



UNIVERSITI PUTRA MALAYSIA

**MAPPING OF THE FUNCTIONAL DOMAINS OF THE NEWCASTLE
DISEASE VIRUS PHOSPHOPROTEIN (P)**

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By

FATEMEH JAHANSHIRI

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**MAPPING OF THE FUNCTIONAL DOMAINS OF THE NEWCASTLE
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January 2006

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The phosphoprotein (P) of the Newcastle disease virus (NDV) plays a central role in the transcription and the replication of the viral genome. Together with the large (L) protein, it forms and stabilizes the active RNA-dependent RNA polymerase complex. It also mediates the interaction between the L protein subunit of this complex with the viral nucleocapsid (NP:RNA) template comprising a nucleocapsid tightly bound RNA genome. The association of P and the unassembled nucleocapsid protein (NP^o) is crucial in ensuring the specific binding of NP to the RNA genome. Although much is known about the interactions between the proteins of the transcriptive complex in the Sendai and vesicular stomatitis viruses, very little work has been done on the transcriptive complex of NDV. Therefore in this study, the yeast two-hybrid system was employed to identify the domains of P protein involved in interaction with itself, NP^o and L proteins. The interaction domain of P with NP:RNA was also localized.

To identify the P protein self-association domain, a total of 11 mutants with successive deletions from either the N- or C-terminal of the *P* gene were generated. Full length P protein (cloned in fusion with the binding domain of the transcriptional activator, BD) as well as each deletion mutant (cloned in fusion with the activation

domain, AD) were co-transformed into the yeast *Saccharomyces cerevisiae* strain L40, separately. To analyze positive interactions, β -galactosidase (β -gal) assay was performed. A high level of β -gal activity was detected in the interaction of P with itself, indicating the ability of NDV P protein to oligomerize. The self-association domain was mapped to the C-terminal half of the protein between amino acid residues 247 to 291.

To map the domains of P involved in the interaction with NP^o, the P protein and its derivatives along with full length NP were co-transformed into the yeast. A strong β -gal activity was observed in the interaction of full length P and NP, indicating a specific interaction between P and NP proteins. Surprisingly, the same region in the C-terminal of P which is important for self-association (residues 247-291) was shown to be involved in the interaction with NP^o as well.

In order to map the regions of L that interact with P, the six functional domains of L, along with full length P were co-transformed into the yeast. However, due to the low expression level of the domains, no β -gal activity was detected in the interactions of the domains with P. Therefore, an *in vitro* co-translation approach was employed to identify P:L interaction domain. The results showed a region located in the C-terminal half of P (amino acids 292-338), just beside the P protein oligomerization domain, is involved in the stabilization of L protein.

Co-immunoprecipitation was performed to investigate the regions of P that interact with NP:RNA template. Purified NP:RNA template along with *in vitro* translated P or its derivatives were separately co-immunoprecipitated using anti-His antibody.

The results showed that the immediate N-terminal of P protein is involved in its interaction with NP:RNA template.

In summary, the current study had delineated the key functional regions of the P. This knowledge will be useful for structural and functional exploration of the NDV P protein.



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

**PEMETAAN DOMAIN-DOMAIN BERFUNGSI PROTEIN FOSFO (P) VIRUS
PENYAKIT NEWCASTLE (NDV)**

Oleh

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Protein fosfo (P) virus penyakit Newcastle (NDV) memainkan peranan utama dalam transkripsi dan replikasi genom virus. Protein ini bersama-sama dengan protein besar (L), membentuk dan menstabilkan kompleks aktif polymerase RNA yang bergantung kepada RNA. Protein P juga membantu dalam interaksi di antara subunit protein L dalam kompleks ini dengan templat nukleokapsid virus (NP:RNA) yang mengandungi nukleokapsid yang bergabung kuat dengan genom RNA. Penggabungan protein P dengan protein nukleokapsid yang tidak berhimpun (NP^o) adalah penting dalam menentukan pelekatan spesifik NP kepada genom RNA. Setakat ini, interaksi di antara protein-protein di dalam kompleks transkriptif virus-virus sendai dan 'vesicular stomatitis' telah banyak diketahui dan bukannya bagi kompleks transkriptif NDV. Oleh itu dalam kajian ini, system dwi-hybrid yis telah digunakan untuk menentukan domain-domain yang terdapat pada protein P yang terlibat dalam interaksi dengan sendiri, protein nukleokapsid dan protein L. Domain yang terlibat dalam interaksi P dengan NP:RNA telah ditempatkan.

Untuk menentukan domain penggabungan sendiri protein P, 11 mutan (Bahagian 3.1.2) dengan pemotongan bersiri dari terminal-N atau C gen P telah dihasilkan. Protein P yang penuh (diklon bersama dengan domain pelekatan pengaktif transkripsi, BD) berserta dengan setiap mutan terpotong (diklon bersama dengan domain pengaktif, AD) telah diko-transformasikan ke dalam *Saccharomyces cerevisiae* strain L40 secara berasingan. Untuk menganalisa interaksi positif, pencerakinan β -galactosidase (β -gal) telah dijalankan. Satu tahap aktiviti β -gal yang tinggi telah dikesan dalam interaksi di antara protein P dengan sendiri, menandakan kebolehan protein P NDV untuk mengoligomer. Domain penggabungan sendiri telah dipetakan kepada terminal C protein ini di antara residu asid amino 247 hingga 291.

Untuk memetakan domain-domain pada protein P yang terlibat dalam interaksi dengan NP, protein P dan terbitannya bersama dengan NP yang penuh telah diko-transformasikan ke dalam yis. Satu aktiviti β -gal yang tinggi telah diperhatikan dalam interaksi di antara P dan NP yang penuh, menandakan interaksi spesifik antara protein-protein P dan NP. Bahagian yang sama pada terminal C protein P yang penting dalam penggabungan sendiri (residu 247-291) turut terlibat dalam interaksi dengan NP.

Bagi memetakan bahagian-bahagian pada protein L yang berinteraksi dengan P, keenam-enam domain berfungsi L, bersama-sama dengan protein P yang penuh telah diko-transformasi ke dalam yis. Walau bagaimanapun, tiada aktiviti β -gal dikesan dalam interaksi antara domain-domain tersebut dengan protein P, kesan daripada tahap ekspresi domain yang rendah. Oleh itu, suatu pendekatan ko-translasi *in vitro* telah digunakan untuk menentukan domain interaksi P:L. Keputusan menunjukkan

bahawa satu bahagian pada terminal C protein P (asid amino 292-338), bersebelahan dengan domain oligomerisasi protein P, terlibat dalam penstabilan protein L.

Ko-immunopresipitasi telah dijalankan untuk mengkaji bahagian-bahagian protein P yang berinteraksi dengan templat NP:RNA. NP:RNA yang tulen bersama dengan protein P atau terbitannya yang ditranslasi secara *in vitro* telah diko-immunopresipitasi secara berasingan menggunakan antibody anti-His. Keputusan menunjukkan bahawa terminal N yang langsung pada protein P terlibat dalam interaksinya dengan templat NP:RNA.

Kesimpulannya, kajian semasa ini telah menggariskan bahagian-bahagian berfungsi yang penting pada protein P. Pengetahuan ini bakal berguna untuk peninjauan struktur dan fungsi protein P NDV.

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I certify that an Examination Committee met on 12th January 2006 to conduct the final examination of Fatemeh Jahanhsiri on her Doctor of Philosophy thesis entitled “Mapping of the Functional Domains of the Newcastle Disease Virus Phosphoprotein (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:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations 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.

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