A new chlamydia-like 16S rDNA sequence from a clinical sample

Chlamydiae constitute an important group of obligate intracellular parasites, causing a variety of diseases in mammals and birds. Among them Chlamydothila pneumoniae has been recognized as a common respiratory pathogen in man and it has been associated to atherosclerosis. Recently, new chlamydia-like organisms have been described, most being responsible for human respiratory infections. In Israel, Simkania negevensis is frequent in infants with bronchiolitis (11) and in adults with community-acquired pneumonia (5, 13). Parachlamydia acanthamoebae, identified as an endosymbiont of an Acanthamoebae sp. isolated from a healthy human nasal mucosa (1), might be a case of atypical pneumonia (2). These organisms form new families within the Chlamydiaceae (4), and they are not recognizable by conventional diagnostic procedures for classic chlamydiae, i.e. Cpb. pneumoniae, Chlamydothila psittaci complex or Chlamydia trachomatis. Therefore their prevalence and diversity in clinical samples is certainly underestimated. For example, using PCR, Ossewaarde & Meijer (14) detected several DNA sequences related to either Simkania or Parachlamyda in respiratory samples, peripheral blood monocytes and in a vessel-wall specimen.

Starting with a human respiratory sample (from broncho-alveolar washings) sent to the laboratory for the diagnosis of viral or chlamydial infection, we detected a new 16S ribosomal DNA sequence belonging to the parachlamydiae. We have named this corvenA4 (GenBank accession no. AF308693).

DNA was extracted from a 300 µl aliquot of the sample by the classic phenol/chloroform method after proteinase K digestion, and the 16S rDNA was amplified by PCR using a pan-chlamydia primer set amplifying almost all the gene (nucleotide positions 40–1485, P. acanthamoebae 16S rDNA numbering). Manipulations were carried out according to recommended guidelines (12) and included negative controls starting from the DNA extraction step. Both strands of the PCR product (~ 1400 bp) were sequenced (three repetitions) using a series of inner primers. The resulting complete sequence was compared to the available corresponding sequences obtained from GenBank using the BLAST server. Sequences were aligned, gaps and ambiguous sites excluded, for a total of 1354 nt. A similarity of 91.7–93.8% was found with the sequences of P. acanthamoebae and two other related amoebal symbionts of Acanthamoeba spp. strains (UWE1 and UWE25), 92.3–92.5% with that of Neochlamydia bartmannellae and related amoebal endosymbionts (Acanthamoeba sp. strains UWC22 and TUME1), 88% with that of Waddha chondrophila, 86.8% with that of S. negevensis, and less than 86.3% with those of Chlamydothila pneumoniae strain TW-183, Cph. psittaci strain NJ1 and C. trachomatis strain Har-13. A matrix of evolutionary distances was derived from the alignment using DNAdist, Jukes & Cantor’s option, and a phylogenetic tree was inferred using Fitch & Margoliash’s criteria. The topological stability of the tree was assessed by bootstrap analysis using consensus to yield a strict majority-rule consensus tree on 300 samples. Our sequence, corvenA4, was shown to be related to, but distinct from, both Parachlamydia and Neochlamydia lineages (Fig. 1). Considering a value of 16S rDNA sequence similarity of at least 95% for two organisms belonging to a same genus (4), it seems probable that corvenA4 was from an organism representing a new genus within the Parachlamydiaceae. We failed to isolate such an organism in cell culture, as evidenced by Giemsa staining of inoculated Vero and HeLa cells. Amoebae were not observed under the microscope, and the sample did not present any evidence for acanthamoebal DNA by PCR using a primer set specific for the 18S rDNA. Therefore a description of this chlamydia-like organism was not possible, as the only evidence of its existence is the 16S rDNA sequence.

The diversity within the family Parachlamydiaceae is increasing. At present, two species and two genera have been validly described: P. acanthamoebae (1) and N. bartmannellae (10). Four other endosymbionts of Acanthamoeba sp. have been identified, probably forming three new species or genera on the basis of 16S rDNA sequence similarities (9). UWC22 was from an Acanthamoeba sp. isolated from a case of amoebic keratitis, while TUME1, UWE1 and UWE25 were from environmental isolates of Acanthamoeba sp. (6, 9). It is interesting to note that the UWC22 and TUME1 strains are closely related to Neochlamydia, that infects...
The role of AAA (ATPases Associated to a variety of cellular Activities) family protease FtsH in bacterial cell division is not known, although mutations in ftsH were found to inhibit cell growth and division (1, 6, 13). Overexpression of heterologous FtsH in Escherichia coli results in the formation of multinucleate filamentous cells due to the abolition of cell septation (8). Further, independent studies on FtsH (15) and FtsZ (2), which is the key regulator of bacterial cell division, have shown that FtsH protease and FtsZ protein are localized to the mid-cell site during septation. FtsZ protein is the prokaryotic homologue of tubulin (5, 10, 12), possessing GTP-dependent polymerization activity (4, 11). Significantly, the AAA family FtsA member katanin disassembles tubulin polymers in an ATP-dependent manner (7). Based on these observations, we reasoned that an interaction similar to that between katanin and tubulin might hold true for FtsH and FtsZ. Therefore, we examined whether the FtsH and tubulin might hold true for FtsH and FtsZ.

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Bacterial cell division protein FtsZ is a specific substrate for the AAA family protease FtsH

The bar indicates estimated genetic distance. Verrucomicrobium sp. strain VeCb1 was used as the outgroup.

Hartmannella and Dictyostelium but does not grow in Acanthamoeba (10).

The possibility of infection of humans by new chlamydia-like organisms deserves additional investigation. The Simkania-related and Parachlamydia-related DNA sequences detected in an abdominal aortic aneurysm and in the peripheral blood monocytes, respectively, by Ossewaarde & Meijer (14) indicate that a variety of such organisms may be present also in human body sites other than mucosas. The passage from a putative amoebal host to mammalian cells may be possible, P. acanthamoebae being cultivated in Vero cells (1). More extensive studies are necessary to evaluate their potential pathogenic role, and might allow demonstration of the reality of such infections, explaining the aetiology of numerous respiratory infections in which no conventional pathogens are found. In vitro studies (7) showed that amoebae infected by parachlamydiaceae exhibit an increased cytotoxic effect on cell cultures. Amoebae and other protists host a variety of intracellular organisms and may act as a reservoir and/or vector for human infection (3), as is the case in Legionella infections. Such amoeba/bacterium systems are very interesting as infectious sources and symbioses in general, and the recent discovery of the ‘pararickettsiae’ endosymbiont of Acanthamoeba spp. following acquisition of bacterial endosymbionts of Acanthamoeba spp. (5) and tubulin might hold true for FtsH and FtsZ.

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The role of AAA (ATPases Associated to a variety of cellular Activities) family protease FtsH in bacterial cell division is not known, although mutations in ftsH were found to inhibit cell growth and division (1, 6, 13). Overexpression of heterologous FtsH in Escherichia coli results in the formation of multinucleate filamentous cells due to the abolition of cell septation (8). Further, independent studies on FtsH (15) and FtsZ (2), which is the key regulator of bacterial cell division, have shown that FtsH protease and FtsZ protein are localized to the mid-cell site during septation. FtsZ protein is the prokaryotic homologue of tubulin (5, 10, 12), possessing GTP-dependent polymerization activity (4, 11). Significantly, the AAA family FtsA member katanin disassembles tubulin polymers in an ATP-dependent manner (7). Based on these observations, we reasoned that an interaction similar to that between katanin and tubulin might hold true for FtsH and FtsZ in prokaryotes as well. To verify this hypothesis, we examined whether the FtsH protease of Escherichia coli (FtsH<sub>Ec</sub>) could degrade FtsZ of E. coli (FtsZ<sub>E</sub>) in vitro.

Incubation of FtsZ<sub>Ec</sub> with FtsH<sub>Ec</sub> showed degradation of the FtsZ<sub>E</sub> protein in an ATP- and Zn<sup>2+</sup>-dependent manner (Fig. 1, lane 2). Absence of ATP or presence of ortho-phenanthroline (OP; chelator of zinc) abolished protease activity (Fig. 1, lanes 3 and 4). Similarly, substitution of ATP with itshydrolysable analogue, ATP-γ-S, also inhibited FtsZ degradation (Fig. 1, lane 5). Identical observations were made upon incubation of the FtsZ protein of Myco-
protein was degraded by FtsH (data not shown), indicating that the recognition and degradation of FtsZ by FtsH is specific.

Here we demonstrate for the first time that FtsH protease degrades the cell-division-initiation protein FtsZ in vitro. The implication of our in vitro studies is that the AAA family protease FtsH could be a proteolytic regulator of the cell-division-initiation FtsZ in vitro. The regulation of FtsZ activity has so far been shown to involve only protein–protein interactions, which prevent mid-cell localization or polymerization of the protein (3, 9), whereas our finding is suggestive of the existence of a proteolytic regulatory mechanism also. Secondly, the fact that FtsH Ec protease degraded FtsZ molecules from two divergent bacterial genera implies that FtsZ could be a substrate for FtsH protease across the bacterial kingdom. Finally, since FtsH is a stress-responsive protease and its mid-cell localization occurs only in a fraction of dividing cells (15), it is logical to presume that the proteolytic regulation of FtsZ by FtsH protease, if it occurs in vitro, might be restricted to specific conditions of bacterial growth.

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