REACTIVATION OF ARGININE GENES UNDER THE INFLUENCE OF POLAR MUTATIONS

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1. Introduction

The genetic loci *arg*E, C, B and H, tightly clustered in that order, respectively specify the enzymes L-ornithine: α-*N*-acetylornithine hydrolase, α-*N*-acetyl-L-glutamate γ-semialdehyde: NADP oxydoreductase (phosphorylating), ATP: α-*N*-acetyl-L-glutamate 5-phosphotransferase and L-argininosuccinate arginine lyase (ref. [1], fig. 1). *Arg*C, B and H are expressed with a strong degree of mutual coordination: the synthesis of the corresponding enzymes is repressible 50 to 70 times [2]; the repressibility coefficient of enzyme E, however, is about 18 [2,3]. The *arg*E nonsense mutations isolated so far do not influence the expression of *arg*C, B nor H. In contrast, mutants carrying certain *arg*EC or CB deletions, or *arg*B and C nonsense or frameshift mutations, exhibit a low potential of enzyme H synthesis [4,5]. These strains utilize ornithine at a rate proportional to this residual production. We aim to show that in these polar mutants, *arg*H may be reactivated by various mutations, which may be detected by an increase of the growth rate in the presence of ornithine. The ornithine-utilizers still argininеless (orn-ut) derivatives have undergone either (1) internal rearrangements of the *arg* cluster which throw some light on the functional organization of the latter or (2) near tandem duplications of the *arg* genes, which appear as chromosomal, although duplication on episomes cannot be excluded [6] as an intermediary step in the process.

2. Results and discussion

Transductions, conjugations [1], enzyme assays and cultures [2] were performed as described previously. All bacterial strains are derivatives of Hfr P4X [1]. The test applied to classify the various Orn-ut derivatives (*sup* genotype) uses a deletion mutant,

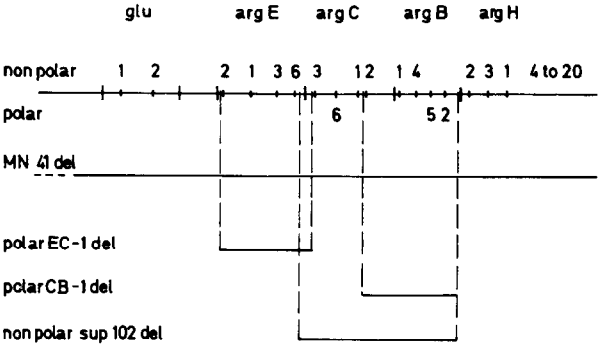

Fig. 1. The *arg*ECBH cluster; polar and non-polar mutations. *Arg*E and *arg*H are about 85% cotransducible.

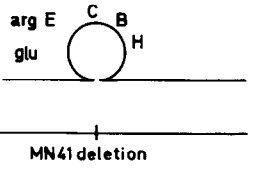

Fig. 2. Assumed configuration of paired genomes in transductions involving MN41 (*glu arg*E to H deletion) as recipient.

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Table 1
Enzyme specific activities in polar *arg* mutants, ORN-ut mutants and *arg*R (depressed) derivatives (average of 3 to 5 independent determinations in each case); n.m.: not measurable.

Strain	Pertinent feature	Supplement to minimal medium		Acetyl-ornithinase (*arg*E)	Phospho-transferase *arg*B)		Arginino-succinase (*arg*H)
P4X	w.t.	arginine	18X	4.6	0.1	54X	0.18
P4XB2	*arg*R	arginine		84.1	4.0		9.7
*arg*B-5	polar	ornithine		40.0	n.m.		0.17
*arg*CB-1	polar	ornithine		41.0	n.m.		0.21
*arg*EC-1	polar	ornithine		n.m.	n.m.		0.10
sup 102	*arg*R$^+$	arginine		n.m.	n.m.	14X	0.7
sup 102	*arg*R	arginine		n.m.	n.m.		9.7

MN41, from which the whole cluster and the linked *glu* locus are absent (ref. [2], fig. 1). The latter strain is used as recipient in transductions with Orn-ut donors. The rationale of these experiments, illustrated in fig. 2, is as follows:

a) Glu$^+$ recombinants are selected; if all (100 to 200 tested) are Orn-ut, the reactivation is inferred to have occurred within the limits of the deleted segment. If only some or no *glu*$^+$ recombinants are Orn-ut, the *sup* mutation is considered as external to the arg group.

b) Orn-ut recombinants are selected and scored (100 to 200) for the Glu phenotype. In case of an internal reactivation, all of them are expected – and actually found – to be Glu$^+$ (case of *sup* 102, see below). The occurrence of Orn-ut Glu recombinants, illustrated by transductions with mutants *sup* 101 and 104, not only shows that the *sup* mutation is external to the *arg* cluster but also that it compensates an argH deletion (fig. 2); all Glu recombinants inherit, indeed, the whole *glu arg* deletion [8]. Absence of Orn-ut recombinants should mean that the *sup* mutation is unlinked to the cluster and unable to compensate the loss of the *arg*H locus. Such external suppressors have also been isolated and are presently under study.

a) *Internal reactivation: sup 102*

This Orn-ut derivative of mutant *arg*CB-1 was found to carry a deletion of the whole *arg*C locus ($\leqslant 0.01\%$ recombination with *arg*C-1, 2, 3 and 4; Met$^+$ as standard) ending in *arg*B and *arg*E; the distal portion of *arg*E affected is so short that acetylornithinase is still partially functional: slow growth (5 hr doubling time) on acetylornithine is still possible.

The synthesis of enzyme H, now proceeding at a high rate, presumably because of the suppression of the frameshift present in the original mutant, exhibits a coefficient of repressibility (14, table 1) much smaller than in the wild-type (54) and similar to the one of enzyme E (18). That the maximal level produced by the *arg*R (derepressed) derivative of *sup* 102 is not limited by the physiological balance of the cell is attested by the still higher values found in *arg*R, *sup* 102 cells having received by conjugation an episome (F14 [7]) carrying a second exemplar of *arg*H. These merodiploids produce twice as much enzyme H (specific activity 16.0) as the *arg*R strain P4XB2 (table 1).

The *sup* 102 deletion thus puts *arg*H directly under the control of *arg*E, through destruction of the *arg*E-C boundary. These features, and the lack of polar effect of amber *arg*E mutants on its neighbours [4,5] show (1) that the decoordination observed between *arg*E and the *arg*CBH group is not an enzymological artefact, (2) that *arg*E and *arg*CBH have the same polarity (clockwise) but, although adjacent, are separated by a punctuation which might be an operator–promotor complex; other possibilities are however investigated, and discussed elsewhere [5].

b) *External sup mutations*

In transductions between MN41 (fig. 2) and *sup* 101 or *sup* 104 mutants (derived from *arg*EC-1 and *arg*B-5, respectively) the occurrence of Glu recombinants points to an external reactivation by a mecha-

nism among the Orn-ut ones able to replace the missing *arg*H function. Their frequency indicates that *sup* 101 and *sup* 104 are 40 to 60% cotransducible with *glu*. We conclude that both *sup* mutations are chromosomal duplications of *arg*H, or *arg*B and H, on the following basis.

(a) The phenotype of all Glu^+ Sup^+ (not Orn-ut) recombinants show that Orn-ut strains will harbour the parental polar defect *and* a normally situated *arg*H locus under its influence. The possibility of a translocation of *arg*H, leaving a deletion at the usual site, is thus excluded.

(b) In both mutants, as in Glu Orn-ut recombinants, enzyme H specific activity is not repressible by arginine (nor by nutrient broth). Maximal or submaximal activities are recorded: 3.6 ± 0.2 for *sup* 104, 7.1 ± 0.7 for *sup* 101 (compare with P4XB2, table 1). Interestingly enough, the analysis of the *sup* 101 strain and of 4 Glu Orn-ut issued from the transduction between MN41 and *sup* 101, shows that the latter mutation also leads to a near maximal and no more repressible production of enzyme B (not measurable in the parental strain *arg*EC-1).

(c) Conjugation experiments between a glu-1 argH-2 F^- strain and the Orn-ut mutants (Hfr's of the P4X type) indicate close linkage to *glu* (40 to 50% Orn-ut among Glu^+) and 1 to 2% with thr-leu (Orn-ut among $Thr\text{-}Leu^+$).

Sup 104 is in first analysis similar to *sup* 100 [8], although considerably more active and occurring in a different strain. *Sup* 101 exhibits the interesting feature of a reactivation affecting 2 genes: this practically excludes the possibility of unknown enzymes taking over the function of *arg*B and H.

Chromosomal duplications are noteworthy if one considers that they may have been a step in the transition from the clustered type of gene organization (frequent in bacteria) to the scattered pattern more often found in higher organisms. It remains to be shown if the Orn-ut genotype of *sup* 100, 101 and 104 imply either: (1) the presence of initiator sites at the beginning of the genes becoming duplicated, (2) the duplication of the latter in nonessential operons, (3) the formation of initiators at the site of insertion in silent DNA regions, or, finally (but may be not exhaustively) the reactivation of cryptic *arg* genes produced by ancestral duplications.

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