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LETTERS TO THE EDITOR

Mechanisms of Increasing Expression of a Yeast Gene in *Escherichia coli*

Escherichia coli strains with increased expression of the cloned yeast *his*3 gene were selected. In some mutants an *E. coli* chromosomal locus is altered; in others the yeast *his*3 sequence is affected. Alterations of the *his*3 sequence include a point mutation, deletion, and IS2 insertion. When IS2 inserts into the yeast sequence, all pre-existing copies of IS2 are maintained in their original location in the *E. coli* chromosome. The results indicate that *E. coli* can employ a variety of mechanisms to increase expression of a foreign gene.

Interspecific transformation may be an important aspect of evolution by providing sources of genetic diversity beyond those available to an organism by interaction with members of the same species. A number of evolutionarily diverse species such as bacteria, yeast, mammalian cells and plants are capable of DNA uptake (Mandel & Higa, 1970; Hinnen *et al.*, 1978; Wigler *et al.*, 1978; Lurquin & Kado, 1977). Genes can be expressed across species barriers. Examples include the expression of rabbit β-globin gene in monkey cells (Mulligan *et al.*, 1979), the expression of the human thymidine kinase gene in mouse cells (Wigler *et al.*, 1978), and the expression of a number of yeast genes coding for intermediary metabolic enzymes in *Escherichia coli* (Struhl *et al.*, 1976; Ratzkin & Carbon, 1977). Though many features of gene expression are conserved in evolutionarily distant species, the regulatory systems of donor and recipient species may differ. The ability to alter the expression of a newly acquired gene would be evolutionarily advantageous.

This study investigates mechanisms by which *E. coli* increases the expression of a newly introduced eukaryotic gene. *E. coli* strains with the *hisB*463 mutation lack imidazoleglycerolphosphate dehydratase activity and therefore require histidine for growth. A 10·1 kb† fragment (Sc2601) has been isolated containing the yeast IGP dehydratase (*his*3) gene (Struhl *et al.*, 1976; Struhl & Davis, 1977). When integrated as a prophage, a bacteriophage λ *his*3 hybrid (λgt4-Sc2601) complements the *hisB*463 mutation; the lysogen *hisB*463 (λgt4-Sc2601), when grown in the absence of histidine, requires expression of the yeast *his*3 gene for histidine biosythesis. The growth rate of *hisB*463 (λgt4-Sc2601) on minimal medium is limited by a lack of histidine, because the expression of the yeast IGP dehydratase is insufficient for wild-type rates of histidine biosynthesis (Struhl *et al.*, 1976). Unlike the genes of the *E. coli his* operon, the expression of the yeast *his*3 gene is not regulated as a function of histidine starvation (Struhl *et al.*, 1980). For these reasons, the lysogen *hisB*463 (λgt4-Sc2601) is

† Abbreviations used: kb, 10^3 base-pairs; IGP, imidazoleglycerolphosphate.

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sensitive to low concentrations of aminotriazole, a competitive inhibitor of IGP dehydratase. Aminotriazole also inhibits *de novo* adenine biosynthesis in *E. coli* (Bond & Akers, 1961). Thus an increase in the expression of yeast IGP dehydratase will increase resistance to aminotriazole only in the presence of adenine. A non-specific resistance to aminotriazole would result in increased resistance regardless of the presence or absence of adenine. A selection and screen based on the above considerations allows the isolation of lysogens which increase expression of the yeast IGP dehydratase.

(a) *Isolation and characterization of* his*3 over-producers*

Seventeen independent isolates of the lysogen *hisB*463 (λgt4-Sc2601) were plated on minimal medium supplemented with 1 mM-aminotriazole and with 0·2 mM-adenine. Colonies appeared at a frequency of 10^{-6}. One aminotriazole-resistant colony from each original lysogen was tested for growth on minimal medium supplemented with 6 mM-aminotriazole in the presence or absence of 0·2 mM-adenine. Ten isolates grew regardless of the adenine supplement; these were presumed to be non-specifically resistant to aminotriazole (possibly permeability mutations). Seven isolates grew only in the presence of adenine; these were presumed to have increased expression of yeast IGP dehydratase.

Mutations either in the bacterial chromosome or in the prophage might result in increased expression. To map the mutations conferring aminotriazole resistance each lysogen was cured of its resident prophage and the prophage of each isolate was induced. The cured cells were reinfected with λgt4-Sc2601, while the induced phage were used to reinfect *hisB*463. New lysogens were selected on minimal medium and tested for growth in the presence of 6 mM-aminotriazole and 0·2 mM-adenine. Of the seven presumptively overexpressing lysogens, four gave rise to cured cells that showed aminotriazole resistance when relysogenized with λgt4-Sc2601; the mutations therefore map on the *E. coli* chromosome. Three isolates, when induced, give rise to derivatives of λgt4-Sc2601 that confer aminotriazole resistance to *hisB*463 cells which harbor them as lysogens. These three phages (λgt4-Sc2682, λgt4-Sc2683, and λgt4-Sc2684) were studied in more detail.

Lysogens of *hisB*463 with λgt4-Sc2682, λgt4-Sc2683, or λgt4-Sc2684 cultured on minimal medium have the same growth rate as *hisB*463 cultured on minimal medium supplemented with histidine (Table 1). *hisB*463 (λgt4-Sc2601) grown on minimal medium had an appreciably slower growth rate. The specific activity of yeast IGP dehydratase in the lysogens of *hisB*463 with the three derivatives is three to eight times that of the lysogen *hisB*463 (λgt4-Sc2601) (Table 1).

Restriction endonuclease cleavage site patterns of λgt4-Sc2682, λgt4-Sc2683 and λgt4-Sc2684 phage DNAs were compared to the parental λgt4-Sc2601 DNA (Fig. 1). Any physical alteration was mapped with respect to the location of the *his*3 gene determined previously (Struhl & Davis, 1979). λgt4-Sc2682 DNA has no detectable alterations. λgt4-Sc2683 DNA has a deletion of 1·4 kb with one endpoint within 0·15 kb of the 5′ end of the IGP dehydratase structural gene. λgt4-Sc2684 DNA has a 1·35 kb insertion with an endpoint within 0·1 kb of the 5′ end of the IGP dehydratase structural gene. This insertion has a *Hin*dIII site.

TABLE 1

Doubling times and IGP dehydratase levels of hisB463 *(λgt4-Sc2601) and aminotriazole-resistant derivatives*

Strain	Doubling Time (h)	IGP dehydratase (spec. act.)
*his*B463	N.D.†	—
*his*B463 (λgt4-Sc2601)	1·6	0·5
*his*B463 (λgt4-Sc2682)	1·2	1·4
*his*B463 (λgt4-Sc2683)	1·2	2·4
*his*B463 (λgt4-Sc2684)	1·2	3·8
*his*B463‡	1·2	<0·1

Growth rates at 30°C in minimal medium were determined as described by Struhl *et al.* (1976). The specific activity of IGP dehydratase was determined by the method of Struhl & Davis (1977).

† N.D., not detectable.

‡ Grown on minimal medium supplemented with 40 μg histidine/ml.

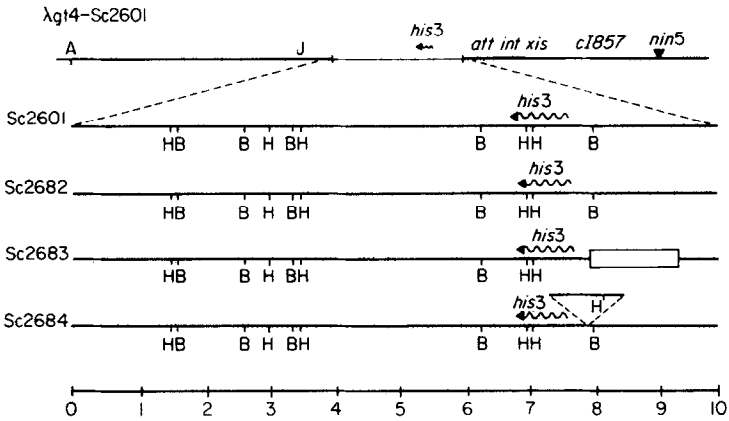

FIG. 1. Sc2682, Sc2683 and Sc2684 DNAs were mapped in relation to *Bam*HI (B) and *Hin*dIII (H) sites in Sc2601 DNA. The mutations were mapped at high resolution with respect to the *his*3 gene (Struhl & Davis, 1980; data not shown). The *Hin*dIII site in Sc2684 is 0·9 kb from the left end of the insertion. The position is consistent with IS2 being in orientation II with respect to the *his*3 gene (Ghosal & Saedler, 1978).

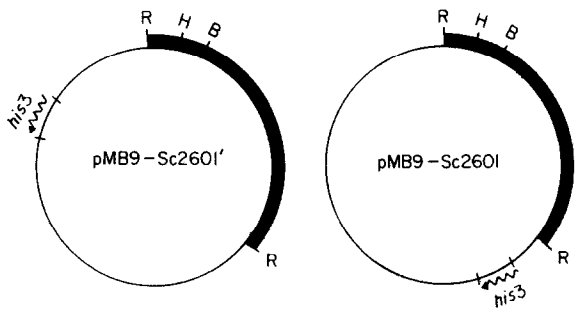

FIG. 2. The orientation of Sc2601 in the plasmids pMB9-Sc2601 and pMB0-Sc2601′ is analogous to the orientation of Sc2682 and Sc2683 in pMB9-Sc2682, pMB9-Sc2682′, pMB9-Sc2683 and pMB9-Sc2683′.

The Sc2682 and Sc2683 DNA fragments were cloned into pMB9 DNA at the *Eco*RI restriction endonuclease cleavage site, as previously described for the Sc2601 fragment (Struhl & Davis, 1977). Two plasmids were isolated for each fragment; they differ in the orientation of the inserted fragment with respect to the vector (Fig. 2). The Sc2682 and Sc2683 fragments were cleaved from the plasmids by *Eco*RI restriction endonuclease, and were re-introduced into the λgt4 vector (Panasenko *et al.*, 1977). Again, two λ-hybrids differing in orientation of the insert to vector, were isolated.

(b) *Increased expression by point mutation*

The level of *his*3 expression in Sc2682 depends upon orientation in both λgt4 and pMB9 vectors. As a prophage in *hisB*463, λgt4-Sc2682 confers resistance to 1 mM-aminotriazole, while λgt4-Sc2682′ does not. Further, *hisB*463 (pMB9-Sc2682) is resistant to 15 mM-aminotriazole, while *hisB*463 containing pMB9-Sc2682′, pMB9-Sc2601, or pMB9-Sc2601′ is resistant to only 10 mM-aminotriazole. Therefore Sc2682 has a mutation that is necessary but not sufficient for aminotriazole resistance.

One model consistent with these results is that the differential expression between prophages of λgt4-Sc2682 and λgt4-Sc2682′ results from transcription originating from a promoter in the vector sequences. *hisB*463 (λgt4-Sc2601′) does not have a higher level of expression than *hisB*463 (λgt4-Sc2601) because of a sequence in the yeast DNA which terminates transcription from this vector promoter; Sc2682 has mutated this terminator. In support of this model, λgt4-Sc2682 has a higher level of *his*3 expression than λgt4-Sc2601 when expression depends on transcription initiated at the λ promoter P_L (Struhl *et al.*, 1980). Because of the structure of normal single lysogens, the most reasonable location for the promoter is in λ DNA between the *Eco*RI restriction endonuclease cleavage site at 54·3% λ and the λ attachment site. "Readthrough" from the *E. coli* biotin operon cannot explain the increased levels of *his*3 expression, as biotin concentration in the medium does not affect the level of aminotriazole resistance.

(c) *Increased expression by deletion*

Sc2683 when cloned in either orientation in λgt4 or pMB9 confers increased aminotriazole resistance when compared to the analogous Sc2601 derivatives (Tables 1 and 2). Therefore, the deletion in Sc2683 is directly responsible for increased expression. Two possible explanations for these results are that either the novel junction of Sc2683 forms a promoter, or that the deletion brings a promoter in the Sc2683 fragment nearer to the *his*3 structural gene.

In addition, the level of aminotriazole resistance exhibited by Sc2683 is partially dependent on the orientation in the vector (Table 2). The orientation dependence may be explained by the model proposed for Sc2682. Because Sc2683 has a deletion, it is likely that the proposed terminator is located in the deleted DNA sequence. Clarke & Carbon (1978) noted deletions associated with increased expression of *arg*4, but did not map the deletion with respect to the *arg*4 gene.

(d) *Increased expression by IS2 insertion*

The size, restriction endonuclease cleavage pattern, and function of the insert in λgt-Sc2684 suggested that the insert was IS2. The Sc2684 fragment has strong

TABLE 2

Levels of aminotriazole resistance

Strain	Aminotriazole concentration (mM) 1	5	10	15	20	25
*his*B463 (pMB9-Sc2601)	+	+	+/−	−	−	−
*his*B463 (pMB9-Sc2601′)	+	+	+/−	−	−	−
*his*B463 (pMB9-Sc2682)	+	+	+	+/−	−	−
*his*B463 (pMB9-Sc2682′)	+	+	+/−	−	−	−
*his*B463 (pMB9-Sc2683)	+	+	+	+	+	+/−
*his*B463 (pMB9-Sc2683′)	+	+	+	+/−	−	−

Cells were tested for growth on minimal medium plates supplemented with 0·2 mM-adenine and the indicated concentration of aminotriazole. A + indicates the ability to form single colonies comparable to those on minimal medium; +/− indicates that the strains could form only small colonies; − indicates that no colonies appeared.

homology to λp*gal*8-490 (Decrombrugghe *et al.*, 1973), which contains IS2, but the Sc2684 fragment does not have homology to λp*gal*8. That IS2 is found inserted very near the 5′ end of the *his*3 structural gene supports the suggestion of Saedler (1977) that "IS2 could be used in evolution as a prefabricated element to allow expression of newly evolved, or previously silent genes if the element becomes transposed to these genes". IS2 was previously reported to be associated with an increase in the expression of the yeast *trp*5 gene in *E. coli* (Walz *et al.*, 1978). However, as the orientation and location of this yeast gene with respect to the inserted IS2 element were unknown, the result did not demonstrate that increased expression was a consequence of IS2 insertion.

(e) *Original chromosomal locations of IS2 are maintained after an insertion event*

λgt4-Sc2684 and λp*gal*8-490 have homology to five *Eco*RI restriction endonuclease-generated fragments from *his*B463 DNA to which λgt4-Sc2601 and λd*gal* do not have homology. This result is consistent with that reported by Saedler & Heiss (1973), who found approximately five copies of IS2 in *E. coli*. The DNA of the original lysogen isolate of *his*B463 (λgt4-Sc2684) has six *Eco*RI fragments which hybridize to λp*gal*8-490 but not to λp*gal*8. The DNA of parental *his*B463 (λgt4-Sc2601) has only five of these *Eco*RI fragments with IS2 homology. The new fragment in *his*B463 (λgt4-Sc2684) comigrates with the Sc2684 fragment. The conservation of the pre-existing copies of IS2 in the insertion of IS2 into λgt4-Sc2601 supports replication-dependent models for the migration of insertion elements (Shapiro, 1979).

(f) *Summary*

E. coli can increase the expression of the yeast *his*3 gene either by mutating a chromosomal locus, or by altering *his*3 and/or adjacent sequences. Three mechanisms of increasing expression of a foreign gene in *E. coli* have been characterized in some detail. These are (1) a point mutation which may alter a terminator for readthrough transcription from non-yeast DNA promoters, (2) a deletion mutation which may create a more efficient promoter, and (3) insertion of IS2 in the orientation conducive

to transcriptional initiation from the mobile element. As the three λ hybrids that have been characterized all have different mutations, it is likely that other mutations could result in increased expression of the yeast *his*3 gene.

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