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Effects of Microgravity on Genetic Recombination in *Escherichia coli*

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Microorganisms, and especially bacteria, have been among the first biological specimens flown in space [1, 3] but, in general, the experiments performed were mostly concerned with the effect(s) of spaceflight, including microgravity, on viability, growth, mutation, basic physiology and gross morphology. To our knowledge, no study has been performed on the effect(s) of microgravity on the mechanisms responsible for the exchange of genetic information between organisms. Thus, it seemed to us of a certain interest to ascertain the possible existence of such effect(s) on the simplest living organisms, the bacteria. It is well known that in bacteria, and probably in the other prokaryotes, there exist three basic mechanisms for genetic recombination. The first mechanism, conjugation or sexuality, involves the oriented transfer of a portion of the chromosome from a donor cell to a recipient cell. Through this mechanism, the latter cell acquires a portion of the donor's chromosome and the genes that are encoded in it. In transduction, a defective bacteriophage attaches a bacterial cell transferring to it the piece of the bacterial chromosome carried in the defective viral particle. Thus, the bacterium acquires the genes carried by the bacteriophage. Finally, in transformation, a fragment of DNA is taken up by a bacterial cell. This DNA may be either a piece of chromosomal DNA from another bacterium or a particle free in the cell cytoplasm, e.g. a plasmid. If the transforming DNA comes from a bacterial chromosome, it becomes integrated in the chromosome of the recipient cell, whereas a plasmid remains, in general, free in the cell's cytoplasm. As outlined in Table 1, the three mechanisms involve three different kinds of interaction: cell with cell in conjugation, cell with bacteriophage in transduction, cell with DNA in transformation. The experiments were divised to ascertain any effect that microgravity could have on these three processes. For convenience of comparison, the three processes were analyzed in the same bacterium, i.e. *Escherichia coli.*

Table 1. Mechanisms of genetic recombination in *E. coli* and types of interaction

Mechanism of recombination	Type of interaction
Conjugation	Cell:cell
Transduction	Cell:bacteriophage
Transformation	Cell:DNA

Experimental Procedure

Standard procedures were followed for the preparation of cells to be used in the conjugation [6], transduction [2] and transformation [4] experiments, except that glycerol was added to all liquid media to give a final concentration of 15% (v/v). The following strains were utilized: *E. coli* C strains C-1091 (*Hfr*-12 *ura*) and C-411 (F^-, *gal-3* T1, *try pur arg str ile thi*) for conjugation, C-1055 (F^+, *his xan str thr*-5 *leu*-5μ_2) for transduction and *E. coli* K-12 strain HB101 (F^-, *hsdS20*(r_B^-, m_B^-), *recA13*, *ara-14*, *proA2*, *lacY1*, *galK2*, *rpsL20*(Sm^r), *xyl-5*, *mtl-1*, *supE44*, λ^-) for transformation. Phage P1 *Kc cc* was prepared according to [4], whereas plasmid pBR322 was prepared following the procedure in [5]. The suspensions containing cells, phage or plasmid DNA were cooled in ice, mixed rapidly, aliquoted in the reaction tubes and frozen in liquid nitrogen. The tubes were stored at −70 °C or in dry ice until transferred to the passive thermal unit in which they were kept at −13 °C for 12 h. After loading on the Biorack, the samples were kept for 6.3 days at −15 °C until they were transferred to the incubator at +37 °C

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Table 2. Effect of microgravity on conjugation. Each experiment consisted of 12 independent 0.4-ml samples and, for each sample, two aliquots were plated on the selection media. The rate of spontaneous revertion to *Ile*$^+$, *Arg*$^+$, *Pur*$^+$ was, in all cases, less than 10^{-8}. The frequency of *Arg*$^+$ and *Pur*$^+$ recombinants relative to *Ile*$^+$ recombinants is reported in parentheses

Marker selected	Flight (μg)	Flight, centrifuge (1 *g*)	Ground, control (1 *g*)	Ground, centrifuge (1.4 *g*)
	Frequency of recombinants/*Hfr*			
Ile$^+$	2.54×10^{-4} (100)	5.71×10^{-4} (100)	4.56×10^{-4} (100)	3.75×10^{-4} (100)
Arg$^+$	1.44×10^{-4} (56)	0.60×10^{-4} (10)	0.51×10^{-4} (11)	0.54×10^{-4} (14)
Pur$^+$	0.33×10^{-4} (13)	0.22×10^{-4} (3.8)	0.18×10^{-4} (3.9)	0.20×10^{-4} (5.3)
	Frequency of recombinants/F$^-$			
Ile$^+$	2.25×10^{-5} (100)	3.80×10^{-5} (100)	2.71×10^{-5} (100)	1.89×10^{-5} (100)
Arg$^+$	1.05×10^{-5} (42)	0.40×10^{-5} (10)	0.30×10^{-5} (11)	0.27×10^{-5} (14)
Pur$^+$	0.29×10^{-5} (12)	0.15×10^{-5} (3.9)	0.10×10^{-5} (3.6)	0.10×10^{-5} (5.2)
Viable cells (cells/ml)				
Hfr (C-1091)	5.5×10^7	2.8×10^7	3.5×10^7	2.4×10^7
F$^-$ (C-411)	6.2×10^8	4.2×10^8	5.9×10^8	4.8×10^8

Table 3. Effect of microgravity on transduction. Each experiment consisted of 12 independent 0.4-ml samples and, for each sample, two aliquots were plated on the selection media. The rate of spontaneous reversion to *His*$^+$, *Thr*$^+$ and *Leu*$^+$ was, in all cases, less than 10^{-8}. The m.o.i. was 0.1

Marker selected	Frequency of transductants (number of transductants/survivors)			
	Flight (μg)	Flight, centrifuge (1 *g*)	Ground, control (1 *g*)	Ground, centrifuge (1.4 *g*)
His$^+$	0.84×10^{-6}	1.03×10^{-6}	0.98×10^{-6}	0.84×10^{-6}
Thr$^+$	1.38×10^{-6}	1.78×10^{-6}	1.76×10^{-6}	1.50×10^{-6}
Leu$^+$	$2{,}69 \times 10^{-6}$	3.03×10^{-6}	2.81×10^{-6}	2.51×10^{-6}
Viable cells (cells/ml)				
C.1055	8.1×10^8	6.4×10^8	7.1×10^8	6.6×10^8

Table 4. Effect of microgravity on transformation. Each experiment consisted of 15 independent 0.4-ml samples and, for each sample, two aliquots were plated on the selection media. The rate of spontaneous mutation to *Amp*^{r} was less than 10^{-8}

Marker selected	Frequency of transformants (number of transformants/μg DNA)			
	Flight (μg)	Flight, centrifuge (1 *g*)	Ground, control (1 *g*)	Ground, centrifuge (1.4 *g*)
Amp^r	1.1×10^3	2.1×10^3	3.1×10^3	0.8×10^3
Viable cells (cells/ml)				
HB101	2.4×10^7	1.7×10^7	3.9×10^7	0.9×10^7

for 3 h. After incubation at +37 °C, the samples were transferred to another passive thermal unit at +4 °C and kept at such temperature until analysis on ground (1.5 days after the incubation at +37 °C). All samples on the flight module and those on the ground, that were run under the same conditions, were analyzed at the same time. After plating aliquots of the incubation mixtures on the appropriate media, the plates were incubated at +37 °C for 24 or 48 h and colonies scored.

With the constraints imposed by the experimental conditions, the frequency of recombinants was in all three types of experiment severely depressed as compared to that observed under standard conditions (in general, two orders of magnitude). However, the number of the recombinant colonies was still significant (100 to 700 colonies/plate in the conjugation experiments, 20 to 130 colonies/plate in the transduction experiments, 5 to 60 colonies/plate in the transformation experiments). The results for each type of experiment are reported in Tables 2 to 4, whereas the statistical analysis is given in Table 5.

Table 5. Nested analysis of variance

Source	Significance		Estimated contribution to total variance [%]
Conjugation			
Isoleucine			
Experiments	$P>0.05$		0
Boxes	$P>0.05$		0
Samples	$P<0.001$		82.9
		Replicates	17.1
Arginine			
Experiments	$P<0.001$		92.3
Boxes	$P>0.05$		0
Samples	$P<0.001$		6.0
		Replicates	1.7
Purines			
Experiments	$P<0.001$		76.8
Boxes	$P<0.01$		8.0
Samples	$P<0.001$		10.2
		Replicates	5.0
Transduction			
Histidine			
Experiments	$P>0.05$		0
Boxes	$P<0.01$		26.9
Samples	$P<0.001$		33.1
		Replicates	40.0
Threonine			
Experiments	$P>0.05$		0
Boxes	$P<0.01$		33.2
Samples	$P<0.001$		35.5
		Replicates	31.3
Leucine			
Experiments	$P>0.05$		0
Boxes	$P>0.05$		0
Samples	$P<0.01$		46.0
		Replicates	54.0

Results

In the case of conjugation, it is clear that whereas no difference exists in the rate of transmission of the first marker (*ile* 0.8 min), the late markers (*arg* and *pur* at, respectively, 27.1 and 38.6 min from the origin of transfer) appear to be transmitted more efficiently under microgravity conditions (Table 2). In microgravity, the number of recombinants for the late markers is, in general, three- to fourfold higher than that in the controls both on flight (1-*g* centrifuge) and on ground (static or 1-*g* centrifuge). Even taking into account the increase in cell number that was observed under microgravity, a finding that would agree with previous results indicating a faster growth rate of bacteria in cultures exposed to microgravity [7], the difference in the transmission of the later characters is still evident. Indeed, as compared to the controls, under microgravity, the frequency of recombinants for the second and the third selected character varied between two- and threefold when it was expressed in relation to the number of F^- cells and close to twofold when calculated in relation to the number of *Hfr* cells. Thus, it appears that microgravity exerts a positive effect on the gradient of transmission, whereas, apparently, it has no effect on the transfer of the first character and hence, presumably, it does not affect the formation of mating pairs or aggregates. Since it is widely believed that the connection between conjugating *Hfr* and F^- cells is easily broken, it seems reasonable that, in microgravity, mating pairs or aggregates, are less likely to be disrupted than in the presence of gravity and thus give rise to higher recombination frequencies for later markers, but not for early ones.

In the case of transduction, no significant difference was observed under any of the tested conditions (Table 3). One has, therefore, to assume that microgravity does not affect the bond between the bacterial cell and the phage particle, possibly because such a complex is more stable and less sensitive to stresses than the one formed between cells in conjugation. In addition, the transfer of DNA from the phage to the bacterial host is much more rapid than that taking place during conjugation

so that, in comparison to conjugation, the length of time during which environmental stresses could interfere with the transduction process is much shorter.

Finally, no conclusion may be drawn in the case of the experiments on transformation (Table 4). In such experiments, variations were observed in the viable counts as well as in the number of transformants. However, no rational pattern could be discerned among the different conditions that could point to any effect, in either direction, of microgravity or, even, to the absence of any effect. It should be remembered that transformation with plasmid DNA in *E. coli* is carried out under rather unphysiological conditions (cell permeability is altered by pretreatment with salts to render cells competent to take up plasmid DNA; uptake of plasmid is independent of incubation temperature, etc.). It is possible that the effects, if any, of microgravity on transformation could have been ascertained using a more physiological system, such as that of *Bacillus subtilis* and chromosomal DNA. Unfortunately, experimental constraints did not allow the utilization of such a system.

In conclusion, our results show that microgravity exerts a positive effect on conjugation, possibly by reducing the frequency of mating interruptions, whereas it has apparently no effect on transduction.

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