Characterizing the DNA Contacts and Cooperative Binding of F Plasmid TraM to Its Cognate Sites at oriT

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Richard A. Fekete‡ and Laura S. Frost§

From the Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

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The F plasmid is a 100-kbp circular plasmid1 found in Escherichia coli and is a paradigm for pilus-mediated conjugation. Transfer proteins required for conjugation have been divided into five categories based on relaxosome formation and DNA transport, pilus assembly, mating pair stabilization, surface
attachment, and regulation (1). The relaxosome is a multiprotein complex that spans the cell envelope and is
responsible for pilus assembly and mating pair formation.

The highest affinity binding sites for F TraM are sbmA and
-B, which are involved in the autoregulation of the traM gene
(9), whereas the lowest affinity site, sbmC, is located nearest to
nic and appears to have an important role in DNA metabolism
during transfer. Removal of sbmA and -B from a cloned oriT
segment decreases transfer by 100-fold, whereas removal of
sbmC further decreases transfer another 100-fold (10). A sec-
ond function of sbmA and sbmB may be to assist binding of
TraM to sbmC, the weakest binding site, which is also the most
critical for conjugation. This is supported by a similar muta-
tional analysis of R100 (an F-like plasmid), where mutagenesis
of the proximal half of sbmC (closest to nic) decreased mating
120-fold, whereas mutagenesis of any of the other TraM binding
sites decreased mating to a maximum of 7-fold (11).

The multiple binding sites may function in a cooperative fash-
ion (12) whereby low concentrations of protein act synergistically
to control DNA-related events. Cooperative binding of TraM to its
binding sites was determined using electrophoretic mobility shift
assays (EMSA)2 (2). TraM-DNA contacts within the sbmA and -C
sites were mapped using hydroxyl radical footprinting. The re-
results suggest that TraM contacts slightly different bases than
predicted and that the pattern of contact by TraM on the DNA
suggests the formation of a nucleosome-like structure, which
might aid in readying the DNA for transfer.

EXPERIMENTAL PROCEDURES

Recombinant DNA Techniques—Restriction enzymes, calf intestinal
alkaline phosphatase, Klenow fragment, T4 polynucleotide kinase, and T4
DNA ligase were supplied by Roche Diagnostics. All procedures were
as described in Ausubel et al. (13) except where noted. Plasmids were
transformed using CaCl2 competent cells (14) or by electroporation
using a Bio-Rad Gene Pulser at 2.5 V, 25 microfarads, and 200 Ω. DNA
fragments used to create plasmid constructs were isolated from acryl-
amide (14) or agarose gels (Qiagen gel extraction kit). All gels were run
in 89 mM Tris, 89 mM borax, 2 mM EDTA, pH 8.0, buffer unless
otherwise indicated. PCR was performed using Vent polymerase (New
England BioLabs) with 20 nmol of dNTP (Roche) and ~500 pmol of
each primer in a 100–µl volume for 30 cycles. Fill-in reactions were done
using Klenow polymerase and 500 pmol of dNTP in 30 µl at 37 °C for 30
min. Plasmids were isolated using the method of Birnboim and Doly
(15) or using Qiagen Miniprep columns.

Construction of Clones Containing TraM Binding Sites—pRF911 was
constructed by phosphorylating ~400 pmol of pRF11 (CTAGGGACG-
CACCCTAGCCGGMCCCTAGCCGGATAC) and RFE12 (CTAGGATAC-
CGCTAGGCGCGCTGCTAGCGTGGC) with T4 polynucleotide ki-

1 The nucleotide sequence of the F plasmid is available in the Gen-
BankTM database under accession number NC002483.
22 The abbreviations used are: EMSA, electrophoretic mobility shift
assay; MCS, multiple cloning site; IHF, integration host factor.
If the association constants for each site are identical, then,

$$\omega = \frac{1}{x} $$

Values of $\omega$ greater than 1 indicate cooperativity.

**Competition Assays to Determine the Specificity of Binding**—The binding sites cloned into pRF911 (sbmA) and pRF940 (sbmABC) were amplified by PCR using primers RFE16 and RFE17 and used as competitor DNA in competitive electrophoretic mobility assays. The non-radioactive DNA was purified from a 1.5% agarose gel and quantified by absorbance at 260 nm. 0.5 fmoles of radiolabeled template was incubated with various amounts of TraM for 15 min at 37°C. Non-radioactive sbmABC (0.5 amol to 500 fmol) was then added in a 1-µl volume and incubated for an additional 15 min at 37°C. Reactions were resolved on a 5% acrylamide gel, and the intensity of bands was determined by phosphorimaging the dried gel.

**Hydroxyl Radical Footprinting**—Approximately 0.5 µg of plasmid DNA (pRF911, pRF920) was digested with PstI and Run on a 1.8% agarose gel. The 156- and 183-bp fragments were excised from the gel and purified. Sequencing reactions were performed using RFE9 (GTGGCGCGGGAGCCCTC) or RFE10 (GCTGATACCTTAACTGGAQ). PCR reactions using the end-labeled primer and 125 pmol of either RFE9 or RFE10 were performed, and the product was run out on a 1.8% agarose gel. The 156- and 183-bp fragments were excised and purified. Radioactivity was quantified using a scintillation counter and a 5000 cpm standard.

**Determination of Cooperativity of Binding**—The presence of cooperativity of binding was assessed using four different but related approaches: 1) Hill plots (12) were constructed by graphing the log of the free DNA concentration divided by that of the total complexed DNA versus the log of the protein concentration. The Hill coefficient ($n_H$), was calculated from the maximum slope of the curve and used to determine cooperative binding ($n > 1$ greater than 1). 2) The breadth of the binding curve was used to determine affinity of TraM for its binding sites according to the method of Freifelder (12) with cooperativity being assessed using four different but related approaches:

$$\omega = \frac{1}{x} $$

**RESULTS**

**TraM Binds sbmA, -B, -C Cooperatively—EMSA was used to determine the affinity of TraM for fragments of DNA containing sbmA, -B, and/or sbmC derived from the F oriT region. The TraM binding sites were cloned individually and in combinations into pBEND2 (Ref. 16, Fig. 1) to give pRF911 (sbmA), pRF918 (half of the sbmA site), pRF920 (sbmC), pRF930 (sbmAB), and pRF940 (sbmABC). Examples of EMSA for these fragments and a negative control (MCS of pBEND2) are shown in Fig. 2, A–F. Shifts above 1700 nM TraM were considered to be the result of nonspecific binding as demonstrated in the negative control (Fig. 2F). TraM bound to sbmA, sbmAB, and sbmABC in an ordered fashion giving rise to two retarded species (complexes I and II) below 1700 nM. A finer gradation in increasing TraM concentrations gave more precisely defined patterns of band retardation for sbmA and sbmABC, which were used to determine association constants and the presence of cooperativity (Fig. 3, A and B). Only two species were initially seen for sbmABC (Fig. 2), which were resolved into three
distinct bands in Fig. 3B (complexes I–III). Increased amounts of TraM gradually decreased the mobilities of \( \text{sbmA} \) and the half \( \text{sbmA} \) site, with a faint intermediate band appearing in the latter (Fig. 2, B and C). This was interpreted as a lower affinity for these sites and indicated that either TraM requires both half sites in cis for high affinity binding or the smaller repeats within \( \text{sbmA} \) are not sufficient for TraM recognition. By calculating the percentage of unbound DNA in each lane, association constants (\( K_a \)) were estimated (1/\([\text{TraM}] \) at 50% unbound, Table I). TraM bound \( \text{sbmA} \), \( \text{sbmC} \), \( \text{sbmAB} \), or \( \text{sbmABC} \) cooperatively because of the presence of two half sites in \( \text{sbmA} \) and \( \text{sbmC} \) as well as multiple sites in the more complex fragments. Consequently, a variety of methods were used to assess cooperative binding (“Experimental Procedures”). Using the EMSA data (Figs. 2 and 3), Hill plots were constructed for \( \text{sbmA} \), \( \text{sbmC} \), \( \text{sbmAB} \), and \( \text{sbmABC} \) and used to determine the Hill coefficient, \( n_H \) (Table I). If \( n_H \) is greater than 1 and less than \( n \), the number of binding sites, cooperativity is assumed. The \( n_H \) value for \( \text{sbmA} \) was 1.7 indi-
cating cooperative binding to *sbmA*, which has two TraM binding sites. A Hill coefficient of 0.9 was found for *sbmC*, which contains also two predicted binding sites, indicating non-cooperative binding. Hill coefficients of 3.25 and 2.9 were found for *sbmAB* (four sites) and *sbmABC* (six sites), respectively, indicating cooperative binding of TraM to both these fragments. The breadth of the binding curve, $\tau$ and $\omega$ values (Table I), also confirmed that TraM bound *sbmA*, *sbmAB*, and *sbmABC* cooperatively, with TraM binding to *sbmABC* with the greatest degree of cooperativity. Calculations for $\omega$ were not done for *sbmA*, because the presence of three shifted species on an EMSA complicated the calculations.

**Competition Assays to Determine Specificity of Binding**—The stabilities of TraM-DNA complexes described above were determined by performing EMSA in the presence of increasing amounts of unlabeled *sbmA* or *sbmABC* competitor DNA. The amount of TraM used to generate either complex I or II(III) was based on the results presented in Fig. 2. Competitive EMSA using *sbmA* as a competitor gave similar results to assays using *sbmABC* and are not shown. TraM bound to *sbmA* (complex I, Fig. 4A), *sbmAB* (complex II, data not shown), or *sbmABC* (complex II(III), Fig. 5) could be competed off at high concentrations of either competitor DNA. In the case of *sbmAB* (data not shown) and *sbmABC* (Fig. 5), complex II was shifted to the position of complex I at the highest concentrations of competitor DNA. Complex I of *sbmAB* or *sbmABC* was extremely stable and could not be competed off over the range of competitor DNA used during a 15-min incubation. TraM bound to *sbmC* was completely competed off with 100-fold excess of competitor DNA, supporting the idea that TraM has a lower affinity for linear DNA containing *sbmC* alone (Fig. 4B).

**DNA Bending Assays**—The ability of TraM to bend DNA was estimated using circular permutation assays (see "Experimental Procedures"). Using primers that bordered the MCS of pBEND2, DNA fragments from pRF911 (*sbmA*) and pRF940 (*sbmABC*) were amplified by PCR and digested with restriction enzymes to generate a nest of fragments of equal length but different mobilities. The DNA was used in each lane with the amount indicated. 0.5 fmol of DNA was used in each lane with the amount specified above each lane.

**Hydroxyl Radical Footprinting**—Previously, DNase I footprinting had delineated sequences (*sbmA*, -B, -C) in the oriT region protected by TraM, which suggested that TraM contacted the upper, retained DNA strand more than the lower, transferred strand. These protected regions were used to derive a consensus sequence, which was thought to be important for TraM recognition of its binding sites (NT(A/G)(G/T)(G/T/G)/GCTA (6)). To determine which nucleotides are contacted by TraM within these binding sites, hydroxyl radical footprinting of the upper and lower strands of DNA containing *sbmA* and *sbmC* was performed (Fig. 7, A and B), and the results from three different gels are summarized in Fig. 8. Unlike the DNase I protection assay, protection of bases in the top and bottom strands was approximately equal. There also appeared to be protection of bases outside the *sbmC* foot-
Cooperativity of F TraM

FIG. 6. Analysis of sbmA (A) and sbmABC (B) bending by TraM.
The fragments were digested with the restriction enzymes indicated and electrophoresed with or without 17 and 6.8 μM TraM. The unbound and bound DNA fragments are indicated by the lower and upper arrows, respectively. The bands below the lower arrow represent small labeled fragments resulting from digestion of the PCR products with different restriction enzymes, which were used as negative controls for TraM binding as well as internal markers for the distance each band had migrated in the gel.

print with a spacing pattern of ~11 bp (Figs. 7B and 8B).

The hydroxyl radical footprint for sbmA did not align precisely with the predicted consensus sequence but was shifted somewhat upstream (solid arrows compared with dashed arrows in Fig. 8A). Although the GC-rich nature of the TraM binding sites is striking, TraM contacted the CT bases within the CTAG motif, repeated twice, 12 bases apart, on both the upper and lower strands of sbmA, suggesting that sequence recognition was dependent on the non-GC bases within this 4-base palindrome.

The pattern of contact between TraM and bases within sbmC appeared to be unrelated to the footprint for TraM and sbmA (Fig. 8B). The strongest bending was in the center of the inverted repeat with both strands of the DNA being protected. This sequence bears no resemblance to the CTAG motif in sbmA suggesting that sequence recognition and protection did not coincide with each other. Radiating out from this central footprint are protected regions spaced approximately every 11 bases toward the 5'-ends of the DNA fragment. The bases protected at these intervals do not in any way resemble the sequences protected in sbmA.

DISCUSSION

An improved purification procedure for TraM has allowed better resolution of shifted bands in EMSA, resulting in the estimation of Kd values that reflect the affinity of TraM for its binding sites. Although TraM is predominately a tetramer upon purification (6, 20, 21), monomer and dimer forms are present as measured by gel exclusion and ultracentrifugation. Resizing purified tetrameric TraM resulted in the reappearance of monomers and dimers, suggesting that the three forms of TraM exist in equilibrium (3).

The cooperative nature of TraM binding, especially to sbmABC, was clearly evident and probably reflects the tetrameric nature of the protein. In binding to sbmA, an initial complex (I) of TraM bound to sbmA could be converted to a single tetramer of TraM bound to both sbmA and sbmB simultaneously (complex II) upon increasing concentrations of TraM. Thus the small amounts of the monomeric or dimeric forms of TraM might initiate the binding process, followed by tetrimerization of TraM during cooperative binding to these sites. Preliminary evidence on estimating the number of TraM molecules bound to sbmA suggested that complex I contains less than a tetramer of TraM whereas complex II does indeed contain a tetramer. More complete studies are currently under way. The number and arrangement of TraM subunits within complex III of sbmABC is not obvious, because TraM is apparently binding up to six sites involving direct and inverted repeats simultaneously.

Analysis of F TraM, bound to its individual binding sites (sbmA and sbmC), showed little bending of the DNA. This is in accord with results for TraM from the F-like plasmid pED208, which had no detectable effect on DNA bending as determined by electron microscopy (22). Instead, IHF, which has two binding sites in oriT bordering sbmC, has been implicated in construction of the relaxosome in F and pED208 (22, 23). The sequence between sbmA and sbmB does not contain an IHF binding site (3), suggesting that TraM could bind sbmA and -B simultaneously, folding the DNA back on itself to form a hairpin. Thus, bending the DNA in the sbmABC region could involve a combination of IHF and looping by TraM.

An interesting phenomenon involved the binding patterns of TraM to sbmA, sbmAB, and sbmABC versus sbmC and a half sbmA site. The binding pattern of the first three gave defined species suggesting the formation of distinct complexes that were composed of specified amounts of protein and DNA. However, binding to the latter two binding sites gave species that gradually increased in size as more TraM was added, a phenomenon that was also seen in all EMSAs at high protein concentrations. This suggests that, as more protein was added, the protein complex bound to the DNA increased proportionately in size, with the added TraM being distributed approximately evenly among all the complexes. The smearing of these species during EMSA also suggested that these complexes were not stable and dissociated during electrophoresis. The striking pattern of protection in the hydroxyl radical footprints occurred at approximately every 11–12 bp. This suggests a model whereby TraM could wrap the DNA around itself to form a large complex with the protein contacting 4–5 bases approximately every 11 bp. This would resemble the nucleosome-like structure proposed for TraK in the RP4 system (24). TraK bends the DNA and wraps it around a multimeric core of protein in a process that is independent of IHF.

Competition assays demonstrated that complex I and II(III) for sbmA, sbmAB, and sbmABC, were found to be highly stable and resist dissociation in the presence of a 1000-fold excess of specific DNA competitor. At very high concentrations of specific DNA competitor, small amounts of dissociation were seen for complex II of sbmAB and sbmABC, which resulted in formation of complex I. Complex I resisted dissociation in all cases suggesting a very stable complex was formed. Binding to sbmC was not as strong, and dissociation was seen when competitor DNA reached a 100-fold excess. Clearly, the presence of sbmA and sbmB stabilizes TraM binding to sbmC. These experiments were conducted using linear DNA templates, however, supercoiling is predicted to have an effect on TraM activity and possibly increase the degree of cooperativity even further, an aspect of TraM function that is currently under study.

Hydroxyl radical footprinting was also performed on TraM bound to sbmA and sbmC to define which bases were protected within the previously published DNase I footprint (6). Footprinting of sbmA showed that TraM had as many points of contact on the upper and lower strands in contrast to the results for DNase I footprinting, which indicated more contact with the upper, retained strand. Modeling TraM onto sbmA suggested that a dimer initially recognizes the sequence CTAG and binds to two adjacent major grooves 12 bp apart on the same face of the DNA (Fig. 8). This would agree with the tetrameric nature of TraM where each dimer within a tetramer would interact with two major grooves at two binding sites (sbmA and sbmB, for instance), in a cooperative fashion.

Footprinting of sbmC showed that TraM made contact with
the top and bottom strands in a symmetrical pattern, although there appeared to be no sequence conservation between the contacted sites. The footprints on the top and bottom strands were approximately equal in intensity and were centered at the ACAACA sequence in the center of the footprint. Interestingly the footprints extended from the central region toward the 5'-ends of the DNA fragment. Two dimers can be modeled onto the sbmC site with each dimer occupying two major grooves on the upper and lower strands, respectively. The dimers would overlap at the center of the inverted repeat giving protection on both strands (Fig. 8B). The spacing of 11–12 bp is suggestive of a phase-dependent process with one face of the DNA repeatedly contacting the protein. A consensus sequence was previously generated from the DNase I footprints by compiling the sequences in all three TraM binding sites (6). However, the hydroxyl radical footprint for sbmA demonstrated that contact with the DNA was shifted slightly upstream toward the palindromic sequence CTAG and that all four CTAG sequences in sbmA were involved (Fig. 8). TraM contacted the DNA every 11–12 bases outside the original DNase I footprint when a high concentration of TraM was used (Fig. 7, A and B). This suggested that TraM aggregates, as seen at high concentrations of protein on EMSA gels, wrapping the DNA around the protein and contacting the same face of the DNA upon each turn.

The importance of multiple binding sites was exemplified by the LacI repressor using a reporter gene containing various combinations of Lac operators (25). These experiments showed that O1 is required for repression, with repression increasing 38- and 24-fold when O2 or O3, respectively, were present. This effect was attributed to cooperativity of binding by Lac repressor tetramers to the DNA. A similar mechanism can be suggested for TraM at oriT. The weak binding sites, sbmC (10) and sbmA in R100 (corresponding to the proximal half of sbmC in F (11)), have been shown to be of critical importance in maximizing transfer efficiency. The increase in affinity of TraM for DNA fragments containing sbmABC versus sbmC alone (almost 10-fold) suggests that sbmAB may assist with loading TraM...
on sbmC, the weakest binding site, a step that might be critical for TraM function and efficient transfer.

We have demonstrated that the intracellular concentration of TraM is critical for transfer (26). As cells enter exponential growth, the transcription of traM diminishes and the levels of TraM in the cell are undetectable by late exponential phase. If sbmC, the site nearest oriT, and ostensibly the most important for transfer, was bound by TraM in a manner that was sensitive to the intracellular concentrations of the protein, the F conjugation apparatus would have engineered a simple, sensitive mechanism for controlling conjugation ability. In wild type F-like plasmids, conjugation is repressed by the fertility inhibition system FinOP (27). F is naturally derepressed and appears to control its fertility via a complex circuitry centered around control of traM transcription by TraY (9) and other host proteins. F could modulate its readiness for transfer at oriT by controlling the amount of TraM in the cell which, in turn, would affect binding of TraM to sbmC, following binding to sbmA and sbmB, in a cooperative manner. This model would predict that binding of TraM to the oriT region, especially sbmC, would affect the local superhelical density of the DNA. This would cause unwinding near oriT, most probably in the AT-rich region upstream of sbmC in sbyA, permitting entry of the TraI helicase and initiating large scale DNA unwinding and transport to the recipient cell. Because TraM has been suggested to bind TraD, the coupling protein (8, 28), the ability to form a nucleosome-like structure at high intracellular concentrations plus its ability to move the DNA into position for transport by binding TraD suggest that TraM might indeed be the "signaling" protein proposed by Willetts and Wilkins in 1984 (29).

REFERENCES

1. Frost, L. S., Ippen-Ihler, K., and Skurray, R. A. (1994) Microbiol. Rev. 58, 162–210
2. Matson, S. W., Nelson, W. C., and Morton, B. S. (1993) J. Bacteriol. 175, 2599–2606
3. Matson, S. W., Sampson, J. K., and Byrd, D. R. (2001) J. Biol. Chem. 276, 2372–2379
4. Nelson, W. C., Howard, M. T., Sherman, J. A., and Matson, S. W. (1995) J. Bacteriol. 177, 28374–28380
5. Fekete, R. A., and Frost, L. S. (2000) J. Bacteriol. 182, 4022–4027
6. Di Laurorenzo, L., Frost, L. S., and Paranchych, W. (1992) Mol. Microbiol. 6, 2951–2959
7. Achtman, M., Willetts, N., and A. J. Clark. (1971) J. Bacteriol. 106, 529–538
8. Disque-Kochem, C., and Dreiseikelmann, B. (1997) J. Bacteriol. 179, 6133–6137
9. Penfold, S. S., Simon, J., and Frost, L. S. (1996) Mol. Microbiol. 20, 549–558
10. Fu, Y. H., Tsai, M. M., Luo, Y. N., and Deonier, R. C. (1991) J. Bacteriol. 173, 1012–1020
11. Abo, T., and Ohtsubo, E. (1995) J. Bacteriol. 177, 4350–4355
12. Freidler, D. (1982) Physical Biochemistry: Applications to Biochemistry and Molecular Biology W. H. Freeman and Co., San Francisco
13. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, J. A. (eds) (1987) Current Protocols in Molecular Biology, and Supplements, John Wiley & Sons, Inc., New York
14. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
15. Birnboim, H. C., and Doly, J. (1979) Nucleic Acids Res. 7, 1513–1523
16. Kim, J., Zwieb, C., Wu, C., and Adhya, S. (1989) Gene 85, 15–23
17. Carey, J. (1991) Methods Enzymol. 208, 103–117
18. Carlson, N. G., and Little, J. W. (1993) J. Mol. Biol. 230, 1108–1130
19. Chatterjee, J., Miyamoto, C. M., and Meighen, E. A. (1996) Mol. Microbiol. 20, 415–425
20. Penfold, S. S. (1995) Transcriptional Regulation of the F Plasmid. Ph.D. thesis, University of Alberta
21. Kupelweiser, G., Schwab, M., Hogenauer, G., Koraimann, G., and Zechner, E. L. (1998) J. Mol. Biol. 275, 81–94
22. Di Laurorenzo, L., Scraba, D. G., Paranchych, W., and Frost, L. S. (1995) Mol. Gen. Genet. 247, 726–734
23. Tsai, M. M., Fu, Y. H., and Deonier, R. C. (1990) J. Bacteriol. 172, 4603–4609
24. Ziegelin, G., Pansegrau, W., Lurz, R., and Lanka, E. (1992) J. Biol. Chem. 267, 17279–17286
25. Oehler, S., Eismann, E. R., Kramer, H., and Muller-Hill, B. (1990) EMBO J. 9, 973–979
26. Frost, L. S., and Manchak, J. (1998) Microbiology 144, 2579–2587
27. Frost, L., Lee, S., Yanch, N., and Paranchych, W. (1989) Mol. Gen. Genet. 218, 152–160
28. Cabezón, E., Sastre, J. I., and de la Cruz, F. (1997) Mol. Gen. Genet. 254, 400–406
29. Willetts, N. S., and Wilkins, B. M. (1984) Microbiol. Rev. 48, 24–41