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A Role of Heat Shock Proteins for Homologous Recombination in *Escherichia coli*

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SUMMARY: Effects of mutations in heat shock protein genes on homologous recombination in *Escherichia coli* were examined by measuring intra-molecular recombinations in the plasmid, pIK43. Recombination frequencies in the *groES*, *groEL* and *dnaK* mutants were decreased 6 to 21% of those of the isogenic wild type strain, at a sub-lethal temperature. Thus, the heat shock proteins GroES, GroEL and DnaK apparently participate in homologous recombination in *Escherichia coli*.

Heat shock proteins induced by exposure of cells to various environmental stresses are required not only for maintenance of intracellular homeostasis but also for normal cell functions, including DNA replication (1-4). Recently, we found that heat shock proteins participate in the regulation of DNA topology; transient relaxation of the plasmid DNA in cells induced by exposure to a high temperature (5) was not recovered in the heat shock gene mutants (T. Mizushima & K. Sekimizu, unpublished). Since DNA topology influences DNA recombination (6), we examined the effect of mutations in heat shock genes on homologous recombination. We report here that *groES*, *groEL* and *dnaK* mutations decrease the intra-molecular recombination in plasmid.

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MATERIALS AND METHODS

Bacterial strains and plasmid: Bacterial strains used were all derivatives of *E. coli* K12 and include MC4100 (*F⁻ araD139 Δ(araF-lac)U169 rpsL150 relA1 flb5301 deoC1 ptsF25 rbsR*), KY1429 (as MC4100 except *rpoH6*(am) *zhf-50*::Tn*10*), KY1472 (as MC4100 except *groES72 zid*::Tn*10*) (M. Kanemori & T. Yura, unpublished), NRK117 (as MC4100 except *groEL44 zje*::Tn*10*), BB1553 (as MC4100 except *ΔdnaK52*::*Cm*^{r} *sidB1*). All these strains were kindly provided by T. Yura. AB1157 (*F⁻ thi-1 his-4 proA2 argE3 thr-1 leuB6 ara-14 lacY1 galK2 xyl-5 mtl-1 supE44 tsx-33 rpsL31*) and plasmid pIK43 were kindly provided by I. Kobayashi.

Manipulation of DNA: Transformation was carried out as described by Hanahan (7), with some modifications.

Determination of recombination frequency: Recombination frequencies were determined as described by Yamamoto *et al.* (8). *E. coli* strains were transformed with pIK43 DNA, under conditions of a single transformation. The entire colonies of the resultant transformants on ampicillin agar plates were inoculated separately into LB medium containing 100μg/ml ampicillin and were left to grow to saturation at 30°C or at 37°C to allow for recombination. Appropriate dilutions of the culture were plated on LB agar plates containing 100μg/ml ampicillin or 50μg/ml kanamycin, and incubation was carried out for 24h at 30°C, after which ampicillin-resistant and kanamycin-resistant colonies were counted. Recombinant frequency, p, was calculated by dividing the number of kanamycin-resistant colony forming units by those of ampicillin-resistant colony forming units. The cell generation number, g, was estimated from the number of ampicillin resistant colonies (8).

RESULTS AND DISCUSSION

We determined the effect of the heat shock gene mutations on homologous recombination using a reporter plasmid, pIK43 (8). pIK43 plasmid DNA has two homologous segments containing neomycin (kanamycin)-resistant genes with deletions on different ends. Homologous recombination between these segments reconstitutes an intact neomycin (kanamycin)-resistant gene, which allows host cells to grow on kanamycin agar plates. The probability of giving rise to one kanamycin-resistant colony forming unit during the generation time of the cell, i.e. recombination frequency per cell generation, a, was calculated by the formula of Stahl (9),

$$p = (1/2)^g \times a$$

Since the *rpoH* gene is required for induction of heat shock proteins (10-14), we compared the recombination frequency in an

rpoH mutant KY1429 with that in its parent strain MC4100. As shown in Table 1, the recombination frequency in the *rpoH* mutant was 13% of that in the wild type strain. This observation suggests that heat shock proteins may possibly have a role in homologous recombination in *Escherichia coli.*

To obtain supportive evidence for our thesis, we examined whether or not *groES*, *groEL* and *dnaK* mutations affect DNA recombination. KY1472 (*groES72*), NRK117 (*groEL44*) and BB1553 (*ΔdnaK52*) cells were transformed with pIK43 at 30°C and grown to saturation at 37°C, a semi-permissive temperature for growth of these mutant strains. The recombination frequencies per cell generation in these mutants were 6, 14 and 21% of that of the wild type strain, respectively (Fig.1). When grown at 30°C, a permissive temperature for these mutants (4, 13), recombination frequencies in these mutants were still less than that in the wild type strain (Fig.1).

We observed that homologous recombinations producing kanamycin-resistant plasmids were reduced by heat shock gene

Table 1. Influence of an *rpoH* mutation on recombination frequency

Strain	Recombination frequency per cell generation ($\times 10^{-5}$) [a]
MC4100 (wild type)	9.4 ± 0.8 [b]
KY1429 (*rpoH*)	1.2 ± 0.2

[a] *E. coli* strains MC4100 and KY1429 (*rpoH6(am) zhf-50*:: Tn *10*) were transformed with pIK43 and the resultant transformant colonies were inoculated separately in LB medium containing 100μg/ml ampicillin and grown to saturation at 30°C to allow for homologous recombination. Appropriate dilutions of the culture were plated on LB agar plates containing 100μg/ml ampicillin or 50μg/ml kanamycin and incubation was carried out for 24h at 30°C, after which ampicillin-resistant and kanamycin-resistant colonies were counted. Recombination frequencies per cell generation were calculated by dividing the ratio of kanamycin-resistant colonies by that of ampicillin-resistant colonies, by generation numbers, as described (8).

[b] Means values with standard deviation are given (3 independent expts.).

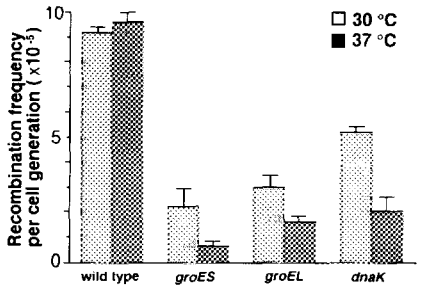

Fig.1. Influence of *groES*, *groEL* and *dnaK* mutations on recombination frequency
E. coli strains MC4100, KY1472 (MC4100 *groES72 zid* ::Tn*10*), NRK117 (MC4100 *groEL44 zje* ::Tn*10*) and BB1553 (MC4100 *dnaK52* ::Cm^r *sidB1*) were used as recipient strains. Transformation, homologous recombination and calculation of recombination frequencies were carried out as described in the footnote to Table 1. Two independent experiments were done and mean values with standard deviation are given.

mutations to about 1/10 of that in the wild type strain. This reduction seems slight, however, taking into consideration that recombination frequencies were determined at a semi-permissive temperature in the *groES*, *groEL* and *dnaK* mutants, and that recombination frequencies per cell generation of pIK43 in a isogenic *recA* mutant and *recF* mutant were 7 and 18% of that of the wild type strain (8), the reduction of recombination frequencies in the *groES*, *groEL* and *dnaK* mutants leads to the notion that heat shock proteins may participate in homologous recombination in *Escherichia coli*.

With regard to the manner in which heat shock proteins function in DNA recombination, at least two mechanisms can be considered: One is that heat shock proteins affect activities of recombination enzymes. These proteins may function in folding of recombination enzymes or in their assembly into oligomeric structures. Another possibility is that heat shock proteins may participate in regulating DNA supercoiling, an event affecting DNA recombination (6). If the latter is the case, it seems reasonable to assume that plasmid DNA carried by the heat shock gene mutants is likely to be more relaxed than that carried by the wild type strain. pUC118 DNA isolated from either KY1429 (*rpoH*), KY1472 (*groES*), NRK117 (*groEL*) or BB1553 (*dnaK*) showed an almost identical superhelical density with that isolated from MC4100 (wild type)

(data not shown). Thus, the latter possibility can probably be ruled out.

In *Escherichia coli*, intra-molecular recombination of plasmid DNA takes place through either the RecE or RecF pathway (15). Yamamoto *et al.* showed that the homologous recombination of pIK43 in the strain AB1157 was performed through the RecF pathway (8). We found that transduction of *groES* or *groEL* mutation into AB1157 led to a reduction in the recombination frequency in pIK43 (data not shown), an observation which strongly suggests that heat shock proteins function in the RecF pathway.

Recombination is important in DNA repair, and heat shock proteins may participate in this process. A defective phenotype of *umuDC*-dependent UV mutagenesis in *groE* mutants has been reported (16). In addition, inductions of GroEL and DnaK proteins occur with UV irradiation and nalidixic acid which damage DNA (17). Our working hypothesis is that heat shock proteins function in repairing the DNA damaged by environment-related stress.

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