VIRULENCE

How do all the sensing systems we've looked at so far come together to control the response of a pathogen to its host and what is the response of the host

3 examples

Vibrio cholerae

Yersinia

Shigella

Vibrio cholerae

Natural inhabitant of aquatic environments

Forms biofilms-attached to plants, zooplankton, insects etc-important for persistance

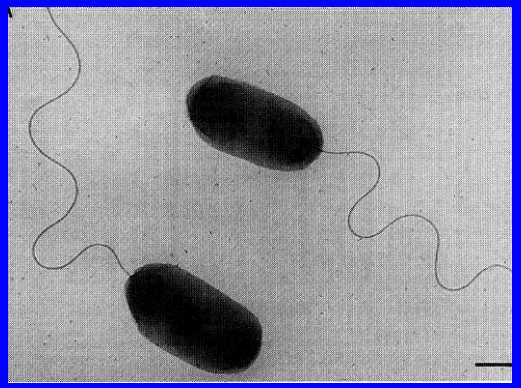
Under conditions of stress (human and bacterial) causes the severe diarrhoeal disease, cholera

2 biotypes, the current one (El Tor) is responsible for the 7th pandemic

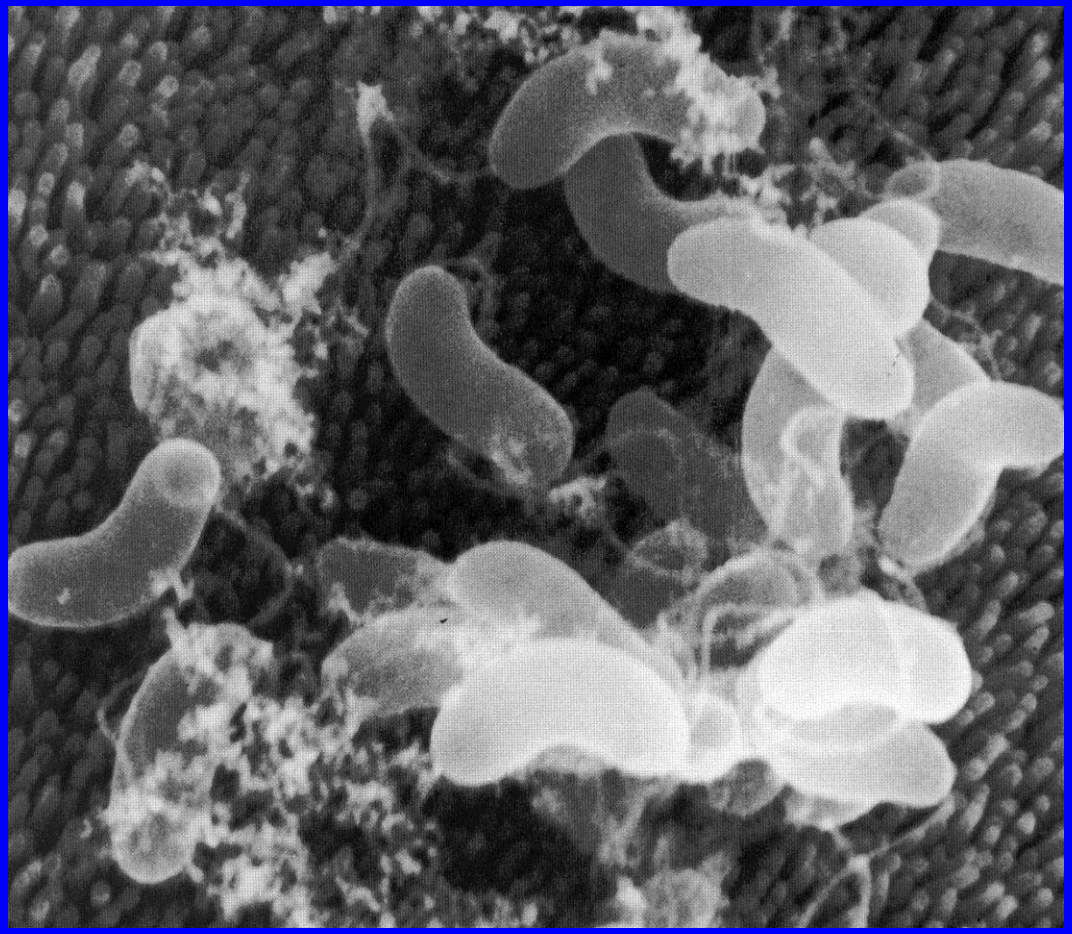
Pathogenicity

MOTILITY

- **Need motility to reach the gut wall**
- **Non-motile strains are non-pathogenic, unless placed on site of adhesion**
- **Inverse relationship between expression of flagella and toxin production, suggesting once at gut wall there's a switch in behaviour**
- **Phosphorelay system (FlrB-FlrC) important for flagellar gene expression and repression of colonization genes**



Pathogenicity

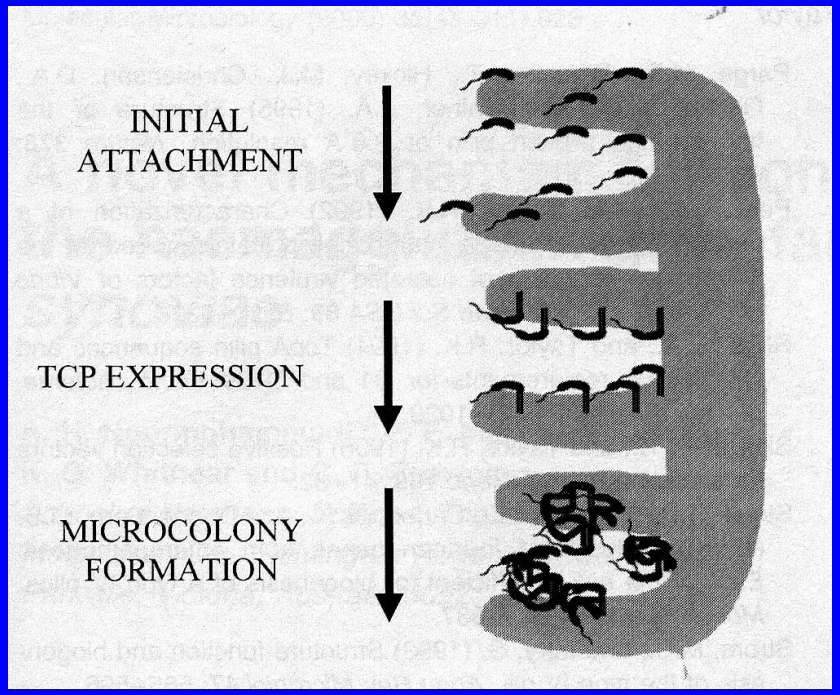


V.cholerae in the small intestine

Major pathogenicity determinant is TCP- the toxin co-regulated pilus.

This is a Type 4 pilus made of repeating subunits of TcpA pilin which forms long filaments which allow formation of bundles and microcolonies in the gut

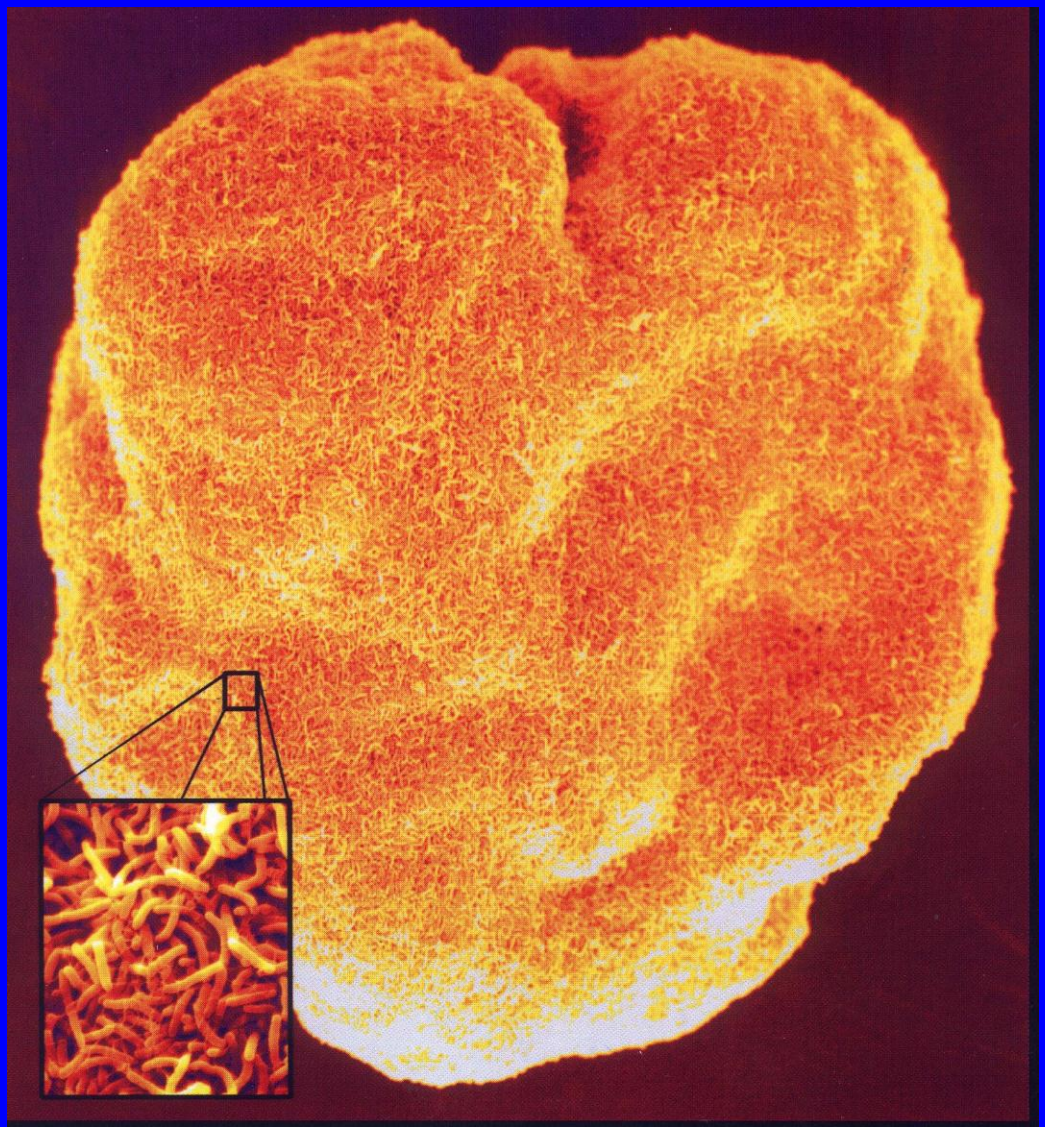
Pathogenicity



V.cholerae microcolony formation

- cells sense the gut wall
- Initial attachment involving mucus adhesion.
- Penetration of gut mucus
- Reduced swimming
- Sensing of local environment
- Expression of TCP and formation of microcolonies
- Microcolonies –resistant to antimicrobial effects in gut and initiate pathogenicity cycle

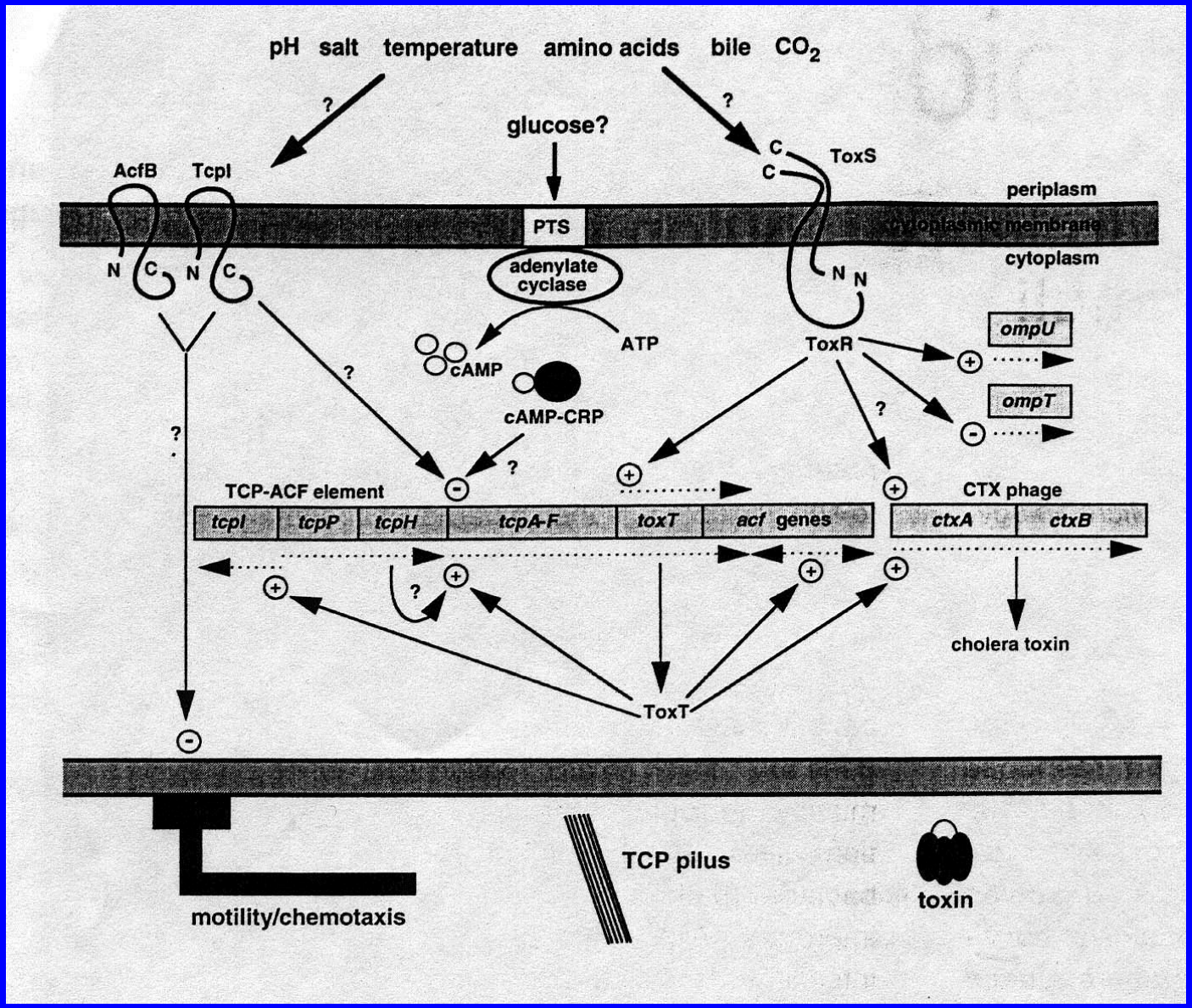
V.cholerae microcolony



ToxR-ToxT-sensing the local environment

- ToxR and Tox S are membrane spanning HPKs
- Receptors Tcp1 and AcfB switch off motility
- Activated by pH 6.5, osmolarity of ~100mM, 37ºC, bile salts and low oxygen –all "gut" signals
- Controls ToxT-a response regulator
- ToxT is a DNA binding RR activating genes from TCP, haemagglutinin, OmpU, haemolysin and cholera toxin production
- Cholera toxin genes show similarity to CTX phage genes and may have arrived by gene transfer (Only pathogenic strains have these genes)

Environmental control of expression



Sequence of infection

- **0-1 hr after ingestion, free swimming cells sense “lumenal “ signals and move to brush border**
- **2-3 hrs cells bind to and penetrate the mucus using induced adhesins and proteases. TCP induced**
- **over 4 hours. Microcolonies produced by TCP interaction. ToxR dependent genes switched on including *ctxAB* coding for CT and haemolysin**
- **Microcolonies are also thought to respond to quorum signals, but this is still unclear (although AI production has been shown, role uncertain)**
- **Production of CT-ADP-ribosylating exotoxin which is transported into epithelial cells causes constitutive activation of adenylate cyclase in epithelial cells resulting in disruption of sodium transport into the cells and subsequent water and electrolyte into intestine.**

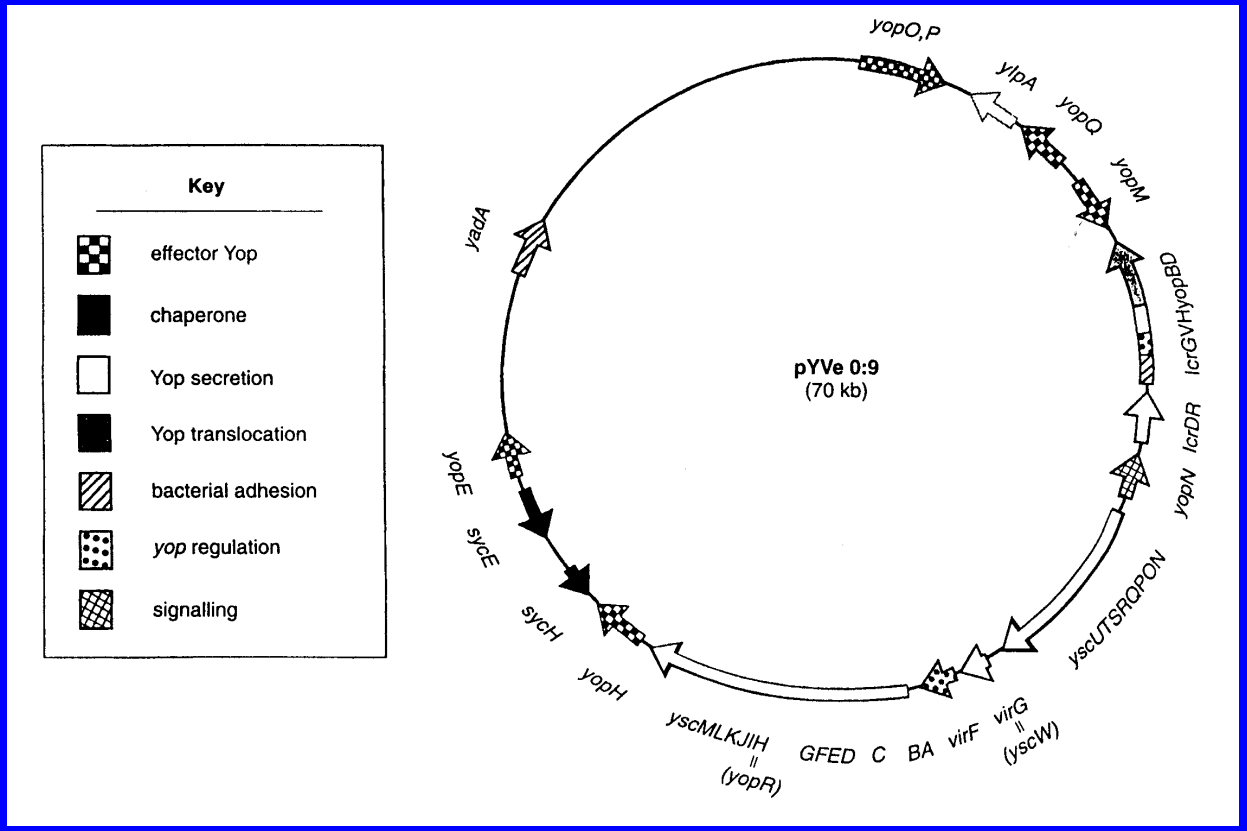
Yersinia

- **Various species: *Y.pestis* causes plague and lives in fleas and rodents, *Y. pseudotuberculosis* lives in water and mammalian gut**
- **Can survive and grow in lymphoid tissue of host**
- **Motile species with a biphasic life style**
- **Virulence genes, which control expression of flagella, invasin, Yops, lipopolysaccharide, regulated by temperature, calcium and quorum sensing**
- **These control the invasion of the host epithelial cells**

Yersinia

- **Bacterium has to sense both a change in environment and contact with a eukaryotic cell membrane for full expression of genes on a 70kb plasmid pYV-the YOP virulon**
- **Virulence genes are often found on either particular region of chromosome or plasmids-called pathogenicity islands (Pais). These may help horizontal transmission.**
- **Communication with host cell uses a Type III secretion pathway. The major transport proteins are closely related to the flagellar export pathway.**
- **Yop toxins not cytotoxic if added extracellularly, need to be inside the eukaryotic cell**

Yop virulon



YopE –cytotoxic

YopH –protein tyrosine phosphatase (antiphagocytic)

YpkA-protein kinase (euk)

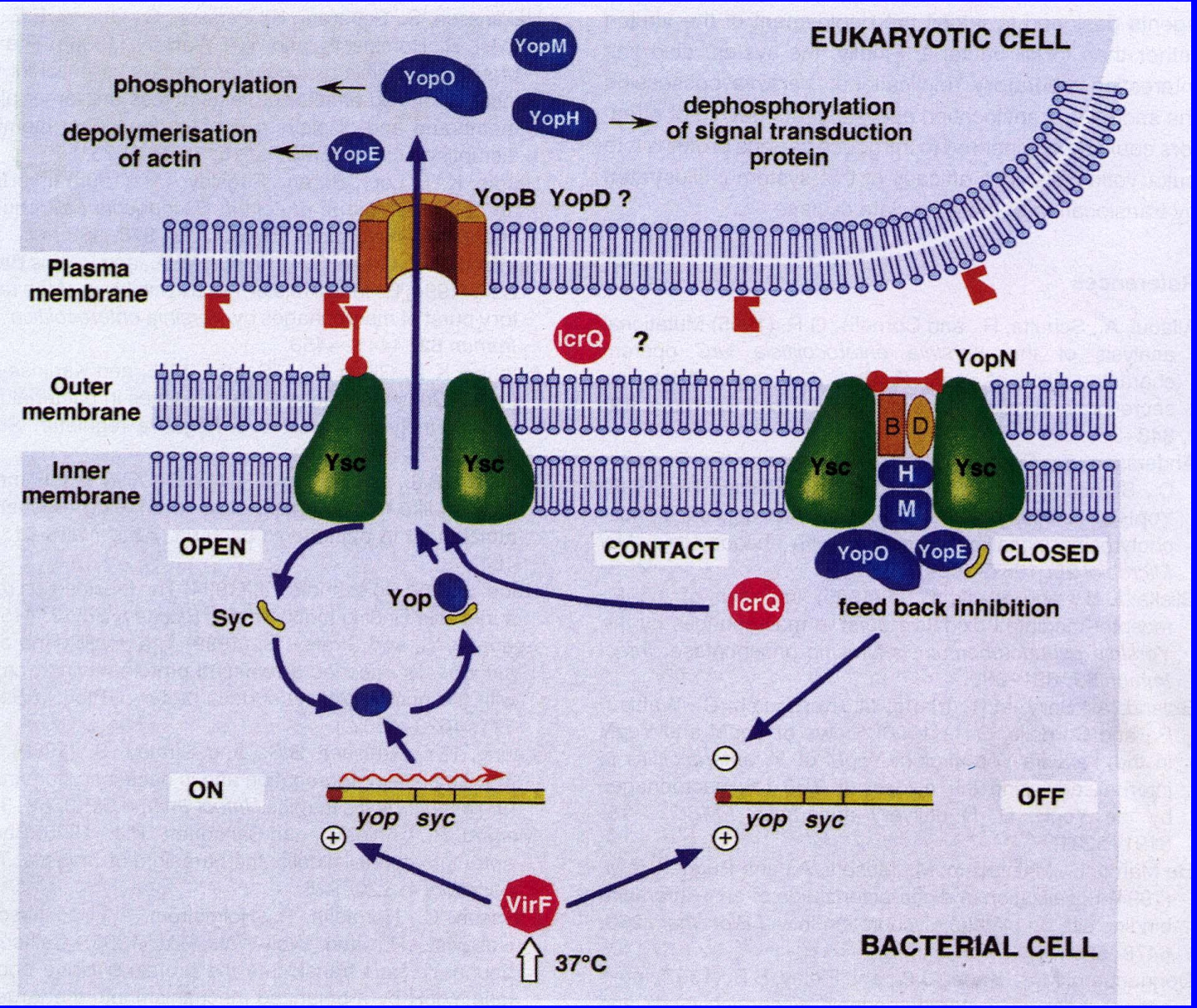
YopJ/P –apoptotic

YopM-thrombin binding factor-prevents blood clotting

Sequence of events

- 37 ^{o}C and Ca $^{2+}$
- VirF-transcriptional activator binds *yop* promoter
- Ysc secretion apparatus and Yop proteins synthesised (Yop stands for Yersinia outer membrane protein)
- If no eukaryotic cell contact YopN blocks Ysc secretion pathway and accumulation of LcrQ inhibits further transcription
- Contact with eukaryotic cell by YopN sensor opens secretion channel at point of contact and LcrQ secreted
- *Yop* genes now fully expressed and Yop proteins transported into eukaryotic cell (Yop proteins have specific chaperone that binds while in bacterium and allows specific transport
- Series of invasin and antiphagocytosis proteins allow invasion of eukaryotic cell

Model of cell-cell interaction

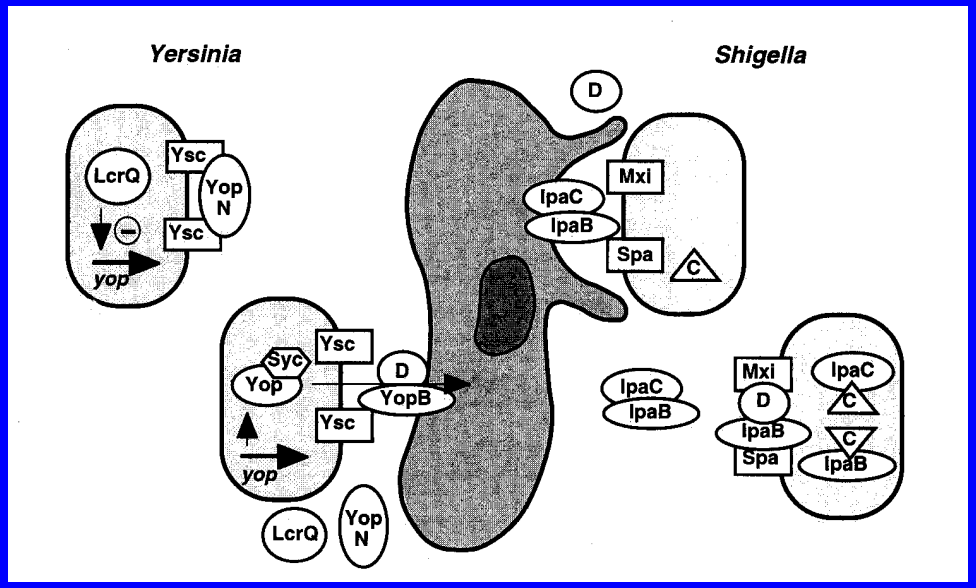


Shigella

- Related to *E.coli*
- Were until recently thought to be non-motile-now found to be motile in the gut and motility helps adhesion to epithelium
- Causes an extreme form of bloody diarrhoea resulting from severe inflamation of bowel resulting from tissue invasion
- *Shigella* invades through M-cells in gut. This involves 30 genes on bacterial side (31kb) on 230kb plasmid and rearrangement of eukaryotic cytoskeleton, followed by engulfment-the Ipa-Ipg system
- *Shigella* are engulfed by macrophages, but rapidly escape from phagosomes into cytoplasm, and induce apoptosis of macrophage and an inflammatory cascade
- Bacteria spread from cell to cell by inducing actin polymerisation that pushes through cell membranes

Comparison between *Shigella* and *Yersinia*

- Type III secretion channel kept shut by YopN
- Accumulation of LcrQ prevents transcription
- Cell contact- YopN released
- Yop proteins secreted into host



- Type III secretion system kept shut by IpaB/D
- Ipa proteins accumulate, protected by chaperone IpgC
- Cell contact releases IpaB/D
- IpaB/C induces membrane ruffling and uptake of cell

Pathogenic sequence

- Virulence depends on temperature, pH (7.4) and osmolarity
- Mutations in EnvZ and/or OmpC reduce virulence (OmpC implicated in cytoskeleton reorganisation and movement within cells)
- CpxA-CpxR-phosphorelay system involved in sensing pH (effect on transcription can be inhibited by temperatures
- VirF is primary virulence gene activator, binding to *virB* promoter. Temperature dependence comes from changes in supercoiling around VirF binding sites. VirF does NOT belong to phosphorelay or quorum family but a family of transcriptional activators responding to physical changes

VirB and VirF control transcription of *ipa* and *ipg* operons

Pathogenic sequence

• IpgC, a specific chaperone, and most other Ipg proteins accumulate in bacterial cytoplasm. IpgC prevents proteolysis (no transcriptional inhibition)

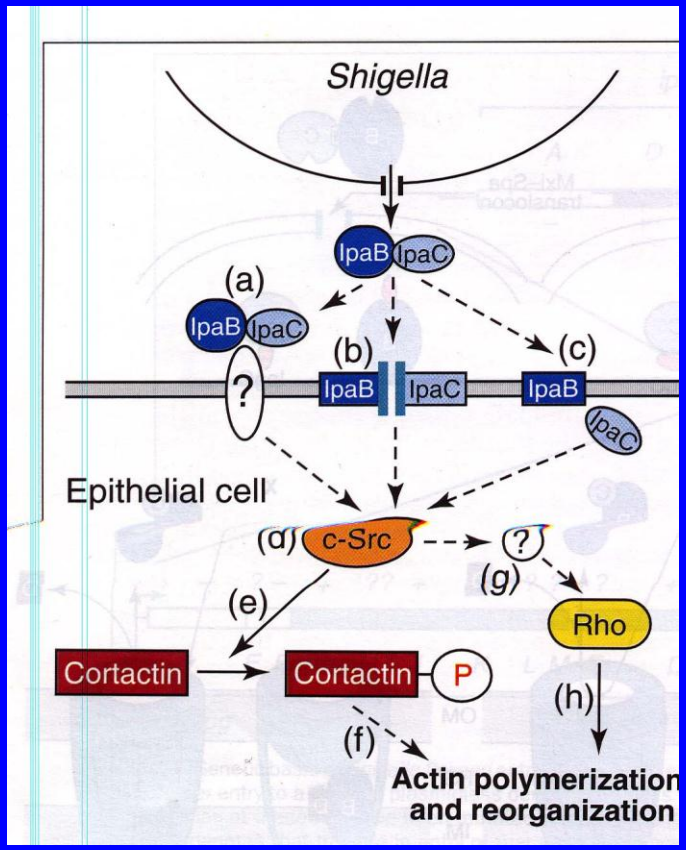
•A type III secretory system is produced (Spa-Mpx). Leakage of accumulated cell proteins is prevented by IpaB and IpaD which work as a “plug”

•On cell contact an inducing signal released IpaB/D

•The local concentration of IpaB and IpaC which is immediately released from the cell induces cell ruffling and engulfment

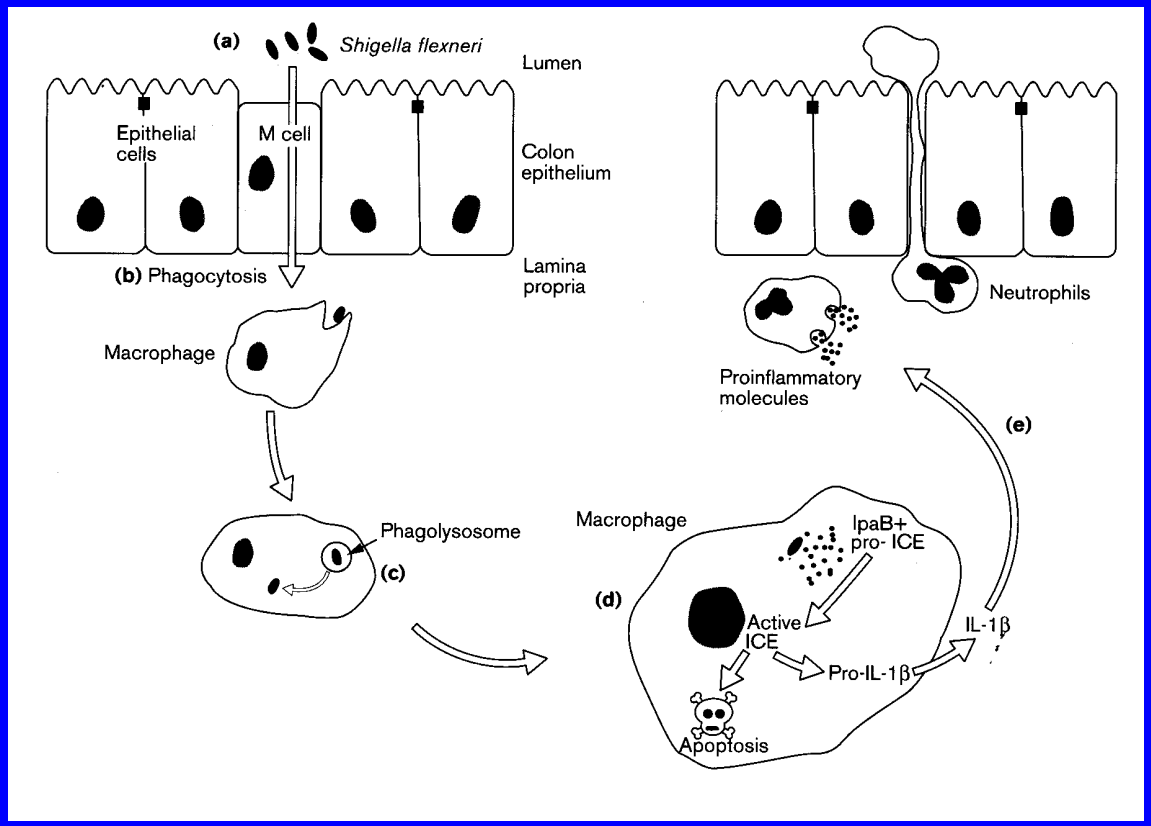
•Once inside the cell the bacterium recruits the cell’s cytoskeleton to produce actin tails which polymerise at great speed, pushing the bacterium through the cell into the neighbouring cell, dividing as it goes

Pathogenic sequence



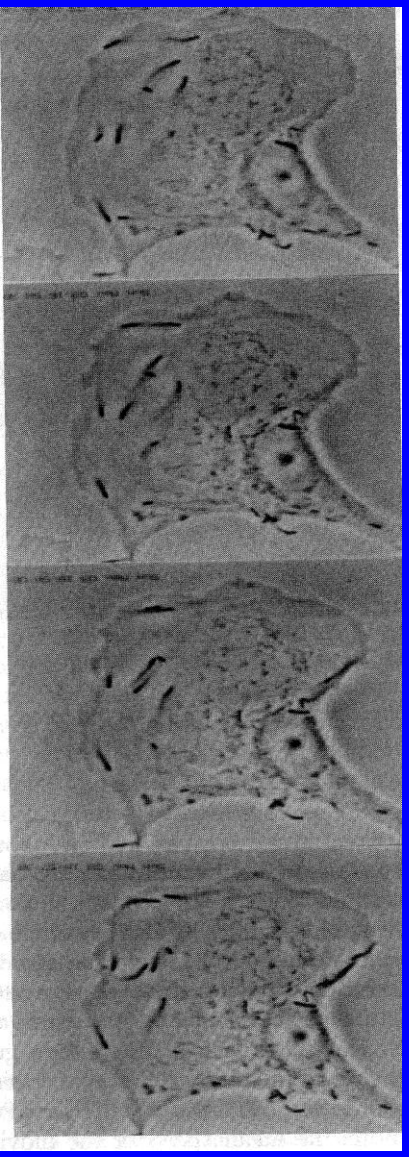
- Cell contact and release of IpaB/C
- Formation of pore in euk cell
- Transmembrane signal activates protooncogene pp60 which causes tyrosine phosphorylation of cortactin (actin binding protein)
- Cytoskeleton reorganised and along with G-protein Rho
- All of which allows cell entry

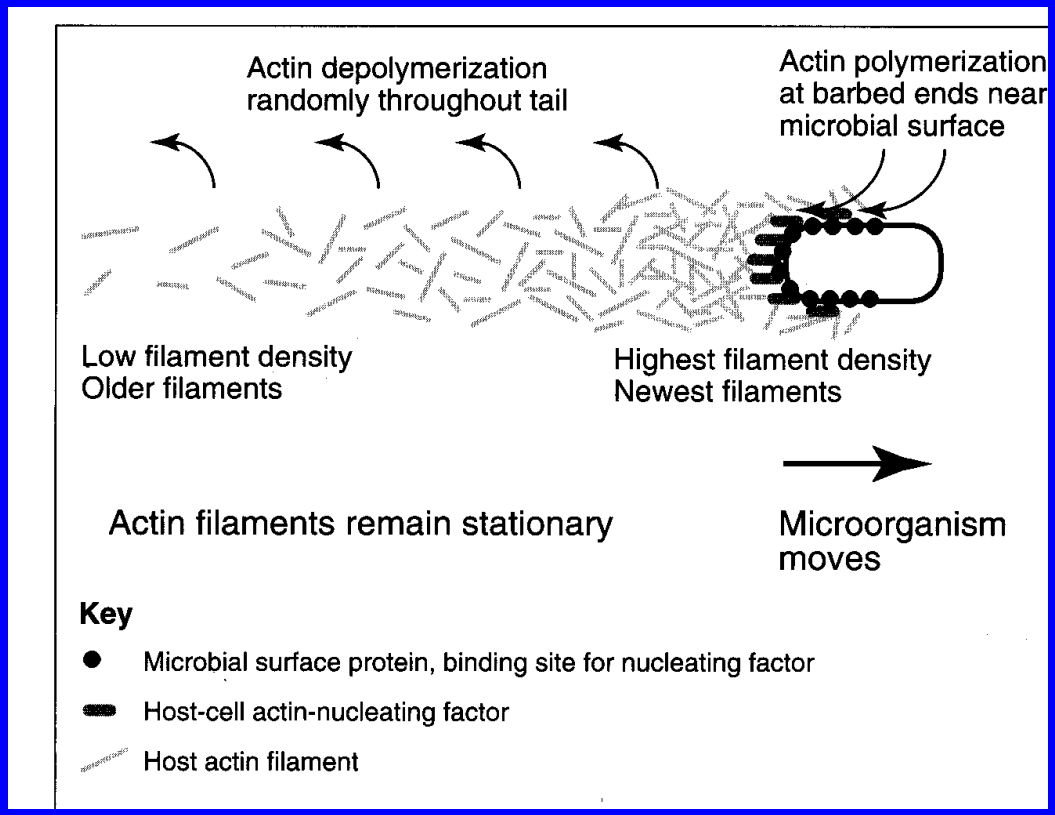
Pathogenic sequence



- On entry in macrophage engulfed by phagolysosome-escapes
- IpaB combines with euk signal to activate apoptosis
- Releases interleukin and activates inflammatory sequence

Actin polymerisation





Up to 100μm/min

- Actin polymerisation used for rapid movement from cell to cell and out of cells into blood stream

- Used by other pathogens e.g *Listeria monocytogenes*-an acid tolerant, salt-tolerant, psychro-tolerant bacterium, wide spread in soil and water

Summary

- Bacteria need to sense where they are-a combination of signals are usually required
- Often a pathogenicity island coding for virulence genes
- Cell contact usually required for response
- TypeIII secretory pathways, similar to flagellar basal bodies, are often required in toxin secretion
- Host systems need to be overcome for proliferation