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# A New Technique for Selection of Sensitive and Auxotrophic Mutants of *E. coli*: Isolation of a Strain Sensitive to an Excess of One-carbon Metabolites

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**Summary.** An *E. coli* strain deleted in the region mal-asd is used for the selection of conditional or auxotrophic mutants. Thermosensitive and auxotrophic strains have thus been isolated on plates. After selection in liquid medium, a strain has been isolated which is sensitive to excess one-carbon metabolites. It carries two mutations, *smg* A[1] (near *met* A and *arg* H), probably identical to *rel* C, and *smg* B (between *asn* and *ilv*), probably part of the *E. coli* membrane ATPase.

In the course of our analyses of the functions of secondary punctuation signals in prokaryotic organisms, we have found that a molecule, derived from the tetrahydrofolate metabolism, acts as a coupling signal essential for important macromolecular syntheses. This has been recognized, for instance, from the observation that growth of the relaxed mutants of *E. coli* is completely inhibited by excess of the one-carbon pool metabolites (Uzan and Danchin, 1976a, b). Such a growth inhibition can easily be obtained *in mineral media* supplemented with serine (100 μg/ml) and either another one-carbon aminoacid (such as glycine or methionine) or a metabolite from the tricarboxylic respiratory cycle. This prompted me to investigate whether it was possible to find other loci than *relA* involved in this inhibition, and also to study its relieving. In this article, I present evidence for two other mutants yielding a strain which cannot grow on a synthetic medium supplemented with serine, methionine and glycine. The study of the metabolic reversion of this inhibition will be presented in a further article (Uzan and Danchin, 1976b), where the complete one-carbon metabolism is developed.

[1] *Abbreviations.* dap: l meso diaminopimelic acid, smg: serine + methionine + glycine, 1:1:1 per weight

The penicillin enrichment procedure is generally used when one needs a selection for sensitive or auxotrophic strains (Miller, 1972). It is grounded on the observation that only *growing* cells are killed by the antibiotic whereas non-growing bacteria are left alive, provided that the mutation(s) they harbor are not lethal. The killing is the consequence of an imperfect wall synthesis yielding cellular lysis by osmotic pressure in hypotonic media. This broadly used technique has two drawbacks: first, it is hardly possible to have enrichments higher than one thousand fold, second the mutants resistant to the antibiotic appear in significant amounts (about one in $10^8$ bacteria). This precludes single selection from non-mutagenized cultures and obliges to do several enrichment cycles, with at least two different antibiotics (usually ampicillin, followed by cycloserine, in semi-anaerobic conditions). Besides, it is not possible, or very difficult, to obtain mutants on plates, which precludes rapid screening of independent mutations.

For all these reasons, I decided to look for a technique which would enable plate selection, at least for auxotrophic or temperature-sensitive mutations, and which would allow a more extensive enrichment of mutants. I use the same basic principle as in penicillin selection, i.e. killing of rapidly growing cells due to abnormal wall synthesis, but, instead of using an external agent, I use a bacterial mutation which leads to the same result. l-mesodiaminopimelic acid (dap) is known as an essential wall constituant in *E. coli*, since it couples the aminoglycosidic backbone to the peptide backbone of murein; I use a diaminopimelic auxotrophic strain, having a chromosomal deletion producing this auxotrophy. Upon growing, this mutant ($asd^-$) lyses in hypotonic media which do not contain added diaminopimelate, and the deletion ensures that there are no detectable spontaneous reversions allowing growth in the absence of dap. Thus one can grow bacteria on a selective medium, liquid

or solid, devoid of dap, then, after an appropriate lag, add diaminopimelate and required metabolites under permissive conditions. Only those bacteria which had been unable to multiply in the former, non-permissive conditions, will yield colonies in the latter. I report hereafter the general applications of the method and the result of the selection of a serine-+methionine+glycine (smg) sensitive mutant of *E. coli*.

## Material and Methods

### *E. coli Strains*

Some of the strains come from *E. coli* genetics stock center, thanks to the courtesy of Dr. B. Bachman. The others are kept in the Institut Pasteur collection, thanks to the care of Miss S. Lacaille.

The mutant used for the enrichment procedure, $MD_3$, has been selected as a maltose deletion by Dr. M. Schwartz (Schwartz, 1966).

The Hfr strains used for rapid mapping come from the collection of Cold Spring Harbor Laboratory, after reisolation by Dr. M. Springer. They are those used in the technique described by Dr. B. Low (Low, 1973).

The strains are listed in Table 1.

### *Selection for Temperature-sensitive Mutations*

Selection plates were used as follows:

– For selection of temperature-sensitive mutants in minimal media, $10^7$ bacteria of strain $MD_3$ were spread onto M63 plates (Miller, 1972) supplemented with histidine, threonine, methionine and lysine (100 µg/ml each), thiamine (5 µg/ml) and 0.4% glucose as a carbon source. The plates were then incubated at 42° C for four days. Care must be taken as to keep the atmosphere of the incubator saturated with humidity: otherwise, plates become slowly drier, which increase the ionic strength and protects the growing cells against lysis.

– For selection of ts mutants in rich media $10^{10}$ $MD_3$ bacteria were spread onto freshly prepared LB plates (Miller, 1972) without dap, and incubated at 42° C for four days with constant humidity.

After the incubation time, a minimal soft agar (0.6% agar) supplemented with diaminopimelate (corresponding to 100 µg/ml per plate, final concentration) was poured gently on the plate (at the temperature of 45° C) and incubation proceeded at 30° C for 24–48 h. Single colonies were picked out with a platinum wire, purified and tested for their thermosensitivity.

**Table 1.** Listing of the parental strains used in this work

| Strain | Genotype | Phenotype |
|---|---|---|
| $MD_3$ | Hfr(G6) *thi his Δ(malA-asd)* | $B_1^-$ $His^-$ $Mal^-$ $Dap^-$ $Homoserine^-$ [a] $Glycerol^-$ |
| Hfr H | Hfr *thi relA* | $B^-$ $Smg^s$ |
| Hfr 6 | Hfr *metB relA mut2 mtl malA* | $Met^-$ $Mtl^-$ $Mal^-$ $\lambda^r$ $Smg^s$ |
| KL 99 | Hfr *thi relA lac* | $B_1^-$ $Lac^-$ $Smg^s$ |
| PK 191 | Hfr *Δ(lac-pro) sup 56* | $Lac^-$ $Pro^-$ $Su^+$ |
| KL 14 | Hfr *thi relA* | $B_1^-$ $Smg^s$ |
| KL 25 | Hfr *supE 42* | $Su^+$ |
| Ra 2 | Hfr *supE 42 malA* | $Mal^-$ $Su^+$ $\lambda^r$ |
| KL 209 | Hfr *sup 53 malB* | $Su^+$ $\lambda^r$ |
| KL 228 | Hfr *thi leu sup 54 lac gal* | $B_1^-$ $Leu^-$ $Lac^-$ $Gal^-$ $Su^+$ |
| KL 16 | Hfr *thi relA* | $B_1^-$ $Smg^s$ |
| KL 983 | Hfr *xyl lac mgl* $P_1$ | $Xyl^-$ $Lac^-$ |
| KL 96 | Hfr *thi relA* | $B_1^-$ $Smg^s$ |
| KL 208 | Hfr | |
| KL 226 | Hfr *relA tonA* $T_2$ | $T_1^r$ $T_2^r$ $Smg^s$ |
| BW 113 | Hfr *metB* | $Met^-$ |
| AB 2002 | Hfr *ilvA argHpurF xyl supE44* | $Ile^-$ $Arg^-$ $Ade^-$ $Xyl^-$ $Su^+$ |
| ER [b] | $F^-$ *thi asn* | $B_1^-$ $Asn^-$ |
| $MDA_3$ [b] | $F^-$ *thi unc* | $B_1^-$ $Succ^-$ $Smg^s$ |
| $MDB_{10}$ [b] | $F^-$ *thi unc* | $B_1^-$ $Succ^-$ $Smb^s$ |
| $MDA_8$ [b] | $F^-$ *thi unc leu thyA deo lacZ strA* | $B_1^-$ $Succ^-$ $Thy^-$ $Leu^-$ $Lac^-$ $Str^r$ $Smg^s$ |
| $MDA_{18}$ [b] | $F^-$ *thi unc leu thyA deo lacZ strA* | $B_1^-$ $Succ^-$ $Thy^-$ $Leu^-$ $Lac^-$ $Str^r$ $Smg^s$ |
| POP 815 [c] | $F^-$ *thi thyA argH metA trpE his strA rpoB* | $B_1^-$ $Thy^-$ $Arg^-$ $Met^-$ $Trp^-$ $His^-$ $Str^r$ $Rif^r$ |
| POP 815 A | id *thyA*$^+$ after $P_1$ transduction | id $Thy^+$ |
| 815 164 | $F^-$ *thi argH metA trpE strA rpoB* (cross of POP 815A with KL 16) | id $His^+$ |
| 1649 | $F^-$ *thi argH trpE ilvA rpoB* (cross of 815 164 with AB2002) | id $Ile^-$ $Str^s$ $Met^+$ |

[a] Homoserine may be replaced by threonine + methionine

[b] Obtained from Dr. J. Daniel (Daniel et al., 1976)

[c] Built by Dr. M. Hofnung

*Selection for Aminoacid Auxotrophy*

The same basic procedure was used for isolating, on plates, mutants which require an aminoacid for growth. The incubation was done at 37° C on M63 supplemented as above, without dap and other aminoacids. After four days of incubation, soft agar containing casaminoacids (Difco) (final concentration 2 mg/ml), tryptophan (final concentration 50 µg/ml) and dap (100 µg/ml final) was poured on the plate, and left for further incubation. After 36 h, colonies were picked out, purified and tested for their auxotrophy.

*Selection for smg Sensitivity*

Sensitivity to excess one-carbon metabolites is expected to occur only in minimal media. The selection procedure has therefore been applied on $MD_3$ bacteria in M63, in the presence of smg (100 µg/ml each). Smg influence is relieved on rich medium, therefore the premissive step of the enrichment used LB broth supplemented with casaminoacids and dap.

It would have been useless, here, to perform a selection on plates since addition of rich media would have revealed not only the smg-sensitive mutants but also, and in a large proportion, auxotrophic mutants. The isolation of the proper mutant was therefore obtained in liquid medium.

The following procedure was repeated five times:

Five millimeters of M63 supplemented with thiamine (5 µg/ml), threonine, histidine, lysine and serine, methionine and glycine at 100 µg/ml each were inoculated with $10^7$ $MD_3$ bacteria and incubated for 24 h at 37° C with aeration. Then 5 ml of LB were added together with casaminoacids (Difco, at a final concentration of 1 mg/ml) and dap (100 µg/ml) and incubation proceeded for 10 h. 0.1 ml of the bacteria were then inoculated to fresh M63 containing dap (100 µg/ml), homoserine (100 µg/ml), histidine (100 µg/ml) and thiamine (5 µg/ml), and incubated for 36 h, in order to eliminate the auxotrophs. 0.05 ml of these bacteria were then submitted to the same cycle.

At the end of this procedure, bacteria were streaked onto LB plates containing dap. Isolated colonies were assayed for their smg sensitivity on M63 supplemented with dap, homoserine, histidine, with and without smg as a control.

## Results

*Isolation of Temperature-sensitive and Auxotrohic Mutants*

Following the procedures described in the Material and Methods section, I isolated 32 colonies on LB; after purification, 19 were found to be thermosensitve since they grew well on LB plates at 30° C, in the presence of dap, whereas they failed to grow at 42° C in the same conditions. The reversion frequency for thermosensitivity was quite variable (from less than $10^{-8}$ to about $10^{-6}$) showing that these mutants, isolated as independent colonies, were most likely to carry different mutations. The frequency of these ts mutants is about $5 \cdot 10^{-9}$.

The efficiency of the enrichment procedure on M63 supplemented as indicated in the Material and Methods section could be confirmed after the isolation of auxotrophic mutants; after purification of 32 independent colonies, 24 were found to be able to grow in the presence of casaminoacids supplemented with tryptophan and dap and not without the aminoacids. A screening on plates supplemented with different aminoacids showed that one had obtained a variety of auxotrophs: 6 $Arg^-$, 6 $Pro^-$, 5 $Trp^-$, 3 $(Ile+Val)^-$, 2 $(Phe+Tyr+Trp)^-$, 1 $Leu^-$, 1 $Gly^-$. The total frequency of auxotrophs was bout $8.10^{-7}$.

*Isolation of a Serine + Methionine + Glycine-sensitive Strain*

The parental strain $MD_3$ grows well in the presence of serine, methionine and glycine in medium M63. A mutant SMD31 has been isolated after five cycles of growth in minimum medium supplemented with smg, in the absence of dap followed by growth in rich medium plus dap, and finally minimum medium plas dap without smg, as described in the Methods section.

The SMD31 mutant is able to grow on M63 supplemented with thiamine (5 µg/ml), homoserine (100 µg/ml), diaminopimelate (100 µg/ml) and histidine (100 µg/ml). It retained its Hfr character, injecting the deletion *Δ (mal-asd)* in the first minutes. This mutant fails to grow on the same medium when serine, methionine and glycine are added (from 20 µg/ml each to 100 µg/ml). However, this inhibition is reversed, at least partly when isoleucine (100 µg/ml) is added together with smg (Uzan and Danchin, 1976b); this relieving does not restore normal growth rate as it is observed in *relA* mutants (Uzan and Danchin, 1976b); however it can be improved when leucine, valine (100 µg/ml) and panthotenate (1 µg/ml) are added together with isoleucine: under such conditions the mutant appears to be unsensitive to smg.

*Genetic Characterization of the Mutant*

In order to perform a rapid mapping of the mutation using the technique described by B. Low, I isolated streptomycin-resistant mutants of SMD31. Among five independent $str^r$ alleles (all found to map in the strA region) only one, SMD31 S4 was still completely sensitive to smg. This shows that a mutation in the 30S subunit of the ribosome may interfere with the mutation(s) producing smg sensitivity.

SMD31 S4 was grown in LB supplemented with dap to stationary phase and aerated then for 10 h. This was done in order to obtain phenocopies of the strain, devoid of male pili (Miller, 1972). 0.1 ml of the culture was poured on an M63 plate supplemented with streptomycine (100 µg/ml), dap, ho-

**Table 2.** $P_1$ transduction into POP 815 A from Smg sensitive mutant

| Donor | Recipient | $Met^+$ | $Rif^+$ | $Smg^s$ | $Arg^+$ | Selection |
|---|---|---|---|---|---|---|
| SMD31 S4 | POP 815 A | 32 | 20 | 9 | 0 | $Met^+$ |
| | | (100%) | (58%) | (26%) | (0%) | |
| ($rif^+$ | | 0 | 21 | 13 | 32 | $Arg^+$ |
| $smg^s$ | | (0%) | (60%) | (38%) | (100%) | |
| $arg^+$ | | 3 | 3 | 3 | 3 | $Met^+$ $Arg^+$ |
| $met^+$) | | | | | | |

One always observes a frequency of Smg sensitive recombinants lower than expected. This is probably due to the high frequency of reversion of this phenotype (Uzan and Danchin, 1976a). The results can tentatively be interpreted as giving the order met A ... rpo B ... smg A ... argH

moserine and histidine (100 mg/ml) together with smg (100 µg/ml each) and a replica of the various Hfr strains, arranged in concentric circles according to their point of injection on the *E. coli* chromosome (Low, 1973), was made on this minimal plate for replica making. Incubation was then performed at 42° C in order to have a clean inhibition by smg.

From growth of recombinants, it was clear that the mutation(s) mapped between the point of injection of KL25 and Hfr H. Some growth of mating pairs derived from Ra2, KL209 and KL228 suggested that the mutation(s) could lie around *ilv* and/or in the *met* B region.

From this result, I could use SMD31 as a Hfr donor in order to inject the mutation into female recipients. Mating was performed with POP815A with selection for $Met^+$ $Arg^+$ or 1649 with selection for $Ile^+$ $Met^+$. All recombinants from POB815A became smg-sensitive, however, with a wide variation in sensitivity and reversion potentiality, about one among ten recombinants being as sensitive as the parent SMD31. This suggested that there might be *two* mutations yielding smg sensitivity instead of one, and this could explain why I had obtained recombinants from KL228 and Ra2 which grew on smg. Indeed, nine among ten of the recombinants from the cross SMD31 × 1649 were as smg-sensitive as the original mutant. In addition, after a selection on M63 plate supplemented with glycerol instead of glucose, I observed that these SMD31 × 1649 recombinants failed to grow on glycerol, as did the recombinant derived from POP815A which had the highest smg sensitivity. Since the only known marker in the ilv region which leads to failure of glycerol utilization is the membrane ATPase (Daniel et al., 1975; Bachman et al., 1976), I tested the ability of the recombinants to grow on L-malate: those which had the highest smg sensitivity failed to grow in the presence of L-malate as a carbon source.

I therefore undertook the characterization of the mutations using $P_1$ transduction. A $P_1$ lysate obtained from SMD31 S4 was used to select for $Met^+$ and/or $Arg^+$ recombinants in POP815A. Three $Met^+$ $Arg^+$ were found; they were also rifampicin-sensitive, as expected since SMD31 S4 was rifampicin-sensitive and smg-sensitive, albeit with a lower efficiency than in SMD31 S4 (high frequency of reversion for resistance). These strains were also $malate^+$. 32 $Met^+$ and 32 $Arg^+$ were also tested for smg sensitivity. Table 2 summarizes the results obtained. The amount of smg-sensitive recombinants is always lower than expected from the gradient $Met^+$ $Rif^+$ or $Arg^+$ $Smg^+$ $Rif^+$, probably because of the high frequency of reversion towards resistance (about $10^{-5}$). However, since no $Smg^s$ $Met^+$ recombinants were found to be also $Rif^r$, whereas a few $Arg^+$ $Smg^s$ recombinants (three) were also $Rif^r$, it is possible to conclude that the $Smg^s$ mutation, smgA, lies between *rpo* B and *arg* H (Bachmann et al., 1976): *met* A *rpo*B *smg*A *arg* H.

In order to characterize the second mutation, the same kind of transductions were done using strain 1649 and selecting for $Ilv^+$. 20% of the recombinants were also smg-sensitive, exhibiting however a slow growth on smg-supplemented plates. In order to have a better characterization of the mutations, I used strain 1649-1 which is a recombinant from a cross SMD31 S4 × 1649, which is $Met^+$ $Ilv^+$ and very sensitive to smg (therefore harbouring the two smg mutations): transduction was performed, using a $P_1$ lysate obtained on strain ER which is auxotrophic for asparagine and selecting, in the presence of asparagine, either for recombinants able to grow on glycerol or

**Table 3**

| | Growth on l-malate | Smg sensitivity |
|---|---|---|
| ER $Asn^+$ | + | − |
| $MDA_3$ | − | + |
| $MDA_8$ | − | ± |
| $MDA_{18}$ | − | + |
| trkD | + | + |

Transduction into ER of different alleles of unc A and trk D. All the mutants are Smg sensitive

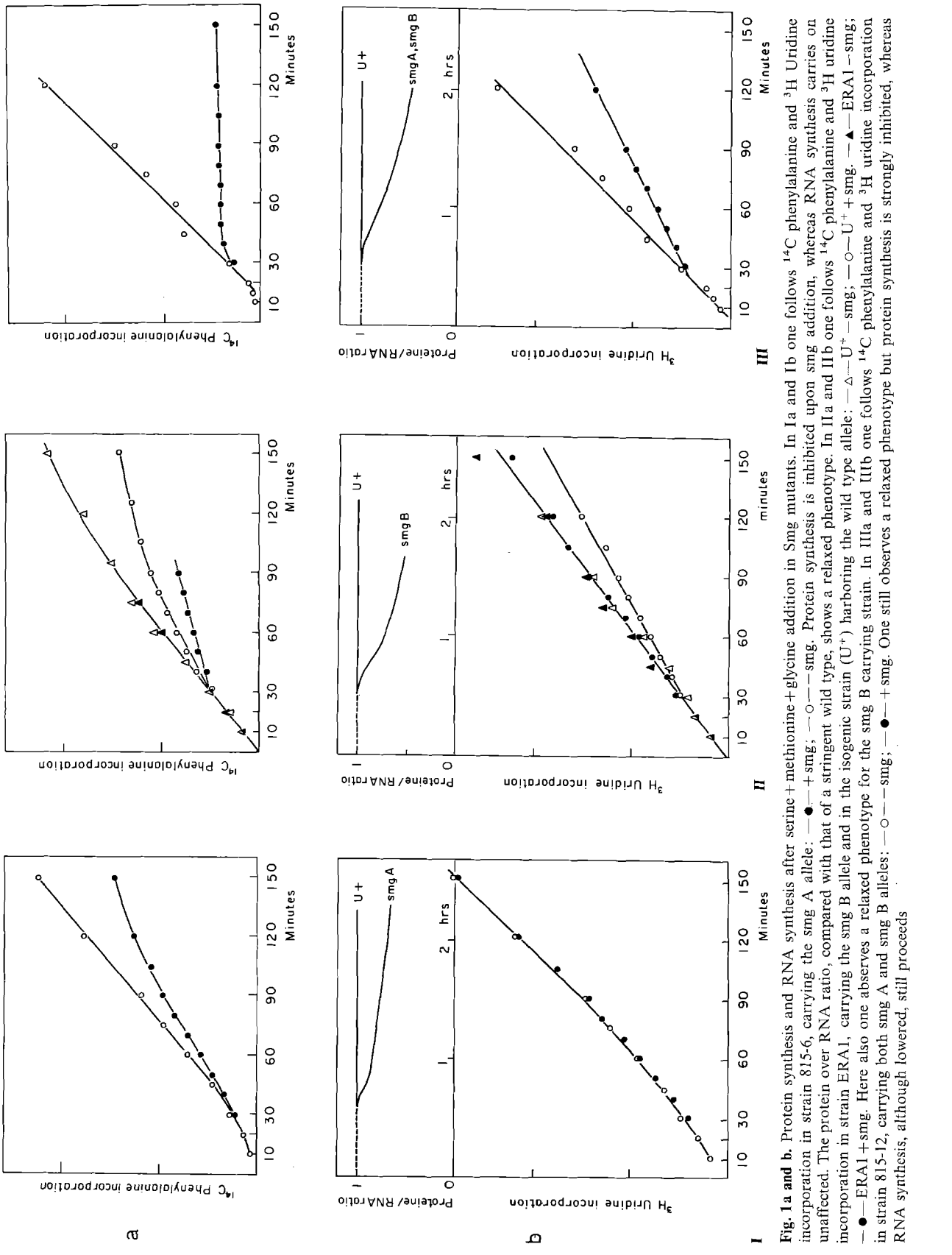


**Fig. 1a and b.** Protein synthesis and RNA synthesis after serine+methionine+glycine addition in Smg mutants. In Ia and Ib one follows $^{14}$C phenylalanine and $^{3}$H Uridine incorporation in strain 815-6, carrying the smg A allele: —●— +smg; —○— smg. Protein synthesis is inhibited upon smg addition, whereas RNA synthesis carries on unaffected. The protein over RNA ratio, compared with that of a stringent wild type, shows a relaxed phenotype. In IIa and IIb one follows $^{14}$C phenylalanine and $^{3}$H uridine incorporation in strain ERA1, carrying the smg B allele and in the isogenic strain ($U^+$) harboring the wild type allele: —△—$U^+$—smg; —○—$U^+$+smg. —▲—ERA1−smg; —●—ERA1+smg. Here also one observes a relaxed phenotype for the smg B carrying strain. In IIIa and IIIb one follows $^{14}$C phenylalanine and $^{3}$H uridine incorporation in strain 815-12, carrying both smg A and smg B alleles: —○— smg; —●— +smg. One still observes a relaxed phenotype but protein synthesis is strongly inhibited, whereas RNA synthesis, although lowered, still proceeds

for smg-resistant clones. In both cases, 30% of the clones were also auxotrophic for asparagine; this shows that the second mutation, smg B, lies near asn (Table 3).

In summary, strain SMD31 possesses two $smg^s$ mutations, one near *asn* and *ilv* and the other between *met* A and *arg* H.

### *Some Physiological Properties of the Mutant*

Since SMD31 harbors two mutations, and since the parental strain exhibit a somewhat slow growth, I decided to study physiological properties after conjugation or transduction.

Four strains were used: 1649-1, which is derived by conjugation from the cross Hfr SMD31 S4 × 1649 after selection for $Ilv^+$ and $Met^+$, and harbors *smg* A and *smg* B; 815-6, which is derived from SMD31 S4 × POP815 after selection for $Met^+$ and $Arg^+$, and harbors *smg* A; 815-12, derived from the same cross but harboring *smg* A and *smg* B. *Smg* B was assayed after transduction from SMD31 S4 into ER and selecting for prototrophy (strain ER Al).

The figure shows the profile of incorporation of $^{14}$C-phenylalanine and $^{3}$H-uridine upon addition of smg in these different strains. One observes that there is some continuation of RNA synthesis after smg addition which leads to RNA accumulation, as it is observed in relaxed mutants. However, two main differences can be seen: first, the inhibition of protein synthesis is very abrupt in the smg mutants compared to the wild type or *relA* mutants second, the RNA accumulation slowly slackens pace whereas it remains in a steady state for a longer time in the *relA* mutants.

In order to support the conclusion that *smg* B could be related to the membrane ATPase complex, several alleles of *unc* A (mutants yielding a defect in this complex) were transduced into ER after selection for $Asn^+$. The *trk* D mutation, which leads to defect in potassium transport (Epstein and Kim, 1971), was also assayed. Table 3 shows that all these mutations yield smg sensitivity, although their behaviour are different in the ATPase assay (Daniel et al., 1975, Epstein and Kim, 1971). Indeed, *trk* D mutant can grow on L-malate as a carbon source.

## Discussion

The penicillin enrichment procedure for isolation of sensitive or auxotrophic mutants of *E. coli* can advantageously be substituted for by the use of a strain requiring diaminopimelate for growth, carrying a deletion yielding the auxotrophy. In particular, the method I described allows for selection of conditional mutants on rich media and might be used for the isolation of drug-sensitive strains. One of the best advantages of the method is the fact that it enables to obtain mutants as isolated independent colonies on plates.

I could not make use of this advantage for the isolation of a strain sensitive to excess one-carbon metabolites, but succeeded in isolating such a mutant. The strain isolated carried two mutations named *smg* A and *smg* B, which map near *ilv* and near *rif* (*rpo* B, Bachmann et al., 1976). Each of these mutations has a relaxed phenotype since the isoleucine deprivation induced by smg (Uzan and Danchin, 1976b) stops protein synthesis but fails to stop RNA accumulation, at least for one hour.

As we have seen, the drawback of the enrichment method in liquid media is that one can collect several mutations during the cycles of enrichment. Indeed a mutant will be better protected against the lethal effect of dap starvation if it stops absolutely its growth. In the case of mutant SMD31, it can seen on the figure that protein synthesis is very efficiently inhibited upon addition of smg, especially in the presence of both mutations *smg* A and *smg* B.

The genetic localization and the interference with some $str^r$ alleles of the mutation *smg* A suggest that it is identical with *rel*C, known to give a relaxed phenotype and supposed to be the gene coding for the ribosomal protein Lll (Block and Haseltine, 1974). Our results thus suggest that there is a coupling between the 30S and 50S subunits of the ribosome, mediated by proteins S12 and L11, and interfering with the synthesis of ppGpp which is necessary for obtaining a correct stringent response.

The locus *smg* B, which appears to be related to the membrane ATPase complex of *E. coli* is more difficult to visualize as directly involved in the I-C pool control of macromolecular syntheses. However, mutants defective in the membrane ATPase have a general defect in the transport of charged metabolites (such as cations, inorganic phosphate, etc., Daniel et al., 1975; Bachmann et al., 1976, other markers near *ilv* and data not shown), and one might speculate that the elimination from the cell of ppGpp could be related to the correct functioning of this ATPase complex; thus a mutation affecting its correct activity could perhaps yield a low level of ppGpp accumulation upon aminoacid starvation. On the other hand, we have observed that the control of cell growth by the I-C metabolism is directly related to the respiration of the cell, since tricarboxylic metabolites (Uzan and Danchin, 1976a), or anaerobic conditions (unpublished observations) lead to a stop of cell growth when the I-C metabolites are added to the medium.

Finally, I had described (Danchin, 1973) that a selection medium consisting of the I-C metabolites, together with antifolic agents such as trimethoprim and sulfonamides yielded mutants falling in several classes: DNA synthesis (Thy A), RNA synthesis ($Rif^r$), protein synthesis ($Str^r$); a fourth class (about 1% of the mutants) fell into another category: their growth was either inhibited by the Krebs cycle metabolites, or they were unable to use these metabolites as carbon sources (unpublished observations). All these results suggest that there is a major relation between the membrane of *E. coli* and the control of macromolecular syntheses by the I-C metabolism.

Further work is in progress to characterize the genetic and biochemical events which are involved in this coupling.

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