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TypA is a virulence regulator and is present in many pathogenic bacteria

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Tyrosine phosphorylation is rare in prokaryotes and, despite its pivotal function in the control of cell division and differentiation in eukaryotes, its biological role in bacteria is poorly understood. In bacteria it has been shown to play a minor role in protein phosphorylation, which suggests that it may have a more significant function elsewhere.[1-3] TypA has gained in interest recently due to the fact that it is tyrosine phosphorylated in certain *Escherichia coli*. Early work in an *E. coli* K-12 *typA* mutant showed that protein expression was altered, thereby suggesting a regulatory role for TypA.[4,5]

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Table 1. Sequence similarity between 39 of the 59 bacterial species known to contain TypA homologues

Species	*E. coli* identity (%)
Escherichia coli K-12 MG1655	100
Escherichia coli Enteropathogenic 0127:H7 strain E2348-69	100
Salmonella typhi	96.8
Salmonella typhimurium	95.9
Salmonella paratyphi	95.6
Escherichia coli 0157	94
Yersinia pestis	89.4
Pasteurella multocida PM70	84.1
Haemophilus influenzae Rd	82.1
Vibrio cholerae	77
Shewanella putrefaciens	76.9
Vibrio parahaemolytica	76.2
Pseudomonas aeruginosa	73.5
Neisseria gonorrhoeae	64
Stenotrophomonas maltophila	64
Neisseria meningitidis MC58	63.9
Bordetella pertussis	63.2
Acidothiobacillus ferrooxidans	62.3
Clostridium acetobutylicum	56.5
Streptococcus pneumoniae	56.3
Streptococcus pyogenes	55.9
Deinococcus radiodurans	55.5
Bacillus subtilis	54.9
Streptococcus mutans	54.9
Campylobacter jejuni NCTC 11168	54.7
Helicobacter pylori J99	54.2
Helicobacter pylori 26695	54.2
Chlorobium tepidum	53.8
Synechocystis PCC6803	53.3
Rickettsia prowazekii	53.2
Clostridium difficile	51.9
Streptococcus agalactiae	51
Porphyromonas gingivalis W83	50.3
Mycobacterium avium	49.1
Mycobacterium bovis	48.7
Mycobacterium tuberculosis H37 Rv	48.7
Mycobacterium tuberculosis CSU#93	48.7
Corynebacterium diphtheriae	48.5
Mycobacterium leprae	47.9

Interestingly, the expression of the global regulator H-NS is increased, as is the expression of the carbon starvation protein CspA and the universal stress protein UspA.[6] H-NS is an important global regulator and thus also plays a role in virulence.[7] CspA is important in responding to environmental stimuli,[8] while UspA is important in responding to various stress stimuli, including antibiotics.[9,10]

Further work in the enteropathogenic *E. coli* (EPEC) derivative MAR001 shows that *typA* has homology with members of the GTPase superfamily such as elongation

factor G.[6] Studies in EPEC MAR001 and EPEC E2348/69 show that TypA is tryosine phosphorylated.[6] However, mixing extracts of TypA from EPEC MAR001 with extracts of TypA from *E. coli* K-12 does not result in tyrosine phosphorylation of the *E. coli* K-12 TypA.[4,6] Interestingly, EPEC E2348/69 *typA* mutants do not grow below room temperature (~20°C).[11]

It has also been shown that *typA* may have a role in the attaching and effacing phenotype of EPEC, thereby impairing its ability to adhere to and invade cultured cells.[12,13] Further evidence suggests that *typA* is a regulator in the absence of the plasmid-encoded regulatory (*per*) genes in EPEC (unpublished data), while recent data also show that TypA regulates the locus of enterocyte effacement (LEE) and *espC* pathogenicity islands in EPEC.[14]

The role of TypA in the virulence of prokaryotes is further supported by work carried out on *Salmonella enterica* serovar *typhimurium*. It has been shown that the protein BipA, a TypA homologue of *S. enterica*, is strongly induced after exposure to human antimicrobial defence protein BPI.[15] BipA is also a member of the GTPase superfamily and shows significant homology to GTP-binding translation elongation factors. Subsequently, it has been shown that BipA and TypA are the same protein.

Based on the studies above, TypA is thought to be a global virulence regulator in *E. coli* and salmonellas. This is interesting as the advent of genomic sequencing indicates that homologues of certain virulence genes are present in many bacterial pathogens. Such studies help to better understand the molecular ecology and population biology of pathogenic and commensal bacteria.

There is now strong evidence to suggest that pathogens acquire genetic material from commensal bacteria, thereby increasing their resistance to antibiotics and improving their survival ability in the environment.[16,17] Importantly, there is evidence to show that the reverse also occurs: commensals acquire genetic material from pathogenic bacteria, thereby increasing their ability to cause disease. Therefore, our ability to understand the importance of genes that have a regulatory role is important, and resulting studies may lead to improved therapies or vaccines.

Thus, it is important to look at the incidence of the *typA* gene across different bacterial genera. If its presence across diverse species can be demonstrated, as well as high levels of homology, then this may help in understanding the biology of the protein.

In this brief study, the importance of the *typA* gene in general was ascertained by determining its presence in other pathogenic and non-pathogenic bacteria. Using a BLAST-translated nucleotide (tblastx) search (http://www.ncbi.nlm.nih.gov/BLAST/), 59 different bacterial species possessing TypA homologues were identified. Of these, 37 were human bacterial pathogens, which suggests that TypA may have an important role in these bacteria. TypA homology ranged from 48% for *Mycobacterium tuberculosis* to 89% for *Yersinia pestis* (Table 1).

Although a number of human pathogens, such as *Chlamydia pneumoniae*, *C. trachomatis*, *Borrelia burgdorferi*, *Mycoplasma pneumoniae* and *Treponema pallidum*, do not possess TypA homologues, these are not typical bacteria. More importantly, pathogens of significant public health importance, such as salmonellas, *Yersinia pestis*, *Haemophilus influenzae* and *Neisseria meningitidis*, show the highest TypA homology.

Although early genome sequencing studies concentrated on bacterial pathogens, this is no longer the case and therefore these bacterial pathogens are truly representative of those possessing TypA homologues. Control measures are available for some of the pathogens but they remain important diseases worldwide, particularly in developing countries where vaccines may not be widely available. Also, many of the pathogens are re-emerging.

TypA homologues exist in many pathogenic bacteria other than *E. coli*, which further suggests that TypA may be involved in virulence gene regulation. Important questions remain to be answered. Why is the gene present in both pathogenic and commensal bacteria? Does the gene play a different role in these bacteria or does *typA* play a defined role in pathogens only? The importance of genetic transfer must also be addressed and whether or not certain conserved regions within the gene are particularly important.

A number of laboratories, including ours, are performing further work in an attempt to answer such questions. TypA, as a global virulence regulator, has potential as a vaccine candidate for a diverse range of human and animal pathogens. While reverse vaccinology is helping to identify vaccine candidates,[18] TypA has already been identified and therefore requires further *in vitro* and *in vivo* analysis to confirm its potential. □

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Molecular typing of Nigerian *Helicobacter pylori* isolates by *glmM* restriction fragment length polymorphism

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Helicobacter pylori is recognised as the causative agent for chronic gastritis and an essential agent in the development of ulcers, and is implicated as a major risk factor for the development of gastric cancer.[1] Several typing techniques are used for epidemiological and clinical purposes and some of these have confirmed the genomic variability of *H. pylori*.[2-4] The techniques most frequently reported for typing are polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis and randomly amplified polymorphic DNA (RAPD) analysis.

A number of authors have reported on the molecular typing of *H. pylori* using the urease A and B genes but few have used *glmM*, formerly *ureC*, for epidemiological typing and for monitoring recrudescence.[5-8] *glmM* RFLP is reliable, highly reproducible and most useful in monitoring reinfection versus recrudescence.[9-11] Although the main role of *glmM* is now recognised to be related to cell-wall synthesis and not to urease activity, it is still used for genotyping.[12]

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Table 1. Restriction enzyme digest profiles of 41 *H. pylori* isolates using PCR-RFLP of the *glmM* gene

			Restriction enzyme pattern	
Isolate No.	Diagnosis	*cagA* status	*Hha*I	*Sau*3A
1	NUD	–	1	1
2	Ulcer	+	1	2
3	Ulcer	+	2	3
4	NUD	+	3	4
5	Ulcer	+	2	5
6	Ulcer	+	1	6
7	Ulcer	+	1	7
8	Ulcer	+	1	8
9	NUD	+	4	1
10	NUD	+	3	1
11	NUD	–	2	1
12	NUD	+	1	1
13	Ulcer	+	2	9
14	NUD	+	1	10
15	NUD	+	3	1
16	NUD	+	4	1
17	Ulcer	+	5	2
18	NUD	+	5	2
19	NUD	+	2	1
20	Ulcer	+	3	4
21	Ulcer	+	1	10
22	NUD	+	1	1
23	NUD	+	1	1
24	Ulcer	+	5	2
25	NUD	+	4	1
26	NUD	+	2	3
27	Ulcer	+	5	3
28	Ulcer	+	3	5
29	NUD	+	1	6
30	Ulcer	–	3	7
31	Ulcer	+	3	10
32	Ulcer	+	1	8
33	NUD	+	1	1
34	NUD	+	4	1
35	NUD	+	5	1
36	Ulcer	+	3	10
37	NUD	+	5	1
38	NUD	+	2	1
39	NUD	+	2	1
40	Ulcer	+	5	10
41	Ulcer	+	5	3

NUD: non-ulcer dyspepsia

Isolates 22 and 23, 10 and 15 and 25 and 34 were from the same patients

This study aims to type Nigerian *H. pylori* strains using a previously described *glmM* PCR-RFLP technique,[9,10] and to examine multiple isolates from the same patients with non-