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## *Short communications*

# The glutamate dehydrogenase structural gene of *Escherichia coli*

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**Summary.** The glutamate dehydrogenase structural gene, *gdhA*, was mapped at 38.6 min on the genetic map and at 1860 kb on the physical map. A detailed map of this region is presented.

**Key words:** *Escherichia coli* – Glutamate dehydrogenase – *gdhA* gene

The enteric bacteria have two primary pathways of assimilatory ammonia metabolism, each leading to formation of glutamate. One pathway involves the enzyme glutamate synthase (GOGAT) together with glutamine synthetase. In *Escherichia coli*, GOGAT is encoded in the *gltBDF* operon at 69.4 min on the genetic map (Bachmann 1983) corresponding to coordinates 3420–3430 on the physical map (Kohara et al. 1987). Glutamate dehydrogenase (GDH) also mediates glutamate synthesis. Its structural gene, *gdhA*, is located at 27 min on the *Salmonella typhimurium* chromosome, close to *pncA* (Rosenfeld et al. 1982). Mutations causing loss of GDH in *E. coli* and in *Klebsiella aerogenes* have been mapped near *trp*, also at about 27 min. In this report I show that the GDH structural gene (*gdhA*) in *E. coli* maps at about 38.6 min, close to *pncA*. This corresponds to the *S. typhimurium* location when a large rearrangement distinguishing the two species from each other is taken into account (Sanderson and Hall 1970).

Mutants that are singly deficient in either pathway have no growth factor requirement, but *glt gdh* double mutants require aspartate or glutamate (Reitzer and Magasanik 1986). Aspartate is convertible to glutamate and is utilized more readily than glutamate (Pahel et al. 1978; Rosenfeld et al. 1982). I observed an aspartate-requiring mutant ($Asp^-$ phenotype) among colonies appearing after plating from a culture sample frozen after 728 generations of glucose-limited growth in a chemostat (Helling et al. 1987). However the mutant, designated RH448, grew without supplementation anaerobically, unlike a bona fide *glt gdh* mutant obtained from the *E. coli* Genetic Stock Center. Enzyme assays showed the mutant to be deficient in GDH, as was the parent strain, JA122. RH448 showed GOGAT activity during anaerobic growth, but not in air. The parent strain had normal GOGAT activity. The mutation affecting GOGAT activity mapped to the region of the *gltBDF* operon and is complemented by the Clarke-Carbon clone bank (Clarke and Carbon 1974) plasmid pLC9–34, containing *gltBDF* (K. Corrado and R. Helling unpublished results).

Mating with a set of Hfr strains (Singer et al. 1989) showed that a gene conferring an $Asp^+$ phenotype to an RH448 derivative (RH461) was transferred by an Hfr (RH470) transferring counterclockwise from 61.5 min as well as by Hfrs transferring the *gltBDF* operon. However no such recombinants were obtained from matings transferring counterclockwise from 35 min, across the putative locus of *gdh* at 27 min.

RH470 (Table 1) was found to transfer the gene for $Asp^+$ after *gyrA* ($Na^r$, at 48 min) and Tn*10* ($Tc^r$, at 43 min) had been transferred by the same donor. Among $Asp^+$ $Kn^r$ recombinants, 58.3% were $Na^r$ and 80.6% were $Tc^r$. Application of an appropriate mapping function (Low 1987) suggested that the gene for $Asp^+$ was located at approximately 38 min.

Transduction experiments showed that the gene for $Asp^+$ cotransferred with a Tn*10* integrated at about 37.4 min (Table 2), at a frequency suggesting that the two loci were over 1 min apart (Low 1987). The *gap* locus is known to map at 39.3 min (Bachmann 1983), or 1880 on the physical map (Branlant and Branlant 1985). If the gene for $Asp^+$ were located clockwise from 37.4 min, it would be expected to map between 37.4 and 39.3, and to cotransduce with *gap*. Indeed the genes did cotransduce at a relatively high frequency (Table 2), and direct assay showed that an $Asp^+$ *gap-3* transductant had GDH but lacked GOGAT. $Asp^+$ transductants arise from incorporation of genes at either of two different regions of the chromosome (at 38 min and at the *gltBDF* operon at 69 min), thus complicating the use of the cotransduction data to estimate distance. Nevertheless it

**Table 1.** Strains

| Strain | Relevant characteristics | Source or reference |
|---|---|---|
| *Escherichia coli*: | | |
| JA122 | $F^-$ *araD139 thi-1 gdh supE44 hss-1(λ)* plus plasmid pBR322Δ5 | Helling et al. (1987) |
| RH448 | $Asp^-$ JA122, lacks plasmid | Clone from frozen sample taken after 728 generations of glucose-limited culture of JA122; Helling et al. (1987) |
| RH542 | *Δ(xthA-pncA)90* | Received from B. Weiss as strain BW9115, a derivative of strain BW9101 of White et al. (1976) |
| RH461 | RH448::Tn*5* ($Kn^r$) | Infection with λL71:Tn*5* (the location of Tn*5* was not determined) |
| RH470 | KL16 *zed-3069*::Tn*10* *gyrA* ($Na^r$, 48 min) | L. Peruski; CAG5055 of Singer et al. (1989); spontaneous $Na^r$ mutant |
| RH474 | *zdh*::Tn*10* | D. Friedman (the Tn*10* is 3′ of *himA* and was used to obtain the *himA* deletion strain K1299 (Friedman et al. 1984)) |
| RH480 | *gap-3* | B. Bachmann (CGSC5585); DF225 of Hillman and Fraenkel (1975) |
| RH481 | *gap-7* | B. Bachmann (CGSC5953; DF240, a derivative of DF234 of Hillman and Fraenkel (1975) |
| Lambda phages: | | |
| 4B8 (328)<br>12H7 | | Y. Kohara, A. Ishihama and T. Nagata; Kohara et al. (1987) |
| Plasmids: | | |
| pLC7-10<br>pLC10-4<br>pLC26-8<br>pLC40-13 | | R. Van Bogelen; Clarke and Carbon (1974) |
| E1923 | | S. Tabata; Tabata et al. (1989) |

$Tc^r$ indicates tetracycline-resistance, $Kn^r$ indicates kanamycin-resistance, and $Na^r$ indicates nalidixic acid-resistance

seemed likely that the gene for $Asp^+$ in the 38 min region was about 30–40 kb to the left of *gap* (as oriented in the map in Fig. 1).

Therefore a set of cloned DNA segments from the *gap* region was examined in order to identify those that contained a gene conferring an $Asp^+$ phenotype on RH448. RH448 infected with λ4B8 gave prototrophic recombinants. Enzyme assays on one such recombinant showed it to contain GDH but to be still deficient in GOGAT. No protrophic recombinants appeared following infection with λ12H7, which contains a cloned DNA segment partially overlapping that in λ4B8 (Kohara et al. 1987). Transformants containing the plasmid pLC10-4 or pLC26-8 were also $Asp^+$, but no prototrophs appeared following transformation with plasmids containing adjacent segments of cloned *E. coli* DNA (pLC7-10 and pLC40-13). The plasmid E1923 contains a cloned *E. coli* DNA segment that completely overlaps the DNA in λ4B8, pLC10-4, and pLC26-8. However transformants of RH448 containing E1923 remained $Asp^-$. Cells with this plasmid are strongly selected against and plasmid loss or rearrangement is observed frequently (S. Tabata, personal communication; my observations), so it is likely that the gene from λ4B8, pLC10-4, and pLC26-8 that converts RH448 to $Asp^+$ was not present in a functional from in the E1923 plasmid that was used.

**Table 2.** Cotransduction of *gdh* and nearby loci

| Donor | Selected (number scored) | Unselected (number observed) | Frequency of cotransduction |
|---|---|---|---|
| RH474 | $Tc^r$ (50) | $Asp^+$ (2) | 0.04 |
| RH474 | $Asp^+$ (252) | $Tc^r$ (4) | 0.02 |
| RH480 | $Asp^+$ (205) | *gap-3* (49) | 0.24 |
| RH481 | $Asp^+$ (516) | *gap-7* (151) | 0.29 |

In every case the recipient was RH461 ($Tc^s$ $Asp^-$) and the vector was P1bt. Transductions were carried out by standard procedures (Lakshmi and Helling 1976). Selection for $Asp^+$ was on minimal medium (Helling et al. 1987) containing succinate (0.2%) plus glycerol (0.2%) as carbon and energy sources. Inability to grow on glucose minimal medium at 40° C indicated the presence of a *gap* mutation. *gap-7* mutants grow on glucose at 30° C but not at 40° C

The sequence of *gdh* has been determined in two laboratories (McPherson and Wootton 1983; Valle et al. 1984). I compared the restriction map of *gdh* and its flanking region with the restriction map of the entire *E. coli* chromosome (Kohara et al. 1987). The two maps coincided at physical coordinate 1860 on the overall map, corresponding to about 38.6 min on the genetic map. The map of the *gdh* locus failed to match the chromosome in the region of 27 min or indeed at any location other than 1860. The segment containing *gdh* is present on λ4B8 and on pLC10-4 and pLC26-8, but is absent from the other clones tested. I conclude that the structural gene for GDH (*gdhA*) is at coordinate 1860, and that RH448 and its parent JA122 are deficient in GDH because of mutation at that locus.

Several other genes known to be in this region of the chromosome have been sequenced. I have correlated the sequences with each other and with the regional restriction map to give the detailed genetic and physical map shown in Fig. 1. Other genes have been reported within this region but have not been mapped precisely. These include a gene suppressing formation of deletions

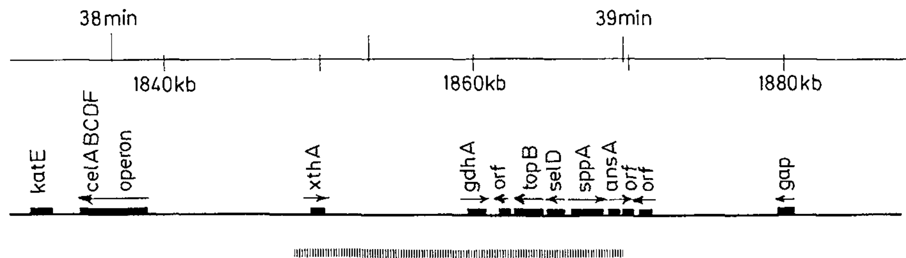


**Fig. 1.** Genetic and physical map of the region containing *gdhA*. The physical map coordinates are from Kohara et al. (1987; the genetic map positions from Bachmann (1983). Gene positions are based on the physical map. Note that 1 min on the genetic map would correspond to 47 kb if there were a strict linear correlation of genetic and physical maps. The *lower line* indicates the known extent of the *xth-pnc* deletion (White et al. 1976). Absence of the functions of *gdh*, *ansA* (or *nit*), and *selA* in the deletion strain but not in the parent was shown, respectively, by direct assay, by inability to use asparagine well as nitrogen source, and by lack of production of gas from glucose anaerobically. References for the sequences are: *celABCDF* (Parker and Hall 1990), *xthA* (Saporito et al. 1988), *gdhA* (McPherson and Wootton 1983; Valle et al. 1984), *topB* (DiGate and Marians 1989), *selD* (*fdhB*; Leinfelder et al. 1990), *sppA* (Ichihara et al. 1986), *ansA* (Jerlström et al. 1989), *gap* (Branlant and Branlant 1985). A restriction map of *katE* is presented in Mulvey et al. (1988). No gene within this segment was included in the recent alignment of about 50% of the *E. coli* data base sequences with the overall physical map (Rudd et al. 1990)

(*topB*?; Yi et al. 1988), a gene for utilization of aspartate as carbon source (*nit*?; Spring et al. 1986), *ackB* (*sppA* or *topB*?), *feo* (Hantke 1987), *pncA*, and in *Salmonella*, the *pncX* gene (Hughes et al. 1983) and the *nit* gene, which controls use of aspartate, asparagine, and many other organic nitrogen sources (Broach et al. 1976). The *pncA* gene is known to map midway between *xthA* and *gap* in *E. coli* (White et al. 1976). On the basis of three point crosses it has been reported to map to the right of *sppA* (Suzuki et al. 1987), and well to the left of *ansA* (Del Casale et al. 1983). These results are inconsistent because sequencing has shown the *E. coli sppA* (Ichihara et al. 1986) and *ansA* (Jerlström et al. 1989) genes to be adjacent (Fig. 1).

The *pncA* gene of *Salmonella* is the second gene of a two-gene operon transcribed divergently from *gdh* (Hill-Chappell et al. 1986) and is located on the opposite side of *gdh* from *nit* (Rosenfeld et al. 1982). Possibly *pncA* is the second gene of the *ansA* operon (Jerlström et al. 1989), and *pncX* in *Salmonella* is the gene for asparaginase I (*ansA*). Both the asparaginase and nicotinamide amidohydrolase (from *pncA*) enzymes are produced constitutively (Jerlström et al. 1989; Hill-Chappell et al. 1986). The amidohydrolase is found in the periplasmic space, and the 5′ end of the gene following *ansA* encodes what appears to be a typical signal sequence for membrane transport (Jerlström et al. 1989). The substrates of the two enzymes have similar structures and the reactions appear to be nearly identical. On the other hand, if *pncA* maps to the left of *ansA* it is presumably close to *gdh*. Genetic distance measurements in this region are not linear (White et al. 1976), and three factor crosses have given incorrect gene order in other regions also (Williams et al. 1988).

Nevertheless there is an alternative explanation for the seemingly contradictory mapping results that does not invoke unusual genetics. *nit* mutants are impaired in utilization of many organic nitrogen sources including asparagine, and it is reasonable to think that the general impairment would also extend to use of nicotinamide. If the putative *pncA* mutant selected and used in mapping studies by Del Casale et al. (1983) were in fact mutant in the *nit* gene, the results could be rationalized with a map order *nit-gdh-sppA-ansA-pncA-gap*. It is plausible that some putative *ansA* mutations are actually *nit* mutations, and this would also confuse mapping.

A well studied deletion extends between *xthA* and *pncA* (White et al. 1976). The deletion mutant is known to lack *sppA* (Suzuki et al. 1987) and also the gene suppressing formation of deletions (Yi et al. 1988). I found that the mutant lacks the functions controlled by *gdh*, *ansA* (or *nit*), and *selD* (formerly *fdhB*; Haddock and Mandrand-Berthelot 1982; Leinfelder et al. 1990) as well (Fig. 1), unlike its parent which is proficient for them. Thus the deletion removes DNA from at least coordinates 1850 through 1868.

These results show unambiguously that *gdhA*, the structural gene for glutamate dehydrogenase, is located at 38.6 min. How then could the location have been assumed to be at 27 min? In part it is because this seemed to correspond to the location of *gdhA* in *Salmonella*, at about 27 min (Rosenfeld et al. 1982). However *Salmonella* and *Escherichia* differ by a large inversion of the segment between about 25–27 and 35–39 min, and by smaller rearrangements near the inversion endpoints (Riley and Krawiec 1986). The current results suggest that *gdh* was included within the overall rearrangement together with the closely linked gene *pncA*, known to be at 25 min in *Salmonella* and at about 38.5 min in *Escherichia*. In *S. typhimurium*, the functional equivalent of *selD* of *E. coli* is *selA* (Leinfelder et al. 1990), which maps in the general region of 21 min (Kramer and Ames 1988) and so is probably also in the rearranged segment.

The primary mapping of *gdh* to about 27 min in *E. coli* (Pahel et al. 1978) was based on a whole-cell, single-colony assay and a total of three cotransductant colonies. In one cross, 2 of 78 $purB^+$ transductants were *gdh*. In a second cross, 1 of 52 $trp^+$ transductants was *gdh*. The same *gdh gltB* strain (Berberich 1972) was used in selecting for the cloned DNA segments containing a $gdh^+$ gene that were sequenced (McPherson and Wootton 1983; Valle et al. 1984). The cloned gene was verified

as the *gdh* structural gene (Sanchez-Pescador et al. 1982; Valle et al. 1983; Mattaj et al. 1982), and marker-rescue experiments suggested that the chromosomal mutation causing loss of GDH activity was in the *gdh* structural gene (Mattaj et al. 1982).

Subsequently Vogler et al. (1989) discovered a new gene (*glmX*) affecting amino sugar metabolism that mapped to 26.8 min, between *trp* and *purB*. However, using the same *gdh gltB* strain as Pahel et al. they found no cotransduction of *gdh* with *trp*, *glmX*, or either of two other genes in this region. They concluded that *gdh* was not located near 27 min. [Note that their Table 5 has errors suggesting cotransduction of *gdh* and *trp*. No cotransduction of *gdh* was found with any gene in this region (J. Lengeler, personal communication)]. I have used that same strain in matings and obtained results consistent with the location of the *gdh* mutation near 38 min, but found no Hfr gene that conferred an $Asp^+$ phenotype and mapped within the region immediately counterclockwise of 35 min. I conclude that the original mapping of *gdh* in *E. coli* was in error, and that subsequent results are consistent with the map location reported in this paper.

The *gdh* gene was also reported to map close to *trp*, at about 27 min, in *K. aerogenes* (Bender et al. 1976). Cotransduction of *gdh* was observed with both *trp* and *pyrF*. Significant numbers of different categories of transductants were obtained and the data are self-consistent. The *gdh* mutation was complemented by an F′ carrying a segment of *E. coli* extending from about 21.5 to 27 min. I have used the same F′ (F′126) in matings with a $Na^r$ derivative of the *E. coli* strain used in the original mapping, and with RH448. The F′ plasmid failed to complement the *gdh* mutation of either *E. coli* recipient. Thus it seems unlikely that the *gdh* mutation of *K. aerogenes* is in the GDH structural gene. The nature of the *K. aerogenes gdh* gene remains to be determined.

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**Note added in proof**

Both a recent map of *E. coli* DNA sequences (Kröger, Wahl and Rice, 1990, Nucleic Acids Res 18:2549) and the recent *E. coli* genetic map (Bachmann, 1990, Microbiol Rev 54:130) include several of the genes shown in Fig. 1 but in incorrect order. Sak, Eisenstark and Touti (1989, Proc Nat Acad Sci USA 86:3271) located *xthA* but in incorrect orientation. *nadE* is an essential gene that may be in this region (Hughes, Olivera and Roth, 1988, J Bacteriol 170:2113. The order and orientation of genes in Fig. 1 is correct with the possible exception of *gap*. The orientation of *gap* could be incorrect because the scarcity of restriction sites at this locus makes correlation with the map of Kohara et al. (1987) difficult.