The Location of a Temperature-sensitive *trans*-Dominant Mutation and Its Effect on Restriction and Modification in *Escherichia coli* K12

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An *Escherichia coli* K12 chromosomal *Eco*RI–*Bam*HI fragment containing a mutant *hsdS* locus was cloned into plasmid pBR322. The *mcrB* gene, closely linked to *hsdS*, was used for selection of clones with the inserted fragment using T4 α gt57 β gt14 and λ vir.*Pvu*II phages; the phage DNAs contain methylated cytosines and hence can be used to demonstrate McrB restriction. For the efficient expression of the *hsdS* gene, a *Bgl*II fragment of phage λ carrying the p_R promoter was inserted into the *Bam*HI site of the hybrid plasmid. Under these conditions a *trans*-dominant effect of the *hsdX*_{ts}⁺^d mutation on restriction and modification was detected. Inactivation of the *hsdS* gene by the insertion of the λ phage *Bgl*II fragment into the *Bgl*II site within this gene resulted in the disappearance of the *trans*-dominant effect. When the cloned *Bam*HI–*Eco*RI fragment was shortened by *Hpa*I and *Eco*RI restriction enzymes, the *trans*-dominant effect was fully expressed. The results indicate that the *X*_{ts}⁺^d mutation is located in the *hsdS* gene. The effect of gene dosage of the HsdS subunit on the expression of *X*_{ts}⁺^d mutation was studied. The results of complementation experiments, using F'-merodiploids or plasmid pBR322 with an inserted *X*_{ts}⁺^d mutation, support the idea that the HsdS_{ts}⁺^d product competes with the wild-type HsdS product, and has a quantitatively different effect on restriction and modification.

INTRODUCTION

Knowledge of the structural and functional organization of the genes coding for type I restriction and modification enzymes is important not only for the elucidation of the mechanisms of regulation of gene activity, but also for studies of protein–protein and protein–nucleic acid interactions (Sain & Murray, 1980; Burckhardt *et al.*, 1981). Type I restriction endonucleases are encoded by three chromosomal genes: *hsdR*, *hsdM* and *hsdS*. Genetic experiments have shown that the corresponding polypeptides R, M and S can be interchanged between related systems and have identified the S subunit as the determinant of the specificity of restriction (Boyer & Roulland-Dussoix, 1969; Hubáček & Glover, 1970; Van Pal & Colson, 1974).

Using a genetic approach we analysed a temperature-sensitive (ts) mutation affecting restriction and modification in *Escherichia coli* K12 (Hubáček & Weiserová, 1980). The results demonstrated its location outside the *hsdR* and *hsdM* genes and its *trans*-dominant (d⁺) nature (Hubáček & Kossykh, 1982). The high frequency of recombination between *hsdR* and *hsdX*_{ts}⁺^d mutations led us to postulate an additional gene to the *hsd* three-gene complex which affects the expression of the R-M phenotype (Hubáček & Weiserová, 1985).

Genetic analysis of the *E. coli* *rglA* and *rglB* restriction systems, which were reported to degrade the DNA of T-even phages when the DNA contained 5-hydroxymethylcytosine in a nonglucosylated state, positioned *rglA* at 25.24 min and *rglB* at 98.39 min on the chromosome

(Ravi *et al.*, 1985). Recently the genetic region involved in McrB (= RglB) restriction of 5-methylcytosine DNA has been cloned and located on a 5500 bp *Bgl*II-*Eco*RI fragment proximal to the *hsd* genes of *E. coli* K12 and closely linked to *hsdS* (Raleigh & Wilson, 1986; Ross & Braymer, 1987).

In the present work, new data on the location of the *hsdX*_{ts}⁺ mutation were obtained by gene cloning techniques, using the *mcrB* gene as a selective marker. The gene dosage of the wild-type HsdS product was found to affect the expression of the *hsdX*_{ts}⁺ mutation and vice versa.

METHODS

Plasmids and bacteriophages. pBR322 (Bolivar *et al.*, 1977), *λvir* and P1CM*clr*100, the thermoinducible mutant of P1CM (Rosner, 1972), were used. Recombinant plasmids pMS_K14, pMS_K41, pMS_KC23, pMS_KV14 and pMS_KV17 were described previously (Zinkevich *et al.*, 1981, 1984). The phage T4 α gt57 β gt14 (obtained from V. I. Taniashin, USSR Academy of Sciences, Puschino on the Oka, USSR) grown on *E. coli* *rglA rglB* was used to estimate McrB (= RglB) and McrA (= RglA) activities. The phage DNA is nonglucosylated and contains 5-hydroxymethylcytosine. The activity of the *mcrB* (= *rglB*) gene was tested using the phage *λvir*. *Pvu*II. The phage was modified during its growth on *E. coli* carrying a plasmid with the *Pvu*II restriction-modification system (kindly supplied by V. M. Kramarov, USSR Academy of Sciences, Puschino on the Oka, USSR). DNA with *Pvu*II methylated sites is the substrate for the action of the *rglB* system (Raleigh & Wilson, 1986).

Bacteria. The *E. coli* strains used are described in Table 1.

Media and microbial methods. Bacterial and phage buffers, M9 medium, L-amino acid and vitamin supplements were used as described by Hubáček & Glover (1970). The complex medium was L-broth solidified with 1.5% (w/v) agar (soft agar with 0.6%, w/v). Antibiotics were added at the following concentrations: ampicillin (Ap), 50 µg ml⁻¹; tetracycline (Tc), 12.5 µg ml⁻¹. Preparation of F'-merodiploids and transduction procedure were as described previously (Hubáček & Weiserová, 1980).

Testing of plating efficiencies on temperature-sensitive mutants. Bacteria (0.2 ml) grown overnight at 30 °C or 42 °C in L-broth were suspended in 3 ml soft agar with 0.01 M-MgSO₄ and mixed with standard dilutions of *λvir*. K or *λvir*. O. At least 16 h growth at 42 °C is needed to obtain the expression of the temperature-sensitive restriction phenotype of strain HfrH-90-XC with the *X*_{ts}⁺ mutation (Hubáček & Kossykh, 1982). The cell suspensions were plated on L-agar immediately after mixing with phage. Restriction was scored with *λvir*. K and *λvir*. O by the method described by Hubáček & Glover (1970). Modification was scored using standard indicator strains *E. coli* K and C; the fraction of K-plating phage produced is calculated as a function of the efficiency of plating (e.o.p.) on *E. coli* C, which is taken as 1.0.

Preparation of DNA. Chromosomal DNA was prepared as described by Marmur (1961). Plasmid DNA for ethidium bromide/CsCl equilibrium density-gradient centrifugation and for rapid small-scale isolation was prepared by the alkaline lysis method (Maniatis *et al.*, 1982).

Table 1. *Escherichia coli* strains

Strain	Hsd phenotype	Other characteristics	Source or reference
Hfr-1	r _K ⁺ m _K ⁺	<i>thr thi</i>	Hubáček & Weiserová (1980)
HfrH-90-XC	r _{ts} ⁺ m _{ts} ⁺	<i>thi hsdX</i> _{ts} ⁺	
HfrH-IV	r _K ⁻ m _K ⁻	<i>thi hsdS</i>	
HfrJC 5088		<i>thr ilv recA56</i>	
AB2463	r _K ⁺ m _K ⁺	<i>thr leu arg pro his thi recA13</i>	Raleigh <i>et al.</i> (1988)
K802	r _K ⁻ m _K ⁺	<i>met gal hsdR2 mcrA mcrB</i>	
K803	r _K ⁻ m _K ⁻	<i>met gal hsdS3</i>	
C	r ⁻ m ⁻	<i>serB</i>	
C600	r _K ⁺ m _K ⁺	<i>thr leu thi</i>	Hubáček (1973)
C600-5K	r _K ⁻ m _K ⁺	<i>thr leu thi hsdR</i>	
C600-IV	r _K ⁻ m _K ⁻	<i>leu thi hsdS</i>	
C600-5K <i>thy</i>	r _K ⁻ m _K ⁺	<i>thr leu thy thi hsdR</i>	
5K-76	r _{ts} ⁺ m _{ts} ⁺	<i>leu thy thi hsdX</i> _{ts} ⁺	<i>thy</i> derivative of C600-5K
5K-76-19	r _{ts} ⁺ m _{ts} ⁺	<i>leu thi hsdX</i> _{ts} ⁺ <i>recA56</i>	Thr ⁺ HsdX _{ts} ⁺ transductant of C600-5K <i>thy</i> <i>recA</i> derivative of 5K-76; this paper
IV-28	r _K ⁻ m _K ⁻	<i>leu thy thi hsdS</i>	<i>thy</i> derivative of C600-IV
IV-28-1	r _K ⁻ m _K ⁻	<i>leu thi hsdS recA56</i>	<i>recA</i> derivative of IV-28
NM273	r _B ⁻ m _B ⁻	<i>hsdR hsdM</i>	Kindly supplied by N. E. Murray, Univ. of Edinburgh, UK
M15		<i>lacZΔM15 recA</i>	Ruther <i>et al.</i> (1981)
P678-54		<i>minA minB</i>	Adler <i>et al.</i> (1967)

Restriction, ligation, and gel electrophoresis. All experiments were done as described by Maniatis *et al.* (1982).

Biochemicals and reagents. Restriction enzymes *Bam*HI, *Eco*RI, *Bgl*II and *Hpa*I, and T4 DNA ligase, were obtained from the Institute of Biochemistry and Physiology of Micro-organisms, USSR Academy of Sciences. *Mlu*I was prepared by I. Gavrilenko (USSR Academy of Sciences, Puschino on the Oka, USSR); *Hind*III was provided by the Institute of Applied Enzymology, Vilnius, USSR. Medium components were obtained from Oxoid.

RESULTS AND DISCUSSION

Cloning of a DNA fragment containing the $hsdX_{ts}^{+d}$ mutation

The following results do not resolve the location of the $hsdX_{ts}^{+d}$ mutation, but they point against its being located outside the *hsd* three-gene complex as had been previously believed (Hubáček & Weiserová, 1980).

E. coli K802 was crossed with the HfrH-90-XC $hsdX_{ts}^{+d}$ donor and after 25 min of conjugation the mating was interrupted by vortex shaking and Gal⁺ recombinants were selected. Among the 40 purified colonies the unselected McrB⁺ phenotype appeared in 12 recombinants with lowered sensitivity to T4 α gt57 β gt14 and λ vir. *Pvu*II-modified phages. The Gal⁺ McrB⁺ recombinants were tested for restriction and modification of phage λ at 30 °C and 42 °C. Table 2 lists the two classes of different R-M phenotypes obtained, $r_{ts}^{+}m_{ts}^{+}$ and $r^{-}m_{ts}^{+}$. All the McrB⁺ recombinants were temperature-sensitive in restriction or modification; three McrB⁺ clones out of 12 were of the $r^{-}m_{ts}^{+}$ phenotype. The *hsdR* mutation is separable by recombination from the *mcrB*⁺ gene and the $hsdX_{ts}^{+d}$ mutation and we can assume that the X_{ts}^{+d} mutation is more closely linked to *mcrB* than the *hsdR* gene. The relatively high frequency of recombination between *hsdR* and *mcrB* mutations could indicate a rather higher frequency of recombination among the mutations located inside the *hsd* complex locus or close to it than we considered previously (Hubáček & Weiserová, 1980).

The structures and properties of recombinant pMS_K14, pMS_KC23 and pMS_KV17 plasmid DNAs used in the following experiments are summarized in Fig. 1. pMS_K14 is pBR322 with the *Eco*RI–*Bam*HI fragment of the *hsd* region containing the intact and very inefficiently expressed *hsdS_K* gene. This DNA, after digestion with *Bam*HI and *Sal*I, served as a recipient of the *Bam*HI–*Sal*I fragment from pMS_K41 with the *hsdM* gene and p_{mod} promoter. Ap^R colonies were selected after ligation and transformation into *E. coli* C. Some of them were found to modify the λ vir. O phage; the plasmid pMS_KC23 was thus obtained with efficiently expressed *hsdS_K* and *hsdM* genes. Restriction analysis has shown that this plasmid contains an *Eco*RI–*Sal*I fragment which consists of a *Bam*HI–*Sal*I fragment from pMS_K41 and an *Eco*RI–*Bam*HI fragment from pMS_K14. Plasmid pMS_KC23 has two recognition sites for *Pvu*II and one each for *Sma*I and *Hpa*I. Their location made it possible to clone *Pvu*II–*Sma*I and *Hpa*I–*Sma*I fragments into the *Pvu*II site of pBR322, resulting in plasmids pMS_KV14 and pMS_KV17.

The recombinant plasmids pMS_K14, pMS_KC23 and pMS_KV17 were transformed into the recipient strain 5K-76-19 ($hsdX_{ts}^{+d}$) and the selected Ap^R clones were tested for restriction and modification at 30 °C and 42 °C (Table 3). Complementation of the wild-type restriction and modification phenotypes was obtained in transformants with plasmid DNA in which the inefficient expression of the *hsdS* gene in plasmid pMS_K14 (Zinkevich *et al.*, 1981; Table 3, expt 1) is improved by fusion with the *Bam*HI–*Sal*I fragment containing the intact *hsdM* gene (plasmid pMS_KC23, Table 3, expt 2). The *Eco*RI–*Sal*I fragment in pMS_KC23, shortened by *Hpa*I and *Sma*I to about 3.8 kb in length and cloned into the pBR322 to give pMS_KV17, carries only two genes, *hsdM* and *hsdS*, which are expressed under the control of the p_{mod} promoter (Loenen *et al.*, 1987); even under these conditions complementation of the wild-type R-M phenotype was obtained (Table 3, expt 3). The results indicate that the genetic region which is involved in the expression of temperature-sensitive restriction and modification is located on the *Hpa*I–*Sma*I fragment, on which the only functional genes are *hsdS* and *hsdM*.

In further experiments we tried to clone the DNA fragment containing the $hsdX_{ts}^{+d}$ mutation. Chromosomal DNA from one of the K802 Gal⁺ McrB⁺ $r_{ts}^{+}m_{ts}^{+}$ recombinants (Table 2) was used as the source of DNA. The *hsdS_K* and *mcrB* genes are carried on an *Eco*RI–*Bam*HI fragment

Fig. 1. Map of the *hsd* and *mcr* genes and the location of recognition sites for different restriction enzymes (E, *Eco*RI; P, *Pvu*II; B, *Bam*HI; H, *Hind*III; Hp, *Hpa*I; Bg, *Bgl*II; Sm, *Sma*I; Sl, *Sal*I). The hybrid plasmids with cloned fragments of the *hsd* region in pBR322 are shown. The relevant modification phenotypes of cells harbouring the hybrid plasmids are: *E. coli* K803(pMS_K14), m_K⁻; *E. coli* NM273(pMS_K41), m_K⁺; *E. coli* C(pMS_KC23), m_K⁺; *E. coli* C(pMS_KV14), m_K⁺; *E. coli* C(pMS_KV17), m_K⁺. The arrows indicate the direction of transcription.

Table 2. Restriction and modification phenotypes of Gal⁺ McrB⁺ recombinants resulting from a cross between HfrH-90-XC (*hsdX*_{ts}⁺^d) as donor and K802 as recipient

Temp. (°C)*	Restriction† λvir. O	Modification† K	Phenotype of recombinants	No. of Gal ⁺ McrB ⁺ clones tested
30	0.003–0.02	1.2–0.8	r _K ⁺ m _K ⁺	9
42	0.1–1.0	0.1–0.004	r _K ⁻ m _K ⁻	
30	0.5–1.0	1.0–0.6	r _K ⁻ m _K ⁺	3
42	0.8–1.0	0.1–0.001	r _K ⁻ m _K ⁻	

* The temperature of cultivation of bacteria before testing restriction and modification.

† Restriction indicates the e.o.p. of λvir. O relative to λvir. K (not included). Modification indicates the e.o.p. of phage produced by recombinants on *E. coli* K and C indicator strains. For each set of results the numbers represent the lowest and highest levels of e.o.p. of phage among the individual clones tested.

Table 3. Restriction and modification phenotypes of transformants obtained by transformation of 5K-76-19 (*hsdX*_{ts}⁺^d) with hybrid pBR322 plasmid DNA

Expt no.	Hybrid plasmid	Temp. (°C)*	Restriction† λvir. O	Modification† K	Phenotype of transformants
1	pMS _K 14	30	0.01	0.5	r _K ⁺ m _K ⁺
		42	1.0	0.01	r _K ⁻ m _K ⁻
2	pMS _K C23	30	0.0003	0.5	r _K ⁺ m _K ⁺
		42	0.0003	1.0	r _K ⁻ m _K ⁻
3	pMS _K V17	30	0.0002	0.7	r _K ⁺ m _K ⁺
		42	0.0003	0.8	r _K ⁻ m _K ⁻

*† For details see the footnotes to Table 2.

(Zinkevich *et al.*, 1981; Ross & Braymer, 1987), on which the *hsdX*_{ts}⁺^d mutation could be located. Chromosomal DNA of K802 Gal⁺ clone no. 6 was therefore digested with *Eco*RI and *Bam*HI, ligated to *Eco*RI/*Bam*HI-digested pBR322 and used to transform CaCl₂-treated *E. coli* K802 cells. The advantage of the close linkage of *hsdX*_{ts}⁺^d and *mcrB* (Table 2) was used for direct selection of McrB⁺ clones. The phage T4αgt57βgt14 (m.o.i. = 1) was added and the cell suspension was plated on L-agar with ampicillin. Among the Ap^R colonies, the clone which

Fig. 2. Electrophoretic analysis of digests of DNA. The source of DNA and enzymes used to digest the DNA were as follows. (a) 1, pVM12, *EcoRI* + *Bam*HI; 2, pVMΔ2, *EcoRI* + *Bam*HI; 3, pMS_K14, *EcoRI* + *Bam*HI; 4, λ DNA, *Mlu*I; 5, pVMΔ2, *Hind*III; 6, pMS_K14, *Hind*III. (b) 1, pVM3, *Hind*III; 2, pVM16, *Hind*III; 3, λ DNA, *Mlu*I. Sizes of some fragments are given to the right of the photographs.

effectively restricted T4 α gt57βgt14 and λvir. *Pvu*II phages was used for further analysis and detection of the unselected HsdX_{ts}⁺^d phenotype. The plasmid DNA was extracted from the transformant, physically analysed and transformed into *E. coli* strain C600 (the plasmid was designated pVM12).

Restriction analysis showed the presence of an *EcoRI*–*Bam*HI fragment (about 6.8 kb) analogous to the fragment in plasmid pMS_K14 isolated previously (Zinkevich *et al.*, 1981); in addition to this fragment a *Bam*HI fragment of unknown origin appeared in pVM12 (Fig. 2a, slot 1). Ap^R C600(pVM12) transformants were tested for restriction and modification of phage λ at 30 °C and 42 °C. The results shown in Table 4, expt 2, demonstrate the slight *trans*-dominant effect of the X_{ts}⁺^d mutation on the expression of the restriction phenotype. To demonstrate the precise location of the temperature-sensitive mutation it was necessary to cut out the additional *Bam*HI fragment from the pVM12 DNA molecule. Digestion with *Bam*HI, ligation, transformation of *E. coli* C600 and restriction analysis of isolated plasmid DNA yielded a hybrid pBR322 plasmid containing only the *EcoRI*–*Bam*HI fragment (Fig. 2a, slot 2); this plasmid was designated pVMΔ2. Functional analysis of the McrB and R-M phenotypes demonstrated the same level of expression of the McrB phenotype as observed with pVM12, while the *trans*-dominant effect of the hsdX_{ts}⁺^d mutation disappeared (Table 4, expt 3).

Table 4. *Restriction and modification of E. coli strains with hybrid plasmids pVM12, pVMΔ2, pVM3, pVM16 and pVM30*

Expt no.	Strain	Temp. (°C)*	Restriction† <i>λ</i> vir.O	Modification† K
1	C600	30	0.0005	0.9
		42	0.0002	1.0
2	C600(pVM12)	30	0.0004	0.7
		42	0.001	1.0
3	C600(pVMΔ2)	30	0.0004	0.8
		42	0.0007	0.6
4	C600(pVM3)	30	0.0005	1.0
		42	0.05	0.06
5	C600(pVM16)	30	0.0001	0.8
		42	0.0004	0.9
6	C600(pVM30)	30	0.0004	0.7
		42	0.5	0.001

*† For details see the footnotes to Table 2.

Analysis of the primary structure of the *hsdM* and *hsdS_K* genes revealed that the transcription of these genes starts from the common promoter p_{mod} (Loenen *et al.*, 1987). It was shown previously (Zinkevich *et al.*, 1981) that the expression of the *hsdS* gene located on the *EcoRI*–*BamHI* fragment cloned in pBR322 is inefficient due to the absence of its own promoter. Plasmid pMS_K14 (Table 3, expt 1) is unable to complement wild-type restriction and modification after transformation into strain 5K-76-19. We can assume, therefore, that in plasmid pVMΔ2 the *hsdX_{ts}⁺* mutation is situated in the *hsdS* gene and the low degree of its *trans*-dominant effect is a result of the inefficient transcription of this gene.

To improve the expression of the *hsdS* gene we put it under the control of the λ p_R promoter. A 2441 bp fragment of *λ*cI857 was inserted into the *BamHI* site of plasmid pVMΔ2 (Fig. 3*a–d*). The resulting plasmid, designated pVM3 (Fig. 3*d*), with the *Bgl*/II fragment in the correct orientation, was distinguished from the plasmids with the opposite orientation by *Hind*III restriction analysis of plasmid DNA and by the *trans*-dominant effect on the restriction and modification phenotype of transformed cells exerted by the *X_{ts}⁺* mutation. Plasmid pVM3 revealed, after *Hind*III restriction, a new fragment (1.4 kb) generated by joining the *BamHI*–*Hind*III part of the cloned *BamHI*–*EcoRI* fragment with the *Bgl*/II–*Hind*III part of the λ -controlling region (Fig. 2*b*, slot 1). The C600(pVM3) Ap^R transformant, temperature-sensitive in restriction and modification, clearly demonstrated the *trans*-dominant effect of the *X_{ts}⁺* mutation on the expression of the R-M phenotype (Table 4, expt 4).

The expression of the *hsdX_{ts}⁺* gene under the control of the λ p_R promoter in *E. coli* P678-54(pVM3) minicells grown at different temperatures was determined as follows. Cell-free homogenates from minicells labelled with ¹⁴C-amino acids were analysed by SDS-PAGE and autoradiography. Computer-assisted image analysis of the resulting autoradiogram allowed quantification of the product of the *hsd* gene among other labelled proteins (Vohradský *et al.*, 1988). The results (Fig. 3*g*) show that, due to the effect of *cI857* on the λ p_R promoter, the formation of the *hsdS* gene product increases when the temperature of cultivation is raised from 28 °C to 37 °C and 42 °C.

In the next experiment the *Bgl*/II λ fragment was inserted in the same orientation into the *Bgl*/II site inside the *hsdS* gene, leading to its interruption (Fig. 2*b*, slot 2; Fig. 3*f*); the resulting plasmid was designated pVM16. In this case the *trans*-dominant effect of the *X_{ts}⁺* mutation disappeared (Table 4, expt 5).

Plasmid pVM3 still carries a large region of the cloned *EcoRI*–*BamHI* fragment downstream of the *hsdS* gene. To exclude the possibility that the *X_{ts}⁺* mutation could be located in this region, the *Hpa*I–*EcoRI* part of the cloned *EcoRI*–*BamHI* fragment was deleted (Fig. 3*e*; plasmid designated pVM30). If the mutation lies in this region, the *trans*-dominant effect of the *X_{ts}⁺*

Fig. 3. (a-f) Restriction maps of the hybrid plasmids pVMA2, pVM3, pVM16 and pVM30. (a) Restriction map of plasmid pVMA2. The internal size scale is in kb. (b) *Bgl*/II fragment of λ phage. (c) *Bam*HI-*Eco*RI chromosomal fragment of pVMA2. (d) Plasmid pVM3: *Bgl*/II fragment of λ phage cloned into the *Bam*HI site of pVMA2. (e) Plasmid pVM30: *Hpa*I-*Eco*RI region of pVM3 deleted. (f) Plasmid pVM16: *Bgl*/II fragment of λ phage cloned into the *Bgl*/II recognition site within the *hsdS*_{ts}⁺ gene. —, pBR322 DNA; —, *Bam*HI-*Eco*RI chromosomal fragment; —, *Bgl*/II fragment of λ phage; ----, *hsdS* gene; - - - - , *mcrB* gene; the arrows indicate the direction of transcription from p_R. Restriction enzymes: E, *Eco*RI; H, *Hind*III; Hp, *Hpa*I; Bg, *Bgl*/II; B, *Bam*HI; Sl, *Sal*I; Ps, *Pst*I.

(g) Level of *hsdS*_{ts}⁺ gene expression at cultivation temperatures of 28 °C, 37 °C and 42 °C. The amount of HsdS protein is expressed as a fraction of the total labelled proteins at the respective temperature.

mutation on restriction and modification should be eliminated. In fact the *trans*-dominant effect was still apparent (Table 4, expt 6), a result that supports the location of the mutation in the *hsdS* gene; no other open reading frame is present in plasmid pVM30 with the *Hpa*I-*Eco*RI deletion (Gough & Murray, 1983).

Level of HsdS and HsdS_{ts}⁺ products in the cell and expression of the R-M phenotype

If the *X*_{ts}⁺ mutation is within the *hsdS* gene, the question arises how it 'controls' the expression of restriction and modification. One can assume that the mechanism of action of the *X*_{ts}⁺ mutation is based on the level of synthesis of the γ subunit, the product of the *hsdS* gene, which is structurally modified, especially at higher temperatures, as a result of the substitution of one amino acid for another in a critical part of the HsdS protein. To compare the effect of different levels of HsdS and HsdS_{ts}⁺ products on the expression of the R-M phenotype, we constructed partial F' diploids with the wild-type or the mutated *hsdS* gene and with the *X*_{ts}⁺ mutation, both situated either on the bacterial chromosome or on the F'-plasmid.

Table 5. *Restriction and modification phenotypes of F' merodiploids*

The F' elements were equally stable at 30 °C and 42 °C; at both temperatures more than 95% of cells carried the F' plasmid at the time of phage *λ*vir. O addition.

Expt no.	Haploid phenotype		Temp. (°C)*	Restriction† <i>λ</i> vir. O	Modification† K	Phenotype of merodiploid
	Recipient	F'				
1	AB2463	90-XC				
	<i>hdsS</i> ⁺	<i>X</i> _{ts} ⁺ ^d				
	<i>r</i> _K ⁺ <i>m</i> _K ⁺	<i>r</i> _K ⁺ <i>m</i> _K ⁺	30	0.003	0.8	<i>r</i> _K ⁺ <i>m</i> _K ⁺
2	IV-28-1	90-XC				
	<i>hdsS</i> ⁻	<i>X</i> _{ts} ⁺ ^d				
	<i>r</i> _K ⁻ <i>m</i> _K ⁻	<i>r</i> _K ⁺ <i>m</i> _K ⁺	30	0.003	0.3	<i>r</i> _K ⁺ <i>m</i> _K ⁺
3	5K-76-19‡	KLF1				
	<i>X</i> _{ts} ⁺ ^d	<i>hdsS</i> ⁺				
	<i>r</i> _K ⁺ <i>m</i> _K ⁺	<i>r</i> _K ⁺ <i>m</i> _K ⁺	30	0.0001	0.7	<i>r</i> _K ⁺ <i>m</i> _K ⁺
4	5K-76-19‡	IV				
	<i>X</i> _{ts} ⁺ ^d	<i>hdsS</i> ⁻				
	<i>r</i> _K ⁺ <i>m</i> _K ⁺	<i>r</i> _K ⁻ <i>m</i> _K ⁻	30	0.0006	1.0	<i>r</i> _K ⁺ <i>m</i> _K ⁺
			42	0.6	0.0003	<i>r</i> _K ⁻ <i>m</i> _K ⁻

*† For details see the footnotes to Table 2.

‡ The recipient strain 5K-76-19 was prepared from C600-5K as a Thr⁺ HsdX_{ts}⁺^d cotransductant to which the *recA* mutation was introduced by conjugation with HfrJC 5088 as donor strain.

The *trans*-dominant effect of the *X*_{ts}⁺^d mutation on the restriction phenotype was obtained in diploids using recipients with wild-type *hdsS* as well as mutated *hdsS* alleles (Table 5, expts 1 and 2), while the effect on K-specific modification was observed only in the diploid with *hdsS*⁻ recipient (Table 5, expt 2) or in *hdsS*⁺ cells with the *X*_{ts}⁺^d mutation located on the hybrid multi-copy pVM3 plasmid (Table 4, expt 4). In reciprocal partial diploids, with the *X*_{ts}⁺^d mutation located on the recipient chromosome, the temperature-sensitive R-M phenotype was detected only in experiment 4, (Table 5), while the diploid with the *hdsS*⁺ allele on the F' element displayed the wild-type R-M phenotype (Table 5, expt 3). The results indicate that the *trans*-dominant effect of the *X*_{ts}⁺^d mutation depends on the ratio of HsdS⁺ and S_{ts}⁺^d products. The higher sensitivity of the expression of restriction to the effect of the HsdS_{ts}⁺^d product as compared to the modification phenotype could reflect different quantitative aspects of interaction of the protein subunits during the formation of R. *EcoK* and M. *EcoK* enzymes or their recognition properties.

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