Spontaneous zygogenesis in *Escherichia coli*, a form of true sexuality in prokaryotes

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A new type of mating, differing from classic conjugation and previously observed in a certain strain of *Escherichia coli* K-12, has also been found in strains derived from ordinary F⁻ cells of *E. coli* K-12 exposed to an exogenous factor originating in an *E. coli* clinical isolate. Immunofluorescence and electron microscopy after single and double labelling of DNA were used to produce evidence in favour of a novel mating mechanism by cell contact at the poles of the bacterial rod. These findings are supported by genetic analyses indicating complete genetic mixing. Unstable complementing diploids were formed, which throw off phenotypically haploid cells, of which some showed a parental phenotype and some were true genetic recombinants. Recombination was observed even when one parent was a UV-inactivated F⁻ RecA⁻ strain. The name 'spontaneous zygogenesis' (Z-mating, for short) is proposed for this kind of mating.

INTRODUCTION

Bacteria are classically described as haploid organisms, but are capable of a form of sexual reproduction known as conjugation, in which there is unidirectional transfer of a copy of the replicating DNA molecule from a donor cell to a recipient cell. Chromosome transfer is usually partial and the transferred donor fragment is unable to replicate unless it is included in a plasmid. Rec-dependent recombination allows inheritance of the transferred alleles while not altering the haploid status (Hayes, 1968; Finnegan; 1976; Porter, 1988).

However, the notion of diploidy in bacteria has appeared occasionally in the literature. Diploidy in *Escherichia coli*, first proposed by Lederberg in 1949 to account for various observations on prototrophs obtained in conjugation experiments with *E. coli* K-12, was confirmed by analyses of clonal pedigrees from isolated single cells (Zelle & Lederberg, 1951). More recently, diploidy has been achieved artificially, by polyethylene-glycol-induced protoplast fusion in various bacteria: *Bacillus megatherium* (Fodor & Alfoldi, 1976), *Bacillus subtilis* (Scheafer et al., 1976), *Providencia alcalifaciens* (Coetzee et al., 1979) and even *Streptomyces* spp. (Hopwood et al., 1977). In *B. subtilis* exfusants, diploidy is most often non-complementing, with one parental chromosome remaining in an inactive state. Non-complementing diploids of this kind show occasional phenotype switching from one parental type to the other (Hotchkiss & Gabor, 1980; Grandjean et al., 1996).

About a decade ago, an *E. coli* K-12 derivative was isolated (MG352), which displayed surprising behaviour (Gratia, 1994). On the one hand it could mate with F⁻ bacteria, although it descended from an F⁻ strain. On the other hand it could exist in two forms with different bacteriophage resistance profiles. The zygotes formed in heterogenic matings, isolated as colonies expressing dominant alleles of both parental strains, were able to retain this complementing phenotype through several rounds of purification on the initial selective medium. A pedigree analysis of one biparental colony yielded MG388, capable of switching from one parental phenotype (all markers simultaneously) to the other. A mutation could occur in either form of the strain without affecting the other.

Strains MG352 and MG388 were interpreted as being capable of mating spontaneously with other bacteria and as carrying two co-replicating genomes, only one being expressed in a given form. They would behave like the *B. subtilis* exfusants described above: sooner or later the expressed parental genome would switch off, but would remain in the cell to be switched on again, as evidenced by a subsequent phenotype switch (Gratia, 1994). These strains have been used in successive crosses generating other strains displaying this same mating behaviour.

It is important to confirm and clarify the conclusions of the Gratia (1994) paper. The aim of the present work is to complete the analysis of this new kind of mating and of its products exhibiting parental, recombinant or mixed phenotype. It will be useful to decipher how the
phenomenon is generated in Szp⁺ strains, such as MG352 or MG388. The results suggest the involvement of an infectious mating factor. They also allow us to rule out artefacts such as cross-feeding between sticky cells. Further information on the mode of this putative new zygote-forming process emerges from a microscopical approach using immunocytochemical methods. In the original report, the process was coined ‘spontaneous zygogenesis’ (Z-mating for short) will be used (according to a suggestion made by David Thaler, Rockefeller University, USA).

**METHODS**

**Bacterial strains.** Table 1 lists the *E. coli* K-12 strains used as Z-mating-promoting (Szp⁺) clones and the strains used as partners in the crosses considered here, with their sources. Besides F⁻ strains, the Hfr P4X, in which the origin of counterclockwise transfer is located at 7 min between lac and pro, was also used in comparative experiments. Strains carry various auxotrophic mutations that do not revert under the conditions used (less than 10⁻⁷ per plated c.f.u.). MG392 is a His⁺ recombinant derived from a NalA⁻ mutant of the F⁻ strain 1485 after a cross with the Szp⁺ strain MG388, itself the result of various recombination events from MG352 (Gratia, 1994). It behaved like MG352 or MG388 in the various subsequent crosses with F⁻ strains. Among other Szp⁺ strains, MG401 and MG402 were created from scratch by exposing cells of F⁻ strains MG301 and A69, respectively, to lysates of an another selective medium was also used, LCA, i.e. a minimal medium supplemented with 5 g lactose 1⁻¹ (substituted for glucose), auxotrophic mutants of this strain (data not shown). The experiment was carried out as follows. *E. coli* strain 84SV cells (10⁶) were starved for 1 h in 100 mM MgSO₄ and exposed to various doses of 260 mJ UV. After the addition of an equal volume of Lennox broth and a 2.5 h incubation, lysis was completed by the addition of chloroform. Part of the lysates was treated with 10 µg DNase ml⁻¹ (pancreatic DNase I; Boehringer) for 20 min at 37 °C and centrifuged at high speed (1 h at 20000 g). Samples of crude lysates (treated with 0–1 % trypsin for 20 min at 37 °C to destroy colicins) and of washed pellets of DNase-treated phage preparations were allowed to adsorb for 20 min onto 10⁻⁶ exponentially growing bacteria to be transformed. Szp⁺ clones, cleared of phage by repeated washings, were detected among the many non-transformed bacteria by mixing them at various concentrations with genetically labelled F⁻ strains; samples of the mixtures were plated on GDA medium (Gratia & Deschuyteneer, 1998; see below), where the parental types could be distinguished on the basis of their ability (yellow colonies) or inability (white colonies) to use lactose. When sectored white and yellow colonies appeared (these being suggestive of a phenotype switch), bacteria isolated from them were tested for the Szp⁺ character.

**Media and chemicals.** Lennox broth (LB; Gibco-BRL) was the main culture medium; blood-agar base (Gibco) was the nutrient agar (NA). The minimal salts medium (MA) used to select prototrophic products of Z-mating was composed of Simmons citrate agar (Difco) supplemented with glucose (2 g l⁻¹) and thiamine (1 µg ml⁻¹). For some tests, MA was supplemented with 200 µg streptomycin sulphate ml⁻¹ (Gibco) or with 20 µg nalidixic acid ml⁻¹ (sodium monohydrate; ICN Biomedicals). In several crosses, the parents differed in their ability to ferment lactose and required substances not included in casein acid hydrolysate; in such cases another selective medium was also used, LCA, i.e. a minimal medium supplemented with 5 g lactose 1⁻¹.

**Table 1. *E. coli* K-12 strains used**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Conjugational phenotype*</th>
<th>Source or derivation†</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG301</td>
<td>lacY metB rpsL</td>
<td>F⁻</td>
<td>ED8654 constructed by W. Brammar from WA803; gift of B. Hohn</td>
</tr>
<tr>
<td>1485/Nx</td>
<td>trpA trpB his nalA</td>
<td>F⁻</td>
<td>N. Franklin; NalA⁻ mutant</td>
</tr>
<tr>
<td>GMS343</td>
<td>lacY galK ardD manA argG mtl xyl rpsL</td>
<td>F⁻</td>
<td>G. Novel; CGSC 5496</td>
</tr>
<tr>
<td>A69</td>
<td>Δ(lacZ proB) rpsL</td>
<td>F⁻</td>
<td>Derived from P90C (Cupples et al., 1990); from R. D’Ari to D. S. Thaler</td>
</tr>
<tr>
<td>KL262</td>
<td>galK pyrD trpC tyrA recA thyA mtl rpsL malT xylA mtlA thi</td>
<td>F⁻</td>
<td>B. Low; CGSC 4322</td>
</tr>
<tr>
<td>P4X/Nx</td>
<td>argH nalA thi</td>
<td>Hfr‡</td>
<td>C. Godard</td>
</tr>
<tr>
<td>MG352</td>
<td>lacY metB rpsL</td>
<td>Szp⁺</td>
<td>Gratia (1994)</td>
</tr>
<tr>
<td>MG392</td>
<td>trpAB nalA</td>
<td>Szp⁺</td>
<td>J. P. Gratia (unpublished); recombinant from cross between 1485/Nx and MG388</td>
</tr>
<tr>
<td>MG401</td>
<td>lacY metB rpsL</td>
<td>Szp⁺</td>
<td>This study; MG301 exposed to 84SV lysate</td>
</tr>
<tr>
<td>MG402</td>
<td>Δ(lacZ proB) rpsL</td>
<td>Szp⁺</td>
<td>This study; A69 exposed to 84SV lysate</td>
</tr>
<tr>
<td>84SV</td>
<td></td>
<td>(Szp⁺)§</td>
<td>J. P. Gratia (unpublished); colicinogenic/lysogenic <em>E. coli</em> clinical isolate</td>
</tr>
</tbody>
</table>

*The F⁻ non-Szp⁺ strains showed a normal efficiency of plating of phage T7 and were unable to mate with each other.
†CGSC: *E. coli* Genetic Stock Center, Yale University, New Haven, CT, USA; W. Brammar, University of Edinburgh; R. D’Ari, Institut Jacques Monod, Paris, France; N. Franklin, Stanford University, USA; B. Hohn, Ciba-Geigy, Basel, Switzerland; G. Novel, IRBA, Université de Caen France; D. S. Thaler, Rockefeller University, USA.
‡Origin of transfer at 7 min between lac and pro; counterclockwise orientation of transfer.
§According to unpublished experiments (see Methods).
100 mg casein acid hydrolysatel−1 (‘peptone 5’; Gibco) and 1 μg thiamine ml−1. LUTA, i.e. Simmons citrate agar supplemented with 5 g lactose l−1 and 40 μg UT mixture (uracil, thymine, tryptophan, tyrosine and thiamine) ml−1 was used in experiments involving KL262 bacteria which are Lac+ and require these substances. In media used to select Z-mating products formed from A69 or its derivative MG402, arginine was always present to avoid suppression of the pro mutation, as recommended by D. Thaler (personal communication). M9 buffer (Miller, 1972) was commonly used for starving, mixing and diluting cell suspensions. GDA medium was used to select candidate Szp+ strains after exposure to the 845V lysate. M9, supplemented with 1·6 g Gibco agar l−1, 2·5 g lactose l−1, 0·1 g glucose l−1, 1 g ‘peptone 5’ l−1, 0·25 g yeast extract l−1, 0·5 mM MgSO4 and 65 mg bromothymol blue l−1, was adjusted to pH 7·2 with approximately 5 mM NaOH. MacConkey agar base (Difco), supplemented with a solution of sugar (lactose, galactose, mannitol or mannose) at a final concentration of 1 % (w/v) at the time of pouring, was used in fermentation tests.

Labelling of the DNA in the thymine-dependent strain KL262 was performed by adding 25 μg 5’-bromo-2-deoxyuridine ml−1 (BrdU; Boehringer Mannheim) for 1–2 h to the culture medium.

**Mating experiments and analysis of Z-mating products.** In the mating experiments described here, the chosen parents bore different auxotrophic markers to allow selection for the disappearance of all or some of the auxotrophies using the appropriate medium described above (the formation of colonies on such media after mixing of two different auxotrophs was also routinely used to identify new Szp+ strains). One parent was chosen as resistant to nalidixic acid or sensitive to UV, and its partner resistant to streptomycin.

Cultures of parental strains growing exponentially in LB were diluted in M9 and mixed at a cell density that did not exceed 2 × 10^7 c.f.u. ml−1. Mixtures were incubated at 37 °C under gentle linear rocking. The incubation time was 20 min before plating on the selective medium, MA, LCA or both. In time experiments cultures were vortexed before plating.

In experiments where F− RecA− mating partner KL262 was UV-irradiated, a UV dose corresponding to a survival of less than 1 × 10^−4 c.f.u. per plated RecA− cell was applied to the F− RecA− bacteria before they were mixed with the Szp+ partner. In such experiments, LUTA, permissive for KL262, was also used.

For the purification of isolates to be analysed under conditions preventing any cross-feeding of sticky cells, bacteria picked up from the selected colony were suspended in 10 ml M9 and these suspensions were strongly vortexed for 30 s. Furthermore, samples were streaked onto the medium used in the initial selection. Two to three colonies per isolated clone were picked up and incubated for 90 min in LB. Cells were allowed to divide for one to two generations up to a cell density of at most 10^9 c.f.u. ml−1, so that possible adhesion between separated cells was limited. In some cases, the composition of these dilute suspensions had to be realigned when changes had occurred within the population. Examples are given in Table 3, where mixed isolates were suspected (P1/2 or P/R). Suspensions were then restreaked onto permissive NA and, after incubation, several colonies were again picked up. Initial suspensions in LB or colonies of the supernumerary step on NA were stabbed onto NA plates with grids, 1 inch apart. After overnight incubation, those plates were replicated onto media appropriate for marker testing (response to an amino acid, fermentation of a sugar, sensitivity to streptomycin, nalidixic acid or UV).

**Cytochemical labelling of DNA, immunofluorescence and electron microscopy.** Small aliquots of overnight cultures of the bacteria to be examined were incubated in M9 with added requirements: 0·2% (w/v) glucose, 1 g ‘peptone 5’ l−1 and 1 μg thiamine ml−1 for the Szp+ strain (MG401) and for the isogenic F− non-Szp+ used as a control (MG301); glucose and all required substances, including thymine, for brief exponential growth of F− RecA− bacteria (strain KL262). The latter culture was centrifuged and incubated in the same medium deprived of thymine (thymine starvation) until the bacteria began to elongate. BrdU was then added to the starved-cell culture at 25 mg l−1 for incorporation into the DNA in place of thymine. As soon as the bacteria were forming long rods or filaments (this was checked under the light microscope), they were centrifuged to eliminate BrdU and mixed for 5 min with either MG401 cells or MG301 cells in 100 ml M9 (final cell density: 1 × 10^9–2 × 10^9 c.f.u. ml−1). The KL262 cell control was simply diluted to this density. The presence of long rods (whose formation was caused by thymine starvation) made it easier to distinguish the two strains in mixtures and also to observe the cells under the light microscope.

For immunofluorescence detection, smears of the different bacterial strains and mixtures were made on slides and fixed for 10 min at −20 °C in acetone, rehydrated, rinsed in water and plunged into boiling water for 5 min before being rapidly placed on ice. The slides were treated for 30 min at 37 °C with an anti-BrdU mAb (Roche Diagnostics) diluted 1:100 in PBS (0·14 M NaCl, 6 mM Na2HPO4, 4 mM KH2PO4) containing normal goat serum (NGS) diluted 1:50 and 0·2% (w/v) BSA (type V; Sigma). After rinsing with PBS containing 1% BSA (w/v), the slides were incubated for 30 min at 37 °C with FITC-conjugated goat anti-mouse antibody (Sigma) diluted 1:100 in PBS containing 0·2% BSA. After several rinses, the slides were mounted with Citifluor AF1 (Agar Scientific).

For immunoelectron microscopy, the cultures and mixtures were centrifuged to form pellets. The latter were fixed for 90 min in a mixture of 4% (w/v) formaldehyde and 0·05% (w/v) glutaraldehyde in 0·1 M Sörensen’s buffer (pH 7·4). After fixation, the cells were washed in Sörensen’s buffer, dehydrated through graded ethanol solutions and finally embedded in Epikote 812. The resin was allowed to polymerize for 2–3 days at 40–45 °C. Ultrathin sections were placed on boiling water for 5 min before being rapidly transferred onto water at 2 °C. For labelling, the sections were incubated for 30 min in PBS (pH 7·2) containing NGS diluted 1:30 and 1% BSA, then rinsed in PBS containing 1% BSA. In the next step, the sections were incubated for 4 h at room temperature with an anti-BrdU mAb, diluted 1:100 in PBS (pH 7·2) containing NGS diluted 1:50 and 0·2% BSA. After washing with PBS (pH 8·2) containing 0·2% BSA, the sections were incubated at room temperature for 2 h with goat anti-mouse IgG coupled to 5 nm colloidal gold particles diluted 1:40 in PBS (pH 8·2) containing 0·2% BSA. After rinsing in PBS (pH 8·2) containing 1% BSA, then in deionized water, they were mounted on collodion-coated nickel grids and stained with uranyl acetate and lead citrate before examination in a JEOL CX100 II electron microscope at 60 kV.

To visualize the general distribution of DNA in the bacteria, we used the *in situ* terminal deoxynucleotidyl transferase-immunogold labelling procedure as described previously (Thiry, 1992).

**RESULTS**

**Typical Z-mating experiments**

In Table 2 we report crosses between Szp+ strains (either the streptomycin-resistant, methionine-requiring MG352 or the nalidixic acid-resistant, tryptophan-requiring MG392, derived from MG388 as described by Gratia, 1994) and
Table 2. Comparative crosses between Szp<sup>+</sup> and F<sup>-</sup> or Hfr non-Szp<sup>+</sup> strains

<table>
<thead>
<tr>
<th>Cross (P1 × P2)</th>
<th>Time</th>
<th>No. of plated minority parent P1 colonies (c.f.u.)*</th>
<th>No. of prototrophic colonies on:†</th>
<th>Segregation‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MA</td>
<td>MA&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>MG301 (P1) (F&lt;sup&gt;-&lt;/sup&gt; non-Szp&lt;sup&gt;+&lt;/sup&gt; metB lacY rpsL) x 1485/Nx (P2) (F&lt;sup&gt;-&lt;/sup&gt; trpAB his nalA)</td>
<td>20 min</td>
<td>8·1 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0 0 0 – – – –</td>
<td>– – – –</td>
</tr>
<tr>
<td>MG352 (P1) (Szp&lt;sup&gt;+&lt;/sup&gt; metB lacY rpsL) x 1485/Nx (P2)</td>
<td>20 min</td>
<td>7·9 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>52 68 0 – –</td>
<td>31 18 8</td>
</tr>
<tr>
<td></td>
<td>20 min</td>
<td>7·2 × 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>4 13 0 0</td>
<td>8 4 2</td>
</tr>
<tr>
<td>MG401 (Szp&lt;sup&gt;+&lt;/sup&gt; metB lacY rpsL) x 1485/Nx</td>
<td>20 min</td>
<td>6·1 × 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>9 29 0 –</td>
<td>19 10 7</td>
</tr>
<tr>
<td></td>
<td>1 min</td>
<td>8·6 × 10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>17 – 0 0</td>
<td>9 3 4</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>3·9 × 10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>185 – 0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>MG392 (Szp&lt;sup&gt;+&lt;/sup&gt; trpAB nalA) x GMS343 (F&lt;sup&gt;-&lt;/sup&gt; argG aroD rpsL)</td>
<td>20 min</td>
<td>7·9 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>101 – 0 0</td>
<td>cf Table 3</td>
</tr>
<tr>
<td>P4X/Nx (Hfr argH nalA) x MG301 (F&lt;sup&gt;-&lt;/sup&gt; metB lacY rpsL)</td>
<td>20 min</td>
<td>1·1 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>26 – 25 0</td>
<td>0 1 0</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>6·4 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>58 – 38 11</td>
<td>0 40 0</td>
</tr>
<tr>
<td>P4X/Nx (Hfr argH nalA) x MG352 (Szp&lt;sup&gt;+&lt;/sup&gt; metB lacY rpsL)</td>
<td>20 min</td>
<td>1·8 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>36 – 2 0</td>
<td>1 10 7</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>2·0 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>59 – 7 2</td>
<td>3 16 9§</td>
</tr>
</tbody>
</table>

*Total amount of the minority parent P1 (Szp<sup>+</sup> or Hfr) c.f.u. spread on three plates of selection medium, each plate having received less than 3 × 10<sup>5</sup> c.f.u.
†MA<sup>+</sup>, MA plus 0·1 % LB; MA + S, MA plus 200 µg streptomycin ml<sup>–1</sup>; MA + Nx, MA plus 20 µg nalidixic acid ml<sup>–1</sup>.
‡Examination of reisolates: P1/2, biparental subclones, phenotypically unstable and yielding parental types; R<sup>+</sup>, prototrophic recombinants stably growing on MA or Met<sup>+</sup> His<sup>+</sup> recombinants growing on MA + tryptophan, having recombined a resistance to either streptomycin or nalidixic acid; L<sup>+</sup>S<sup>R</sup>, Lac<sup>+</sup> RpsL<sup>+</sup> recombinants, prototrophic or not, fermenting lactose on McConkey + 500 µg streptomycin ml<sup>–1</sup>.
§Among the total number of tested isolates (46), 32 were composed partly or completely of RpsL<sup>+</sup> cells and 22 comprised Lac<sup>+</sup> cells.
other strains with reciprocal combinations of antibiotic-related phenotypes. Although colonies appeared on MA at a noticeable frequency (up to approx. 1-5 per 10⁴ cells of the minority parent MG352 or MG392), not a single colony appeared when either antibiotic was included in the selective medium (Table 2, rows 2–4).

In a classic conjugation experiment it would be very odd for a recipient strain not to retain its streptomycin resistance marker [streptomycin is commonly used in conjugation experiments for counter-selection of an RspL⁺ donor strain (Miller, 1972; Finnegan, 1976)], nor to incorporate the nalidixic acid resistance marker of the donor strain if the donated DNA fragment is long enough [nalidixic acid can be used to interrupt conjugation without preventing the formation of recombinant colonies (Zipkas & Riley, 1976)] and vice versa.

The prototrophs formed through Z-mating behaved as if they were zygotes expressing the dominant alleles of both parental genomes and were sensitive to both antibiotics because the resistance mutations, rpsL (Lederberg, 1951) and nalA (Hane & Wood, 1969), are recessive. This view has been challenged, however, on the ground that extreme stickiness of Szp + cells, with cross-feeding between cells, could give the same result. However, if the explanation is that cells simply stick together, genetic recombination of their genomes should not happen. Now, recombinants did appear among reisolates, some of which exhibited the Lac + phenotype of one parent and the resistance to streptomycin of the other parent (Table 2, last column). This shows that the antibiotic resistance mutations were present in the initial prototrophic suspected zygotes, and their expression in subclones displaying a recombinant phenotype establishes them as true genetic recombinants, in contrast to the doubly sensitive prototrophs from which they derived.

Hfr × F⁻ crosses provided instructive and complementary information.

(1) No transfer gradient was observed when mating between the Hfr donor and F⁻ Szp + partner was interrupted at different times, as briefly mentioned by Gratia (1994). As shown in Table 2 (penultimate row), when Hfr strain P4X (argH, lac + rpsL +), in which the origin of transfer is located between lac and pro, was crossed with F⁻ strain MG301 (metB lacY rpsL), transfer of metB + was about four times lower after 20 min than after 1 h (the prototrophic colonies were then twice as numerous for half the amount of plated bacteria). On the contrary, the number of colonies on plates smeared with the same amount of mixed Hfr and Szp + MG352 was not very different, whatever the co-incubation time before plating of vortexed samples (Table 2, last row).

(2) Selection on medium MA containing either streptomycin or nalidixic acid did not prevent formation of colonies, at least those that were expected to result from partial chromosome transfer by the Hfr donor, i.e. at relatively high frequency when P4X was crossed with the F⁻ parent and only for a minor part of the conjugants formed at the expense of the Szp + partner.

(3) As expected for the cross of P4X with the F⁻ non-Szp + strain MG301, Met + Arg + recombinants were formed at a low frequency on account of the close location of parental mutations argH and metB limiting the area of crossing-over, and the reisolates (referred to in the last column of Table 2) were stable. Among the 58 examined colonies, 10 inherited the streptomycin sensitivity allele of the donor, but none of them received the distal Lac + marker. The situation was entirely different when P4X was crossed with the Szp + MG352. Transcomplementation of metB and argH by wild-type alleles on respective chromosomes gathered in the Z-mating product was suitable for selecting transient complementing diploids. Accordingly, the colonies formed were phenotypically unstable. Among the 46 tested reisolates, 29 contained bacteria having inherited the streptomycin-sensitivity allele, and up to 22 were found to contain Lac + cells (which were not revertants, as the mutation was almost non-reverting in all F⁻ strains used, including MG352).

Z-mating, a new property acquired in E. coli K-12

The initial Szp + strain, MG352, was isolated during work where the K-12 F⁻ strain MG301 was exposed to phages that had passed through several strains, the first one being a mutant selected for its resistance to the colicins produced by 84SV (J.-P. Gratia, unpublished). One can suggest that MG352 might have resulted from exposure of MG301 to an exogenous genetic factor, initially released by strain 84SV and passed through several strains. This led to an attempt at isolating new Szp + strains by exposing F⁻ strains, e.g. auxotrophic lactose-non-fermenting strains MG301 and A69, to various amounts of different preparations of UV-induced 84SV lysates (see Methods). Infected bacteria were mixed with tryptophan-requiring lactose-fermenting strain 1485/Nx. Diluted samples plated on GDA showed a very low number of sectored colonies (at most 0-1% among Lac⁻ colonies of the minority parent). To check the transforming effect of the lysate, bacteria out of three sectored colonies from each mixture were plated on GDA again. The percentage of sectored colonies was then increased, i.e. 5% of the Lac⁻ colonies or even more. Lac⁻ bacteria issued from such sectored colonies were tested for their ability to give rise to prototrophic colonies with 1485/Nx on MA or LCA. Two to four isolates per series of 6–12 tested mixed clones proved positive. The new Szp + strains, MG401 and MG402, were obtained in this way and were indistinguishable from MG301 and A69, from which they respectively derived, except that they were endowed with the property to generate the formation of prototrophic colonies upon mixing with various F⁻ auxotrophic bacteria, including 1485/Nx.

Besides colicins, the 84SV lysate contained plaque-forming phage. The latter did not appear to be responsible for the
conversion to the Szp\(^+\) status. Indeed, MG401 and MG402 were unable to produce this phage and remained sensitive to it, which means that they were not lysogenized and rules out them being phage-resistant mutants. Conversely, lysogenization of various sensitive bacteria with this phage appeared not to confer the ability to promote Z-mating: more than 60 isolates, which resulted independently from infection of MG301 and other F\(^-\) bacteria (e.g. 1485) with the phage contained in 25 plaques, tested negative for this ability. Crude lysates of 84SV and DNase-treated preparations were both efficient in the conversion to the Szp\(^+\) state. Hence, the Z-mating determinant present in the 84SV lysate might be carried by defective phage particles, not by the plaque-forming phage or by free DNA. Protease-sensitive proteins were not involved either, since trypsin or papain was used to avoid killing of the bacteria by the colicins present in the crude lysates.

MG401 was compared to non-Szp\(^+\) MG301, from which it derived, and to MG352 in crosses with F\(^-\) nalidixic-acid-resistant strain 1485/Nx (Table 2). Plating of the mating mixtures at the expense of Szp\(^+\) MG401, unlike the F\(^-\) non-Szp\(^+\) initial strain, MG301, gave rise to colonies on MA. They behaved like MG352 and could give rise to prototrophic colonies at an even higher frequency, up to \(5 \times 10^{-4}\) per c.f.u. of the minority parent on MA (and up to \(2 \times 10^{-3}\) on LCA; data not shown). Again, in these crosses, no colony appeared on MA supplemented with streptomycin or nalidixic acid, even when the parental strains were co-incubated for 1 h before plating with the added antibiotic. This and the behaviour of the Z-mating products make the possibility plausible that the new strains were Szp\(^+\), like MG352 and derivatives, including MG392.

### Table 3. Subclonal analysis of products selected for complementation after Z-mating

<table>
<thead>
<tr>
<th>1. Cross: P1 (MG392: Szp(^+); Trp(^-) Nal(^-)) x P2 (GMS343: F(^+); Arg(^-) Aro(^-) Lac(^-) Gal(^-) Man(^-) Mtl(^-) RpsL(^-))</th>
<th>MA(^*)</th>
<th>LCA(^*)</th>
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<tbody>
<tr>
<td>2. Selection:</td>
<td></td>
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<tr>
<td>3. Marker scoring of: lacY galK trp aroD manA nalA argG rpsL mtl</td>
<td>Frequency(^\dagger)</td>
<td></td>
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<td><em>(8)(\dagger)</em></td>
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<table>
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<tr>
<th>Type isolate($)</th>
<th>P1</th>
<th>P2</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>P1/2</th>
<th>P1</th>
<th>P2</th>
<th>R8</th>
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<th>P/R</th>
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<td>Frequency(^\dagger)</td>
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<td>MA(^*)</td>
<td>LCA(^*)</td>
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<td>+/−/+</td>
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<td>−/+</td>
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<tr>
<td>Frequency(^\dagger)</td>
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<tr>
<td>MA(^*)</td>
<td>LCA(^*)</td>
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<tr>
<td>Frequency(^\dagger)</td>
<td>0.19</td>
<td>&lt;0.02</td>
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</table>

\*See text for selection and subcloning.
\(^\dagger\)Frequency expressed as a fraction of the total number of total isolates.
\(\dagger\)Loci are in the order of their positions (indicated in minutes) according to Bachmann (1987).
\$P1 and P2, the two parental types: R, stable recombinants; P1/2, biparental subclones; P/R, unstable subclones yielding a parental and a recombinant type.
| Marker of parent P1; | Marker of parent P2. | Marker of parent P2.

### Genetic recombination

Crosses between Rec\(^+\) partners (some 326 have been performed) consistently yielded evidence of recombination and the results depicted in Table 2 (last column) are not exceptional. Subclonal analysis was performed in the cross between MG392 and the streptomycin-resistant F\(^-\) strain GMS343 marked at various loci throughout the whole chromosome (Table 3). This F\(^-\) partner cannot use lactose and requires shikimic acid and arginine; Z-mating products could be selected on LCA for use of lactose as well as for biosynthesis of shikimic acid, absent from the acid
hydrolysate of casein. About three subclones of each initial clone purified on selective medium, MA or LCA, were subjected to scoring procedures described in Methods. After the resolation step on NA, 72 subclones derived from 39 colonies isolated on MA, and 48 subclones derived from 21 colonies isolated on LCA were tested for parental markers. They appeared to fall into three categories: those whose descendants all displayed one parental type (P1 or P2), those whose descendants all showed a recombinant type (R1–R7) and ‘mixed subclones’ whose descendants displayed different phenotypes: either the two parental types (P1/P2) or one parental and one recombinant type (P/R). These mixed subclones were restreaked on NA and the resulting single colonies were retested. As shown in the last rows of the table, the ‘subsubclones’ fell into the following categories: parental P1 or P2, or recombinant (R8, R9) for subclones of the ‘P1/P2’ subclones, and P2 or a new recombinant phenotype (R10) for subclones of the P/R subclones. It should be stressed that some recombinants might arise through remating within mixed subclones.

The results in Table 3 are incompatible with unidirectional transfer of a DNA segment short enough to be transferred during a 20 min co-incubation of parental cells (with 1 min vortexing afterward). In classic conjugation, total unidirectional chromosome transfer takes about 100 min and is rare because conjugation is frequently interrupted. Assuming that the F' strain was the ‘recipient’ of such a transfer, it should have incorporated markers corresponding to only a short donor fragment starting at the origin of transfer. In actual fact, several recombinants show incorporation of ‘donor’ markers scattered from 8 min to 89 min on the E. coli chromosome. In some cases, ‘donor’ markers are the majority. What is more, colonies were obtained on MA even when the co-incubation time was reduced to 1 min prior to vortexing in similar experiments (Table 2, row 5). In the light of these data, it seems much more reasonable to assume that there was no donor and no recipient, but that at some point both genomes were present in the same cell at the same time and thus free to recombine at any location.

Does extreme stickiness plus post-plating conjugation account for the results? This possibility is unlikely and to counter it we used two complementary approaches. In a first series of experiments, an F' RecA - strain was used, so as to preclude unidirectional transfer of a segment through Hfr-mediated conjugation. In one of these experiments, this strain was inactivated prior to plating, so that it could not cross-feed. Afterwards, mating mixtures were examined by immunofluorescence and electron microscopy to visualize mating more directly.

**Rescue of a partner inactivated prior to mixing, an argument for cytoplasmic contact between mated cells**

In plasmid-mediated conjugation, crosses between an Hfr Rec + and an F' RecA - partner are infertile (donor markers introduced into the recipient by Hfr-type transfer cannot integrate into the chromosome for lack of a functional Rec system), unless the selected donor markers is/are transferred into the RecA + recipient on an F' plasmid (Low, 1968). In contrast, crosses between an Szp + strain and a RecA + strain are fertile: MG388 derives from such a cross (Gratia, 1994). This is confirmed by the experiment depicted in Table 4, where the Rec - F' strain KL262 was crossed with the Rec + Szp + strain MG402. Such a cross gave rise to colonies displaying the Lac - Trp + Pyr + Thy + phenotype. They were formed on LCA at quite a high frequency: \(1.8 \times 10^{-3}\) per Szp + cell (Table 4, row 4). The number of KL262 c.f.u., however, appeared to drop somewhat upon mixing with MG402, possibly because of cell aggregation.

But this experiment provides additional important information. Rows 7–10 of Table 4 show what happened when the RecA + partner KL262 was exposed to UV light prior to mixing with the Szp + strain [the inability of KL262 cells to effect recombination is due to a defect in DNA repair and is thus associated with high sensitivity to UV (Clark, 1973)]. Controls included KL262 irradiated alone (row 5) and irradiated KL262 mixed with the non-Szp + strain A69 (bearing the same markers as MG402). Quantitative analysis of the results, which were the same whether KL262 was irradiated prior or at any time during co-incubation, yielded interesting findings.

(i) Colonies appeared on LCA only when MG402 was present in the plated cell suspension. Their number increased in parallel with the concentration of MG402 in the mating mixture (Table 4, columns 7–9; rows 8–10 versus rows 5–7).

(ii) The survival rate of unmixed irradiated KL262 was only \(1.9 \times 10^{-5}\) (Table 4, column 5, row 5), but when KL262 was mixed with MG402, colonies appeared on LUTA at a rate up to 50 times higher. Their number increased with the amount of MG402 in the mating mixture (column 5, rows 8–10). In contrast, the A69 + KL262 control simply mirrored the survival rate of KL262 (column 5, rows 6–7). Increasing the amount of A69 did not increase KL262 survival. Strain MG402 would thus appear to ‘rescue’ its irradiated partner (or at least the genome of the latter) through Z-mating. (Mg401 gave rise to similar observations but MG402 was preferred in this study on account of its non-reversible lacZ-proB deletion.)

It should be noted that on LUTA the cells of non-irradiated KL262, the survivors of irradiation, and KL262 cells mixed with A69 cells all formed large colonies of uniform size in less than 24 h. The colonies formed by the c.f.u. of the Z-mating mixture were of variable size after a longer period of incubation. It is also important to note that KL262-like cells contained in colonies appearing on LUTA were indistinguishable from the KL262 parent, even with regard to UV sensitivity. We interpret this to mean that rescue was due to the early and transient presence of a functional Rec system capable of repairing the DNA damage caused...
Table 4. Quantitative analysis of crosses between Szp<sup>+</sup> Rec<sup>+</sup> MG402 and F<sup>−</sup> RecA<sup>−</sup> KL262

Rescue of UV-inactivated KL262 cells by the Rec system of MG402.

<table>
<thead>
<tr>
<th>Sample</th>
<th>1. No. of P1 cells&lt;sup&gt;*&lt;/sup&gt;</th>
<th>2. No. of P2 cells&lt;sup&gt;*&lt;/sup&gt;</th>
<th>3. No. of colonies on LUTA†</th>
<th>4. No. of colonies P2-like cells (4/3)</th>
<th>5. Frequency of P2 rescue per P1‡</th>
<th>6. Frequency of P2 rescue per P1‡</th>
<th>7. No. of clones with prototrophs§</th>
<th>8. Ratio (7/4)</th>
<th>9. Ratio (7/2)</th>
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</thead>
<tbody>
<tr>
<td>1. A69</td>
<td>6·1·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>2. MG402</td>
<td>6·3·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3. A69 + KL262</td>
<td>1·4·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5·0·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>4·7 (±0·5)·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>9·4·10&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>&lt;1·10&lt;sup&gt;−3&lt;/sup&gt;</td>
<td>–</td>
</tr>
<tr>
<td>4. MG402 × KL262</td>
<td>1·6·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5·0·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3·4 (±0·6)·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>6·8·10&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>–</td>
<td>2·9·10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>8·5·10&lt;sup&gt;−4&lt;/sup&gt;</td>
<td>1·8·10&lt;sup&gt;−3&lt;/sup&gt;</td>
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</tr>
<tr>
<td>5. KL262/UV</td>
<td>–</td>
<td>5·0·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>9·3 (±2·5)·10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1·9·10&lt;sup&gt;−5&lt;/sup&gt;</td>
<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>6. A69 + KL/UV</td>
<td>1·4·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5·0·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>8·5 (±0·9)·10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1·7·10&lt;sup&gt;−5&lt;/sup&gt;</td>
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<td>–</td>
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<tr>
<td>7. A69 + KL/UV</td>
<td>5·6·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5·0·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>8·0 (±1·0)·10&lt;sup&gt;3&lt;/sup&gt;</td>
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<tr>
<td>8. MG402 × KL/UV</td>
<td>1·6·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5·0·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>2·0 (±0·1)·10&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>2·9·10&lt;sup&gt;−4&lt;/sup&gt;</td>
<td>–</td>
</tr>
<tr>
<td>9. MG402 × KL/UV</td>
<td>3·2·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5·0·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3·1 (±0·4)·10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6·2·10&lt;sup&gt;−4&lt;/sup&gt;</td>
<td>9·4·10&lt;sup&gt;−4&lt;/sup&gt;</td>
<td>6·9 (±1·9)·10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2·2·10&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>2·2·10&lt;sup&gt;−4&lt;/sup&gt;</td>
<td>–</td>
</tr>
<tr>
<td>10. MG402 × KL/UV</td>
<td>6·4·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5·0·10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>4·5 (±0·5)·10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>9·0·10&lt;sup&gt;−4&lt;/sup&gt;</td>
<td>6·9·10&lt;sup&gt;−4&lt;/sup&gt;</td>
<td>1·3 (±1·0)·10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2·9·10&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>2·0·10&lt;sup&gt;−4&lt;/sup&gt;</td>
<td>–</td>
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</table>

<sup>*</sup>P1, A69 or MG402; P2, KL262; grown separately in LB, then diluted in M9 (alone or with a partner strain, as indicated) and plated after a 20 min incubation.

†In the tests presented in rows 5–10, the KL262 bacteria were irradiated prior to mixing. The UV dose was sufficient to cause 99·9 % inactivation of phage T2 in assays on KL262 cells.

‡Frequency of rescued cells capable of growth on the KL262-permissive medium. The number of counted colonies minus the number of KL262 survivors was divided by the number of plated P1 cells.

§Mean number of colonies containing prototrophic cells, either putative diploids transiently growing among various segregants in small colonies or recombinants forming larger colonies. They appeared on LCA after long incubation, either by direct selection or indirectly after replica plating from colonies formed on LUTA (mean ±SD of 12 plated samples, i.e. two direct and two indirect selections, three plates per selection).
by UV irradiation. This is consistent with the view that Z-mating involves not only total genetic mixing (the chromosome of the Rec+ MG402 parent being transiently expressed or not in the diploid) and cytoplasmic contact between mated cells (pre-synthesized RecA protein being introduced in this way).

(iii) Although the number of colonies on lactose agar increased with the concentration of MG402 in the mating mixture, the number of colonies formed per Szp+ cell tended to decrease. This may reflect the fact that the optimal density for Z-mating appears to be in the range of 10^5–10^6 cells ml^{-1} (J.-P. Gratia, unpublished). It is noteworthy that the optimal density for classic conjugation is about 100 times higher (Achtman et al., 1978).

Whether KL262 was irradiated or not, a variable proportion of the bacteria from each clone selected directly or indirectly on LCA (Table 4, column 7) continued to grow on this medium. They behaved as if they were MG402-like bacteria that expressed wild-type alleles substituting for the lacZ-proB deletion, while keeping and expressing the wild-type alleles of pyrD, trpC and thyA scattered from 21 to 61 min. In 32% of the examined prototrophic recombinants, all the non-selected markers scattered from 17 (gal) to 81 min (mtl) from the Szp+ MG402 parent were inherited en bloc. These combined selected and non-selected markers represent 70% of the Szp+ chromosome.

In summary, this experiment again demonstrated genetic mixing, under circumstances that argue heavily against any kind of F-mediated DNA transfer. The experiment combined crossing of two strains tested for their F- character, the use of a RecA partner for the Szp+ strain and UV inactivation of that partner. Nevertheless, colonies displaying a ‘complementing’ phenotype appeared at quite a high frequency and the recombination profile observed in subclones is incompatible with transfer of a short DNA fragment (as on an F' plasmid).

**Microscopical visualization of cell fusion**

To visualize morphologically the fusion between bacteria, we labelled the DNA of the thymine-dependent strain KL262 with BrdU. This was then mixed with the F- strain MG301 or with the Szp+ derivate MG401 and the results were analysed by immunofluorescence microscopy. When the strain KL262 was incubated for 1–2 h in media containing BrdU, almost all the cells were labelled (~98%, n = 500). When the BrdU-labelled strain KL262 was mixed with the unlabelled strain MG301 or MG401, a significant proportion of cells (50 and 30%, respectively) exhibited clear labelling. The labelling appeared as multiple dots dispersed along the bacterium (Fig. 1a, b). By contrast, no label occurred on strains MG301 or MG401 alone, nor when the primary antibody was omitted.

Careful examination of labelled cells with the corresponding phase-contrast clearly revealed further that the labelling appeared sometimes incomplete (Fig. 1c, d). In this case, one extremity of the bacterial rod, or rarely both ends, was unlabelled. A quantitative analysis of the labelling was realized on three different slide smears of strain KL262 and mixtures. The results indicated that partially labelled cells were three times more frequent in KL262+MG401 mixtures (40–85 ± 5–25%, n = 500) than in KL262+MG301 mixtures (10–73 ± 3–77%) or in KL262 alone (15–16 ± 0–32%). A Student’s t test revealed furthermore that the difference between the percentages of partially labelled cells obtained in KL262 alone and in KL262+MG401 mixtures was significant at the level of probability of 0·01%. By comparison, the difference between the percentage of

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**Fig. 1.** View of BrdU-labelled KL262 DNA in MG401+KL262 mixtures, as observed by immunofluorescence microscopy. The label covers completely (a, b) or partially (c, d) the length of the bacterium. (a’–d’) Phase-contrast images. Bar, 3 μm.
partially labelled cells obtained in KL262 alone and in KL262 + MG301 mixtures was insignificant at the level of probability of 0-1%. The partially labelled cells observed in KL262 alone were likely to correspond to cells having only incorporated BrdU into a part of their genome.

To assure that the partial fluorescent labelling did not correspond to bacteria joined end to end, but rather to fused bacteria with loss of the membranous structures separating both parental DNAs, we developed the same strategy at the ultrastructural level. However, to facilitate the identification of completely or partially labelled cells in mixtures, our in vivo incorporation of BrdU into bacterial DNA was combined with the in situ terminal deoxynucleotidyl transferase (TdT)-immunogold labelling procedure, a method allowing study of the general distribution of DNA in biological material with high specificity and high sensitivity (Thiry, 1999). In the TdT technique, TdT in the presence of modified nucleotides elongates DNA ends generated by sectioning. The labelled sites present at the surface of ultrathin sections were then visualized by an indirect immunogold labelling procedure. The detection of BrdU incorporated in vivo was realized on one face of the ultrathin sections with 5 nm gold particles and the TdT method was applied on the opposite face of the same section with 10 nm gold particles. Under these experimental conditions, the comparison of the distribution of both markers at the surface of ultrathin sections allows us to see whether the bacterial genome is completely or partially labelled. Three types of labelling were found in the cultures and mixtures. In the first of them, only large gold particles were found (Fig. 2a). This labelling thus provided the general distribution of the bacterial genome. Label was detected over several ribosome-free areas of the bacteria. These areas of low electron density contain numerous filaments and would be likely to correspond to the many fluorescent spots seen at the optical level. In the second type of labelled cells (Fig. 2b), both large and small gold particles were observed over all the ribosome-free areas of the bacteria. Finally, in the third type of labelled cells (Fig. 2c), large and small gold particles were observed; however, ribosome-free areas situated near to one bacterial extremity were only labelled with large gold particles and not with small gold particles, indicating that the bacterium was partially labelled. In this case, these single-labelled areas were never separated from the double-labelled areas by any membranous structure.

DISCUSSION

Z-mating versus stickiness and classic conjugation

Classic conjugation in bacteria involves the unidirectional transfer of a copy of the replicating DNA molecule from a donor cell to a recipient cell (for reviews, see Hayes, 1968; Clark, 1973; Finnegan, 1976; Porter, 1988). Mobilization of the donor chromosome is mediated by a conjugative plasmid and involves transposable elements. Complete chromosome transfer takes about 100 min, but usually transfer is only partial because of frequent interruption of the conjugation process. The transferred donor fragment is unable to replicate unless it is included in a plasmid. Donor genes are thus detected in the merozygote when Rec-dependent recombination occurs.

The results presented here show, in agreement with our previous paper (Gratia, 1994), that Z-mating is very different and suggest that Z-mating brings both parental genomes together in the same cell. They rule out Hfr-type transfer and argue strongly against F’ transfer. The evidence supporting this conclusion can be summarized as follows.

Z-mating yields apparently complete zygotes

One way to isolate Z-mating products is to select for complementing diploidy, as manifested by the disappearance of recessive parental markers: auxotrophies, resistance to streptomycin and nalidixic acid (Table 2) or even a missing Rec function (Table 4). It should be stressed that crosses between an Szp+ and an F’- partner invariably yield such ‘complementing’ phenotypes, no matter how many markers are included in the parental strains and no matter how scattered they are throughout the E. coli chromosome.

These complementing diploids are distinguishable from true recombinants by their phenotypic instability: although purified on the initial selective medium, they rapidly yield monoparental or recombinant types in which recessive markers have re-emerged in various combinations. In this context, the behaviour of the streptomycin and nalidixic acid markers (Tables 2 and 3) is noteworthy: the mating products initially isolated were sensitive to both antibiotics, but monoparental types and recombinants resistant to one or both markers were obtained among their descendants. The recombination profiles observed are incompatible with unidirectional transfer of a short DNA fragment from a ‘donor’ to a ‘recipient’.

It has been proposed that a possible stickiness of Szp+ cells could account for the appearance of a ‘complementing’ phenotype and for the emergence of parental types at a later time, as cells might cross-feed each other in a cell cluster. In that case, cross-feeding would be prevented if an antibiotic killed one of the parents, so that the colonies that were formed in the absence of the drugs would be due to ‘fake prototrophs’, apparently sensitive to both streptomycin and nalidixic acid in our experiments. However, cells merely sticking together might eventually yield monoparental types, but never recombinants, and a Rec+ cell sticking to a Rec− cell could not rescue the latter from UV-inflicted damage to its DNA (as in Table 4).

Another possible interpretation of our findings has been that extreme stickiness plus post-plating genetic recombination might account for our phenotype data. The experiment depicted in Table 4 argues against this hypothesis: we
ruled out any Hfr-type transfer by mating an Szp\textsuperscript{+} strain with a UV-sensitive F\textsuperscript{-} Rec\textsuperscript{-} parent since this cross was fertile. We can also reasonably rule out F\textsuperscript{-}-mediated transfer, as an analysis of the recombinants issued from the initial mating products revealed a profile incompatible with transfer of a short DNA fragment from the Szp\textsuperscript{+} parent to its F\textsuperscript{-} partner. What is more, mating products were selected at only a fivefold lower frequency when the UV-sensitive F\textsuperscript{-} Rec\textsuperscript{-} parent was irradiated prior to mixing under conditions that normally kill it (survival rate 1\texttimes 10\textsuperscript{-5}). The irradiated partner appeared to be ‘rescued’ through mating with the Szp\textsuperscript{+} parent and recombinants again appeared in the descendants. Events believed to occur in such crosses are represented schematically in Fig. 3.

**Fig. 2.** Ultrastructural identification of BrdU-labelled KL262 DNA in MG401 + KL262 mixtures (as revealed by small gold particles; small arrowheads). The general distribution of the bacterial DNA is also detected by the TdT method (as revealed by large gold particles; large arrowheads). A comparison of the two labelling patterns reveals BrdU-unlabelled (a), completely labelled (b) and partially labelled (c) DNA in mixtures. Bar, 0.2 \textmu m.
Cell fusion: a likely mechanism

Z-mating appears to be a very rapid process, yielding products that resist vortexing after 1 min co-incubation, whereas, in classic conjugation, vortexing disrupts the mating pairs and, after the same limited time, only 1% of the donor genome would have time to be transferred (Low, 1965). Experiments involving UV-irradiated RecA² cells show that the Rec system contributed by the Rec + parent functions in Z-mating products, at least transiently. The high frequency of rescue of the UV-irradiated RecA² parent and the fact that Lac + prototrophs occur only about five times less frequently when the F² Lac + parent is lethally irradiated further suggest that functional complementation of the recA mutation could be quite rapid, rather than depending solely on transcription and translation of a transferred wild-type gene in a dying cell with a severely damaged genome. The observation that KL262-like cells present in the colonies formed on LUTA remained as sensitive to UV as the parental cells rules out any gene transfer mechanism leading to stable expression of a functional recA + gene. This suggests that preformed RecA protein, introduced along with the Rec + genome into the initial mating product, might play a role in allowing DNA repair and hence rescue of UV-sensitive cells and genetic recombination. It is an argument in favour of a close contact at cytoplasmic level.

A large variety of immunocytological methods are now available for studying DNA in situ in biological material (Thiry, 1999). These methods, combined or not with molecular biotechnology, are powerful tools for studying the structure and function of DNA. In the present study, we applied some of them, for the first time in microbiology, to distinguish one parental genome from another. Immunofluorescence microscopy with BrdU labelling of the F² partner showed that a significant proportion of the F² cells in Z-mating mixtures remained unlabelled at one extremity of a bacterial rod, or occasionally at both extremities. Electron microscopy with double labelling reinforced our findings obtained by immunofluorescence microscopy. Importantly, no partition could be seen between the BrdU-labelled and -unlabelled DNA of cells in Z-mating mixtures.

All these cytological data strongly suggest a mechanism of cell fusion or, at least, close contact between parental cells at the cytoplasmic level, allowing passage of DNA and gene products (e.g. the RecA protein). These highly instructive and demonstrative results also highlight the rod extremities as the site of cell–cell binding, as schematically represented in Fig. 3. The frequency of putative mated cells was rather high (up to 30%), in agreement with results presented elsewhere, showing the instability of complementing diploidy and suggesting that the number of diploids initially formed could be much higher than indicated by the number of visible colonies formed on minimum agar (J.-P. Gratia, unpublished).

Fig. 3. Schematic representation of events occurring in a mixture of Szp + bacteria and UV-irradiated F RecA + cells. Parental cells are represented with their expressed chromosome. Mating is promoted at the junction area between parental cells owing to an alteration of the poles of the Szp + cell (thick black bar). Presumptive exchange of preformed proteins through cytoplasmic contact (or transient expression of both chromosomes) allows survival of the irradiated RecA - cell. Both parental chromosomes are present in the zygote and in part of its descent; their complementation is responsible for growth on MA or LCA selective medium. Among bacteria composing the colonies formed, P1/2 is a biparental subclone whose progeny consists of one or both parental forms. P2-like bacteria have recovered their initial sensitivity to UV. P/R is issued from a recombination event (r.e.) within the zygotic clone and contains the chromosome of parent P1 expressed in a P1-type segregant and a recombinant chromosome expressed in recombinant P1 : 2.
It has recently been claimed that transformation of *E. coli* K-12 cells by electroporation may induce a kind of ‘conjugation’. When parent cells contain different plasmids, electric treatment produces new cell lines containing both plasmids (Tyrin et al., 1997). In the cited work, it seems that the process analysed includes a transient ‘multicellular agglomerate’ stage interpolated as leading to cell fusion.

Our data pointing to diploidy are reminiscent of what happens when *Bacillus subtilis* protoplasts are fused. Spontaneous fusion of *E. coli* K-12 protoplasts (or possibly spheroplasts?), either F− or F+, was not observed when looked for (Lederberg & St Clair, 1958). In the present case, our Szp+ cells mate spontaneously without prior conversion to protoplasts. This might mean that Z-mating requires outer-membrane components not normally present or functional in *E. coli* K-12.

**Nature and origin of the Z-factor**

In this study we have sought to reconstruct the emergence of the initial Szp+ strain. The presence of a transmissible Z-factor in a clinical isolate of *E. coli* (84SV) was suspected on the basis of unpublished observations (J.-P. Gratia, unpublished). This interpretation is supported by the possibility of creating Szp+ strains by exposing ordinary F− strains to an 84SV lysate. Perhaps this unidentified factor encodes membrane components enabling Szp+ strains to attach to other strains and to promote membrane fusion. There have been attempts to isolate pure stocks of plaque-forming phage particles capable of transmitting the Szp+ character, using the 84SV lysate and sensitive bacteria. These attempts have failed. The putative Z-factor might be transduced by a defective phage particle and a thorough analysis of the 84SV lysate is currently under way to determine whether this is so.

**Conclusions**

We have shown that the mechanism formerly suggested by Lederberg (1949) for bacterial conjugation, i.e. fusion leading to the exchange of genetic material, may exist in the bacterial world, even though it had not been confirmed in any *E. coli* K-12 strain studied since that time. In hundreds of crosses we have not observed failure of a strain displaying the Szp+ character to mate with another *E. coli* K-12 strain. Z-mating has even been observed between Szp+ *E. coli* and other species of enterobacteria (Gratia & Deschuyteneer, 1998; J.-P. Gratia, unpublished). It is the first time that this aspect of the prokaryotic world has been revealed. It opens up exciting prospects in both bacteriology and cell research and for applications in biotechnology.

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