Jpn. J. Vet. Res., **39**, 167–177 (1991)

CHARACTERIZATION OF DNA FRAGMENT FROM *CHLAMYDIA PSITTACI* AVIAN STRAIN WHICH SHOWS HIGH HOMOLOGY WITH *HYP*B GENE OF *CHLAMYDIA*

Chiaki SATO[1], Atushi KATUMATA[2], Ikuo TAKASHIMA[1] and Nobuo HASHIMOTO[1]

SUMMARY

A study was performed to characterize DNA fragment No. 17 of *C. psittaci* strain P–1041 which encoded 42 KD β-galactosidase fusion protein with type-specific antigenecity. Sequence determination identified a partial open reading frame that spanned about 1,200b. p. nucleotides. Screening the literatures for the nucleotide and deduced amino acid sequences revealed extensive similarity between the DNA fragment of P–1041 and two chlamydial *hyp*B genes. This DNA showed 91.5% homology with *C. psittaci* GPIC *hyp*B gene in nucleotide sequence and 96.4% homology in deduced amino acid sequence. The *hyp*B gene of *C. trachomatis* serovar A and the P–1041 DNA fragment showed 81.2% and 91.3% homology in nucleotide and amino acid sequences, respectively. Dot enzyme-linked immunosorbent assay, for the products of deleted DNA fragments defined the coding region for type-specific antigenic polypeptide. In addition, the P–1041 DNA fragment carried a sequence highly homologous (>49%) with other bacterial and plant genes called chaperonin which responds to various stress in cells. From these results, the P–1041 DNA fragment was found to be a part of *hyp*B gene and to encode the region critical for type-specific antigenicity.

Key words: *Chlamydia psittaci*, heat-shock gene

INTRODUCTION

The genus *Chlamydia* is a procaryotic pathogen which is an obligate intracellular parasite of eucaryotic cells and causes a wide variety of syndromes in mammalian and avian hosts (2, 12, 13). This gram negative bacteria has a unique life cycle in which two distinct forms, an infectious, metabolically inactive form elementary body (EB) and a non-infectious, metabolically active form reticulate body (RB) can be defined (1, 9).

[1] Department of Veterinary Public Health, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, 060, Japan

[2] Institute for Chemical Research, Kyoto University, Kyoto, 611, Japan

Despite this unique developmental cycle and considerable medical importance, relatively little is known of the molecular basis for biological properties of chlamydia. At present, only a limited number of chlamydial genes have been cloned.

Recently, Morrison et al. cloned and sequenced *hyp* genes of *Chlamydia psittaci* GPIC strain and *Chlamydia trachomatis* serovar A and suggested that *hyp* gene products were responsible for the hypersensitivity of ocular chlamydial infections (6, 7, 8). It was shown that the hyp gene contained two open reading frames (ORFs), *hyp*A and *hyp*B, coding 20KD and 57KD proteins, respectively. These two proteins have been identified as members of a stress response protein family which is widely conserved among bacteria and plants with common antigenic properties. Proteins such as *Escherichia coli* GroEL, *Coxella burnetii* 60KD Hsp, and plant Rubisco subunit binding protein are included in this family which is classified as chaperonin (3, 15). Although the avian strain of *C. psittaci* is mostly responsible for human psittacosis, little has been published about molecular cloning of the gene of this species. Furthermore, *hyp*B was reported to encode the antigenic epitope common to the chlamydial genus.

We recently cloned DNA fragments from *C. psittaci* pigeon strain (P–1041) expressing 42KD β-galactosidase fusion protein with type-specific antigenicity, as revealed by the reactivity only with homologous antiserum to the *C. psittaci* P–1041 strain (11). In this report, sequence analysis was performed to define the characteristics of the P–1041 DNA fragment expressing 42KD protein, showing the DNA fragment to have highly homologous sequence with the chlamydial *hyp*B genes. Furthermore, deletion fragments were generated from the P–1041 DNA fragment and their products were examined for antigenicity, to the reveal coding region critical for type specific antigen.

MATERIALS AND METHODS

Bacteria, bacteriophages and plasmids: *Chlamydia psittaci* avian strain P–1041 was isolated from feral pigeons in our laboratory. Cultivation and purification of chlamydia and extraction of DNA has been followed by ref. 4 and 16. Plasmid pUC19 was used initially for cloning of chlamydial DNA fragments. Subclones in the M13 phages, mp18 and mp19, were used for DNA sequencing as well as plasmids pUC18 and pUC19. The plasmids, phages, and *E. coli* host strain JM109 were kindly provided by the Department of Genetic Biochemistry, Faculty of Science, Hokkaido University, Sapporo.

Antisera to chlamydia: Antisera against chlamydia were prepared in mice. An emulsion of purified EB suspension was injected subcutaneously. The immunizations were carried out at weeks 0, 2 and 3. Antisera were collected one week after the final immunization.

DNA preparation and cloning: The methods of preparation and cloning of chlamy-

dial DNA have been followed by ref. 4. The purified EBs were treated with 1% (W/V) sodium dodecyl sulfate (SDS) and 20mM EDTA for 1hr at 37°C, followed by digestion with proteinase K (0.1mg/ml) for 2hr at 37°C. The DNA was isolated by phenol-chloroform extractions and ethanol precipitation. RNA was removed by treatment with 0.1mg/ml ribonuclease A for 1hr at 37°C. Phenol-chloroform extraction and ethanol precipitation were repeated, and the final DNA pellet was suspended 10mM Tris-HCl buffer containing 1mM EDTA, pH 8.0. Total DNA from *C. psittaci* P–1041 was partially digested with *Sau*3AI and ligated to *Bam*HI–digested plasmid pUC19. These recombinant plasmids were introduced to *E. coli* JM109. Ampicillin-resistant white colonies were screened for expression of chlamydial proteins with dot-ELISA.

Dot-ELISA for immuno-screening: Standard methods were used for enzyme-linked immunoassay (17). Briefly, the transformed *E. coli* were transferred to the nitrocellulose filter. After lysis by chloroform, products of each colony were allowed to react with anti-chlamydia homologous antiserum that was absorbed with host *E. coli* proteins. The filter was than washed, and the bound antibodies were reacted with peroxidase-labeled antimouse immunoglobulin goat serum (CAPPEL, Co., Ltd., West Chester, PA.). The chromogen-3, 3'-diaminobenzidine tetrahydro-chloride was used for colour development.

Enzymes and radiolabeled compounds: Restriction endonucleases were purchased from TAKARA SHUZO Co., Ltd (Kyoto, Japan) or NIPPON GENE Co., Ltd (Toyama, Japan), and the manufacturer's directions for their use were followed. The radio-labeled triphosphate, [α–32p] dCTP, was obtained from AMERSHAM JAPAN Co., Ltd (Tokyo, Japan). All enzymes were used according to the instructions provided by the manufacturers.

DNA sequencing and sequence analysis: Both strands of the DNA were sequenced by the primer extention dideoxy-chain termination procedure by Sangar et al. (10). The nested deletions were generated by the DNase I/Shotgun cloning method and each deleted fragment was inserted in pUC18 and pUC19 for double strand sequencing (5). In addition, some of fragments were cloned into M13 phages for sequencing. Sequence analysis was done using the DNASIS programs (HITACHI Software Engineering Co., Ltd. Yokohama, Japan), and the homologous sequence was searched among the sequences submitted in the GenBank and EMBL nucleic acid sequence data bank.

Determination of coding region for chlamydial antigenic polypeptide: Each of the recombinant plasmids containing sequence-deleted fragment generated by DNase I/ Shotgun cloning methods was tested for the expression of antigenic proteins. Characterization of the products of these clones was carried out by dot ELISA as described above for immuno-screening using homologous and heterologous antisera (17).

RESULTS

Characterization of recombinant clones: Chlamydial DNA was inserted into pUC19, and recombinant plasmid was used to transform *E. coli*. Ampicillin-resistant white colonies expressing β-galactosidase fusion protein were screened by dot ELISA. Only two of over 8,000 colonies tested were positive giving strong signal, and one of them was further characterized. The product of the recombinant was previously shown to be 42KD β-galactosidase fusion protein by western blotting and to have type-specific antigenicity by dot ELISA (11).

The plasmid containing chlamydial DNA inserts at approximately 1.7Kbp was

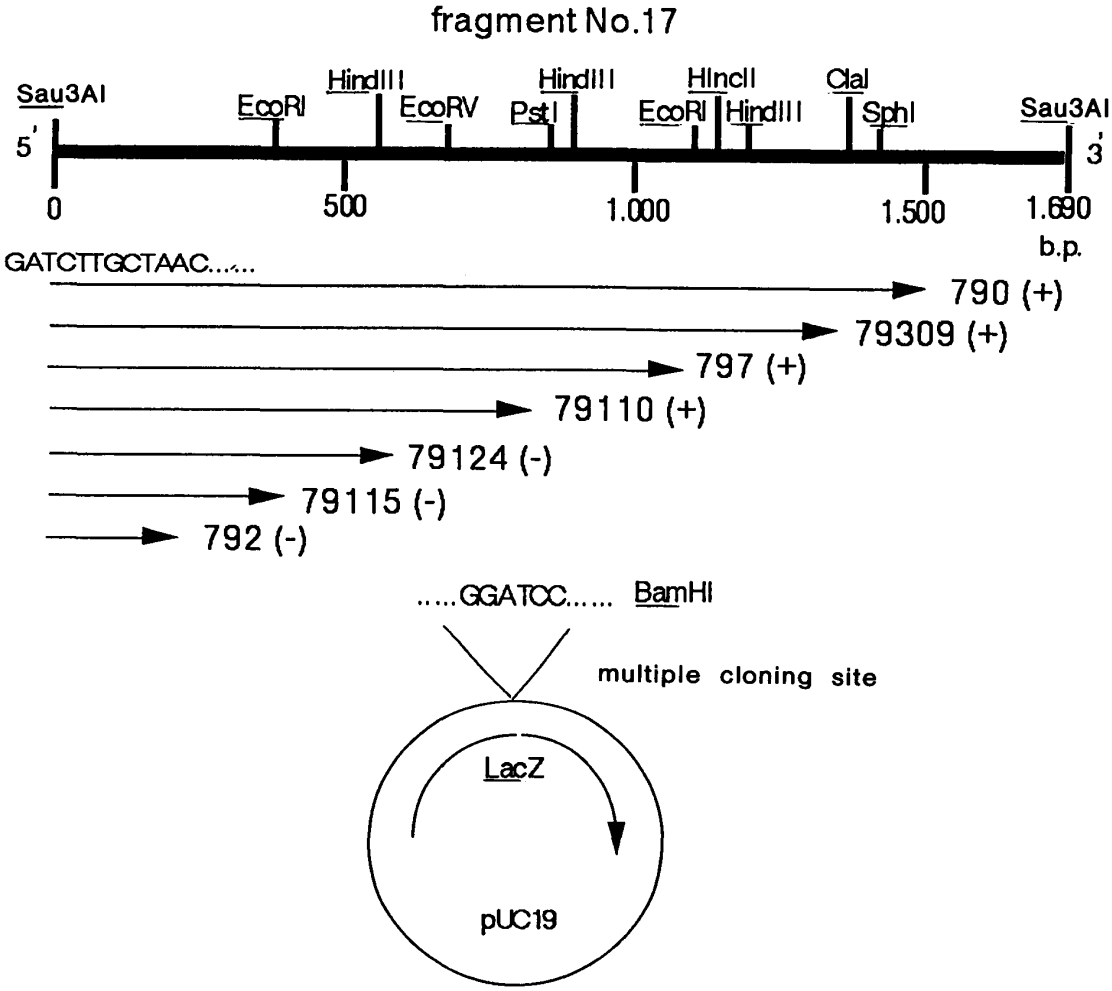

Fig. 1 Restriction map of P-1041 DNA fragment No.17 and antigenic expression of the deleted fragments. Deleted fragments from pUC19 produced immuno-reactive polypeptide (+) or nonimmunoreactive polypeptide (−).

digested with several restriction enzymes and mapped as followed by ref. 14. The restriction map of this fragment is shown in Fig. 1.

Nucleotide sequence and deduced amino acid sequence: The nucleotide and deduced amino acid sequences are shown in Fig. 2. A partial ORF of ca. 1,250b. p. nucleotides was identified and an amino acid sequence of 417 residues was predicted approximately as a 44.8KD polypeptide. This is close to the estimated molecular

```
   1
  TAGAGGATCAAATCAAAAAAATTAGCAAACCTGTACAACATCATAAAGAAATAGCTCAAGTAGCCACCATTTCT
     E  D  Q  I  K  K  I  S  K  P  V  Q  H  H  K  E  I  A  Q  V  A  T  I  S
  76
  GCTAACAACGATTCTGAAATTGGTAATCTCATCGCCGAAGCCATGGAAAAAGTTGGAAAAAATGGCTCCATTACT
   A  N  N  D  S  E  I  G  N  L  I  A  E  A  M  E  K  V  G  K  N  G  S  I  T
 151
  GTTGAGGAAGCTAAAGGTTTCGAAACTGTTCTCGATGTTTTTGAAGGTATGAATTTCACCCGAGGATACCTTTCT
   V  E  E  A  K  G  F  E  T  V  L  D  V  V  E  G  M  N  F  N  R  G  Y  L  S
 226
  AGCTACTTCTCTACAAATCCTGAGACACAAGAATGTGTGTTAGAAGAAGCTCTTGTGCTTATCTACGACAAAAAA
   S  Y  F  S  T  N  P  E  T  Q  E  C  V  L  E  E  A  L  V  L  I  Y  D  K  K
 301
  ATTTCCGGAATTAAAGATTTCCTTCCAGTTTTACAACAGGTAGCAGAATCCGGACGTCGCCTATTAATTATTGCT
   I  S  G  I  K  D  F  L  P  V  L  Q  Q  V  A  E  S  G  R  P  L  L  I  I  A
 376
  GAAGATATCGAAGGCGAAGCTCTAGCTACTTTAGTAGTCAACAGACTACGTGCAGGATTCAGAGTCTGCGCAGTT
   E  D  I  E  G  E  A  L  A  T  L  V  V  N  R  L  R  A  G  F  R  V  C  A  V
 451
  AAAGCTCCTGGATTTGGAGATAGAAGAAAAGCTATGTTAGAAGACATTGCTATCTTAACCGGTTGCCAACTTATC
   K  A  P  G  F  G  D  R  R  K  A  M  L  E  D  I  A  I  L  T  G  G  Q  L  I
 526                Hind III
  AGCGAAGAGCTTGGTATGAAGCTTGAAAACACCACTTTATCCATGCTAGGAAAAGCTAAGAAAGGCATCGTTTCT
   S  E  E  L  G  M  K  L  E  N  T  T  L  S  M  L  G  K  A  K  K  V  I  V  S
 601
  AAAGAAGACACAACAATTGTTGAAGGTCTCGGAAACAAAGAAGATATCGAAGCTCGATGCGAAATTATCAAAAAA
   K  E  D  T  T  I  V  E  G  L  G  N  K  E  D  I  E  A  R  C  E  N  I  K  K
 676
  CAAATCGAAGACAGCACTTCTGACTACGATAAAGAAAAACTCCAACAACGTTTAGCAAAACTTTCTGGAGGCGTA
   Q  I  E  D  S  T  S  D  Y  D  K  E  K  L  Q  E  R  L  A  K  L  S  G  G  V
 751
  GCTGTAATCCGTGTAGGAGCTGCTACAGAAATTGAAATGAAAGAGAAAAAAGACCGAGTAGATGACGCTCAACAT
   A  V  I  R  V  G  A  A  T  E  I  E  M  K  E  K  K  D  R  V  D  D  A  Q  H
 826        Pst I
  GCAACTCTTGCTGCAGTTGAAGAAGGAATCTTGCCTGGTGGTGGTACACGTTTAGTTCGTTGTATCCCTACTTTA
   A  T  L  A  A  V  E  E  G  I  L  P  G  G  G  T  A  L  V  R  C  I  P  T  L
 901 Hind III
  GAAGCTTTCATTCCTGTTCTTACAAATGAAGATGAACAAATCGGAGCACGTATTGTTCTTAAAGCATTATCCGCT
   E  A  F  I  P  V  L  T  N  E  D  E  Q  I  G  A  R  I  V  L  K  A  L  S  A
 976
  CCTTTAAAACAAATCGCAGCAAATGCAGGTAAAGAAGGTGCTATCATCTGCCAACAAGTGCTCTCTCTCGCTTCT
   P  L  K  Q  I  A  A  N  A  G  K  E  G  A  I  I  C  Q  Q  V  L  S  L  A  S
1051
  AACGAAGGCTACGATGCTTATCGCGATGCTTACACCGATATGATTGAAGCAGGAATTCTCGACCCAACTAAAGTC
   N  E  G  Y  D  A  Y  R  D  A  Y  T  D  M  I  E  A  G  I  L  D  P  T  K  V
1126
  ACACGTTGTGCTTTAGAGAACGCAGCTTCTGTAGCTGGCCTTCTATTAACAACAGAAGCTTTAATTGCTGATATT
   T  R  C  A  L  E  N  A  A  S  V  A  G  L  L  L  T  T  E  A  L  I  A  D  I
```

```
1201
 CCTGAAGAGAAATCCTCTTCTGTTCCAGCAATGCCAGGCGCAGGAATGGAGTATTAATCCCCTATGTAGAGAACT
   P  E  E  K  S  S  S  V  P  A  M  P  G  A  G  M  D  Y  *
1276
 TCTTCTCTATTCTTACAAGGTCTGGGTTCATCTATCTCAAGAAGAAGGGGGACCTTTTTCATTTTGTAATATTTC
1351
 TTTCTTCATCGATGTTAGAAACTAAGATAAACACAATCTCATCTGC--ATGCTTAAACTTCTAAAAAATTTATTT
                                                   M  L  K  L  L  K  N  L  F
1424
 TTGTTAGCATTCTGCATTGCTGCCTACTTTTGGATTCGTAAAGAAAGCATTGTCGAGCACTGGTTATCTGCAAAA
   L  L  A  F  C  I  A  A  Y  F  W  I  R  K  E  S  I  V  E  H  W  L  S  A  K
1499
 TTACATACTCAAGTCACCGTAGGAAGAGTCTCCCCGAGAACTTCAGGAATGAAAATCCGTTACGTATGTATTCAT
   L  H  T  Q  V  T  V  G  R  V  S  P  R  T  S  G  M  K  I  R  Y  V  C  I  H
1574
 AACCCCATGCCATCGGAACGTTTCCCCTATGCCATGGAAATCGAATACGTCAACCTGCGCTTCTCTCTGATTTCT
   N  P  M  P  S  E  R  F  P  Y  A  M  E  I  E  Y  V  N  L  R  F  S  L  I  S
1649
 ATGCTTTTGTCGAAAAAAATAGAAATCTCAGATCCCCGGGTACC
   M  L  L  S  K  K  I  E  I  S  D  P  R  V
```

Fig. 2 Nucleotide sequence and deduced amino acid sequence of *C. psittaci* DNA fragment No. 17. The first base of 5' end of the P–1041 gene is numbered as 1. Asterisk indicates the stop codon, and underline indicates the initiation codon of the putative second ORF.

mass of 42KD obtained by SDS-polyacrylamide gel electrophoresis of the product previously shown (11). There was no putative termination structure after the stop codon of the ORF. In addition, there seemed to be another ORF started, from a distance of 139 bases downstream from the stop codon of the first ORF. Furthermore, this suppositional second ORF had no putative promoter site nor ribosome binding site.

Antigen-coding region of the fragment and the cross-reactivity of the expressed antigen with the antisera against the other chlamydial strains: To investigate the antigen coding region, the nested deletions were generated by the DNase I/shotgun cloning methods and each of the products of the deletion DNA fragment was tested on the antigenicity (Fig. 1). The dot-ELISA analysis revealed that 79110 of the fragment, a region containing ca. 800 bases starting from 5'end, expressed an immunoreactive product. However, nearby 79124 of the P–1041 DNA fragment, a region containing ca. 650 bases starting from 5'end, produced non-immunoreactive polypeptide.

This result suggests that epitope area located in the region from ca. 650 to ca. 800 position. Furthermore, these immunoreactive product from each deleted fragments reacted only with homologous antiserum to P–1041 strain, and not with heterologous antisera to 9 *C. psittaci* strains and 2 *C. trachomatis* strains (11).

Table 1 Percent homology of nucleotide and deduced amino acid sequence among chlamydial hypB genes and other bacterial and higher plant chaperonin genes.

	C. psittaci P-1041 DNA	C. psittaci GPIC hypB gene	C. trachomatis A hypB gene	Cox. brunetii hsp60 gene	E. coli groES gene	T. aesticum Rubisco binding protein gene
C. psittaci P-1041 DNA		91.5* (96.4)	81.2 (91.3)	61.1 (59.1)	59.4 (56.9)	56.5 (49.2)
C. psittaci GPIC hypB gene			80.9 (93.0)	61.2 (61.4)	60.0 (60.8)	57.2 (51.0)
C. trachomatis A hypB gene				62.1 (61.3)	61.1 (60.6)	57.4 (50.8)
Cox. brunetii hsp60 gene					68.5 (75.5)	54.7 (49.5)
E. coli groES gene						55.8 (49.5)
I. aesticum Rubisco binding protein gene						

Asterisk indicate nucleotide sequence homology and ( ) indicate amino acid sequence homology.

Homology of nucleotide sequence and the deduced amino acid sequence of the fragment with those of hypB and chaperonin genes: To assess the function of the chlamydial gene, nucleotide and amino acid homology searches were carried out on the published sequence data of organisms. The P-1041 DNA fragment was extensively and highly homologous to two of the chlamydial *hyp* genes (Table 1). With one of these, the *C. psittaci* GPIC *hyp*B gene, the P-1041 DNA showed 91.5% homology in nucleotide sequence and 96.4% homology in amino acid sequence. Another *hyp* gene isolated from *C. trachomatis* serovar A showed 81.2% and 91.3% homology in nucleotide and amino acid sequence with the DNA from P-1041, respectively. Variable regions among the amino acid sequence were found at two locations, residues 184 to 229, and residues 340 to 380. It was observed that 5 to 10 residue substitutions occurred among three chlamydial sequences (Fig 3).

```
                10        20        30        40        50        60        70        80
P-1041 DQIKKISKPVQhHKEIAQVATISANNDSEIGNLIAEAMEKVGKNGSITVEEAKGFETVLDVVEGMNFNRGYLSSYFSTNP
GPIC   .E.......................................................................
A      ........................A.....................................................A...

                90       100       110       120       130       140       150       160
P-1041 ETQECVLEEALVLIYDKKISGIKDFLPVLQQVAESGRPLLIIAEDIEGEALATLVVNRLRAGFRVCAVKAPGFGDRRKAM
GPIC   ................................................................................
A      ........D..................I.............................I.G.....................

               170       180       190       200       210  *1   220       230       240
P-1041 LEDIAILTGGQLISEELGMKLENTTLSMLGKAKKVIVSKEDTTIVEGLGNKEDIEARCENIKKQIEDSTSDYDKEKLQER
GPIC   ........................A.....................R..H..S...S...................
A      ......................AN.A...................M.E..AL.....S........S...........

               250       260  *2   270       280       290       300       310       320
P-1041 LAKLSGGVAVIRVGAATEIEMKEKKDRVDDAQHATLAAVEEGILPGGGTALVRCIPTLEAFIPVLTNEDEQIGARIVLKA
GPIC   ................................................................I...............
A      ....................................I..............I.........L.M...............

               330       340       350       360       370       380       390       400
P-1041 LSAPLKQIAANAGKEGAIICQQVLSLASNEGYDAYRDAYTDMIEAGILDPTKVIRCALENAASVAGLLLLTTEALIADIPE
GPIC   ........................RS.S.....L.........................S...................
A      ..................F...M.RSA......L.......L.......A....S...S..............E...

               410
P-1041 EKSSSVPAMPGAGMDY
GPIC   .....A..........
A      ..PAAA..........
```

Fig. 3 Deduced amino acid sequence alignment of the P-1041 chaperonin-like protein and other chlamydial *Hyp*B proteins. Comparison was made among P-1041; *C. psittaci* strain avian P-1041, GPIC; *C. psittaci* strain guinea pig inclusion conjunctivitis and, A; *C. trachoamtis* serovar A. Dot indicate sequence identity. 3' end of deletion-fragments are represented by 1 with asterisk for fragment 79124 and 2 for fragment 79110.

In addition, the P–1041 DNA fragment showed a highly homologous sequence with several genes of chaperonin family from other bacteria and higher plants (Table 1).

Discussion

In this report, DNA fragment No. 17 of a *C. psittaci* avian strain P–1041, was cloned and characterized for nucleotide sequencing and the expression of protein. The results show that the corresponding region of ORF of the P–1041 DNA was extensively and highly homologous to *hyp*B genes of *C. trachomatis* serovar A and *C. psittaci* GPIC strain both in nucleotide and amino acid sequence.

Furthermore, it can be supposed that the cloned DNA of P–1041 is contained in chaperonin gene family known to code for the stress-response protein of other bacteria and plants. Chaperonin, protein product of this gene family, has been proposed to play a essential role for folding certain polypeptide and their correct assembling into oligomeric structure (3). Therfore, it may be possible that the cloned DNA fragment of P–1041 as well is related to the assembly of the protein during its unique developmental cycle.

The antigen coding region in the P–1041 DNA fragment was defined to locate from ca. 650 position to ca. 800 position in the nucleotide sequence by fragment deletion analysis. The product of the P–1041 DNA seemed to be type-specific, since the polypeptide had no cross-reactivity against 11 antisera to heterologous chlamydial strains icluding 9 *C. psittaci* and 2 *C. trachomatis* strains (11). On the other hand the HypB polypeptide was reported to be the chlamydial genus-common antigen (6, 7, 8). It may be possible that there are more than two epitopes in the HypB protein; a genus-common one, located in the region further upstream the cloned fragment, and a type-specific one, located in the cloned DNA of P–1041. Another possible explanation for antigenic specificity of the product is that the other common epitopes have been expressed, but are hidden by other proteins sterically. Deduced amino acid comparison showed that the variable region in amino acids was found at the region of 184 to 229 corresponding closely to 3' end of deleted fragment 79124. This region may be related to type-specific epitopes since the product of fragment 79110 was antigenic, while the fragment 79124, lacking the variable region, was not. Additionally, the P–1041 DNA did not have any putative transcriptional termination structure, nor the promoter site in the noncoding region between first and second putative ORFs. Therefore, the *hyp*B-like first ORF and the second ORF may be cotranscribed as an operon in the P–1041 gene.

Since the DNA fragment of *C. psittaci* pigeon strain cloned in this study has a highly homologous sequence with *hyp*B genes of two other chlamydial strains, it is necessary to examine whether the product is also responsible factor for the hypersensitivity or other pathological events of *C. psittaci* infection in avian hosts.

ACKNOWLEDGMENTS

We would like to thank Dr. Mitsuru Takanami, Institute for Chemical Reserch, Kyoto University, for his valuable advice and discussion.

REFRENCES

1) BECKER, Y. (1978): The *Chlamydia*: molecular biology of procaryotic obligate parasites of eucaryotes. Microbiol. Rev., **42**, 274–306
2) GRAYSTON, J. T., KUO, C. C., WANG, S. P. and ALTMAN, J. (1986): A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. N. Engl. J. Med. **315**, 161–168
3) HEMMINGSEN, S. M., WOOLFORD, C., VAN DER VIES, S. M., TILLY, K., DENNIS, D. T., GEORGOPOULOS, C. P., HENDRIX, R. W. and ELLIS, R. J. (1988): Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature (London), **333**, 330–334
4) HYYPIA, T., LARSEN, S. H., STAHLBERG, T. and TERHO, T. (1984): Analysis and detection of Chlamydial DNA. J. Gen. Microbiol., **130**, 3159–3164
5) LIN, H. -C., SHAU-PING and WILCOX, G. (1985): An improved DNA sequencing strategy. Anal. Biochem., **147**, 114–119
6) MORRISON, R. P., LYNG, K. and CALDWELL, H. D. (1989): Chlamydial disease pathogenesis. Ocular hypersensitivity elicited by a genus-specific 57-kD protein. J. Exp. Med., **169**, 663–675
7) MORRISON, R. P., BELLAND, R. J., LYNG, K. and CALDWELL, H. D. (1989): Chlamydial disease pathogenesis. The 57-kD chlamydial hyper sensitivity antigen is a stress response protein. J. Exp. Med., **170**, 1271–1283
8) MORRISON, R. P., SU, H., LYNG, K. and YUAN, Y. (1990): The *Chlamydia trachomatis* hyp opreron is homologous to the groE stress response operon of *Escherichia coli*. Infect. Immun., **58**, 2701–2705
9) MOULDER, J. W. (1982): The relation of basic biology to pathogenic potential in the genus *Chlamydia*. Infection, **10**, 1–9
10) SANGER, F., NICKLEN, S. and COULSON, A. R. (1977): DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA, **74**, 5463–5467
11) SATO, C., KATUMATA, A., TAKASHIMA, I. and HASHIMOTO, N. (1991): Cloning and antigenic expression of two genes from *Chlamydia psittaci* avian strain. FEMS Microbiol. Left., **80**, 201–206
12) SCHACHTER, J. (1978): Chlamydial infections. N. Engl. J. Med. **298**, 428–435, 490–495, 540–549
13) SHEWEN, P. E. (1980): Chlamydial infection in animals: a review. Can. Vet. J., **21**, 2–11
14) SMITH, H. O., and BIRNSTIEL, M. L. (1976): A simple method for DNA restriction site mapping. Nucleic. Acids Res., **3**, 2387–2398
15) VODKIN, M. H. and WILLIAMS, J. C. (1988): A heat shock operon in *Coxiella burnetii* produces a major antigen homologous to a protein in both *Mycobacteria* and *Escher-*

ichia coli. J. Bacteriol., **170**, 1227–1234

16) WAGAR, E. A. and STEPHENS, R. S. (1988): Developmental form-specific DNA-binding proteins in *Chlamydia* spp. Infect. Immunol., **56**, 1678–1684

17) ZANG, Y. -X., STEWART, S., JOSEPH, T., TAYLOR, H. R. and CALDWELL, H. D. (1987): Protective monoclonal antibodies recognize epitopes located on the major outer membrane protein of *Chlamydia trachomatis*. J. Immunol., **138**, 575–581