Site-directed mutagenesis and phylogenetic comparisons of the *Escherichia coli* Tus protein: DNA-protein interactions alone can not account for Tus activity

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**Abstract** The Tus protein of *Escherichia coli* is capable of arresting DNA replication in an orientation-dependent manner when bound to specific sequences in the bacterial chromosome called *Ter* sites. Arrest of DNA replication has been postulated to occur either by a barrier mechanism, where Tus acts as a physical block to replication fork progression, or through protein-protein interactions between Tus and some component of the replication fork. A previous mutational analysis of Tus suggested that the amino acids in the L1 loop might play a role in replication arrest. Site-directed mutagenesis of amino acids in the L1 loop and other amino acid residues on the “non-permissive” face of Tus was performed to identify residues that affected Tus function. One mutant, E47Q, gave results that are inconsistent with the barrier model, showing a greater affinity for the *Ter* site (with a $t_{1/2}$ of 348 min versus 150 min for wild-type Tus) but a reduced ability to arrest DNA replication in vivo. In addition to the site-directed mutagenesis studies, the *tus* genes of *Salmonella*, *Klebsiella*, and *Yersinia* were sequenced and the proteins expressed in *E. coli* to assess their ability to arrest DNA replication. The results presented here support a role for protein-protein interactions in Tus function, and suggest that residues E47 and E49 participate in replication fork arrest.

**Keywords** *Escherichia coli* · DNA replication · Replication arrest · Tus · Site-directed mutagenesis

**Introduction**

The Tus protein of *Escherichia coli* is a DNA-binding protein that possesses the unique ability to arrest DNA replication when bound to its DNA recognition sequence, called a *Ter* site. The Tus-Ter complex is asymmetric and forms a one-way impediment to the progression of replication forks, stopping replication forks that approach from one side of the complex, but allowing replication forks approaching from the other side to pass through. In *E. coli*, ten *Ter* sites (TerA–TerJ) are distributed equally between the two halves of the chromosome, and are located opposite oriC in the terminal third of the chromosome. All of the *Ter* sites are oriented so as to permit passage of DNA replication forks moving in the origin-to-terminus direction, but prevent replication forks from traveling from the terminus to the origin. Thus, Tus-Ter complexes form a trap for replication forks and confine replication termination to the terminus region of the chromosome (for reviews, see Hill 1996; Wake and King 1997; Bussiere and Bastia 1999).

Over the last decade, the Tus-Ter complex has been well characterized. Tus binds as a monomer to the *Ter* site (Coskun Ari et al. 1994; Sista et al. 1991) and forms a very stable complex [$t_{1/2} = 150–300$ min, depending on buffer conditions; Gottlieb et al. 1992; Skokotas et al. 1995]. Tus binds *Ter* DNA in a novel two-domain structure with interstrand $\beta$-sheets connecting the domains (Kamada et al. 1996). A positively charged cleft is formed between the two domains and the *Ter* site lies within this cleft. The main regions of the protein involved in DNA recognition are residues in each of the interdomain $\beta$-strands. These $\beta$-strands lie partially within the major groove of the *Ter* DNA and contain 18 of the 42 amino acid residues that contact the DNA. The remaining 24 residues that participate in DNA binding...
are scattered along the length of the protein between residues 50 and 302.

One proposed mechanism by which Tus poses a directional block to replication fork progression is the barrier model, which postulates that the binding of Tus to Ter is sufficient to halt DNA replication. The possibility that DNA binding alone could account for the polar activity of Tus was first suggested by the observation that Tus could block the activity of various unrelated DNA helicases (Lee et al. 1989). Subsequent DNA binding and footprinting studies of the Tus-Ter complex demonstrated that Tus was very tightly bound to the Ter site, contacting both DNA strands of the Ter site at the non-permissive end of the complex, but associating with only one DNA strand on the permissive side (Gottlieb et al. 1992). It was proposed that tight binding of Tus to both strands at the non-permissive face could prevent DNA unwinding when replication forks approached from this direction, but permit invasion of the binding site from the permissive end since only one strand was contacted. The solution of the Tus-Ter co-crystal by Kamada et al. (1996) gave rise to a more sophisticated explanation for the barrier model. It was postulated that α-helices that protrude in front of the interdomain β-sheets on the non-permissive face of Tus protect the main DNA-binding domain from invasion by DNA unwinding proteins. More recently, Neylon et al. (2000) proposed a model suggesting that steric hindrance due to insertion of the non-permissive face of Tus into the channel formed by hexameric DnaB helicase could account for helicase blockade.

The notion that DNA binding alone can account for Tus activity has been challenged by several investigators who proposed that Tus arrests DNA replication through protein-protein interactions (Khatri et al. 1989; Hiasa and Marians 1992; Skokotas et al. 1994, 1995). The protein-protein interaction model is based primarily on in vitro experiments in which purified Tus showed a differential ability to block the DNA unwinding activity of helicases (Khatri et al. 1989; Hiasa and Marians 1992). However, in vivo evidence for the protein-protein interaction model is lacking and, to date, no direct evidence for an interaction with the main replicative helicase of E. coli, DnaB, has been published. The only findings from in vivo experiments that are consistent with the protein-protein interaction model come from a mutant, E49K, that binds Ter sites as well as wild-type Tus does, but has reduced replication arrest activity (Skokotas et al. 1995). The E49K mutation maps to a domain of Tus called the L1 loop, which consists of amino acid residues 42–52 and constitutes a highly charged domain that is located at the DNA-protein interface on the non-permissive face of the Tus-Ter complex. Because TusE49K binds to Ter sites with normal affinity, the reduction in arrest activity was postulated to result from perturbation of a protein-protein interaction between the mutant Tus and some component of the replication fork (Skokotas et al. 1995).

In an effort to identify other amino acid residues in Tus that reduce replication arrest activity without compromising DNA-binding activity and to provide additional support for the protein-protein interaction model, we introduced site-directed mutations at several positions on the non-permissive face of Tus, concentrating primarily on the L1 loop. The resulting mutants were tested for Ter binding activity, ability to arrest DNA replication in vivo, and ability to inhibit DnaB-catalyzed unwinding of duplex DNA substrates. The tus genes from other members of the Enterobacteriaceae family (Salmonella, Klebsiella, and Yersinia) were also sequenced and assayed in E. coli for in vivo replication arrest activity, in order to identify conserved residues and amino acids that can be substituted without affecting replication arrest activity. Of the mutant Tus proteins examined in this study, two with mutations in the L1 loop (E47Q and E49A) showed a combination of traits inconsistent with the barrier model: an increased affinity for Ter but reduced replication arrest activity in vivo. These results are most consistent with the protein-protein interaction model and suggest that E47 and E49 play an important role in the arrest of DNA replication by Tus.

### Materials and methods

#### Bacterial strains

All E. coli strains used in these studies are derivatives of MG1655. TH838 was constructed by making a deletion of tus (Δtus805) in which the tus gene was replaced with a res-npt-res cassette (Kristensen et al. 1995). The Δtus::res-npt-res deletion was crossed into the chromosome of MG1655, and the strain was cured of the npt marker (M. Valjavec-Gratian and T. M. Hill, unpublished data). TH831 contains the InvTer::spc cassette (Sharma and Hill 1995), Δtus805, and Δarc1076 (M. Valjavec-Gratian and T. M. Hill, unpublished data). TH457 is Δtus2474; TH835 is Δtus2474 Δara714 leu::Tn10.

The species and strains used for the tus genes from Enterobacteriaceae were Escherichia coli (MG1655), Salmonella typhimurium (LT2), Salmonella enteritidis (ATCC 13076), Klebsiella pneumoniae (ATCC 11296), Proteus mirabilis (UND strain), Serratia marcescens (UND strain), Pseudomonas aeruginosa (ATCC 10145), Yersinia enterocolitica (UND strain), Y. pestis (EV7657) and Neisseria sicca (ATCC 9913).

#### Plasmids

Plasmid pBAD33tus was constructed by inserting the 1.3-kb fragment encoding the tus gene from pBAD18tus (formerly pBADtus; Sharma and Hill 1995) into pBAD33 (Guzman et al. 1995). Plasmid pBAD33tusA173T was constructed by PCR amplification of the A173T mutant tus gene from TH349 (Skokotas et al. 1994), cloning the 1.1-kb PCR fragment into the CiaI/EcoRV sites of Bluescript SK+ , and then transferring the mutant tus gene into pBAD18 using SalI/EcoRI ends. The tusA173T gene was then cloned into pBAD33 via a NruI+ HindIII digestion. pBAD vectors containing the tus gene from the Enterobacteriaceae species were constructed by gel-purifying the tus PCR products, digesting with EcoRI and HindIII, and inserting the fragments into pBAD18 or pBAD33 that had been digested with the same enzymes. Plasmids pBAD18STtus, pBAD18SETtus, pBAD18KOtus and pBAD18YPtus contain the tus gene from S. typhimurium, S. enteritidis, K. pneumoniae, and Y. pestis, respectively.