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A NEW MATURATION-DEFECTIVE MUTANT IN BACTERIOPHAGE T4

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ABSTRACT

Jen-Leih Wu (1976). *A New Maturation-Defective Mutant In Bacteriophage T4.* Bull. Inst. Zool., Academia Sinica **15**(2): 65-72. A new temperature-sensitive mutant of bacteriophage T4 which is located between gene 33 and 34 has been isolated. Based on the phenotypes of DNA synthesis, serum-blocking power, temperature shift-up experiment, RNA-DNA hybridization competition and complementation test, *ts*333 was thought to be a mutant of third maturation-defective gene of bacteriophage T4.

Bacteriophage T4 expresses its gene functions intracellularly in a well-controlled sequence after infection[1,2]. In the stage immediately after infection, most of the T4-specific proteins synthesized are those needed for phage DNA metabolism[2,8]. After T4 DNA replication has been initiated, the synthesis of most of the early proteins is gradually shut off and the late proteins involved in phage morphogenesis, maturation and cell lysis are synthesized[4]. Pre-replicative and post-replicative mRNAs are classified as early and late mRNAs based on the time of their appearance[3,5]. T4 early mRNA is transcribed from the l-strand DNA while the late mRNA is transcribed from the r-strand DNA[6]. The early cistrons are interspersed along the segment of the genome while late mRNAs are transcribed predominantly from a separate segment[7].

In general, late gene expression depends on viral DNA replication[8,9] and the two regulatory polypeptides of genes 55 and 33.[10,11] The host RNA polymerase must be modified for the switch from early to late gene transcription. Modification of this enzyme requires addition of three T4-induced polypeptides[10,11] and T4-induced sigma factors[12], covalent addition of ADP to α-subunit[13], phosphorylation of α-subunit[14], and a structural change in β'-subunit[15]. The maturation-defective mutant, lacking the function of gene 55 or gene 33, synthesizes DNA, early mRNA but not late mRNA. It has been proved that block in late protein synthesis was correlated with block in transcription of late mRNA[3,16]. Except genes 55 and 33 polypeptide, there is a third polypeptide associated with host RNA polymerase[10,11]. Thus the isolation of third maturation-defective gene of bacteriophage T4 was presented in this paper. Based on the complementation test, DNA synthesis, serum-blocking power, temperature shift-up experiment, RNA-DNA hybridization competition and genetic mapping, *ts*333 is thought to be a third maturation-defective gene of bacteriophage T4.

MATERIALS AND METHODS

(A) Bacteria and bacteriophages

Escherichia coli K strain CR63 was used as a permissive host for preparing stocks of T4 amber mutants. *E. coli* B strain Tr201, a low thymine-requiring mutant obtained from G. R. Greenberg, was used as a nonpermissive host for amber mutants (B. Dale, Ph. D. Thesis, Univ. of Michigan, Ann Arbor, 1968). *E. coli* BB and *E. coli* BO21 were also used as nonpermissive hosts. T4D was used as wild type bacteriophage. T4 mutants used were *am*N134 (gene 33), *am*BL292 (gene 55), *am*C5 (gene 59), *am*N58 (gene 34), *am*A453 (gene 32) and *ts* P7 (gene 32) were used in various experiments.

(B) Media

There are several kinds of media used in experiments for different purposes:

(i) *1×CT⁻ and 1×CT⁺ media*[17]

1×CT⁻ medium contained 0.2% glucose and 0.1% casein hydrolysate in 1× salt solution, which was prepared by 50 fold dilution of 50× salt solution. 50× salt solution contained 10 g of $MgSO_4 \cdot 7H_2O$, 100 g of citric acid·H_2O, 500 g of K_2HPO_4 and 175 g of $NaNH_4HPO_4 \cdot 4H_2O$ in 670 ml of H_2O. 1×CT⁻ medium containing 5 μg/ml of thymine was named as 1×CT⁺ medium. These media were used for growing *E. coli* B Tr201 and also used for radioactive thymine-labeling experiment.

(ii) *M9 medium*

M9 medium contained 7.0 g of $Na_2HPO_4 \cdot 2H_2O$, 3.0 g of KH_2PO_4, 0.5 g of NaCl, 1.0 g of NH_4Cl, 10^{-3} M $MgSO_4$, 10^{-4} M $CaCl_2$ and 4.0 g of glucose in 1 liter of H_2O.

(iii) *3×D medium*

The enriched medium was used for preparing phage lysates and for growing bacteria for phage plating. 3×D medium was prepared by mixing sterilized solution A (containing 9 g of KH_2PO_4, 21 g of Na_2HPO_4, 30 g of casein hydrolysate, 26 g of glycerol and 60 mg of gelatin in 1,900ml of H_2O) and sterilized solution B (containing 0.6 g of $MgSO_4 \cdot 7H_2O$ and 0.6 ml of 1 M $CaCl_2$ in 100 ml of H_2O).

(C) Phage crosses

The procedure used for phage crosses was described by Tessman with some modification[19]. *E. coli* was grown to 5×10^8 cells/ml in 3×D medium and infected with an equal volume of phage mixture (T4D+*ts*-mutant) in the presence of D, L-tryptophan (20 μg/ml). The multiplicity of infection (MOI) was four for T4D and one for *ts*-mutant. The infected bacteria were aerated at 25° for 6 min and diluted with 3×D medium by 10^4 fold. 5 ml of the diluted infected-bacteria were aerated at 25° for 130 min, then lysed with a few drops of chloroform.

(D) Measurement of kinetics of DNA synthesis

An overnight culture of *E. coli* B Tr201 was diluted 50-fold with 1×CT⁺ medium and grown to a concentration of 5×10^8 cells/ml. These cells were centrifuged and the cell pellet was resuspended to a concentration of 1×10^9 cells/ml in 1×CT⁻ medium containing 40 μg/ml of D, L-tryptophan. An equal volume of phage suspension was added at a multiplicity of 5 phage particles per bacterium. Three min after infection, [^{14}C] thymine (1 μCi/ml, 42 mCi/mmol) was added. The radioactivity in acid-insoluble fractions was determined by pipetting 0.1 ml of a sample onto a 25-mm disk of Whatman No. 3 filter paper.

(E) Temperature shift-up

E. coli BB was grown in 3×D medium to 5×10^8 cells/ml, then centrifuged and resuspended in 1× medium. The bacteria (2×10^9 cells/ml) were infected with *ts*-mutant at an MOI of 0.5 in the presence of tryptophan. Phage adsorption took place at 25° for 6 min. Anti-T4 serum was added for another 4 min. Growth was initiated by diluting the infected bacteria 100 fold into 3×D medium and this was regarded as the zero time. Shift-up from 25° to 42° was performed at different time after infection. phage yield was measured at 90 min after the time of shifting.

(F) ^{14}C-Amino acid incorporation

E. coli. B was grown in M9 medium at 37° and infected with phages at 41.5° At various time after infection, the culture was pulse-labeled

with L-[^{14}C] amino acids (0.25 μCi/50μg/ml) for 4 min. The radioactivity in acid-insoluble fraction was determined.

(G) Serum-blocking power of bacteriophage T4

E. coli. BB was grown in 3×D medium to 5×10^8 cells/ml, then infected by T4 at 42° with an MOI of 0.5. At different time after infection, chloramphenicol was added (50 μg/ml), then the cells were lysed by adding chloroform. 0.6ml of phage-infected cell lysate and 0.2 ml of T4D (5×10^7 phage particles/ml) were mixed and incubated at 37°. 0.2 ml of anti-T4 serum was added ($k=1.6$) and mixed well. Ten min later, 0.1 ml of phage-serum mixture were diluted to 10 ml. The survival phage particles were counted by plating on agar plate.

(H) RNA-DNA hybridization competition

The method of preparing phage DNA and RNA was described by Bolle *et al.*[3]. For RNA-DNA hybridization competition, 1 μg/ml of [^{3}H] RNA, 20 μg/ml of denatured T4D DNA and varying amount of unlabelled RNA (0-1,000 μg/ml) were annealed at 66° in 0.3 M NaCl, 0.03 M sodium citrate in small stoppered tubes (0.4 ml in total) for 6 hours. Samples were then transferred to approximately 15 ml of 0.5 M KCl, 0.01 M Tris-HCl (pH 7.3) and filtered through nitrocellulose filter, washed with 80 ml of additional solvent, followed by 5 ml of 80% ethanol, dried and counted.

RESULTS

(A) Isolation of *ts*-revertant

T4 *ts*-revertant which is temperature-sensitive was isolated from *am*N134 (gene 33) when plated on *E. coli.* BB. T4 *ts*333 was isolated and crossed with wild-type T4D three times for the purification of this mutant. In the preliminary spot test, *ts*333 could complement with *am*N134 or *am*BL292 (gene 55) (Table I). This means that *ts*333 carries different mutational locus from gene 33 or gene 55. The stronger evidence was carried out by the determination of burst size (Table II). When *ts*333 and *am*N134 or *am*BL292 were infected together and let the phage produce under non-permissive condition (*E. coli.* BB, 42°), the burst size were over 400. However, when *ts*333, *am*N134 or *am*BL292 were infected alone, the burst size was very low. T4 *ts*333 and *am*N134 or *am*BL292 could compensate each other for their own defective functions. Thus, *ts*333 has a mutational site which is different from gene 33 or gene 55.

TABLE I
Complementation test of *ts*333

	*ts*333	*am*N134	*am*BL292
*ts*333	−	+	+
*am*N134	+	−	+
*am*BL292	+	+	−

Note: (i) Host: *E. coli* BB;
(ii) Temperature: 41°;
(iii) +: Complementation;
(iv) −: No complementation

TABLE II
Burst size of complementation test

Phage	Titer (PFU/ml)*	Burst size**
*am*N134	2.83×10^9	11.3
*am*BL292	2.35×10^9	9.4
*ts*333	1.85×10^9	7.4
*ts*333+*am*N134	105×10^9	420
*ts*333+*am*BL292	107×10^9	428

* PFU: plaque forming unit
** Burst size= $\frac{\text{titer}}{2.5\times10^8}$

(B) Temperature sensitivity

T4 *ts*333 is a temperature-sensitive mutant as shown in Table III. This mutant had an PFU

TABLE III
Temperature sensitivity of *ts*333

	Titer (PFU/ml)	
	25°	41°
*ts*333	5.0×10^9	0×10^8
*am*N134	6.6×10^9	6.5×10^9
*am*BL292	6.0×10^9	6.0×10^9

of 5×10^9/ml at 25°, but no plague formed at 41°. Either *am*N134 or *am*BL292 was not temperature-sensitive.

(C) DNA synthesis

The kinetics of DNA synthesis in T4-infected *E. coli* was studied at 25° and 41.5°. [^{14}C] thymine incorporation into TCA (trichloroacetic acid)-insoluble fraction of T4-infected cells was used as an index of DNA synthesis. T4 *ts*333 DNA synthesis was initiated at 4 min after infection at 41.5° (Fig. 1) and initiated at 10 min after infection at 25° (Fig. 2). T4 *ts*333 has the same type of DNA synthesis as maturation-defective mutants, *am*N134 and *am*BL292. All of these mutants have phenotype of continuous DNA synthesis. This indicates that *ts*333 may be defective in either DNA-maturation or late gene expression, but not directly involved in either DNA synthesis or synthesis of precursors.

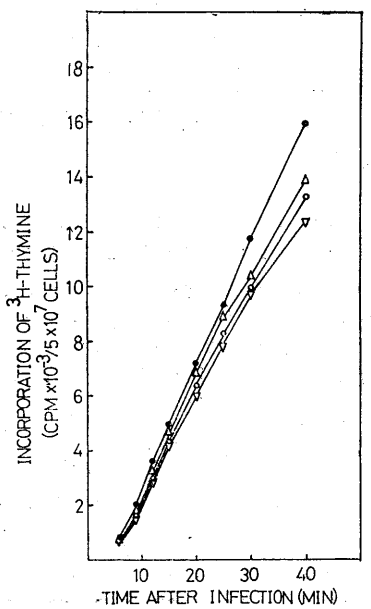

Fig. 1. DNA synthesis of T4D, *ts*333, *am*N134 infected *E. coli* at 41.5°.
●—●, T4D; △—△, *am*BL292; ○—○, *ts*333; ▽—▽, *am*N134.

(D) Effect of temperature shift-up

In order to know when *ts* 333 starts to express its gene function, the temperature shift-up experiment was performed. *E. coli* B cell cultures were infected with *ts*333 under permissive temperature (25°), then shift to nonpermissive temperature (42°) at different time after infection and the phage yields were determined after 90 min of further incubation. As shown in Fig. 3, *ts*333 could not produce significant phage yield before 15 min after infection, but increased steadily after this time. This means that gene function of *ts*333 starts to express after 15 min of infection. The T4 gene 32 mutant, *ts*P7, belongs to an early gene function and started to increase its phage yield after 15 min of infection at 25° (Fig. 4). This result indicates that *ts*333 belongs to an early gene.

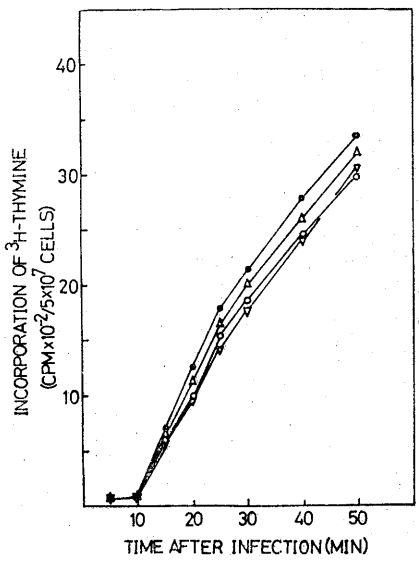

Fig. 2. DNA synthesis of T4D, *ts*333, *am*BL292 and *am*N134 infected *E. coli* at 25°.
●—●, T4D; △—△, *am*BL292; ○—○, *ts*333; ▽—▽ *am*N134.

(E) Pattern of amino acids incorporation

The rate of protein synthesis during the development of bacteriophage T4 was studied by the incorporation of L-[^{14}C] amino acids mixture. After phage infection at an M. O. I. of 5 to *E. coli* B021, the phage-induced proteins were labeled with [^{14}C] amino acids for 4 min at

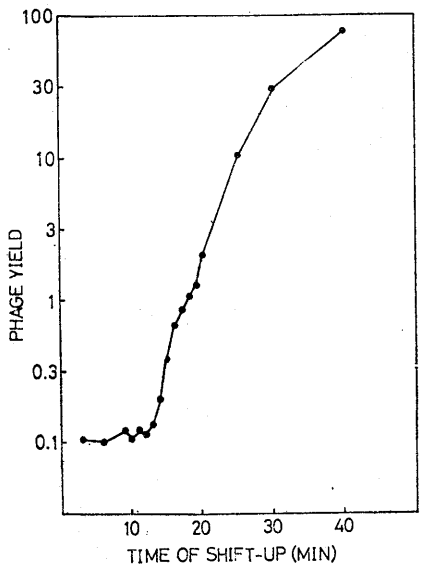

Fig. 3. Temperature shift-up experiment of *ts*333.

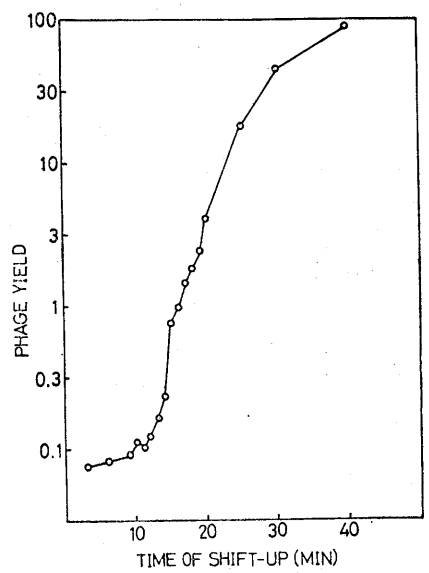

Fig. 4. Temperature shift-up experiment of *ts*p7.

different time after infection at 41.5°. As shown in Fig. 5, the incorporation of wild type T4D increased immediately after infection and reached

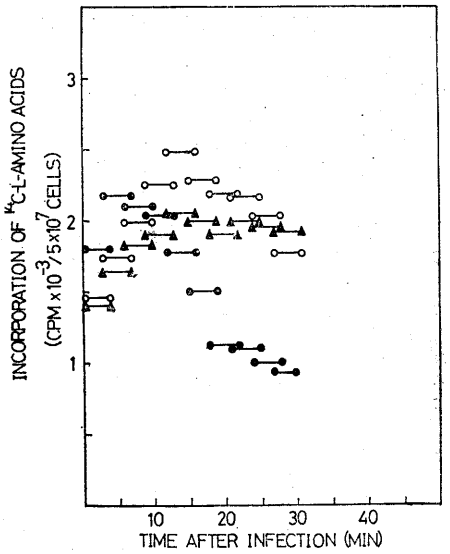

Fig. 5. Amino acid incorporation into T4-infected *E. coli*.
○—○, T4D; ▲—▲, *ts*333; ●—●, *am*N134.

a maximum between 12 to 16 min, then decreased. T4 *am*N134 (gene 33) showed a fastest synthesis rate in the early stage (3 to 7 min), then decreased drastically. In the case of *ts*333-infected cells, the [^{14}C] amino acid incorporation rate increased after infection and reached a plateau after 12 min of infection. This means that *ts*333 is defective in the synthesis of some late RNA, thus the protein synthesis in late stage is affected.

(F) Serum blocking power

Antibodies against viruses have a general property of reacting with the viruses to form a non-infectious antibody-virus complex. This property can be used as a measurement of phage structural proteins formation. T4D or *ts*333 infected *E. coli* B at 42° and its lysate was added to a known amount of T4D phage suspension for the test of serum-blocking power competiton. After 40 min of *ts*333 infection at 42°, there was no competition with T4 phage particles (Fig. 6). Apparently, *ts*333 can not synthesize structural proteins for its maturation

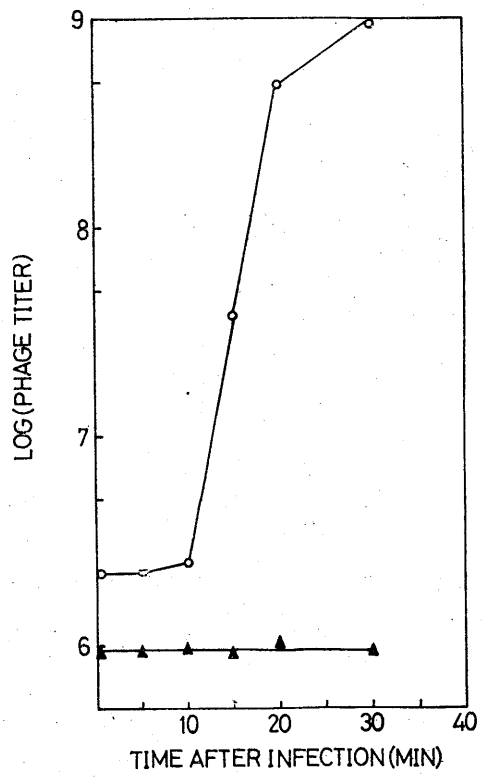

Fig. 6. Serum-blocking power of T4D and *ts*333 infected *E. coli*.
○—○, T4D; ▲—▲, *ts*333.

at non-permissive temperature. On the other hand, there is structural proteins synthesis in T4D infected cells, as shown by the increase of phage titer in the antibody reaction (Fig. 6).

(G) Competition of RNA-DNA hybridization

The percentage of RNA-DNA hybrid formation is able to show the homology between RNA and DNA[18]. Late RNA of T4D was labeled with [^{3}H] uridine between 14 to 19 min (42°) or 16 to 21 min (30°) after infection. T4 *ts*333 unlabeled late RNA was prepared under the above condition and used as competitor. In RNA-DNA hybridization competition experiment, *ts*333 unlabeled RNA (42°, 18 min) showed very little competition with [^{3}H] T4D RNA (42°, 14-19 min). Even on the addition of 1,000 μg/ml of unlabeled *ts*333 RNA, there still

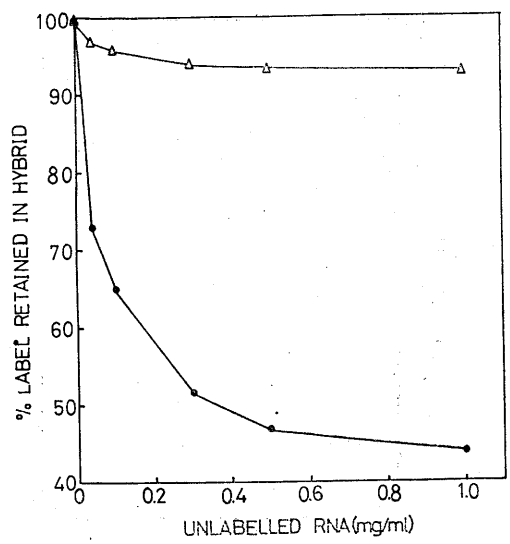

Fig. 7. RNA-DNA hybridization competition.
△—△: T4D ^{3}H-RNA (42°, 14-19 min) +*ts*333 RNA (42°, 18 min).
●—●: T4D ^{3}H-RNA (30°, 16-21 min) +*ts*333 RNA (30°, 20 min).

had 93% of [^{3}H] T4D RNA retained in the hybrid (Fig. 7). This is a good indication that *ts*333 has much less or no late RNA synthesis. However, when *ts*333 RNA was isolated at 20 min after infection at 30°, it could compete the hybrid formation between [^{3}H] T4D RNA (30°, 16-21 min) and T4 DNA. 65% of [^{3}H] T4D RNA retained in RNA-DNA hybrid on the addition of 100 μg/ml of *ts*333 RNA and reduced to 44% on the addition of 1,000 μg/ml of *ts*333 RNA. These results indicate that the defective function of *ts*333 is related to late mRNA synthesis.

(H) Genetic mapping

Genetic locus of *ts* 333 in T4 chromosome

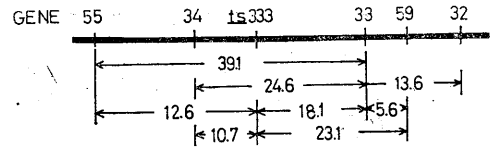

Fig. 8. Genetic locus of *ts*333. Percent of recombination was listed below the map and was calculated as (*ts*$^+$-*am*$^+$ recombinants/total progeny) ×200%.

was determined by two-factor crosses. The *ts*333 used for mapping has been purified by crossing with wild type T4D seven times. As shown in Fig. 8, *ts*333 was located between gene 33 and 34. It was 10.7% away from gene 34 and 18.1% away from gene 33.

DISCUSSION

In the early stage after T4 infection, *E. coli* RNA polymerase is used for the transcription of early mRNA from 1-strand DNA.[6] After early gene products have been synthesized, the late mRNA is transcribed from r-strand DNA by modified *E. coli* RNA polymerase[6]. It has been found that three T4-induced polypeptides are bound to host RNA polymerase.[12] Two of them are the gene products of maturation-defective genes, gene 55 and gene 33[10,14]. The third polypeptide with rn MW of 15,000 daltons is an unidentifed gene product[10,11]. From these observation, the existance of a third maturation-defective gene in T4 chromosome can be speculated.

In order to prove that *ts*333 is a new maturation-defective gene mutant, the genetic and biochemical evidene was presented.

(A) T4*ts*333 is a new gene

The complementation test indicated that *ts*333 is netither the mutant of gene 33 nor gene 55 (Table I and Table II). By genetic mapping, it clearly showed that the mutational site of *ts*333 was located between gene 33 and 34 (Fig. 8). Since no gene has been assigned between gene 33 and gene 34[20], *ts*333 is an unidentified gene in T4 chromosome.

(B) T4 *ts*333 is a maturation-defective mutant

For the identification of *ts*333 as a maturation-defective mutant several criteria were performed.

(i) *cotinuous DNA synthesis*

Only the mutant whose gene product participates in DNA-synthesizing apparatus or nucleotide(s) precursor synthesis can affect phenotype of DNA synthesis[21]. Gene 55 and gene 33 are related to DNA maturation after synthesis and late gene transcription. Thus, gene 55 and gene 33 have normal phenotype of continuous DNA synthesis. T4 *ts*333 has continuous DNA synthesis under both permissive and non-permissive temperature (Fig. 1 and Fig. 2).

(ii) *belongs to an early gene*

Temperature shift-up experiment indicates that *ts*333 is a typical early gene (Fig. 3). Thus, the gene product of *ts*333 can express in the early stage after infection and exert its function in the late stage. This characteristic is the same as gene 55 and gene 33[21].

(iii) *no late mRNA synthesis*

T4 *ts*333 late mRNA synthesized under non-permissive temperature could not compete with T4D late mRNA in RNA-DNA hybridization experiment (Fig. 7). This is a good indication that function of *ts*333 gene is related to late mRNA transcription. Same characteristic has been found in gene 55 mutant[8].

(iv) *no late protein synthesis*

The rate of protein synthesis of *ts*333 decreased earlier than that of wild-type T4D (Fig. 5). Serum-blocking power experiment also indicates that no structural protein is synthesized in *ts*333-infected cells (Fig. 6). Therefore, the effect of *ts*333 mutation is on late mRNA transcription, then late protein synthesis. The above abnormal phenotype are the same as gene 55 and gene 33. From these results, it can be concluded that ts333 is a third maturation-defective gene in bacteriophage T4.

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噬菌體 T4 之新成熟缺陷突變種

吳 金 洌

介於噬菌體 33 號及 34 號基因之間的新成熟缺陷突變種已被分離出來。根據 DNA 合成，血清阻止之能力，溫度升高實驗，RNA-DNA 雜交之競爭以及互補試驗等表現型，*ts*333 被認爲是噬菌體 T4 之第三個成熟缺陷基因之突變種。

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SCIENTIFIC NOTES

An Autoradiographic Study of Prostaglandin E_1 and/or Its Metabolites in Mouse Uterus Tissue

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Prostaglandins (PG) have recently become the subject of extensive research. It has shown that prostaglandins are widely distributed in animal tissues. Recent advances have resulted in various hypotheses as to the action of these substances. The testing of these hypotheses is under way in a number of laboratories by either diffusing or injecting the prostaglandins into the animals. Observation that prostaglandins strongly stimulate smooth muscle has led to many studies of their effects on the uterus. These studies have been made both *in vitro* and *in vivo*. Most of the *in vitro* studies were concerned with the response of human myometrail strips to prostaglandins. The myometrial strip from a pregnant woman is often contracted by PGE_1, though higher doses can cause inhibition[2,3]. Most of the *in vivo* studies of human reproduction systems were done on volunteers. Bygdeman's group[12] reported that intravenous infusions of PGE_1, PGE_2 and $PGF_{2\alpha}$ induce abortion. Karim[8] reported that $PGF_{2\alpha}$ can be used to induce labor. Prostaglandin E_1 has also been used with success in termination of Pregnancy[4]. Further studies on the localization of the prostaglandins or their metabolites in uterus tissues after injection will yield valuable information which can be used to determine their pharmacological effects on human reproduction system.

Grain density autoradiography is beleived to be a useful tool and straight forward method for quantitative distribution studies. Liquid scintillation counting can be used for distribution studies, but in this case the classification of regions has to be made before the experiment is started. Using grain density autoradiography, however, one can classify the regions for counting according to the distribution of the grains on the autoradiogram so that a more valuable conclusion can be obtained. Moreover, the grain density autoradiogram provides a very precise location of radioactivity.

Relatively few papers have been published concerning the use of radioactivity labelled prostaglandins to study their tissue distribution in animals following injection. Liquid scintillation counting was used by Nakano[11] for subcellular localization in tissue and plasma distribution of intravenously injected ^3H-PGE_1 in rats and dogs. A similar counting technique was employed by Samuelsson[13] to study the organ distribution of radioactivity at different times after subcutaneous injection of ^3H-PGE_1 into female rats. Hansson and Samuelsson[7] reported an autoradiographic study using ^3H-PGE_1 and Green *et al.*[5] discussed an autoradiographic study using ^3H-$PGF_{2\alpha}$. Both of these studies involved the use of sagittal sections to indicate the distribution of radioactivity in various tissues. Grain density autoradiography for PGE_1 in mouse kidney was reported by Chen *et al.*[1]. Presently a similar quantitative technique for the distribution of radioactivity in various zones of mouse uterus following ^3H-PGE_1 injection was investigated.

MATERIALS AND METHODS

The solution of prostaglandin-E_1 [5,6-^{3}H(N)] (in a 7:3 ethanal-water mixture) was obtained with a stated specific activity of 68.5 Ci/mM (New England Nuclear). The radiochemical purity was determined by thin layer chromatography to be greater than 95%. The preparation of the injected solution was reported previously[1]. Fifty microcuries of ^{3}H-PGE_1 were injected into through tail vein of each of three 20-g Swiss-Cox female mice. A fourth mouse was served as control.

The three ^{3}H-PGE_1 injected mice, labelled as No. 1, No. 2 and No. 3, were sacrificed by ether at 20 min, 40 min and 45 hr correspondingly after injection, and the uteri were removed and frozen immediately by dipping into isopentane which was cooled by liquid nitrogen[6]. Tissue were sectioned in a thickness of 10 μ at -20°C.

The tissue sections were transfered from the microtome knife to slides which had been previously coated by dipping into kodak NTB liquid emulsion according to the method developed by Morris[9]. All slides were placed in light-tight plastic boxes containing dessicant and stored at -4°C for the duration of the exposure periods as listed in Table 1.

TABLE 1

Experimental Prameter.

Three mice labelled as No. 1, No. 2 and No. 3 are sacrificed at different time after injection. The autoradiograms made from different mouse have different exposure time.

Mouse No.	Time Between Injection and Sacrifice	Exposure Time of Autoradiogram (days)
1	20 min	8
2	40 min	24
3	45 hr	106

After the desired exposure the grain density emulsion slides were photographically processed and histologically stained using the procedure of Skierkowski[14] The distribution of radioactivity in the grain density autoradiograms was determined by microscopic counting of the number of exposed silver grains located over specific uterus regions using an oil-immersion objective (1000×). A grid was placed in the eyepiece of the microscope that superimposed an area of 2500 μ^2. Equally spaced fields in each region were chosen for grain counting. In each region the mean was calculated and the Students' statistic[10] was used to determine the 95% confidence interval of the mean. The mean grain density and its 95% confidence interval in each region were normalized by dividing by the number of days of exposure.

RESULTS AND DISCUSSION

The distribution of radioactivity was examined in various region of uterus tissue sections. When viewed with a microscope the violet stained nuclei could be very easily distinguished from the pink stained cytoplasm. Each region was recognized by its characteristic histological structure and staining. The normalized mean grain density versus region of uterus is plotted in Fig. 1. The 95% confidence intervals are shown as error bars on the histogram. It is shown in Fig. 1 that circular smooth muscle myometrium had the higest radioactivity. The radioactivity in the longitudinal smooth muscle myometrium is higher than that in endometrium. The distribution of grain density in each region was found to be more or less uniform. Background grain density was found to be considerably lower than the grain density on most tissue regions.

Sample from mouse No. 3, which was sacrificed at 45 hr after injection, the radioactivity was so low in uterus tissue that very long exposure time was needed. Unfortunately, a long exposure not only accumulates a high background but also introduces errors as a result of latent image fading. Therefore, the results of mouse No. 3 were only considered as reference information and not for quantitative comparison. However, it is very clear from results No. 3 that the radioactivity in uterus was very

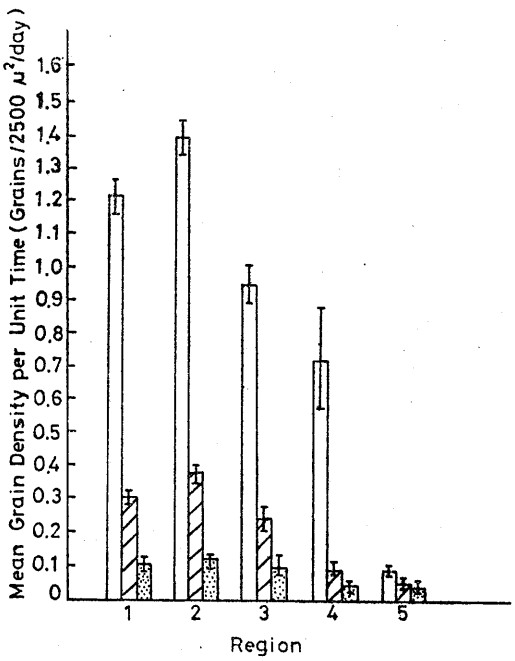

Fig. 1. Histogram of Uterus Grain Density Measurements. The normalized mean grain density of each region is plotted. Results from three mice are plotted together in this figure, ☐ representing results from mouse No. 1, ▨ from mouse No. 2 and ⁞⁞ from mouse No. 3. The 95% confidence intervals are shown as error bars in each mean.

Regions

1. Endometrium.
2. Circular Smooth Muscle of the Myometrium.
3. Longitudinal Smooth Muscle of the Myometrium.
4. Emulsion Region Immediately Adjacent to the Tissue Section.
5. Background of the Emulsion at a Considerable Distance from the Tissue Section.

low at 45 hr after injection.

In order to examine the rat of removal of radioactivity, the net mean grain density and the percentage decrease of the net mean of mouse No. 2 from that of mouse No. 1 in each region were calculated and are given in Table 2. From this table one can see that the percentage decreases in endometrium, circular smooth muscle of myometrium and longitudinal smooth muscle of myometrium are similar. Therefore the distribution of radioactivity in various regions of the uterus remains constant between 20 and 40 minutes after injection. In other words, the binding ability of these three regions are close to each other.

The degree of precision for location of radioactivity of a grain density autoradiogram depends upon how detailed the histological structure can be deduced from the histological staining. It also depends upon the fixation of the compound in the tissue, because translocation of radioactivity during preparation of the autoradiogram may give considerable error in the results. This was prevented in this research by quickly freezing the tissue immediately after it was removed from the animal. The results of this research are acceptable because the region which was considered was quite large in comparison to the possible translocation during sectioning. If a very small region is being considered, such as a nucleus, than a further test of the degree of prevention of translocation would have to be made.

It was found that if the grain density was too high then counting errors occured as a result of counting grains more than once. However, as long as the grain density was below about 150 grains/2500 μ^2, the grain counting is very reproducible. For those regions in which the grain density was greater than 150 grains/2500μ^2 a large number of fields were counted so that satisfying statistical results could be obtained. This can be seen from Fig. 1. The 95% confidence interval was small compared to the mean.

Since prostaglandins have such a wide spectrum of pharmacological effects more autoradiographic studies of their distribution in organs after injection seem warranted. For example, a distribution study in lungs might be helpful in understanding the metabolism of prostaglandins in the lungs. Also., a comparison

TABLB 2.

Percentage Change of the Net Mean Grain Density in Various Uterus Regions.

The percentage decrease of the net mean of mouse No. 2 from that of mouse No. 1 in the same region is given as percentage change of each region.

Region	Net Mean Grain Density Mouse No. 1	Net Mean Grain Density Mouse No. 2	% Change
Endometrium	1.127	0.246	78%
Circular Smooth Muscle of Myometrium	1.315	0.322	76%
Longitudinal Smooth Muscle of Myometrium	0.858	0.181	79%

of the distribution of PGE_2 in the uterus with that of PGE_1 would be interesting since PGE_2 has been found to be very active at inducing labor.

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以放射自動印相法研究前列腺素 E_1 及其代謝物在小鼠子宮之分佈

王 淑 霞

此研究工作爲用黑點密度自動印相 (grain density autoradiography) 之方法測得前列腺素 (prostaglandin) E_1，以及/或它的代謝物在小鼠子宮組織的分佈。實驗結果顯示在子宮肌膜的環狀平滑肌裏黑點的密度最大。而在子宮肌膜的環狀平滑肌，縱走平滑肌以及子宮內膜三區域內放射性在注射後 20 分鐘到 40 分鐘隨時間降低的比例相似。

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SCIENTIFIC NOTES

A Preliminary Report on the Effect of Thyroidectomy on Rat Hypothalamic Gonadotropin-Releasing Hormone (GnRH) Contents

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Previous investigations on adenohypophyseal gonadotropins in thyroidectomized (Tx) female rats indicates lowered in LH content but varied in FSH[1,7~9]. It is understood that gonadotropins is under the control of hypothalamic gonadotropin-releasing hormone (GnRH). Thus, it is essential to study the variations of hypothalamic GnRH after the removal of thyroid.

MATERIALS AND METHODS

Forty Sprague-Dawley female rats reared in this laboratory, in a controled condition (room temperature: 23±1°C; light on 6:00 AM to 6:00 PM), were randomly divided into two groups, the intact control and Tx groups. The rats were on chicken feed (Taiwan Sugar Corp., Taipei) and tap water *ad lib* before and after Tx. One month after the operation, the Tx rats were subjected to vaginal inspection for proestrus. Rats of Tx and control groups that demonstrated proestrus were sacrificed by exsanguiation through dorsal aorta under light ether anesthesia at the time between 10:00 AM to 12:00 noon. The hypothalamic tissue were removed within 3 min after anesthesia. The hypothalami were dehydrated in precooled acetone (−20°C), which was refreshed twice within two days. The hypothalamic tissue was dissected with the approximations of the demarcations of optic chiasma, anteriorly; optic tracts, laterally; mammillary bodies, posteriorly and in a thickness of 5 mm. Control rats were sacrificed at a similar age. Hypothalamic extracts (HE) was prepared by a modification of the method introduced by Derry[2] and Steiner *et al.*[6]. After partial evaporation of acetone, groups of 20 hypothalamic tissue from the control or Tx were homogenized in cold acetone (−20°C) in a all-glass homogenizer. The resulting fine powder was then extracted with 0.1 N HCl at 4°C. The homogenate was then transfered to a pointed centrifuge tube and centrifuged at 1500 g for 60 min. The supernatant was neutralized with 1 N NaOH and the resulting cloudy precipitate removed by centrifugation at 1000 g for 30 min at 4°C. Remaining supernatant was lyophilized. The HE preparations were store in vaccum vials and reconstituted by double distilled water at the time for radioimmunoassay (RIA). The assay method of Koch *et al.*[4] was adapted for the present investigation.

Synthetic GnRH (LH-RH, Calbiochem Co., San Diago, USA) were utilized for both standard and radioiodination. Radioiodine (^{125}I Radiochemical Center, Amersham, England) was reacted with GnRH catalyzed by Chloramine-T and separated in Sephadex G-25 column. Anti-GnRH (Miles Laboratories, Ind., USA) in 1:50 dilution were served for 1st antibody and anti-rabbit

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globulin from sheep (Gibco Co., New York, USA) were used as 2nd antibody in present RIA. The sample GnRH level were determined by a logit transformed linear correlation equation of a standard curve.

RESULTS AND DISCUSSIONS

Synthetic unlabled GnRH of 5 pg to 10,000 pg were used to construct standard curves (Fig. 1). The minimum reliable measurements are in the range of 25 to 30 pg, while the actual quantity of GnRH of HE samples in assay is 400 pg in minimum. The inhibition curve of HE is showed in Fig. 1, the parallelism is evident.

In the present study, HE were from the pool

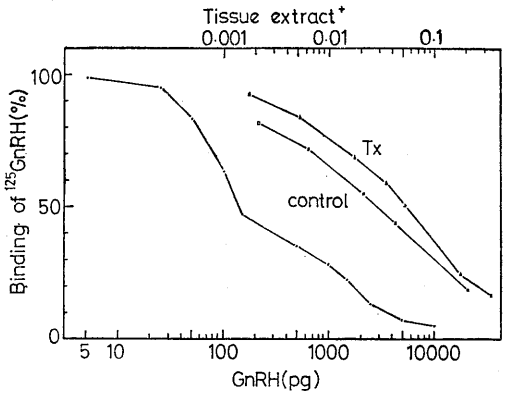

Fig. 1. Inhibition of binding of ^{125}GnRH to Gn RH antiserum by (1) unlabeled GnRH, (left) (2)Tx rat hypothalamic extract (right) and (3)intact rat hypothalamic extract (center). Anti GnRH-BSA serum used at 1:50 dilution. (+) Units indicate number of hypothalami from which the extract assayed was derived.

of 20 hypothalami. With 25 replicates, the averge of intact rats GnRH is 7.61 ± 1.33 ng/ hypothalamus and that of Tx rats, 4.72 ± 0.86 ng. By student t-test, the significant level is $p<0.01$. The randomized selection for rats of the present study may, at least, eliminate some of the individual variations that may contribute to the difference. Treatments designated to investigate on individual variations are in progress. The buffer dilution of the HE in assay may also influence the present estimated difference. An analysis of variance indicates that the difference of GnRH level between control and Tx is not due to the dilution procedure but the difference of treatment ($F=12.41$; $df=1, 32$; $p<0.01$). At this point, it is rather safe to believe that the estimated difference is reliable.

The accumulated data indicated repeatedly a lowered LH in adenohypophyses but varied in FSH level after thyroidectomy[1,7,8,9]. The present estimation on GnRH lowering considered to be responsible for the lowering of adenohypophyseal LH as estimated at the time of 10:00 AM to 12:00 noon of proestrus day, when no obvious pituitary hormonal fluctuation is predicted. This indicates the level of availibility of GnRH in hypothalamus. One component of the availibility must depend heavily on activity of synthesis. It is suggested that the production of GnRH is influenced by biogenic amine[5], and the thyroxine is proposed to have a regulatory effect on LH at the level of hypothalamus[3]. Thus, it is possible, a deficiency of thyroid hormone may influence the GnRH producing process generated by biogenic amine and in turn lowering LH content in pituitaries.

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甲狀腺切除雛鼠下視丘性釋激素之影響

萬家茂　陳幼輝

甲狀腺切除後一個月，將雌鼠之下視丘部份在動情前期日早上 10:00 至 12:00 間取出，用放射免疫測定法測量其萃取液中性釋激素 (GnRH) 之含量。經 25 次重覆測定，測得切除甲狀腺組每個下視丘中 GnRH 平均含量爲 4.72±0.86 ng 而對照組爲 7.61±1.33 ng。其可能之解釋爲，當甲狀腺素缺乏時，會影響下視丘中生源胺 (biogenic amine) 對於 GnRH 之促生過程；而此 GnRH 下降之結果，也可能是切除甲狀腺後腦下腺中 LH 下降之原因。