Deoxyribonucleate Binding and Transformation in *Mycoplasma laidlawii*

By C. E. FOLSOME

*Department of Microbiology, University of Hawaii,
Honolulu, Hawaii*

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SUMMARY

Mycoplasma laidlawii organisms are capable of binding in deoxyribonuclease-resistant form high molecular weight double- or single-stranded homologous or heterologous deoxyribonucleate in amounts of $0.1-1.0 \times 10^{-15}$ g./organism, in a temperature-dependent mode. The maximum amounts of bound DNA ranged from 25 to 125 molecules of 5×10^6 d. Transformation of streptomycin-sensitive recipients to streptomycin resistance under conditions of maximum DNA uptake was relatively rare, being about 2-4-fold higher than spontaneous mutation. Ultraviolet (u.v.) inactivation studies showed that no caffeine-inhibitable u.v. dark-repair mechanism was present. Photoreactivation was demonstrable; the PR cross-sector was 0.39. From these results it was inferred that *M. laidlawii* is unable to be genetically transformed because of the absence of one or more steps which occur between irreversible DNA uptake and genetic integration. A model system which incorporates the above findings and suggests that donor DNA functions transiently in recipients is presented.

INTRODUCTION

Mycoplasma laidlawii organisms possess a circular single DNA molecule of 800×10^6 d. (H. J. Morowitz, personal communication) and thus are at least three times less complex genetically than *Escherichia coli* (2500×10^6 d.; Cairns, 1963). Conditional lethal mutants of *M. laidlawii* can be isolated with ease (Folsome & Folsome, 1966). In general the organism, by virtue of its genetic simplicity and lack of metabolic versatility, seems to be an ideal subject for a complete genetic analysis ultimately directed towards saturation of the chromosome with conditional lethal mutant cistrons.

The present paper summarizes experiments upon deoxyribonucleate (DNA) binding, transformation, and ultraviolet radiation (u.v.) inactivation in *Mycoplasma laidlawii*. It shows that the organisms bound large amounts of DNA, that genetic transformation frequencies were very low, and that the organisms possessed no demonstrable caffeine-inhibitable dark-repair mechanism. From these results it might be concluded that *Mycoplasma laidlawii* possesses no demonstrable recombination enzyme(s).

METHODS

Organisms. *Mycoplasma laidlawii* strain A, (ATCC 14089) was obtained from the American Type Culture Collection in 1965. This culture was passaged through 10 subcultures before use: at each stage a typical single isolated colony was used as the inoculum. A spontaneous streptomycin-resistant mutant was isolated (resistant to at least 200 µg. streptomycin/ml.).

Media. Tryptose (T) broth or agar plates containing, g./l. distilled water: 20, Bacto-tryptose; 5, NaCl; 5, Na acetate; 5, tris; 1, glucose; 10, (or 4 for soft agar) Davis New Zealand agar (when required), adjusted to pH 8.0 with 6 N-HCl. Difco PPLO serum fraction was added to all sterile cooled media to a final concentration of 0.5% (v/v). Streptomycin, at a final concentration of 50 or 10 $\mu\text{g./ml.}$, was used as indicated below in soft agar overlays.

Colony-forming unit counts and transformant assays were done by suitably diluting and pipetting 0.01 ml. samples on to the surface of tryptose agar (T) plates. After 4 hr, 1.5 ml. soft agar streptomycin overlays were poured on T agar plates to select for *str-r* organisms. Plates were incubated 2–5 days at 37° and resultant colonies were counted with the aid of a Zeiss-Jena SMXX stereomicroscope at $\times 40$ magnification. The errors for this system of assays were not in excess of 8%, as measured by computation of the standard deviation of many replicate assays.

DNA was isolated from 1 l. *Mycoplasma laidlawii* or 1 l. *Escherichia coli* exponential phase cultures according to the method of Marmur (1961).

[2-¹⁴C]Thymidine labelled DNA was prepared from *Escherichia coli* 15 T⁻ cultures grown in 1 l. volumes of thymidine-supplemented minimal medium (after Strelzoff, 1962) which contained 100 to 250 μC of [2-¹⁴C]thymidine. [2-¹⁴C]Thymidine-labelled DNA was isolated from *Mycoplasma laidlawii* cultures grown in 1 l. volumes of Razin & Cohen (1963) defined medium in which the thymidine concentration was decreased to 10 $\mu\text{g./ml.}$ and to which was added 250 μC of [2-¹⁴C]thymidine. The working specific activity of both labelled purified DNA preparations ranged from 15,000 to 25,000 counts/min./extinction unit at 260 m μ (one extinction unit equals 50 $\mu\text{g.}$ native purified DNA.)

[¹⁴C]DNA-binding studies. Volumes of up to 25 ml. of a reaction mixture of organisms and labelled DNA were filtered through a 0.22 μ average pore size Millipore GS filter, treated as required, and washed with 30 ml. ice cold ultra-violet buffer. Ultra-violet buffer contained, per l. distilled water: tris 5 g., NaCl 5 g., Na acetate 5 g., MgSO₄·7H₂O 10⁻³ M, at pH 8.0. The extinction of this buffer was 0.12 at 2537 Å and 10 mm. path length. Organisms were essentially all retained on the surface of the filter. Air-dried filters were fastened to 1.25 in. planchets and the radioactivity measured with an ultra-thin window gas-flow Geiger detector using a Baird-Atomic model 135 scaler. Background counts were 10–11/min.; all count data presented here have been corrected for background.

Genetic transformation conditions were based upon the results of the DNA binding studies. Aerated 37° T broth exponential phase *str-s* recipient organisms were exposed to purified DNA isolated from *str-r* donors. DNAase and MgCl₂ were added after 30 min. to final concentrations of 10–50 $\mu\text{g./ml.}$ and 10⁻³ M respectively. Treated organisms, incubated for various times under non-selective conditions in T broth or on T plates, were then assayed for total colony-forming units on T agar plates, and for *str-r* transformants on streptomycin 10 $\mu\text{g./ml.}$ agar plates or on T agar on top of which was overlaid streptomycin soft agar overlays containing streptomycin 10 or 50 $\mu\text{g./ml.}$ S plates were incubated at 37° for 2–3 days, then at 30° for an additional 4 days. T plates were incubated at 37° for 2–3 days.

Ultra-violet inactivation studies. These were done with *Mycoplasma laidlawii* or *Escherichia coli* under similar standard conditions: source, single 8 W General Electric germicidal low-pressure mercury lamp (G8T5); distance from material, 40 cm. from

lower edge of lamp tube; temperature, 20°; *Mycoplasma laidlawii* or *Escherichia coli* exponential phase cultures were diluted 1/10 into u.v. buffer and irradiated with various doses in dim yellow light with agitation as 5 ml. volumes contained in the bottom of a 60 × 10 mm. sterile plastic Petri dish, covered until irradiation began with a Pyrex top coated with aluminium foil. Under these conditions a 21 sec. u.v. dose corresponded to one lethal hit for *Escherichia coli* F-(λ)⁸ exponential phase organisms in u.v. buffer. Irradiated organisms were quickly diluted in T broth or caffeine-supplemented T broth (caffeine 0.75 mg./ml.) and immediately plated on to T or caffeine supplemented (0.75 mg./ml.) T agar plates. Both T and T caffeine (TC) agar plates were incubated at 30° in light-tight boxes; a replicate T agar plate series was immediately exposed to two 15 W daylight fluorescent lamps at a 20 mm. distance for 4 hr before incubation to permit photoreactivation (PR).

RESULTS

DNA binding

Kinetic studies. Two 60 ml. exponential phase *Mycoplasma laidlawii* cultures were added to equal volumes of warmed T broth supplemented with 0.5% Difco yeast extract and incubated 30 min. at 37°. To one culture was added [¹⁴C]*Escherichia coli* DNA (at a final concentration of 12 mg./ml.), to the second [¹⁴C]*Mycoplasma laidlawii* DNA (at a final concentration of 15 µg./ml.), and the mixtures incubated. Periodically 10 ml. volumes were withdrawn and filtered. At each time, and for each culture, two filter pads were prepared and washed; one pad was then exposed to and washed further in 10% trichloroacetic acid at 4° to precipitate nucleic acids. Figure 1 depicts the kinetics of binding of labelled DNA to intact organisms and to the TCA-precipitable fraction. These results indicate that the *Mycoplasma laidlawii* organisms bound homologous or heterologous DNA equally well. At 37° binding occurred slowly; a plateau was reached by 30–40 min. The amount of activity bound was the same for the intact-organism suspension or for the acid precipitable fraction and suggested that bound DNA was not extensively degraded; however, the label might have been degraded and re-incorporated. The maximum amount of DNA bound/organism at 37° was, for these experiments, about 30% of the organism's total haploid DNA content, or about 0.4×10^{-15} g. Many experiments of this type, with either homologously or heterologously labelled DNA, demonstrated that the maximum amount of DNA bound ranged from 0.2 to 1.0×10^{-15} g./colony-forming unit. This range of bound DNA can be expressed as 25 to 125 molecules of size 5×10^6 d., since both the general method of DNA extraction and related experiments made in our laboratory upon DNA-sucrose zone sedimentation indicated the molecular weight range of DNA preparations to be from 5 to 10×10^6 d.

Temperature dependence. Standard volumes of exponential phase culture were mixed with *Escherichia coli* [¹⁴C]labelled DNA and incubated for 30 min. at 4°, 30°, 37°, 40°. At the end of this time 4 ml. samples from each mixture were filtered, the filters washed and counted. Results are shown in Fig. 2. These results showed that DNA uptake was a temperature dependent process, possibly enzymic.

Permanent and transient binding of DNA. For this experiment a suspension of exponential phase organisms at 5×10^8 /ml. was mixed with *Escherichia coli* labelled DNA (364 c./min./µg., 6 µg./ml. final concentration) and incubated 30 min. at 37°.

Two 4 ml. volumes of culture were filtered. Ten ml. of warmed u.v. buffer were added to the surface of one filter; 10 ml. of u.v. buffer containing DNAase 50 $\mu\text{g./ml.}$ in 10^{-3} M-Mg were added to the second filter. Both filters were incubated at 37° for 20 min. Then both filters were washed with chilled u.v. buffer, dried and counted.

Organisms upon both control and DNAase-treated filters possessed equal radioactivity (725 c./min./ 2×10^9 colony-forming unit). This binding was equivalent to 8.3% of the total input label; the amount of DNA bound per colony forming unit was 1.0×10^{-15} g. Apparently no detectable transient binding occurred; bound DNA

Fig. 1

Fig. 2

Fig. 3

Fig. 1. The uptake of homologous or heterologous $[2-^{14}\text{C}]$ -labelled DNA into intact *Mycoplasma laidlawii* A organisms or into cold acid precipitable fractions. Homologous labelled DNA: intact recipients at 3.7×10^8 colony-forming units/ml. were exposed to labelled DNA 12 $\mu\text{g./ml.}$ of specific activity of 59 c./min./ $\mu\text{g.}$ Maximum amount of label bound/organism after 40 min. was 0.41×10^{-9} $\mu\text{g.}$ (●) intact organisms; (○) acid precipitable fraction. Heterologous labelled DNA: (□) Recipient organisms at 3.7×10^8 colony-forming units/ml. were exposed to *Escherichia coli* labelled DNA at 15 $\mu\text{g./ml.}$ and a specific activity of 375 c./min./ $\mu\text{g.}$ Maximum amount of label bound/organism was 0.39×10^{-9} $\mu\text{g.}$

Fig. 2. Temperature dependence of DNA binding by *M. laidlawii* A. Recipient organisms at 5×10^8 /ml. were exposed to *E. coli* [^{14}C] DNA at 8.3 $\mu\text{g./ml.}$ and a specific activity of 380 c./min. $\mu\text{g.}$ at the temperatures indicated for a time of 30 min. Then 4 ml. volumes were filtered and retained organisms washed, dried and counted.

Fig. 3. Competition between labelled and unlabelled DNA. [^{14}C]-labelled DNA at fixed amount (15.8 $\mu\text{g./ml.}$), various amounts of unlabelled DNA, and 5 ml. of suspension of 5×10^8 /ml. exponential phase *M. laidlawii* organisms were incubated 30 min. at 37° . Then 4 ml. volumes were filtered, and the retained organisms washed, dried and counted.

was present in a form inaccessible to DNAase. However, weak interactions between DNA and organisms would not be observed by the above experiment because of the way in which the organisms were washed. Evidence for weak transient binding is discussed below.

Competition between [^{12}C] and [^{14}C]labelled DNA's. For this experiment exponential phase organisms 5×10^8 /ml. were exposed for a standard 30 min. period at 37° to

mixtures containing a fixed amount of [^{14}C]*Escherichia coli* DNA at 15.8 $\mu\text{g./ml.}$ and 380 c./min./ $\mu\text{g.}$, and variable amounts of unlabelled *Escherichia coli* DNA (from 0 to 26 $\mu\text{g./ml.}$). The amounts of radioactivity retained per 4 ml. sample of filtered organisms were plotted against the fraction of radioactive DNA to give Fig. 3. These results indicated that unlabelled DNA and [^{14}C] DNA competed equally for binding to *Mycoplasma laidlawii* organisms. [^{14}C]DNA binding was decreased by 50% when equal concentrations of unlabelled DNA were present.

Binding of single-stranded DNA. One ml. *Escherichia coli* [^{14}C]DNA at 19 $\mu\text{g./ml.}$ (360 c./min./ $\mu\text{g.}$) was divided into two equal portions. One portion was heated 15 min. in a boiling water bath, then plunged into acetone ice for 5 min.; this treatment, according to Doty, Marmur, Eigner & Schildkraut (1960) thermally denatures DNA from double-stranded to single-stranded molecules. This treatment yielded a 25% hyperchromic shift in u.v. (260 $\text{m}\mu$) extinction. Five ml. volumes of exponential phase cultures at 5×10^8 colony-forming units/ml. were added to denatured and to native DNA's at 37° for 30 min. Four-ml. samples of these mixtures were filtered, washed and the radioactivity on the filter measured. Control (native) DNA-treated cultures bound 1.14 $\mu\text{g.}$ (427 c./min./ 2×10^9 organisms), denatured DNA-treated suspensions bound 1.12 $\mu\text{g.}$ (403 c./min./ 2×10^9 organisms). One may conclude that *Mycoplasma laidlawii* bound native or denatured DNA equally well under the same conditions, and for 30 min. exposure. Kinetic studies upon single-stranded DNA binding were not done; it is not possible to conclude that the uptake of both molecular species occurred with the same rate.

Dependence on high molecular weight DNA for binding. [^{14}C]*Escherichia coli* DNA at 170 $\mu\text{g./ml.}$ (61, 370 c./min./ml.) was subjected to strong shearing forces in a Waring blender at 4° under N_2 gas for various lengths of time. One ml. samples of sheared DNA were removed, mixed with 4 ml. recipient culture at 5×10^8 organisms/ml., incubated 30 min. at 37°; 4 ml. were filtered, washed, and radioactivity measured. Figure 4 shows the results, which indicate that maximal DNA binding required high molecular weight DNA. Binding was decreased sixfold with a blending time as short as 1.5 min.

Concentration effect. [^{14}C]*Escherichia coli* DNA (specific activity 370 c./min./ $\mu\text{g.}$; final concentrations from 12 to 0.12 $\mu\text{g./ml.}$) was added to recipients at 5×10^8 organisms/ml. After 30 min. 37° incubation 4 to 25 ml. samples were taken from each reaction mixture. The results are plotted in Fig. 5 as log fraction of input label bound against log concentration of DNA/ml. reaction mixture. The slope of the line is -0.43. Hence the relative amount of DNA bound per organism was greater at higher DNA concentrations and lower at lower DNA concentrations. The amount of DNA bound varied exponentially with DNA concentration, but not proportionately. These data suggest that weak transient binding of DNA did occur and that effective contacts between organisms and DNA molecules occurred with exponentially greater probability at higher than at lower DNA concentrations.

Control experiments. Numerous control experiments were used to test the reproducibility and accuracy of the millipore filter method of collecting and washing recipient organisms. [^{14}C]labelled DNA alone at concentrations from 10 $\mu\text{g.}$ to 300 $\mu\text{g./ml.}$ (specific activity 420 c./min./ $\mu\text{g.}$) was passed through a millipore GS filter. After washing with u.v. buffer, no radioactivity exceeding background values was detectable. Recipient organisms at known titre were filtered and the filtrate

assayed directly for colony forming units to determine whether any organisms would pass through the filter; no colonies (fewer than $1/10^7$) were detected. Thus the filtration and washing procedure as used here retained essentially all viable organisms and there was no non-specific retention of DNA to the filter. Samples of thermally denatured DNA were not retained upon the filter.

Transformation

The data obtained from transformation experiments are summarized in Table 1. Clearly, transformation to *str-r* did seem to occur; but only with great rarity, since transformants were only about 2-4 times more frequent than spontaneous mutation to *str-r*. Control experiments in which no *str-r* DNA was present (Expt. I), or in

Fig. 4

Fig. 5

Fig. 6

Fig. 4. DNA binding dependence on high molecular weight. Labelled DNA was blended at 4° under N_2 for various times, removed, mixed with recipient organisms of *M. laidlawii* for 30 min. at 37° , and the organisms then filtered, washed, dried and counted.

Fig. 5. Effect of DNA concentration. Log of input DNA concentration against log of fraction of input label bound.

Fig. 6. Ultraviolet inactivation of *M. laidlawii*. A. Log-frequency survivors plotted against u.v.-dose for *M. laidlawii* incubated (O) in dark at 30° , (Δ) in dark on caffeine T agar at 30° , ($\square$) exposed 4 hr to light for photoreactivation. *E. coli* K-12 F-(λ)⁻ dark survival is also depicted ($\times$). *M. laidlawii* organisms were filtered through a 0.65μ Millipore filter to remove clumps before u.v.-irradiation.

which *str-r* DNA was first exposed to DNAase (Expt. IV) indicated that the increased frequency of *str-r* colonies was specifically due to *str-r* donor DNA and not to a less specific effect of DNA upon recipient organisms.

About 120 transformation experiments have been made following protocols similar to those of Table 1. Three of these experiments showed transformation frequencies of 10^{-4} when control *str-r* colony frequencies were as low as 10^{-7} . However, two other experiments of this group showed the converse results; control *str-r* frequencies of

10^{-4} to 10^{-5} and *str-r* DNA recipient frequencies of 10^{-6} to 10^{-7} . Hence, the possibility does exist, although slight, that genetic transformation can occur, provided the correct combinations of elusive experimental parameters are present. A more probable explanation of the data is: (1) that true genetic transformation does not usually occur; (2) that the commonly observed low level of transformants is due to some other process (see below); (3) that the occasionally high values for 'transformants' observed are due to the random occurrence of 'jackpots' of *str-r* mutants descendant from an early spontaneous *str-r* mutant in either *str-r* DNA-exposed or control cultures.

Table 1. Genetic transformation of *Mycoplasma laidlawii* A

For all experiments reported in this table <i>str-s</i> recipient exponential phase organisms at 5×10^8 to 1×10^9 colony-forming units/ml. were exposed to DNA for 30 min. at 37°; DNAase (50 µg./ml.) and MgSO ₄ ·7H ₂ O (to 10^{-3} M) were added, and the mixture was either (a) diluted and pipetted onto T agar, incubated at 37° C for 4 hr, then overlaid with 1 ml. streptomycin soft T agar (Streptomycin 50 µg./ml.) and incubated at 37° for 3–4 days, or (b) the broth mixture was incubated a further 100 min. at 37°, and then pipetted on to streptomycin 10 µg./ml. T agar plates and incubated for 3–4 days at 37°.			
Experiment	Donor DNA conc. and source	Frequency of <i>str-r</i> colonies	Ratio exptl./control
I. <i>str-s</i> recipients exposed under standard conditions. DNAase treated organisms plated on T agar and overlaid with streptomycin (50 µg./ml.) soft agar after 4 hr	Control, no DNA	4.9×10^{-7}	—
	Control, <i>str-s</i> DNA 8 µg./ml.	5.0×10^{-7}	—
	<i>str-r</i> DNA 10 µg./ml.	2.1×10^{-6}	4.2
	<i>str-r</i> DNA 5 µg./ml.	1.5×10^{-6}	3.0
	<i>str-r</i> DNA 0.1 µg./ml.	6.6×10^{-6}	13.2
II. <i>str-s</i> recipients as Expt. I	Control, no DNA	1.7×10^{-7}	—
	<i>str-r</i> DNA 20 µg./ml.	3.2×10^{-7}	1.9
	<i>str-r</i> DNA 5 µg./ml.	3.0×10^{-7}	1.8
III. <i>str-s</i> recipients exposed under standard conditions, then incubated in broth 100 min. and plated on to streptomycin (10 µg./ml.) T agar plates	Control, no DNA	5.9×10^{-5}	—
	<i>str-r</i> DNA 5 µg./ml.	1.1×10^{-4}	1.9
	<i>str-r</i> DNA 5 µg./ml. (duplicate)	2.0×10^{-4}	3.4
IV. Repeat of Expt. III, organisms plated on streptomycin (10 µg./ml.) T agar plates	Control, DNAase + <i>str-r</i> DNA 6 µg./ml.	1.4×10^{-4}	—
	Control, no DNA	1.3×10^{-4}	—
	<i>str-r</i> DNA 6 µg./ml.	2.4×10^{-4}	1.8
V. Repeat of Expt. III	Control, no DNA	2.2×10^{-4}	—
	<i>str-r</i> DNA 4 µg./ml.	3.15×10^{-4}	1.4

None of the experiments reported here has ruled out the distinct possibility that the transformants to *str-r* might have arisen by some means other than recombination between donor *str-r* DNA and recipient *str-r* chromosome. For example, the observed increase in *str-r* transformants might have been due directly to spontaneous mutation occurring at normal frequencies in recipient organisms which were permitted (as a result of incorporation of *str-r* DNA) to undergo additional partial growth on streptomycin agar plates. Such an occurrence could generate data similar to those presented in Table 1. Functional transformation has been observed to occur for pneumococcus by Evans (1964), has been discussed by Folsome (1964), and does appear to exist in *Mycoplasma laidlawii* (T. H. Iha & Folsome, 1966, unpublished experiments).

In addition to the experiments recorded in Table 1, many other experiments have been made in which the effects of: (a) culture age from early exponential to early stationary phase, (b) pH value from 6.0 to 9.0, (c) presence or absence of Mg^{2+} and/or Ca^{2+} , (d) temperature from 25° to 40°, (e) various defined media, (f) aerated or N_2 gassed recipients, (g) u.v.-irradiation of recipients to 0.20 survivors have been assessed. In these experiments, without exception, transformation frequencies were not increased. The conditions for optimal transformation thus far are those summarized in Table 1. Concurrently with the above experiments, a search for other modes of genetic exchange was made: a *str-r* strain was cultured with a chloramphenicol resistant (*chl-r*) strain; at various times samples were spread on chloramphenicol streptomycin agar plates in an effort to detect double recombinant, *chl-r str-r*, colonies. The results of over 15 of these experiments were uniformly negative.

Table 2. Ultraviolet (u.v.) sensitivities of *Mycoplasma laidlawii* A under various post-u.v.-irradiation conditions, and of *Escherichia coli*

Organism	Post-u.v.-condition	Inactivation rate at asymptote ($-dS/dD$)
<i>M. laidlawii</i> A	Dark	-2.27
	Dark plus caffeine	-2.27
	Photoreactivation	-1.39
<i>E. coli</i> K-12- $F(\lambda)^-$	Dark	-0.53

PR cross sector (Dulbecco, 1954) = a , where $a+b=1$, and $b = -dS/dD^{(PR)} / -dS/dD^{(Dark)} = -1.39 / -2.27 = 0.61$, $a = 0.39$.

Inactivation by ultraviolet irradiation

Survival of *Mycoplasma laidlawii* as a function of u.v. dose under three post-irradiation conditions was measured; the results are shown in Fig. 6. The three post-u.v. conditions were (a) dark incubation on T agar plates, (b) dark incubation upon TC caffeine 750 $\mu g./ml.$ agar plates, (c) immediate exposure of T agar plates to light for 4 hr for photoreactivation (PR).

There was evidence: (1) for extreme u.v.-sensitivity of *Mycoplasma laidlawii*, (2) that no caffeine-inhibitable dark reactivation appeared to exist (see Metzger, 1964), (3) that PR could be clearly demonstrated (see Table 2). The PR cross-sector (Dulbecco, 1950) was 0.39 (see Table 2 for mode of calculation). This value means that 0.39 of the u.v. lesions at asymptote were capable of photoreactivation. As comparison one might note that coli-phages possess PR cross-sectors ranging from 0.2 for phage T4 to 0.65 for phage T7 (Dulbecco, 1950). The value for the PR cross-sector reported here for *Mycoplasma laidlawii* (0.39) is a minimal one, since the PR light exposure may or may not have been maximal.

Notable was the extreme u.v.-sensitivity of *Mycoplasma laidlawii* as compared to *Escherichia coli*: the ratio of inactivation rates at asymptote was 4.3 ($-2.27 / -0.53$, see Table 2). If one normalizes the u.v.-sensitivities of these two organisms to a sensitivity per unit DNA, then *Mycoplasma laidlawii* was actually $4.3 \times \sim 3 = 13$ times more sensitive. Although this relative sensitivity might be the result of other unknown processes it is consistent with the postulate that *Mycoplasma laidlawii* possesses no u.v. dark reactivation mechanism. Most interesting, however, was the evidence for the lack of caffeine-inhibitable dark repair mechanism. With regard to this charac-

teristic, *Mycoplasma laidlawii* appears to be similar to host-cell-reactivationless (HCR-) mutants of *Escherichia coli* (Sauerbier, 1964) and to u.v.-sensitive mutants of *Escherichia coli* K-12 (Howard-Flanders & Theriot, 1966). Also, transformable *Bacillus subtilis* does show a caffeine-inhibitable DR mechanism (Metzger, 1964).

DISCUSSION

Mycoplasma laidlawii organisms were capable of binding high molecular weight single-stranded or double-stranded DNA to a maximum amount of 0.1 to 0.2 of the organism's genomic DNA content within 30 min. and in a temperature-dependent manner. Bound DNA appeared to remain as high molecular weight material for at least 60 min., as shown by its acid precipitability. One must not discount the possibility that bound DNA was degraded and re-incorporated into newly synthesized recipient DNA.

The comparatively long period required for DNA binding (30 min.) and the apparent DNA exponential concentration-dependence suggested by the results in Fig. 5 can be explained by postulating that concentration-dependent transient binding of DNA occurred within very short time intervals, and that actual DNA entry into the cell required the longer times observed here. The nature of this transient binding must be such that transiently bound DNA is washed off the organisms by the methods used for washing the filter pads. Thus, the experiments on DNA binding were not optimally designed to detect weak transient binding and served mainly to indicate that, once firmly bound, DNA was inaccessible to DNAase and was either within the organism or was held on its surface in a configuration resistant to DNAase attack.

Mycoplasma laidlawii binding of single-stranded DNA also occurred; thus this system, excluding competence, appeared in many ways similar to *Hemophilus influenzae* (Barnhart & Herriott, 1963; Postel & Goodgal, 1966). Although the same amount of ^{14}C label was bound for both native and denatured DNA at 30 min., no kinetic data were available. As a consequence, one cannot state whether similar or different binding rates apply.

One can summarize the stages of DNA binding by listing this most probable sequence of events: (1) transient and quick binding of DNA by weak forces, (2) permanent binding requiring an enzymic mechanism, essentially complete by 30 min. Implicit in the above sequence is the inference that the culture is uniformly competent at least by step 2 and that competence is the normal state of exponential phase cultures, or that transient binding induces competence.

In light of the ability of *Mycoplasma laidlawii* to bind DNA, the experiments on genetic transformation were most surprising since, except for spurious instances, 'transformant' frequencies of only 2-4 times higher than spontaneous mutation were recorded. Two possibilities may be considered: (1) DNA cannot penetrate the organism, but is bound to the surface in a nuclease resistant configuration; (2) DNA can enter the organism, but except for rare instances cannot be genetically recombined into the genophore due to absence of a recombination mechanism. In the event that DNA could not penetrate, one would not expect to observe even the low level of transformants reported. Since the slight transformation which did occur was the specific result of *str-r* DNA, non-penetration could only account for the results if a small subfraction of the population bound DNA internally, and a large fraction

bound DNA in a DNAase resistant form externally. Several experiments on the survival of *str-r* recipients which had bound *str-r* DNA in streptomycin broth have indicated that bound DNA can function (Folsome, unpublished). Thus, for purposes of this discussion, it will be assumed that most bound DNA was internally located.

Were DNA to enter the cell, the question remains as to why transformant frequencies were *str-r* DNA-specific, yet low. The model which best accounts for the result is as follows: (a) Within short spans of time, DNA transiently and weakly binds to the surface of recipient organisms. (b) Recipients normally possess an enzymic mechanism by which exogenous DNA can enter. (c) Once within the organism, the ultimate fate of donor DNA is to be degraded, since *Mycoplasma laidlawii* requires all nucleosides in defined media (Tourtellotte, Morowitz & Kasimer, 1964). Since degradation of DNA does not appear to occur rapidly, it will be assumed that exogenous DNA can function transiently while in recipients. Perhaps exogenous DNA can also become rarely and randomly rescued by the same mechanism through which residual recombination occurs in recombination deficient *Escherichia coli* (Clark & Margules, 1965) or ϕ x 174 phage which are growing within recombination deficient *Escherichia coli* (Tessman, 1966); (d) Recipient organisms are permitted to survive and grow to a greater extent on streptomycin agar plates as a result of the prior functioning of *str-r* DNA; (e) The outcome of the sequence of postulated events is that recipients of *str-r* DNA would show a greater frequency of *str-r* spontaneous mutants than control cultures since they undergo greater residual growth under selective conditions.

In this fashion a large part, or all, of the 2-4-fold excess of observed *str-r* colonies resulting from *str-r* DNA treatment of recipients could be due to transitory functional transformation, and not due to genetic integration of *str-r* DNA into *str-s* genophores. It is also possible that a fraction of the *str-r* 'transformants' was the result of the random and rare marker rescue of *str-r* DNA.

Consistent with these possibilities are the results obtained from u.v.-inactivation studies, by which it was shown that *Mycoplasma laidlawii* lacked a caffeine-inhibitable dark reactivation mechanism. From these data one could infer that *M. laidlawii* is similar to recombination-deficient u.v.-sensitive mutants of *Escherichia coli* K-12 which specifically lack a recombination mechanism. The model presented above views *M. laidlawii* as a natural case of a recombination-deficient organism and resolves the results of experiments upon DNA binding, transformation, and u.v. inactivation. Other preliminary experiments have been made in this laboratory in which *M. hominis* H-39, *M. gallisepticum*, and *M. laidlawii* B have been examined for caffeine-inhibitable dark repair mechanisms. To date, these species resemble *M. laidlawii* A and appear not to possess this type of u.v. dark repair system. In an attempt to select for a variant of *M. laidlawii* A which did possess a u.v. dark repair system, survivors of 3 cyc. of 60 sec. u.v.-irradiation were isolated, but showed no difference from the parental strain as regards dark repair.

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