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# Characterization of the *din* (damage-inducible) and *tin* (temperature-inducible) Genes Isolated from *Escherichia coli*

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# 대장균에서 분리된 *din* (damage-inducible)과 *tin* (temperature-inducible) 유전자들의 특성

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**ABSTRACT: Mu *d1*(Ap *lac*) bacteriophage can be used to search for genes which are members of a common regulatory network without having to know the functions of the genes in advance. Aim was for obtaining the loci in the SOS network as well as temperature inducible loci. For this purpose, *recA441* allele was used. This allele encodes a thermosensitive *recA* gene product; thus, the *recA441* allele can be activated upon temperature upshift without by external DNA damage. Approximately 10,000 colonies were screened, and then searched for the colonies which expressed $\beta$-galactosidase higher level at 42°C than at 30°C . The strains identified fell into two classes; (i) ones in which the increased expression was *recA*$^+$ *lexA*$^+$-dependent, that is, *din*(damage-inducible) genes which were due to the activation of *recA441* allele and (ii) ones in which the increased expression was *recA*$^+$ *lexA*$^+$-independent and only temperature-inducible, *tin* genes. Rough mapping position was obtained for these genes.**

**KEY WORDS □ Chromosomal *lac* fusion, *din*(damage-inducible) genes, *tin*(temperature-inducible) genes, Mu *d1*(Ap *lac*) bacteriophage**

The past several years have seen an explosive growth in our understanding of the molecular strategies employed by cells in responding to challenges to their genetic material. DNA damage presents a very serious challenge to a cell because of the possibilities that it may give rise to mutations or lead to death. Cells have evolved a wide variety of conceptually different strategies to repair damaged DNA accurately (Walker, 1984; Paek *et al.*, 1987).

In recent years it has become clear that *Escherichia coli* has at least four different regulatory networks of genes whose expression is induced in response to treatments with DNA-damaging agents; (i) SOS response, (ii) adaptive response, (iii) heat-shock response, (iv) oxidative DNA damage repair response (Demple and Halbrook, 1983). These regulatory networks are not actually totally exclusive of one another. For example, Krueger and Walker (1984) observed that at least some subset of heat shock proteins (including GroEL and DnaK) were induced by UV and nalidixic acid which were known to be the SOS-inducing agents. Furthermore, this induction of *groEL* and *dnaK* by UV and nalidixic acid was found to be controlled by the *htpR* gene product, a positively acting element required for expression of the heat shock genes (Neidhardt and VanBogelen, 1981; Neidhardt *et al.*, 1983; Paek and Walker, 1987; Yamamori and Yura, 1980). However, it is not clear at this point that generation of an inducing signal for heat shock involves any type of DNA damage.

In an effort to dissociate the physiological complexity of the SOS responses from the issue of their regulation, Kenyon and Walker (1980) successfully used Mu *d1*(Ap *lac*) bacteriophage to identify *din*(damage-inducible) loci whose expres-

**Table 1.** *Escherichia coli strains and plasmids used in this study.*

| Strain/Plasmid | Description | Source/Reference |
|---|---|---|
| GW1000 | *recA441 sulA11 lacU169 thr-1 leu6 his-4 argE ilv(ts) galk2 rpsL31* | Kenyon and Walker (1980) |
| K165 | F *lac*(am) *trp*(am) *pho*(am) *mal*(am) *rpsL sup4*$^{o}$ *htpR*(am) | Cooper and Ruettinger (1975) |
| MH71 | *malPQ*::Tn5 | T. Silhavy |
| GW4723 | As K165, but *malPQ*::Tn5 | P1 transduction K165×P1 (MH71) |
| GW4799 | As GW4792, but *htpR*(am) *malPQ*::Tn5 | P1 transduction GW4792×P1 (GW4723) |
| MAL103 | F Mu *d1*(Ap *lac*) Mu *C*$^{+}$ *Δ(proAB-lac)* XIII, *rpsL1* | B. Wanner |
| HFr's | Various origins and directions of transfer | Laboratory collection |
| pGW600 | Tc$^{r}$, Mu C$^{+}$, pBR322 replication origin | C.J. Kenyon |
| pKP12 | Cm$^{r}$, *htpR*$^{+}$, pACYC184 replication origin | Paek and Walker (1986) |

Tc$^{r}$: tetracyclin resistant, Cm$^{r}$: chloramphenicol resistant

sion was increased by a variety of SOS-inducing treatments. Thus, one is able to search for genes which are members of a common regulatory network without having to know the functions of the genes in advance.

I decided to search for the additional loci in the SOS network as well as temperature inducible loci. For this purpose, I took advantage of the *recA441* allele (formerly called *tif-1*), which leads to constitutive expression of SOS genes at 42°C (Paek and Walker, 1986; Walker, 1984).

## MATERIALS AND METHODS

### Bacterial strains and plasmids

Bacterial strains used in this work are described in Table 1. Derivatives containing the *htpR165* allele were constructed by P1 transduction, using a linked *malPQ*::Tn5 for selection. P1 transductions were performed basically as described by Miller (1972). The presence of the *htpR165* mutation was judged by the temperature sensitivity at 42°C (Cooper and Ruettinger, 1975).

### Media and reagents

Bacteria were routinely grown on LB medium at 30°C, unless otherwise stated. Supplemented M9 /glucose minimal medium were used in the case of labeling and β-galactosidase assay. Kanamycin (Km) (25 μg/ml), ampicillin(Ap) (50 μg/ml), tetracyclin (Tc) (20 μg/ml) and chloramphenicol(Cm) (25 μg/ml) were used to select the strains. X-gal(5-bromo-4-chloro-3-indol-β-D-galactoside) was used to screen for β-galactosidase activity (Miller, 1972).

### Mu *d1*(Ap *lac*) insertional mutagenesis

The insertional mutagenesis to generate the *lac* fusions was done by using the Mu *d1*(Ap *lac*) bacteriophage. This phage has two key features which make it so useful; (i) Mu *d1*(Ap *lac*) carries a gene coding for Ap$^{R}$ so that Ap$^{R}$ colonies can be selected. (ii) Mu *d1*(Ap *lac*) can generate operon fusions in a single step. It is a derivative of bacteriophage Mu and was constructed by Casadaban and Cohen (1979). To prepare Mu *d1* (Ap *lac*) lysate, the strain MAL103 which is a Mu *d1*(Ap *lac*) and Mu Cts double lysogen was used. After preparing the lysate by temperature upshift, the lysate was used within a week to avoid the rapid decrease in the titer of the phage stock that is seen with time. The strain (GW1000, See Table 1) was infected with serial dilution of Mu *d1* phage, pooled, stabilized by transforming with a plasmid overproducing the Mu repressor (pGW 600), plated on LB/ampicillin/X-gal plate, incubated at 30°C, replica plated at 30°C and 42°C on the same plates and screened for the colonies which showed bluer color at 42°C than at 30°C. To discriminate between *din* and *tin* strains, exponentially growing curtures at 30°C were exposed to 15 J/m$^{2}$ UV and the β-galactosidase assay was carried out.

### Other methods

The Hfr's with various origins and directions of transfer were in the laboratory collection and were used for the Hfr quick mapping (Low, 1973) of *din* and *tin* strains. The β-galactosidase assay was carried out essentially as described by Miller (1972) with a slight modification: Cells were assayed within three hours after their removal from the curture. 40 μl CHCl$_3$ were added to the tube containing Z buffer, and the solution was vortexed for 10 seconds and then kept at room temperature for ten minutes. The solution was warmed to 29°C and 4 mg/ml ONPG(o-nitrophenyl-β-D-galactoside) solution was added to

**Table 2.** *Rough mapping data of din and tin strains by Hfr mapping*

| Strains | Description | Mapping position |
|---|---|---|
| GW4750 | As GW1000, but *dinG*::Mu *dI*(Ap *lac*) | 23′-42′ |
| GW4760 | As GW1000, but *dinH*::Mu *dI* (Ap *lac*) | 23′-42′ |
| GW4770 | As GW1000, but *din1*::Mu *dI* (Ap *lac*) | 9′-12.5′ |
| GW4780 | As GW1000, but *dinJ*::Mu *dI* (Ap *lac*) | 43′-60′ |
| GW4792 | As GW1000, but *tin101*::Mu *dI* (Ap *lac*) | 9′-13′ |
| GW4793 | As GW1000, but *tin102*::Mu *dI* (Ap *lac*) | 13′-32′ |
| GW4794 | As GW1000, but *tin103*::Mu *dI* (Ap *lac*) | 22′-43′ |
| GW4795 | As GW1000, but *tin104*::Mu *dI* (Ap *lac*) | 58′-61′ |

start the reaction.

## RESULTS AND DISCUSSION

Several Mu *dI*(Ap *lac*) generated chromosomal fusions in which β-galactosidase expression was induced by temperature upshift in an *htpR*$^+$-dependent fashion were isolated.

These insertions were originally isolated during a screen for the additional loci in the SOS network as well as temperature- inducible loci. I took advantage of a *recA441*(formerly termed *tif-1*) strain in this screen. This *recA* allele encodes a thermosensitive *recA* gene product which can be activated upon temperature upshift without any external DNA damage. Thus SOS responses are induced just upon temperature upshift (Walker, 1984). The Mu *dI*(Ap *lac*) bacteriophage was used to generate random *lac* operon fusions in this strain, approximately 10,000 colonies were screened, and then colonies which expressed β-galactosidase higher level at 42℃ than at 30℃ were searched for. The identified strains fell into two classes; (i) ones in which the increased expression was *recA*$^+$ *lexA*$^+$-dependent, that is, *din* (damage-inducible) genes (Kenyon and Walker, 1980) which were due to the activation of *recA441* allele and the (ii) ones in which the increased expression was *recA*$^+$ *lexA*$^+$-independent and only temperature-inducible. The second class was named as *tin* (temperature-inducible) fusions. Five independent din genes were identified. One of these Mu *dI*(Ap *lac*) insertion was at the *dinD* locus (Kenyon and Walker, 1980), judging from the mapping data and the induction kinetics of β-galactosidase (data not shown). The approximate mapping data of other *din* genes are on Table 2. Also 8 independent *tin* genes were identified. These *din* and *tin* strains showed 3 fold to 11 fold maximum induction after temperature upshift from 30℃ to 42℃. It took about 2 hours to reach the maximum induction. Recently it was discovered that polII was encoded by the *dinA* gene (Bonner *et al.*, 1990), which had been identified previously as a DNA damage-inducible Mu *dI*(Ap *lac*) gene fusion of unknown function (Kenyon and Walker, 1980). So far all the other

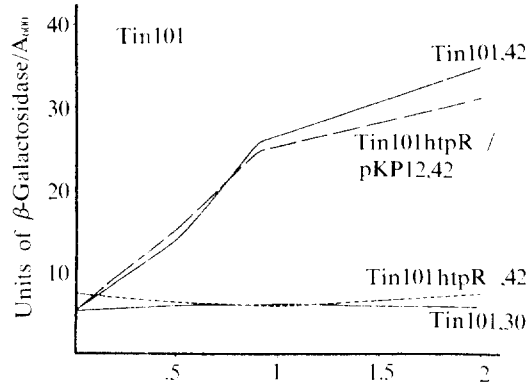


**Fig. 1.** *Induction of β-galactosidase activity in tin101 (GW4792), tin101 htpR (GW4799), GW4799/ pKP2 strains before (30℃) and after (42℃) temperature upshift. For the convenience, all of 30℃ assays were designated as Tin101, 30. The strains showed similar basal level. Sample was taken up to 2 hour.*

*din* or *tin* genes were not identified. In the future further intensive characterization of *din* and *tin* genes may localize those as of already known function.

In 4 of the 8 *tin* strains, the induction of β-galactosidase by temperature upshift was *htpR*$^+$-dependent. That is, when the *htpR*165 mutation was introduced into these strains, the induction of β-galactosidase by a temperature upshift to 42℃ was abolished. The introduction of the *htpR*$^+$-containing plasmid (pKP12) (Table 1 and also Paek and Walker, 1986) into these double mutants (*tin htpR165*) restored the β-galactosidase induction. A typical induction is shown in Figure 1. By this means it was clearly shown that this induction event was *htpR*$^+$-dependent. These 4 *htpR*$^+$-dependent loci were roughly mapped by Hfr quick mapping (Table 2). Even though the induction of these genes were *htpR*$^+$-dependent, I do not think that any of these genes identified in this screen correspond to the known heat shock genes that have been extensively studied. Two classes of observations lead me to this

conclusion; (i) The induction kinetics of these *tin* genes were much slower than those of known heat shock genes. That is, the maximum induction of known heat shock genes reaches within 5 to 7 minutes and decreases sharply (Neidhardt *et al.*, 1984), whereas that of *tin* genes takes about 2 hours. It is not surprising that the screening only could give rise to the *tin* class. The screening system could have biased for the genes which showed such a prolonged response and which were nonessential for cell survival at 42℃. (ii) On the autoradiogram of SDS-polyacrylamide gel of $^{35}$S-methionine labeled total cell extract, I could not detect any known heat shock protein which was missing in these mutants (data not shown).

The *htpR* gene has been shown to function as a sigma factor for heat shock gene promoters (Grossman *et al.*, 1984) and to work mainly at the transcriptional level (Yamamori and Yura, 1980, 1982). The *tin* genes identified in this study may represent a class of genes whose *htpR*$^+$-dependence differs from that of known heat shock genes such as *groEL*, *lon* and *dnaK*. For example, induction of the *tin* genes could require a positively-acting transcription factor that is induced in an *htpR*$^+$-dependent fashion as part of the heat shock response.


## 적 요

박테리오페지 *Mu dI*(Ap *lac*)을 이용하여 공통조절군에 속하는 유전자들을 유전자 자체의 기능을 미리 알 필요없이 얻을 수 있다. 본 실험에서는 *recA441* allele을 이용하여 SOS반응군에 속하는 유전자들뿐만 아니라 온도상승에 의해 유도되는 유전자들을 동시에 찾는 시도를 하였다. 약 10,000개의 colony 중에서 30 에서보다 42 에서 β-galactosidase가 많이 생성되는 것을 선별하였고 이들은 두 group으로 나누어졌다. 첫번째는 *din*(damage-inducible) 유전자군에 속하며 *recA441* allele의 활성화에 따른 것으로 *recA*$^+$ *lexA*$^+$ 조절기작에 의존한다. 두번째는 *recA*$^+$ *lexA*$^+$ 조절기작과 무관하게 온도상승에 의해 유도된 유전자들로 *tin*(temperature-inducible)유전자들이라 명명했다. 특히 tin유전자중 일부는 htpR$^+$ 조절기작에 따르는 것으로 밝혀졌다.


## REFERENCES


1. **Bonner, C.A., S. Hayes, K. McEntee and M.F. Goodman,** 1990. DNA polymerase II is encoded by the DNA damage-inducible *dinA* gene of *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 7663-7667.
2. **Casadaban, M.J. and S.N. Cohen,** 1980. Lactose genes fused to exogenous promoters in one step using a Mu-lac bacteriophage: *in vivo* probe for transcriptional control sequences. *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 4530-4533.
3. **Cooper, S. and T. Ruettinger,** 1975. A temperature-sensitive nonsense mutation affecting the synthesis of a major protein of *Escherichia coli*. *Mol. Gen. Genet.*, **139**, 167-176.
4. **Demple, B. and J. Halbrook,** 1983. Inducible repair of oxidative DNA damage in *Escherichia coli*. *Nature*, **304**, 456-458.
5. **Grossman, A.D., J.W. Erickson and C.A. Gross,** 1984. The *htpR* gene product of *E. coli* is a sigma factor for heat-shock promoters. *Cell*, **38**, 383-390.
6. **Kenyon, C.J. and G.C. Walker,** 1980. DNA-damaging agents stimulate gene expression at specific loci in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A., **77**, 2819-2823.
7. **Krueger, J.H. and G.C. Walker,** 1984. *groEL* and *dnaK* genes of *Escherichia coli* are induced by UV irradiation and nalidixic acid in an *htpR*$^+$-dependent fashion. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 1499-1503.
8. **Low, B.,** 1973. Rapid mapping of conditional and auxotrophic mutations in *Escherichia coli* K-12. *J. Bacteriol.*, **113**, 798-812.
9. **Miller, J.H.,** 1972. in Experiments in Molecular Genetics, Cold Spring Laboratory, New York.
10. **Neidhardt, F.C., R.A. VanBogelen and V. Vaughn,** 1984. Genetics and regulation of heat-shock proteins. *Annu. Rev. Genet.*, **18**, 295-329.
11. **Neidhardt, F.C., R.A. VanBogelen and E.T. Lau,** 1983. Molecular cloning and expression of a gene that controls the high-temperature regulon of *Escherichia coli*. *J. Bacteriol.*, **153**, 597-603.
12. **Neidhardt, F.C. and R.A. VanBogelen,** 1981. Positive regulatory gene for temperature-controlled proteins in *Escherichia coli*. *Biochem. Biophys. Res. Commun.*, **100**, 894-900.
13. **Paek, K.-H., C.C. Dykstra, D.E. Shevell, J.R. Battista, L. Marsh and G.C. Walker,** 1987. Global responses of *Escherichia coli* to DNA damage and stress, in Phosphate Metabolism and Cellular Regulation in Microorganisms, Torriani-Gorini, A., F.G. Rothman, S. Silver, A. Wright and E. Yagil (ed.) pp. 295-299. American Society for Microbiology, Washington, D.C.
14. **Paek, K.-H. and G.C. Walker,** 1987. *Escherichia coli* dnaK null mutants are inviable at high temperature. *J. Bacteriol.*, **169**, 283-290.
15. **Paek, K.-H. and G.C. Walker,** 1986. Defect in expression of heat shock proteins at high temperature in xthA mutants. *J. Bacteriol.*, **165**, 763-770.
16. **Paek, K.-H.,** 1986. Genetic and Molecular studies of *Escherichia coli* heat shock response. Ph.D.

Thesis, M.I.T.
17. **Walker, G.C.,** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage on *Escherichia coli*. *Microbiol. Reviews*, **48,** 60-93.
18. **Yamamori, T. and T. Yura,** 1980. Temperature-induced synthesis of specific proteins in *Escherichia coli*: evidence for transcriptional control. *J. Bacteriol.*, **142**, 843-851.

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