# Relationship Between Transformation Frequency and Gene Function in the Histidine Degrading Enzymes of Bacillus subtilis

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## Summary

The relationship between gene function and recombination has been investigated by transforming Bacillus subtilis mutants having lost the ability to synthesize a functional histidase with DNA isolated from a histidase positive strain both in the presence and absence of histidine. Two histidase structural mutants were investigated. With one of these as recipient, the relative frequency of histidase transformants increased when histidine was added to the transformation medium. However, when the second mutant was used as recipient, the addition of histidine had no effect on the relative frequency of histidase transformants. The further observation that the relative transformation frequency was increased by the addition of histidine in an apparently pleiotropic negative mutant raises further doubts concerning any direct coupling between genetic recombination and gene function.

## Introduction

The relation between gene function and genetic recombination has been investigated in different systems with contradictory results. Helling[1] has reported that recombination in the arabinose operon of E. coli is increased when inducer is present. However, Shestakov et al.[2] find that induction has no effect on recombination in the lactose operon.

In an attempt to resolve this conflict, we have investigated the relation between genetic recombination and gene function by DNA mediated transformation of Bacillus subtilis mutants having lost the ability to synthesize functional histidase. Recipient bacteria were transformed with

DNA from a histidase positive, sulfanilamide resistant strain both in the presence and in the absence of histidine, an inducer of the enzymes involved in the degradation of histidine. The consequences of the addition of histidine were determined by comparing the frequency of histidase positive ($H^+$) transformants with the frequency of sulfanilamide resistant ($SA^r$) transformants.

The control of histidase synthesis in B. subtilis has been described by Hartwell and Magasanik.[3,4] The genetic system was further studied by Chasin and Magasanik.[5] Three mutations ($H_1$, $H_2$, $H_3$) in the histidase structural gene were shown to be closely linked. A mutation conferring resistance to catabolite repression ($R_1$) and a mutation conferring partial constitutivity ($C_1$) were shown to be closely linked to the structural gene for histidase. A pleiotropic negative mutation ($P_1$) resulting in the loss of three enzymes involved in histidine degradation (histidase, urocanase, and forminoglutamic acid hydrolase) was described. They were unable to demonstrate any recombination between $P_1$ and $R_1$.

## Materials and Methods

Bacterial strains:

1. SB25-113 ($H_3^-$, $R_1^-$, $C_1^-$, $SA^s$): This strain, described by Chasin and Magasanik[5], was obtained from B. Magasanik. It should be noted that the $C_1$ mutation confers only partial constitutivity: the rate of histidase synthesis in an $H^+C_1^-R_1^-$ strain is increased three-fold by histidine addition.[5] The presence of the $R_1^-$ mutation was necessary in order to permit induction in the presence of glucose, the latter being required for the growth of competent populations.

2. SB25-16 ($P^-$, $SA^S$): SH-1 ($H^+$, $R_1^-$, $C^+$), obtained from B. Magasanik, was mutagenized by exposure to UV and a mutant lacking histidase activity was isolated. The mutant is also unable to make urocanase (< 5% of wild type), so we referred to it as pleiotropic negative. Transformation of this mutant with wild type DNA ($H^+$, $R^+$) did not yield any $H^+R_1^-$ recombinants (< 0.5%), indicating that this mutation, like that described by Chasin and Magasanik[5], is closely linked or allelic to $R_1$. $H^+$ transformants were observed when SB25-16 was transformed with $H_1^-$ DNA, indicating that we are not dealing with a deletion. For the purpose of obtaining high levels of transformation, the P mutation was transferred into SB25 (a high competence strain) by transformation of SB25 with $P^-$ DNA.

3. SB25-14 ($H_1^-$, $R_1^-$, $C^+$, $SA^S$): SB25-1 ($H^+$, $R_1^-$, $C^+$) was transformed with SH-4 DNA ($H_1^-$, $R^+$, $C^+$). Both strains were obtained from B. Magasanik. Histidase negative transformants were isolated. These were then tested for the presence of the $R_1^-$ marker by transforming with $168^+$ DNA ($H^+R^+C^+$) and looking for recombinants which were $R^-H^+$. One of the histidase negative recombinants which was found to be $R_1^-$ was designated SB25-14. The presence of the original $H_1^-$ allele in this strain was confirmed by the failure to observe $H^+$ transformants when SB25-1 was transformed with SH-4 DNA, although $SA^r$ transformants occurred with normal frequency.

Experimental procedure:

Cultures were grown to competence by standard procedures[6] in LC-2 (Spizizen minimal salts; glucose 0.5%; 0.02% casamino acids; phenylalanine, tryptophan, tyrosine and histidine at 20 γ/ml; parahydroxybenzoic acid and paraaminobenzoic acid at 0.02 γ/ml). The culture was then divided into two aliquots, and histidine was added to one at a final concentration

of 5 x $10^{-4}$ M. DNA (1 γ/ml) was added immediately and transformation continued for 30 minutes, at which time it was terminated by addition of deoxyribonuclease. $H^+$ transformants were scored according to Chasin and Magasanik.[5] $SA^r$ transformants were scored according to Cahn and Fox.[6]

## Results

The three strains described were transformed both in the presence and in the absence of histidine with DNA isolated from an $H^+SA^r$ strain ($168^+SA^r$). The average results of several identical experiments with each recipient are presented in Table 1. The relative frequency of histidase transformants ($H^+/SA^r$) was increased by the addition of histidine in the cases of SB25-113 and SB25-16. However, in the case of SB25-14, the $H^+/SA^r$ transformant ratio was not affected by the addition of histidine.

Table 1

| Recipient Strain | Genetic Markers | $H^+/SA^r$ Transformant Ratio Without Histidine Addition | With Histidine Addition | Number of Determinations |
|---|---|---|---|---|
| SB25-113 | $H_3^-$, $R_1^-$, $C_1^-$ | 0.26±0.06 | 0.83±0.25 | 8 |
| SB25-16 | $P^-$ | 0.046±0.006 | 0.21±0.02 | 5 |
| SB25-14 | $H_1^-$, $R_1^-$, $C^+$ | 0.64±0.12 | 0.81±0.16 | 4 |

The indicated recipients were transformed with wild type DNA ($H^+$, $SA^r$) in the presence or absence of histidine as described in the text. The figures given for the $H^+/SA^r$ transformant ratios are average values from several identical experiments with different competent cell preparations. Standard deviations are given.

The chemical specificity of the stimulation of transformation by histidine addition was examined in experiments in which amino acids other than histidine were added individually to transformation media which lacked histidine. SB25-113 was used as the recipient; valine, proline, lysine, and serine were added at 50 γ/ml. The $H^+/SA^r$ ratio was independent of any of these amino acid additions, and was the same as that observed under the standard experimental conditions when histidine was absent from the media. These results suggest that, when it occurs, the stimulation of histidase transformation by histidine is specific for that amino acid, just as induction of histidase synthesis is a specific property of histidine.

Although the absolute frequency of $SA^r$ transformants was not markedly affected by the presence of histidine, the yield occasionally increased by as much as a factor of two. It was therefore desirable to show that the consequence of the addition of histidine was due to a specific increase in the frequency of $H^+$ transformants. SB25-113 was transformed, both in the presence and in the absence of histidine, with DNA isolated from an erythromycin resistant ($ery^r$), histidase positive strain obtained from B. Magasanik. The $H^+/ery^r$ transformant ratio responded to the presence of histidine in the same manner as did the $H^+/SA^r$ transformant ratio.

The effect of the presence of histidine on recombination events occurring within the histidase region of the genome was examined by transforming SB25-113 and SB25-14 with DNA from $168^+SA^r$ ($R^+H^+$), and scoring for $R^-H^+$ recombinants.[5] In the case of SB25-113 the relative frequency of $R^-H^+$ recombinants was increased to the same extent as was the frequency of $H^+$ transformants by the addition of histidine. In the case in which SB25-14 was used as a recipient, the relative frequency of $R^-H^+$ recombinants was

not influenced by the addition of histidine.

## Discussion

The data suggest that there is no simple relationship between transformation frequency and induction or repression of the structural gene containing the marker to be transformed. In the case of SB25-113, a partially constitutive strain, the relative frequency of $H^+$ transformants is increased when histidine is present in the transformation media. In contrast, addition of histidine has no effect on the relative transformation frequency of strain SB25-14, which bears a different mutation in the structural gene for histidase and which is not constitutive. Furthermore, the relative transformation frequency is increased by the addition of histidine in a presumably pleiotropic negative mutant in which neither the structural genes for histidase or for urocanase appear to function. Since this mutant cannot synthesize these enzymes either in the presence or in the absence of inducer, any simple relationship between gene function and genetic recombination is placed in further doubt.

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