



**UNIVERSITI PUTRA MALAYSIA**

**NUCLEOCAPSID (NP) AND PHOSPHO-(P) PROTEINS OF  
NEWCASTLE DISEASE VIRUS: IDENTIFICATION OF REGIONS  
ON NP THAT FORM PARTICLES AND INTERACT WITH P**

**KHO CHIEW LING**

**FSAS 2003 21**

**NUCLEOCAPSID (NP) AND PHOSPHO-(P) PROTEINS OF  
NEWCASTLE DISEASE VIRUS: IDENTIFICATION OF REGIONS  
ON NP THAT FORM PARTICLES AND INTERACT WITH P**

**KHO CHIEW LING**

**DOCTOR OF PHILOSOPHY  
UNIVERSITI PUTRA MALAYSIA**

**2003**



**NUCLEOCAPSID (NP) AND PHOSPHO-(P) PROTEINS OF NEWCASTLE  
DISEASE VIRUS: IDENTIFICATION OF REGIONS ON NP THAT FORM  
PARTICLES AND INTERACT WITH P**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfillment of the Requirements for the Degree of Doctor of Philosophy**

**May 2003**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**NUCLEOCAPSID (NP) AND PHOSPHO-(P) PROTEINS OF  
NEWCASTLE DISEASE VIRUS: IDENTIFICATION OF REGIONS  
ON NP THAT FORM PARTICLES AND INTERACT WITH P**

By

**KHO CHIEW LING**

**May 2003**

**Chairperson: Professor Datin Khatijah Yusoff, Ph.D.**

**Faculty: Science and Environmental Studies**

The nucleocapsid protein (NP) of Newcastle disease virus (NDV) plays an important role in the replication of the viral genomic RNA. The NP is closely associated with the viral phosphoprotein (P) and this association is crucial in ensuring the specific binding of NP to the viral RNA. In order to have a better understanding of the structure and functions of the NP, deletion mutagenesis was carried out to characterise and localise regions involved in NP-NP and NP-P interactions.

The NP and a fusion derivative (NP<sub>cfus</sub>) containing a hexa histidine tag at its C-terminus were produced abundantly in *Escherichia coli*. These proteins were fractionated on sucrose gradient centrifugation and microscopic analysis showed that both the NP and NP<sub>cfus</sub> proteins self-assembled predominantly into ring-like particles with the diameter of  $24 \pm 2$  nm around a central hole of  $7 \pm 1$  nm. Some of these ring-like particles stacked together to form herringbone-like particles which are heterogenous in length with a diameter of  $20 \pm 2$  nm

and a central hollow of  $5 \pm 1$  nm. Fusion of the C-terminal end to 29 amino acids inclusive of the *myc* epitope and His-tag did not impair ring assembly but inhibit the formation of the long herringbone particles. Immunogold labelling of the ring-like particles with the anti-*myc* antibody showed that the C-terminus of the NP<sub>cfus</sub> protein is exposed on the surface of the particles.

The essential subunit of NDV nucleocapsid is the NP, a polypeptide with 489 amino acids. In order to identify the contiguous sequence on NP that self-assembles into ring- or herringbone-like particles, a total of 11 N- or C-terminally deleted NP mutants were constructed and self-assembly studied in *E. coli* showed that a large part of the N-terminus of the NP encompassing amino acids 1 to 375, was required for the formation of herringbone-like particle. In contrast, the C-terminal end covering amino acids 376 to 489 was dispensable for the formation of this particle. Nevertheless, a region located between amino acids 376 to 439 may play a role in regulating the length of the herringbone-like particle.

As NP and some of its mutants assemble into particles, this feature was exploited to carry or display foreign peptides. Hepatitis B virus core antigen (HBcAg) and the C-terminal fragment of the N protein of Nipah virus were separately fused to a truncated NP protein, NP<sub>ΔC391</sub>, and expressed in *E. coli*. Antigenicity analysis of the chimeric proteins by using ELISA showed that the foreign peptide was at least partially exposed on the surface of the chimeric protein particles.

An *in vitro* binding assay was established to identify the regions on NP that interact with the P. A highly interactive region was located at the first 26 amino acids of the N-terminus of NP. The interaction between these two proteins remained strong even with the removal of 114 amino acids from the C-terminal end of NP. It is most likely that the last 49 amino acids of the NP might form another contact region for P, but is not as important as the N-terminal end.

As a whole, this study has provided a valuable insight into the structure of the NP as well as the delineation of its key functional regions. This knowledge will be useful for detailed exploration of the NP protein.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PROTEIN NUKLEOKAPSID (NP) DAN FOSFO (P) PENYAKIT  
SAMPAR AYAM: PENENTUAN BAHAGIAN NP YANG  
MEMBENTUK PARTIKEL DAN BERINTERAKSI DENGAN P**

Oleh

**KHO CHIEW LING**

**Mei 2003**

**Pengerusi: Profesor Datin Khatijah Yusoff, Ph.D.**

**Fakulti: Sains dan Pengajian Alam Sekitar**

Protein nukleokapsid (NP) virus penyakit Sampar Ayam (NDV) memainkan peranan penting dalam replikasi RNA genomik virus. Protein NP berkait rapat dengan fosfoprotein (P) virus dan penyatuan ini adalah amat penting untuk memastikan protein NP hanya berikat secara spesifik kepada RNA virus. Untuk memahami struktur dan fungsi NP dengan lebih terperinci, mutagenesis potongan telah dijalankan dan mengenal pasti bahagian yang terlibat dalam interaksi NP-NP dan NP-P.

NP dan satu terbitan gabungan ( $NP_{cfus}$ ) yang mengandungi tag enam histidin pada terminal C telah dihasilkan dengan banyak dalam *Escherichia coli*. Protein tersebut telah diasingkan dengan pengemparan kecerunan sukrosa. Analisis mikroskopik menunjukkan kedua-dua protein NP dan  $NP_{cfus}$  gabung sendiri membentuk partikel seperti cincin dengan diameter luaran berukuran  $24 \pm 2$  nm mengelilingi satu lubang tengah yang berdiameter  $7 \pm 1$  nm. Seseengah partikel berbentuk cincin ini bertimbun dan membentuk

struktur menyerupai “herringbone” yang mempunyai saiz yang berlainan dengan diameter luaran berukuran  $20 \pm 2$  nm dan lubang tengah yang berdiameter  $5 \pm 1$  nm. Pencantuman 29 asid amino termasuk epitop *myc* dan tag-histidin pada terminal C protein NP tidak mengganggu pembentukan struktur cincin tetapi menghalang pembentukan struktur panjang “herringbone”. Pelabelan imuno-emas partikel cincin dengan antibodi anti-*myc* menunjukkan terminal C protein NP<sub>cfus</sub> adalah terdedah di permukaan partikel cincin ini.

Subunit utama nukleokapsid NDV adalah NP, polipeptida yang mengandungi 489 asid amino. Untuk menentukan turutan asid amino protein NP yang diperlukan bagi pembentukan struktur cincin dan “herringbone”, sejumlah 11 mutan NP yang samada terminal N atau C-nya disingkirkan telah dibina dan kajian penyatuan dalam *E. coli* menunjukkan sebahagian besar terminal N protein NP yang merangkumi asid amino dari 1 hingga 375 adalah diperlukan bagi pembentukan struktur “herringbone”. Sebaliknya, bahagian terminal C protein NP meliputi asid amino dari 376 ke 489 tidak diperlukan bagi pembentukan struktur ini. Namun begitu, kawasan yang terletak di antara asid amino 376 dan 439 mungkin memainkan peranan dalam mengawal saiz struktur “herringbone”.

Oleh sebab NP dan sesetengah mutannya membentuk partikel, ciri ini telah dieksploit untuk membawa atau mempamerkan peptida asing. Antigen teras virus hepatitis B (HBcAg) dan fragmen terminal C daripada N protein



virus Nipah telah digabungkan kepada terminal C protein NP terpotong, NP<sub>ΔC391</sub> dan diekspres dalam *E. coli*. Analisis keantigenan protein kacukan dengan menggunakan teknik ELISA menunjukkan sebahagian peptida asing terdedah pada permukaan partikel kacukan.

Satu asai pengikatan protein *in vitro* telah digunakan untuk mengenalpasti bahagian protein NP yang berinteraksi dengan protein P. Satu bahagian NP protein yang berinteraksi kuat dengan protein P telah dikenalpasti terletak pada permulaan 26 asid amino dari terminal N protein NP. Interaksi di antara kedua-dua protein ini tetap tinggi walaupun sejumlah 114 asid amino dari terminal C protein NP telah disingkirkan. Berkemungkinan besar 49 asid amino pada terminal C protein NP membentuk satu kawasan yang berinteraksi dengan P, tetapi ia tidak sepenting terminal N protein NP.

Secara keseluruhan, kajian ini telah membekalkan maklumat terperinci mengenai struktur NP dan juga bahagian berfungsi pada protein tersebut. Pengetahuan ini adalah amat berguna untuk mengkaji protein NP dengan lebih mendalam pada masa yang akan datang.

## ACKNOWLEDGEMENTS

Indeed there are many wonderful people who have contributed significantly throughout the whole course of my study up to the completion of my thesis writing. I owe a great deal to them.

First and foremost, I wish to express my most sincere acknowledgement to my supervisors: **Prof. Datin Dr. Khatijah Yusoff**, for her valuable guidance, generosity and freedom throughout the entire research and thesis writing; **Assoc. Prof. Dr. Tan Wen Siang**, a full of passion scientist, for his endless encouragement, thoughtful comments and helpful discussions; **Dr. Tey Beng Ti**, for his trust and helpful suggestions.

I would like to convey my sincere thanks to Chui Fong, Kok Lian, Sing King, Amir and Pria for their advice, suggestions and moral support whenever I faced problems in the experiments. I must also express my deepest thanks to Swee Tin and Eddie Chia for their invaluable help and accompany me in the later stage of my experiments. I thank Dr. Majid Eshagi for his useful comments and discussions in the study of protein interactions. Not forgetting, to all the colleagues in the Virology Laboratory of the Department of Biochemistry and Microbiology, especially Thong Chuan, Rafidah, Geok Hun, Lalita and Suhana, I wish to thank them for their kindness and making my time in the laboratory an enjoyable one.

I am grateful to staff of the Electron Microscopic Unit of Universiti Putra Malaysia, especially Mr. Ho and Ms Azila, for their help in the early stage of protein microscopy analysis. Many thanks also to Mr. Ragnathan from the Electron Microscopic Unit of University Malaya, who has patiently checked through many of my carbon coated grids.

I wish to express my deepest appreciation to my parent, brothers and sisters for their unconditional love and support.

Finally, I would like to thank the Ministry of Science, Technology and Environment of Malaysia for providing me the National Science Fellowship.



I certify that an Examination Committee met on 13<sup>th</sup> of May 2003 to conduct the final examination of Kho Chiew Ling on her Doctor of Philosophy thesis entitled "Nucleocapsid (NP) and Phospho-(P) Proteins of Newcastle Disease Virus: Identification of Regions on NP that form Particles and Interact with P " in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**JANNA ONG ABDULLAH, Ph.D.**

Lecturer  
Department of Biochemistry and Microbiology  
Faculty of Science and Environmental Studies  
Universiti Putra Malaysia  
(Chairperson)

**KHATIJA YUSOFF, Ph.D.**

Professor  
Department of Biochemistry and Microbiology  
Faculty of Science and Environmental Studies  
Universiti Putra Malaysia  
(Member)

**TAN WEN SIANG, Ph.D.**

Associate Professor  
Department of Biochemistry and Microbiology  
Faculty of Science and Environmental Studies  
Universiti Putra Malaysia  
(Member)

**TEY BENG TI, Ph.D.**

Lecturer  
Department of Chemical and Environmental Engineering  
Faculty of Engineering  
Universiti Putra Malaysia  
(Member)

**MARY JANE CARDOSA, Ph.D.**

Director  
Institute of Health and Community Medicine  
Universiti Sarawak Malaysia  
(Independent Examiner)



---

**GULAM RUSUL RAHMAT ALI, Ph.D.**  
Professor / Deputy Dean,  
School of Graduate Studies,  
Universiti Putra Malaysia.

Date: 19 JUN 2003

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

**KHATIJA YUSOFF, Ph.D.**

Professor  
Department of Biochemistry and Microbiology  
Faculty of Science and Environmental Studies  
Universiti Putra Malaysia  
(Chairperson)

**TAN WEN SIANG, Ph.D.**

Associate Professor  
Department of Biochemistry and Microbiology  
Faculty of Science and Environmental Studies  
Universiti Putra Malaysia  
(Member)

**TEY BENG TI, Ph.D.**

Lecturer  
Department of Chemical and Environmental Engineering  
Faculty of Engineering  
Universiti Putra Malaysia  
(Member)



---

**AINI IDERIS, Ph.D.**  
Professor / Dean,  
School of Graduate Studies,  
Universiti Putra Malaysia.

Date: 11 JUL 2003

## DECLARATION

I hereby declare that the thesis is based on my original work except for equations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

  
\_\_\_\_\_  
(KHO CHIEW LING)

Date: 30/5/2003

## TABLE OF CONTENTS

	<b>Page</b>
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xx
 <b>CHAPTER</b>	
1. INTRODUCTION	1
2. LITERATURE REVIEW	5
2.1 Newcastle Disease Virus	5
2.1.1 Virus Classification	6
2.1.2 Virion Structure	6
2.1.3 Viral Genome	10
2.2 Protein in Transcriptase Complex	12
2.2.1 Nucleocapsid Protein (NP)	12
2.2.2 Phosphoprotein (P)	16
2.2.3 Large Polymerase Protein (L)	17
2.3 Virus Replication	18
2.4 Study of Protein Interaction	19
2.4.1 <i>In vivo</i> Approach	20
2.4.2 <i>In vitro</i> Approach	23
2.5 Mutagenesis Approach for Domain Mapping	24
2.5.1 Classical Approach	24
2.5.2 Deletion Mutagenesis	25
2.6 Protein Carriers	26
3. MATERIALS AND METHODS	28
3.1 Preparation of Virus	28
3.1.1 Source of Virus	28
3.1.2 Virus Propagation and Harvesting	28
3.1.3 Virus Purification	29
3.2 Genomic RNA Extraction	30
3.3 Construction of Recombinant Plasmids	31
3.3.1 RT-PCR	31
3.3.2 Purification of Amplified PCR Products	33
3.3.3 Preparation of Competent <i>E. coli</i> Cells	34
3.3.4 TOPO TA Cloning	34

3.3.5	Transformation	35
3.4	Identification of Recombinant Plasmid	35
3.4.1	Plasmid Extraction	35
3.4.2	PCR	37
3.4.3	Sequencing	37
3.5	Co-transformation of NP and P Genes	38
3.6	Construction of NP Deletion Mutants	38
3.7	Construction of NP Chimeric Clones	42
3.7.1	Amplification of tHBcAg and Nipah-n cDNA Fragments	42
3.7.2	Directional Ligation	43
3.8	Protein Expression and Analysis	44
3.8.1	Protein Expression	44
3.8.2	SDS-PAGE	44
3.8.3	Western Blotting	46
3.8.4	Large Scale Production of Recombinant Proteins	48
3.8.5	Sucrose Gradient Centrifugation	49
3.8.6	The Bradford Assay	49
3.8.7	Nucleic Acid Extraction from Herringbone-like Particles	50
3.9	Electron Microscopic Analysis	51
3.9.1	Negative Staining	51
3.9.2	Immunogold Labelling	51
3.10	Virus Disruption Assay	52
3.11	Determination of the Antigenicity of Chimeric Proteins by Direct ELISA	52
3.12	<i>In vitro</i> Transcription and Translation	53
3.12.1	Plasmid Preparation	53
3.12.2	<i>In vitro</i> Transcription	53
3.12.3	<i>In vitro</i> Translation	54
3.13	Development of NP-P Protein Binding Assay	54
3.13.1	pH Optimisation	55
3.13.2	Determination of the Optimum Amount of NP Protein Required for Coating on a Well	56
3.13.3	Time Course Experiment	56
3.13.4	Interaction of NP Mutants and P Proteins	57
4.	RESULTS	58
4.1	Production of Recombinant Proteins	58
4.1.1	RT-PCR and Construction of Recombinant Plasmids	58
4.1.2	Nucleotide Sequence Analysis of NP and P Genes	61
4.1.3	Expression of Recombinant Proteins	67
4.2	Characterisation of NP Protein	72
4.2.1	Purification of NP Protein	72
4.2.2	NP Self-assembles into Ring- and Herringbone-like Particles	72



4.2.3	C-terminal end of NP is Exposed Outside the Ring-like Particles	76
4.2.4	Reducing and Non-Reducing SDS-PAGE	77
4.3	Localisation of NP-NP Interaction Regions	80
4.3.1	Expression of N- and C-terminal NP Deletion Mutants	80
4.3.2	Protein Purification of NP Deletion Mutants	84
4.3.3	Self-assembly Domain of NP Protein	93
4.3.4	Herringbone-like Particles Encapsidate RNA	95
4.3.5	Fusion Effect on the Structure of Long Herringbone-like Particles	100
4.3.6	Construction and Production of NP Chimeric Proteins	102
4.3.7	TEM Analysis of Chimeric Proteins	106
4.3.8	Antigenicity Analysis	110
4.4	Interaction of NP and P Proteins	112
4.4.1	Co-expression of NP-P <sub>cfus</sub> Proteins	112
4.4.2	P Protein Co-migrates with NP Protein	114
4.4.3	TEM Analysis of NP-P <sub>cfus</sub> Protein Complex	116
4.4.4	<i>In vitro</i> Transcription and Translation of P Protein	118
4.4.5	Optimisation of Protein Binding Assay	118
4.4.6	Regions of NP that Interact with P	120
5.	DISCUSSION	126
5.1	Production and Characterisation of Nucleocapsid Protein	126
5.2	Identification of Nucleocapsid Protein Self Assembly Region	130
5.3	Evaluation of a Truncated NP Mutant as a Potential Carrier Molecule	135
5.4	Production of P Protein and its Effect on NP Self Assembly	138
5.5	Localisation of NP-P Protein Interaction Region	142
6.	CONCLUSION AND FUTURE PROSPECTS	147
	REFERENCES	150
	BIODATA OF THE AUTHOR	161
	PUBLICATIONS	162

**LIST OF TABLES**

<b>Table</b>		<b>Page</b>
1	Oligonucleotides Used in Amplification of NP and P Coding Regions	32
2	Oligonucleotides Used in the Sequencing of the NP Coding Region	39
3	Oligonucleotides Used in the Sequencing of the P Coding Region	39
4	Oligonucleotides Used in Generating the NP Deletion Mutants	41
5	Truncated NP Proteins and Their Respective Structural Features	97

## LIST OF FIGURES

Figure		Page
1	Electron Micrographs of NDV Particles Purified from Allantoic Fluid	7
2	Schematic Diagram of a Virion Structure of NDV	8
3	A Schematic Representation of the NDV Genome	11
4	Agarose Gel Electrophoresis (1.5% v/w) of RT-PCR Products of the Coding Regions of NP and P Genes	59
5	Determination of the Orientation of the NP and P Coding Regions	60
6	Nucleotide and Deduced Amino Acid Sequences of the P Coding Region of NDV Strain AF2240	62
7	Nucleotide and Deduced Amino Acid Sequences of the NP Coding Region of NDV Strain AF2240	63
8	Four Highly Conserved Segments of NP Proteins of <i>Paramyxoviridae</i>	66
9	SDS-12% Polyacrylamide Gel Analysis and Coomassie Blue Staining of the Expressed NP and NP <sub>cfus</sub> Proteins	68
10	Western Blot Analysis of the Expressed NP and NP <sub>cfus</sub> Proteins	69
11	Western Blot Analysis of the Expressed P and its Derivative P <sub>cfus</sub> Proteins	71
12	SDS-12% PAGE and Coomassie Blue Staining of Ammonium Sulphate Precipitated NP and NP <sub>cfus</sub> Proteins	73
13	Protein Sedimentation Profiles of NP and NP <sub>cfus</sub> Proteins	74
14	Electron Micrographs of the Purified NDV Recombinant NP and NP <sub>cfus</sub> Proteins that form Ring- and Herringbone-like Particles	75
15	Immunogold Labelling Analysis of NP <sub>cfus</sub>	78
16	Analysis of NP Protein under Reducing and Non-reducing Conditions	79

17	Schematic Representation of the Deletion mutants of NP Protein	81
18	Agarose Gel Electrophoresis (1.5% v/w) of NP Gene Deletion cDNA Fragments	82
19	Western Blot Analysis of Truncated NP Proteins	83
20	Ammonium Sulphate Precipitation of Truncated NP Proteins	85
21	Western Blot Analysis of Ammonium Sulphate Precipitated Truncated Protein NP <sub>ΔC366</sub>	86
22	Protein Sedimentation Profiles of NP <sub>ΔC464</sub> and NP <sub>ΔC440</sub> Proteins	88
23	Protein Sedimentation Profiles and SDS-12% PAGE of NP <sub>ΔC405</sub> and NP <sub>ΔC391</sub> Proteins	89
24	Protein Sedimentation Profiles and SDS-12% PAGE of NP <sub>ΔC380</sub> and NP <sub>ΔC375</sub> Proteins	90
25	Protein Sedimentation Profiles and SDS-12% PAGE of NP <sub>ΔN26</sub> and NP <sub>ΔN122</sub> Proteins	91
26	Protein Sedimentation Profiles and SDS-12% PAGE of NP <sub>ΔC245</sub>	92
27	Electron Micrographs of C-terminal Truncated NP Proteins that form Herringbone-like Particles	94
28	Electron Micrographs of Negative Stained (2% uranyl acetate) N-terminal Truncated NP Proteins that were unable to form Herringbone-like Particles	96
29	Agarose Gel (1%) Electrophoresis of Nucleic Acid Extracted from Ring- and Herringbone-like Particles	99
30	Protein Sedimentation Profile of NP <sub>ΔC391fus</sub> Protein	101
31	Electron Micrograph of 2% Uranyl Acetate Stained NP <sub>ΔC391fus</sub> Protein	103
32	Agarose Gel (1.5% w/v) Electrophoresis of PCR Amplified Truncated Core Antigen and Partial N Gene of Nipah Virus (Nph-n)	103
33	A Schematic Representation of the NP Chimeric Proteins (NP <sub>ΔHBcAg</sub> and NP <sub>ΔNph-fus</sub> )	105

34	Western Blot Analysis of Chimeric Proteins	105
35	Protein Sedimentation Profile of NP <sub>ΔHBcAg</sub> Chimeric Protein	107
36	Protein Sedimentation Profile of NP <sub>ΔNph-fus</sub> Chimeric Protein	108
37	Electron Micrographs of Chimeric Proteins	109
38	Antigenicity Analysis of the Inserted Peptides in Chimeric Proteins	111
39	Western Blot Analysis of Co-expressed NP and P <sub>cfus</sub> Proteins	113
40	SDS-PAGE and Immunoblotting Analysis of Co-expressed NP-P Protein Complex	115
41	Protein Sedimentation Profiles of NP Protein and NP-P <sub>cfus</sub> Protein Complex	117
42	Electron Microscopic Analysis of NP-P <sub>cfus</sub> Protein Complex	117
43	<i>In vitro</i> Translation Product of P Gene	119
44	Identification of Optimal pH for Binding	121
45	Determination of NP Concentration Required to Saturate the Solid Phase	122
46	Time Course Experiment of [ <sup>35</sup> S]-methionine-P Binding to NP-coated Wells	123
47	Localisation of NP Region Required for the Association with P <i>In vitro</i>	125

**LIST OF ABBREVIATIONS**

ATP	adenosine triphosphate
BCP	1-bromo-3-chloro-propane
BCIP	5-bromo-4-chloro-3-indolyl phosphate
bp	base pair
BSA	bovine serum albumin
cDNA	complementary deoxyribonucleic acid
Ci	curie
CITE	cap-independent translation enhancer
cpm	counts per minute
C-terminus	carboxy terminus
DEPC	diethylpyrocarbonate
DNA	deoxy-ribonucleic acid
DNase	deoxyribonuclease
DTT	1, 4-dithiothreitol
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
h	hour
HBcAg	hepatitis B core antigen
IPTG	isopropyl- $\beta$ -D-thiogalactopyranoside
kb	kilobase
kDa	kilodalton
L	large protein

LB	Luria Bertani
$\mu\text{g}$	microgram
$\mu\text{l}$	microlitre
$\mu\text{M}$	micromolar
M	molar
mA	milliampere
min	minute
$M_r$	relative molecular mass
mRNA	messenger RNA
NBT	nitro blue tetrazolium
NDV	Newcastle disease virus
NEP-gel	sodium-phosphate-EDTA-gelatin buffer
ng	nanogram
nm	nanometre
NP	nucleocapsid protein
NP-40	nonidet P40
nt	nucleotide
N-terminus	amino terminus
OD	optical density
ORF	open reading frame
P	phosphoprotein
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline

pH	<i>Puissance hydrogene</i>
PCR	polymerase chain reaction
RBC	red blood cell
RNA	ribonucleic acid
RNase	ribonuclease
rNTP	ribonucleoside triphosphate
rpm	revolutions per minute
RT-PCR	reverse transcriptase-polymerase chain reaction
s	second
SDS	sodium dodecyl sulphate
SV5	simian virus 5
TAE	Tris-acetate-EDTA buffer
TBS	Tris-buffered saline
TEMED	tetramethyl ethylenediamine
U	unit
UV	ultraviolet
VLP	virus-like particle
vol	volume
VSV	vesicular stomatitis virus
w/v	weight/volume



## CHAPTER 1

### INTRODUCTION

Protein-protein interactions are involved in essentially all cellular processes. Many major research topics in biology such as DNA replication, transcription, translation, protein trafficking, cell cycle control, signal transduction, and intermediary metabolism are cellular events in which protein complexes have been implicated as essential components (Phizicky & Fields, 1995). Therefore, a comprehensive understanding of protein-protein interactions as well as the elucidation of their functional domains has become one of the major goals of modern biology. With the advent of recombinant DNA technology, the study of protein-protein interactions and subsequently the localisation of protein interaction domains have become more feasible than ever. Now, it is possible to clone virtually any genes of interest, and translate them into proteins by inserting the genes into appropriate expression systems. Using the approach of site-directed mutagenesis, the nucleotide sequence of a gene can then be modified to make any conceivable variant of the original protein. These techniques have launched the new technology of protein engineering, which has had a major impact on the study of protein structure, function and stability (Creighton, 1993).

Like many other negative-sensed RNA viruses, the RNA genome of Newcastle disease virus (NDV) encodes a core of three polypeptides common

