TRANSFECTION ENHANCEMENT BY ULTRAVIOLET IRRADIATION[1]

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The bacteriophage SP82, isolated by Green (1), was shown by him to contain a DNA which transfects competent B. subtilis in such a way that the number of plaques formed is proportional to the third or fourth power of the DNA concentration. In this report we show that if competent cells are given 100 ergs/mm^2 of ultraviolet light, the transfection is enhanced about 100-fold. Such irradiated cells also yield a first-power dependence on DNA concentration entirely similar to that found by Green using helper phages.

Green ascribed the marked dependence on DNA concentration to the need for genetic recombination to produce plaques, despite the fact that the DNA in question had the sedimentation properties of intact DNA. Accordingly, the requirement for several molecules and their recombination indicated that the DNA may have been degraded after uptake by the competent cells.

Green provided some evidence for this hypothesized degradation by showing that superinfection with an intact helper phage produces a greater plaque-formation by the DNA, and the plaque-formation then varied as the first power of the DNA concentration. The question was thereby raised as to why intact incoming phage DNA escapes the action of the hypothesized degrading factors. There would seem to be two general kinds of models for the escape.

First, there could be degrading enzymes (DNAses?) which are inhibited or repressed by a very early event triggered by the phage-injected DNA. Second,

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the phage-injected DNA could be immune to the degradation because of some special ending or coating which prevents activity of, respectively, exonucleases or endonucleases.

If we assume the first explanation, the incoming transfecting DNA might be protected by providing a pool of competing DNA to trap the degrading enzymes. We sought to provide this pool (without adding to the total DNA of the already complicated situation) by irradiating the competent cells with ultraviolet light (UV). Such irradiation is known (2) to inhibit transformation, but could well be innocuous or stimulatory for transfection since the phage can presumably function independently of the cell DNA.

MATERIALS AND METHODS

SP82 and its host, B. subtilis, strain SB1, were obtained through the courtesy of Dr. D. M. Green. Dr. Eunice Kahan kindly sent several of her temperature sensitive SP82 mutants.

Competent B. subtilis, strain SB1, were prepared according to the recipes of Spizizen and co-workers (3,4). The pre-competent cells were frozen in a dry-ice-acetone bath and stored either at -96 degrees C or in a liquid nitrogen freezer. One ml samples were thawed by adding 10 ml of the appropriate Spizizen medium and incubated at 37 degrees with vigorous shaking for 90 minutes. Competent cells were placed in an iced, sterile petri dish, and given various doses of UV from a GE G4H4 germicidal lamp 30 cm above the dish. Transfecting DNA was added immediately. Delay in adding the DNA produced a lowering of the number of plaques formed. Controls showed no effect due to icing of the cells.

The DNA was prepared from concentrates of liquid broth lysates of wild-type and mutant SP82, using a single phenol extraction at room temperature and very gentle shaking for about 5 minutes, after which the suspension was centrifuged at 5,000 g for 10 minutes. The removed water phase was centrifuged in the cold 5 minutes at 10,000 g. To the supernate was added 2 to 2.5 volumes of room temperature ethanol, and DNA was removed by spooling on a glass rod. The DNA,

dissolved in 1/10 SSC, was dialyzed overnight against SSC. Such preparations retained activity for the several weeks during which they were used for experimentation. The DNA was checked to ensure the absence of infectious phages and for having a 260/280 absorption ratio close to 2.

Our standard procedure was to add (usually) 0.1 ml of a 25 microgram/ml DNA solution to 0.5 ml of competent cells and to incubate, with gentle shaking, at 37 degrees C for 50 minutes before assaying for plaque-forming cells by plating on SB1 seed. Experiments were done in a room with yellow lights to avoid possible complications due to photoreactivation.

RESULTS AND DISCUSSION

The results of a typical UV experiment are shown in figure 1.

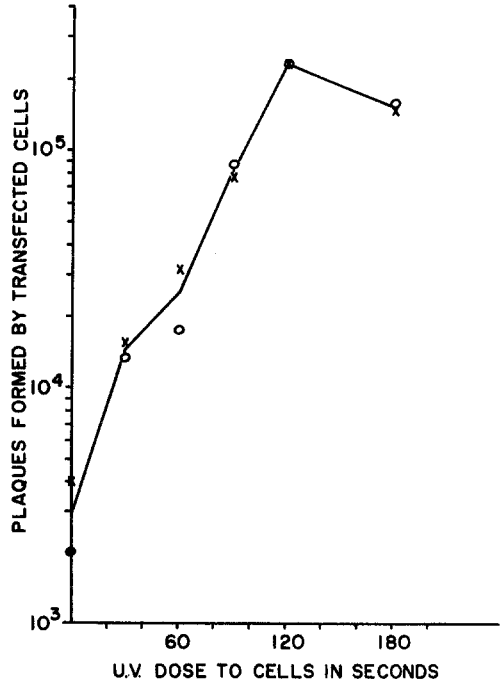

Fig. 1: Competent B. subtilis cells were irradiated for various times and 0.5 ml aliquots incubated with 2.5 micrograms of DNA from phage SP82 for 50 minutes before being diluted and plated to measure plaque-forming centers. The two experiments shown were done on successive days.

The transfected plaque-forming units (PFU's) increase rapidly with dose, reaching a peak at 90 to 120 seconds of UV (corresponding to about 120 to 160 ergs/mm^2). In the experiment shown, the maximum increase in PFU's is by a factor of about 50 over that obtained with unirradiated cells. In most experiments, such a factor was found. In some others, the increase was much greater, but the reason was not that the peak was much higher but rather that the unirradiated cells gave a lower than typical transfection.

The concentration dependence of the transfection was also altered by the irradiation. A typical experimental result is shown in figure 2.

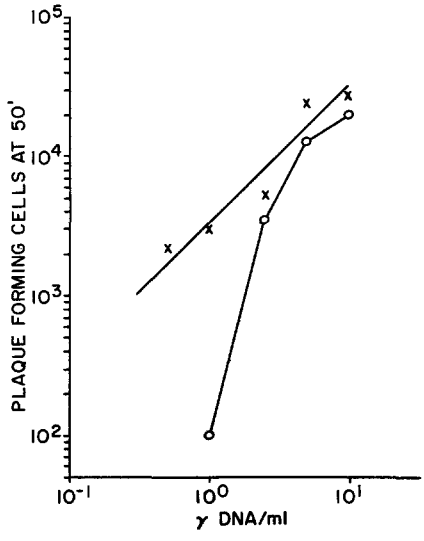

Fig. 2: The relationship between the number of transfectants and the concentration of the DNA in the incubation mixture. The response of the unirradiated cells follows a 3rd or 4th power dependence; that of irradiated cells is directly proportional to DNA concentration.
o - unirradiated cells
x - cells given about 120 ergs/mm^2 of ultraviolet light.

The response of unirradiated cells is at least third-power with respect to DNA concentration; that of the irradiated cells is higher and approximately first-power, thereby resembling closely the effect of the helper phage as measured by Green.

Finally, we measured the survival of competent B. subtilis and found a survival curve similar to but slightly steeper than that of non-competent cells; the dose to give an average of one cell-lethal-hit was 12 seconds of UV. Accordingly, peak transfection of irradiated cells is occuring after 5 to 8 cell-lethal-hits, corresponding to a cell survival of 1% to 0.05%. The competent cells are at a concentration of 1-2 X 10^8/ml, so the survivors numbered between 5 X 10^4 and 2 X 10^6 cells/ml, a number quite similar to the number of successful transfections. Further studies are needed to decide if the equality of the numbers is more than coincidental.

ACKNOWLEDGEMENTS

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REFERENCES

1. D.M. Green. (1964). J. Mol. Biol. 10,438-451. Infectivity of DNA Isolated from Bacillus subtilis Bacteriophage, SP82.

2. I. Mahler, personal communication.

3. C. Anagnostopoulos and J. Spizizen. (1961). J. Bact. 81, 741-746. Requirements for Transformation in Bacillus subtilis.

4. F.E. Young and J. Spizizen. (1963). J. Bact. 86, 392-400. Incorporation of Deoxyribonucleic Acid in the Bacillus subtilis Transformation System.