-dependent expression of the *virBp: lacZ* fusion. In contrast, transformants containing the non-lacP-driven *A. tumefaciens* RpoA construct pPS1.3R did not exhibit any blue color on MG/L or induction medium containing X-Gal and acetylsyringone.

To obtain a quantitative value for expression of the *virBp: lacZ* fusion, β-galactosidase activities were determined for DH5α containing pSL204 and either pHO98, pPS1.3, or...
pPS1.3R (Table 2). Both pPS1.3 and pHO98 conferred significant expression of virBp::lacZ in induction medium supplemented with 55 mM glycerol and 200 μM acetosyringone. The increases in β-galactosidase activity were approximately 4.6- and 30-fold, respectively, for pHO98 and pPS1.3, relative to the control DH5α containing pSL204 only. In contrast, there was no increase in β-galactosidase activity when pPS1.3R was used relative to that of the control. Deletion of either the virA or virG gene in pSL204, pSJ0101, and pSJ0102, resulted in no expression of the virBp::lacZ gene (data not shown), suggesting a VirA-VirG-dependent phenomenon. Interestingly, significantly reduced β-galactosidase activity was observed when cultures were grown in induction medium supplemented with 55 mM glucose and 200 μM acetosyringone (~95% reduction with pPS1.3). A similar level of reduction was seen when the strains were grown in induction medium supplemented with both glucose and glycerol as well as acetosyringone (Table 2).

The inhibitory effect of glucose suggested to us the possible role of catabolite repression. Indeed, vir gene expression was abolished when the induction assays were carried out with a crp deletion mutant strain of E. coli, M182Δcrp, containing pSL204 and pPS1.3, whereas vir gene expression in the parent strain M182 containing pSL204 and pPS1.3 was similar to that in DH5α (Table 3). To test whether the cyclic AMP (cAMP) receptor protein (CRP) affects the virB promoter directly or the lac promoters that drive the expression of virA, virG, and rpoA, DH5α containing pSL204 and pPS1.3 was tested for the glucose effect in the presence of 30 mM cAMP, which activates genes under the control of CRP. Indeed, using 30 mM cAMP, a high level of β-galactosidase activity was induced in DH5α harboring pTZ18R where lacZα is under the control of the lac promoter (data not shown). As shown in Fig. 2, virBp::lacZ expression required both cAMP and acetosyringone, whereas cAMP or acetosyringone alone had no effect. These results suggest that the inhibitory effect of glucose is likely due to the catabolite repression of the lac promoter, which drives the expression of virA, virG, and rpoA genes, rather than to a direct effect on the virB promoter.

**Effect of other virulence gene inducers on the expression of virBp::lacZ in DH5α.** It is known that vir genes of different A. tumefaciens strains respond to different sets of phenolic compounds depending on the origin of the VirA molecules. The virA of pSL204 is derived from strain A348, whose vir gene expression is activated by acetosyringone, acetovanillone, and syringaldehyde but not by hydroxyacetophenone (29). The ability of DH5α containing pSL204 and pPS1.3 to express virBp::lacZ in response to these inducing compounds was evaluated. DH5α containing pSL204 with or without pPS1.3 were grown in induction medium amended with glycerol and with one of the following inducers: acetosyringone, acetovanillone, hydroxyacetophenone, or syringaldehyde. Significant levels of virBp::lacZ expression were obtained with DH5α containing pPS1.3 when acetosyringone and syringaldehyde were present. Low-level expression was detected in the presence of acetovanillone, and no induction was seen when hydroxyacetophenone was present (Table 4). Similarly, a Ti plasmidless A. tumefaciens strain, A136, harboring pSL204 responded strongly to acetosyringone and syringaldehyde and weakly to acetovanillone, but not at all to hydroxyacetophenone (Table 4).

**Inducer-dependent virBp::lacZ expression in DH5α is affected by pH, temperature, and choice of media.** To assess whether the expression of the virBp::lacZ in E. coli is responding to the same environmental signals that affect vir expression in A. tumefaciens, the effect of variations in temperature and pH were examined. When DH5α cells containing pSL204 with
or without pPS1.3 were grown in induction medium containing acetosyringone and glycerol at 28 or 37°C, high-level expression was observed at 28°C but not at 37°C (Fig. 3). When the same strains were assayed for virB::lacZ expression in induction medium at pH 6.0 and 7.0, there was approximately a 36% reduction in β-galactosidase activity when the bacterium was grown at pH 7.0 compared to when it was grown at pH 6.0 (Fig. 4).

To evaluate the effect of rich, complex medium on virB::lacZ expression, induction assays were carried out in MG/L and LB media at pH 6.0 in the presence or absence of acetosyringone. Overall, expression levels were significantly lower than what was obtained with induction medium supplemented with glycerol. The levels of induction were approximately 4.5- and 7-fold with MG/L and LB media, respectively, lower than what was obtained with induction medium supplemented with acetosyringone. Overall, expression levels were significantly lower than what was obtained with induction medium supplemented with glycerol. The levels of induction were approximately 4.5- and 7-fold with MG/L and LB media, respectively, compared to that of the control without acetosyringone (Fig. 5).

**Effect of chvE on virB::lacZ expression.** To evaluate whether chvE from A. tumefaciens can increase virB::lacZ expression in E. coli, we introduced pTCR2.4, which contains lacp-driven chvE and lacp-driven rpoA of A. tumefaciens into DH5α containing pSL204 and compared virB::lacZ expression with that of DH5α containing pSL204 and pPS1.3. To avoid the inhibitory effect of the glucose on the lac promoter driving the expression of virA, virG, and rpoA, 30 mM cAMP was added to the induction medium. As shown in Fig. 6, the

![FIG. 3. Effect of temperature on the expression of the virB::lacZ fusion in E. coli. Strain DH5α harboring both pSL204 and pPS1.3 was grown for 18 h in induction medium (pH 6.0) containing 55 mM glycerol at either 28 or 37°C. Acetosyringone (AS) was added where indicated at a final concentration of 200 μM. β-Galactosidase activity values are averages from three replicates.](http://jb.asm.org/)

![FIG. 4. Effect of pH on the expression of virB::lacZ expression in E. coli. Strain DH5α containing both pSL204 and pPS1.3 was grown for 18 h in induction medium, at pH 6.0 or 7.0, containing 55 mM glycerol with (+) or without (–) 200 μM acetosyringone (AS). β-Galactosidase activity values are averages from three replicates.](http://jb.asm.org/)

![FIG. 5. Effect of growth media on the expression of virB::lacZ expression in E. coli. Strain DH5α containing both pSL204 and pPS1.3 was grown for 18 h in induction medium (pH 6.0) containing 55 mM glycerol (IM) or MG/L or LB medium at pH 6.0. Acetosyringone (AS) was added where indicated at a 200 μM final concentration. β-Galactosidase activity values are averages from three replicates.](http://jb.asm.org/)
essential role in the transcription of many operons in *E. coli* controlled by transcriptional regulators such as FNR (51), GalR (11), MarA (20), MerR (9, 26), MetR (18), OxyR (48), OmpR (17), Rob (21), SoxS (19), UhpA (39), and TyrR (28). Recently, it has become clear that RpoA also plays an essential role in transcription in other bacterial systems such as *Bacillus subtilis* (35, 38), *Bordetella pertussis* (6, 46), *Rhodospirillum rubrum* (13), *Pseudomonas putida* (33), and *Vibrio fischeri* (47).

Similar to results obtained for other transcriptional regulators in other bacterial systems, the results of the domain swap indicate the importance of the C-terminal domain of *A. tumefaciens* RpoA in VirG-mediated transcription from *virB* promoters (35, 46). The ability of pENACN to allow *virB*:lacZ expression indicates that the residues essential for transcription are located in the C-terminal 89 amino acids of *A. tumefaciens* RpoA. Interestingly, deletion of eight residues from the C terminus of *A. tumefaciens* RpoA abolished *virB*:lacZ transcription, indicating an essential role for these residues. However, when these eight residues were added to the C terminus of *E. coli* RpoA, we did not obtain any transcription, indicating that while this region is essential for transcription, other determinants in the C-terminal region are also required. As expected, deletion of the C-terminal 94 amino acids from *A. tumefaciens* RpoA (pADC) or replacement with the C-terminal domain of *E. coli* RpoA (pANEC) failed to result in any expression of the *virB*:lacZ fusion. The reduced *virB*:lacZ expression obtained with the wild-type His-tagged *A. tumefaciens* RpoA indicates that the N-terminal histidine residues are interfering with RpoA function. Interestingly, pENACN gave intermediate levels of *virB*:lacZ expression even though it contains the N-terminal domain of *E. coli* RpoA from pECH, which contains an N-terminal His tag plus the C-terminal domain of *A. tumefaciens*. This suggests that an N-terminal His tag negatively affects *A. tumefaciens* RpoA function to a greater degree than for *E. coli* RpoA, at least at the *virB* promoter.

Another purpose of this study was to determine if we could reconstitute wild-type, inducer-dependent *A. tumefaciens* virulence gene expression in the heterologous host *E. coli*. In a previous study (31), we were unable to detect acetylcytosine-mediated expression of *virB*:lacZ in *E. coli* MC4100 using the constructs pHO98 (*lacp*:rpoA) and pSL107 (*lacp*:virA/G, *virBp*:lacZ). The pSL107 plasmid used in that study was not stable and resulted in high-frequency spontaneous mutations, probably due to the presence of two replicons (ColE1 and IncP origin) (data not shown). In response to this finding, we constructed pSL204, which also contains *lacp*-driven *virA* and *virG*, *virBp*:lacZ, and in contrast to pSL107, only the IncP origin. This allowed us to utilize the high-copy-number plasmid pPS1.3 in our attempts to reconstitute inducer-dependent *virBp*:lacZ expression in *E. coli*. Introduction of either pHO98 or pPS1.3 into DH5α containing pSL204 resulted in significant expression of the *virBp*:lacZ fusion in the presence of acetosyringone. The higher level of expression observed with pPS1.3 most likely is the result of an increase in the copy number of *rpoA* compared to that in pHO98.

The expression of virulence genes in *A. tumefaciens* is known to be affected by changes in temperature (23) and pH (32), with 28°C and pH 5.5 being optimal. The thermosensitive nature of virulence expression in *A. tumefaciens* is due to a reversible inactivation of VirA protein (23). Similar to what is observed in *A. tumefaciens*, we have also seen *virBp*:lacZ expression at 28°C, while no expression was observed at 37°C. In addition, we demonstrated that *virBp*:lacZ expression in DH5α is also affected by pH, with a 36% decrease in expression at pH 7.0 compared with that seen at pH 6.0. We did not use pH 5.5, since the growth of DH5α was adversely affected by this pH. Taken together, these results reinforce our conclusion that we have obtained inducer-dependent *virBp*:lacZ expression in *E. coli* and that the VirA-VirG signaling mechanism appears to be functioning.

Our observation that the choice of sugar greatly influences *virBp*:lacZ expression in DH5α is indicative of catabolite repression. To confirm this, we utilized the isogenic *E. coli* strains M182 and M182Δcpr (3, 4, 8), which differ only in the presence of the cAMP receptor gene *cpr*. Our observation that *virBp*:lacZ expression in M182Δcpr was dramatically reduced in induction medium amended with glycerol confirms the requirement of CRP. Since the addition of cAMP alone in the induction medium did not induce *virBp*:lacZ expression, the CRP may not directly activate the *virB* promoter. The fact that both cAMP and acetosyringone are needed for *virBp*:lacZ expression suggests that the CRP is required for the expression of *virA, virG*, and *rpoA* that are under the control of the *lac* promoter, a well-characterized promoter that requires activation by CRP. An alternative promoter that is not influenced by CRP or any other factors might be needed to further clarify this.

One unresolved question in virulence gene expression is the exact mechanism of sensing of phenolic inducers by the VirA-VirG system. The two possibilities are either a direct binding of the inducer by VirA or an intermediate receptor protein that binds the inducer and then interacts with VirA. Although the
genetic evidence supporting direct binding of inducers by VirA has been reported (29, 30), all attempts to demonstrate direct binding by VirA have been unsuccessful. We were able to demonstrate significant expression of virBp: lacZ in response to acetosyringone, syringaldehyde and, to a lesser extent, acetovanillone but not to hydroxysacetophenone. This result provides further evidence that inducers may be recognized directly by VirA. However, we cannot rule out the possibility that E. coli may contain homologues of A. tumefaciens receptors for the phenolic inducers. In the case of sugar effect, given the fact that E. coli encodes a ChvE homologue, the profound effect of ChvE and sugar on virBp: lacZ expression was somewhat unexpected. Apparently, VirA can be sensitized only by sugar-bound ChvE but not by the glucose-binding protein of E. coli which shares significant amino acid homology with ChvE (S).

Taken together, these results provide conclusive evidence that we have indeed reconstituted inducer-dependent vir gene expression in E. coli. However, the level of expression in E. coli is still significantly less than what is usually observed in A. tumefaciens (29, 30). One possible explanation for the relatively low induction in E. coli may be the E. coli sigma factors, which are inefficient in recognizing the vir gene promoters. It is conceivable that E. coli sigma factors may have a lower affinity for the virB promoter than sigma factors from A. tumefaciens. Although the vegetative sigma factor from A. tumefaciens has been identified (42), it is unclear whether this or an alternative sigma factor is involved in vir gene transcription. It is also likely that the relatively low expression is a consequence, at least in part, of RNA polymerase-containing endogenous E. coli RpoA subunits. One approach to resolve this would be to engineer E. coli RpoA such that it is able to activate virBp: lacZ expression. The results of our domain swap experiments have demonstrated that it is possible to obtain a hybrid RpoA molecule that will function at vir promoters, although the effect of such hybrids on the transcription of E. coli genes is unclear. In a recent study, Carbonetti et al. (6) reported that, in B. pertussis, overexpression of RpoABp reduced the transcription of Bvg-activated virulence genes. It was suggested that excess RpoABp could be active with Bvg and prevent interaction with virulence promoters. Since we previously reported that RpoA from A. tumefaciens, but not from E. coli, is able to interact with VirG (31), it may be that the constructs we are using result in cellular levels of A. tumefaciens RpoA that bind VirG and prevent optimal transcription. Although the current level of expression in E. coli is relatively low, it may still be sufficient to begin studies on A. tumefaciens processes in E. coli such as T-DNA transfer. Studies are under way to address these possibilities.

ACKNOWLEDGMENTS

We thank Nigel Savery of the University of Bristol for the generous gift of E. coli strains M182 and M182ap.

This work is supported by NSF grant MCB-972227.

REFERENCES


