DUPLICATION OF A GENE BELONGING TO AN ARGININE OPERON OF *ESCHERICHIA COLI* K-12

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AMONG the structural determinants of the enzymes involved in arginine biosynthesis in *Escherichia coli*, four are clustered in the order *argE*, *argC*, *argB*, *argH*, and probably adjacent (GLANSDORFF 1965, Figure 1); the synthesis of enzyme E is however not strictly coordinated with the synthesis of enzymes C, B and H (BAUMBERG, BACON and VOGEL 1965; GLANSDORFF and SAND 1965). The expression of the 3 latter loci has been found polarized from left to right (GLANSDORFF and SAND 1965, SAND and GLANSDORFF 1967), the fate of *argE* giving rise to conflicting interpretations (*ibid.*, GLANSDORFF 1967, VOGEL *et al.* 1967). More recent work supports the view that the cluster contains 2 successive operons, both polarized from left to right: *argE* and *argC*, *B*, *H* (CUNIN *et al.* 1968, SAND *et al.* in preparation).

A cell harbouring a strongly polar *argC* or *argB* mutation has its potential of argininosuccinase (enzyme H) synthesis drastically lowered; as a consequence it grows very slowly in minimal medium supplemented with ornithine. This property was used to investigate by which ways a cell could recover from the polar effect of a mutation without being forced to revert to wild phenotype.

Ornithine utilizers (Orn-ut), but still Arg$^-$ derivatives of polar mutant *argB2* were found to occur spontaneously, at a frequency of about 10^{-5}, in sharp contrast with the frequency of reversion to wild phenotype (10^{-10}). One of these strains (MN8C), growing as well on ornithine as the non-polar *argB* mutants, was studied extensively. This communication adds further experimental support to the conclusion reached in a preliminary account of this work (GLANSDORFF 1966), namely that MN8C carries a duplication of *argH*.

MATERIALS AND METHODS

Genetic and enzymological techniques: see GLANSDORFF (1965) and GLANSDORFF and SAND (1965). *Abbreviations used* (DEMEREC *et al.* 1966): *arg* = arginine; *glu* = glucose; *met* = methionine; *orn* = ornithine; *pur* = purine; *xyl* = xylose; *Sm* = streptomycin; *R* = resistance; *S* = sensitivity; *F*$^-$ = genetic recipient; *Hfr* = genetic donor; capital letters: arginine loci nomenclature (MAAS *et al.* 1964; GLANSDORFF, SAND and VERHOEF 1967).

The same letters will frequently be used to designate the enzymes coded by the *argB*, *argC*, *argE* and *argH* genes. The gene-enzyme correspondence illustrated in Figure 1, is as follows: ATP: α-N-acetyl-L-glutamate 5-phosphotransferase (*argB*); α-N-acetyl-L-glutamate γ-semialdehyde NADP oxydoreductase (phosphorylating) (*argC*); L-ornithine α-N-acetylornithine lyase (*argE*); L-argininosuccinate arginine lyase (EC 4.3.2.1) (*argH*).

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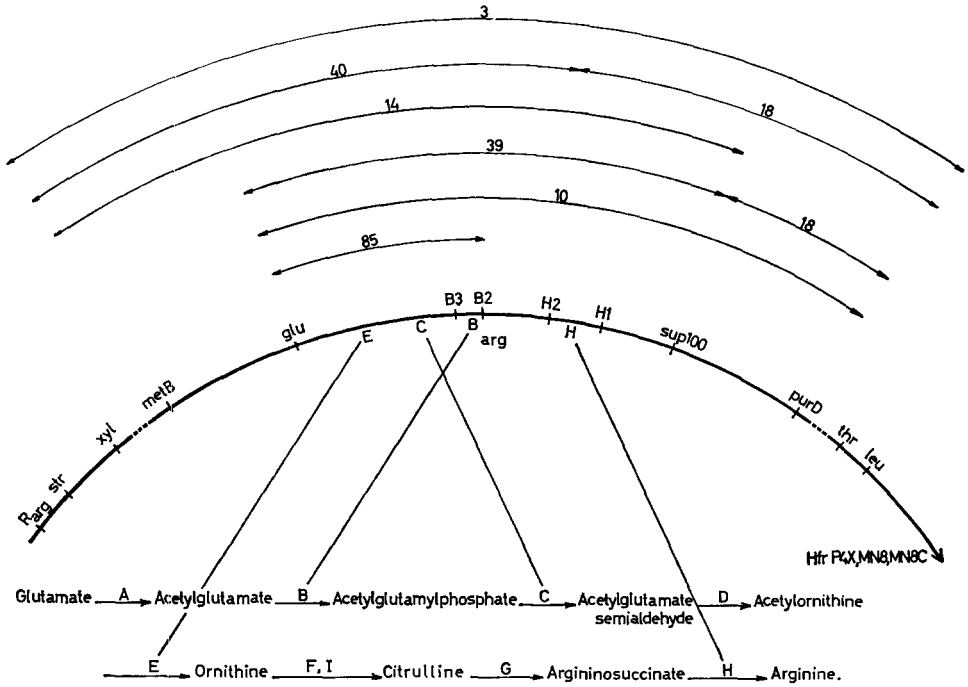

FIGURE 1.—The *argECBH* cluster and neighbouring loci. Map (not to scale) and gene-enzyme relationship for the arginine pathway. Numbers = cotransduction indexes (%, see Table 3 and references in the text).

Strains: The properties of the strains—all derivatives of *E. coli* K-12—are listed in Table 1. *met*$^+$, *glu* and *purD* derivatives of MN8 and MN8C were prepared by transduction between the latter strains and 145M1, PA214 and AT11–31.

RESULTS

We shall first describe the enzymic properties of MN8C. The genetical analysis of the strain will follow immediately.

Enzymatic constitution of MN8C: We have measured the specific activities of five enzymes of the arginine pathway in repressible (R^+_{arg}) and non-repressible (R^-_{arg}) derivatives of MN8C, the parental polar mutant MN8 and the wild-type P4X, under various growth conditions. The results are listed in Table 2. While the repressible and non-repressible derivatives of MN8 and P4X respond as expected to the presence or the absence of arginine in the medium, the response of MN8C exhibits a striking singularity: the specific activities of enzymes E, C, B and ornithine transcarbamylase parallel the values obtained with MN8 but the synthesis of enzyme H is no more repressible by arginine, nor by any of the components present in broth (containing bactotryptone and yeast extract, see GLANSDORFF 1965).

The mutation undergone by MN8 to give the Orn-ut strain MN8C thus seems to have specifically released argininosuccinase synthesis from the control exerted

TABLE 1

List of the strains

Strain	Source	*thr-leu*	*his*	*thi*	*pur*	*met*	*xyl*	*glu*	*arg*	Arg	Sm	Mating activity	R_{arg}°°
P4X	*	+	+	+	+	*B*	+	+	+	+	S	*Hfr*	+
P4XB2	*	+	+	+	+	*B*	+	+	+	+	S	*Hfr*	—
MN8	**	+	+	+	+	*B*	+	+	B2	Arg⁻ Orn-slowut°	S	*Hfr*	+
145M1	**	+	+	+	+	+	+	+	H1	Arg⁻	S	*Hfr*	+
342G1	**	—	—	—	+	+	+	+	H2	Arg⁻	R	*F*⁻	+
MN41	**	+	+	+	+	*B*	+	(del. *glu-argE-CBH*)		Glu⁻-Arg⁻	S	*Hfr*	+
MN8C	**	+	+	+	+	*B*	+	+	(see text)	Arg⁻ Orn-ut	S	*Hfr*	+
P678R2	**	—	—	—	+	+	—	+	+	+	R	*F*⁻	—
PA214	***	—	—	—	+	+	+	—	+	+	S	*F*⁻	+
AT11-31	***	—	+	—	D	+	—	+	+	+	R	*Hfr*	+

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** This laboratory. 145M1, MN41, MN8 and MN8C are all derivatives of Hfr P4X; the origin of transfer characteristic of this strain is represented on Figure 1.

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° *argB2* : complete phenotype is : Arg⁻ and ornithine slowutilizator (Orn-slowut) (GLANSDORFF and SAND 1965; GLANSDORFF 1966).

°° R^+_{arg} R^-_{arg} : alleles of the regulatory gene of arginine biosynthesis, wild type and constitutively derepressed, respectively (MASS 1961).

TABLE 2

*Enzymic constitution of R^+_{arg} and R^-_{arg} derivatives of MN8C, P4X and MN8. Activities in µmoles/hr/mg protein**

Strain	Relevant phenotype	Addition to minimal medium	Acetylornithine lyase (locus *argE*)	Oxydoreductase (locus *argC*)	Phosphotransferase (locus *argB*)	Arginosuccinate lyase (locus *argH*)	Ornithine transcarbamylase (loci *argF, I*)
o	Arg⁻, Orn-slowut, R^-_{arg}	arginine	60	3.5	n.m.	0.03	1640
O	Orn-ut, R^-_{arg}	arginine	59	2.4	n.m.	2.0	1680
MN8C	Orn-ut, R^+_{arg}	arginine	2.2	0.07	n.m.	2.8	0.9
	Orn-ut, R^+_{arg}	ornithine	6	0.16	n.m.	1.9	53
	Orn-ut, R^+_{arg}	broth	..	..	..	2.0	..
P4X	Arg⁺, R^+_{arg}	..	13.6	0.7	0.9	1.5	90
P4XB2	Arg⁺, R^-_{arg}	arginine	56	3.2	3.8	7.4	1100

* : Growth conditions and enzyme assays : see GLANSDORFF and SAND (1965). L-ornithine or L-arginine, when added : 100µg/ml. R^+_{arg}, R^-_{arg} : alleles of the regulatory gene of arginine biosynthesis, wild type and constitutively derepressed, respectively (MAAS 1961).

o : R^-_{arg} (Arg⁻, Orn-slowut), Xyl⁺, SmR recombinant between MN8 and P678R2.

O : R^-_{arg} (Arg⁻, Orn-ut), Xyl⁺, SmR recombinant between MN8C and P678R2.

n.m. : not measurable.

by arginine on the synthesis of the enzymes of the pathway; indeed, the regulation of the synthesis of enzymes E, C and ornithine transcarbamylase remains unaffected by the mutation.

argH is the distal locus of an operon; the behavior of MN8C is thus not readily explained by assuming that an operator locus specific of *argH* has been affected. The next section describes our attempts to understand the genotype underlying the Orn-ut phenotype and the conclusions that can be drawn.

Genetic analysis of MN8C: The following experiment shows that the Orn-ut phenotype of MN8C is due to the occurrence in MN8 of a mutation which can easily be separated from the mutation *argB2*. In other words, this additional mutation may be considered as a "suppressor" of the latter one, but a "suppressor" affecting only the polar effect of *argB2* not the primary consequence of this mutation (lack of enzyme B).

The genotype of MN8C is argB2, sup-100: A cross was carried out between MN8C (Hfr, Orn-ut, Xyl^+, SmS) and P678R2 (F^-, Arg^+, Xyl^-, SmR). Xyl^+ recombinants were isolated after 60 minutes of mating at 37°C. They turned out to be either Arg^- and Orn-slowut (*argB2*; 12.5%), Arg^+ (60%) or Arg^-, Orn-ut (27.5%).

MN8C thus still contains the mutation *argB2*. Besides, the 12.5% of Arg^-, Orn-slowut recombinants suggest that the additional mutation (provisionally called *sup-100*) is linked to *argB2*, but sufficiently distant to be external to the arginine cluster. These points are established by the results of the experiments reported in the next section.

Interrupted matings performed between the Hfr MN8C and a F^- *argB2* recipient (obtained among the Xyl^+ recombinants of a cross between P678R2 and MN8) show that *sup-100* enters the F^- cell 25 to 30 minutes after mixing the conjugants. This result is compatible with the previous ones and the following analysis; it also shows that the *sup-100* mutation is transmitted as a chromosomal marker.

Localization of the sup100 *mutation:* The *sup100* mutation has been mapped by transductions carried out between *metB*, *glu* or *purD* derivatives of MN8 and MN8C. The results are listed in Table 3.

The *sup100* mutation was found cotransducible with *glu* (about 40%) and *metB* (about 14%) at frequencies lower than *argB* and *argH* (75 to 85% with *glu*, 35 to 40% with *metB*; GLANSDORFF 1965). These data point to the location of *sup100* at the right of the arginine cluster, between *argH* and *purD*; indeed, *purD* is 18% cotransducible with *argH2* (DE VLIEGHERE and VANDERWINKEL 1968), 18% with *argB2*, 3% with *metB* (GLANSDORFF 1967) and 10% with *glu*; *purD* is only 18% cotransducible with *sup100* and, although the results as a whole give a clear idea of the order of the markers investigated, it remains to be explained why the *purD—sup100* and *purD—argH* linkage estimates do not significantly differ from each other. It should be stated here that the cotransduction indexes obtained between *sup100* and other markers are not considered as distance estimates strictly comparable to those obtained between the reference markers themselves; indeed the nature of the *sup100* mutation (see below and

TABLE 3

Transduction analysis of the met—purD *region*

Linkage estimated	Recipient	Donor	Number of unselected versus selected recombinants	Percent
metB—sup100	*argB2*	*metB, argB2, sup100*	27 *metB*/223 *sup100*	12.1
metB—sup100	*glu1, argB2*	*metB, argB2, sup100*	20 *metB*/127 *sup100*	15.7
glu —sup100			54 Glu+/127 *sup100*	42.5
glu —sup100			23 *sup100/70* Glu+	32.8
purD—sup100	*purD, argB2*	*metB, argB2, sup100*	35 Pur+/207 *sup100*	16.9
purD—sup100			45 *sup100*/240 Pur+	18.7
purD—argB2	*purD, argB2*	wild type	14 Pur+/119 Arg+	11.7
purD—argB2			22 Arg+/88 Pur+	25.0
purD—glu	*glu1, argB2*	*purD*	19 *purD*/175 Glu+	10.8
purD—argH2	(DE VLIEGHERE and VANDERWINKEL 1968)			18.0
purD—glu	(ibidem)			10.0

DISCUSSION) is likely to modify the probability of crossing-over in its immediate vicinity.

The next experiment had a double aim: to confirm the location of the *sup100* mutation outside the arginine cluster and at the same time to determine if this mutation is able to compensate a total loss of capacity of enzyme H synthesis.

We have attempted to construct a strain which would carry a deletion of the *glu, argECBH* cluster and the *sup100* mutation. Experiments devised as controls for the construction of such strains show that in transductions between a (*glu, argECBH*) deletion recipient MN41 and the wild type, the *glu-arg* segment is always incorporated as a whole; indeed, these transductions do not yield any Glu^+Arg^- nor Glu^-Arg^+ recombinants (less than 1 among 2000 Glu^+ or Arg^+ recombinants, respectively). Yet, from a transduction between the same recipient and MN8C, 60% of the Arg^-Orn-ut recombinants are Glu^-, thus keeping the deletion.

It follows that the *sup100* mutation is indeed external to the arginine cluster, although close to it, and enables the cell to overcome a total loss of capacity of enzyme H synthesis.

Nature and refined mapping of the sup100 *mutation:* The range of hypotheses which *a priori* could have explained the "suppression" of polarity exhibited by MN8C is considerably narrowed by the foregoing results. At this stage, two classes of possible explanations seem to be left to us, both of them in accordance with the fact that the synthesis of the enzyme responsible for the splitting of argininosuccinate in MN8C is no more repressible by arginine: (1°) The *sup100* mutation is a transposed or duplicated *argH* locus; (2°) The *sup100* mutation opens a new pathway able to transform argininosuccinate into arginine.

The latter possibility seems very unlikely indeed : (a) *sup100* mutations have not been obtained so far from *argH* mutants plated in the presence of ornithine; (b) the rather high frequency of occurrence of *sup100* mutations (10^{-5}) is more compatible with the recombination events likely to be involved

in the production of chromosomal rearrangements than with the narrow range of mutations which could open a pathway bypassing argininosuccinase; (c) similar Km estimates were obtained for the enzyme detected in the wild type and MN8C : 3.0 and 2.0 $\times$ 10^{-4}M argininosuccinate, respectively (if the difference between the values was significant—which is doubtful—it would mean that the enzyme of MN8C has even more affinity for argininosuccinate than the wild-type argininosuccinase).

The following—and last—experiments were intended to investigate which chromosomal rearrangement (transposition or duplication) may underlie the Orn-ut phenotype.

With the results, reported at the beginning of this section, of the cross between P678R2 and MN8C, we already had a strong indication that MN8C had not undergone a transposition of *argH*. If MN8C contained a transposed *argH*, with a concomitant deletion at the normal site of this locus, then the formation of the Xyl$^+$, Arg$^-$, Orn-slowut recombinants would have required a crossing-over within that portion of the *argB* locus which extends from *argB2* to the distal extremity of the gene; indeed, in this eventuality the Orn-slowut phenotype requires the association in the same genome of the *argB2* mutation carried by MN8C and the functional *argH* locus of P678R2. This can be easily visualized by substituting *argH*$^+$ for *argH1* in the upper right part of Figure 2. The crossing-over should thus have occurred in a segment which represents less than 1.5% recombination, as estimated previously (GLANSDORFF 1966) in a parallel mating experiment involving the *argB3* and *argH2* mutations (Figure 1). Yet, in the mating P678R2 $\times$ MN8C, as much as 12.5% of the Xyl$^+$ recombinants were *argB2*, Orn-slowut.

More decisive evidence in favour of the duplication hypothesis is provided by the next experiments, which give a detailed topographical analysis of the *glu-arg-sup100* segment in MN8C. If *sup100* is a duplicated rather than a transposed *argH* locus, two specific predictions can be made, both illustrated by Figure 2:

(1) In a transduction between MN8C (recipient) and an *argH* mutant (donor), 3 classes of Arg$^+$ recombinants are expected : *argB*$^+$, *argH*$^+$, *sup*$^+$ recombinants (having regained the repressibility of argininosuccinase synthesis), (*argB*$^+$, *argH*, *sup100*) and (*argB*$^+$, *argH*$^+$, *sup100*). If MN8C carried a transposition, *argB*$^+$, *argH*$^+$, *sup100* recombinants could not be formed; a class of recombinants endowed with a repressible argininosuccinase synthesis (*argB*$^+$, *argH*$^+$) might develop but only if the segment between *argB* and the transposed *argH* locus (Figure 2 : upper right) is dispensable under the growth conditions employed. The simultaneous recovery of the latter 2 classes would thus exclude the possibility of a transposition.

Consequently, MN8C (*metB*, Arg$^-$, Orn-ut) has been transduced by phage grown on 342G1 (Met$^+$, *argH2*) and 145M, (Met$^+$, *argH1*). Table 4 compares the numbers of Arg$^+$ and Met$^+$ recombinants obtained with those recovered when transducing MN8 (*metB*, Arg$^-$, Orn-slowut) with the same phage suspensions. The mere observation of the results confirms that the *sup100* mutation compensates the absence of a functional *argH* locus; indeed, if *argB*$^+$, *argH*,

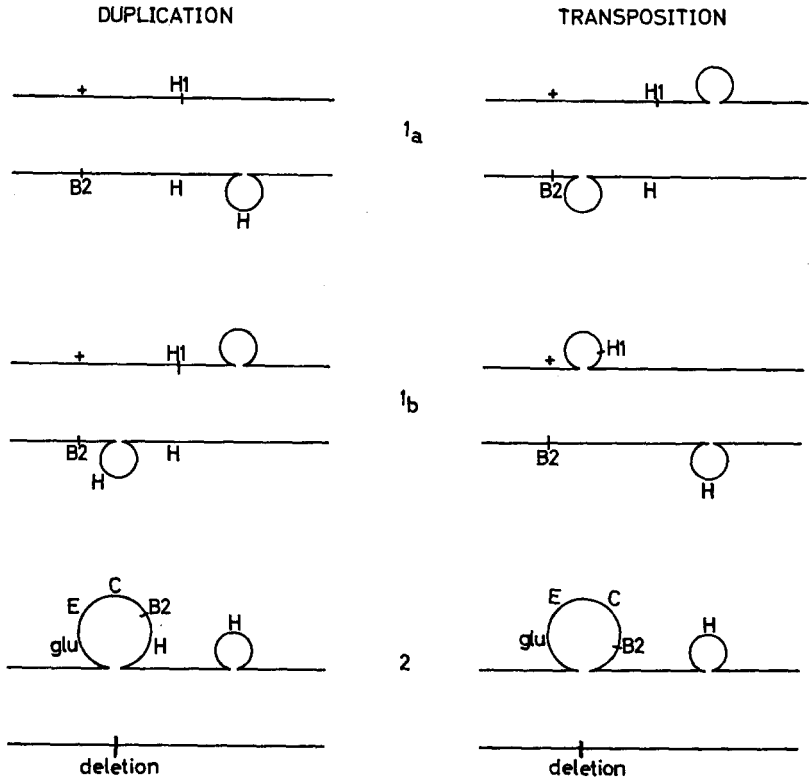

FIGURE 2.—Possible pairing relationships between the chromosomes of MN8C and 145M1 (*argH1*) (1a and 1b) or the chromosomes of MN41 (*glu-argECBH* deletion) and MN8C (2) in 2 eventualities; left : MN8C carries a duplicated *argH* in the vicinity of the arg operon; right : MN8C carries a transposition of this locus in the same region.

sup100 recombinants are Arg$^+$ then the number of Arg$^+$ recombinants must be higher when MN8C rather than MN8 is transduced by phage grown on *argH* strains. A 4.5 fold difference is observed (standardized against the number of Met$^+$ recombinants) when *argH1* is used as donor; with *argH2*, a 19 fold increase is found. This result is in accordance with the fact that the interval *argH1*—

TABLE 4

Comparison of the members of Arg+ and Met+ recombinants issued from transductions between MN8 or MN8C and argH1 *or* argH2

Transduction		Recombinants (per ml)		
Recipient	Donor	*Arg+*	*Met+*	*Arg+/Met+*
MN8 (*metB, argB2*)	*argH1*	4120	43000	0.096
MN8 (*metB, argB2*)	*argH2*	1620	70000	0.023
MN8C (*metB, argB2, sup100*)	*argH1*	16200	36500	0.443
MN8C (*metB, argB2, sup100*)	*argH2*	12800	29100	0.440

argB2 is 3 times as long as the interval *argH2—argB2* (GLANSDORFF 1966). Moreover, the ratios of Arg^+/Met^+ recombinants issued from the transductions MN8C × 145M1 (*argH1*) and 342G1 (*argH2*) are equal (0.44); if $argB^+$, *argH*, *sup100* organisms are Arg^+ this result is expected, since the value of the latter ratio must then be related only to the distance *argB2—sup100*.

Fifty Arg^+ recombinants of the MN8C × 145M1 transduction were then purified in order to detect among them the possible occurrence of $argB^+$, $argH^+$, sup^+ and $argB^+$, $argH^+$, *sup100 recombinants*. After growth in minimal medium and minimal medium supplemented with 200 μg of L-arginine per ml, they were submitted to a tentative identification according to their argininosuccinase specific activity.

4 $argB^+$, $argH^+$, sup^+ recombinants were readily recognized thanks to their regained repressibility of argininosuccinase synthesis. 39 recombinants appeared to be $argB^+$, *argH1*, *sup100*, since their specific activity was practically the same as in MN8C, whether arginine had been added to the culture or not. 7 recombinants were surmised to be $argB^+$, $argH^+$, *sup100*—thus carrying 2 functional *arg* loci—since they exhibited the same activity as MN8C after growth in the presence of arginine, and 50 to 100% more after growth in unsupplemented minimal medium. However, as a 50% difference between enzyme measurements performed on separate cultures does not carry much conviction by itself, the genetic identification of the latter class of recombinants was carried out, as reported in the next paragraph.

Arg^+, *argH*, *sup100* recombinants, if used as donors in transductions involving MN41 (*glu*, *argECBH* deletion) as recipient, are expected to produce 2 classes of Glu^+ recombinants : Glu^+, Arg^- (genotype glu^+, *argH*, sup^+) and Glu^+, Arg^+ (genotype glu^+, *argH*, *sup100*); $argB^+$, $argH^+$, *sup100* recombinants, submitted to the same test, must give rise only to Glu^+, Arg^+ recombinants (either glu^+, $argH^+$, sup^+ or glu^+, $argH^+$, *sup100*). By this method, 2 of the presumed $argB^+$, $argH^+$, *sup100* recombinants were identified unambiguously. (The 5 other ones were not tested and considered of the same genotype by analogy). As control, 2 of the 39 recombinants, supposed to be $argB^+$, *argH*, *sup100* were tested and gave the expected results. It may be seen that the relative proportions of the different classes of Arg^+ recombinants are in accordance with the other *argB—argH—sup100*.

The simultaneous occurrence of $argB^+$, $argH^+$, sup^+ and $argB^+$, $argH^+$, *sup100* recombinants thus discards the possibility of MN8C having undergone a transposition of *argH* and strongly favours the duplication hypothesis. The next experiment provides an independent confirmation of this conclusion.

(2) The second prediction is illustrated at the bottom of Figure 2. In a transduction between MN41 (*glu*, *argECBH* deletion) as recipient and MN8C as donor, the expected Arg phenotype of the Glu^+ recombinants will differ whether one considers that the donor carries a duplication of *argH* or a transposition with a concomitant deletion at the normal site of this locus; in both cases will the Glu^+ recombinants produced be Arg^- but only in the eventuality of duplication will some of them exhibit the Orn-slowut phenotype (about 60% as estimated from

the experiments discussed above, under the heading "*Localization of the* sup100 *mutation*"), the other ones being Orn-ut. In the eventuality of a transposition of the Glu^+ recombinants about half must be Arg^- Orn-ut; the other half must be Arg^- and totally unresponsive to ornithine. Among 25 Glu^+ recombinants selected in the presence of arginine, 13 were Arg^-, Orn-ut and 12 had the Arg^-, Orn-slowut phenotype expected if MN8C carries a duplicated *argH* locus.

DISCUSSION

The *sup-100* mutation, harboured by the Orn-ut strain MN8C, suppresses the polar effect exerted by the mutation *argB2* on the gene *argH*. Having successively shown that *sup-100* is easily separated from *argB2* and external to the *argECBH* cluster, we have presented evidence pointing against the occurrence in MN8C of a mutation opening a new pathway of ornithine utilization when the expression of the *argH* gene is repressed.

One could consider two possibilities of this type in order to explain MN8C : either the transformation of a lyase unrelated to the arginine pathway into an enzyme able to split argininosuccinate, or the mutation of an hypothetical cryptic *argH* locus, already present, towards a functional form. The first of these possibilities at least, is rendered extremely unlikely by the nearly identical affinities for argininosuccinate of the MN8C and the wild-type enzymes; in addition, more recent observations (ELSEVIERS *et al.* 1968 and in preparation) show that organisms where two genes (*argB* and *H*) are under the influence of a polar mutation, may give rise to Orn-ut derivatives where both genes appear duplicated. Against both possibilities stands the fact that Orn-ut variants have not been obtained from *argH* mutants plated on ornithine. Although the hypothesis of a reactivation of cryptic genes should be kept in mind, particularly when considering some recent speculations (HOPWOOD 1967; MCFALL and MAAS 1967; GLANSDORFF, SAND and VERHOEF 1967), the bulk of the evidence presented in this paper—including the topographical analysis of the *metB—argECBH* region in MN8C—points to the *sup100* mutation being a duplication of *argH* in the immediate vicinity of the *arg* cluster. It is certainly not a tandem duplication *sensu stricto;* indeed, the duplicated *argH* locus appears disconnected from its counterpart by a segment of the chromosome; this conclusion derives not only from the topographical analysis presented in Table 3 and in the last experiments of the previous section, but also from the non-repressibility of argininosuccinase synthesis in MN8C. It is not possible, at this stage, to assess how much of the DNA normally adjacent to *argH* has been duplicated with it, nor to predict the consequences of such a rearrangement on linkage estimates in this segment of the chromosome. Since, however, the cotransduction index of *purD* and *sup100* does not substantially differ from the *argH—purD* index, it is possible that the segment between the duplicated locus and *purD* is the same as the one which normally joins *argH* and *purD* in the wild type. On the other hand, this result might be a coincidence; the duplicated locus could be closer to *purD* than *argH* in the wild type, but increase the crossing over frequency in its vicinity.

The genetically detectable consequence of this chromosomal rearrangement is found associated with the bacterial chromosome (see *Genetic analysis of MN8C*, conjugation experiments); in this respect, it differs from the duplication of operons on episomes (MATSUSHIRO *et al.* 1962; AMES, HARTMAN and JACOB 1963). The most obvious way to visualize the duplication of *argH* is to invoke unequal crossing overs between two bacterial chromosomes, by analogy with the generation of the *Bar* genotype in Drosophila (STURTEVANT 1925). As Orn-ut variants occur as frequently in a population of F^- derivatives of MN8 as in cultures of the original *Hfr* strain, the double crossing-over required could be either the bacterial equivalent of a somatic crossing-over or the result of genetic transfer mediated by an undetected episome.

An important consequence of the *sup100* mutation is the non-repressibility of enzyme H synthesis by arginine, the enzyme being produced at a very substantial rate. This can be understood in at least two ways, between which it is not yet possible to make a choice.

1. The duplicated *argH* locus has been inserted in a neighbouring operon, turned on under our experimental conditions. The latter, however, have included measurement of enzyme H specific activity after growth in broth containing bactotryptone and yeast extract, (Table 1), which does not influence the rate of enzyme H synthesis in Orn-ut strains. The hypothesis further requires that the displacement of *argH* has not impaired any function essential to growth; this does not fit too well with the idea that the conjectural operon controlling the duplicated locus is turned on, producing more argininosuccinase than the wild type. (It can not be excluded, of course, that the specific activity measured reflects the insertion of *argH* in an operon which is turned off but has a very high basal activity).

2. The duplicated locus functions independently. In the line of current ideas on the control of gene activity, we may conceive that the absence of an operator locus does not preclude the expression of a gene provided that a promotor (JACOB, ULLMAN and MONOD 1964) is present. In the present case the required promotor could normally belong to the *argH* locus or it could have been generated at the proximal end of *argH* by the chromosomal rearrangement undergone by Orn-ut strains. Indeed, promotors could be relatively simple sequences easily generated by mutation, as evidenced by the RAX *try* mutants isolated by MARGOLIN and BAUERLE (1966). (We suggest that some of the RIX *try* mutants isolated by the same authors are in fact homologous to the Orn-ut mutants.)

The duplication of *argH* is of interest in another respect (GLANSDORFF 1966). A comparison of the genetic topologies of bacteria and higher organisms, mainly the fungi, suggests that the grouping of genes into operons is a more frequent feature in the former organisms than in the latter ones. The duplication (or transposition) of genes brought under the influence of polar mutations could have played a role in the scattering of genes originally grouped into operons; the present one not only occurs at a substantial frequency but is selected whenever the mutant is exposed to arginine starvation in the presence of a suitable inter-

mediate of the biosynthesis (in the present case, acetylornithine, ornithine or citrulline).

SUMMARY

Through a mutation (*sup-100*) which appears as duplicating *argH*, the distal gene of an arginine operon, that gene escapes the influence of a polar *argB* mutation. This phenomenon is discussed in relation to current ideas on the control of gene activity and is visualized as a possible intermediary step between the clustered and scattered types of gene organization.

LITERATURE CITED

AMES, B. N., P. E. HARTMAN, and F. JACOB, 1963 Chromosomal alterations affecting the regulation of histidine biosynthetic enzymes in Salmonella. J. Mol. Biol. **7**: 23–42.

BAUMBERG, S., D. F. BACON, and H. J. VOGEL, 1965 Individually repressible enzymes specified by clustered genes of arginine synthesis. Proc. Natl. Acad. Sci. U. S. **53**: 1029–1032.

CUNIN, R., D. ELSEVIERS, G. SAND, H. FREUNDLICH, and N. GLANSDORFF, 1968 Organisation fonctionelle d'un groupe de loci arginine contigus chez *Escherichia coli*. Arch. Intern. Phys. Biochim. **76** (in press).

DEMEREC, M., E. A. ADELBERG, A. J. CLARK, and P. E. HARTMAN, 1966 A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**: 61–76.

DE VLIEGHERE, M., and E. VANDERWINKEL, 1968 Physiologie et génétique du chemin glyoxylique et de la malate synthase propre à l'utilisation du glycolate chez *Escherichia coli*. European J. Biochem. **5**: 81–90.

ELSEVIERS, D., R. CUNIN, G. SAND, and N. GLANSDORFF, 1968 Reaktivering van genen onder de invloed van polaire mutaties. Arch. Intern. Phys. Biochim. **76** (in press).

GLANSDORFF, N., 1965 Topography of cotransducible arginine mutations in *Escherichia coli* K-12. Genetics **51**: 167–179. —— 1966 Le contrôle génétique des biosynthèses de l'arginine et du carbamoylphosphate chez *Escherichia coli*. Thesis, University of Brussels, Belgium.—— 1967 Pseudoinversions in the chromosome of *Escherichia coli* K-12. Genetics **55**: 49–61.

GLANSDORFF, N., and G. SAND, 1965 Coordination of enzyme synthesis in the arginine pathway of *Escherichia coli* K-12. Biochim. Biophys. Acta **108**: 308–311.

GLANSDORFF, N., G. SAND, and C. VERHOEF, 1967 The dual genetic control of ornithine transcarbamylase synthesis in *Escherichi coli* K-12. Mutation Res. **4**: 743–751.

HOPWOOD, D. A., 1967 Genetic analysis and genome structure in *Streptomyces coelicolor*. Bacteriol. Rev. **31**: 373–403.

JACOB, F., A. ULLMAN, and J. MONOD, 1964 Le Promoteur, élément génétique nécessaire à l'expression d'un opéron. Compt. Rend. Acad. Sc. Paris **258**: 3125–3128.

MAAS, W. K., 1961 Studies on repression of arginine biosynthesis in *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. **26**: 183–191.

MAAS, W. K., R. MAAS, J. M. WIAME, and N. GLANSDORFF, 1964 Studies on the mechanism of repression of arginine biosynthesis in *Escherichia coli*. I. Dominance of repressibility in zygotes. J. Mol. Biol. **8**: 359–364.

MCFALL, E., and W. K. MAAS, 1967 Regulation of enzyme synthesis in microorganisms. In: *Molecular Genetics*, II. Edited by J. H. TAYLOR, Academic Press, New York.

MARGOLIN, P., and R. H. BAUERLE, 1966 Determinants for regulation and initiation of expression of tryptophan genes. Cold Spring Harbor Symp. Quant. Biol. **31**: 311–320.

MATSUSHIRO, A., S. KIDA, J. ITO, K. SATO, and F. IMAMOTO, 1962 The regulatory mechanism of enzyme synthesis in the tryptophan biosynthetic pathway of *Escherichia coli* K-12. Biochem. Biophys. Res. Comm. **9**: 204–207.

SAND, G., and N. GLANSDORFF, 1967 L'opéron arginine d'*Escherichia coli*. Archiv. Int. Phys. Bioch. **75**: 568–569.

STURTEVANT, A. H., 1925 The effects of unequal crossing-over at the *Bar* locus in Drosophila. Genetics **10**: 117–147.

VOGEL, H. J., S. BAUMBERG, D. F. BACON, E. E. JONES, L. UNGER, and R. H. VOGEL, 1967 Gene-ribosome-enzyme organization in the arginine system of *Escherichia coli*. In *Organizational Biosynthesis*, edited by H. J. VOGEL, J. O. LAMPEN and V. BRYSON, Academic Press, New York.