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PHYSICAL PROPERTIES OF BACTERIOPHAGE Xp10 GENOME

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Abstract

Xp10 is a virulent phage of ***Xanthomonas campestris*** pv. ***oryzae***. The phage has an icosahedral head with a diameter of 56.7±0.2 nm and a tail with the length of 107.0 ±0.2 nm. The coat was composed of eight proteins with the molecular weight of 258,000, 150,000, 147,000, 128,000, 90,000, 46,000, 43,800 and 24,200 daltons respectively. The Xp10 genome is linear double stranded DNA with cohesive ends and 46.4 kilobase pairs in size. No nick was found in the DNA molecules. The G-C content of Xp10 DNA was 58.1%. The restriction enzyme cleavage patterns of nine endonucleases, ***Bam*** HI, ***Eco*** RI, ***Hin***d III, ***Kpn*** I, ***Sal*** I, ***Sma*** I, ***Sst*** II, ***Xba*** I and ***Xho*** I were studied by agarose gel electrophoresis. The physical map of five enzymes, including ***Bam*** HI, ***Sma*** I, ***Sst*** II, ***Xba*** I and ***Xho*** I, was constructed.

Key words: Phage Xp10; genome.

Introduction

The phage Xp10 was isolated from phage infected culture of *Xanthomonas campestris* pv. *oryzae*, a pathogenic bacterium of rice blight. The general properties of this phage have been reported (Kuo *et al.*, 1967). After infection with Xp10, the host RNA polymerase was inhibited and a new rifampicin-resistant RNA polymerase was produced (Liao, 1985). Similar phenomena have been described in other phages such as T7, T3, gh-1 and sp-6 (Chamberlin, 1974; Brunokvskis, 1979; Chakraborty, *et al.*, 1973; Towle *et al.*, 1975; Jolly, 1979; Butter and Chamberlin, 1980). The morphology and the genome size of T7, T3, gh-1 and sp-6 are almost identical. Morphologically, Xp10 is biger than T7, T3, gh-1 and sp-6, and also has a longer tail. Recently, a phage coded RNA polymerase was isolated from Xp10 infected cells (Liao, 1985). The molecular size of this new RNA polymerase is also 98,000 daltons,

which is almost identical with these of T7, T3, gh-1 and sp-6. It seems that the system for the control of gene transcription in Xp10 infected cells is very unique. The structure of Xp10 genome was studied in this report for the purpose of further achievement on understanding of phage gene expression. Our study here includes the analysis of phage Xp10 coat proteins, the conformation, the Tm value, the G-C content, and the physical map of Xp10 genome.

Materials and Methods

Bacteria, Phage and Media

Xanthomonas campestris pv. *oryzae* and its phage Xp10 were reserved in our laboratory. Potato-sucrose (PS) medium used for the growth of the bacterium and the phage was described by Yang and Kuo (1984).

Growth and Purificatian of Xp10

For preparation of a large quantity of Xp10 phage, host bacteria were grown in PS medium with shaking at 28°C. Overnight culture at a density of 5×10^8 cells/ml was infected with Xp10 at a multiplicity of 10. After 8 h incubation, cells were lysed by the phage. The cell debris was removed by centrifugation. Twenty mM of calcium chloride was added to the supernatant and the mixture was incubated at 4°C for 1 h. The precipitate was removed by centrifugation, then polyethylene glycol 6,000 at final concentration of 6% was added to supernantant at 4°C. After 2 h incubation, the precipitate containing phage particles was harvested by centrifugation at 90,000 g for 1 h. The phage pellet was dissolved in Tris-Ca solution (10 mM Tris-HCl, pH 8.0, 20 mM $CaCl_2$). The undissolved materials were removed by centrifugation at 20,000 g for 30 min.

Phages were further purified by CsCl gradient centrifugation. CsCl was added into the supernantant at final concentration of 0.786 g/ml and subjected to centrifugation at 102,000 g at 15°C for 44 h. A clear phage band located at about the middle of the centrifuge tube was harvested. The phage suspension was dialyzed 3 times against Tris-Ca solution to remove CsCl and stored in refrigerator.

Purification of Xp10 DNA

Phage DNA purification followed the procedure described by Jackson (1982). The purified phage was dialyzed 2 times against formamide solution (200 mM Tris-HCl pH 8.5, 20 mM EDTA-Na_2, 50% formamide) for 12 h, one time aganist TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA-Na_2) containing 2 M NaCl, and followed by TE buffer containing 1 M NaCl. Finally, the Xp10 DNA was dialyzed 2 times against TE buffer.

Determination of the Melting Temperature of Xp10 DNA

The measurement of DNA melting temperature was performed as described by Dove and Davidson (1962). Xp10 DNA was dissolved with SSC buffer (150 mM NaCl, 15 mM sodium citrate, pH 7.0). DNA at a concentration of approximately 30 μg/ml was added in UV spectrophotometer chamber the temperature in which was raised constantly by electronic heater.

Enzyme Digestion of Xp10 DNA

For all enzyme reactions, 20 μl of reaction mixture containing 1 μg of Xp10 DNA and 1 unit of enzyme were incubated at 37°C for 1 h. The reaction buffer of S1 nuclease was 30 mM NaAc, pH 4.6, 50 mM NaCl and 1 mM $ZnSO_4$. For exonuclease III, the reaction buffer was 5 mM Tris-HCl pH 8.0, 10 mM 2-mercaptoethanol and 5 mM $MgCl_2$. The reaction buffers of the restriction endonucleases were prepared by following the instruction provided by Bethesda Research Laboratories Inc. (Maryland, USA). To determine the sizes of DNA fragments, DNA digests were fractionated by agarose gel electrophoresis. Lambda DNA digested with *Eco* RI and *Hin*d III reectively and φX174 DNA digested with *Hae* III were used as size markers.

Determination of Xp10 Protein Coat

Twenty μl of Xp10 phage was mixed with 20 μl of dissociation solution (125 mM Tris-HCl, pH 6.5, 2% SDS, 5% 2-ME, 50% sucrose). The mixture was boiled for 3 min to denature proteins. Samples were applied onto 7.5% SDS-polyacrylamide gel, and then run at 100 volts for 5 h (Hames, 1981). Protein bands were visualized by silver staining procedures as described by Merril *et al.* (1981). Standard proteins used for molecular weight determination were myosin, α-galactosidase, phosphorylase B, bovine serum albumin, ovalbumin (BRL), β-chymotrypsinogen, α-lactoglobulin, lysozyme and cytochrome C (Bio-Rad).

Results

The Phage Particle and Its Protein Coat

Electron microscopic observation of purified phages showed that phage particles had an isometric, icosahedral head with 56.7±0.2 nm diameter. The tail was 107.0±0.2 nm in length. There was no basal plate on the distal end of tail (Fig. 1). The number and the size of coat proteins were analysed with SDS-polyacrylamide gel electrophoresis. Eight protein bands were detected. The molecular weights were 258,000, 150,000, 147,000, 128,000, 90,000, 46,000, 43,800 and 24,200 daltons, respectively (Fig. 2). Among these proteins the protein which had molecular weight of 258,000 was unusually large.

Melting Temperature of Xp10 DNA

The melting temperature, Tm, was linearly related to the average DNA base composition. Data obtained from the $\Delta OD/\Delta T$ against temperature plotting revealed that the Tm of Xp10 DNA was 92.5°C. The Tm value could be converted to G-C content under standard conditions by the equation: $Tm = 16.6 \log [Na^+] + 0.41 (G\text{-}C\%) + 81.5$ (Davis, 1980). Calculation based on this formula, the G-C content of Xp10 DNA was 58.1%.

The Form of Xp10 DNA

Exonuclease III catalyses the stepwise 3′–5′ removal of 5′-mononucleotides from double stranded DNA. DNA in linear form can be digested by this enzyme, while the circular form can not. Xp10 DNA was digested by exonuclease III. Aliquots of incubate were withdrawn at different time intervals and analysed by agarose gel electrophoresis. The result is shown in Fig. 3. Xp10 DNA showed a typical pattern for a linear form. The size of Xp10 DNA was gradually decreased with the incubation time.

To confirm the linearity of phage genome, Xp10 DNA was digested with two endonucleases. In theory, for a circular DNA the number of DNA fragments from double digestion equals to the total number of DNA fragments generated by two single digestion. For a linear DNA, however, the number of DNA fragments from double digestion should be one band less than that of single digestion (Maniatis *et al.*, 1982). When Xp10 DNA was doublely digested with *Sma* I and *Xba* I, nine fragments were obtained. When Xp10 DNA was digested with *Sma* I and *Xba* I respectively, the total number of two digestion were ten (Fig. 5). Again, the conformation of Xp10 DNA was proved to be linear.

To prove the absence of any nick in Xp10 DNA, the Xp10 DNA was digested with S1 nuclease which cleaves the nick on double stranded DNA (Goding & Russell, 1982). The S1 nuclease digests of Xp10 DNA were taken at different time intervals and analysed. There was no difference between Xp10 DNA and S1 nuclease treated Xp10 DNA (data not shown).

Endonucleases Cleavage Patterns of Xp10 DNA

The Xp10 DNA was digested with nine restriction endonucleases. They were *Bam* HI, *Eco* RI, *Hind* III, *Kpn* I, *Sal* I, *Sma* I, *Sst* II, *Xba* I and *Xho* I. The cleavage patterns are shown in Fig. 4. Eight fragments from *Bam* HI, ten from *Eco* RI, ten from *Hind* III, eleven from *Sal* I, three from *Sal* I, three from *Sma* I, ten from *Sst* II, seven from *Xba* I and six from *Xho* I were obtained. The sizes of DNA fragments were determined by the size against mobility plotting method as described by Southern (1979). The genome size is about 46.4 kilobase pairs.

Physical Map of Xp10 Genome

The order of restriction fragments produced by endonuclease was deduced from (1) the identification of terminal fragments, (2) the identification of neighboring fragments by partial digestion and (3) the overlapping fragments obtained by double digestion.

To identify terminal fragments of Xp10 genome, the native DNA was treated with T4 DNA ligase, and then subjected to restriction enzyme digestion. Two terminal fragments resulted from the linear DNA digests would disappear in the ligated DNA digests and a new fragment equal to the total size of the two terminal fragments would be found on 0.5% agarose gel (unpublished data). In Fig. 5, lane 5 was the native linear Xp10 DNA digested with *Xba* I, 6 bands were observed and designated as Xba-A, Xba-B etc. Lane 4 is the digestion pattern of ligated Xp10 DNA from which Xba-C and Xba-E were missing and a new band comigrated with Xba-A was found. Therefore, the Xba-C and the Xba-E were the terminal fragments. Using the same method, the terminal fragments of other restriction enzymes could be determined. They were F and B fragments for *Xho* I, A and B fragments for *Sma* I, A and D fragments for *Bam* HI, and D and B fragments for *Sst* II. The other of restriction enzyme could be tentatively determined as CDABFGE for *Xba* I, FDCEAB for *Xho* I, BCA for *Sma* I and DCBFA′GEA for *Bam* HI by partial digestion patterns of various endonucleases (Table 2). These orders were further confirmed by double digestion (Table 3, Fig. 6).

Table 1. *Fragment sizes of Xp10 DNA digests*

The fragment sizes are presented in kilobase pairs {kbp}. Data in I were obtained from restriction pattern analysis, while II were deduced from calculation according to the equation: {fragment size from I/sum of fragments in I} × average genome size.

Fragment	*Bam* HI		*Sma* I		*Sst* II		*Xba* I		*Xho* I	
	I	II	I	II	I	II	I	II	I	II
A	9.09	9.10	29.40	28.80	8.38	8.38	13.43	13.60	20.93	20.40
A′	9.09	9.10								
B	8.18	8.45	12.55	12.55	7.59	7.65	9.35	9.35	11.22	11.22
C	6.72	6.93	4.96	5.07	7.25	7.25	7.71	7.61	7.03	7.17
D	4.96	4.96			6.40	6.44	6.97	7.25	3.96	3.92
E	3.69	3.87			6.29	6.27	6.06	6.06	1.90	1.98
F	2.75	2.96			6.02	6.00	2.47	2.40	1.67	1.69
G	1.09	1.07			2.16	2.23	—	0.16		
H					1.36	1.35				
I					0.46	0.45				
J					0.38	0.38				
Sum	45.57	46.44	46.91	46.42	46.29	46.40	45.99	46.43	46.17	46.38

Table 2. *Fragment sizes of partial digests of Xp10 DNA*

Bam HI		*Sma* I		*Sst* II		*Xba* I		*Xho* I	
Size	Fragments	Size	Fragment	Size	Fragment	Size	Fragment	Size	Fragment
25.50	A+A'+E+F+G	34.72	A+C	17.70	B+C+G	27.80	A+C+D	31.40	A+B
23.08	A+A'+E+G,	29.40	A	14.40	A+F	24.76	A+B+F	20.40	A
	B+C+D+F	17.21	B+C	12.70	D+E	22.67	A+B	13.90	A+B+C+D
19.30	A+B+F, B+C+D	12.26	B	10.30	A+G	20.88	A+D	12.51	C+D+E
14.90	B+C, A+E+G	4.86	C	9.69	C+G	19.50	B+E+F	11.22	B
12.75	A'+E			8.38	A	17.87	C+D	9.16	C+E
11.46	C+D, B+F			7.59	B	13.43	A	7.08	C
10.06	A+G			7.25	C	11.91	B+F	5.51	D+F
9.09	A, A'			6.40	D	9.35	B	3.77	D
8.50	B			6.24	E	8.72	E+F	1.86	E
6.73	C			6.02	F	7.71	C	1.65	F
4.95	D, E+G			2.16	G	6.97	D		
3.69	E			1.36	H	6.21	E+G*		
2.67	F			0.45	I	6.06	E		
1.09	G			0.38	J	2.64	F+G*		
						2.47	F		

* The G fragment was confirmed by comparing the patterns of complete digestions and double digestions.

Table 3. *Sizes of Xp10 DNA fragments obtained from double digestions in kbp*

The figures in the parentheses give the number of fragments with the same size.

Fragment	*Bam* HI/ *Sma* I	*Bam* HI/ *Sst* II	*Bam* HI/ *Xba* II	*Bam* HI/ *Xho* I	*Sma* I/ *Sst* II	*Sma* I/ *Xba* I	*Sma* I/ *Xho* I	*Sst* II/ *Xba* I	*Sst* II/ *Xho* I	*Xba* I/ *Xho* I
1	9.09(2)	7.63	6.03	9.09(2)	8.41	11.15	17.20	8.06	8.41	13.31
2	7.03	6.01	5.66	6.35	7.65	9.35	11.48	6.80	7.65	6.99
3	5.19	5.52(2)	5.13	5.76	7.21	7.66	6.89	6.47	6.27	6.03
4	5.01	4.87	4.84	3.30	6.44	6.10	3.92	6.14	5.80	5.13
5	3.94	3.78	4.33	2.95	6.01	4.90	2.96	5.72	4.18	3.90
6	2.96	2.84	3.82	2.10	3.30	2.90	2.04	5.07	3.88	2.56
7	2.85	2.48	3.76	1.98	2.83	2.61	1.70	2.23	3.03	2.39
8	1.09	2.22	2.89	1.84	2.23	2.24		1.67	2.23	2.00
9		1.43	2.66	1.69	1.36			1.42	1.72	1.92
10		1.35	2.56	1.07	0.45			1.17	1.43	1.73
11		1.07	2.39	0.74	0.38			0.54	0.78	
12		0.67	1.07	0.66				0.48	0.45	
13		0.46						0.38	0.38	
14		0.38								
Sum	46.24	46.21	45.13	46.62	46.32	46.91	46.09	46.14	46.21	45.96

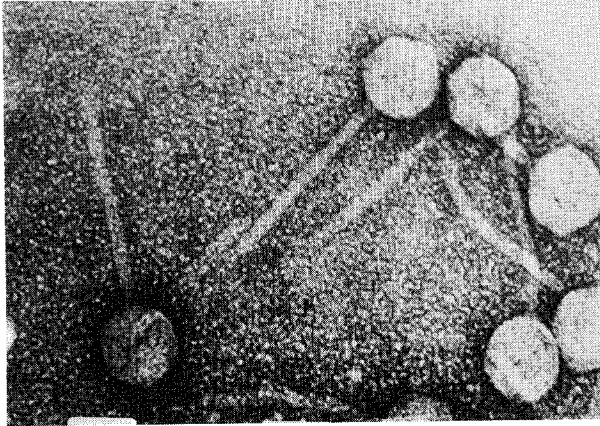

Fig. 1. Electron micrograph of Xp10.
The phage was stained with 1% uranyl acetate. 300,000×.

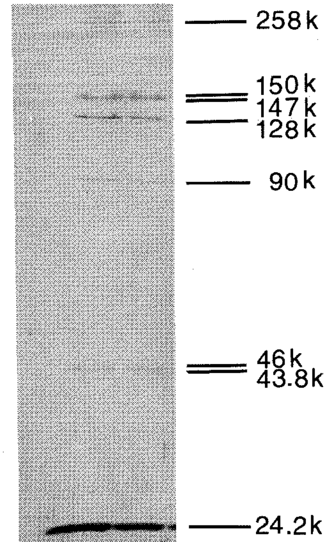

Fig. 2. Phage Xp10 coat proteins. Electrophoresis was run at 100 volts. Protein bands were visualized by silver staining.

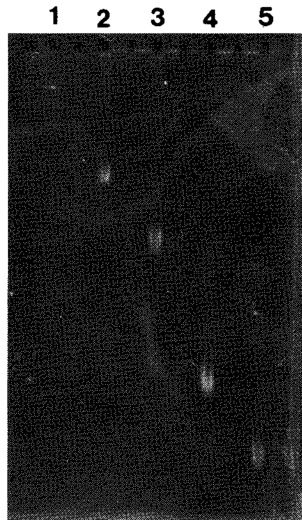

Fig. 3. Exonuclease III digestion patterns of Xp10 DNA.
Xp10 DNA was treated with exonuclease III for different time intervals and analyzed by agarose gel electrophoresis. DNA in lanes are (1) 0′ (2) 10′ (3) 20′ (4) 45′ and (5) 60′ digestion.

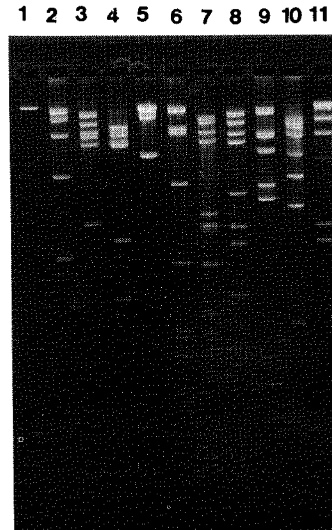

Fig. 4. Digestion patterns of Xp10 DNA.
Xp10 DNA was digested by 5-fold excess amount of endonucleases and then fractionated by electrophoresis in 1% agarose gel at 50 volts for 12 h. Lanes contain Xp10 DNA (1) undigested DNA, (2) ***Xho*** I, (3) ***Xba*** I, (4) ***Sst*** II, (5) ***Sma*** I, (6) ***Sal*** I, (7) ***Kpn*** I, (8) ***Hind*** III, (9) ***Eco*** RI and (10) ***Bam*** HI digested. Lane (11) contains lambda DNA digested with ***Hind*** III.

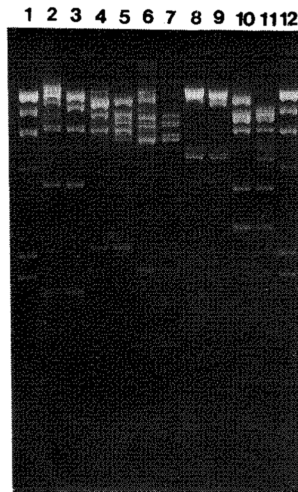

Fig. 5. Digestion patterns of ligated and native Xp10 DNA.
Xp10 DNA was ligated with T4 ligase then digested with different endonuclease. Lanes contain DNA *Hind* III/lambda DNA (1, 12), ligated Xp10 digested *Xho* I (2), native Xp10 DNA digested with *Xho* I (3), ligated Xp10 DNA digested with *Xba* I (4), native Xp10 DNA digested with *Xba* I (5), ligated Xp10 DNA digested with *Sst* II (6), native Xp10 DNA digested with *Sst* II (7), ligated Xp10 DNA digested with *Sma* I (8), native Xp10 DNA digested with *Sma* I (9), ligated Xp10 DNA digested with *Bam* HI (10), native Xp10 DNA digested with *Bam* HI (11).

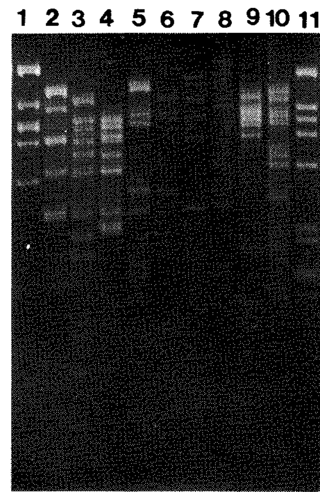

Fig. 6. Restriction patterns of Xp10 DNA double digests.
Xp10 DNA was consequently digested with two endonucleases. Prior to the second enzyme digestion, the first enzyme was removed by phenol extraction. Lane 1 is lambda DNA digested by *Eco* RI (1), Lanes 2-11 are Xp10 DNA digested by *Xho* I/*Xba* I (2), *Xho* I/*Sst* II (3), *Xba* I/*Sst* II (4), *Xho* I/*Sma* I (5), *Xba* I/*Sma* I (6), *Sst* II/*Sma* I (7), *Xho* I/*Bam* HI (8), *Xba* I/*Bam* HI (9), *Sst* II/*Bam* HI (10), *Sma* I/*Bam* HI (11), respectively.

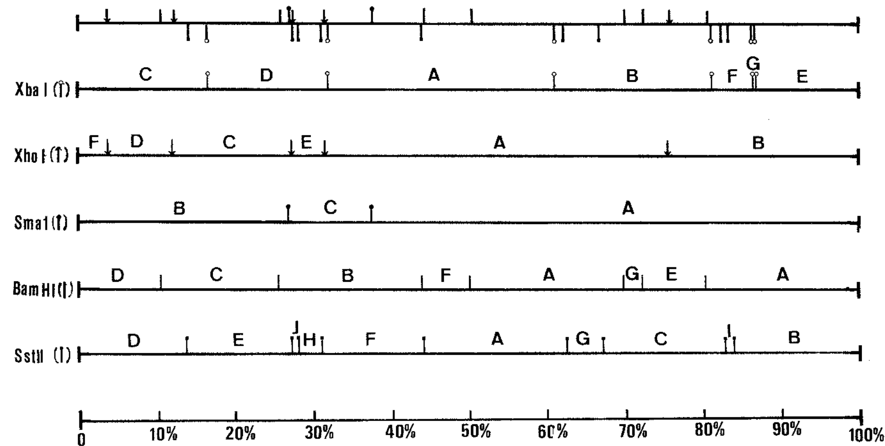

Fig. 7. The physical maps of Xp10 DNA.
The maps were derived from partial and double digestion patterns. The upper line shows the complete map for all restriction sites indicated in the individual maps. The lowest line shows the percentage of total genome.

In order to determine the left terminus of restriction fragments, the double digestion patterns of each pair of enzymes were compared with the overlapping fragments. Using this approach, the physical maps of *Xba* I, *Xho* I, *Bam* HI and *Sma* I were constructed.

By using the partial digestion pattern analysis, we had difficulty to determine the tentative order of *Sst* II fragments, however, comparing the double digestion patterns of *Sst* II with *Bam* HI, *Sma* I, *Xba* I and *Xho* I, the map of *Sst* II could be obtained. The complete physical maps of Xp10 DNA for restriction enzymes *Bam* HI, *Sma* I, *Sst* II, *Xba* I and *Xho* I are shown in Fig. 7. The determination of the left terminus of Xp10 genome has been confirmed by the transcription map of Xp10 RNA polymerase on Xp10 genome. The Xp10 polymerase transcribed only right most 70% of the late gene of Xp10 genome (Liao, 1985).

Discussion

After *X. campestris* pv *oryzae* was infected with Xp10, the host RNA polymerase is used to transcribe the early phage genes, and a new phage coded RNA polymerase is induced to transcribe the late phage genes. The molecular size of Xp10 RNA polymerase is 98,000 daltons (Liao 1985). Similar phenomena have been reported in the phages such as coliphage T7 and T3 (Chamberlin and Krishnapillai, 1974), *Pseudomonas* phage gh-1 (Towle *et al.*, 1975) and *Salmonella* phage sp-6 (Butter and Chamberlin 1982). Compared the morphology and genome size of Xp10 with those of T7, T3, gh-1 and sp-6 they were quite different: Morphelogically T7, T3, gh-1 and sp-6 are almost identical (Butter and Chamberlin 1982). These phages have a isometric particles with the diameter of 63 nm and a very short tails with the length of 15 nm. The Xp10 has a isometric particles with the diameter of 56.7 nm and long tail with the length of 107.0 nm. In addition the genome size for T7 is 39 Kb and Xp10 is 46.4 Kb. Therefore Xp10 is slightly larger than T7.

The Xp10 coat is composed of eight proteins. One of these proteins has an unusually large molecular weight, which is about 258,000 daltons. One small protein with the molecular weight of about 24,200 daltons is composed of about 90% of the total amount of the whole protein coat.

The G-C content of Xp10 DNA is about 58.1%. It is quite high as compared with the other phages, such as lambda, T1, T3, T5, and T7 with G-C content of 51, 51, 43, 53 and 46%, respectively (Carl *et al.*, 1962).

The Xp10 DNA was digested with restriction endonucleases: *Bam* HI, *Sma* I, *Sst* II, *Xba* I and *Xho* I, and 8, 3, 10, 7 and 6 fragments were produced, respectively. The digested fragments range in size from 0.1 to 28.8 kilobase pairs (Table 1). The physical map of Xp10 genome is constructed from the digestion pattern. The average molecular size of Xp10 genome is about 46.4 kilobase pairs.

In our experiments, the reactions of restriction enzymes on Xp10 DNA should be stopped at 70 °C and then followed by quick cooling, otherwise, the minor bands will be found on agarose gel analysis. After the treatment with DNA ligase, linear form of Xp10 DNA is converted into circular form or contactemers which is proved by the restriction endonucleases digestion, and the minor bands described above disappeared (Fig. 5). The results verify that the termini of Xp10 DNA are cohesive ends.

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Literatures Cited

Brunokvskis, I. and W. C. Summers. 1979. The process of infection with coliphage T7. V. Shutoff of host RNA synthesis. Virol. **45**: 224-231.

Butter, E. T. and M. J. Chamberlin. 1982. Bacteriophage sp-6-specific RNA polymerase. I. Isolation and characterization of the enzyme. J. Biol. Chem. **257**: 5772-5778.

Carl, L. S., J. Marmur, and P. Doty. 1962. Determination of the base composition of doxyribonucleic acid from its buoyant density in CsCl. J. Mol. Biol. **4**: 430-443.

Chakraborty, P. R., P. Sarker, H. H. Huang, and U. Maitra. 1973. Studies on T3-induced RNA polymerase. III. Purification and characterization on T3-induced RNA polymerase from bacteriophage T3-infected ***E. coli*** cells. J. Biol. Chem. **248**: 6637-6646.

Chamberlin, P. R. and V. Krishnapillai. 1974. Characterization of T7-specific ribonucleic acid polymerase. J. Biol. Chem. **248**: 2235-2244.

Davis, W., D. Botstein, and J. R. Roth. 1980. A Manual for Genetic Engineering. Advanced bacterial genetics. Cold Spring Horbar, New York, 222 pp.

Dove, M. F. and N. Davidson. 1962. Cation effects on the denaturation of DNA. J. Mol. Bio. **5**: 467-478.

Goding, C. R. and W. C. Russell. 1982. S1 sensitive sites in adenovirus DNA. Nucl. Acid Res. **11**: 21-36.

Hames, B. D. 1981. An Introduction to Polyacrylamide Gel Electrophoresis. ***In*** B. D. Hames and D. Rickwood (eds.), Gel Electrophoresis of Proteins. IRL Press, New York, pp. 1-91.

Jackson, P. 1982. DNA purification. Bethesda Research Laboratories, Inc. Focus. 4(2): 12.

Jolly, J. F. 1979. Program of bacteriophage gh-1 DNA transcription in infected ***Pseudomonas putida***. J. Virol. **30**: 771-776.

Kuo, T. T., T. C. Huang, R. Y. Wu, and C. M. Yang. 1967. Characterization of three bacteriophages of ***Xanthomonas oryzae***. Bot. Bul. Aca. Sinica **8**: 390-397.

Liao, Y. D. 1985. Control of Gene Transcription in Phage Xp10 Infected Cells. Ph D. thesis, National Taiwan University, Taipei, Republic of China, 152 pp.

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor. New York, 454 pp.

Merril, C. R., D. Goldman, S. A. Sedman, and M. H. Ebert. 1981. Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. Science **211**: 1437-1438.

Southern, E. 1979. Gel Electrophoresis of Restriction Fragments. ***In*** R. Wu (ed.), Methods in Enzymology, Vol. 68. Academic Press, New York, pp. 152-176.

Towle, H. C., J. F. Jolly, and J. A. Boezi. 1975. Purification and characterization of bacteriophage gh-1-induced DNA-dependent RNA polymerase from *Pseudomonas putida.* J. Biol. Chem. **250**: 1723-1733.

Yang, M. K. and T. T. Kuo. 1984. A physical map of the filamentous bacteriophage Cf genome. J. General Virol. **65**: 1173-1181.

細菌噬菌體 Xp10 基因體之物理特性

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Xp10 為水稻白葉枯病病原菌 (*Xanthomonas campestris* pv. *oryzae*) 之病毒性噬菌體。此噬菌體具有二十面體之頭部，直徑為 56.7±0.2 nm，而尾部長則為 107.0±0.2 nm。其外殼由八個蛋白質所組成，分子量分別為 258,000，150,000，147,000，128,000，90,000，46,000，43,800 及 24,200。Xp10 的基因體為直線形雙股的去氧核醣核酸，而兩端具有粘合性。基因體大小約 46.4 Kb。基因體分子上未發現有斷口。利用九種不同的核酸限制酶來剪切 Xp10 的去氧核醣核酸，在洋菜膠上分析其分布型式。以其中五種核酸限制酶，*Bam* HI, *Sma* I, *Sst* II, *Xba* I 及 *Xho* I，拼出 Xp10 基因體之核酸限制酶物理圖譜。