The ygdP and apah genes of Salmonella enterica serovar Typhimurium (S. Typhimurium) encode two unrelated dinucleoside polyphosphate (Np,N) hydrolases. For example, YgdP cleaves diadenosine tetraphosphate (Ap4A) producing AMP and ATP, while ApaH cleaves Ap3A producing 2ADP. Disruption of ygdP, apah individually, and disruption of both genes together reduced intracellular invasion of human HEp-2 epithelial cells by S. Typhimurium by 9-, 250-, and 2000-fold, respectively. Adhesion of the mutants was also greatly reduced compared with the wild type. Invasive capacity of both single mutants was restored by transcomplementation with the ygdP gene, suggesting that loss of invasion was due to increased intracellular Np,N. The normal level of 3 μM adenylated Np,N (Ap,N) was increased 1.5-, 3.5-, and 10-fold in the ygdP, apah and double mutants, respectively. Expression of the putative ptsP virulence gene downstream of ygdP was not affected in the ygdP mutant. Analysis of 19 metabolic enzyme activities and the ability to use a range of carbohydrate carbon sources revealed a number of differences between the mutants and wild type. The increase in intracellular Np,N in the mutants appears to cause changes in gene expression that limit the ability of S. Typhimurium to adhere to and invade mammalian cells.

The dinucleoside polyphosphates (Np,N)1 are a ubiquitous family of nucleotides found at micromolar to submicromolar concentrations in which two nucleoside moieties are linked 5′-5′ through a polyphosphate chain containing from two to seven phosphoryl groups. The most widely studied are diadenosine 5′,5′-P1,P3-triphosphate (Ap3A) and diadenosine 5′,5′-P1,P4-tetraphosphate (Ap4A), for which several functions have been suggested, although none yet conclusively proved (1–3). In Escherichia coli, Ap4A has been proposed to couple DNA replications to cell division (4, 5) and to participate in stress responses by modulating protein refolding by chaperones (3, 6, 7).

1 The abbreviations used are: Np,N, dinucleoside 5′,5′-Pn,Pn-polyphosphate; Ap,N, diadenosine 5′,5′-Pn,Pn-tetraphosphate (other compounds are abbreviated similarly); Ap,A, adenine(5′)-polyphosphate(5′)-nucleoside; S. Typhimurium, Salmonella enterica serovar Typhimurium; WT, wild type; ApA and related adenyalted dinucleotides (e.g. Ap3N and ApN,N, where N = any nucleoside) are synthesized mainly by aminoacyl-tRNA synthetases, although other ligases have been shown to synthesize them in vitro (8, 9). In Gram-negative bacteria, the predominant enzyme believed to be responsible for ApA hydrolysis is the symetrically cleaving diadenosine tetraphosphatase, ApaH. This enzyme, which is active toward many Np,N nucleotides (n ≥ 3), degrade ApA to two moles of ADP and Ap,A to ADP and ATP (10–12) and is structurally related to serine/threonine protein phosphatases (13, 14). Deletion of the E. coli apah gene leads to a 10 to 100-fold increase in intracellular Ap,N (15, 16).

Recently, a second prokaryotic dinucleoside polyphosphate hydrolase was discovered. The IalA protein from the invasive pathogen Bartonella bacilliformis is a member of the Nudix (nucleoside diphosphate linked to X) hydrolase family and hydrolyzes ApA asymmetrically to AMP and ATP and Ap,A to ADP and ATP (17, 18). It is closely related to the eukaryotic ApA hydrolases, particularly those from plants, which also hydrolyze many Np,N species, where n ≥ 4 (12, 18). The ialA gene has been implicated indirectly in the process of cellular invasion by this bacterium; expression of B. bacilliformis ialA in non-invasive E. coli renders it invasive (19). Furthermore, the orthologous ygdP gene from E. coli K1 may be required for the invasion of human brain microvascular endothelial cells as its expression is up-regulated by invasion-enhancing growth conditions and down-regulated by invasion-repressing conditions (20). YgdP and the related InvA protein from Rickettsia prowazekii preferentially hydrolyze ApA (21, 22). Since the intracellular levels of several Ap,N species are known to increase substantially under conditions of oxidative stress (23, 24), we previously suggested that the ability to metabolize Np,N may be necessary for invasion in the face of an oxidative attack by the invaded cell (18). If that were so, then the apah gene would be expected to be essential for invasion as well. Indeed, a DNA fragment from the oral pathogen Actinobacillus actinomycetemcomitans that confers an invasive ability on E. coli contains the apah gene (25).

We have, therefore, examined the effects of deleting the ygdP and apah genes both singly and doubly on the invasive ability of Salmonella enterica serovar Typhimurium (S. Typhimurium), a facultative intracellular parasite that can invade and multiply within various cell types, including phagocytes and epithelial cells, and so establish a chronic infection. Our results provide the first direct evidence of the involvement of these bacterial genes in intracellular invasion.

**Experimental Procedures**

Reagents, Bacterial Strains, and Plasmids—Recombinant human ApA hydrolase was prepared as described for the Caenorhabditis elegans enzyme (26). Strains and plasmids used and produced in this...
study are listed in Table I. Plasmids were introduced into E. coli by transformation and into S. Typhimurium strain LT2 by electroporation using a Bio-Rad Gene Pulser II.

DNA Manipulation and Analysis—DNA ligation, restriction analysis, and gel electrophoresis were carried out as described by Sambrook et al. (28).

Cloning, Expression, and Purification of ApaH and YgdP—The coding regions of the apaH and ygdP genes were PCR-amplified from S. Typhimurium genomic DNA. ApaH was amplified using the 5′-ATTATATAGGCGAATCTCATC-3′ coding region of the adaH gene (11) and the 3′-primer HP2 (5′-CGCGACCAGAATAAAACGT-3′) containing an NdeI site and inserted after restriction digestion between the NdeI and BamHI sites of pET15b (Novagen) to give pET-ApaH. YgdP was amplified using the 5′-primer HP3 (5′-GCTCGAGTAGTTT-3′) containing an NcoI site and the 3′-primer HP4 (5′-CATGTCTAGACTTAGATGTGATGCTGGTCA-3′) containing a XhoI site and inserted after restriction digestion between the XhoI and Xhol sites of pET32b (Novagen) to give pET-YgdP. For expression, plasmids were transformed into E. coli BL21(DE3). The His-tagged recombinant proteins were expressed and purified on NiNTA-agarose resin (Sigma) as described previously (29).

Construction of apaH and ygdP Disruption Cassettes—Gene disruption cassettes were generated by PCR according to Wach et al. (30). An apaH::kan cassette was constructed as follows. First, a 306-bp 5′-segment of the apaH gene was amplified from S. Typhimurium genomic DNA using primer HP1 (5′-TCGCGAGGTGGGACACCA-3′) corresponding to part of the 3′-end of the coding region of the adaH gene and primer HP2 (5′-CGCGAGGTGGGACACCA-3′). The 3′-primer HP3 (5′-TGCGAGGTGGGACACCA-3′) where HP1 corresponds to part of the 5′-end of the non-coding region of the coding region of the adaH gene, and HP2 the 5′-end of the coding region of the adaH gene and HP3 the 5′-end of the non-coding region of the kanamycin resistance gene from pUC4K. A 285-bp 3′-segment of the apaH gene was also amplified using the 5′-primer HP3 (5′-GCTCGAGTAGTTT-3′) containing an NcoI site and the 3′-primer HP4 (5′-CATGTCTAGACTTAGATGTGATGCTGGTCA-3′) containing a XhoI site and inserted after restriction digestion between the XhoI and Xhol sites of pET32 (Novagen) to give pET-YgdP. For expression, plasmids were transformed into E. coli BL21(DE3). The His-tagged recombinant proteins were expressed and purified on NiNTA-agarose resin (Sigma) as described previously (29).

Construction of apaH::kan cassette was generated as follows. First, a 306-bp 5′-segment of the adaH gene was amplified from S. Typhimurium genomic DNA using primer HP1 (5′-TCGCGAGGTGGGACACCA-3′) corresponding to part of the 3′-end of the coding region of the adaH gene, and primer HP2 (5′-CGCGAGGTGGGACACCA-3′). The 3′-primer HP3 (5′-TGCGAGGTGGGACACCA-3′) where HP1 corresponds to part of the 5′-end of the non-coding region of the coding region of the adaH gene, and HP2 the 5′-end of the non-coding region of the kanamycin resistance gene and HP3 corresponds to part of the 3′-end of the non-coding region of adaH. In the second step, the kanamycin resistance gene cassette in pUC4K using primer HP1, HP4, and the two PCR products from the first step as primers to give the apaH::kan cassette in which the kanamycin resistance gene is flanked by >250 bp segments of the adaH gene. A ygdP::kan disruption cassette was produced in a similar manner using primers YP1 (5′-CATGTCTAGACTTAGATGTGATGCTGGTCA-3′), YP2 (5′-TCGCGAGGTGGGACACCA-3′) corresponding to part of the 3′-end of the coding region of the ygdP gene, and YP3 (5′-GCTCGAGTAGTTT-3′) containing an NcoI site and the 3′-primer YP4 (5′-CATGTCTAGACTTAGATGTGATGCTGGTCA-3′) containing a XhoI site and inserted after restriction digestion between the XhoI and Xhol sites of pET32 (Novagen) to give pET-YgdP. For expression, plasmids were transformed into E. coli BL21(DE3) and the cassettes purified after gel electrophoresis using a Qiagen purification kit. Cassettes were then ligated into the cut suicide vector pMRS101 (31). The ligation mixture was transformed into E. coli K12 CC118 (Δpir) and the resulting plasmids, pTrc-ApaH and pTrc-YgdP, were amplified from S. Typhimurium genomic DNA using primer HP1 (5′-ATTATATAGGCGAATCTCATC-3′) containing an NdeI site and inserted after restriction digestion between the NdeI and BamHI sites of pTrc Hs2-TOPO vector (Invitrogen) and propagated in TOP10 E. coli to yield the plasmids pTrc-ApaH and pTrc-YgdP. Purified plasmids were then electroporated into S. Typhimurium strains STY201 and STY202 and transformants selected on LB agar plates containing 75 µg/ml ampicillin and 50 µg/ml kanamycin.

Invasion and Adhesion Assays—HEp-2 epithelial cells and U937 macrophage-like cells were maintained in Eagle’s minimal essential medium (Invitrogen) with 5% (v/v) fetal calf serum and 0.15% Na2HCO3. HEp-2 cells were seeded on coverslips in vials at 1 × 105 cells/coverslip and grown overnight at 37 ºC in 1 ml of medium. U937 cells were split into 1 ml cultures at a density of 2 × 105 cells/ml and grown with 50 µg/ml gentamicin. For invasion experiments, 4 × 105/ml cells were incubated at 37 ºC in 1 ml of 5% CO2 for 3 h. For invasion assays, cells were incubated for 1 h with 25 µg/ml gentamycin, washed five times with 1 ml of phosphate-buffered saline, then lysed in 0.5 ml 0.5% sodium deoxycholate. Lysates were diluted in phosphate-buffered saline and the viable count determined on LB agar plates (containing 50 µg/ml kanamycin for mutants). For adhesion assays, incubation with gentamycin was omitted. In addition, HEp-2 monolayers were washed with phosphate-buffered saline after adhesion, fixed with methanol, and stained with a 10% solution of Giemsa prior to examination by light microscopy.

Metabolic Phenotype—Nineteen different enzyme activities of the WT and mutants strains were assayed by the API ZYM kit (bio-Mérieux, Basingstoke, UK). The ability of the WT and mutant strains to metabolize a variety of different carbohydrates was determined using the API 50 CH system (bio-Mérieux). This system tests assimilation, oxidation, and fermentation of the carbohydrate sources.

Expression and Assay of ApaH and YgdP—Cells from 50 ml cultures of S. Typhimurium (WT and mutants) in mid log phase (OD 0.6–0.7) were collected by rapid centrifugation (5000 × g for 5 min). Pellets were resuspended in 5 ml of ice-cold 0.4 M trichloroacetic acid and shaken for 15 min. Neutralization, alkaline phosphatase digestion, and purification of the dinucleotide-containing fraction were as described previously (25). The freeze-dried extract was dissolved in water and applied to the 100 µl lysis in 1 ml of 5% (v/v) NaOH, pH 7.7, 5 mM magnesium acetate and triplicate 25-µl samples each mixed with 25 µl of luciferin/luciferase ATP-monitoring reagent (Bio-Ori). After measuring the background luminescence, 1 ng of recombinant human ApaH hydrolyase was added to generate ATP and the increase in luminescence determined. This generalized assay measures all ATP-generating nucleotides of the form ApnNp, where n > 4. Samples (10 µl) of the neutralized acid extract were also retained for luminoetric ATP determination before alkaline phosphatase digestion. These were mixed with 90 µl of 30 mM Hepes-NaOH, pH 7.7, 5 mM magnesium acetate, and triplicate 25-µl samples each added to 25 µl of luciferin/luciferase ATP-monitoring reagent and the luminescence determined.

Previous ATP assays were used to calculate the N/ATP ratio and the intracellular ATP concentration was determined at 37 ºC. For WTS and mutant strains, the intracellular ATP concentration was determined at 37 °C by reverse transcription-PCR (27). The N/ATP ratio was calculated using the intracellular ATP concentration, which was measured using the luciferase-based ATP assay (25).

ApaH activity was determined as described previously (26). The activity of ApaH with Ap5A was measured by reverse transcription-PCR. Total S. Typhimurium RNA (1 µg, DNase-treated) was incubated at 70 °C for 5 min with 20 pmol of reverse primer (5′-CGCGGACGAGAATAACAGGTCC-3′) in 11 µl of water, then incubated at 37 °C for 5 min in a final

GATC-3′) and the 3′-primer (5′-TGGATCAGTTTCTGGCAGAGG-3′). Both PCR products were cloned into the pTrc Hs2-TOPO vector (Invitrogen) and propagated in TOP10 E. coli to yield the plasmids pTrc-ApaH and pTrc-YgdP. Purified plasmids were then electroporated into S. Typhimurium strains STY201 and STY202 and transformants selected on LB agar plates containing 75 µg/ml ampicillin and 50 µg/ml kanamycin.
Fig. 1. Purity and kinetic analysis of recombinant ApaH and YgdP hydrolases. A, 15% SDS-polyacrylamide gel stained with Coomassie Blue and containing molecular mass standards (Sigma) as indicated on the left (lane 1); lysate of E. coli BL21(DE3) cells containing pET-ApaH and induced for 3 h with 1 mM isopropyl-1-thio-D-galactopyranoside (lane 2), 2 μg of purified His-tagged ApaH (lane 3), lysate of E. coli BL21(DE3) cells containing pET-YgdP and induced for 3 h with 1 mM isopropyl-1-thio-D-galactopyranoside (lane 4), and 0.9 μg of purified His-tagged thioredoxin-YgdP fusion protein (lane 3). B, Michaelis-Menten plots for recombinant ApaH (filled circles) and YgdP (open circles) with Ap₄A as substrate.

Volume of 19 μl containing 4 μl of Moloney murine leukemia virus buffer (MBI Fermentas), 1 mM concentration of each dNTP, and 20 units RNase inhibitor. First strand cDNA was synthesized by adding 1 μl (200 units) of Moloney murine leukemia virus reverse transcriptase (MBI Fermentas) and incubating at 42 °C for 60 min. One μl of this was amplified in a final volume of 20 μl containing 20 pmol of forward (5'-GATCATTCAAGCTGGCCAC-3') and reverse primers, 0.1 mM concentration of each dNTP, 2.5 mM MgCl₂, 2.5 units of Taq polymerase, and 2 μl of Taq buffer (MBI Fermentas).

**RESULTS**

**Properties of the ApaH and YgdP Proteins**—To confirm that the S. Typhimurium ApaH and YgdP proteins had the enzymatic activities predicted from their sequences, they were cloned and expressed in E. coli BL21(DE3) cells: ApaH in pET15b as a His-tagged 33.6-kDa protein and YgdP in pET32b as a His-tagged thioredoxin fusion protein of total mass 39.2 kDa. When purified to homogeneity (Fig. 1A), the enzymes had the expected activities. ApaH efficiently hydrolyzed Ap₄A, Ap₅A, and Ap₆A, always producing ADP as one product, while YgdP hydrolyzed the same nucleotides, with a preference for Ap₅A, like the E. coli and R. prowazekii enzymes (21, 22), and always producing ATP as one product. Both enzymes followed Michaelis-Menten kinetics with all substrates tested; representative plots for the hydrolysis of Ap₄A by both enzymes are shown in Fig. 1B. Kinetic constants were calculated by non-linear regression. Kₘ and kₘ₄ values for ApaH for Ap₄A and Ap₅A were 37 μM and 37 s⁻¹ and 14 μM and 33 s⁻¹, respectively, similar to the E. coli enzyme when assayed under the same conditions (10, 11). Kₘ and kₘ₄ values for YgdP for Ap₄A, Ap₅A, and Ap₆A were 18 μM and 18 s⁻¹, 22 μM and 32 s⁻¹, and 54 μM and 0.8 s⁻¹, respectively.

ApaH, YgdP, and Ap₅A in WT and Mutant Cells—ΔapaH (STYA201) and ΔygdP (STYY202) null mutants and a ΔapaH ΔygdP double null mutant (STAYAY203) were generated by replacement of the genes with antibiotic resistance cassettes (Table I). Gene deletion was confirmed by PCR and by measurement of Ap₅A hydrolytic activities in cell extracts. ApaH and YgdP have predicted pl values of 4.8 and 10.0, respectively. Thus, they can be measured independently after separation by batch anion-exchange chromatography at pH 7.5. Table II confirms the absence of the enzymes in the appropriate mutants. The total concentration of nucleotides of general structure Ap₅A (n > 4) was also measured using a luciferase-based assay in which human Ap₅A hydrolase is used to generate ATP from Ap₅A (n > 4) compounds. WT cells had 3.6 μM Ap₅A, which compares favorably with previous figures of 3 μM in both S. Typhimurium (23) and E. coli (15). Deletion of ygdP led to a slight, 1.5-fold increase in Ap₅A and deletion of apaH to a 3.5-fold increase, while deletion of both genes led to a 10-fold increase. These results show that both YgdP and ApaH contribute to control of the Ap₅A pool in S. Typhimurium. As non-adenylated N₃P₃, which are not detected by the assay, are also substrates for these two hydrolases, it is likely that their levels also increase. Hence the Ap₅A pool measurements provide a rough indication of the effects of deleting the hydrolase genes but do not yet convey the detailed picture.

**Invasion**—The ability of the mutants to invade HEP-2 epithelial cells was determined using a gentamicin protection assay (36). Deletion of ygdP (STYY202) reduced invasion by 9-fold compared with the WT, while deletion of apaH (STYA201) reduced invasion by 250-fold. Deletion of both genes (STYY203) produced a dramatic 3000-fold reduction (Fig. 2). Importantly, transformation of both STYY202 and STYA201 with the YgdP expression plasmid pTrc-YgdP restored full invasive capacity. This strongly suggests that loss of invasion is primarily related to a common property of the genes but do not yet convey the detailed picture.

**Results**

**Cell Growth, Morphology, and Motility**—Apparent doubling times in LB determined from optical density measurements were similar for WT, STYA201, and STYY202 (20–22 min). However, STYA201 showed a much longer lag-phase after inoculation compared with the others (4.5 versus 1.5 h) and did not attain as high a final cell density. Microscopic examination showed STYA201 formed long filaments, 20–30-times normal
length. Filamentous growth has previously been observed as a consequence of apaH deletion in E. coli (16).

Metabolic Activities—When tested for expression of a range of metabolic enzymes using the API ZYM and API 50 CH systems, WT cells were found reproducibly (n = 3) to not express cystine arylamidase, α-glucosidase, or β-glucuronidase activity, whereas such activity was detectable in each of the three mutants. The α-glucosidase activity was relatively lower in STYAY203 compared with STYA201 and STYAY203. No differences were observed in the expression of the remaining enzymes (alkaline phosphatase, C4 esterase, C8 esterase/lipase, C14 lipase, leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol phosphohydrolase, α-galactosidase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase). With regard to carbohydrate utilization the only differences were between the WT and STYA201 and STYAY203. The WT and STYAY203 strains were able to utilize myo-inositol and n-tagatose, whereas STYA201 and STYAY203 could not (n = 3). These results indicate that apaH and ygdP deletion has substantial but specific effects on the cells.

PtsP Expression—In S. Typhimurium and other enterobacteria, ygdP is in an operon with the downstream ptsP gene. E. coli ptsP encodes Enzyme PII, a component of a P-enolpyruvate-dependent phosphotransferase system believed to be involved in the regulation of RpoN-dependent operons (37). PtsP has been shown to be a virulence factor in Pseudomonas aeruginosa (38) and Legionella pneumophila (39), hence, it was important to show whether insertional deletion of ygdP had also disrupted ptsP expression. Reverse transcription-PCR analysis showed that ptsP expression was unaffected. An identical PCR
creases in Np
intracellular survival by restricting potentially deleterious in-
logue, IalA, was required for intracellular invasion to enhance
Previously, we suggested that the
vincing argument for the involvement of Np
combating host cell-mediated oxidative stress. The most con-
subsequently reduce their ability to adhere to and then invade
product was amplified from WT and STYY202 total RNA using
ptsP gene-specific primers (Fig. 4). Thus, the phenotype of
STYY202 is not simply due to loss of PtsP expression.

Discussion
Many genes and gene products are required for the success-
fual invasion of mammalian cells by S. enterica and similar bacteria (40, 41). To these we can now add ygdP and apaH.
Previously, we suggested that the B. bacilliformis YgdP homologue, IalA, was required for intracellular invasion to enhance
intracellular survival by restricting potentially deleterious in-
creases in NpN levels caused by oxidative attack by the
invaded cell (18). This hypothesis was based on the well estab-
lished increases in the levels of NpN in cells exposed to
oxidising agents (23, 42). However, the data here show that, at
least in S. Typhimurium, an increase in NpN has profound
effects on the bacteria in the absence of mammalian cells which
subsequently reduce their ability to adhere to and then invade
the cells. This does not support a role for these enzymes in
combating host cell-mediated oxidative stress. The most con-
vincing argument for the involvement of NpN in the invasive
phenotype is the transcomplementation of the ΔapaH mutant
by ygdP.

The increases in total ApN measured in the mutants were
less than initially expected, given the 10–100-fold increase
previously reported upon deletion of the E. coli apaH gene
alone (15, 16). However, it is not known if ygdP is expressed in
the E. coli strains used in these studies; its lack of discovery
until recently (and then only as a recombinant product) sug-
gests that it may not be. In contrast, it is clearly expressed in
S. Typhimurium LT2 (Table II). The modest 3.5-fold increase in
ApN upon apaH deletion appears to be sufficient to cause the
filamentous, non-invasive phenotype. However, it is possible
that this overall figure for ApN (comprising predominantly
ApN) hides a more dramatic rise in one or more specific minor
species that is/are primarily responsible for the phenotype or
that a non-adenylated, and therefore undetected, NpN is the
critical species involved. A detailed analysis of the specific
NpN content of the mutants is clearly required.

Of the two NpN hydrolases, loss of ApaH has much the
greater effect. In E. coli, deletion of apaH has previously
been shown to cause filamentation and to decrease transcription of
motility and chemotaxis genes by inhibiting expression of the
RpoF alternative sigma factor (16). RpoF is itself regulated by
the cAMP/cAMP-binding protein complex via the flhDC master
operon (43), and since the transcription of other catabolite-
repressible, cAMP/CAP-controlled genes such as lacZ and galK
is also substantially reduced, increased NpN may directly
interfere with the production or function of the cAMP-CAP
complex (16). RpoF also regulates flagellar operons in S. Typhi-
murium (44), so similar changes may occur in S. Typhi-
murium when NpN is increased. Indeed we have observed a
complex pattern of changes in flagellar and fimbral expression
in the mutants.2 This could be a major factor in the loss of
adhesion and invasion and is currently under investigation.

With regard to a possible functional connection between
ygdP and ptsP, both of which are virulence genes and which
together comprise an operon, it is possible that increased NpN
may affect PtsP (Enzyme INtr) directly by binding to its regu-
laratory domain (37). Expression of ygdP along with ptsP may be
necessary to ensure the proper control of NpN concentration
under conditions where the activity of Enzyme IStr is required.
Thus, increased NpN may affect the transcription of RpoN-, as
well as RpoF-, regulated genes. Such genes are significant in
number (43, 45). What is clear is that extensive but specific
metabolic changes have occurred in the null mutants as a
consequence of an increase in some NpN species. This is
shown by the changes in metabolic enzymes and carbohydrate
utilization that would be expected consequences of changes in
transcription factor activity. In R. prowazekii, the YgdP homol-
ogue InvA is co-expressed with a putative two-component re-
ponse regulator protein Rrp (46). A clear role for InvA in the
entry of rickettsiae into animal cells has not yet been shown, so
it is too early to draw a comparison between these two rather
different systems.

It will now be of interest to determine whether the above
effects of ygdP and apaH deletion on the invasion of cultured
cells extend to a reduction in virulence in an animal model
system. If so, then YgdP and particularly ApaH, which has no
known mammalian orthologue, may represent useful targets

2 R. M. La Ragione, M. J. Woodward, T. M. Ismail, C. A. Hart, and
A. G. McLennan, unpublished data.
for new antibacterial agents. Substrate analogue inhibitors of
E. coli ApaH have already been synthesized that could serve as
starting points for the design of inhibitors of intracellular
invasion (47, 48).

Acknowledgments—We are grateful to H. E. Allison and J. R.
Saunders for advice.

REFERENCES

1. McLennan, A. G. (ed) (1992) ApA and Other Dinucleoside Polyphosphates,
CRC Press Inc., Boca Raton, FL.
2. McLennan, A. G. (2000) Pharmacol. Ther. 87, 73–89
3. McLennan, A. G., Barnes, I. D., Blackburn, G. M., Brenner, C., Guranowski,
A., Miller, A. D., Rovira, J. M., Rotllan, P., Soria, B., Tanner, J. A., and
Sillers, A. (2001) Drug Dev. Res. 52, 249–259
4. Nishimura, A., Moriya, S., Ukai, H., Nagai, K., Wachi, M., and Yamada, Y.
(1997) Genes Cells 2, 401–413
5. Nishimura, A. (1998) Trends Biochem. Sci. 23, 157–159
6. Johnston, D. B., and Farr, S. B. (1991) EMBO J. 10, 3897–3904
7. Fuge, E. K., and Farr, S. B. (1993) J. Bacteriol. 175, 2321–2326
8. Brevet, A., Chen, J., Levecque, F., Plateau, P., and Blanquet, S. (1989) Proc.
Natl. Acad. Sci. U. S. A. 86, 8275–8279
9. Sillers, A., and Sillers, M. A. G. (2000) Pharmacol. Ther. 87, 91–102
10. Plateau, P., Fromant, M., Brevet, A., Gesquière, A., and Blanquet, S. (1985)
Biochemistry 24, 914–922
11. Guranowski, A., Jakubowski, H., and Holler, E. (1983) J. Biol. Chem. 258,
14784–14789
12. Guranowski, A. (2000) Pharmacol. Ther. 87, 117–139
13. Barton, G. J., Cohen, P. T. W., and Barford, D. (1994) Eur. J. Biochem.
220, 225–237
14. Lohe, D. L., Denu, J. M., and Dixon, J. E. (1995) Structure (Lond.) 3, 987–990
15. Levecque, F., Blanchin-Roland, S., Fayat, G., Plateau, P., and Blanquet, S.
(1995) J. Mol. Biol. 212, 319–329
16. Farr, S. B., Arnosti, D. N., Chamberlin, M. J., and Ames, B. N. (1989) Proc.
Natl. Acad. Sci. U. S. A. 86, 5010–5014
17. Conyers, G. B., and Bessman, M. J. (1999) J. Biol. Chem. 274, 1203–1206
18. Cartwright, J. L., Britton, P., Minnick, M. F., and McLennan, A. G. (1999)
Biochem. Biophys. Res. Commun. 256, 474–479
19. Mitchell, S. J., and Minnick, M. F. (1995) Infect. Immun. 63, 1552–1562
20. Badger, J. L., Wess, C. A., and Kim, K. S. (2000) Mol. Microbiol. 36, 174–182
21. Bessman, M. J., Walsh, J. D., Dunn, C. A., Swaminathan, J., Weldon, J. E., and
Shen, J. Y. (2001) J. Biol. Chem. 276, 37834–37838
22. Gaywee, J., Xu, W., Radulovic, S., Bessman, M. J., and Azad, A. F. (2002) Mol.
Cell. Proteom. 1, 179–185
23. Bochner, B. R., Lee, P. C., Wilson, S. W., Cutler, C. W., and Ames, B. N. (1984)
Cell 37, 225–232
24. Lee, P. C., Bochner, B. R., and Ames, B. N. (1983) J. Biol. Chem. 258,
6827–6834
25. Siaerela, M., Asikainen, S., Alahuuusa, S., and Fives-Taylor, P. (1998) Anaerobe
4, 139–144
26. Abdelghany, H. M., Gasmi, L., Cartwright, J. L., Bailey, S., Rafferty, J. B., and
McLennan, A. G. (2001) Biochem. Biophys. Acta 1550, 27–36
27. Herrero, M., de Lorenzo, V., and Timmis, K. N. (1990) J. Bacteriol. 172,
6557–6567
28. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A
Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring
Harbor, NY
29. AbdelRahim, S., and McLennan, A. G. (2002) BMC Biochemistry
http://www.biomedcentral.com/1471-2091/3/5
30. Wach, A., Brachat, A., Pühmann, R., and Philippsen, P. (1994) Yeast 10,
1793–1808
31. Sarkar, M. R., and Cornelius, G. R. (1997) Mol. Microbiol. 23, 409–411
32. Murphy, G. A., Halliday, D., and McLennan, A. G. (2000) Cancer Res. 60,
2342–2344
33. Bochner, B. R., and Ames, B. N. (1982) J. Biol. Chem. 257, 9759–9769
34. Plateau, P., Fromant, M., Kepes, F., and Blanquet, S. (1987) J. Bacteriol. 169,
419–424
35. Prescott, M., Thorne, N. M. H., Milne, A. D., and McLennan, A. G. (1992) Int.
J. Bacteriol. 24, 565–571
36. Fletcher, J. N., Embaye, H. E., Getty, B., Bitt, R. M., Hart, C. A., and
Saunders, J. R. (1992) Infect. Immun. 60, 2229–2236
37. Babus, R., Reizer, J., Paulsen, I., and Saier, M. H. (1999) J. Biol. Chem. 274,
26185–26191
38. Tan, M.-W., Rahme, L. G., Sternberg, J. A., Tompkins, R. G., andAusubel,
F. M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 2408–2413
39. Higa, F., and Edelstein, P. H. (2001) Infect. Immun. 69, 4782–4789
40. Galan, J. E. (2001) Annu. Rev. Cell Dev. Biol. 17, 53–86
41. Galan, J. E. (1996) Curr. Top. Microbiol. Immunol. 209, 43–60
42. Lee, P. C., Bochner, B. R., and Ames, B. N. (1983) Proc. Natl. Acad. Sci.
U. S. A. 80, 7496–7500
43. Helmann, J. D. (1991) Mol. Microbiol. 5, 2875–2882
44. Ohnishi, K., Kutsukake, K., Suzuki, H., and Iino, T. (1990) Mol. Gen. Genet.
223, 139–147
45. Merrick, M. J. (1993) Mol. Microbiol. 10, 903–909
46. Gaywee, J., Radulovic, S., Higgins, J. A., and Azad, A. F. (2002) Infect. Immun.
70, 6346–6354
47. Guranowski, A., Starzynska, E., Taylor, G. E., and Blackburn, G. M. (1989)
Biochem. J. 262, 241–244
48. McLennan, A. G., Taylor, G. E., Prescott, M., and Blackburn, G. M. (1989)
Biochemistry 28, 3868–3875