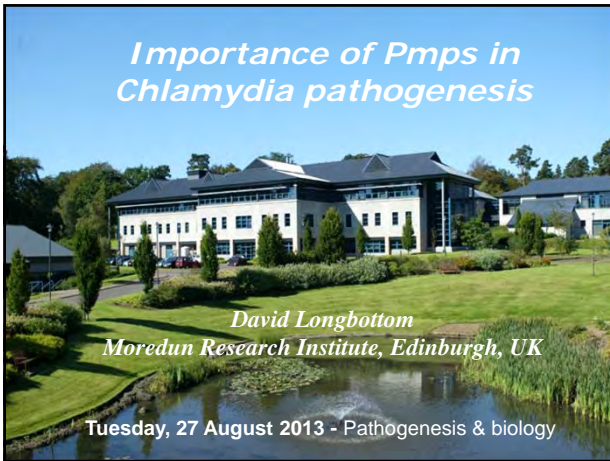


## Importance of Pmps in *Chlamydia* pathogenesis



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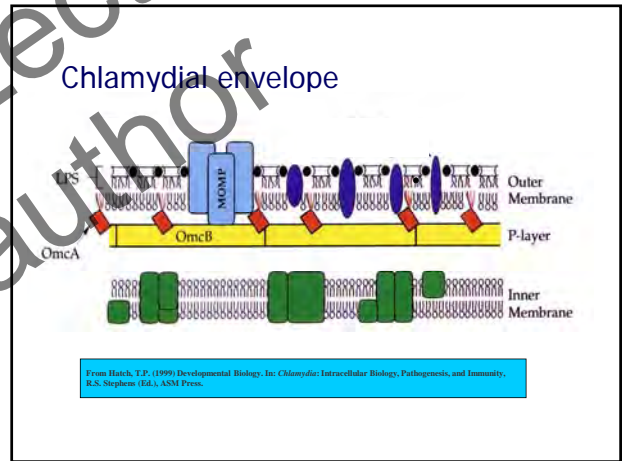
Tuesday, 27 August 2013 - Pathogenesis & biology

### Family Chlamydiaceae

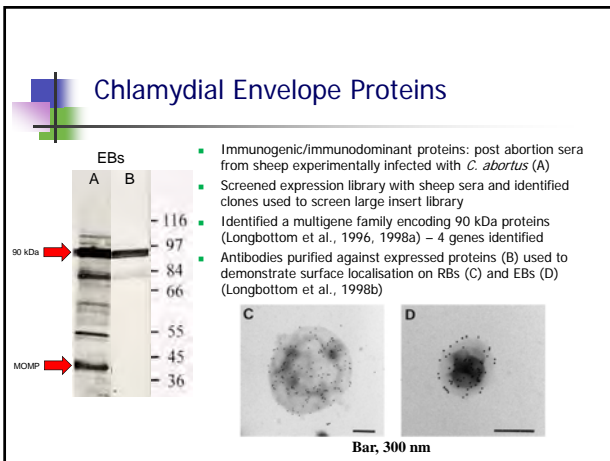
| Chlamydia sp.         | Typical Host         | Infection/Disease  |
|-----------------------|----------------------|--|
| <i>C. trachomatis</i> | Humans               | STI, PID, salpingitis, trachoma  |
| <i>C. pneumoniae</i>  | Humans, Koala, Horse | Pneumonia, bronchitis  |
| <i>C. muridarum</i>   | Mice, hamsters       | Respiratory, genital, (Model System)   |
| <i>C. caviae</i>      | Guinea pig           | Ocular, genital, (Model System)  |
| <i>C. felis</i>       | Cats                 | Conjunctivitis   |
| <i>C. psittaci</i>    | Birds, poultry       | Respiratory  |
| <i>C. abortus</i>     | Ruminants, swine     | Abortion   |
| <i>C. suis</i>        | Swine                | Enteric, respiratory, reproductive   |
| <i>C. pecorum</i>     | Ruminants, swine     | Enteritis, conjunctivitis, metritis, mastitis, polyarthritis, pneumonia, encephalomyelitis |

### Pregenomic era (prior to 1998)

- Caldwell et al., 1975: *C. trachomatis*-specific polypeptide antigen of 155 kDa – promise for serodiagnosis of LGV infections
- Batteiger & Rank, 1987: 84 kDa outer membrane protein recognised by sera from guinea pigs genitally infected with *C. caviae*
- Campbell et al., 1990: Antibodies to a 98 kDa *C. pneumoniae* antigen were detected in patients with proven *C. pneumoniae* infection
- Cevenini et al., 1991: immunogenic 89 kDa protein of *C. abortus*
- Souriau et al., 1994: highly immunogenic 80-90 kDa proteins of *C. abortus* EBs with sera from post-abortion ewes

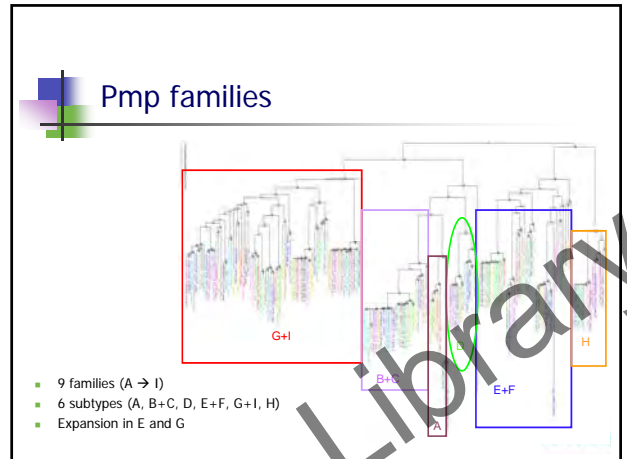
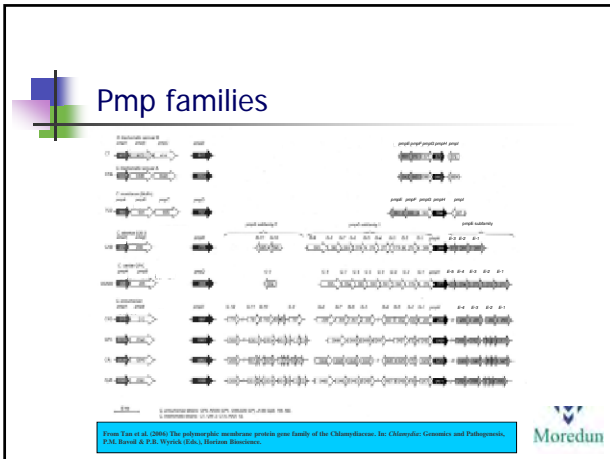
### Chlamydial Envelope Proteins



- Immunogenic/immunodominant proteins: post abortion sera from sheep experimentally infected with *C. abortus* (A)
- Screened expression library with sheep sera and identified clones used to screen large insert library
- Identified a multigene family encoding 90 kDa proteins (Longbottom et al., 1996, 1998a) – 4 genes identified
- Antibodies purified against expressed proteins (B) used to demonstrate surface localisation on RBs (C) and EBs (D) (Longbottom et al., 1998b)

### Postgenomics

| Species               | No. Pmps  | References   |
|-----------------------|-----------|--|
| <i>C. trachomatis</i> | 9 (A → I) | Stephens et al., 1998; Carlson et al., 2005                        |
| <i>C. pneumoniae</i>  | 21        | Kalman et al., 1999; Read et al., 2000; Shirai et al., 2000        |
| <i>C. muridarum</i>   | 9         | Read et al., 2000  |
| <i>C. caviae</i>      | 18        | Read et al., 2003  |
| <i>C. abortus</i>     | 18        | Thomson et al., 2005; Sait et al., 2012                            |
| <i>C. felis</i>       | 20        | Azuma et al., 2006   |
| <i>C. psittaci</i>    | 21        | Voigt et al., 2011; Van Lent et al., 2012; Seth-Smith et al., 2012 |
| <i>C. pecorum</i>     | 15        | Mojica et al., 2011  |
| <i>C. suis</i>        | 9?        | unavailable  |



- ### Pmp evolution
- Present in Chlamydiaceae not in other members of Chlamydiales
  - Acquisition of pmp genes likely precedes divergence of Chlamydiales into Chlamydiaceae – critical survival factor in vertebrate hosts?
  - Likely that expansion into 6 pmp subtypes occurred in an ancestor common to all Chlamydiaceae and further diversification occurred as a result of intra- and inter-pmp recombination
  - Expansion occurring later as chlamydial hosts themselves evolved
  - Differential expansion and genetic plasticity likely implies a critical role for pmp subtypes in chlamydial pathogenesis
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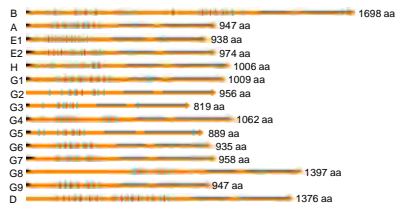
- ### Pmp structural features
- In silico analysis – secondary structure similar to autotransporter proteins of Type V secretion system (Henderson & Lam, 2001)
  - Type V secretion system
    - Essential components of bacterial virulence/pathogenesis
    - Adhesins, toxins, enzymes and mediators of motility that interact directly with host cell
    - Modulate host immune response
    - e.g. *B. pertussis* pertactin, *Y. enterocolitica* YadA, *E. coli* EspP (SPATE), *E. coli* AIDA-1
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- Moredu

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  - Autotransporters
    - Signal sequence
    - Passenger domain
    - M (middle/linker) domain
    - β-barrel domain
- 
- Taken from Henderson et al. (2004) Type V Protein Secretion Pathway: the autotransporter story. Microbiology and Molecular Biology Reviews 68: 692-744
- Moredu

- ### Pmp structural features
- Structural prediction supported by modelling and proteomic observations
    - Putative β-barrel & passenger domains
    - Proteolytic processing of some Pmps
    - Immunogenicity of passenger domain
- 
- 
- C. abortus* Pmp13G threaded to hmw1 of *H. influenzae*
- 
- β helix passenger domain
- Moredu

## Pmp features

- Multiple repeat motifs **GGAI(I,L,V)** and **FXXN** – similar to those in OmpA of *Rickettsia* spp and proteins involved in interactions with eukaryotic cells
- Leader sequence – surface localisation
- Trp residues in carboxy terminal part of protein and terminal Phe – outer membrane location

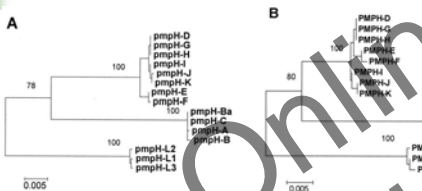


## Single copy Pmp subtypes

- Pmps A, B (C), D & H
- Relative genetic distance between members across species are conserved, suggesting function has remained stable
  - Most essential biological activity?
- PmpD suggested to function as an adhesin – Cpn/Ctr neutralisation studies (Wehrl et al., 2004; Crane et al., 2006)
- PmpA – well conserved across species – not interrupted by mutation – essential gene? – but little expression observed??



## Single copy Pmp subtypes



nucleotide (A) and inferred amino acid (B) sequences of *pmpH*

- PmpH – RFLP profile (Cfr) parallels the ocular, genital and LGV disease groups suggesting a role in pathogenesis (Stothard et al., 2003)



## Pmp expansion & function

- Pmps E (F) & G (I) in contrast show much more variability
  - multifunction and/or role in niche specificity?
- Pmp-specific responses vary in host organisms
- Pmps stimulate both innate and adaptive immune responses
  - Pmps B, D & G-6 of Cpn stimulate CD4+ T cells (Mygind et al., 2004)
  - Pmps B & D of Cpn also stimulate proinflammatory cytokine/chemokine production (IL-6 & CCL2) by endothelial cells through activation of the NF- $\kappa$ B pathway (Niessner et al., 2003; Wehrl et al., 2004)
  - PmpD of Cpn stimulates IL-8 production (Wehrl et al., 2004)



## Pmp expansion & function

- Why differences in expansion between species?
  - Reflect different fundamental functions (multifunction) and possibly subcellular location (niche specificity)?
  - Diversity, expression and function evolved in response to specific demands of host environment?
  - Higher selective immune pressure for these variants to emerge?
- Does the most essential Pmp biological activity reside in the 'single' Pmps, while the expanded subfamilies may serve as 'decoys' from interference by external threats??
- Evidence for variation in gene and protein expression?



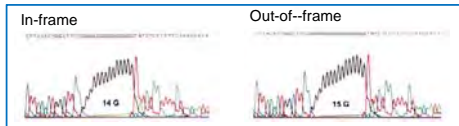
## Pmp expression

- Antigenic variation similar to *opa* gene family in *Neisseria gonorrhoeae*
- Genotypic evidence
  - Frameshift mutations
  - Single nucleotide polymorphisms (SNPs)
  - Deletions
  - Insertions
- Variable expression
  - Transcriptional analysis
  - Protein analysis – proteomics/antibody detection



## Genotypic variation

- Frameshift mutations, point mutations and out-of-frame deletions and insertions may produce non-functional or truncated genes whose products are likely not folded or translocated
- Frameshifts often observed in G tracts in some Pmps suggesting a slip strand mispairing mechanism during replication

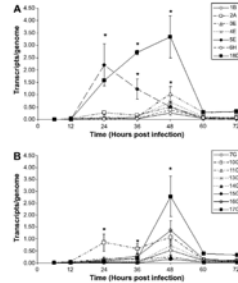


- Intragenic in-frame deletions, insertions and duplications may produce new variants with distinct antigenic properties and functions

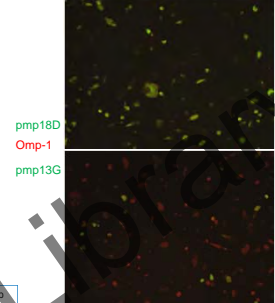


## Variable expression

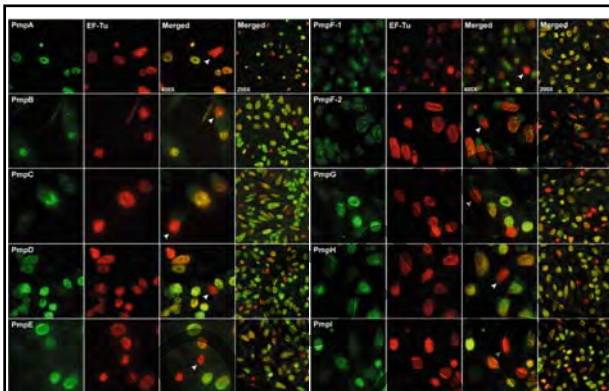
C. abortus – pmp transcriptional analysis



C. abortus – pmp protein expression



Wheelhouse et al., 2009; Wheelhouse et al., 2012a,b



Variable expression of the nine Pmps in *in vitro* grown *C. trachomatis* serovar E (Tan et al., 2010)

## Pmps: Functional role

- Differences in expression levels between *C. trachomatis* and *C. abortus*
- Greater heterogeneity in *C. abortus* both in terms of the timing and proportion of inclusions expressing each protein
- Support multifunctional role and a role in antigenic variation
- Providing antigenic diversity in the face of differing host adaptive immune responses
  - Strains with less diversity (*C. trachomatis*) cause mostly localised infections and may thus not be exposed to the same immunological and environmental pressures.
  - Species causing systemic infections (eg *C. pneumoniae*, *C. abortus*) may require expanded pmpG family to evade systemic host responses or adapt to multiple environments with additional modifications further expanding the antigenic repertoire of these proteins



Thank you for  
your attention