In Vitro Repair of UV-Irradiated Micrococcus luteus Bacteriophage N1 Transfecting DNA

INGA MAHLER, JEANNE GEORGE, AND LAWRENCE GROSSMAN

Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154

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Calcium-treated UV-sensitive, host cell reactivation\(^{+}\) strains of Micrococcus luteus are infected with UV-irradiated N1 DNA. In strains lacking UV endonuclease, in vitro treatment of the irradiated DNA results in transfection enhancement.

The transfection of Escherichia coli protoplasts with UV-irradiated \(\phi X174\) RF DNA (replicative form I) is subject to host cell reactivation (hcr). The survival of the irradiated, double-stranded DNA is substantially lower in UV-sensitive (UV\(^{-}\), hcr\(^{-}\) hosts than in UV\(^{+}\) hcr\(^{+}\) hosts (3, 14). Rösch et al. (15) first reported that treatment of irradiated RF DNA in vitro with crude extracts prepared from cells of Micrococcus luteus resulted in a marked enhancement in the survival of the treated DNA in hcr\(^{-}\) hosts. These results were confirmed and extended by other authors (17, 18), who reported enhancement or partial in vitro repair of irradiated \(\phi X174\) RF DNA treated with \(M.\) luteus extracts and purified T4 endonuclease V.

As shown by Taketo et al. (17), an increase in biological activity of irradiated \(\phi X174\) RF DNA occurs when this activity is assayed on the following \(E.\) coli UV\(^{-}\) mutants: \(wurA\), \(wurB\), \(wurC\), and \(wurD\). One might assume that these strains all share a common defect, i.e., the absence of a functional UV endonuclease. However, an examination of the characteristics of these mutants suggests the presence of some in vivo endonuclease activity in \(wurC\) and \(wurD\) after cellular exposure to UV light or mitomycin C (2, 5, 12, 16).

Although certain strains of \(M.\) luteus can be made competent to take up transforming DNA (6, 7), we have not succeeded in infecting such competent cells with bacteriophage DNA. However, we have found that strains of \(M.\) luteus can be transfected after treatment with calcium ions (9). Cells grown to a viable count of \(5 \times 10^{7}\) ml were passed through a membrane filter (HA, 0.45 \(\mu\) m; Millipore Corp.) and washed with 0.1 M Tris buffer, pH 7.5. The cells were resuspended in 0.2 M CaCl\(_2\) in 0.1 M Tris to 1/10 of their former volume. A 0.2-ml amount of

the cell suspension was added immediately to 10 \(\mu\)g of N1 DNA (phage N1 was a gift from J. Mayo), incubated at room temperature for 10 min, and plated by the soft agar technique (1) by using ML (Micrococcus lysodeikticus media) (11) plates and soft agar. Wild-type \(M.\) luteus (ATCC no. 4698) was used as the indicator strain.

The level of transfection of UV-irradiated N1

![Graph showing the transfection of UV-irradiated N1 DNA]({http://jvi.asm.org/})

FIG. 1. N1 bacteriophage DNA prepared by the phenol method (10) was irradiated under a 15-W germicidal lamp. The output, 42 cm from the source, was 11 ergs/mm\(^2\). DNA (10 \(\mu\)g) was added to 0.2 ml of calcium-treated cells incubated at room temperature for 10 min, and the mixture was plated by the soft agar method (1). Symbols: 0, wild type, hcr\(^{+}\) host; , DB\(_{11}\), hcr\(^{-}\) host.

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Table 1. Origin and phenotypic properties of transfected *M. luteus* strains

<table>
<thead>
<tr>
<th>Designation</th>
<th>Origin</th>
<th>UV sensitivity</th>
<th>Host cell reactivation N1 phage</th>
<th>UV endonuclease level&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Transfection enhancement by UV endonuclease&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>ATCC no. 4698</td>
<td>Resistant</td>
<td>+</td>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>DB&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Wild-type&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Sensitive</td>
<td>-</td>
<td>Not detectable</td>
<td>+</td>
</tr>
<tr>
<td>DB&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Wild-type&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Sensitive</td>
<td>-</td>
<td>Not detectable</td>
<td>+</td>
</tr>
<tr>
<td>DB&lt;sub&gt;200&lt;/sub&gt;</td>
<td>DB&lt;sub&gt;1&lt;/sub&gt; cells transformed with wild-type DNA</td>
<td>Sensitive</td>
<td>-</td>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>DB&lt;sub&gt;6&lt;/sub&gt;</td>
<td>UV irradiation of wild-type cells</td>
<td>Sensitive</td>
<td>-</td>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>DB&lt;sub&gt;a&lt;/sub&gt;</td>
<td>UV&lt;sup&gt;e&lt;/sup&gt;NI&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Sensitive</td>
<td>-</td>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>DB&lt;sub&gt;22&lt;/sub&gt;</td>
<td>UV irradiation of wild-type cells</td>
<td>Sensitive</td>
<td>-</td>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>DB&lt;sub&gt;22&lt;/sub&gt;</td>
<td>Wild-type&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Sensitive</td>
<td>-</td>
<td>Normal</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Procedures for the isolation of UV mutants has been reported (7).

<sup>b</sup> The UV endonuclease levels of *M. luteus* strains were determined by several methods (2, 4, 8).

<sup>c</sup> For transfection enhancement, an endonuclease specific for UV-irradiated DNA was isolated from *M. luteus* by using a modification of the method previously described (4). This enzyme does not adsorb to DEAE-cellulose and can be separated from another closely associated endonuclease (14).

<sup>d</sup> Received from S. Okubo.

<sup>e</sup> Mutagen used is *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chem. Co.).

Table 2. Effect of nuclease and cell extracts on infectivity of UV-irradiated N1 DNA surviving fraction

<table>
<thead>
<tr>
<th>Strains tested</th>
<th>UV-irradiated N1 DNA</th>
<th>Treatment of UV-irradiated N1 DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UV endonuclease</td>
</tr>
<tr>
<td>Wild type</td>
<td></td>
<td>7.4 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>DB&lt;sub&gt;1&lt;/sub&gt;</td>
<td></td>
<td>2.6 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>DB&lt;sub&gt;200&lt;/sub&gt;</td>
<td>3.8 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>3.9 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*</sup>N1 DNA was exposed to 450 ergs/mm<sup>2</sup> at 257 nm. The irradiated DNA was incubated (i) with 0.36 U of purified UV endonuclease for 45 min; (ii) with 10 pl of cellular extracts (4 mg of protein per ml) for 15 min; and (iii) with DNase, 2 x 10<sup>-5</sup> mg/ml in 0.01 M Tris/0.01 M MgCl<sub>2</sub>, all at 37°C.

DNA using an hcr<sup>+</sup> and an hcr<sup>-</sup> host is presented in Fig. 1. As shown previously with most coliphage DNAs, the surviving number of PFU is higher on an hcr<sup>+</sup> strain as compared with a host unable to carry out host cell reactivation.

The origin, some of the characteristics, and the transfection enhancement of several *M. luteus* hosts is shown in Table 1. Of the strains examined, only DB<sub>1</sub> and DB<sub>2</sub>, which lack UV endonuclease, showed enhanced phage production after UV-irradiated N1 DNA had been reacted with highly purified UV endonuclease. The other five hcr<sup>-</sup> mutants possess normal levels of this enzyme and contain single-strand breaks in vivo after cellular exposure to UV irradiation (8, 13). Presumably, they are capable of carrying out the incision hydrolysis provided by external UV endonuclease treatment.

Figure 2 shows the extent of the enhancement obtained with the enzyme-negative DB<sub>1</sub> strain. It is clear (Fig. 2) that no enhancement occurs when a UV<sup>+</sup> strain is used. However, with *M. luteus*, unlike *E. coli*, no transfection enhancement results when the host is UV<sup>-</sup> and hcr<sup>-</sup> but possesses normal levels of UV endonuclease. Thus, the enzyme-stimulated enhancement of irradiated N1 DNA can be observed only in those strains in which UV sensitivity can be
correlated with an absence of functional UV endonuclease.

As shown in Table 2, transfection enhancement can be achieved with either purified enzyme, crude extracts prepared from a wild-type strain, or extracts prepared from a UV− but UV endonuclease + strain. The enhancement phenomenon in *M. luteus* appears to be as specific as it is in *E. coli* (17)—no enhancement results when irradiated DNA is treated with either pancreatic (Worthington Biochemical Corp.) DNase or an extract prepared from an enzyme-defective strain.

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