Host proteins can stimulate Tn7 transposition: a novel role for the ribosomal protein L29 and the acyl carrier protein

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The bacterial transposon Tn7 is distinguished by its ability to insert at a high frequency into a specific site in the Escherichia coli chromosome called attTn7. Tn7 insertion into attTn7 requires four Tn7-encoded transposition proteins: TnsA, TnsB, TnsC and TnsD. The selection of attTn7 is determined by TnsD, a sequence-specific DNA-binding protein. TnsD binds attTn7 and interacts with TnsABC, the core transposition machinery, which facilitates the insertion of Tn7 into attTn7. In this work, we report the identification of two host proteins, the ribosomal protein L29 and the acyl carrier protein (ACP), which together stimulate the binding of TnsD to attTn7. The combination of L29 and ACP also stimulates Tn7 transposition in vitro. Interestingly, mutations in L29 drastically decrease Tn7 transposition in vivo, and this effect of L29 on Tn7 transposition is specific for TnsABC+D reactions.

Keywords: acyl carrier protein (ACP)/ribosomal protein L29/target site-selection/transposon Tn7

Introduction

Transposable elements are discrete DNA segments that can translocate between sites within a genome. Although transposable elements can usually insert into many different sites, most transposons do exhibit some degree of target-site selectivity (reviewed by Craig, 1997). The bacterial transposon Tn7 is unusual in that it can utilize two distinct classes of target sites using two different transposition reactions. Using one class of target sites, Tn7 inserts at a high frequency into a specific site called attTn7 found in the chromosomes of many bacteria; alternatively, Tn7 also inserts at a low frequency into another class of non-attTn7 sites (Barth et al., 1976; Lichtenstein and Brenner, 1981; Craig, 1991; Wolkow et al., 1996).

Multiple Tn7-encoded proteins, TnsA, TnsB, TnsC and TnsD (TnsABC+D), mediate Tn7 transposition into attTn7 (Rogers et al., 1986; Waddell and Craig, 1988; Kubo and Craig, 1990; Bainton et al., 1993). TnsD, a sequence-specific DNA-binding protein, is responsible for selecting the attTn7 target site (Waddell and Craig, 1988; Kubo and Craig, 1990; Bainton et al., 1993) and subsequently for recruiting the core transposition machinery to the target DNA. This machinery is a oligomeric complex containing both TnsA and TnsB, (May and Craig, 1996; Samovskiy et al., 1996) and TnsC, the transposition regulator (Stellwagen and Craig, 1997). Thus, the role of TnsD in Tn7 transposition is to position the core transposition machinery at the Tn7 insertion site.

The binding of TnsD to attTn7 exerts considerable control over Tn7 transposition into attTn7. Previous studies have demonstrated that the initiation of the chemical steps, i.e. cleavage and joining steps, of the transposition reaction requires the recognition of attTn7 by TnsD. We have previously proposed that cleavage at the Tn7 ends occurs only after the formation of a nucleoprotein complex containing TnsABC+D, attTn7 target DNA and Tn7 ends (Bainton et al., 1991, 1993). Although the selection of an appropriate target DNA takes place early in Tn7 transposition reaction, not all transposons do so at an early step. In contrast to Tn7, Tn10 selects its target sites at a late step in the transposition reaction; the Tn10 transposase does not interact with or capture the target DNA until the complete excision of the Tn10 element (Sakai et al., 1995; Kleckner et al., 1996; Sakai and Kleckner, 1997). Regardless of the stage at which the target DNA enters the transposition reaction, the interaction of the transposase with appropriate DNA targets is a vital step in transposition.

Host-encoded proteins can also influence target-site selection. For example, the recruitment of the yeast retrovirus-like Ty3 element to its preferred insertion sites located within the promoters of genes transcribed by RNA polymerase (pol III), occurs through the direct interaction of the Ty3 integrase and the pol III transcription factors TFIIB and TFIIIC (Kirchner et al., 1995). Do host proteins also influence the target-site selectivity of Tn7? There has been no obvious requirement for host proteins in Tn7 transposition. Indeed, efficient reconstitution of Tn7 transposition in vitro has been accomplished using highly purified Tn7-encoded transposition proteins (Bainton et al., 1993). However, it has been observed in previous work that the addition of crude extract to the transposition reaction can stimulate Tn7 transposition in vitro (Bainton et al., 1991) and more specifically that host proteins can stimulate TnsD binding to attTn7, suggesting that host proteins may be involved in Tn7 transposition (Bainton et al., 1993).
We have also found that L29 has a substantial impact on TnsD binding to attTn7, suggesting that the interactions between L29, ACP and TnsD–attTn7 complexes may contribute to high-frequency insertion of Tn7 in vivo. To identify the host proteins that influence TnsD–attTn7 complex formation, we fractionated crude extracts (see Materials and methods) and evaluated fractions for their ability to promote TnsD–attTn7 complex formation at each purification step using the appearance of altered TnsD–attTn7 complexes as an assay. Fractionation of cell extracts over a phosphocellulose (PC) column separated the host activity into two components. One component bound to the PC column, PCbound, and contained a host protein(s) that could alter the migration of TnsD–attTn7 complexes. The TnsD–attTn7 complexes formed in the presence of the PCbound factor had migration patterns that were equivalent to TnsD–attTn7 complexes formed in the presence of unfractonated crude extract. In contrast, the other host component, PCflow through, enhanced TnsD binding to attTn7 in the presence of the PCbound factor, but could not influence TnsD–attTn7 complex formation alone.

The PCbound fraction was further fractionated by cation exchange (MonoS) and gel filtration (Superose 12), which resulted in Component 1. We found that Component 1 alone could alter the binding of TnsD to attTn7, resulting in the formation of a new slower-migrating TnsD–attTn7 complex (Figure 2B, lane 3). We also found that the formation of Component 1 + TnsD–attTn7 complexes could be greatly stimulated when the PCflow through component, which was identified as ACP (see below), was present in the attTn7 binding reactions (Figure 2C, lanes 5–11). Analysis of the Superose 12 fractions by SDS–PAGE and Coomassie Blue staining revealed that a small polypeptide of ∼7.0 kDa (Figure 2A) coincided with fractions displaying Component 1 host activities. The identity of the 7.0 kDa protein was determined using amino-terminal sequencing; the first 12 amino acids of this protein were identified as MKAKELREKSV. A protein homology search found this sequence to be identical to the ribosomal protein L29.

The PCflow through factor(s) were further fractionated by anion exchange (MonoQ) and two components, 2A and 2B, were identified that could influence the formation of L29 + TnsD–attTn7 complexes. Components 2A and 2B were fractionated once more by gel filtration (Superose 12) and active host fractions were evaluated by SDS–PAGE and Coomassie Blue staining. Component 2A was identified as an ∼15.0 kDa protein which was found to coincide with fractions that could greatly enhance TnsD binding to attTn7 in the presence of L29 (Figure 3A, lanes 3–8, and B, lanes 13–18). In contrast, Component 2B was identified as an ∼30.0 kDa protein which was found to coincide with fractions that caused the formation of several distinct slower-mobility L29 + TnsD–attTn7 complexes (Figures 4A, lanes 2–7, and B, lanes 13–18). Note that neither Components 2A nor 2B alone can influence the binding of TnsD to attTn7 (Figures 3B, lanes 5–11, and 4B, lanes 5–11).

To determine the identity of the proteins present in both Components 2A and 2B, N-terminal sequencing was used. Surprisingly, we obtained identical amino acid sequences (MTIEERVKKIIG) for both Components 2A and 2B, despite their different fractionation profiles and different observed molecular weights. A protein homology search found this protein sequence to be a perfect match to the Escherichia coli ACP. Components 2A and 2B were identified as ACP monomers [(ACP)1] and covalent dimers of ACP [(ACP)2], respectively (see below).

In summary, the fractionation of the host-cell extracts...
revealed that L29 causes the formation of slower-migrating TnsD–attTn7 complexes, and that (ACP)₁ and (ACP)₂ collaborate with L29 to stimulate binding of TnsD to attTn7 more than 20-fold compared with the binding of TnsD to attTn7 in the absence of host factors.

Our purification procedure uses two different E.coli strains based on findings in our initial purification attempts. Note that we purified ACP from an L29⁻ strain which contained a deletion of 48 nucleotides within rpmC, the gene encoding L29, to prevent the contamination of ACP with wild-type L29. Since ACP is an essential protein, no ACP null mutants are available: therefore, L29 cannot be purified in the absence of wild-type ACP. Hence, we are unable to exclude the possibility that trace amounts of ACP exist in the L29 preparations.

**Two forms of ACP can influence TnsD binding to attTn7**

As described above, (ACP)₁ (Component 2A) and (ACP)₂ (Component 2B) can both alter the formation of L29 + TnsD–attTn7 complexes. (ACP)₁ + L29 enhances the binding of TnsD to attTn7 >20-fold, causing the formation of a slower-migrating TnsD–attTn7 complex (Figure 5B, lane 6). In contrast, (ACP)₂ + L29 causes the formation of several other distinct slower-migrating TnsD–attTn7 complexes; the formation of the higher-order complexes suggests the multiple ACP molecules may be capable of interacting with TnsD when bound to attTn7 (Figure 5B, lane 7). (ACP)₂ + L29 + TnsD–attTn7 complexes can sometimes be detected in the presence of unfractionated extract (data not shown).

(ACP)₂ consists of two (ACP)₁ molecules linked covalently through disulfide bonds (Rock and Cronan, 1981; reviewed by Magnuson et al., 1993). (ACP)₂ can be converted into (ACP)₁ in the presence of the reducing agent DTT. To demonstrate whether (ACP)₂ was indeed the form of ACP present in Component 2B, we evaluated the effects of (ACP)₂ on L29 + TnsD–attTn7 complexes in the presence and absence of DTT, which promotes the conversion of (ACP)₂ into (ACP)₁. We observed that when DTT was present in the binding reaction, (ACP)₂ resulted in the formation of (ACP)₁ + L29 + TnsD–attTn7 complexes that had migration patterns that were identical to TnsD–attTn7 complexes formed in the presence of (ACP)₁ + L29 (Figure 5B, lanes 6–8). We also found that the migration pattern of the (ACP)₂ polypeptide when treated with DTT is the same as that of (ACP)₁, as evaluated by SDS–PAGE (Figure 5A, lane 2). Thus, two
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Fig. 3. (A) Analysis of (ACP)$_1$ fractions by SDS–PAGE and Coomassie Blue R250 staining. Lanes 1–8 contained ~30 µl of Superose 12 fractions #58–64. (B) Analysis of the host activity of the (ACP)$_1$ fractions. Dilutions of 1:10 of Superose 12 fractions #58–64 (lanes 5–18) were analyzed in TnsD binding reactions using the gel mobility-shift assay. Lanes 2–18 contained 6.7 ng of TnsD. L29 (20 ng) is present in lanes 3, 4 and 12–18. Lane 4 contained ~1.5 µg of the MonoQ load (phosphocellulose flow-through). The reaction conditions were the same as those in Figure 1 and are described in Materials and methods. The results shown are of a scanned gel.

distinct forms of ACP [(ACP)$_1$ and (ACP)$_2$] can influence the formation of L29 + TnsD-attTn7 complexes; the ability of (ACP)$_1$ and (ACP)$_2$ to form L29 + TnsD-attTn7 complexes that have different mobilities on non-denaturing polyacrylamide gels suggests that ACP is a constituent of the slower-migrating TnsD-attTn7 complexes.

L29 and ACP are present in the TnsD-attTn7-host complexes

We have presented evidence suggesting that both L29 and ACP are involved in stimulating TnsD binding to attTn7. Are L29 and ACP present in the TnsD-attTn7-host complexes? The fact that purified L29 causes a shift in mobility strongly suggests that it is a component in this complex; ACP, however, causes no additional shift. Therefore, to further examine this question, tritium ($^3$H)-labeled (ACP)$_1$ was used in the TnsD-binding reactions with unlabeled attTn7 DNA fragments. The mobility of the TnsD-attTn7 complexes that have different mobilities on non-denaturing polyacrylamide gels suggests that ACP is a constituent of the slower-migrating TnsD-attTn7 complexes.

We also detected a novel complex on non-denaturing polyacrylamide gels in attTn7 binding reactions containing only L29$_{HH}$, a modified version of L29 containing a histidine purification tag and the HSV epitope tag at its C-terminus, and $[^3$H](ACP)$_1$, suggesting that these two proteins can interact without TnsD (Figure 6, lane 14). Although attTn7 DNA is present in the binding reaction, we found that neither L29 nor ACP is able to bind to attTn7 without TnsD (data not shown). Note that these slower-migrating complexes are not present in binding reactions containing $^{32}$P-labeled attTn7 (Figure 6, lane 9). Thus, it is likely that L29 and ACP can interact through direct protein–protein interactions. In contrast, we have found that interactions between TnsD, L29 and ACP require the presence of the attTn7 target DNA, suggesting that stimulation in TnsD binding to attTn7 by L29 and ACP may also involve protein–DNA interactions (data not shown).

To demonstrate that L29 [63 amino acids (aa)] interacts with TnsD-attTn7 complexes, we evaluated the ability of L29$_{HH}$ (63 + 26 aa) to alter the formation of TnsD-attTn7 complexes. We found that L29$_{HH}$ + TnsD-attTn7 complexes migrated more slowly than L29 + TnsD-attTn7 complexes (Figure 7A, lane 6 versus lane 2). The formation of L29$_{HH}$ + TnsD-attTn7 complexes of altered
mobility provides evidence that L29 is indeed a component of the TnsD–attTn7-host complexes formed in the presence of crude extracts. The formation of L29HH + TnsD–attTn7 complexes was also stimulated in the presence of (ACP)1 (Figure 7A, lane 7). Note that along with the L29HH + TnsD–attTn7 complexes, another protein–DNA complex is present in the TnsD-binding reaction that migrates to a position identical to that of L29HH + TnsD–attTn7 complexes. This is likely to result from both L29HH and wild-type L29 being present in the L29HH protein preparation; on Coomassie-Blue-stained SDS–polyacrylamide gels both L29HH and wild-type L29 are visible (data not shown). The presence of wild-type L29 in the L29HH preparation suggests that L29 can form multimers, although we have no evidence indicating that the multimeric state of L29 is critical to its role in Tn7 transposition.

L29 was further determined to influence TnsD–attTn7 complex formation by: (i) showing that host extracts made from a strain containing an internal deletion of 16 amino acids within L29, L29Δ9-24, could no longer stimulate the formation of TnsD–attTn7 complexes (Figure 7B, lane 6); (ii) establishing that the combination of purified L29 and the L29Δ9-24 extract stimulates TnsD binding to attTn7 to a level that is indistinguishable from that of unfractionated wild-type host extracts (Figure 7B, lane 7); and (iii) demonstrating that L29 (provided by K. Neirhaus) that was purified from a different purification procedure causes the formation of a protein–DNA complex whose migration pattern is identical to that of L29 + TnsD–attTn7 complexes that is formed in the presence of our L29 preparation (Figure 7B, lane 5). Together, these observations strongly suggest that L29 is a constituent of TnsD–attTn7 complexes formed in the presence of crude extract and is indeed involved in stimulating TnsD–attTn7 complex formation.

L29 and ACP can stimulate Tn7 transposition in vitro

We demonstrated above that L29 and ACP can affect TnsD binding to attTn7. Can L29 and ACP also influence Tn7 transposition? To determine whether L29 and/or ACP can alter Tn7 transposition in vitro, the effects of both host proteins on Tn7 insertion into an attTn7-containing target were evaluated in a reconstituted system using purified Tn7-encoded transposition proteins. The Tn7 transposition in vitro reaction is very efficient under standard reaction conditions, and thus does not permit the effects of L29 and ACP on Tn7 transposition to be adequately evaluated. However, we found that when the amount of TnsD in the transposition reaction is limiting, an effect of L29 and ACP on Tn7 transposition to be detected; other reaction conditions tested did not have notable effects on the transposition reaction (data not shown). It should be noted that the combination of L29 and ACP causes an increase of at least three-fold (for the 1/3X and 1/9X TnsD dilutions) in Tn7 insertion into the attTn7 target site (Figure 8, lanes 7–16). The molar amounts of L29 and ACP present in transposition reactions are similar to TnsD levels; i.e. the amount of L29 and ACP needed to stimulate Tn7 transposition in vitro is not...
We have also examined the effect of L29 on Tn7 transposition in vivo. L29 plays a role in Tn7 transposition in vivo, as evidenced by the fact that stimulation of TnsD binding to attTn7 by L29 and/or ACP results in enhanced Tn7 transposition. Notably, neither L29 nor ACP alone can alter Tn7 transposition in vitro (Figure 8, lanes 5 and 6 versus lane 7).

**L29 plays a role in Tn7 transposition in vivo**

We have also examined the effect of L29 on Tn7 transposition into the chromosomal attTn7 site in vivo. This was accomplished by evaluating the level of Tn7 insertions into attTn7 in the wild-type L29 and the L29Δ9-24 strains by probing a Southern blot with a DNA probe specific for the attTn7 target site (data not shown). We found that a considerable fraction (1-3%) of the chromosomal attTn7 sites of the wild-type L29 strain received an Tn7 insertion; comparable levels of insertions have been observed for other strains (DeBoy and Craig, 1996). We were unable to detect Tn7 insertions into the chromosomal attTn7 site of the L29Δ9-24 strain, indicating at least a 9-fold decrease in transposition. However, we found that when the L29 mutation was complemented by supplying the cells with wild-type L29 on a plasmid, the level of transposition into the chromosomal attTn7 site was restored to that observed in the wild-type L29 strain. This finding indicates that the defect in Tn7 transposition is due to L29.

We also evaluated the effects of L29 on Tn7 transposition using an assay that monitors Tn7 transposition from the chromosomal attTn7 site into a conjugable plasmid containing the essential attTn7 sequences (see Materials and methods). In cells containing wild-type L29, Tn7 transposition into attTn7 occurs at a high frequency (Table I). Strikingly, in the L29Δ9-24 strain, Tn7 insertion into attTn7 decreased >100-fold (Table I). We also evalu-
ated the effects of an L29 mutation on the non-TnsD Tn7 transposition pathway that directs Tn7 insertions to non-attTn7 target sites. Tn7 insertion into non-attTn7 target sites was similar in both the wild-type L29 and the L29<sup>Δ9-24</sup> strains. These results indicate that L29 plays an important role in Tn7 transposition into attTn7 within the cell. We have not yet been able to evaluate the effect of ACP on Tn7 transposition; since ACP is an essential protein, addressing this question is technically challenging.

These results support the view that L29 has an important role in directing Tn7 insertion in vitro into target DNAs, plasmid or chromosomal, that contain the TnsD binding site, i.e. attTn7, but not non-attTn7 target sites.

**Discussion**

The transposon-encoded protein TnsD is responsible for directing Tn7 insertions into the chromosomal site. attTn7: TnsD binds specifically to attTn7 and recruits the rest of the transposition machinery to this target site. In this work, we report the identification of two host-encoded proteins that together stimulate TnsD binding to attTn7. We also provide evidence that these host factors stimulate Tn7 transposition in vitro. Interestingly, a host factor(s) also influences Tn7 transposition in vivo.

**L29 and ACP can stimulate TnsD binding to attTn7**

We have identified two host proteins, L29 and ACP, that collaborate to stimulate the binding of TnsD to attTn7. L29 is a relatively small (63 aa) basic protein that binds to 23S ribosomal RNA and is also a component of 50S ribosomal subunits (Bitar, 1975; Urlaub et al., 1995; Wittmann-Liebold et al., 1995; Noller and Nomura, 1996). The function of L29 in protein synthesis is not known; as such, it is not clear whether L29 is essential for cell viability. However, a strain containing an internal deletion of 16 amino acids, L29<sup>Δ9-24</sup>, is viable (Dabbs, 1979). Ribosomal proteins have been implicated in cellular processes such as replication, transcription, RNA processing and DNA repair (reviewed by Wool, 1996). Notably, this is the first report of a ribosomal protein participating in bacterial transposition.

ACP is a small (77 aa) acidic protein that functions as a carrier of various fatty acids in several biosynthetic pathways, including fatty acid biosynthesis (reviewed by Magnuson et al., 1993), phospholipid biosynthesis (reviewed by Cronan and Rock, 1996), lipopolysaccharide biosynthesis (reviewed by Raetz, 1996) and activation of hemolysin (Issartel et al., 1991). The ACP polypeptide is post-translationally modified through the transfer of 4′-phosphopantetheine (4′-PP) from coenzyme A to Ser36 of ACP, forming (ACP)<sub>1</sub>. (ACP)<sub>1</sub> is further modified at the terminal sulphydryl moiety by the enzymatic attachment of various fatty acids, which leads to the formation of several different acylated derivatives of (ACP)<sub>1</sub>. (ACP)<sub>1</sub> can also form covalent dimers with itself through the formation of disulfide bonds, forming (ACP)<sub>2</sub> (Rock and Cronan, 1981; Magnuson et al., 1993).

We report here that ACP + L29 results in the formation of TnsD–attTn7 complexes that are similar in their mobilities (on non-denaturing polyacrylamide gels) to the TnsD–attTn7-host complexes that are formed in the presence of crude extracts. Since we found that L29 + unmodified ACP (the ACP polypeptide which lacks the 4′PP moiety and was a gift from C. Walsh) can also stimulate TnsD binding to attTn7, it seems unlikely that the prosthetic groups of modified forms of ACP play a role in the formation of L29 + TnsD–attTn7 complexes (data not shown). Thus, it is most likely that a region common to all ACP molecules is responsible for interacting with L29 + TnsD–attTn7 complexes. It should be noted that we have not directly evaluated acylated derivatives of ACP for their effects on TnsD binding to attTn7; therefore, we cannot speculate on the potential role of acyl–ACPs on Tn7 transposition.

The evidence presented here suggests that both L29 and ACP can associate with TnsD when it is bound to attTn7. We found that an altered form of L29 causes the formation of TnsD–attTn7 complexes that migrate more slowly than L29 + TnsD–attTn7 complexes, providing evidence that L29 is indeed a component of the TnsD–attTn7-host complexes. We have also shown that ACP is a constituent of the TnsD–attTn7-host complexes by demonstrating that slower-mobility TnsD–attTn7 complexes can be visualized in the presence of [³H](ACP)<sub>1</sub> + L29.

**L29 and ACP can stimulate Tn7 transposition**

Many features of Tn7 transposition into attTn7 in vivo have been realized using an in vitro transposition system, a highly efficient reaction requiring only purified TnsABC+D, the mini-Tn7 element and attTn7-containing target DNAs. Prior to this work, there was no obvious role for host proteins in Tn7 transposition in vitro, although a stimulation of Tn7 transposition in vitro has been detected in the presence of host extracts. We have demonstrated here that L29 and ACP can enhance Tn7 transposition into attTn7 in vitro at least three-fold. It should be noted, however, that both L29 and ACP are necessary to detect any increase in the amount of simple insertion products produced; thus, the host activities of L29 and ACP in Tn7 transposition in vitro are dependent upon each other. It is important to note that the in vitro transposition assay was developed and optimized without host proteins. Thus, it may be that our standard assay conditions are not conducive for studying the effects of host proteins on Tn7 transposition in vitro; it is possible that more dramatic effects can be observed under other assay conditions.

ACP has also been reported to stimulate nicking at the 3′ ends of Tn3 by the purified Tn3 transposase, although it is not clear whether ACP alters the binding of the Tn3 transposase to the 3′ ends (Maekawa et al., 1996). Here we have been able to show a direct effect of ACP on Tn7 transposition in vitro. Owing to the essential role of ACP in vivo, its effects on Tn7 transposition in vivo have not yet been evaluated.

Most interestingly, L29 was found to play a vital role in Tn7 transposition in vivo. Tn7 transposition into attTn7 decreases >100-fold when intact L29 is absent from cells. Moreover, L29 specifically affects Tn7 transposition reactions involving TnsD; this specificity seems reasonable, since L29 was identified based on its role in enhancing TnsD binding to attTn7.

**L29 and ACP may play a regulatory role in Tn7 transposition**

Tn7 transposition is a tightly regulated process. The selection of appropriate target sites controls the initial
steps of the transposition reaction. TnsD is responsible for selecting the attTn7 target site by binding to a specific sequence within attTn7 (Waddell and Craig, 1988; Bainton et al., 1993). It has been previously established by DNA footprinting analysis that TnsD interacts with TnsC, thus recruiting the TnsA+B transposase bound to the Tn7 ends to attTn7. Thus, a nucleoprotein complex, containing TnsABC+D, attTn7 and the mini-Tn7 ends, is essential for promotion of Tn7 transposition into attTn7.

In this work, we have demonstrated that the combination of L29 and ACP enhances the formation of TnsD–attTn7 complexes and stimulates Tn7 transposition in vitro. Moreover, we have observed that L29 is extremely important for high-frequency Tn7 insertion into attTn7 in vivo. Hence, host proteins may indeed play an essential role in Tn7 transposition within the cell.

By what mechanism might L29 and ACP influence Tn7 transposition? The fundamental activity of L29 and ACP is to increase the apparent affinity of TnsD for attTn7. Perhaps L29 and ACP promote conformational changes within TnsD that allow TnsD to bind more stably to attTn7, thereby increasing the frequency of Tn7 transposition into attTn7. It is possible that L29 and ACP directly function as an anchor, securing TnsD to attTn7 through protein–protein and/or protein–DNA interactions. We have presented some evidence here showing potential protein–protein interactions between L29 and ACP, whether L29 and/or ACP can bind to attTn7 when bound to TnsD remains to be established.

Why might L29 and ACP be involved in Tn7 transposition in vivo? One attractive hypothesis is that L29 and ACP modulate Tn7 transposition into attTn7 by signaling to Tn7 when cellular conditions are favorable or unfavorable for insertion. An intriguing but unexamined issue is the possibility that the amount of L29 available to Tn7 can influence transposition. An intriguing but unexamined issue is the possibility that the amount of L29 available to Tn7 can influence transposition. It is possible that L29 and ACP directly function as an anchor, securing TnsD to attTn7 through protein–protein and/or protein–DNA interactions. We have presented some evidence here showing potential protein–protein interactions between L29 and ACP, whether L29 and/or ACP can bind to attTn7 when bound to TnsD remains to be established.

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The transposase TnsD, which is a member of the lambda family of transposases, is essential for the transposition of Tn7 into the host chromosome. TnsD is a 108-kDa protein that contains a helicase domain and a transposase domain. The transposase domain is responsible for catalyzing the transposition reaction, while the helicase domain is involved in解开DNA supercoils.

**Materials and methods**

**Bacterial strains**

L29 was identified from crude extract prepared from HN1125 [E.coli galK2 hupA16::KanR hupB11::TetR supO strR] (Wada et al., 1988). HN1151 [E.coli galK2 supO strR] is the isogenic parent of HN1525 (Bachmann, 1972). ACP and (ACP)2 were identified from crude extract prepared from AM111-5 [E.coli gdhA2 his-95 metB relA1 rpmC rna-19 spoT1] (Dabs, 1979). A19 [E.coli gdhA2 his-95 metB relA1 rna-19 spoT1] is the isogenic parent of AM111-5.

Host crude extracts made from the following strains were used to evaluate their effect on TnsD binding to attTn7. Plviir transduction was used to transfer each mutation into the same strain background (Miller, 1972). The HU strain was made by P1 transduction of hupA16::KanR hupB11::TetR from HN1525 into HN1151. The FIS strain was created by P1 transduction of hisB::kanB from OH76 into HN1511. The HIF strain was made by P1 transduction of hisC::kanB from BD784 and himA::Tet from BD783 (laboratory strains) into HN1151. The HS- strain was made by transduction HN1111 with a hns205::TetPlviir lysate (Dersch et al., 1993).

The following strains were used in the mating-out assay. PS164 was made by P1 transduction of attTn7:Tn7 from OH14 (Hughes, PhD thesis) into a AM111-5 strain background. PS165 was made by P1 transduction of Tn7 from OH14 into an A9 strain background. Several strains, PS166, PS167, PS168 and PS169, served as donors in the mating-out assay. PS166 consists of PS164 containing pOX38–Gen (a derivative of the conjugable F factor which has gentamycin resistance, Gen), and PS167 consists of PS164 containing pOX38–attTn7 (pOX–GencontainingattTn7sequences).PS168isPS165containingpOX38–Gen and PS169 PS165 containing pOX38–attTn7. CW51 [E.coli F ara arg lac pro X11 recA56 nalR1 mfi] was the recipient strain used in all matings (Waddell and Craig, 1988).

**PCR primers**

The following DNA primers were used during the cloning of TnsDHis and L29d (see below). NLC129, 5’-TAAGTGGTACAGCTATGGAGACACTGCC-3’; NLCl86, 5’-CGAGATGACGCCAAAGACCCAGAGTTGGTACAGCGG-3’; NLCl287, 5’-GGGAAATTCTATGAAAGCAAAGAACGCGCTCCGAGGAAAGGCGG-3’; NLCl288, 5’-CCGCGTCGAGACCCCCCTCTCTGTTGAATAAAGCTTAAACGG-3’. **Affinity purification of TnsDHis**

TnsDHis contains all 508 amino acids of Tn7 (with the exception of a change in the second amino acid of Gly to Ser) fused to a 15-aminocarboxylic acid (Aaa) linker containing a 10-residue C-terminal histidine tag; its activities are indistinguishable from those of authentic TnsD (data not shown). The TnsD expression plasmid, pPLS24, was constructed by inserting the Ncol–SphI fragment from pKAO41 (Orle and Craig, 1991) and the Sph–BanHI PCR fragment amplified from pKAO41, which was obtained using PCR primers NLC129 and NLCl86, into the Ncol–BanHI site of pGDI108 (Graeye et al., 1984).

The TnsDHis protein was purified from NCM33 [E.coli K-12 (λ)] lacZ::TnsDhis®] (Bainton et al., 1993). L29d was purified from BL21(λDE3) (Novagen). Tritium-labeled ACP was prepared from SJ16 [E.coli F panD zad2200:Tn16 metB relA1 λ34 gsrA216] (Jackowski and Rock, 1981).

**L29 and ACP can stimulate Tn7 transposition**
Tris pH 7.9, 500 mM NaCl, 10% glycerol). TnsD·His was eluted from the column with Buffer C (200 mM imidazole, 20 mM Tris pH 7.9, 500 mM NaCl, 10% glycerol). Peak fractions were pooled, dialyzed against 50 mM KCl, 50 mM Tris–HCl (pH 8.0), 1 mM EDTA, 2 mM DTT and 25% glycerol, and stored at −80°C. Approximately 25 μg/g of cells were collected; the final TnsD·His preparation was >95% pure.

**Purification of L29 and ACP**

We identified the ribosomal protein L29 and the ACP by fractionating a crude cell extract from HN1525 and AM111-5, respectively. Cells were grown at 37°C to an OD_{600} = 1.0 in LB medium. All purification steps were performed at 4°C, unless otherwise specified. For each gram of cell pellet, four milliliters of lysis buffer (10 mM potassium phosphate pH 7.0, 1 mM EDTA, 5 mM β-mercaptoethanol (β-ME), 0.1 mM PMSF, 500 mM NaCl) was used to resuspend the cell pellets. The cells were sonicated and the cellular debris was removed by centrifugation at 26,000 g for 30 min at 4°C. Polyethylenimine (PEI) pH 7.9 was added to a final concentration of 0.5% over 10 min, and the mixture was centrifuged for 15 min at 26,000 g. The host extract was boiled while stirring for two min and the denatured proteins were removed by centrifugation at 12,000 g for 15 min. The remaining proteins were precipitated with ammonium sulfate, which was added to 100% saturation. Lysis buffer was used to resuspend the ammonium sulfate pellet, which was dialyzed overnight in a low-salt buffer (10 mM potassium phosphate pH 7.0, 1 mM EDTA, 5 mM β-ME, 0.1 mM PMSF).

The lysate was then loaded onto a 2.5 ml phosphocellulose column (Whatman P11) and bound proteins were eluted over a 50 ml linear gradient, 75–769 mM potassium phosphate (pH 7.0). Fractions containing host factors that caused the formation of a low-mobility TnsD·His·His tag complex (active fractions) eluted in ~300 mM potassium phosphate. The active fractions were pooled, dialyzed into 50 mM potassium phosphate, 50 mM NaCl, and 1 mM EDTA, and loaded onto a MonoS HR 5/5 column (Pharmacia). Proteins that bound to the MonoS column were eluted over a 20 ml linear gradient of 50-555 mM NaCl and the host activity was recovered at around 320 mM NaCl. The active fractions were pooled and concentrated using the Centricon-3 centrifugal concentrators (Amicon) and further fractionated using a Superose 12 HR 10/30 column (Pharmacia) column with Buffer C (200 mM imidazole, 20 mM Tris pH 7.9, 500 mM NaCl) was used to resuspend the cell pellets. The cells were sonicated and the cellular debris was removed by centrifugation at 26,000 g for 30 min. The resulting cleared lysate was filtered through a 0.45 μm syringe filter. The filtrate was then loaded onto a Ni^{2+}–Sepharose B column (Novagen) and the column was washed with the same buffer and with 100 mM imidazole, 20 mM Tris pH 7.9, 500 mM NaCl. The L29·HH fraction was eluted with 200 mM imidazole, 20 mM Tris (pH 7.9), 500 mM NaCl, dialyzed overnight into 10 mM sodium phosphate, 1 mM EDTA, 0.1 mM PMSF, 5 mM β-ME, and loaded onto a Mono Q HR 5/5 column. L29·HH eluted at ~300 mM NaCl. Wild-type L29, expressed from the chromosomal rpmC gene, co-eluted with the L29·HH.

**Preparation of trinitium-labeled ACP**

H-labeled ACP was prepared essentially as described by Rock and Cronan (1981), using E. coli strain SJ16 (PafΔ), a gift from John Cronan.

**Gel mobility-shift assay**

The effect of host proteins on binding of TnsD to attTn7 was evaluated using a gel-mobility shift assay as described by Bainton et al. (1993). All of the reaction mixtures (20 μl final volume) contained buffer components (50 mM Tris–HCl pH 8.0, 140 mM KCl, 1 mM EDTA, 1.8 mM dithiothreitol (DTT), 9.8% (v/v) glycerol, 340 μg/ml bovine serum albumin (BSA), 17 μg/ml sheared salmon-sperm DNA, ~0.1 pmol of labeled attTn7 DNA fragments) plus 6.7 ng TnsD and various amounts of each host protein or purified protein. In experiments evaluating H-labeled ACP, ~0.5 pmol of unlabeled attTn7 DNA fragments were used. All reactions were incubated for 20 min at room temperature and electrophoresed through 8.75% polyacrylamide gels (29:1 acrylamide:N,N′-methylene-bisacrylamide) in Tris-borate–EDTA (TBE) buffer at 11.5 V/cm for 3–4 h. The gels were dried under a vacuum and exposed to X-ray film or PhosphorImager screen. Gels containing H-ACP, were treated with the autoradiography enhancer Enlightening (DuPont) prior to drying and exposure to X-ray film.

**In vitro transposition assay**

The in vitro transposition reactions were performed essentially as described by Bainton et al. (1993). Reaction mixtures (100 μl volume) contained 0.25 mM mPMN dNTP (mPMN dATP, mPMN dCTP, mPMN dGTP, mPMN dTTP), 25 mM HEPES, 2 mM EDTA, 2 mM DTT, 2.5 mM Tris pH 8.0, 2.0 mM ATP, 50 μg/ml BSA, 100 μg/ml RNA and TnsD–His, 3 nM TnsB–His, 10 nM TnsC, various concentrations of TnsD·His, ~14 nM L29, ~11 nM ACP and 15 mM magnesium acetate (MgAc). The reaction mixtures (96 μl), which included everything except the MgAc, were assembled on ice and allowed to incubate at 30°C for 30 min. MgAc (4.0 μl) was added to 15 mM and the reaction mixtures were incubated for an additional 30 min. Reactions were stopped by phenol/chloroform (1:1 v/v) extraction, the DNA was ethanol precipitated, digested with ScaI, and analyzed by agarose gel electrophoresis and Southern hybridization using a DNA probe specific for the mini-Tn7 element. The blots were examined by autoradiography and PhosphorImager screens (Molecular Dynamics).

**Mating-out assay**

The effect of L29 on Tn7 transposition was evaluated using the mating-out assay (Waddell and Craig, 1988). To evaluate the influence of L29 on low-frequency Tn7 insertion into non-attTn7 sites, we used as a target a derivative of the conjugable F plasmid, pOX38–Gen. To evaluate the effect of L29 on high-frequency Tn7 insertion into attTn7, we used as a target a derivative of pOX38–Gen containing the attTn7 DNA sequences, which will be referred to as pOX38–attTn7. Insertions into pOX38–Gen and pOX38–attTn7 were evaluated in both the L29 wild-type (L29WT) and the L29 mutant (L29Δ9–24) strains. The donor strains contained a chromosomal copy of Tn7, which confers resistance to trimethoprim (Tm R) and either pOX38–Gen or pOX38–attTn7, which are resistant to gentamycin (Gen R). The recipient strain, CW51, was resistant to nalidixic acid (Nal R) and rifampicin (Rif R). The total number of recipient cells obtaining an F plasmid was determined by selecting for Nal R Rif R Gen R colonies. Recipients obtaining F plasmids with Tn7 insertions were detected by selecting for Nal R Rif R Gen R colonies. The transposition frequency was calculated by dividing the number of recipient cells that received an F plasmids containing an Tn7 insertion by the total number of recipient cells that received an F plasmid.

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References


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