COMMENTARY

Is the hoopla over CPAF justified?

DOI: 10.1111/2049-632X.12211

It may be worth asking whether all the hoopla over CPAF is justified. Our view is: yes, it is. As in any active scientific area, not every proposal regarding CPAF function has held up. The recent developments are very encouraging: we now have a much better idea of what CPAF can do, but also what it does not do. Our current knowledge can be summarized as follows: Chlamydiae produce a potent protease with unusual characteristics that is made by both pathogenic and environmental Chlamydiae, which likely transfers from the chlamydial inclusion into the cytosol, and which has the potential to cleave both bacterial and host proteins. We have recently and in more detailed fashion contributed to the discussion on CPAF in two commentaries (Conrad et al., 2013; Hacker, 2014) and will limit our comments here to several issues that have come up recently, which we think are important.

Where is CPAF located in the infected cell?

A critical question is whether CPAF is cytosolic or found only in the inclusion. If it is in the inclusion, then it almost certainly targets a chlamydial component; if in the cytosol, then it very likely modulates a host cell function. The view provided by Bavoil and Byrne (2014) in their commentary points out potential flaws in the techniques used in the past to demonstrate cytosolic localization of CPAF. We wish to reference our recent immuno-EM study of CPAF during productive infection and in penicillin-treated cultures (penicillin suppresses growth and Chlamydia assumes an aberrant morphology). This study finds that CPAF is clearly localized in the cytosol during productive infection but not in penicillin-treated cultures, where it was found only in the inclusion (Dille et al., 2014). Although we do not provide final proof and other problems may emerge, the study is additional evidence in favour of cytosolic localization of CPAF under physiological conditions. Thus, this case is not resolved at present. It is clear that more CPAF is released during inclusion rupture (Snively et al., 2014) [however, at least for C. trachomatis, rupture may not be necessary for propagation (Hybske & Stephens, 2007)]. CPAF-mediated proteolysis of host cell substrates may therefore occur at a low level; rather than degrading host proteins aggressively, it may exert a subtle function. Indeed, the typical, clinically invisible chlamydial infection runs its course without much thunder, which would be consistent with a discrete mode of action for CPAF (see also below).

Is CPAF a virulence factor?

The definition of CPAF as a virulence factor – a term broadly employed by Bavoil and Byrne – deserves consideration. In a common definition, a virulence factor is anything that permits or facilitates infection by a bacterial pathogen, and in this sense CPAF is certainly a virulence factor. However, for an obligate intracellular bacterium, even a housekeeping gene will be a virulence factor by this definition, so CPAF may not be specifically linked to virulence. However, using CPAF mutants, we have gained important information about CPAF; in particular, that its loss reduces growth. But this observation at this stage is limited to effects on the rapidly propagating C. trachomatis L2-strain in a tumour cell line in vitro (Snively et al., 2014). Under other conditions, the effect of CPAF may be more or less pronounced. In particular, the effect of CPAF may be different under the hypoxic conditions of the genital tract (Roth et al., 2010), it may be different for other serovars and species of Chlamydia and different again in the conditions found in chronic infections.

The existence of CPAF seems close to inseparable from the obligate intracellular lifestyle of Chlamydia as it is present from human pathogenic to amoeba-adapted species such as Parachlamydia. CPAF could have different functions in different bacteria, but there is no obvious reason why that should be the case. If we assume for the moment that Parachlamydia CPAF has the same function as Chlamydia CPAF, then two issues emerge.

First, the requirements for infection and growing in amoebae, on one hand, and in human cells, on the other, are bound to be very different. However, there is likely some overlap of core requirements. Thus, many interactions with signalling pathways that have been described for human cells are unlikely or impossible in amoebae, such as inhibition of apoptosis and activation of NF-κB (Hacker, 2014). Nonetheless, both environmental and pathogenic species must meet the requirements of life within an inclusion, such as embedding it into the cytoskeleton and delivery of nutrients to the bacteria inside the inclusion, and the growth of the inclusion membrane as the bacteria proliferate. Assuming that CPAF may have a function that is conserved between the species, then a function related to life and replication within the inclusion seems plausible.

Secondly, chlamydial infection is slow and, for the most part, silent. Genital infections with C. trachomatis may sometimes be purulent but often the infection goes unnoticed and may persist for over a year without any symptoms (although this may be less the case for ocular infections). Infections of at least adults with C. pneumoniae probably produces mild or no symptoms, and animals infected with their adapted chlamydial species show few signs of disease. Amoebae, as far as we can tell, do not suffer when harbouring their chlamydial species and may be able to
maintain a symbiotic relationship. Even though the immune system can interfere with infection, it is fair to argue that natural infection of epithelial cells with Chlamydia is mostly a low-key affair. Taking this into consideration and with the benefit of hindsight, it could be argued that it is implausible for Chlamydia to secrete a potent protease that plays havoc with host cell function and digests numerous host cell proteins to a large extent.

And what now?

We know very little about CPAF. We know that it is conserved within the Chlamydiae (with the exception of Simkania) (Collingro et al., 2011) and that it is not essential for C. trachomatis L2 infection but enhances the generation of infectious progeny. Although others may disagree, we also believe that CPAF is partly cytosolic.

What strategies may we employ to further understand its role? An obvious approach for understanding the function of a protease is to identify potential substrates. We have carried out experiments to identify cleavage events when CPAF is expressed ectopically in uninfected human cells, and we are still scratching our heads over the 3000 or so cleavage sites identified (L. Volceanov, O. Schilling, G. Häcker, unpublished results). It may be more promising to identify a function through the use of CPAF mutants. After all, many cell biological events are known to occur during infection, such as modifications of vesicular transport, alterations to the cytoskeleton, and reorganization of the Golgi apparatus (not only the Golgi), and recognition of the bacteria by the host cell’s innate immune response; as well as the stress response of Chlamydia (changing into ‘aberrant bodies’ but often recovering) when exposed to various stimuli, and the ability of Chlamydiae to grow in different cells (including insect cells) and under different conditions. Thus, testing the host and bacterial response using CPAF-deficient mutants may be the most promising approach for the future.

It is clear that the road will not be easy. We can already foresee experimental problems arising from the growth defect in CPAF-deficient strains. Very likely the deficiency will result in retarded development of the inclusion, which would simply delay all of the events that occur during normal infection. It would also be desirable to have clean deletions of CPAF, if possible also in other strains, including C. muridarum which could be tested in vivo in a mouse model.

Science does not have to be easy, and we look forward to these challenges. Science can also benefit from mistakes, and CPAF is an example. It may seem like a tragedy when a beautiful hypothesis is slain by an ugly fact, as Thomas Huxley said, but it is certainly more beautiful to gain clear knowledge.

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References


