The distinctive cell division interactome of *Neisseria gonorrhoeae*

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Abstract

**Background:** Bacterial cell division is an essential process driven by the formation of a Z-ring structure, as a cytoskeletal scaffold at the mid-cell, followed by the recruitment of various proteins which form the divisome. The cell division interactome reflects the complement of different interactions between all divisome proteins. To date, only two cell division interactomes have been characterized, in *Escherichia coli* and in *Streptococcus pneumoniae*. The cell division proteins encoded by *Neisseria gonorrhoeae* include FtsZ, FtsA, ZipA, FtsK, FtsQ, FtsI, FtsW, and FtsN. The purpose of the present study was to characterize the cell division interactome of *N. gonorrhoeae* using several different methods to identify protein-protein interactions. We also characterized the specific subdomains of FtsA implicated in interactions with FtsZ, FtsQ, FtsN and FtsW.

**Results:** Using a combination of bacterial two-hybrid (B2H), glutathione S-transferase (GST) pull-down assays, and surface plasmon resonance (SPR), nine interactions were observed among the eight gonococcal cell division proteins tested. ZipA did not interact with any other cell division proteins. Comparisons of the *N. gonorrhoeae* cell division interactome with the published interactomes from *E. coli* and *S. pneumoniae* indicated that FtsA-FtsZ and FtsZ-FtsK interactions were common to all three species. FtsA-FtsW and FtsK-FtsN interactions were only present in *N. gonorrhoeae*. The 2A and 2B subdomains of FtsA*Ng* were involved in interactions with FtsQ, FtsZ, and FtsN, and the 2A subdomain was involved in interaction with FtsW.

**Conclusions:** Results from this research indicate that *N. gonorrhoeae* has a distinctive cell division interactome as compared with other microorganisms.

**Keywords:** Cell division, Interactome, *N. gonorrhoeae*, Protein-protein interaction, Bacterial two-hybrid assay, Surface plasmon resonance, GST pull-down, FtsA domains

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**Background**

Cell division is essential for bacterial survival. In *Escherichia coli* (Ec), normal cell division is driven by the formation of an FtsZ-ring at the division site [1], followed by the recruitment of other essential proteins, which together form the divisome [2]. Genes encoding most cell division proteins are located in a conserved region, the division and cell wall (dcw) cluster [3]. dcw clusters have been identified in most bacterial species, including *E. coli*, *Bacillus subtilis* (Bs), *Streptococcus pneumoniae* (Sp), *Caulobacter crescentus* (Cc) and *Neisseria gonorrhoeae* (Ng) [4–7]. Although the gene organization of the dcw cluster varies in different bacteria species [8], proteins involved in the cell division process are relatively conserved [9, 10].

*E. coli* encodes ten essential cell division proteins, including FtsZ, FtsA, ZipA, FtsK, FtsQ, FtsB, FtsL, FtsW, FtsI, and FtsN [11, 12]. Assembly of the FtsZ-ring structure is initiated with the polymerization of FtsZ, driven by GTP hydrolysis, at the mid-cell [13]. FtsA and ZipA are recruited by FtsZ and anchor FtsZ to the inner membrane [14]. After the recruitment of FtsK, a DNA translocase involved in DNA segregation [15–17], the protein complexes FtsQ-FtsB-FtsL and FtsW-FtsI are localized to the septal ring, sequentially [15, 18]. Recent studies showed that the FtsQ-FtsB-FtsL complex serves as a signal sensor which promotes cell wall remodeling necessary for cell constriction [19]. FtsI is a high-molecular-
weight transpeptidase that cross-links glycan strands. The FtsW-FtsI complex is part of the peptidoglycan synthesis machinery, and FtsW, a lipid II flippase, transports the cell wall precursor across the membrane [20, 21]. FtsN is recruited as the last essential division protein that initiates cell constriction [22].

Using a bacterial two-hybrid (B2H) assay, an E. coli cell division protein-protein interaction network, the cell division interactome, which included 16 interactions between 10 cell division proteins, was identified [23, 24]. The cell division interactome of S. pneumoniae was also characterized using a combination of B2H and co-immunoprecipitation assays [25]. A total of 17 interactions was observed among nine cell division proteins of S. pneumoniae which included FtsZ, FtsA, FtsK, DivL, DivC, FtsL, FtsW, and PBP2x [25]. To date, E. coli and S. pneumoniae are the only two organisms with characterized cell division interactomes [23–25].

N. gonorrhoeae is a Gram-negative diplococcus that causes gonorrhea in humans [26]. Previous studies on N. gonorrhoeae cell division focused on its Min system which localizes FtsZ to the mid-cell, and FtsZ [27–29]. N. gonorrhoeae also contains a dcw cluster which encodes 5 cell division proteins - FtsZ, FtsA, FtsQ, FtsW, and FtsI [7]. Other non-dcw cluster division proteins encoded by N. gonorrhoeae include ZipA, FtsK, and FtsN. As compared to E. coli, N. gonorrhoeae lacks FtsB and FtsL [7].

To investigate the cell division interactome in N. gonorrhoeae, its cell division protein interactions were identified using a combination of B2H and glutathione S-transferase (GST) pull-down assays, as well as surface plasmon resonance (SPR). We identified nine interactions among the eight cell division proteins tested. We also identified the subdomains of FtsANg involved in its interaction with FtsQNg, FtsZNg, FtsNNg, and FtsWNg. Comparison of the cell division interactomes of E. coli, S. pneumoniae and N. gonorrhoeae indicates that N. gonorrhoeae possesses a distinctive cell division interactome.

Methods

Strains and growth conditions

The bacterial strains and plasmids used in this study are shown in Table 1. E. coli DH5α and XL1-Blue were used as hosts for cloning. E. coli BL21 (DE3) and C41 (DE3) were used as hosts for protein purification. E. coli R721 was used in B2H assays [30]. E. coli DH5α, XL1-Blue, BL21 (DE3) and C41 (DE3) were grown in Luria-Bertani (LB) medium (BD Difco, Sparks, MD), for 16–18 h (hr), at 37 °C. E. coli R721 was grown under the same conditions and incubated at 34 °C, as described previously [24].

N. gonorrhoeae CH811 was grown on GC medium base agar (GCMB, Oakville, ON), supplemented with Kellogg’s defined supplement (GCMBK, 40 g D-glucose, 1 g glutamine, 10 ml of 0.5% ferric nitrate and 1 ml of 20% cocarboxylase), at 35 °C, in a humid environment, with 5% CO₂, for 18 to 24 h [31].

When required, the following concentrations of antibiotics were added to LB medium: 100 μg/ml ampicillin (Sigma, Oakville, ON) or 50 μg/ml kanamycin (Sigma). For B2H assays, 34 μg/ml chloramphenicol (Sigma), 30 μg/ml kanamycin, and 50 μg/ml ampicillin were added to LB medium.

DNA manipulations

N. gonorrhoeae CH811 genomic DNA was purified using a QIAamp® genomic DNA kit (Qiagen, Mississauga, Ontario, Canada). DNA samples were stored at −20 °C. Oligonucleotides for polymerase chain reaction (PCR) amplifications were synthesized by Invitrogen (Table 2; Burlington, Ontario, Canada). PCRs were performed in a GeneAmp® PCR system 9700 (Applied Biosystems, Foster City, CA, USA) as follows: 4 min (min) at 94 °C, 30 cycles of denaturation for 1 min at 94 °C, annealing for 45 s (s) at 55 °C, extension for 1.5 mins at 72 °C, and 10 mins at 72 °C. PCRs were carried out in 100-μl (final volume) mixtures comprising 71.5 μl double-distilled H₂O (ddH₂O), 10 μl of 10× PCR buffer [15 mM MgCl₂, 4 μl of 10 mM deoxynucleoside triphosphate (dNTP), 2 μl of each primer (0.2 μg/μl), 0.5 μl of Taq DNA polymerase (5 U/μl; New England Biolabs, Ontario, Canada)], and, 10 μl of purified N. gonorrhoeae CH811 genomic DNA suspension.

Bacterial two-hybrid assays

The method developed by Di Lallo et al. [24] was used for all B2H assays. ftsA, ftsK, ftsQ, ftsI, ftsW, and ftsN were amplified from N. gonorrhoeae CH811 by PCR using the primer pairs P1/P2, P3/P4, P5/P6, P7/P8, P9/P10, and P11/P12 (Table 2), respectively. PCR amplicons were digested with BamHI and SalI and ligated into previously digested pclp22 and pcl434 vectors, to produce pclp22-A, pclp22-K, pclp22-I, pclp22-W, pclp22-Q, pclp22-N, pcl434-A, pcl434-K, pcl434-I, pcl434-W, pcl434-Q, and pcl434-N (Table 3). zipANg was amplified from N. gonorrhoeae CH811 genomic DNA using the primer pair P13/P14 (Table 2); the PCR amplicons was digested with BglII and BamHI, and ligated into pre-digested pclp22 and pcl434 to produce pclp22-Δ, pclp22-Δ-K, pclp22-Δ-I, pclp22-W, pclp22-Q, pclp22-N, pcl434-Δ, pcl434-Δ-K, pcl434-Δ-I, pcl434-Δ-W, pcl434-Δ-Q, and pcl434-Δ-N constructs were generated previously [32].

The expression of ftsANg, ftsZNg and zipANg from B2H constructs was verified by Western blot analysis using appropriate antibodies prepared in our lab using previously described methods [33]. These proteins were expressed from the vectors under the conditions tested (data not shown). The expression of these proteins indicated that any negative B2H interactions involving them was not a function of lack of expression.
To ascertain what subdomains of FtsA<sub>Ng</sub> interacted with gonococcal cell division proteins FtsZ<sub>Ng</sub>, FtsQ<sub>Ng</sub>, FtsW<sub>Ng</sub>, or FtsN<sub>Ng</sub>, six previously created truncations of the protein (T1, T2, T3, T4, T5, and T6; Additional file 1: Figure S1) were used [33]. Plasmid constructs for B2H assays were previously generated [33].

B2H assays were performed as described previously [24]. This assay is based on the reconstitution of a chimeric repressor that binds to the 434/P22 hybrid operator and represses the expression of a downstream lacZ gene in E. coli R721. Each gene tested for a potential interaction was cloned into pcI<sub>p22</sub> and pcI<sub>434</sub> and recombinant constructs were transformed into E. coli R721 either singly or in combination. N. gonorrhoeae FtsZ self-interaction was used as positive control. R721 without plasmids and single plasmid transformants were used as negative controls. R721 without plasmids had a β-galactosidase activity of 2504 ± 34 Miller units. The β-galactosidase activity of each combination was compared to that of R721. Values of less than 50% (<1250 Miller Units) indicate a positive interaction between two proteins, while values of more than 50% (>1250 Miller Units) indicate a negative interaction [24]. Statistical analyses were performed using the unpaired Student t-test. Standard deviations were determined for the mean value of Miller units where three independent experiments were performed.

Table 2 Primers designed in this study

| Primer name | Sequences (5′-3′) |
|-------------|------------------|
| P1          | FtsA-reBamHI     | GCCGGGATCCTCAAGAGGTGTCTTTCAATCC |
| P2          | FtsA-fwSalI     | GCCGCAGCACATTCAAGACACAGCAGAAAAGTAC |
| P3          | fwSalI-fsK      | CGCGGTCAAGATTTTAGATCATGTTCTATCCTATAGT |
| P4          | reBamHI-fsK     | CGCGGGATCCATGATCTCAAGACAGTCTCAAGACAGCAG |
| P5          | fwSalI-ftsQ     | CGCGGTCAAGATTTTAGATCATGTTCTATCCTATAGT |
| P6          | reBamHI-ftsQ    | CGCGGGATCCATGATCTCAAGACAGTCTCAAGACAGCAG |
| P7          | fwSalI-fsI      | CGCGGTCAAGATTTTAGATCATGTTCTATCCTATAGT |
| P8          | reBamHI-ftsI    | CGCGGGATCCATGATCTCAAGACAGTCTCAAGACAGCAG |
| P9          | fwSalI-ftsW     | CGCGGTCAAGATTTTAGATCATGTTCTATCCTATAGT |
| P10         | reBamHI-ftsW    | CGCGGGATCCATGATCTCAAGACAGTCTCAAGACAGCAG |
| P11         | fwSalI-ftsN     | CGCGGGATCCATGATCTCAAGACAGTCTCAAGACAGCAG |
| P12         | reBamHI-ftsN    | CGCGGGATCCATGATCTCAAGACAGTCTCAAGACAGCAG |
| P13         | fwBglII-ZipA    | CGCGGGATCCATGATCTCAAGACAGTCTCAAGACAGCAG |
| P14         | reBamHI-ZipA    | CGCGGGATCCATGATCTCAAGACAGTCTCAAGACAGCAG |
| P15         | FtsA-reEcoRI-pET30a | ATATCAGAATCTACTCTCTGAGGTGGTTCTCTCAATCC |
| P16         | FtsA-fwBglI-pET30a | AGCCCGACATGATGGAACACAGCAGAAGATACATC |
| P17         | fwBglII-ftsQ-pET30a | AGCCCGACATGATGGAACACAGCAGAAGATACATC |
| P18         | reEcoRI-ftsQ-pET30a | ATATCAGAATCTACTCTCTGAGGTGGTTCTCTCAATCC |
| P19         | FtsZ-fwBglI-II-pET30a | AGCCCGACATGATGGAACACAGCAGAAGATACATC |
| P20         | FtsZ-reEcoRI-pET30a | AGCCCGACATGATGGAACACAGCAGAAGATACATC |
| P21         | fwFtsA-BamHI-GST | CGCGGGATCCATGGAACACAGCAGAAGATACATC |
| P22         | fwEcoRI-FtsN    | GACGGATCTAGTGGAGGAATTTTCCAAATCCC |
| P23         | reXhoI-FtsN     | GACGGATCTAGTGGAGGAATTTTCCAAATCCC |
**Construction and purification of his-fusion proteins**

For His-fusion constructs, full-length ftsA, ftsQ, and ftsZ were PCR-amplified from *N. gonorrhoeae* CH811 genomic DNA using primer pairs P15/P16, P17/P18 and P19/P20 (Table 2), respectively. PCR amplicons were digested with EcoRI and BglII and ligated into pre-digested pET30a, to create pETA, pETQ, and pETZ. Plasmid pETA was transformed into *E. coli* C41 (DE3) and plasmids pETQ and pETZ were transformed into *E. coli* BL21 (DE3). The overexpression of all fusion proteins was induced with 400 μM IPTG, at 30 °C, for 2 h. Purification of His-FtsA Ng, His-FtsZNg, and His-FtsQ Ng was completed using His•Bind® Resin (EMD Millipore, Billerica, MA), following the manufacturer's instructions. His-FtsZNg was further treated with thrombin protease (EMD Millipore, Billerica, MA), overnight, at 4 °C, to cleave the N-terminal His tag. Thrombin was removed using 100 μl of p-aminobenzamidine-agarose (Sigma #A7155). FtsZ was dialyzed against MES buffer (50 mM MES, 300 mM KCl, 10 mM MgCl₂, pH 7.5) prior to use in FtsZ polymerization experiments [34].

| Plasmid | Relevant genotype | Source/Reference |
|---------|-------------------|------------------|
| pcIp22  | pC132 derivative carrying N-terminal end of P22 repressor | [30] |
| pcI434  | pACYC177 derivative carrying N-terminal end of 434 repressor | [30] |
| pcIp22-A | pcdp22 derivative carrying the ftsANg gene | This study |
| pcI434-A | pcI434 derivative carrying the ftsANg gene | This study |
| pcIp22-K | pcdp22 derivative carrying the ftsKNg gene | This study |
| pcI434-K | pcI434 derivative carrying the ftsKNg gene | This study |
| pcIp22-Q | pcdp22 derivative carrying the ftsQNg gene | This study |
| pcI434-Q | pcI434 derivative carrying the ftsQNg gene | This study |
| pcIp22-I | pcdp22 derivative carrying the ftsINg gene | This study |
| pcI434-I | pcI434 derivative carrying the ftsINg gene | This study |
| pcIp22-W | pcdp22 derivative carrying the ftsWNg gene | This study |
| pcI434-W | pcI434 derivative carrying the ftsWNg gene | This study |
| pcIp22-N | pcdp22 derivative carrying the ftsNNg gene | This study |
| pcI434-N | pcI434 derivative carrying the ftsNNg gene | This study |
| pcIp22-Z | pcdp22 derivative carrying the ftsZNg gene | [32] |
| pcI434-Z | pcI434 derivative carrying the ftsZNg gene | [32] |
| pcIp22-AT1 | pcdp22 derivative carrying the ftsANg gene fragment encoding amino acids 1–162 | [33] |
| pcIp22-AT2 | pcdp22 derivative carrying the ftsANg gene fragment encoding amino acids 1–194 | [33] |
| pcIp22-AT3 | pcdp22 derivative carrying the ftsANg gene fragment encoding amino acids 1–230 | [33] |
| pcIp22-AT4 | pcdp22 derivative carrying the ftsANg gene fragment encoding amino acids 231–301 | [33] |
| pcIp22-AT5 | pcdp22 derivative carrying the ftsANg gene fragment encoding amino acids 302–414 | [33] |
| pcIp22-AT6 | pcdp22 derivative carrying the ftsANg gene fragment encoding amino acids 351–414 | [33] |
| pcI434-AT1 | pcI434 derivative carrying the ftsANg gene fragment encoding amino acids 1–162 | [33] |
| pcI434-AT2 | pcI434 derivative carrying the ftsANg gene fragment encoding amino acids 1–194 | [33] |
| pcI434-AT3 | pcI434 derivative carrying the ftsANg gene fragment encoding amino acids 1–230 | [33] |
| pcI434-AT4 | pcI434 derivative carrying the ftsANg gene fragment encoding amino acids 231–301 | [33] |
| pcI434-AT5 | pcI434 derivative carrying the ftsANg gene fragment encoding amino acids 302–414 | [33] |
| pcI434-AT6 | pcI434 derivative carrying the ftsANg gene fragment encoding amino acids 351–414 | [33] |
| pET30a | KanR P7-6Xhis | EMD Millipore, Billerica, MA |
| pETA | pET30a derivative carrying the ftsANg gene | This study |
| pETQ | pET30a derivative carrying the ftsQNg gene | This study |
| pETZ | pET30a derivative carrying the ftsZNg gene | This study |
| pGEX2T | AmpR Plac::gst::lacIq | Amersham Bioscience, Uppsala, Sweden |
| pGEXA | pGEX2T derivative carrying the ftsANg gene | This study |
| pGEXN | pGEX2T derivative carrying the ftsANg gene | This study |
GST pull-down assay
For GST fusion constructs, full-length ftsA and ftsN were PCR-amplified, from *N. gonorrhoeae* CH811, using primer pairs P21/P18 and P22/P23 (Table 2), respectively. The ftsA amplicon was digested with BamHI and EcoRI and ligated into pre-digested pGEX2T, to create pGEXA (Table 3). The ftsN amplicon was digested with EcoRI and Xhol and ligated into pre-digested pGEX2T, producing pGEXN (Table 3). Plasmids pGEXA and pGEXN were transformed into *E. coli* C41 (DE3) and *E. coli* BL21 (DE), respectively. Overexpression of GST-FtsA and GST-FtsN was accomplished by induction with either 400 μM or 800 μM of IPTG, respectively, at 30 °C, for 2 h. Purification of GST-FtsA and GST-FtsN was carried out using GST•Bind™ Resin (EMD Millipore, Billerica, MA), following the manufacturer’s instructions.

Purified GST-fusion and His-fusion proteins were incubated with pre-equilibrated GST•Bind™ Resin in phosphate buffered saline (PBS) buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 0.5% Triton-X100, 1 mM DTT, pH 7.9) at 4 °C overnight. Pre-purified GST was used as a negative control. The pre-bound resin was collected by centrifugation and washed in PBS three times. Bound proteins were dissociated from resin by adding 5X Laemmli buffer, separated by electrophoresis on 10% sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE), and identified by Western blot using polyclonal anti-GST or anti-6 × His antibodies (Thermo Scientific; Waltham, MA), sequentially.

For FtsANg-FtsZNg interactions, the GST pull-down assay was performed in MES buffer (50 mM MES-NaOH, 50 mM KCl, 10 mM MgCl₂, 0.5% Triton-X100, 1 mM ATP, 2 mM GTP, pH 7.5) [34]. To promote the polymerization of FtsZNg necessary for this interaction, FtsZNg was treated with 2 mM GTP and 1 mM ATP, as described previously [34], before mixing with GST-FtsANg and GST•Bind™ Resin.

All GST pull-down assays were performed minimally in duplicate.

FtsZ polymerization assays
FtsZNg polymerization was measured by 90° angle light scattering using a Dynapro-MS800 instrument (Wyatt Technology Corporation) with a wavelength of 310 nm and a slit width of 0.5 mm. MES buffer is optimal for FtsZ polymerization which is required to observe an FtsA-FtsZ interaction [34, 35]. FtsZNg (~6 μM) in MES buffer (50 mM MES-NaOH, 50 mM KCl, 10 mM MgCl₂, pH 7.5) was injected into a 45 ul quartz cuvette and warmed to 30 °C, prior to the measurement. Data were collected, for 4 min, from unpolymerized FtsZNg to establish a baseline. GTP was then added to a final concentration of 2 mM and data were collected every 5 s for 25 min. Data were recorded and analyzed using Dynamics v5 software.

Negative stain electron microscopy was used to visualize FtsZNg polymers. 5 μl of FtsZ (6 μM) with, or without, GTP (final concentration 2 mM) was incubated, at 30 °C, for 5 min. The mixture was placed on a carbon-coated copper grid (400 mesh size) for 2 min and then blot dried. The grid containing FtsZNg was stained with 1% uranyl acetate, blotted, and air-dried for 3 h. Polymers were visualized and photographed using a Hitachi transmission electron microscopy HT7700.

Surface plasmon resonance (SPR)
Protein interactions were examined by SPR using a Bio-Rad XPR36 (Bio-Rad Laboratories) instrument and a ProteOn™ HTE Sensor Chip (Bio-Rad Laboratories). The chip surface was regenerated by injection of 0.5% SDS, 50 mM NaOH, 100 mM HCl and 300 mM EDTA, at a flow rate of 30 μl/min, for 120 s. Activation was performed using 500 μM of NiSO₄.

For FtsANg-FtsZNg and FtsANg-FtsQNg SPR experiments, ligands (i.e. His-FtsNNg for FtsANg-FtsNNg, and His-FtsQNg for FtsANg-FtsQNg interactions) were immobilized onto the sensor chip at a concentration of 200 nM. A two-fold dilution series of the analyte (FtsANg), in PBS buffer with Tween-20 (PBST; 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 0.1% BSA, 0.05% Tween-20, pH 7.9), was injected at a flow rate of 30 μl/min over the surface of the chip for 120 s. This was followed by an injection of PBST buffer for 300 s. Negative controls comprised a reference channel flowed with PBST buffer, and a chip surface immobilized with either FtsQNg or FtsNNg flowed with GST in PBST.

For the FtsANg-FtsZNg interaction, the SPR binding assay was performed using MES buffer, supplemented with 0.1% BSA, 0.05% Tween-20, and 1 mM ATP were added with the pH adjusted to 7.5. FtsANg was immobilized on the chip surface as described above. Each 120-s injection of polymerized FtsZNg was followed by an injection of supplemented MES buffer for 300 s for dissociation. Negative controls included a reference channel which was flowed with MES buffer containing 2 mM GTP, and the FtsANg immobilized chip surface flowed with GST in supplemented MES instead of polymerized FtsZNg.

All SPR data was analyzed using ProteOn Manager™ (Bio-Rad Laboratories). The sensorgram (i.e. a graph of the response unit versus time) was first substracted by the response units (RU) of the reference channel, with no immobilized ligands, to reduce the non-specific binding signals between analyte and empty chip surface. Then, the sensorgram was substracted with the RU signal with running buffer and ligand immobilized on the chip. Association and disassociation constants were obtained using the Langmuir 1:1 kinetic fit model, by nonlinear regression, using ProteOn Manager™. Each protein pair was tested minimally in duplicate.
Results
Identification of N. gonorrhoeae cell division protein interactions by bacterial two-hybrid assay
Using B2H assays, we investigated 28 potential interactions among eight gonococcal divisome proteins including FtsZ, FtsA, ZipA, FtsK, FtsI, FtsW, and FtsN. The results (Table 4) show that nine interactions, FtsZ-FtsA, FtsZ-FtsK, FtsZ-FtsW, FtsA-FtsK, FtsA-FtsQ, FtsA-FtsW, FtsA-FtsN, FtsI-FtsW, and FtsK-FtsN, displayed a residual $\beta$-galactosidase activity lower than 50%, indicating a positive interaction between these proteins in N. gonorrhoeae. The interaction between FtsA$_{Ng}$ and FtsN$_{Ng}$ had the lowest residual $\beta$-galactosidase activity (24%), indicating the strongest interaction. This was followed by FtsA$_{Ng}$-FtsK$_{Ng}$ (30%), FtsN$_{Ng}$-FtsK$_{Ng}$ (31%), FtsI$_{Ng}$-FtsW$_{Ng}$ (35%), FtsZ$_{Ng}$-FtsW$_{Ng}$ (39%), FtsA$_{Ng}$-FtsZ$_{Ng}$ (40%), FtsZ$_{Ng}$-FtsK$_{Ng}$ (41%), FtsA$_{Ng}$-FtsW$_{Ng}$ (45%), and FtsA$_{Ng}$-FtsQ$_{Ng}$ (48%) interactions. ZipA$_{Ng}$ did not directly interact with other cell division proteins as the residual $\beta$-galactosidase activity of all interactions was above 50% (Table 4).

GST pull-down of FtsA$_{Ng}$-FtsQ$_{Ng}$, FtsA$_{Ng}$-FtsZ$_{Ng}$ and FtsA$_{Ng}$-FtsN$_{Ng}$ interactions
To confirm the results of selected B2H assays, we examined several interactions (i.e. FtsQ$_{Ng}$-FtsA$_{Ng}$, FtsA$_{Ng}$-FtsN$_{Ng}$, FtsA$_{Ng}$-FtsZ$_{Ng}$) using GST pull-down assays. GST pull-down results (Fig. 1a) showed that His-FtsQ$_{Ng}$ was pulled down by GST-FtsA$_{Ng}$, but not GST itself (negative control), indicating an interaction between FtsA$_{Ng}$ and FtsQ$_{Ng}$. Using similar evaluation criteria, we ascertained that His-FtsA$_{Ng}$ pulled down FtsZ$_{Ng}$ and FtsN$_{Ng}$ because of the conflicting results observed with B2H and GST pull-down assays. GTP was added to promote FtsZ$_{Ng}$ polymerization (Additional file 2: Figure S2). The sensorgram indicated that FtsZ$_{Ng}$ interacted with FtsA$_{Ng}$ at concentrations of 6 $\mu$M and 12 $\mu$M (Fig. 2a), but not at concentrations lower than 6 $\mu$M (data not shown). Kinetic analysis showed that the FtsA$_{Ng}$-FtsZ$_{Ng}$ interaction had a slow association ($k_a = 3.56 \times 10^2$ M$^{-1}$ s$^{-1}$) and a significant

| Interaction     | pclP22 ZipA | FtsZ | FtsA | FtsK | FtsI | FtsQ | FtsW |
|-----------------|------------|------|------|------|------|------|------|
| FtsZ            | 71±2.62%   | 28±0.27% |
| FtsA            | 77±1.67%   | 40±2.46% |
| FtsK            | 78±2.45%   | 41±2.06% | 30±2.53% |
| FtsI            | 83±3.21%   | 91±3.92% | 83±3.10% | 76±2.00% |
| FtsQ            | 100±4.04%  | 73±2.15% | 48±2.59% | 88±3.09% | 90±2.82% |
| FtsW            | 87±2.95%   | 39±2.66% | 45±3.29% | 100±2.62% | 32±1.31% | 100±4.72% |
| FtsN            | 100±2.78%  | 76±1.25% | 24±1.66% | 31±0.67% | 88±3.21% | 97±2.10% |

Table 4 Interactions between eight cell division proteins in N. gonorrhoeae as determined by B2H assay

By comparison to positive controls (E. coli R721 without plasmids), interactions with less than 50% of residual $\beta$-galactosidase activity (framed) were considered as positive. FtsZ$_{Ng}$ self-interaction was used as a positive control. The numbers represent percentage of mean $\beta$-galactosidase activity, ± standard deviation.

*Statistically significant ($P \leq 0.05$); NS: not statistically significant ($P > 0.05$)
disassociation activity (kd = 5.31 × 10\(^{-3}\) s\(^{-1}\)), giving a KD value of 14.9 μM. This suggested that the interaction between FtsAN\(_G\) and FtsZ\(_N\) was likely transient. When GTP was absent from the FtsZ\(_N\) protein solution, no binding was detected between FtsAN\(_G\) and FtsZ\(_N\) (data not shown). The sensorgram of the interaction between FtsAN\(_G\) and the negative control (GST) also showed no binding activity (Fig. 2b), indicating the specificity of the SPR results for the interaction of FtsAN\(_G\) with FtsZ\(_N\).

For the SPR analysis of the FtsAN\(_G\)-FtsQ\(_N\) interaction, FtsAN\(_G\) was tested using various concentrations (from 31.25 nM to 250 nM; Fig. 2c). At 0 s, the association of FtsAN\(_G\) and FtsQ\(_N\) was observed immediately following injection of the FtsAN\(_G\) solution onto the FtsQ\(_N\)-labeled chip surface, with a rapid increase of response units (ka = 2.72 × 10\(^5\) M\(^{-1}\) s\(^{-1}\); Fig. 2c). This indicated a fast binding event between the two proteins. Disassociation between FtsAN\(_G\) and FtsQ\(_N\) was not significant (kd = 4.09 × 10\(^{-3}\) s\(^{-1}\)), suggesting this interaction was strong and stable (KD = 15.1 nM). The negative control, using non-interacting GST, did not cause any change in the response units (Fig. 2d).

The FtsAN\(_G\)-FtsN\(_N\) interaction was observed with an increasing concentration of FtsAN\(_G\) (62.5 nM, 125 nM, 250 nM and 500 nM; Fig. 2e). His-FtsN\(_N\) had a binding affinity (KD) of 53.3 nM with FtsAN\(_G\). The association and disassociation constants were 1.15 × 10\(^5\) M\(^{-1}\) s\(^{-1}\), and 6.16 × 10\(^{-3}\) s\(^{-1}\), respectively (Fig. 2e), indicating a strong interaction between FtsAN\(_G\) and FtsN\(_N\). The injection of non-interacting GST onto the FtsN\(_N\) immobilized chip surface did not cause any change in the response units (Fig. 2f).

### The 2A and 2B subdomains of FtsAN\(_G\) interacts with FtsZ\(_N\), FtsQ\(_N\), FtsW\(_N\) and FtsN\(_N\)

Since FtsAN\(_G\) interacted with FtsZ\(_N\), FtsQ\(_N\), FtsW\(_N\), and FtsN\(_N\), we further examined the interaction regions of FtsAN\(_G\) with these four proteins using B2H assays. Based on FtsAN\(_G\) homology modeling, six FtsAN\(_G\) truncations (T1-T6) were created (Additional file 1: Figure S1), which contained one or more FtsAN\(_G\) subdomains [33]. FtsZ\(_N\) self-interaction was used as a positive control. And negative controls included E. coli R721 without plasmids or carrying each single recombinant B2H vector in which the gene of interest had been cloned. FtsAN\(_G\) truncations T3, T4, and T5 interacted with FtsZ\(_N\) and FtsN\(_N\) (Figs. 3 and 4, blue bars). FtsAN\(_G\) truncations T1, T2, and T6 did not show an interaction with these proteins (Figs. 3 and 4, green bars). The T4 and T5 truncations included the 2B and 2A subdomains of FtsAN\(_G\), suggesting that these subdomains of FtsAN\(_G\) interacted with both FtsZ\(_N\) and FtsN\(_N\). The T3 construct contained also contained the 2A subdomain of FtsAN\(_G\), as compared to truncations T1 and T2, indicating that this subdomain was also involved in interactions with FtsZ\(_N\) and FtsN\(_N\). FtsQ\(_N\) interacted only
with the T4 and T5 truncations of FtsANg (Fig. 5, blue bars), indicating that the 2B and 2A2 subdomains, but not the 2A1 subdomain, were required for the FtsANg-FtsQNg interaction. Only the T5 truncation of FtsANg interacted with FtsWNg, suggesting that 2A2 subdomain was involved in the interaction with FtsWNg (Additional file 3: Figure S3). In summary, these results showed that the 2A1, 2A2 and 2B subdomains of FtsANg are required for its interaction with FtsQNg and FtsZNg. The FtsANg 2A2 and 2B subdomains are required for interaction with FtsQNg and the 2A2 subdomain is involved in the interaction with FtsWNg.

Discussion

The N. gonorrhoeae cell division interactome described in our study is the third cell division interaction network identified in bacteria, in addition to E. coli and S. pneumoniae (Fig. 6a) [23–25]. Compared to the other two interactomes (Fig. 6b and c), fewer interaction protein pairs are identified in N. gonorrhoeae (Fig. 6a). Only nine interactions are present among the eight divisome

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**Fig. 2** SPR measurement for N. gonorrhoeae FtsA-FtsZ, FtsQ-FtsA and FtsA-FtsN interactions. 

a 6 and 12 μM of FtsZNg were analyzed for interaction with FtsANg. 

b Negative interaction between FtsANg and GST; 

c FtsANg at different concentrations (31.25, 62.5, 125 and 250 nM) were measured for binding affinity to FtsQNg. 

d Negative interaction between FtsQNg and GST; 

e FtsNNg at different concentrations (62.5, 125, 250 and 500 nM) was analyzed for interaction with FtsANg. 

f Negative interaction between FtsNNg and GST. Association and disassociation constants were obtained using the Langmuir 1:1 kinetic fit model by nonlinear regression using ProteOn Manager™ (Bio-Rad Laboratories)
Fig. 3 Interactions between FtsA\textsubscript{Ng} truncations (T1, T2, T3, T4, T5 and T6) and FtsZ\textsubscript{Ng} (Z) by B2H assays. R721 without plasmids and single transformants were used as negative controls. R721 without plasmids had a $\beta$-galactosidase activity of $2504 \pm 34$ Miller units. FtsZ\textsubscript{Ng} self-interaction was used as a positive control. Values of less than 50% ($<1250$ Miller Units) indicate a positive interaction between two proteins (blue bars) while values of more than 50% ($>1250$ Miller Units) indicate a negative interaction (green bars) positive and negative controls are labeled in white (white bar).

Fig. 4 Interactions between FtsA\textsubscript{Ng} truncations (T2, T3, T4, T5 and T6) and FtsN\textsubscript{Ng} (N) by B2H assays. Values of less than 50% ($<1250$ Miller Units) indicate a positive interaction (blue bars) while values of more than 50% ($>1250$ Miller Units) indicate a negative interaction (green bars).
Fig. 5 Interactions between FtsA<sub>Ng</sub> truncations (T2, T3, T4, T5 and T6) and FtsQ<sub>Ng</sub> (Q) by B2H assays. Values of less than 50% (<1250 Miller Units) indicate a positive interaction between two proteins (blue bars) while values of more than 50% (>1250 Miller Units) indicate a negative interaction between the two proteins (green bars).

Fig. 6 Cell division interactomes of a N. gonorrhoeae, b E. coli [23, 24], and c S. pneumoniae [25]. Red lines indicate common interactions; blue lines indicate unique interactions in N. gonorrhoeae.
proteins tested in *N. gonorrhoeae*, while *E. coli* and *S. pneumoniae* have 21 and 17 interactions among ten and eight divisome proteins, respectively [24, 25].

The development of all three cell division interactomes was based on interaction data obtained from the same B2H system [24, 25]. The *E. coli* interactome was developed using B2H results exclusively while the *S. pneumoniae* study also applied co-immunoprecipitation to verify selected B2H positive interaction pairs [24, 25]. In our study, we used a combination of GST pull-down and surface plasmon resonance to further study selected positive B2H interactions.

Two interactions, FtsA-FtsZ and FtsZ-FtsK, are conserved in the cell division interactomes of *N. gonorrhoeae*, *E. coli*, and *S. pneumoniae* (Fig. 6, red lines). The FtsA-FtsZ interaction is a common interaction in prokaryotes [24, 25, 39–41]. Both our B2H and SPR results confirmed this interaction in *N. gonorrhoeae*. A proper ratio between FtsA and FtsZ is crucial for the interaction in *E. coli* [42] and our SPR results support this finding: FtsANg interacts with FtsZNg only when its concentration is higher than 6 μM (Fig. 3b), indicating that the interaction requires a critical concentration threshold. Our SPR results further showed that interaction between FtsANg and FtsZNg was transient, a result warranting further study to fully understand its implications for divisome formation in *N. gonorrhoeae*. Unexpectedly, the GST pull-down assay, an in vitro assay, did not detect an FtsANg-FtsZNg interaction. We believe that this “false negative” in vitro result was caused by the requirement of a membrane/solid surface support for the interaction to anchor FtsA [35, 43, 44].

The interaction of FtsZ with FtsK has been observed in *N. gonorrhoeae*, *E. coli*, *S. pneumoniae*, *B. subtilis* and *C. crescentus* [24, 25, 45, 46]. The C-terminus of FtsK is required for proper DNA segregation in *E. coli* [47]. The absence of an FtsZ-FtsK interaction in both *E. coli* and *C. crescentus* caused abnormal chromosome segregation and cell filamentation [45, 48]. This suggests that the FtsZ-FtsK interaction connects the cell division process with chromosome segregation, by ensuring that the replicated chromosome is cleared from the division site.

The FtsA-FtsW interaction has been observed only in *N. gonorrhoeae* (Fig. 6, blue lines). Since FtsW is a membrane protein and difficult to purify, we did not verify the interaction by GST pull-down and SPR assays. However, we performed additional B2H assays to identify which subdomains of FtsA were involved in its interaction with FtsW (Additional file 3: Figure S3) and showed that the 2A2 subdomain of FtsA strongly interacts with FtsW (Additional file 3: Figure S3). FtsW, an inner membrane protein, is required in *E. coli* for the recruitment of FtsI and the translocation of the cell wall precursor, lipid II [20, 21, 49, 50]. An FtsL-FtsW protein interaction has been observed in *E. coli*, *Streptomyces coelicolor*, and *Mycobacterium tuberculosis* [21, 51, 52]. Interestingly, we discovered that FtsLNg only interacts with FtsWNg, suggesting that its localization may depend on this protein.

The importance of the unique FtsKNg-FtsNNg interaction in *N. gonorrhoeae*, as determined by B2H, is not clear (Fig. 6, blue lines). In *E. coli*, FtsN is the last protein, of ten essential cell division proteins, recruited to the division site to initiate cell constriction [53, 54]. A previous study suggested that *E. coli* FtsN and FtsK stabilize the Z-ring cooperatively, without direct interactions [55]. Since the FtsK-FtsN interaction is present in *N. gonorrhoeae*, their joint involvement in gonococcal cell division requires further investigation.

ZipANg did not interact with any other gonococcal cell division protein. In *E. coli*, ZipA only interacts with FtsZ, and is required for downstream protein recruitment, including FtsK, FtsQ, FtsL, and FtsN [24, 56]. One report suggested that ZipANg is a homologue of the *E. coli* protein with high similarity in its key domains [57]. Although ZipANg complemented a conditional *zipA* mutant in *E. coli*, it did not fully restore a wild type phenotype in this strain [57]. Given these data, the role of ZipA in gonococcal cell division remains to be elucidated.

In *N. gonorrhoeae*, the existence of FtsLNg is unclear due to its low homology with *E. coli* FtsL [58]. An open reading frame (ORF) located between *mraW* and *ftsI* in the *dcw* cluster of *N. gonorrhoeae* was reported by Francis et al. [7] and they reported that it was not a coding ORF. Snyder et al. [58] named the same ORF *ftsL*. Because this ORF shares only 17% amino acid similarity to its *E. coli* homologue, we considered that it was not a functional ORF and did not test its interaction with other gonococcal cell division proteins.

*N. gonorrhoeae* lacks FtsB [7]; thus, the protein complex FtsQ-B-L, present in other species, such as *E. coli*, *S. pneumoniae* and *B. subtilis*, would not be formed in *N. gonorrhoeae* [59–61]. This protein complex has been described as a bridge connecting FtsK and the FtsL-FtsW complex in *E. coli* [18]. A recent study suggests that the *E. coli* FtsQ-B-L complex acts as a signal transmitter for cell wall remodeling and constriction, which is mediated by direct interactions with the FtsL-W complex and FtsN [19]. In *S. pneumoniae*, the FtsQ homologue, DivIB, interacts with FtsKsp, FtsLsp, and FtsWsp [25]. Interestingly, our B2H data show that FtsQNg only interacts with FtsANg, suggesting that the function of FtsQNg in cell division in *N. gonorrhoeae* may be distinct.

There are several models for bacterial cell constriction. One *E. coli* model suggests that the force that drives constriction comes from septal peptidoglycan synthesis [62]. In this model, the FtsANg-FtsNEc interaction
activates peptidoglycan synthesis by direct or indirect interaction with FtsL [63]. Another *E. coli* model suggests that the energy generated from FtsZ-mediated GTP hydrolysis drives cell constriction [43]. We observed an FtsANg-FtsNNg interaction in *N. gonorrhoeae*. However, there is no further evidence supporting either model of cell constriction in *N. gonorrhoeae* at this time.

The non-essential proteins, FtsENg and FtsXNg, are also implicated in cell division in *N. gonorrhoeae* [64]. Similarly, in *E. coli*, FtsE and FtsX are non-essential for cell division under conditions of high osmotic pressure [65]. Gonococcal FtsE and FtsX have high similarity in amino acid sequence to known homologues in other species [64]. In *E. coli*, the interaction between FtsE and FtsZ has a regulatory effect on the Z-ring [65]. Future research could focus on revealing the effects of FtsENg and FtsXNg in cell division in *N. gonorrhoeae*.

The major issue interpreting B2H assay results is the empirical cut-off of 50% residual β-galactosidase activity used to discriminate positive and negative interactions. In particular, values close to the cut-off could be interpreted as either false positive or negative results. To validate our B2H results, we used other B2H interactions to test which subdomains of FtsANg interacted with FtsZNg, FtsNNg and FtsQNg. We determined that the 2A and 2B subdomains of FtsANg interacted with FtsZNg, FtsQNg, and FtsNNg. We also evaluated some positive interactions obtained by B2H using SPR and GST pull-down assays. The SPR method detects and measures weak or transient interactions, in real-time, with high sensitivity [66]. The SPR method showed a transient FtsANg-FtsZNg interaction. GST pull-down assays, on the other hand, are ideal in detecting strong protein-protein interactions, as weak interactions may dissociate during the assay [67]. We consider this to be a reasonable explanation for our failure to confirm when the interaction of FtsANg with FtsZNg when using a GST pull-down assay.

To date, most of studies on cell division have been focused on model organisms (i.e. the Gram-negative rod *E. coli* and the Gram-positive rod *B. subtilis*) due to the abundant availability of tools for genetic manipulation [62]. Research on cell division in non-model organisms is expanding, and this includes studies with *N. gonorrhoeae* [7, 27]. For example, *Chlamydia trachomatis*, which lacks FtsZ, requires an actin-like protein, MreB, for cell division [68]. A gene cluster encoding three cell division proteins, named MldA, MldB, and MldC, was identified only in *Clostridium difficile* and its closely related bacteria [69]. Results from studies using non-model organisms suggest that cell division mechanisms are complex and vary in different organisms, reflecting vast biological diversity.

**Conclusions**

In our research, we discovered that nine interactions among eight cell division proteins defined the cell division interactome of *N. gonorrhoeae*. In comparison with the published cell division interactomes of *E. coli* and *S. pneumoniae*, FtsA-FtsZ and FtsZ-FtsK interactions were common to all three bacteria. FtsK-FtsN and FtsA-FtsW interactions were only present in *N. gonorrhoeae*, suggesting that they play different roles in the cell division of this microorganism. ZipANg did not interact with any other cell division proteins tested in this study, indicating that its role may differ as compared to its *E. coli* homologue. We also determined that the subdomains of FtsANg which interacted with FtsQNg, FtsZNg, FtsWNg, or FtsNNg differed from its *E. coli* homologue. This suggests that *N. gonorrhoeae* possesses a distinctive cell division interactome, and likely a different mechanism of cell division as compared to *E. coli* and other organisms.

**Additional files**

**Additional file 1: Figure S1.** Schematic representation of *N. gonorrhoeae* ftsA and its truncations [33], T1 (162aa, Met1-Ile162) contained the N-terminal 1A and 1C domains of ftsA, T2 (194aa, Met1-Val194) included the N-terminal 1A, 1C and 1A domains of ftsA, T3 (250aa, Met1-Ile250) included the N-terminal 1A, 1C, 1A and 1A domains of ftsA, T4 (71aa, Pro231-Glu301) contained the 2B domain of ftsA, T5 (114aa, Ile301-Leu414) contained the 2A, and 1A C-terminal domains of ftsA, T6 (64aa, Ala351-Leu414) contained the 1A C-terminal domain of ftsA (DOX 30 kb).

**Additional file 2: Figure S2.** FtsZNg polymerization assays. FtsZNg polymers visualized by transmission electron microscope with (A) or without (B) 2 mM GTP in MES buffer (50 mM MES-NaOH, 50 mM KCl, 10 mM MgCl2, pH 7.5) at 30 °C. Solid arrows indicate FtsZNg polymers. Scale bar indicates 100 nm. (C) Light scattering of FtsZNg polymerization (6 μM) in MES buffer. (DOX 211 kb).

**Additional file 3: Figure S3.** Interactions between FtsANg truncations (T2, T3, T4, T5 and T6) and FtsWNg (W) by B2H assay. Values of less than 50% (<1250 Miller Units) indicate a positive interaction between two proteins (blue bars) while values of more than 50% (>1250 Miller Units) indicate a negative interaction (green bars). (DOX 66 kb).

**Abbreviations**

B2H: Bacterial two-hybrid; Bs: Bacillus subtilis; Cs: Caulobacter crescentus; dw: division and cell wall; DLS: Dynamic light scattering; Ec: Escherichia coli; Ng: Neisseria gonorrhoeae; ORF: Open reading frame; Sp: Streptococcus pneumoniae; SPR: Surface plasmon resonance; TEM: Transmission electron microscopy; Y2H: Yeast two-hybrid.

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Availabiity of data and materials

The data from this report are included within the article. Datasets used in the current study are available upon request.

Authors' contributions

YZ and YL participated in the experimental design, implementation and data analysis. YZ also wrote the first draft of manuscript. JRD designed and supervised the entire project and was responsible for the final submission of the manuscript. All authors contributed to manuscript revisions. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Ethics approval is not required for this study. Consent to participate is not applicable.

Consent for publication

The authors declare that they have no competing interests.

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