Molecular Characterization of Important Regions of the Lumpy Skin Disease Virus Genome

by

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Preface

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Through Him
With Him
In Him

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In this study, almost 29 000 bp of the capripoxvirus lumpy skin disease virus genomic DNA were sequenced, analysed and compared with the sequences of other poxviruses. *PstI* clones -E, -M and -K represent 12 530 bp of the left hand terminal region, whereas *PstI* clones -F and -G represent 16 356 bp, from the central region of the genome. The terminal regions of poxvirus genomes are of particular interest and importance, as they contain the genes required for host range specificity and virulence, while the central regions encode the essential genes for viral replication and survival within the host. It was first necessary to sub-clone *PstI* clones into smaller fragments, after precise restriction mapping and the development of cloning strategies for each of the *PstI* clones. Forty-six non-overlapping subclones were consequently generated. The recombinant bacterial clones were sequenced in the forward and reverse directions with M13 vector primers, and edited by using Apple Macintosh Sequence Navigator™ sequence analysis software. To assemble the non-overlapping LSDV subclones, the edited DNA sequences

were first translated to amino acid sequences in six reading frames, after which open reading frames (ORFs) of at least 50 amino acids were identified visually, and blasted against the SwissProt protein database. Homologous poxvirus proteins were then used as templates to assemble the LSDV DNA fragments into continuous sequences. Oligonucleotides were designed from the DNA sequences to sequence the regions spanning the gaps between clones. Further analysis included the identification of specific protein motifs (SMART), and the identification of the regulatory promoter sequences. Thirty four LSDV ORFs are encoded in the sequenced regions, representing a diverse group of potential proteins involved in functions such as viral transcription and mRNA synthesis, nucleotide metabolism, protein modification, structural functions, cellular functions and most importantly, host-related functions. Additionally, one LSDV ORF without homology to any other poxvirus protein has been identified, suggesting that this protein is possibly involved in the limitation of LSDV to bovine cell infection.

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ABBREVIATIONS

A	-	adenine
Å	-	angstrom
A_{260}	-	absorbency at 260 nanometer
aa	-	amino acid
AD	-	Anno domini
AMP	-	ampicillin
ANK	-	ankyrin repeat motif
ATPase	-	adenosine triphosphatase
bp	-	base pair
°C	-	degrees Celcius
C	-	cytosine
$CaCl_2$	-	calcium chloride
CAM	-	chorioallantoic membrane
CAP	-	capripox virus
CHO	-	chinese hamster ovary
CIP	-	calf intestinal phosphatase
CPV	-	cowpox virus
dNTP	-	2'-deoxyribonucleoside-5'-triphosphate
ddNTP	-	dideoxynucleotide triphosphate
dH_20	-	distilled, sterilised water
DMSO	-	dimethyl sulfoxide
dNTP	-	deoxynucleotide triphosphate
DNA	-	deoxyribonucleic acid
ds	-	double stranded
DSPc	-	dual specificity phosphatase, catalytic domain
DTT	-	dithiothreitol

E. coli	-	*Escherichia coli*
EDTA	-	ethylene diamine tera-acetate
EGF	-	epidermal growth factor
EPG	-	electropherogram
EPV	-	entomopoxvirus
EtBr	-	ethidium bromide
EtOH	-	ethanol
Fig.	-	figure
FPV	-	fowlpox virus
FTP	-	file transfer protocol
G	-	guanine
GP	-	goatpox virus
h	-	hour
IG	-	immunoglobulin
IL	-	interleukin
IFN	-	interferon
ITR	-	inverted terminal repeat
kbp	-	kilobase pairs
KCl	-	potassium chloride
kD	-	kilodalton
LB	-	Luria-Bertani
LD_{50}	-	lethal dose 50
LSD	-	lumpy skin disease
LSDV	-	lumpy skin disease virus

M	-	molar
MCP	-	monocyte chemotactic protein
MCS	-	multiple cloning site
MCV	-	molluscum contagiosum virus
mM	-	millimolar
$MnCl_2$	-	manganese chloride
mRNA	-	messenger ribonucleic acid
MsEPV	-	melanopus sanguinipes entomopox virus
MYX	-	myxoma virus
µg	-	microgram
µl	-	microlitre
mg	-	milligram
MHC	-	major histocompatibility complex
min	-	minute
ml	-	millilitre
NaOAc	-	sodium acetate
NaOH	-	sodium hydroxide
ng	-	nanogram
nt	-	nucleotide
OD	-	optical density
OIE	-	Office International des Epizooties
ORF	-	open reading frame
OV	-	orf virus
OVI	-	Onderstepoort Veterinary Institute
p	-	plasmid
Pipes	-	Piperazine-N, N'- bis[2-ethane-sulfonic acid]
Ppi	-	inorganic pyrophosphate

PTPc	-	Protein tyrosine phosphatase, catalytic domain
RE	-	restriction endonuclease
RNA	-	ribonucleic acid
RNAse A	-	ribonuclease A
RPOL	-	ribonucleic acid polymerase
rpm	-	revolutions per minute
RVFV	-	rift valley fever virus
S	-	seconds
SFV	-	shope fibroma virus
SP	-	sheeppox virus
SPV	-	swinepox virus
str	-	strain
T	-	thymine
TBE	-	tris borate EDTA
TEMED	-	tetramethylethylenediamine
TK	-	thymidine kinase
TNF	-	tumor necrosis factor
U	-	unit
UNIX	-	uniplexed information and computing system
UV	-	ultraviolet
VAR	-	variola virus
VETF	-	viral early transcription factor
VGF	-	virus growth factor
VV	-	vaccinia virus