

Target Site Selection by Tn7: *att*Tn7 Transcription and Target Activity

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The bacterial transposon Tn7 inserts at high frequency into a specific site called *att*Tn7, which is present in the chromosomes of many bacteria. We show here that transcription of a nearby gene, *glmS*, decreases the frequency of Tn7 insertion into *att*Tn7, thus providing a link between Tn7 transposition and host cell metabolism.

Tn7 and the *att*Tn7 site. Most transposable elements insert into the bacterial chromosome at multiple DNA sites and only at low frequency. In contrast, Tn7 displays high-frequency, site-specific insertion into a single locus (3, 4). Subsequent molecular analysis revealed that Tn7 inserts in a single orientation into this target site, called an attachment site, or *att*Tn7, at a specific nucleotide position downstream of the *glmUS* operon, which encodes two proteins involved in cell wall biosynthesis (9, 10, 16) (Fig. 1).

The Tn7-encoded protein TnsD is the key component of the transposition machinery which directs site-specific insertion of Tn7 into *att*Tn7. TnsD recognizes a 35-bp DNA segment within the protein-coding region of *glmS* (2, 15), which is essential for *att*Tn7 target activity (2, 11). Tn7 insertion actually occurs in the transcriptional terminator of the *glmUS* operon, approximately 25 bp away from the TnsD binding site (8).

Transcription and target activity. Target activity is the capacity of a particular DNA site to attract a transposon insertion. There are several processes that contribute to the target activity of *att*Tn7, including the positive influence of host factors that augments binding of TnsD to *att*Tn7 (13) and the negative influence of long-range target immunity effects that discourages Tn7 from inserting into *att*Tn7 (7). The specific point of Tn7 insertion and the TnsD binding site are both located within transcribed sequences of the *glmUS* genes (Fig. 1). We asked whether target site transcription positively or negatively influences target activity of *att*Tn7. We hypothesized that modulation of *glmUS* expression influences *att*Tn7 target activity and hence Tn7 transposition, thereby connecting Tn7 to host metabolism. Previous experiments using *att*Tn7-containing plasmids showed that the frequency of Tn7 insertion is equivalent in the presence or absence of a strong *lac* promoter (8). Efficient *att*Tn7 target activity was also found to be independent of transcription orientation (11) and of transcription termination (8). Since some features of these high-copy-number plasmid targets might bypass the effect of transcription on Tn7 insertion, we have reexamined whether transcription in

the *att*Tn7 target site modulates Tn7 insertion by using low-copy-number targets.

Transcription of potential target DNAs has been found to block insertion by other transposons (5, 6).

Evaluating the target activity of *att*Tn7 in the chromosome or an F plasmid. We have previously described a method to evaluate *att*Tn7 target activity by directly measuring “empty” and “filled” *att*Tn7 sites by using a Southern blot assay (7). Insertion frequency is expressed as the percentage of *att*Tn7

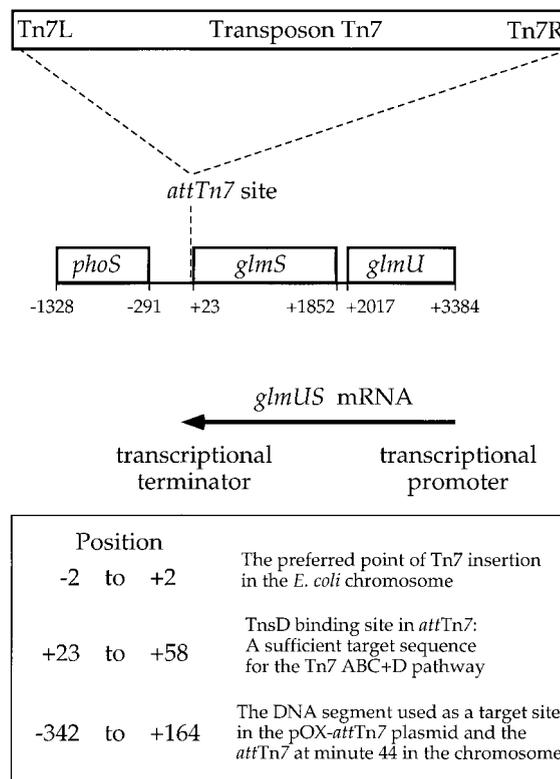


FIG. 1. The preferred site for Tn7 insertion in the *E. coli* chromosome, *att*Tn7. The center of the 5-bp sequence (–2 to +2) duplicated upon Tn7 insertion is designated position 0. Although Tn7 inserts into the transcriptional terminator of the *glmUS* genes, a small 35-bp DNA segment (+23 to +58) in the coding region of *glmS* is sufficient for site-specific insertion. The diagram also indicates the preferred orientation of Tn7 insertions in *att*Tn7; the right end (Tn7R) joins proximally to the target sequences near the *glmS* gene.

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Preferred Tn7 insertion site in *attTn7* sequence

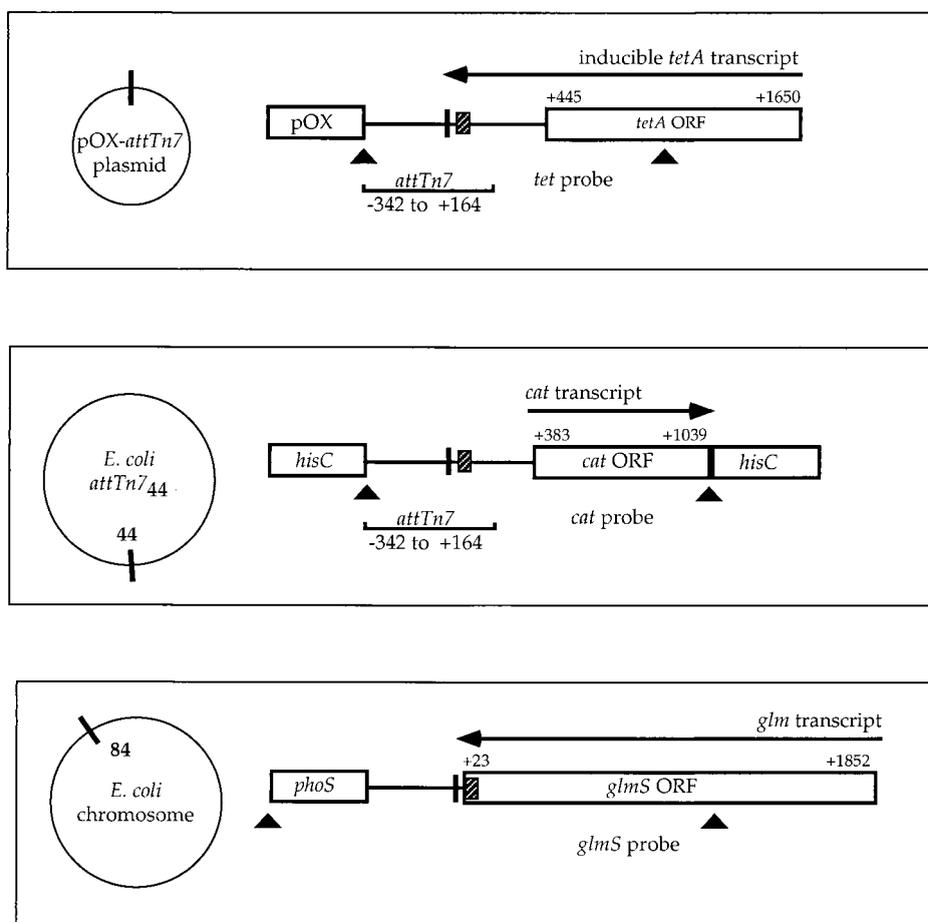
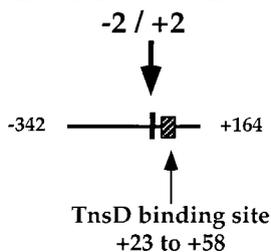


FIG. 2. Physical maps and transcriptional activities for the *attTn7* target site at the native position in the *E. coli* chromosome (*attTn7*₈₄), at a different location in the chromosome (*attTn7*₄₄), or in a plasmid target (pOX-*attTn7*). The target sites in *attTn7*₄₄ and pOX-*attTn7* contain sequences from -342 to +164 of *attTn7*₈₄, including the TnsD binding site (shaded box), which is sufficient to direct site specific insertion of Tn7. Each panel shows the combinations of restriction sites (arrowheads) used to generate the hybridization probes used for Southern analysis of Tn7 insertion in that particular target site; the *cat* probe was used to detect insertion into *attTn7*₄₄ and the *tet* probe was used to detect insertion into pOX-*attTn7*.

sites which are filled by a Tn7 insertion. In addition to its natural location at minute 74 in the *Escherichia coli* chromosome, the *attTn7* site has been introduced at minute 44 in the chromosome (7) and in an F-plasmid derivative, pOX-*attTn7* (1, 14). The new chromosomal and plasmid target sites contain ~500 bp (-342 to +164) of *attTn7* (Fig. 2). Thus, we can simultaneously evaluate the target activity for each of these sites in individual cell populations by using the appropriate combination of restriction enzyme digests and DNA probes for Southern blot analysis (Fig. 2).

Inhibition of *attTn7* target activity by a high level of transcription in a plasmid target. We have taken advantage of the pOX-*attTn7* plasmid target to reexamine whether target site transcription influences Tn7 insertion. The *attTn7* site in this low-copy-number plasmid is flanked by a tightly regulated *tetA* gene which can be induced by the presence of tetracycline, promoting transcription across the *attTn7* site. Cells were grown in conditions that switch “off” or “on” transcription through the *attTn7* site, and transposition was examined under these different conditions. As a control, transposition into

TABLE 1. Frequency of Tn7 transposition into pOX-*att*Tn7 or control site when transcription in pOX-*att*Tn7 is off or on^a

Test group	% of target sites with a Tn7 insertion					
	Transcription off			Transcription on		
	pOX- <i>att</i> Tn7	Control <i>att</i> Tn7 ₄₄ site	Ratio	pOX- <i>att</i> Tn7	Control <i>att</i> Tn7 ₄₄ site	Ratio
Population 1	3.1	1.4	2	0.5	1.4	0.4
Population 2	3.3	1.4	2	0.6	1.4	0.4
Population 3	3.9	1.5	3	0.6	1.4	0.4
Population 4	3.6	1.5	2	0.6	1.4	0.4

^a Southern blot analysis of Tn7 transposition from *att*Tn7₈₄::Tn7 in the chromosome to a control site, *att*Tn7₄₄, and a pOX-*att*Tn7 plasmid in cells grown on Luria-Bertani plates without (transcription off in pOX-*att*Tn7) or with (transcription on in pOX-*att*Tn7) tetracycline. Total DNA from ~10⁸ cells of each clonal population was examined for Tn7 inserts.

*att*Tn7₄₄, which lacks a tetracycline-inducible promoter, was evaluated simultaneously (Fig. 2).

We found that transposition into pOX-*att*Tn7 was substantially reduced when transcription across *att*Tn7 from the tetracycline promoter occurred and that this is a local effect, not global. We did not observe a significant change in the absolute amount of Tn7 insertion into the control site (*att*Tn7₄₄) when cells were grown in the presence or absence of tetracycline (Table 1). In contrast, Tn7 insertion into pOX-*att*Tn7 changed more than fivefold in the same cells in the presence of tetracycline. Therefore, tetracycline inhibits Tn7 only locally, at the *att*Tn7 target site downstream of the tetracycline-inducible promoter.

Increase of *att*Tn7 target activity by a small (threefold) decrease in transcription from the *glm*US genes in the chromosome. We also examined whether a modest change in the transcriptional activity of the *glm*US genes influences the target activity of *att*Tn7₈₄. Studies of *glm*US have found that gene expression can be experimentally modulated threefold by growing cells in the presence or absence of the amino sugar glucosamine, a GlmS metabolite (12). We have confirmed that growing cells in amino sugars reduces transcription through *att*Tn7₈₄; cells containing promoterless *lacZY* genes in the *att*Tn7 site produced threefold less β-galactosidase activity when grown in M9 minimal media supplemented with 0.4% *N*-acetylglucosamine (data not shown).

To test if a threefold variation in endogenous transcription through *att*Tn7₈₄ influences the activity of that DNA site as a target for Tn7, we compared the levels of Tn7 insertion in cells grown in M9 minimal media in the presence or absence of an amino sugar supplement. To establish that the amino sugar supplement only influences Tn7 insertion into the nearby *att*Tn7₈₄ site, we also simultaneously evaluated Tn7 insertion into the *att*Tn7₄₄ control site. We have found an inverse correlation between the transcriptional activity of *att*Tn7₈₄ and the frequency of Tn7 insertion into that chromosomal target site (Fig. 3). Target activity increases when target site transcription is decreased; that is, Tn7 insertion increases threefold when amino sugars are present. In contrast to the observed effect of amino sugars on the target activity of *att*Tn7₈₄, the amino sugar supplement does not increase the level of Tn7 insertion into *att*Tn7₄₄ in the same cells. Thus, the effect of the amino sugar supplements on *att*Tn7₈₄ target activity reflects a local, rather than global, effect.

We do not know yet what kinds of cellular events in actively growing cells might affect the target activity of *att*Tn7₈₄ through changes in *glm*US transcription. The *glm*US genes en-

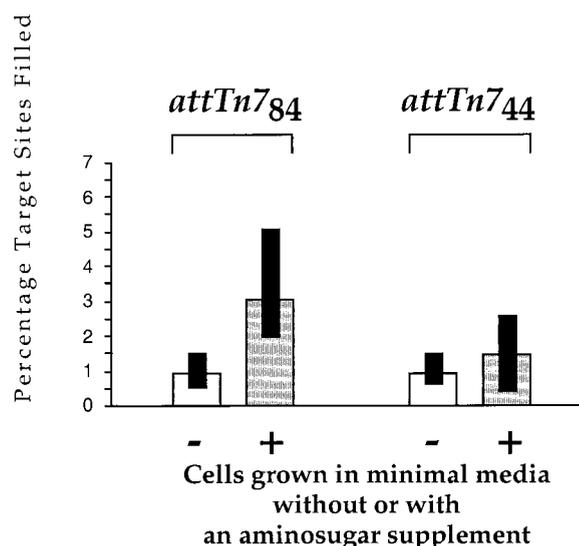


FIG. 3. Tn7 insertion from pOX-*att*Tn7 into the chromosomal *att*Tn7₈₄ site increases when target site transcription is reduced by a small (threefold) increment. Tn7 insertion into *att*Tn7₈₄ and the control site *att*Tn7₄₄ was measured simultaneously by Southern blot analysis of genomic DNA with target site probes. The frequency of transposition into both target sites in each clonal population was calculated as the percentage of DNA molecules that have a Tn7 insertion; columns indicate the mean values, and black bars indicate the range of the highest and lowest values. The figure displays the level of Tn7 insertion (y axis) in cells grown in minimal media with amino sugars (+) (17 populations) relative to the level of Tn7 insertion in cells grown without amino sugars (+) (16 populations).

code proteins involved in cell wall biosynthesis which are essential for cell growth under laboratory conditions. We propose that *glm*US expression, and thus *att*Tn7₈₄ target activity, might be linked to cellular growth and the availability of nutrients in the environment.

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