Requirement for *Vibrio cholerae* Integration Host Factor in Conjugative DNA Transfer

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The requirement for host factors in the transmission of integrative and conjugative elements (ICEs) has not been extensively explored. Here we tested whether integration host factor (IHF) or Fis, two host-encoded nucleoid proteins, are required for transfer of SXT, a *Vibrio cholerae*-derived ICE that can be transmitted to many gram-negative species. Fis did not influence the transfer of SXT to or from *V. cholerae*. In contrast, IHF proved to be required for *V. cholerae* to act as an SXT donor. In the absence of IHF, *V. cholerae* displayed a modest defect for serving as an SXT recipient. Surprisingly, SXT integration into or excision from the *V. cholerae* chromosome, which requires an SXT-encoded integrase related to λ integrase, did not require IHF. Therefore, the defect in SXT transmission in the *V. cholerae* IHF mutant is probably not related to IHF's ability to promote DNA recombination. The *V. cholerae* IHF mutant was also highly impaired as a donor of RP4, a broad-host-range conjugative plasmid. Thus, the *V. cholerae* IHF mutant appears to have a general defect in conjugation. *Escherichia coli* IHF mutants were not impaired as donors or recipients of SXT or RP4, indicating that IHF is a *V. cholerae*-specific conjugation factor.

SXT was originally isolated in 1993 from a clinical isolate of *Vibrio cholerae*, the causative agent of the diarrheal disease cholera (31). SXT belongs to the diverse class of prokaryotic mobile genetic elements known as ICEs (integrative and conjugative elements). ICEs integrate into their hosts' chromosomes and are transmissible by conjugation. The ~100-kb SXT genome contains sequences encoding the element's conjugal transfer and chromosome integration machineries, genes that confer resistance to multiple antibiotics, as well as many genes of unknown function (5). Recently, it has become clear that SXT is part of a family of highly related ICEs that all have very similar integration and conjugation genes but differ in the genes that are not important for their transmissibility, such as antibiotic resistance genes (6).

SXT integrates in a site-specific fashion in the chromosomal gene *prfC*, which encodes a translation regulation protein, peptide chain release factor 3 (RF3). SXT integration disrupts the 5' end of *prfC* but provides a novel promoter and 5' coding sequence to enable production of a functional RF3 (19). SXT is excised from the chromosome to form a circular but non-replicative extrachromosomal intermediate that is thought to be the substrate for conjugative transfer. The mechanism of SXT integration into and excision from the chromosome appears to be similar to that of the lambdoid phages (7, 19). SXT integration and excision require an SXT-encoded tyrosine recombinase, Int, which is related to the λ family of recombinases. Excision also requires the SXT-encoded recombination directionality factor, Xis. Although the SXT Xis is unrelated to

the λ Xis, it appears to inhibit integration and promote excision as does λ Xis (7).

In the laboratory, SXT is transmissible by conjugation to a variety of gram-negative bacteria. The SXT conjugal transfer genes are distantly related to those found in the F plasmid (5), and thus it is likely that the basic mechanisms underlying transmission of these two elements are similar. Studies of F plasmid transmission have led to several key insights into the mechanism of conjugative DNA transfer (reviewed in reference 23). Conjugation requires cell-to-cell contact initially made by pili, which are cell-surface appendages encoded by the conjugative plasmid. Through a poorly understood process, these contacts become stabilized and a mating pair is formed that is hypothesized to contain a pore or channel for DNA transfer between the two partnering cells. Following mating pair formation, one strand of the F plasmid is cleaved and single-stranded F plasmid DNA is transported to the recipient cell. The second strand of DNA is then synthesized in both the donor and recipient cells, resulting in stable maintenance of the plasmid in each cell.

Although transmissible elements such as phage λ and the F plasmid encode many of the factors necessary for their own maintenance and dissemination, they also depend upon host-encoded proteins for regulation of some of their key functions. Some of these host proteins are referred to as "nucleoid" proteins, a group of abundant DNA binding proteins that modulate the structure of the chromosome (21). These proteins also serve as both positive and negative regulators of transcription, replication, and recombination (24). Two of these nucleoid proteins, the integration host factor (IHF) and the factor for inversion stimulation (Fis), influence both F plasmid and λ phage biology (reviewed in references 13 and 14). IHF is a heterodimeric protein whose subunits are encoded by the *himA* and *himD* genes, while Fis is a homodimeric protein encoded by the *fis* gene. IHF helps regulate expression of F

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TABLE 1. *E. coli* and *V. cholerae* strains and plasmids used in this study

Strain or plasmid	Genotype or phenotype	Reference or source
<i>E. coli</i> strains		
CAG18539	MG1655 <i>lacZU118 lacI42::Tn10</i>	29
BI533	MG1655 <i>Nal^r</i>	18
SM10 λ pir	F ⁻ <i>recA::RP4-2Tc::Mu Kn^r λpir</i>	Laboratory collection
β 2155	RP4-2Tc::Mu Δ <i>dapA::(erm-pir) thrB1004 pro thi strA hsdS lacZ</i> Δ M15 (F' <i>lacZ</i> Δ M15 <i>lacI^a</i> <i>traD36 proA⁺ proB⁺</i>)	11
JO641	β 2155 SXT	This study
J63	RP4 Ap ^r Kn ^r Tc ^r	Laboratory collection
SM604	MG1655 <i>himA</i> Δ el83-Tn10	R. Johnson
MC1061	F ⁻ <i>araD139</i> Δ (<i>ara-leu</i>)7696 <i>galE15 galK16</i> Δ (<i>lac</i>)X74 <i>rpsL</i> (Sm ^r) <i>hsdR2</i> (r _K ⁻ m _K ⁺) <i>mcrA mcrB1</i>	32
BW25113	<i>lacI^a rnaB₁₁₄ ΔlacZ_{WJ16} hsdR514</i> Δ <i>araBAD</i> _{AH33} Δ <i>rhaBAAD</i> _{LD78}	9
JO212	BW25113 SXT Δ <i>floR</i>	5
<i>V. cholerae</i> strains		
N16961	O1 (El Tor) biotype clinical isolate	Laboratory collection
SM463	N16961 <i>lacZ</i> Δ <i>himA</i>	This study
SM465	N16961 <i>lacZ</i> Δ <i>fis</i>	This study
SM480	N16961 SXT	This study
SM482	SM463 SXT	This study
SM484	SM465 SXT	This study
SM697	N16961 pBAD18-Kn	This study
SM698	SM463 pBAD18-Kn	This study
SM699	SM463 pSM660	This study
SM737	SM697 SXT	This study
SM738	SM698 SXT	This study
SM739	SM699 SXT	This study
SM782	SM463 pSM774 RP4	This study
SM783	N16961 pBAD18-Cm RP4	This study
SM784	SM463 pBAD18-Cm RP4	This study
SM795	SM463 pSM793	This study
SM796	SM795 SXT	This study
Plasmids		
pSM660	pBAD18-Kn <i>V. cholerae</i> <i>himA</i>	This study
pSM774	pBAD18-Cm <i>V. cholerae</i> <i>himA</i>	This study
pSW25T	<i>oriR6K mobRP4 Cm^r</i>	11
pVI64A	pSW25T P _{BAD} <i>int^{SXT} attP/prfC Kn^r</i>	V. Burrus
pXis	pBAD-TOPO <i>xis</i>	7
pCVD442	<i>oriR6K mobRP4 sacB Ap^r</i>	12
pSM419	pCVD442 <i>fis</i> -based deletion fragment	This study
pSM420	pCVD442 <i>himA</i> -based deletion fragment	This study
pSM793	pBAD18-Kn <i>E. coli</i> <i>himA himD</i>	This study

conjugation genes and stimulates the TraI-mediated cleavage of its origin of transfer (*oriT*) (15, 28). Additionally, IHF is required for both integration and excision of λ , and the Fis protein stimulates λ excision (3, 25, 26, 30).

Little is known about the role of host-encoded proteins in the biology of ICEs. Here we explored the role of IHF and Fis in SXT transmission. We found that a *V. cholerae* IHF mutant had a markedly reduced capacity to donate SXT, even though SXT integration and excision were not influenced by the lack of IHF. Unexpectedly, this effect was host specific; *Escherichia coli* IHF mutants were fully competent for SXT transfer. The *V. cholerae* IHF mutant appears to have a generalized defect in conjugative transfer, as transfer of RP4, an unrelated conjugative element, was also reduced in *V. cholerae* Δ *himA*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The bacterial strains and plasmids used in this study are described in Table 1. These strains were grown in Luria-Bertani (LB) broth at 37°C on a roller drum incubator. Growth of *V. cholerae* strains was measured in a BIO-TEK plate reader. Antibiotics were used at the

following concentrations: streptomycin, 200 μ g/ml; sulfamethoxazole, 160 μ g/ml; trimethoprim, 32 μ g/ml; kanamycin 50 μ g/ml; nalidixic acid, 40 μ g/ml; ampicillin, 100 μ g/ml for *E. coli* and 50 to 80 μ g/ml for *V. cholerae*; chloramphenicol, 20 μ g/ml for *E. coli* and 5 μ g/ml for *V. cholerae*; and tetracycline, 12 μ g/ml for *E. coli* and 1 μ g/ml for *V. cholerae*. Where indicated, bacterial cultures were supplemented with 0.002% DL- α , ϵ -diaminopimelic acid (DAP), 0.2% glucose, or 0.02% arabinose.

Plasmid and strain construction. The *V. cholerae* strains created here were derived from N16961, the sequenced El Tor biotype clinical isolate (17). SM463 (N16961 Δ *himA*) contains an internal, in-frame deletion of 222 bp from positions 39 to 260 with respect to the start of the *himA* coding region. SM465 (N16961 Δ *fis*) contains an internal deletion of 207 bp from positions 44 to 250 with respect to the start of the *fis* coding region. Deletions in the *himA* and *fis* loci of N16961 were created by allele exchange using derivatives of the counterselectable, *pir*-dependent vector pCVD442 (12). We performed splicing overlap-extension (SOE) PCR, as previously described (20), to create allele exchange vectors pSM419 and pSM420 for the *fis* and *himA* loci, respectively. N16961 chromosomal DNA was used as a template for the SOE PCR. These SOE PCR products contained approximately 500 bp of DNA upstream and downstream of the region on the chromosome to be deleted. The PCR products were first cloned into pCRII-TOPO (Invitrogen) and then subcloned into the XbaI and SacI restriction sites of pCVD442. The *E. coli* strain SM10 λ pir was used to mobilize pSM419 and pSM420 into N16961. The Δ *fis* and Δ *himA* N16961 strains were isolated as

described previously (10). In both cases, the deletions were verified by PCR with primers flanking the deleted regions.

Expression plasmids pSM660 and pSM774 contain the *V. cholerae* *himA* in pBAD18-Kn and pBAD18-Cm, respectively (16). These plasmids were constructed by first using PCR to amplify from N16961 chromosomal DNA a fragment extending from 39 bp upstream of the start of *himA* translation to 14 bp downstream of the *himA* stop codon. The upstream primer contained a *SacI* restriction site, and the downstream primer contained an *XbaI* site. This PCR product was cloned into pCRII-TOPO and then subcloned into the *SacI* and *XbaI* sites of either pBAD18-Kn or pBAD18-Cm. The expression plasmid pSM793 contains *E. coli* *himA* and *himD* in pBAD18-Kn. To construct this plasmid, PCR was used to amplify DNA fragments containing *himA* and *himD* from MG1655. The *E. coli* *himA* fragment contained sequences extending from 26 bp upstream of the start of the coding region to 28 bp beyond the stop codon, while the *E. coli* *himD* fragment contained sequences extending from 101 bp upstream of the start of *himD* translation to 17 bp downstream of the *himD* stop codon. Each PCR product was cloned into pCRII-TOPO. The *himA* fragment was subcloned into the *SacI* and *XbaI* sites of pBAD18-Kn. The *himD* fragment was subsequently cloned into this resulting vector in the *XbaI* and *PstI* sites.

Bacterial conjugation. Conjugation assays were performed by mixing equal volumes of overnight cultures of donor and recipient strains. In conjugation assays with strains containing the pSM660, pSM774, or pSM793 expression plasmids, overnight cultures were grown with 0.02% arabinose. Cell mixtures were concentrated by centrifugation and then resuspended in a 0.1 volume of LB broth. This cell suspension was applied to a membrane filter on an LB plate supplemented with DAP or glucose, if necessary. All conjugation assays were performed at 37°C. Matings with SXT were carried out for 6 h; matings with RP4 into or from *V. cholerae* were conducted for 3 h and for 15 min with *E. coli* mating pairs. Cells were collected in 2 ml of LB, and serial dilutions were plated on the appropriate selective media to determine the numbers of donors, recipients, and exconjugants.

SM10 λ pir was used to mobilize pVI64A to N16961 and SM463 (N16961 Δ *himA*). These matings were performed in the presence of glucose to prevent integration of the plasmid into the donor strain. Prior to selecting for exconjugants, the cells were exposed to 0.2% arabinose for one hour to induce expression of the SXT Int. To measure excision of pVI64A from N16961 and SM463 (N16961 Δ *himA*), pXis was transformed into the *V. cholerae* pVI64A exconjugants in the presence of glucose to repress *xis* expression. These transformants were then grown to an optical density at 600 nm of $\sim$ 0.6, induced for expression of Int and Xis with arabinose for one hour, and scored for loss of the plasmid antibiotic resistance markers.

Real-time quantitative PCR assays for relative quantification of *attB*. Real-time quantitative PCR assays were performed to measure the percentage of cells in a culture that contained unoccupied SXT *attB* sites. In each case, the amount of *attB* was normalized to the amount of chromosomal DNA in each sample by amplifying the *recA* locus by real-time quantitative PCR. Primer design, reactions, and analysis were performed as previously described (7). Primers to amplify the *attB* region of *V. cholerae* are VattBF (5' CGAAGTATCAAAGCGC CGTAC 3') and VattBR (5' ATCGTGGTTTACCCGCG 3'), and primers to amplify the *recA* locus of *V. cholerae* are VrecAF (5' AAAGGCTCCATCAT GCGC 3') and VrecAR (5' CCGGTCGAAATGGTTTCTACA 3').

RESULTS

IHF promotes SXT transfer to and from *V. cholerae*. To investigate the role of the host-encoded proteins IHF and Fis in SXT transmission, we constructed mutations in *himA* (the α subunit of IHF) and *fis* in *V. cholerae* strain N16961, which is a sequenced El Tor biotype clinical isolate. Strain SM463 (referred to as *V. cholerae* Δ *himA*) contains a deletion of *himA* and strain SM465 (referred to as *V. cholerae* Δ *fis*) contains a deletion of *fis* (Table 1). We tested growth of *V. cholerae* Δ *himA* and *V. cholerae* Δ *fis* in rich media. *V. cholerae* Δ *fis* grew nearly identically to wild-type N16961 but had a slightly longer initial lag phase. *V. cholerae* Δ *himA* also had a longer lag phase and did not reach as high a saturation density as the wild-type strain (Fig. 1). Thus, the *himA* mutation appears to have a modest effect on *V. cholerae* growth. These results are similar to what has been reported for *fis* and *himA* in *E. coli* (8, 14, 22).

FIG. 1. Growth of *V. cholerae* Δ *himA* and Δ *fis* mutants. Growth of *V. cholerae* strains N16961 (wild type [wt]), SM463 (N16961 Δ *himA*), and SM465 (N16961 Δ *fis*) were measured in LB broth at 37°C. Growth of each strain was assayed in triplicate, and the average absorbances and standard deviations of each time point are presented.

We tested whether *V. cholerae* Δ *himA* and *V. cholerae* Δ *fis* could serve as SXT recipients. Due to the low transfer frequency of SXT from *V. cholerae* to *V. cholerae*, an SXT-bearing *E. coli* strain was used as the SXT donor in these experiments. The frequency of SXT transfer to *V. cholerae* Δ *himA* was $\sim$ 20-fold reduced compared to a wild-type N16961 recipient (Fig. 2). Furthermore, the reduction in the recipient capacity of the *himA* mutant could be complemented by expressing *himA* from a plasmid (pSM660), indicating that this defect is due to a lack of *himA*. The conjugation defect of the *V. cholerae* *himA* mutant could also be complemented by expressing the *E. coli* IHF genes (*himA* and *himD*) from a plasmid (pSM793). In contrast to IHF, Fis does not appear to influence N16961 acquisition of SXT; *V. cholerae* Δ *fis* and N16961 were equally capable as SXT recipients (Fig. 2).

We observed a more severe conjugation defect when the donor cells lacked IHF. IHF appears to be required for SXT transmission from N16961, as transfer of SXT from *V. cholerae* Δ *himA* was undetectable (Fig. 2). The limit of detection in this assay is approximately 7×10^{-9} ; therefore, SXT transfer from *V. cholerae* Δ *himA* is reduced at least 250-fold compared to wild-type *V. cholerae*. SXT transfer from *V. cholerae* Δ *himA* was restored to wild-type levels when *himA* was supplied in *trans* from an expression vector, indicating that the observed defect in SXT transfer is due to the mutation in the *himA* locus. The transfer defect of the *V. cholerae* *himA* mutant could also be partially complemented by expressing the *E. coli* IHF genes from a plasmid, strongly suggesting that the *V. cholerae* and *E. coli* IHF proteins can function in a similar fashion to enable SXT transfer from N16961. Transfer of R391 (an SXT-related ICE) from the *himA* mutant was also undetectable (data not shown). Thus, the requirement for IHF for ICE transmission from *V. cholerae* is not limited to SXT; this result is not surprising because R391 and SXT share 95% identity over the genes that mediate integration, excision, regulation, and conjugative transfer of these elements (4). In contrast to IHF, Fis is not required for SXT transfer from N16961; *V. cholerae* Δ *fis* donated SXT at frequencies similar to that of

FIG. 2. Transfer of SXT into and from Δ *himA* and Δ *fis* *V. cholerae* mutants. SXT transfer frequency is expressed as the number of exconjugants per the number of recipient cells when SXT is donated from *E. coli* to *V. cholerae*; when SXT is donated from *V. cholerae* to *E. coli*, SXT transfer frequency is expressed as the number of exconjugants per the number of donor cells. The *E. coli* SXT donor was the *dapA* strain JO641. The *V. cholerae* recipient strains were: N16961 (*Vc wt*), N16961 Δ *himA* (*VcΔhimA*), and N16961 Δ *fis* (*VcΔfis*). The plasmids were all derivatives of pBAD18-Kn with either no insert, the *V. cholerae himA* (pSM660), or the *E. coli himA* and *himD* (pSM793). The *V. cholerae* SXT donor strains were the same as the *V. cholerae* recipient strains except that they contained SXT. The *E. coli* recipient was strain CAG18439. The averages and standard deviations of at least three independent assays are shown. The asterisk indicates that the frequency of exconjugant formation was below the limit of detection ($\sim 7 \times 10^{-9}$).

wild-type *V. cholerae* (Fig. 2). Thus, Fis does not appear to influence SXT transmission to or from *V. cholerae*.

IHF is not required for integration or excision of SXT. Given the similarity between the λ and SXT integrases and the requirement for IHF in integration and excision of λ DNA from the *E. coli* chromosome, we initially suspected that the diminished capacity of the *V. cholerae himA* mutant to transfer or receive SXT reflected a role for IHF in SXT excision and integration. To determine whether the observed defect in the capacity of the *V. cholerae* IHF mutant to act as an SXT recipient was due to a decreased frequency in SXT integration into the chromosome, we utilized pVI64A, a plasmid containing the minimal SXT DNA elements required for integration into the chromosome (V. Burrus, unpublished). The pVI64A plasmid is based on the *pir*-dependent vector pSW23T (11) and carries both the SXT *attP* region and the SXT *int* under control of an inducible promoter. After conjugative transfer from a *pir*⁺ host to a *pir* host, Kan^r exconjugants can only be isolated if pVI64A integrates into the chromosome; in nearly all cases, integration occurs via recombination between the chromosomal attachment site (*attB*) and a homologous sequence on the plasmid (*attP*) and is catalyzed by the SXT integrase (reference 7 and V. Burrus, unpublished data). By utilizing this minimal SXT integration system, we are bypassing any effects IHF may have on the expression of SXT-encoded genes. Following mobilization of pVI64A from the *pir*⁺ *mob* RP4⁺ host SM10 λ *pir*, the frequencies of formation of N16961 (pVI64A) and *V. cholerae* Δ *himA* (pVI64A) exconjugants were nearly identical (Fig. 3). In addition, PCR assays for the SXT inte-

gration junction (*attL* and *attR*) sequences in wild-type and *V. cholerae* Δ *himA* pVI64A exconjugants (four each) revealed that pVI64A was integrated into the SXT *attB* site, the 5' end of *prfC*. Together these findings indicate that IHF is not required for SXT integration. These data also suggest that the IHF mutant recipient is fully competent to receive and process DNA transferred by the RP4 conjugation system present in the donor cells in this assay.

To determine whether pVI64A was capable of being excised from the chromosome in the Δ *himA* background, pXis (7), a plasmid containing the SXT *xis* under the control of an inducible promoter, was introduced into a wild-type and a *V. cholerae* Δ *himA* pVI64A exconjugant. Because pVI64A cannot replicate in *V. cholerae*, its excision from the chromosome ultimately results in plasmid (Kan^r) loss. Following expression of Xis, 97% $\pm$ 2% of the wild-type cells lost pVI64A, whereas 75% $\pm$ 16% of the Δ *himA* cells lost the plasmid. Thus, IHF is not required for pVI64A excision from the chromosome. The slightly lower excision frequency observed in the *V. cholerae* Δ *himA* strain may be attributable to differences in the levels of Xis between the wild-type and Δ *himA* backgrounds, as the pXis plasmid appeared to be less stable in the Δ *himA* background.

To confirm that SXT excision from the chromosome occurs in the absence of IHF, we used real-time quantitative PCR to measure SXT excision in wild-type and *V. cholerae* Δ *himA* strains. The amount of *attB* relative to a single-copy chromosomal locus (*recA*) was quantified in chromosomal DNA preparations of wild-type N16961 SXT⁺ and *V. cholerae* Δ *himA*

FIG. 3. IHF is not required for integration of pVI64A. Integration of pVI64A, a mobilizable replication-deficient plasmid containing the SXT *attP* site and the SXT *int* under the control of an inducible promoter, was tested in a conjugation assay. The frequency of exconjugant formation was calculated by dividing the number of exconjugants by the number of recipients. SM463 is the N16961 Δ himA strain. The averages and standard deviations of three independent conjugation assays are shown.

SXT⁺. In this assay, *attB* is only detected if SXT has excised from the chromosome (7). The amount of unoccupied *attB* was 0.47% $\pm$ 0.21% in the wild type and 0.31% $\pm$ 0.15% in *V. cholerae* Δ himA. This small reduction in the percent *attB* in the *himA* strain versus the wild type is not statistically significant (*P* = 0.1889, Student *t* test). These results indicate that IHF is not required for SXT excision. Furthermore, because excision relies on both the SXT-encoded *Int* and *Xis* proteins, these results suggest that the levels of *Int* and *Xis* are not dramatically affected in the IHF mutant. If this is the case, the reduced frequency of SXT exconjugants when *V. cholerae* Δ himA was the recipient is not likely attributable to reduced levels of *Int*, which is required for SXT integration.

IHF influences RP4 transfer to and from *V. cholerae*. Since the deficiency in SXT transfer from *V. cholerae* Δ himA does

not appear to be attributable to a critical role for IHF in SXT recombination with the chromosome, we explored if the *himA* deletion caused a generalized conjugation defect in N16961. To test this possibility, we measured transmission of the broad-host-range conjugative plasmid RP4 into and from *V. cholerae* Δ himA. Unlike SXT, RP4 does not integrate into the chromosome; however, RP4 transfers through a process that is presumed to be mechanistically similar to that of SXT. RP4 transfer from an *E. coli* donor to *V. cholerae* Δ himA was over 30-fold lower than that for wild-type N16961 (Fig. 4). Furthermore, transfer of RP4 from *V. cholerae* Δ himA was reduced over 200-fold compared to the wild type. The frequency of RP4 transfer from *V. cholerae* Δ himA was restored to wild-type levels when *himA* was supplied in *trans* from an expression plasmid. These results are similar to those found with transfer

FIG. 4. Transfer of RP4 into and from Δ himA *V. cholerae*. The frequency of exconjugant formation was calculated by dividing the number of exconjugants by the number of recipients when assaying RP4 transfer into *V. cholerae* and by dividing the number of exconjugants by the number of donors when measuring RP4 transfer from *V. cholerae*. The *E. coli* RP4 donor strain was J63. The *V. cholerae* recipient strains were N16961 (Vc wt) and N16961 Δ himA (Vc Δ himA). The plasmids were all derivatives of pBAD18-Cm with either no insert or the *V. cholerae* *himA* (pSM774). The *V. cholerae* RP4 donor strains were the same as the *V. cholerae* recipient strains except that they contained RP4. The *E. coli* recipient of RP4 was MC1061. The averages and standard deviations of three independent conjugation assays are shown.

FIG. 5. Influence of IHF on conjugal transfer of SXT and RP4 into and from the *E. coli* IHF mutant. The frequency of SXT or RP4 transfer is shown as the number of exconjugants divided by the number of recipients when the mutant was used as the recipient, and the frequency is presented as the number of exconjugants divided by the number of donors when the IHF mutant strain was used as the donor. The SXT donor strain is JO212 and the RP4 donor strain is J63. The recipient strains are as follows: BI533 (MG1655, wild type), SM768 (MG1655 *himA*), CAG18439 (*E. coli* Tet^R), or MC1061 (*E. coli* Sm^R). The averages and standard deviations of three independent conjugation assays are shown.

of SXT into and from *V. cholerae* Δ *himA* and suggest that the *himA* deletion confers a general defect in conjugation to N16961.

IHF is not required for conjugation in *E. coli*. Even though SXT was originally isolated from a *V. cholerae* O139 strain, in the laboratory this ICE is transmissible to many gram-negative species (31). We explored if *himA* is also required for SXT transmission to and from *E. coli*. The *V. cholerae* IHF α and IHF β subunits are 86% and 81% identical to the respective *E. coli* orthologs. To our surprise, no defect in either SXT or RP4 transmission was detected in *E. coli* strain MG1655 *himA* Δ 83-Tn10 (Fig. 5). The frequency of RP4 or SXT exconjugant formation was equivalent to that of the wild type when the *himA* mutant strain was either the recipient or donor of either conjugative element. In the case of *E. coli*, the IHF β subunit encoded by *himD* was capable of functioning without the IHF α subunit (*himA*); an *E. coli* *himA* *himD* double mutant was also assayed as a recipient and donor of SXT. Like the *himA* single mutant strain, the frequency of exconjugant formation was approximately the same as that of the wild type when this *himA* *himD* double mutant was either the recipient or donor of SXT (data not shown). These results are in marked contrast to what we observed in the *V. cholerae* Δ *himA* mutant.

DISCUSSION

We found that the host-encoded nucleoid protein IHF is required for efficient transfer of SXT and RP4 to and from *V. cholerae* but not to and from *E. coli*. In contrast, Fis did not influence transfer of SXT to or from *V. cholerae*. The reason(s) why IHF limits SXT and RP4 transfer in a species-specific manner is not clear. However, since the reductions in the frequencies of transfer of SXT, an ICE, and RP4, a noninte-

grative but conjugative element, to and from Δ *himA* *V. cholerae* were similar, it is clear that IHF's role in promoting DNA recombination does not explain our observations. Instead, our findings suggest that IHF facilitates the capacity of *V. cholerae* to act as a donor and perhaps as a recipient in conjugation.

Deletion of *himA* from *V. cholerae* did not appreciably alter the frequency of SXT integration into or excision from the *V. cholerae* chromosome. In contrast, IHF is required for integration and excision of λ and closely related bacteriophages into and from the *E. coli* chromosome. IHF is thought to perform a primarily architectural role in λ recombination, bending the DNA and permitting assembly of recombinogenic complexes (2). SXT and λ encode similar tyrosine recombinases that are thought to catalyze recombination between these elements and chromosomal DNA. The similarity of the likely active site of the SXT integrase to the active sites of the λ type integrases suggests that the fundamental chemistry of SXT recombination is similar to that described for recombination of λ DNA with its chromosomal target. We suspect that differences in the architecture of the nucleoprotein complexes formed during SXT and λ recombination explain the different requirements for accessory factors in the integration and excision of these mobile elements. In fact, sequence analysis (based on the *E. coli* IHF consensus DNA binding site) suggests that there is no IHF binding site in the region of SXT that recombines with the chromosome. It is possible that SXT integration and/or excision require accessory factors other than IHF or Fis. Excision from the *E. coli* chromosome of the conjugative transposon Tn916, which also encodes a tyrosine recombinase, has been shown to be stimulated by HU (8), a nucleoid protein that shares structural homology to IHF and some overlapping functions (27).

The *himA* deletion had a more potent effect on the capacity of *V. cholerae* to donate SXT or RP4 than to acquire these conjugative elements. Transfer of SXT or RP4 from the IHF mutant was reduced at least several hundred-fold compared to wild-type *V. cholerae*. In contrast, the capacity of the IHF mutant to serve as a recipient of these elements was only reduced 20- to 30-fold compared to the wild type. In the conjugation assays performed here, there is probably sufficient time for newly formed exconjugants to serve as donors of SXT or RP4. Thus, it is possible that the comparatively small reduction in the frequency of *V. cholerae* Δ *himA* to acquire SXT or RP4 may at least in part reflect this mutant's inability to donate newly acquired elements. This idea is supported by our experiments using pVI64A shown in Fig. 3. In this assay, transfer of pVI64A depends on the RP4 conjugation system expressed from the chromosome of the donor cell (and not from the mobile element). Therefore, newly formed exconjugants are unable to serve as donors. The frequencies of pVI64A exconjugants were approximately equal using either wild-type or Δ *himA* *V. cholerae* as recipients, suggesting that the *V. cholerae* IHF mutant may not be impaired in its capacity to serve as a recipient of conjugative elements.

We believe it is unlikely that IHF plays a direct role in transfer of SXT and RP4 from *V. cholerae*. One reason we suspect IHF does not directly affect SXT or RP4 transfer is that *E. coli* IHF mutants were not defective in acquisition or transfer of these elements. The *V. cholerae*-specific requirement for IHF for transmission of SXT and RP4 was unexpected, as the *E. coli* and *V. cholerae* IHF proteins appear to be highly similar. In fact, the *E. coli* IHF protein can complement the *V. cholerae* IHF mutant for transfer of SXT. Thus, it seems unlikely that the *E. coli* and *V. cholerae* IHF proteins could interact with these mobile element in different ways in their respective hosts.

The *V. cholerae*-specific requirement for IHF in the transfer of SXT and RP4 is difficult to explain. It is possible that *E. coli* codes for an additional factor (not present in *V. cholerae*) that can substitute for IHF in its absence. Another possible explanation is that the IHF regulon differs between *E. coli* and *V. cholerae* and that an important regulator of conjugation is IHF dependent only in *V. cholerae*. In *E. coli*, IHF is a global regulator of transcription; microarray analyses revealed that *E. coli* IHF directly or indirectly affects the transcription of over 100 genes of diverse functions (1). Microarray analyses suggest that this appears to be the case in *V. cholerae* as well (S. M. McLeod and M. K. Waldor, unpublished data). In *V. cholerae*, IHF may regulate a host factor that is required for transmission of both SXT and RP4. The identity of this factor awaits future investigation. Overall, there appears to be differences in the conjugation capacities of *V. cholerae* and *E. coli*. The transfer frequencies of both RP4 and SXT are significantly higher between *E. coli* cells than between *V. cholerae* cells or between *V. cholerae* and *E. coli*. Perhaps *E. coli* has both IHF-independent as well as IHF-dependent conjugation pathways, whereas *V. cholerae* has only IHF-dependent pathways.

Although self-transmissible mobile genetic elements contain genes that encode machinery necessary for their maintenance and transmission, it is evident that these elements also depend on host-encoded functions. Given the complex biology of conjugation and the degree of interaction that must occur between

element- and host-encoded factors, it is amazing that many mobile genetic elements have a broad host range. The data presented here suggest that relatively subtle changes in host cell physiology can significantly impact the host range of SXT and RP4.

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