

## ABSTRACT

The Gifsy phage family consists of three lambdoid bacteriophages that are similar in sequence and can lysogenize serovars of *Salmonella enterica* (*S. enterica*). All three Gifsy prophages are found in *S. enterica* serovar Typhimurium (*S. typhimurium*) strain ATCC14028s. Lambdoid phages, such as the Gifsy phages, enter the bacterial chromosome via phage-mediated site-specific recombination. This type of recombination requires two DNA target sites (*attP* on the phage DNA and *attB* on the bacterial DNA), the phage encoded catalytic Int protein, and accessory factors provided by the host. The Gifsy-1 Int belongs to the large tyrosine family of recombinases but is grouped into a small and less studied subfamily than Lambda Int or P22 Int. The other member of this subfamily is the Int of phage  $\phi 80$ , an *Escherichia coli* (*E. coli*) phage similar in organization and DNA sequence to Gifsy-1. The *attP* and *attB* regions required for Gifsy-1 integration, as well as the sequence of the gene that encodes Int (*int*), are known.

This work determined the host factors required for Gifsy-1 integration via an *in vivo* electroporation assay. Cells that contained intact HU, but a non-functional IHF subunit ( $\alpha$  or  $\beta$ ), could not form detectable integrants in this assay. This result indicates a need for IHF in order to observe integrative events. Bacterial strains containing IHF with either a HU- $\alpha$  or HU- $\beta$  subunit could form lysogens; cells that contained neither the  $\alpha$  nor the  $\beta$  subunit of HU could not. These results indicate HU is also required for Gifsy-1 integration, but homodimeric HU can substitute for the heterodimeric form. Gifsy-1 integration is atypical in its requirement for two host factors.

An additional unusual aspect of Gifsy-1 integration is the existence of only one IHF binding site on the Gifsy-1 *attP* DNA. This site was localized to the 5' side of the core recombination region (core) by gel mobility shift assays. A DNase I nuclease protection assay, or footprint, confirmed the presence of the site and identified the bases bound by IHF (5' AAAAA-8bp-TATGAA-4bp-CAT 3'). Sequence alignment of the *attP* IHF binding site with the IHF consensus site revealed four mismatches, three of which are confined to the extreme 3' end.

Deletion mutants of the Gifsy-1 *attP* at the 3' end revealed the presence of a sequence important for integration between bases +112 and +189. Due to the 5' location of the IHF binding region and comparison with other site-specific recombination systems, this was designated as a potential Int arm-type binding site. The location of this region 3' to the core and away from the IHF binding site means another bending protein would be needed to bend the DNA and allow the Int proteins to interact with the core and arm sites. This would explain why Gifsy-1 requires both IHF and HU for integrative recombination and how Gifsy-1 can function with one IHF site as opposed to the multiple sites found in other systems. In addition to the host factor work, the putative Gifsy-1 *xis* gene was cloned and shown to be functional by an electroporation assay.