The Role of Transcription in Lactococcal Phage Replication

A Thesis Presented in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Microbiology

at Massey University, Palmerston North, New Zealand

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Abstract

The involvement of transcription in DNA replication has been demonstrated in mitochondria, bacteria, plasmids and phages. Previous studies indicated that transcription may also be required for the prolate-headed lactococcal phage c2 DNA replication in *L. lactis*.

In this study, the role of transcription in origin of DNA replication function was examined. A model system was used, in which the intergenic region of phage c2, presumably containing the origin of DNA replication, supported the replication of a plasmid in *Lactococcus lactis*. Within this region there is an active promoter that produces several non-coding transcripts. This allowed the importance of this early promoter 1 (P_E1) and the length and sequence (and presumably therefore secondary structure) of the P_E1 transcripts in replication to be investigated. It was demonstrated that a functional promoter (but not necessarily wildtype P_E1) and a specific length and sequence of the P_E1 transcripts are required for c2 origin function.

The transcription start site of the P_E1 transcripts in the plasmid system was determined by primer extension analysis and was identical to the transcription start site in the phage itself. The P_E1 transcripts made in the replicating plasmids were detected and quantified by Northern blots, and processing of the transcripts was shown by RNase protection analysis. However, no transcripts of the expected size were detected in the non-functional origins cloned in a plasmid able to replicate in *L. lactis*. Possible secondary structures of the wildtype and modified P_E1 transcripts were modelled using several different computer programs.

Lactococcal proteins were shown to bind to the P_E1 transcripts by RNA gel shifts and North-Western blots. Affinity purification and amino-terminal sequencing were used to identify one such protein with similarity to the ribosomal protein S1.

Growth curves of *L. lactis* containing the various replicating plasmids did not show any major differences in the ability of the *L. lactis* cells to grow. To characterize the plasmids further, the relative amount of plasmid DNA per lactococcal cell was determined. It was also demonstrated, by using a high copy number plasmid that harboured the c2 origin, that the c2 origin does not confer a Per phenotype.

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Abbreviations

All abbreviations and units used in this thesis and not specified in this list are standard SI-units.

aa	amino acid
ABI	Applied Biosystems
amp	ampicillin resistance gene
APS	ammonium persulphate
AT	content of deoxyadenylate and deoxythymidylate in DNA
ATP	adenosine triphosphate
BHI-A	brain heart infusion agar
BHI-B	brain heart infusion broth
BLAST	basic local alignment search tool
BSA	bovine serum albumin
CAPS	3-(cyclohexylamino)-1-propanesulfonic acid
cDNA	complementary DNA
cfu	colony forming units
cm	chloramphenicol resistance gene
cos	cohesive
cpm	counts per minute
CTP	cytidine triphosphate
DEPC	diethylpyrocarbonate
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxynucleoside triphosphate
ds	double-stranded
DTT	DL-dithiothreitol
ECL	enhanced chemiluminescence (Amersham)
EDTA	ethylenediaminetetraacetic acid
EMSA	electrophoretic migration shift assay
ery	erythromycin resistance gene

FP	forward primer
g	acceleration equivalent to the earth's gravity (9.806 ms^{-2})
G+C	content of deoxyguanylate and deoxycytidylate in DNA
HEPES	N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)
IPTG	isopropyl-β-D-thiogalactopyranoside
kan	kanamycin resistance gene
LB	Luria Bertani broth
LBA	Luria Bertani agar
MCS	multiple cloning site
MIC	minimum inhibitory concentration
MOPS	3-(N-morpholino) propanesulphonic acid
mRNA	messenger RNA
n/a	not applicable
OD	optical density at specified wavelength in nanometers
ON	overnight
ORF	open reading frame
ori	origin of replication
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
Per	phage encoded resistance
pfu	plaque forming units
PIPES	piperazine-N,N'-bis(2-ethanesulfonic acid)
Pos.	position
PVDF	polyvinylidene difluoride
RNA	ribonucleic acid
RNase	ribonuclease
RP	reverse primer
rpm	revolutions per minute
RT	room temperature
S.D.	standard deviation
SDS	sodium dodecyl sulphate
ss	single-stranded

SSC	saline-sodium citrate buffer
TBE	Tris borate EDTA buffer
TE	Tris EDTA buffer
TEMED	N,N,N',N' tetramethylethylenediamine
tet	tetracycline resistance gene
T_m	melting temperature
Tris	Tris(hydroxymethyl)methylamine
tRNA	transfer RNA
U	unit
UTP	uridine triphosphate
UV	ultraviolet (light)
vol	volume
w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside
ΔG	amount of energy required for or released by a reaction [Kcal/mol]
Ω	transcriptional and translational terminator derived from bacteriophage T4 gene

In addition, the conventional one-letter codes for amino acids, deoxyribonucleosides and ribonucleosides were applied:

amino acids: A, R, N, D, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y, V for alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine respectively.

deoxyribonucleosides: A, C, G, T for deoxyadenylate, deoxycytidylate, deoxyguanylate and deoxythymidylate respectively.

ribonucleosides: A, C, G, U for adenylate, cytidylate, guanylate and uridylate respectively.

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