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Effect of Temperature on Translocation Frequency of the Tn3 Element

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The effect of temperature on the translocation frequency of the Tn3 element was investigated. The temperature optimum for translocation of Tn3 was in the range from 26 to 30°C. At temperatures above 30°C, the translocation frequency decreased rapidly and linearly; at 36°C it was only 5% of the frequency observed at 30°C. The duration and reversibility of the temperature effect were utilized to demonstrate a requirement for protein synthesis in the translocation process.

Transposable genetic elements (Tn elements) are discrete segments of DNA capable of translocation from one genome to another (for reviews, see references 4, 8, and 12). We recently reported (10) a study of the frequency and specificity of translocation between plasmids of one such element, Tn3 (2), which codes for resistance to ampicillin (9). In that study we were unable to detect translocation of Tn3 at 45°C, whereas the frequency of Tn3 translocation at 37°C was much lower than that at 30°C. In the present investigation, we have more fully defined the temperature sensitivity of Tn3 translocation and have used it to investigate the role of protein synthesis in the translocation event.

MATERIALS AND METHODS

Bacterial plasmids used in this study are described in Table 1. Antibiotics and concentrations (in micrograms per milliliter) used were as follows: ampicillin (Ap), 20; tetracycline (Tc), 10; kanamycin (Km), 20; streptomycin (Sm), 10; and chloramphenicol (Cm), 100, when used for plasmid amplification. Covalently closed circular plasmid DNA was isolated with Triton X as previously described (13). Translocation frequencies were estimated by the "alternative" method (more fully described in reference 10). Briefly, the recipient plasmid was introduced by transformation (5) into *Escherichia coli* C600 (1) containing the donor plasmid. At time zero (i.e., immediately after the 42°C heat pulse step in the transformation procedure), samples of the transformation mixture were transferred to flasks containing 100 ml of L broth (LB) (11) prewarmed to the desired temperature(s). After 2 h of incubation, streptomycin was added to select transformant cells that contained the recipient replicon. After overnight growth at the desired temperatures (at least 25 generations), plasmid DNA was extracted and used to transform *E. coli* C600 cells; transformants were selected on nutrient agar plates (Difco Laboratories) containing streptomycin and ampicillin, or streptomycin alone. Tetracycline resistance of these Sm^r Ap^r transformants was examined by transferring samples of individual colonies from streptomycin-ampicillin plates to tetracycline plates. Translocation frequency was calculated by determining the fraction of Sm^r colonies (indicated by numbers on the streptomycin plates) that were concurrently Ap^r and Tc^s (such colonies have been shown previously [10] to be due to transformation by a single RSF1010 molecule containing a Tn3 element). The fraction obtained was divided by 8.7 (the molecular weight of RSF1010 is 5.8 $\times 10^6$, or 8.7 kilobases [kb] [10]) to express the frequency of translocation events per kb of recipient DNA. Single colonies were tested for colicin production by transferring cells to nutrient agar plates with toothpicks. After overnight incubation at 37°C, plates were exposed to chloroform for 5 min, placed open on the bench for 10 min, and overlaid with approximately 2×10^7 to 5×10^7 *E. coli* C600 cells in 3 ml of L-soft agar (LB containing 0.7% agar [Difco]). After an additional overnight incubation, a ring of lysis of the bacterial lawn surrounded colicin-producing colonies. Restriction endonucleases *Eco*RI and *Hin*dIII were purchased from Bethesda Research Laboratories. Gel electrophoresis with 1% agarose was as described previously (7), using Tris-acetate buffer (40 mM Tris-hydrochloride [pH 7.8], 5 mM sodium acetate, 1 mM sodium EDTA).

RESULTS

Effect of temperature on Tn3 translocation frequency. The donor and recipient plasmids used in this study were pSC175 and RSF1010, respectively. Translocation frequencies were determined as indicated above by using the alternative procedure described previously (10). The frequency of translocation of Tn3 was studied at temperatures ranging from 23 to 37°C. Three separate experiments were performed. Each experiment yielded a series of translocation frequencies determined at different temper-

atures (Table 2). Our previous studies (10) had indicated that variation in translocation frequency occurs from experiment to experiment; to minimize the effects of such variation, we used the same competent recipient cell preparation to assay the translocation frequencies of all temperature points of an experiment, and we assayed each experiment on at least three separate occasions, using a different competent cell preparation on each occasion. To compare the three separate assay results, we assigned the translocation frequency of Tn*3* at 30°C in each experiment a maximum value of 100%, and we expressed the frequencies observed at other temperatures relative to this value.

TABLE 1. *Plasmids used*

Plasmid	Antibiotic resistance[a]	Comments	Reference
pSC175	Tc, Ap	Is pSC101 containing Tn*3*	9
RSF1010	Sm		6
ColE1::Tn*3*	Ap	Amplified by Cm	C. A. Miller (unpublished data)
pACYC139	Km	Amplified by Cm	3

[a] Tc, Tetracycline; Ap, ampicillin; Sm, streptomycin; Km, kanamycin; Cm, chloramphenicol.

The results of these experiments are summarized in Table 2 and shown in Fig. 1. As observed previously (10), there was considerable variation in translocation frequency at any one temperature when the same DNA preparation was assayed on different occasions. Despite this variation, the temperature sensitivity of translocation of Tn*3* is clearly demonstrated by our data. Temperature changes in the range from 26 to 30°C appear to have little effect on translocation frequency, in contrast to the dramatic decrease in frequency observed when the temperature is

TABLE 2. *Effect of temperature on translocation frequency*[a]

Temp (°C)[b]	Expt no. 1	Expt no. 2	Expt no. 3	Avg %[c]
23				
a		108		80 ± 22
b		50		
c		70		
d		92		
26				
a	111			98 ± 16
b	107			
c	76			
29				
a	120			90 ± 20
b	72			
c	77			
30				
a	100	100	100	100
b	100	100	100	
c	100	100	100	
d		100		
31				
a	84	91		75 ± 12
b	68	77		
c	49	65		
d		94		
33				
a	48	91	53	44 ± 18
b	22	84	42	
c	23	36	47	
d		45		
34				
a			41	40 ± 3
b			43	
c			36	
35.5				
a			11	10 ± 2
b			7	
c			12	
36				
a	6			4.5 ± 1.5
b	3			
c	<4			
37				
a	<2			
b	<4			
c	<6			

[a] Translocation frequencies were estimated and normalized as described in the text. Translocation frequencies at 30°C were (in terms of translocation events per kb of recipient DNA) 1.8×10^{-4} to 4.4×10^{-4}.

[b] In each experiment, temperatures of all water baths were measured with the same thermometer, both before and after overnight incubation. Experiments in which temperatures varied overnight were discarded. For each experiment, the set of plasmid DNA samples representing the different temperature points of that experiment were assayed on at least three (e.g., a through c) or four (e.g., a through d) separate occasions (that is, three or four competent cell preparations).

[c] Average, with standard deviation, of all relevant normalized frequencies for each temperature.

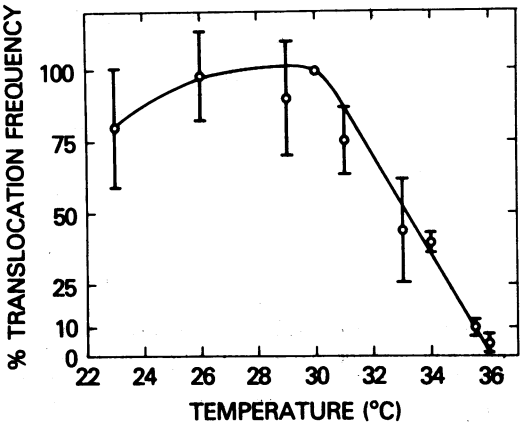

FIG. 1. *Effect of temperature on translocation frequency.*

elevated above 30°C (Fig. 1); at 36°C, the frequency of translocation is about 5% of that seen at 30°C. These results indicate that under the experimental conditions employed, the optimum temperature for translocation of Tn*3* is in the range from 26 to 30°C.

Duration and reversibility of temperature inhibition effect. The duration and reversibility of the effect of temperature on translocation of Tn*3* was investigated in the following experiment. The RSF1010 plasmid was introduced by transformation into *E. coli* C600 carrying plasmid pSC175. Immediately after the heat pulse step of the transformation procedure, equal samples containing 10^4 transformant cells were added to two flasks containing LB, which had been preincubated at 30 or 36°C. After 2 h of incubation at 30 or 36°C to allow expression of antibiotic resistance genes, streptomycin and tetracycline were added and incubation was continued. After overnight growth (approximately 25 to 30 generations), plasmid DNA was extracted from the bacteria in each flask, and translocation frequency was estimated as described above.

To investigate the kinetics of the low frequency of translocation observed after overnight growth at 36°C (as shown in Table 2), we diluted the 36°C culture 200-fold into LB containing streptomycin and tetracycline and allowed it to grow for an additional 25 or 50 generations at 36°C (i.e., a total of approximately 50 or 75 generations of growth after the initial transformation, respectively). Plasmid DNA was extracted from both cultures, and translocation frequencies were again determined. The results of this experiment (Table 3) indicate that after introduction of a recipient plasmid into a cell carrying a Tn*3* donor plasmid, the maximum frequency of translocation normally observed at 30°C is obtained within 25 to 30 generations of overnight growth (and remains constant thereafter as previously shown [10]). However, extended incubation of an identical culture at 36°C for as many as 75 generations did not lead to a significant increase in translocation frequency and still yielded a frequency of translocation less than 1/10 of the frequency observed after 25 to 30 generations at 30°C. These findings do not allow us to conclude whether the low frequency of translocation observed at the higher temperature is simply the result of a slower rate of translocation or whether it reflects a shift in the apparent translocation equilibrium.

For examination of the reversibility of the temperature effect, the culture grown for 75 generations at 36°C was diluted 10^5-fold into LB preincubated at 30°C and grown overnight at 30°C for an additional 25 generations, after which the translocation frequency was estimated. The optimal translocation frequency was obtained within 25 generations of growth at 30°C (Table 3), demonstrating rapid and complete reversibility of the temperature effect.

Role of protein synthesis in the translocation event. We used the temperature sensitivity of Tn*3* translocation to investigate the role of protein synthesis in the translocation event. Because of the possibility that replication of donor and recipient plasmids might be required for translocation to occur, two compatible plasmids, both of which are capable of replicating in the presence of the protein synthesis inhibitor chloramphenicol, were selected for use in this experiment. Both plasmids were introduced by transformation into the same bacterial cell, which was then grown to logarithmic phase at a temperature (42°C) that inhibits translocation. Chloramphenicol was added, and the temperature of incubation was lowered to 30°C; the translocation frequency was assayed after overnight growth at this (permissive) temperature to determine whether translocation occurs during

TABLE 3. *Effect of time on temperature inhibition of translocation*

Generations	Temp (°C)	$Tc^a/Sm^r\ Ap^r\ Sm^a$	Translocation frequency[b]
0–25	30	27/406	1.5×10^{-4}
0–25	36	3/490	0.99×10^{-5}
25–50	36	2/515	1.1×10^{-5}
50–75	36	13/574	1.5×10^{-5}
75–100	30	45/385	2.0×10^{-4}

[a] The number of Tc^s colonies observed over the number of $Sm^r\ Ap^r$ colonies screened for tetracycline resistance.

[b] Translocation frequency was calculated as described in the text.

plasmid replication in the absence of continued protein synthesis.

The donor of Tn*3* in this experiment was ColE1::Tn*3*, which was constructed in our laboratory by C. A. Miller, using a selected translocation procedure (10). The recipient of Tn*3* was pACYC139, a 6.8-kb, P15A-derived plasmid that contains a kanamycin resistance gene and that is compatible with ColE1 and its derivatives (3). Replication of both plasmids within the same cell was shown previously to occur in the presence of chloramphenicol (3).

To obtain a clone of cells containing both donor and recipient plasmids, we transformed *E. coli* C600 with a mixture of ColE1::Tn*3* and pACYC139 DNA species. Subsequent steps were carried out at 42°C (unless otherwise indicated) to inhibit translocation (Fig. 1 and Table 2). After 2 h of incubation, the transformation mixture was plated on prewarmed nutrient agar plates containing kanamycin and ampicillin and incubated overnight. A single colony that contained both plasmids (see below) was selected, inoculated into 20 ml of prewarmed LB, and incubated overnight. A 3-ml amount of this culture was added to each of two flasks containing 200 ml of prewarmed LB, and incubation was continued until an optical density at 600 nm of 0.4 was obtained, at which time chloramphenicol (100-μg/ml final concentration) was added to one of the flasks (culture 2). Both flasks were incubated overnight at 30°C with shaking.

Plasmid DNA was extracted from the two flasks, and the translocation frequency was estimated by the alternative method. *E. coli* C600 was transformed by plasmid DNA representing both cultures, and selection for transformants was carried out on nutrient plates containing kanamycin, to determine recipient plasmid number, and on nutrient plates containing kanamycin and ampicillin. Km^r Ap^r colonies were transferred to nutrient plates and tested for colicin production. Km^r Ap^r colicin-negative colonies indicated the number of recipient plasmid molecules in the population that had received Tn*3*. The results of these experiments (Table 4) indicate that inhibition of protein synthesis at 30°C (culture 2) severely reduced the translocation frequency to a level less than 1% of that observed at the same temperature in a culture (culture 1) lacking chloramphenicol.

Four Km^r Ap^r colicin-negative colonies were purified, and their DNA was isolated and subjected to agarose gel electrophoresis after digestion with restriction endonucleases *Eco*RI and *Hin*dIII. All four plasmid preparations were identical by this analysis, each containing a single plasmid with a molecular weight of approximately 12 kb with one *Hin*dIII restriction site and no *Eco*RI site (Fig. 2, slots 7 and 6, respectively). Such a molecular weight and restriction pattern are characteristic of a pACYC139::Tn*3* replicon, as distinct from the donor plasmid ColE1::Tn*3*, which contains an *Eco*RI site but lacks a *Hin*dIII site. This is shown in Fig. 2, slots 1 through 4, where the plasmid preparations from cultures 1 and 2 were electrophoresed after digestion with *Eco*RI or *Hin*dIII. Each can be seen to consist of two plasmids having molecular weights and endonuclease restriction patterns characteristic of the donor plasmid, ColE1::Tn*3* (approximately 11.5 kb, *Eco*RI positive and *Hin*dIII negative), and the recipient plasmid, pACYC139 (6.75 kb, *Eco*RI negative and *Hin*dIII positive).

TABLE 4. *Role of protein synthesis in translocation of Tn3*

Culture	Chloramphenicol	Km^r Ap^r colonies		Translocation frequency[a]
		Screened	Colicin negative	
1	−	320	63	1.0×10^{-3}
2	+	744	0	$<1.1 \times 10^{-5}$

[a] Translocation frequency was estimated by determining the fraction of recipient molecules that contained the Tn*3* transposon (number of Km^r Ap^r colicin-negative colonies divided by the number of Km^r colonies) and dividing by the molecular weight of the recipient plasmid pACYC139 (6.75 kb) to express the translocation frequency as the number of translocation events per kb of recipient DNA.

DISCUSSION

The experiments reported here were designed to characterize more fully the temperature sensitivity of translocation of Tn*3* that was reported briefly in a previous study (10). Using as donor a pSC101 plasmid containing Tn*3* (pSC175) and plasmid RSF1010 as recipient, we demonstrated that the temperature optimum for translocation of Tn*3* is in the range from 26 to 30°C. Above 30°C, the translocation frequency decreases rapidly and linearly such that, at 36°C, it is approximately 5% of the frequency observed at 30°C. We detected no translocation at 37°C in the experiments reported in Table 2, presumably because we did not scan greater numbers of Sm^r Ap^r transformants for tetracycline resistance.

Our current findings and those reported previously (10) indicate that translocation is an actively controlled process. Recent evidence indicating the existence of a Tn*3*-encoded repressor substance that regulates the frequency of translocation of Tn*3* (S. N. Cohen, M. J. Casadaban, J. Chou, and D. Tu, Cold Spring Harbor

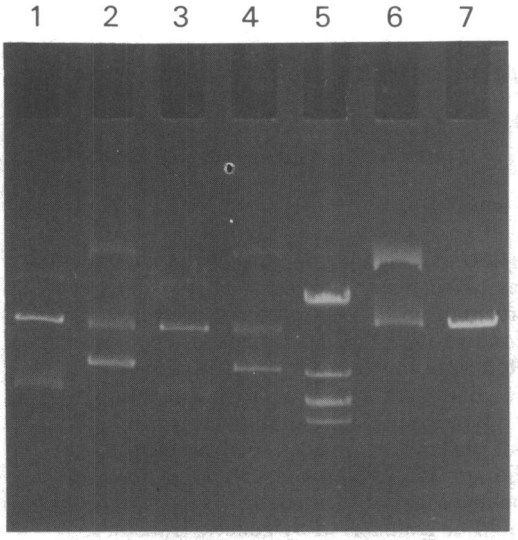

FIG. 2. *Restriction endonuclease analysis of donor, recipient, and translocated plasmids (see text). Agarose gel electrophoresis was carried out as described in the text. DNA samples from culture 1 (slots 1 and 2), culture 2 (slots 3 and 4), and a Km^r Ap^r colicin-negative colony (slots 6 and 7) were digested with EcoRI (slots 1, 3, and 6) or HindIII (slots 2, 4, and 7). Slot 5 contained EcoRI-digested lambda DNA as marker.*

Symp. Quant. Microbiol., in press) is consistent with this interpretation. The experiment reported in Table 3 suggests that the inhibitory effect of temperature was readily reversible, resulting in an optimal translocation frequency within 25 generations of growth after the temperature was lowered from 36 to 30°C. Moreover, a similar duration and reversibility of the temperature effect have been observed with plasmids other than those used in the experiment reported in Table 3 (P. Kretschmer and E. Schmader, unpublished data), leading us to believe that the observed temperature effect is characteristic of the Tn*3* translocation process per se, rather than a characteristic of the plasmids chosen in these experiments as donor and recipient.

The duration and reversibility of the temperature effect were utilized to investigate whether concurrent protein synthesis is required in the translocation event. A temperature of 42°C was used to severely inhibit Tn*3* translocation between two plasmids coexisting in the same cell; a protein synthesis inhibitor (chloramphenicol) was added, and the occurrence of translocation was determined after transfer of the culture to a temperature that permits translocation. Table 4 shows that the culture lowered to 30°C in the absence of chloramphenicol (culture 1) had at least a 100-fold-greater translocation frequency than the one lowered to 30°C in the presence of chloramphenicol (culture 2). Since we have shown that pACYC139::Tn*3* molecules isolated at 30°C replicate in the presence of chloramphenicol (unpublished data), the result shown in Table 4 indicates that protein synthesis is required during the Tn*3* translocation event.

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LITERATURE CITED

1. **Bachman, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
2. **Campbell, A., D. Berg, D. Botstein, R. Novick, and P. Starlinger.** 1976. Abbreviated rules of nomenclature for transposable elements of DNA. Nature (London) **263**:734.
3. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
4. **Cohen, S. N.** 1976. Transposable genetic elements and plasmid evolution. Nature (London) **263**:731–738.
5. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *E. coli* by R-factor DNA. Proc. Natl. Acad. Sci. U.S.A. **69**:2110–2114.
6. **Guerry, P., J. van Embden, and S. Falkow.** 1974. Molecular nature of two nonconjugative plasmids carrying drug resistance genes. J. Bacteriol. **117**:619–630.
7. **Helling, R. B., H. M. Goodman, and H. W. Boyer.** 1974. Analysis of endonuclease R·*Eco*RI fragments of DNA from lambdoid bacteriophages and other viruses by agarose-gel electrophoresis. J. Virol. **14**:1235–1244.
8. **Kleckner, N.** 1977. Translocatable elements in procaryotes. Cell **11**:11–23.
9. **Kopecko, D. J., and S. N. Cohen.** 1975. Site-specific *recA*-independent recombination between bacterial plasmids: involvement of palindromes at the recombinational loci. Proc. Natl. Acad. Sci. U.S.A. **72**:1373–1377.
10. **Kretschmer, P. J., and S. N. Cohen.** 1977. Selected translocation of plasmid genes: frequency and regional specificity of translocation of the Tn3 element. J. Bacteriol. **130**:888–899.
11. **Silver, R. P., and S. N. Cohen.** 1972. Nonchromosomal antibiotic resistance in bacteria. V. Isolation and characterization of R factor mutants exhibiting temperature-sensitive repression of fertility. J. Bacteriol. **110**: 1082–1088.
12. **Starlinger, P., and H. Saedler.** 1976. IS-elements in microorganisms. Curr. Top. Microbiol. Immunol. **75**: 111–152.
13. **Timmis, K. F., F. Cabello, and S. N. Cohen.** 1974. Utilization of two distinct modes of replication by a hybrid plasmid constructed *in vitro* from separate replicons. Proc. Natl. Acad. Sci. U.S.A. **71**:4556–4560.