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Variation in the *Hin*dIII Restriction Fragments of DNA from the Chinese Tian Tan Strain of Vaccinia Virus

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SUMMARY

*Hin*dIII fragments D to P of DNA from a Chinese vaccine strain (Tian Tan) of vaccinia virus have been molecularly cloned into the plasmid pAT153 at the unique *Hin*dIII site. The Chinese strain DNA differs from a non-vaccine American strain (WR) in having an additional *Hin*dIII fragment (P). Twelve *Hin*dIII D clones and 12 *Hin*dIII F clones of the Chinese strain were analysed by digestion by *Eco*RI, *Bam*HI, *Pst*I and *Xho*I. Two forms of D (designated a and b) and of F (a and b) were demonstrated. In each the differences were detected as the presence of an additional *Eco*RI site, in Da and Fa. The *Hin*dIII Fa and Fb fragments of the Chinese strain were shown to differ significantly from the WR strain in their restriction site maps.

In recent years, interest has arisen in the use of the vaccinia virus genome as a eukaryotic expression vector for the construction of live recombinant vaccines directed against both human and veterinary infectious diseases or for the synthesis of biological products. Panicali & Paoletti (1982), Panicali *et al.* (1983) and Mackett *et al.* (1982) successfully adapted site-specific recombination between the *Hin*dIII F or J fragments of vaccinia virus DNA flanking the foreign DNA and homologous sequences in replicating viral DNA to allow insertion of the foreign DNA into infectious progeny. Biological activity of recombinant vaccinia virus expressing hepatitis B virus surface antigen (Smith *et al.*, 1983), influenza virus haemagglutinin (Panicali *et al.*, 1983) and herpes simplex virus thymidine kinase (Panicali & Paoletti, 1982) have been demonstrated. These authors, however, used the Western Reserve (WR) strain of vaccinia virus, which has not been used for human vaccination. The safety of the recombinant vaccinia viruses as human live vaccines awaits further investigation. In this paper we describe the cloning of the *Hin*dIII fragments D to P of the Chinese Tian Tan strain of vaccinia virus, the variation in the *Hin*dIII D and F fragments, and the distinct restriction map of the *Hin*dIII F fragment of the Tian Tan strain, as compared with that of the WR strain.

Tian Tan strain vaccinia virus was obtained from Dr Zheng-ren Cheng of the National Vaccine and Serum Institute. Stock virus was passaged in primary chick embryo fibroblasts. Virus DNA was purified from virus particles by the method of Mackett & Archard (1979). Digestion of DNA of the Chinese strain with *Hin*dIII (Boehringer) results in the formation of 16 fragments ranging in size from approximately 0·8 to 50 kb. The size of the whole virus DNA was estimated to be 190 kb. Except for the two end fragments (B and C) and an internal fragment (A), they were suitable for cloning in the unique *Hin*dIII site of pAT153 (Twigg & Sherratt, 1980). The cloning strategy was to 'shotgun' a *Hin*dIII digest of vaccinia virus DNA, and select ampicillin-resistant, tetracycline-sensitive colonies, which were probed with authentic virus DNA, ^{32}P-labelled by nick translation. Plasmid DNAs in positive colonies were isolated and analysed by agarose gel electrophoresis after *Hin*dIII digestion.

Further identification of recombinant plasmids was made by comparing their *Hin*dIII fragments with *Hin*dIII fragments of authentic vaccinia virus DNA in 0·7% agarose gel electrophoresis. Ethidium bromide-stained gels are shown in Fig. 1(*a*). In every case, the fragments from cloned DNA co-migrated with virion DNA fragments. Southern hybridization

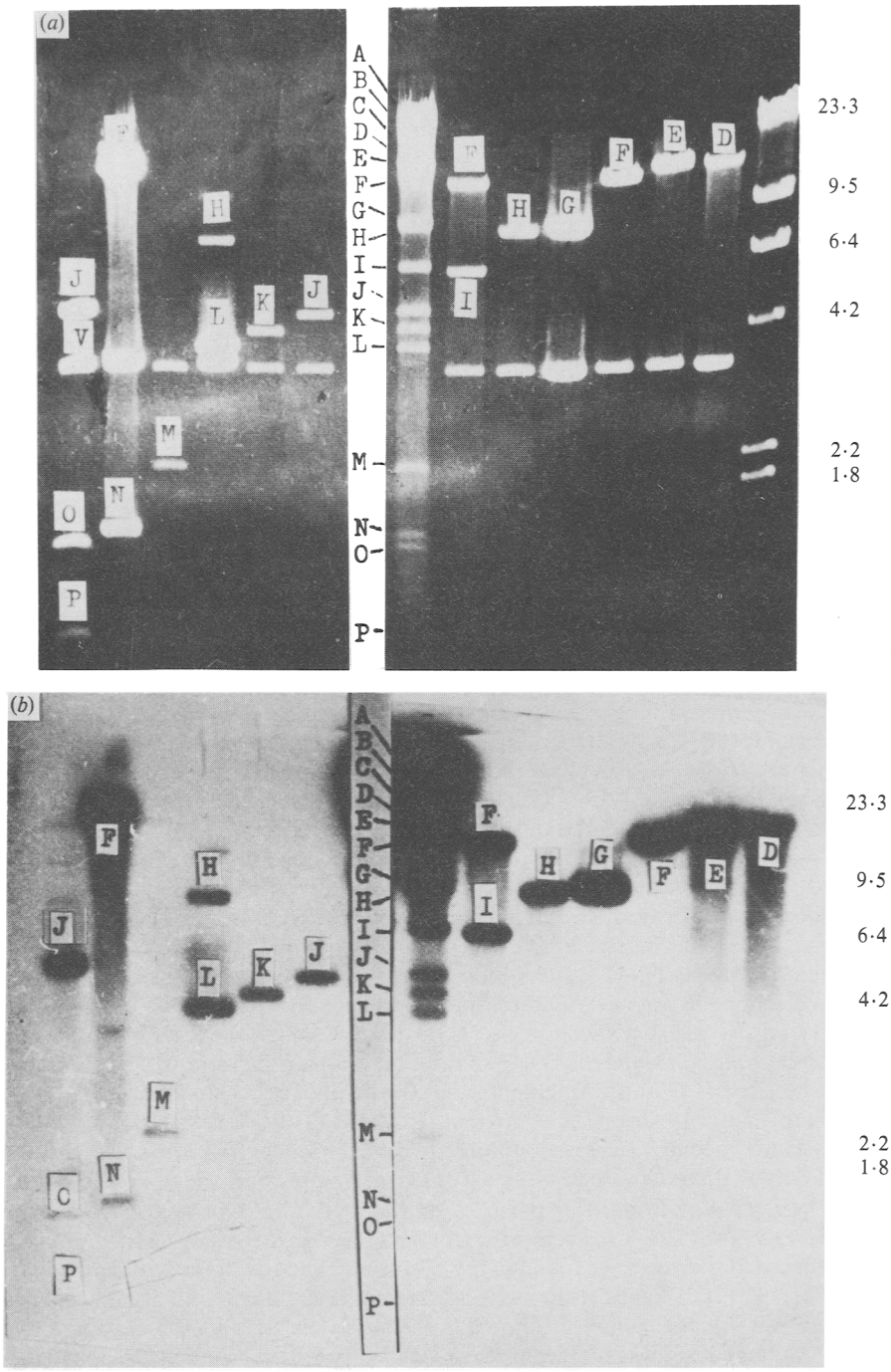

Fig. 1. (*a*) Recombinant plasmids containing *Hin*dIII restriction fragments from the Tian Tan strain of vaccinia virus were digested with *Hin*dIII. The digests were then analysed by electrophoresis on 0·7% agarose gels. Restricted vaccinia virus Tian Tan DNA is included in the centre lane as a reference, and each fragment is lettered. The position of the pAT153 vector (3·6 kb) on the gel is clear in each lane. The marker lane (λ DNA, *Hin*dIII-digested digests) is on the right (kb). (*b*) The same gel, Southern hybridized with ^{32}P-labelled authentic vaccinia virus DNA.

Table 1. *Main enzyme fragments within cloned* Hind*III D, E and F fragments of vaccinia virus Tian Tan strain genome*

*Hin*dIII fragment ...	Da	Db	E	Fa	Fb
Clone no.	17 528	57	5 915	5 559	441 778
	166 688	135	254 940	710 639	755 963
	440 840		396 758	721 775	854 1527
	473 890		575 1329		
	659 937		842		
Enzyme					
*Bam*HI	8·1*	8·1	7·5	7·6	7·6
	3·2	3·2	7·0	5·0	5·0
	2·8	2·8	0·7	0·6	0·6
	1·3	1·3			
*Eco*RI	5·4	5·4	3·2	6·9	6·9
	4·0	3·1	3·0	6·2	3·4
	2·4	2·4	2·0		2·8
	1·2	1·2	2·0		
	0·9	0·9	1·4		
	0·8	0·9	0·95		
	0·7	0·8	0·95		
		0·7	0·85		
			0·7		
*Pst*I	6·3	6·3	15·1	4·3	4·3
	5·1	5·1		3·5	3·5
	4·0	4·0		2·9	2·9
				2·4	2·4
*Xho*I	14·0	14·0	15·1	8·6	8·6
	1·4	1·4		4·5	4·5

* Size (kb) of restriction fragments of *Hin*dIII fragments in numbered clones cleaved by other enzymes.

of the same gels with ^{32}P-labelled virus DNA is shown in Fig. 1 (*b*). *Hin*dIII D, E, F, G, H, J, K and M were cloned separately. Some recombinants, however, contained multiple *Hin*dIII fragments of vaccinia virus DNA (*Hin*dIII F–I, H–L, F–N and J–O–P).

Each of the recombinant DNAs was subjected to double digestion with *Hin*dIII and at least one other restriction nuclease known to cut that segment of virion DNA. Thus, by three criteria (co-migration, hybridization and location of an additional site), the cloned DNA fragments were identical to restriction fragments obtained by cleavage of authentic vaccinia virus DNA.

In an attempt to study variation in several *Hin*dIII fragments of the Chinese strain, 12 D, 9 E and 12 F clones were digested with *Bam*HI, *Eco*RI, *Pst*I and *Xho*I. The main fragments resulting are shown in Table 1. It was demonstrated that there are two forms of *Hin*dIII D clone, designated Da and Db, which differ in that Da has an additional *Eco*RI site. *Hin*dIII F clones were also found in two forms, Fa and Fb, differing in that Fb has an additional *Eco*RI site. Da and Db, and separately Fa and Fb, were shown to be homologous by cross-hybridization.

Finally, differences between the *Hin*dIII F fragments of the Tian Tan and WR strains (the latter was obtained from W. K. Joklik) were examined. Restriction sites were mapped by a series of multiple digestions, or partial digestions of DNA fragments which had been 3′-labelled with [α-^{32}P]ddATP (Maniatis *et al.*, 1982). The 12 restriction maps obtained are shown in Fig. 2. Both WR and Tian Tan clones were in the same *Escherichia coli* strain (HB101). Compared with WR, the Tian Tan F clones are characterized by the possession of an extra *Bam*HI site, an extra *Xho*I site and an extra *Pst*I site, and by the absence of an *Eco*RI site (in Fa only) and a *Cla*I site.

The sizes of 13 *Hin*dIII fragments of the Tian Tan strain were found to be similar to those from the WR strain, with the exception of one fragment (P), which is not present in WR (Panicali *et al.*, 1981). The Copenhagen strain also lacks this fragment, but it is present in the Lister strain (Drillien & Spehner, 1983; Mackett & Archard, 1979). *Hin*dIII digests of the Lister strain appeared quite different from those of the Tian Tan strain on fractionation by electrophoresis in 0.7% agarose (Zheng-ren Chen, personal communication).

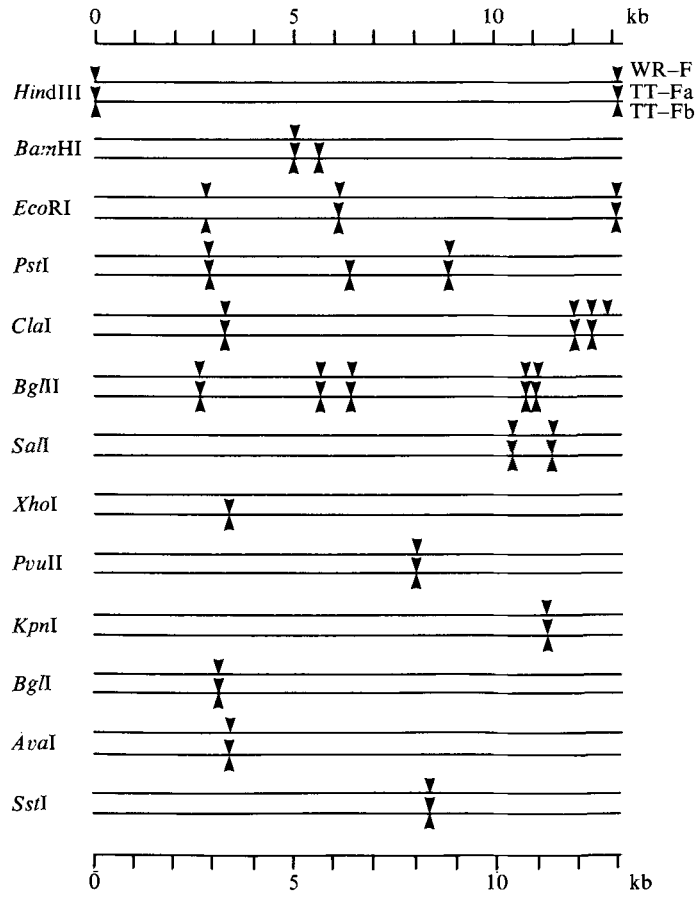

Fig. 2. Summary of the restriction sites mapped within *Hin*dIII F fragments from Tian Tan and WR strains of vaccinia virus. Restriction sites were mapped by a series of multiple digestions or by a partial digestion of DNA fragments, 3′ end-labelled with [α^{32}P]ddATP. WR-F, *Hin*dIII F fragment from the WR strain of vaccinia virus; TT-Fa and TT-Fb, *Hin*dIII Fa and Fb fragments from the Chinese Tian Tan strain of vaccinia virus.

The *Hin*dIII D fragment is located in the central region of the virus genome, which is considered to be highly conserved (Mackett & Archard, 1979; Drillien & Spehner, 1983). The occurrence of two forms of the *Hin*dIII D fragments in Tian Tan virus stocks suggests that some point mutations can occur in this conserved region.

The *Hin*dIII F fragment of the Chinese strain is significantly different from that of WR, judged by their restriction maps. It is worth noting that the variable *Bam*HI, *Eco*RI, *Pst*I and *Xho*I sites are located around the F promoter (Panicali *et al.*, 1983), and this should be taken into account when using this strain for construction of recombinant vaccinia virus strains.

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