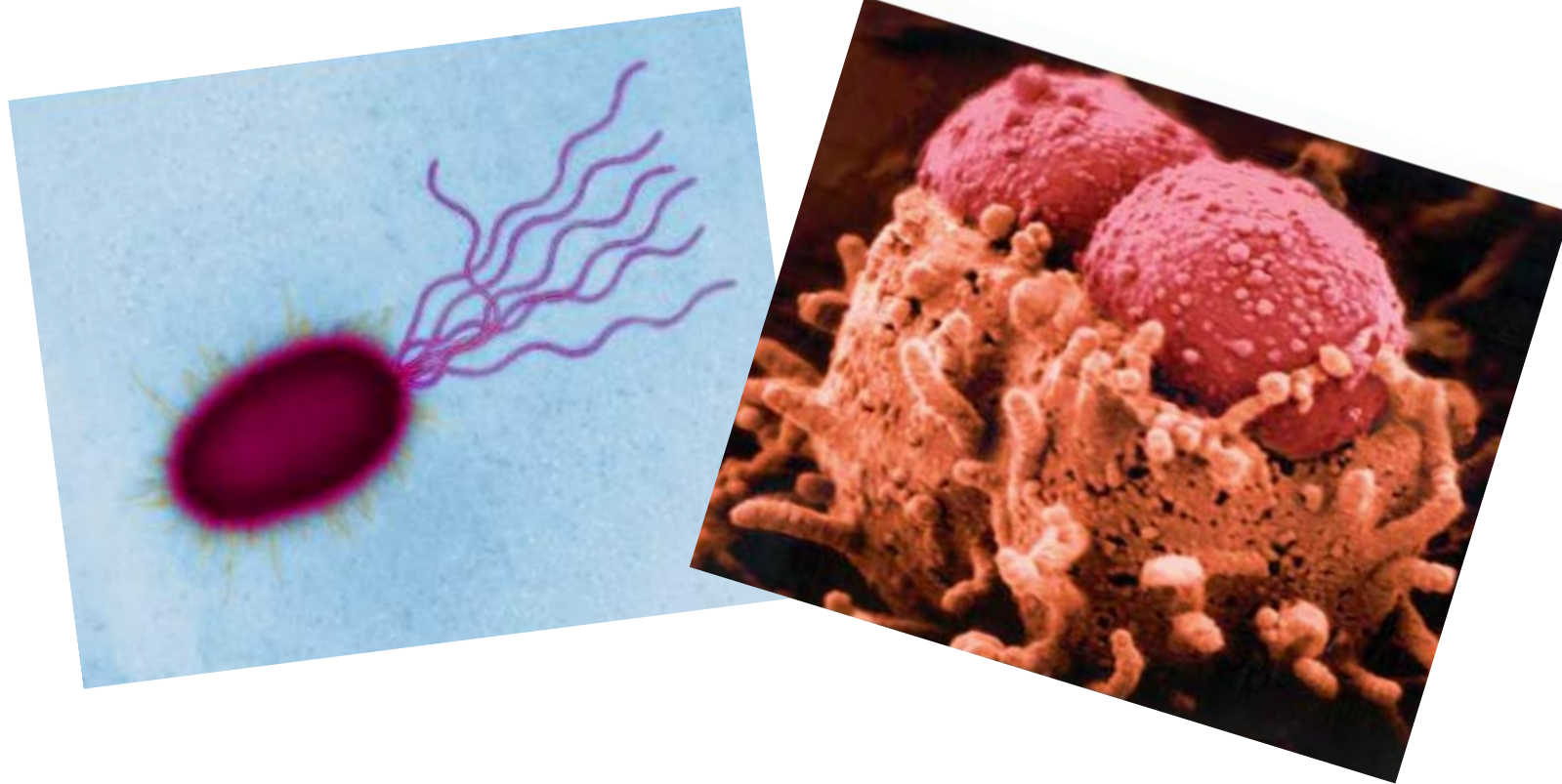
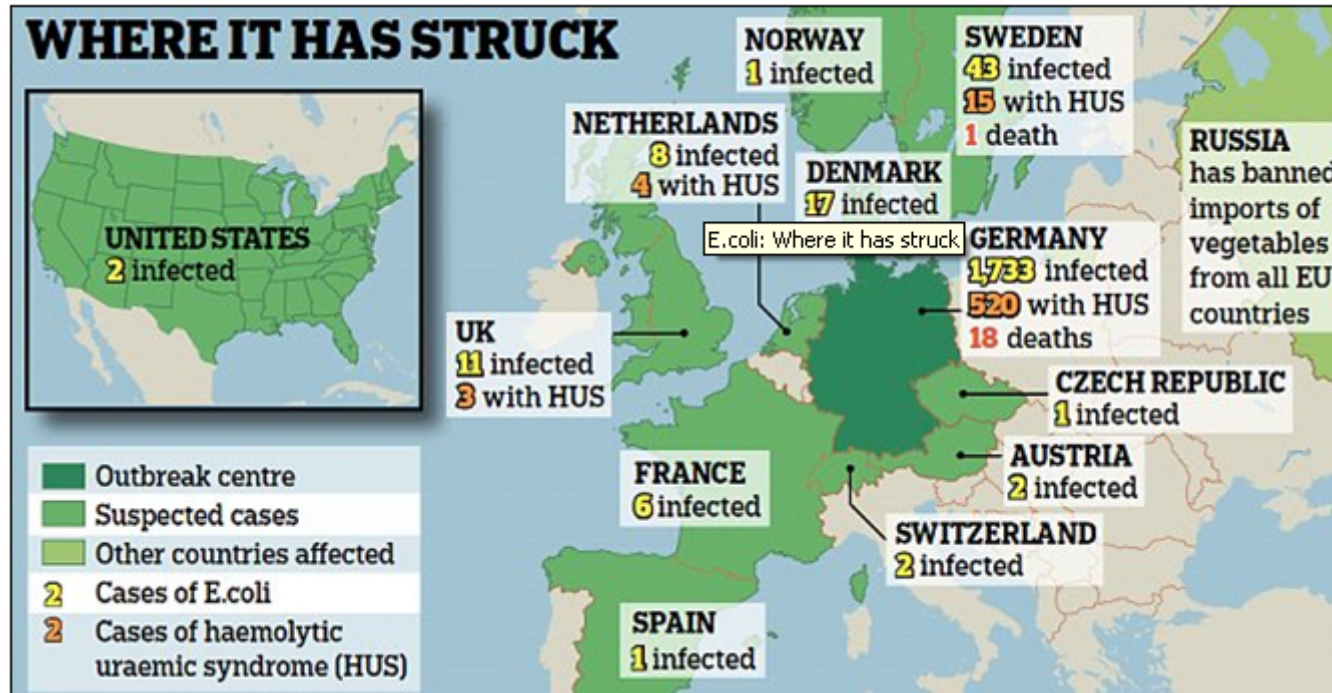


Biology of pathogenic *Escherichia coli*



Why a lecture on pathogenic *E. coli* ?

⇒ outbreak in May-June 2011 : **3507** Europeans + **2** Americans contaminated
39 deaths



- bean sprouts

- new pathogenic *E. coli* : EAHEC for enteroaggregative haemorrhagic *Escherichia coli*

Overview

Introduction

I- Non pathogenic *Escherichia coli*

II- Pathogenic *E. coli*

II-1 Commons features

II-2 Gastrointestinal infections : EHEC

II-3 2 new pathogenic *E. coli* : EAHEC and AIEC

III- Laboratory diagnosis

IV- Antibiotic therapy

Conclusion

Selected reading

INTRODUCTION

Escherichia coli :

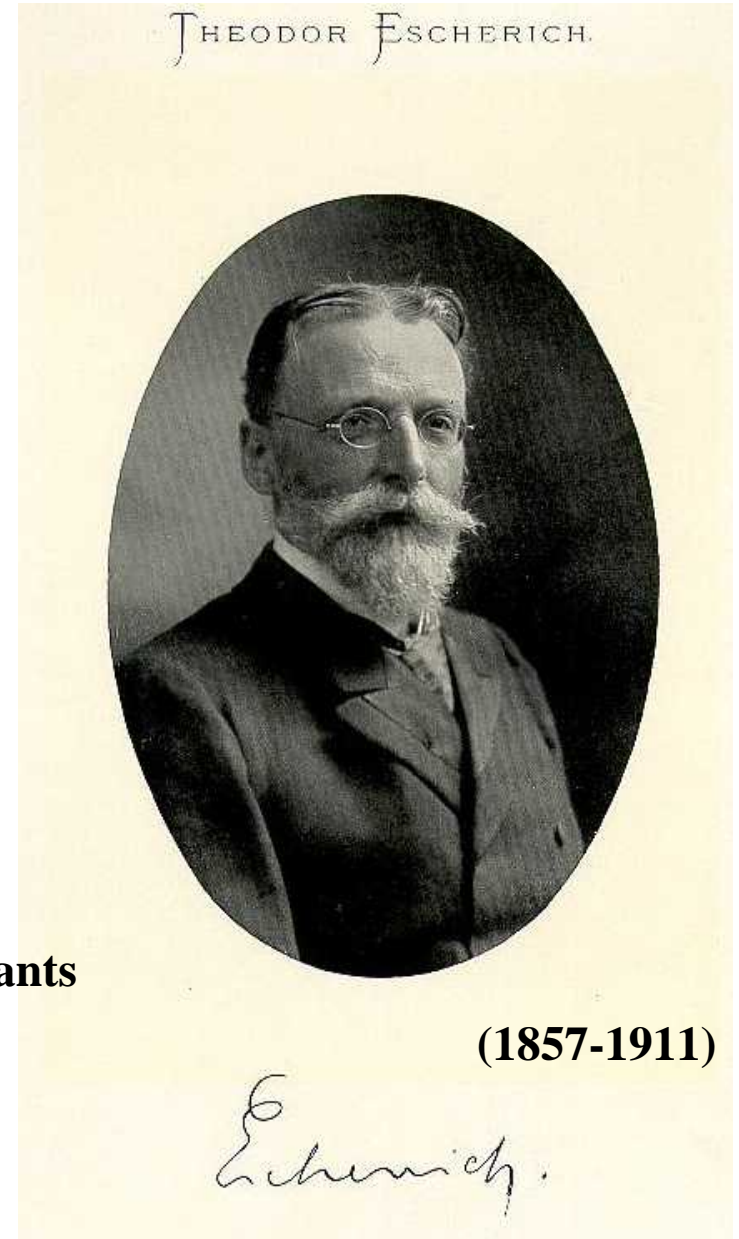
- member of the **normal intestinal microflora** of humans and others mammals
- **model** organism, laboratory workhorse
- important species in **biotechnology** :
 -
 -
 -
 -
- highly **versatile** and frequently deadly **pathogen**

Non pathogenic *E. coli*

- discovered in 1885

- 1919 : *Escherichia coli*

- 1935 : a strain of *E. coli* was shown to be the cause of an outbreak of diarrhoea among infants



Non pathogenic *E. coli*

- member of the **normal intestinal microflora**
- **colonization** of the GI tract of most warmed-blooded animals within hours or a few days after birth
- **adhesion** to mucus of the large intestine/colon
- benefit to their hosts : **beneficial symbiotic relationship**

Non pathogenic *E. coli*

- *Enterobacteriaceae* family (the enteric bacteria)

- **Gram negative** bacterium

- facultative anaerobic

- shape :

long :

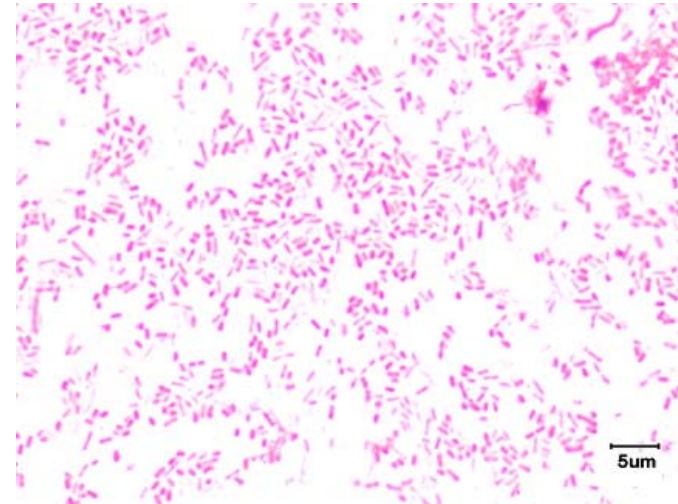
diameter :

-optimal growth :

good **adaptation** to its characteristic habitats
response to environmental signals

- flagella, peritrichous arrangement

- 1997 : complete **genome** sequence



Non pathogenic *E. coli*

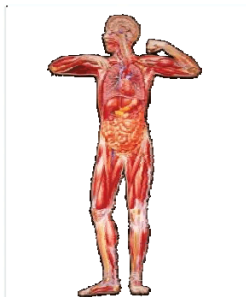
ARTICLE

The Complete Genome Sequence of *Escherichia coli* K-12

Frederick R. Blattner,* Guy Plunkett III,* Craig A. Bloch, Nicole T. Perna, Valerie Burland, Monica Riley, Julio Collado-Vides, Jeremy D. Glasner, Christopher K. Rode, George F. Mayhew, Jason Gregor, Nelson Wayne Davis, Heather A. Kirkpatrick, Michael A. Goeden, Debra J. Rose, Bob Mau, Ying Shao

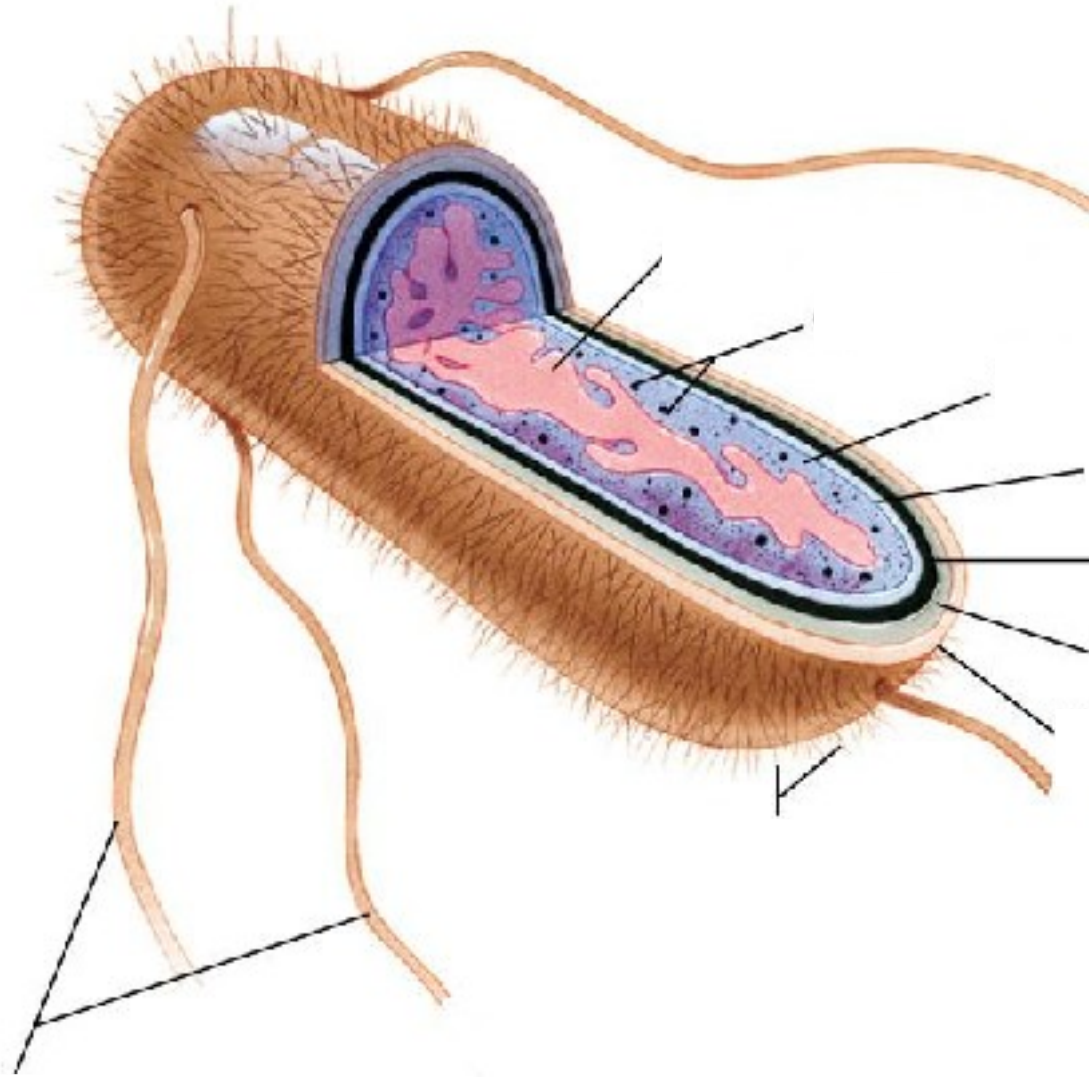
The 4,639,221–base pair sequence of *Escherichia coli* K-12 is presented. Of 4288 protein-coding genes annotated, 38 percent have no attributed function. Comparison with five other sequenced microbes reveals ubiquitous as well as narrowly distributed gene families; many families of similar genes within *E. coli* are also evident. The largest family of paralogous proteins contains 80 ABC transporters. The genome as a whole is strikingly organized with respect to the local direction of replication; guanines, oligonucleotides possibly related to replication and recombination, and most genes are so oriented. The genome also contains insertion sequence (IS) elements, phage remnants, and many other patches of unusual composition indicating genome plasticity through horizontal transfer.

www.sciencemag.org • SCIENCE • VOL. 277 • 5 SEPTEMBER 1997



: 3.4 billion– base pair

Non pathogenic *E. coli*



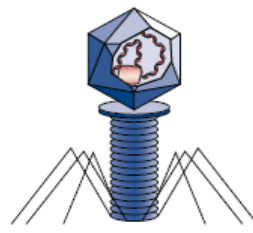
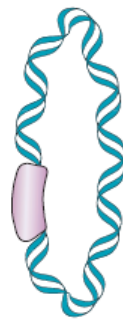
Pathogenic *E. coli* : common features

- acquisition of **specific virulence attributes**

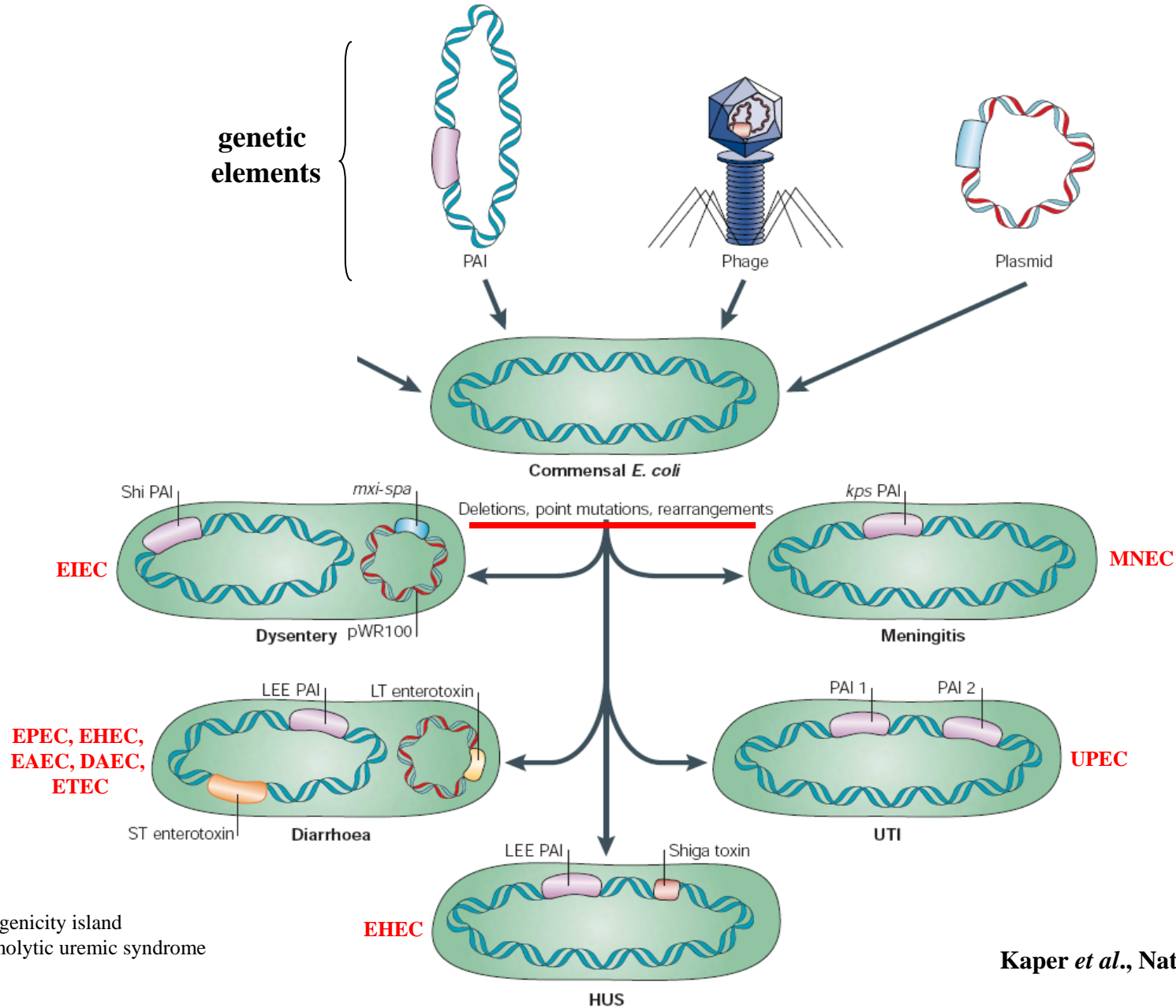
⇒ **increased ability to adapt to new niches :**

⇒ **cause a broad spectrum of disease**

- encoded on **genetic elements**



Pathogenic *E. coli* : common features



PAI : pathogenicity island
 HUS : haemolytic uremic syndrome

Kaper *et al.*, Nature reviews, 2004

Pathogenic *E. coli* : common features

- 2 infections :

⇒ enteric/diarrhoeal diseases :

EPEC (enteropathogenic *E. coli*)

EHEC (enterohaemorrhagic *E. coli*)

ETEC (enterotoxigenic *E. coli*)

EAEC (enteroaggregative *E. coli*)

DAEC (diffusely adherent *E. coli*)

EIEC (enteroinvasive *E. coli*)

EAHEC (enteroaggregative haemorrhagic *E. coli*)

AIEC (adherent-invasive *E. coli*)

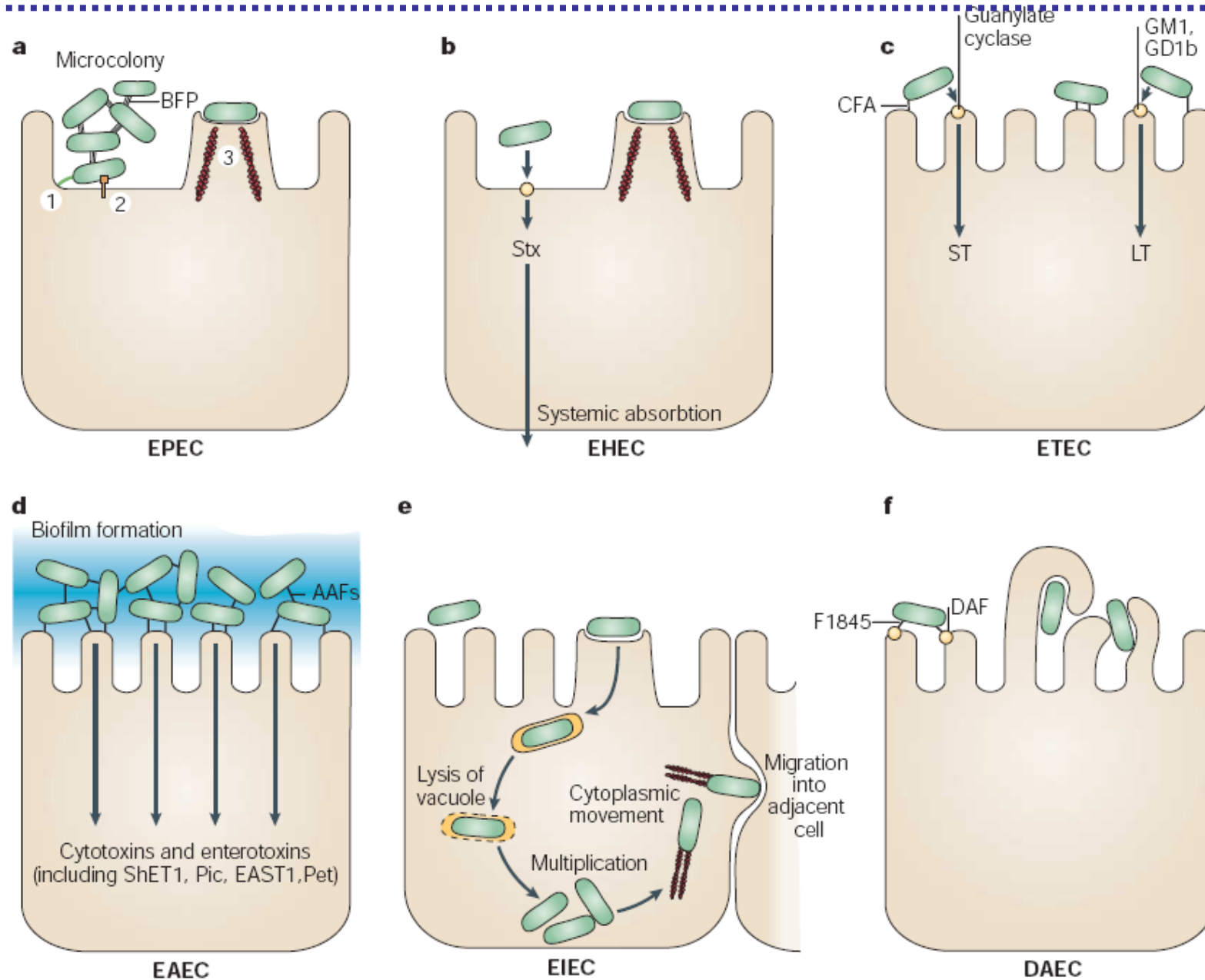
⇒ extra-intestinal diseases:

- urinary tract infections (UTIs) : **UPEC** (uropathogenic *E. coli*)

- sepsis/meningitis : **MNEC** (meningitis-associated *E. coli*)

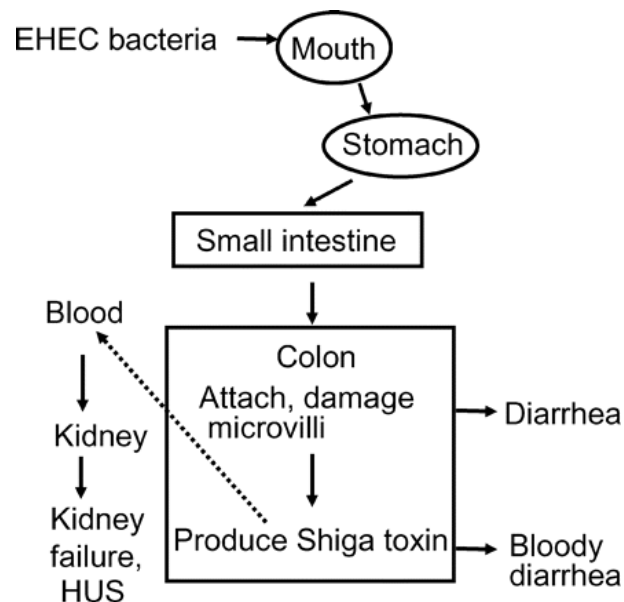
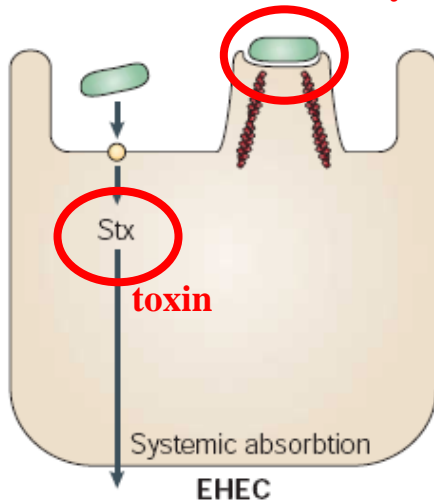
classification based on their unique virulence factors and identification only by these traits

Gastrointestinal infections



EHEC : enterohaemorrhagic *E. coli*

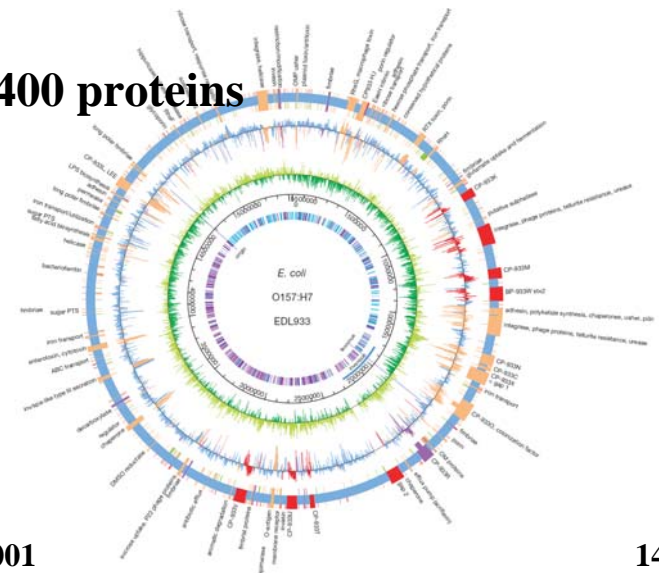
- adhesin
- secretion system



Gyles, J. Anim Sci., 2001

- in the popular press : **Hamburger *E. coli***
Hamburger disease
- **O157:H7 serotype**
- reservoir of EHEC : **bovine intestinal tract**
- infectious dose : **10-100 bacteria**
- mild diarrhea,
hemorrhagic colitis,
hemolytic uremic syndrome (**HUS, 5-10%**)

- genome : **5.5 mbp, 5400 proteins**



Perna *et al.*, Nature, 2001

EHEC : enterohaemorrhagic *E. coli*

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Q&A: E. coli O157

News Front Page
World
UK
England
Northern Ireland

How e-coli spreads from livestock to humans



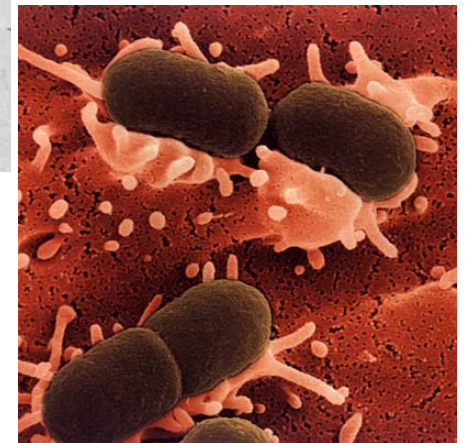
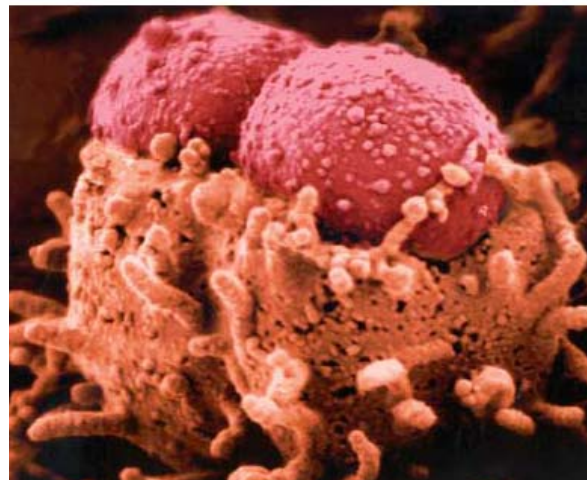
EHEC infection

- attaching and effacing lesion : intimate adhesion, cytoskeletal changes

⇒ effacement of intestinal microvilli and pedestal-like structures



Frankel *et al.*, Mol mic., 1998
Nougayrede *et al.*, Cell mic., 2003
Kaper *et al.*, Nature reviews, 2004



PAI of EHEC

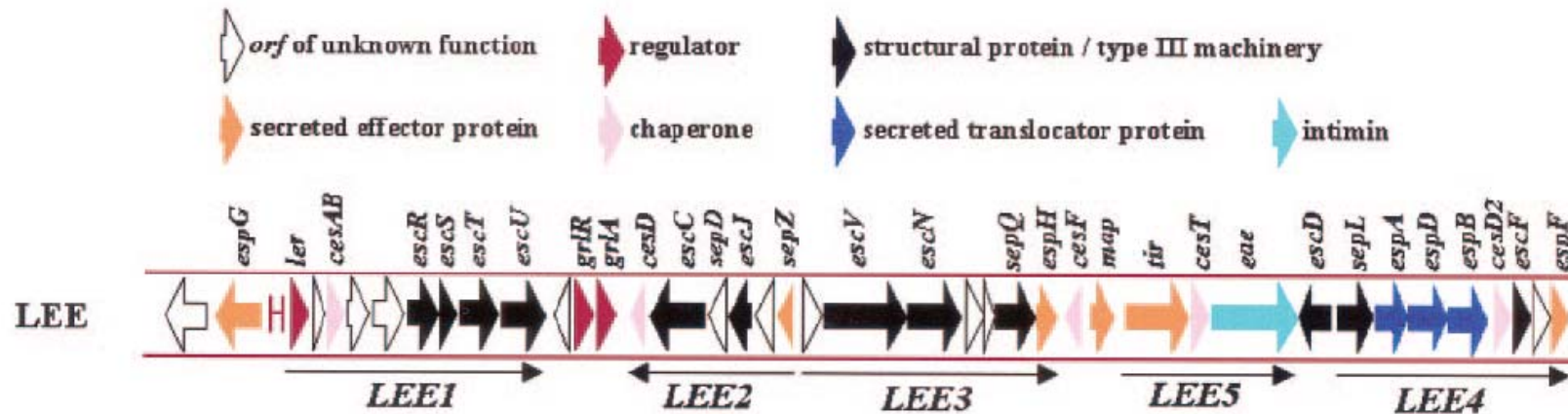
- 35-kbp PAI : LEE (Locus of Enterocyte Effacement)

Proc. Natl. Acad. Sci. USA
 Vol. 92, pp. 1664–1668, February 1995
 Microbiology

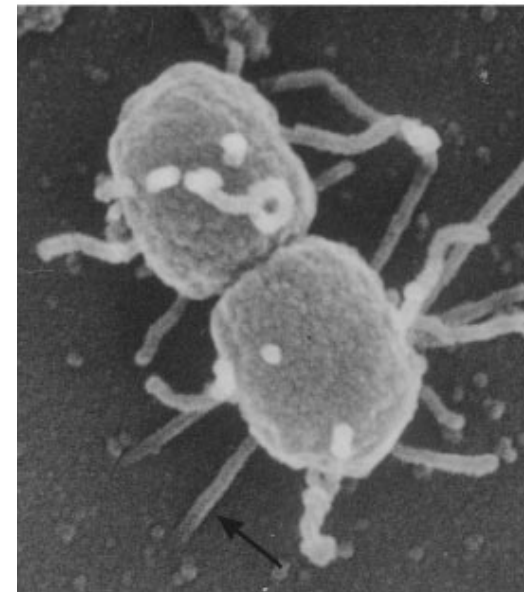
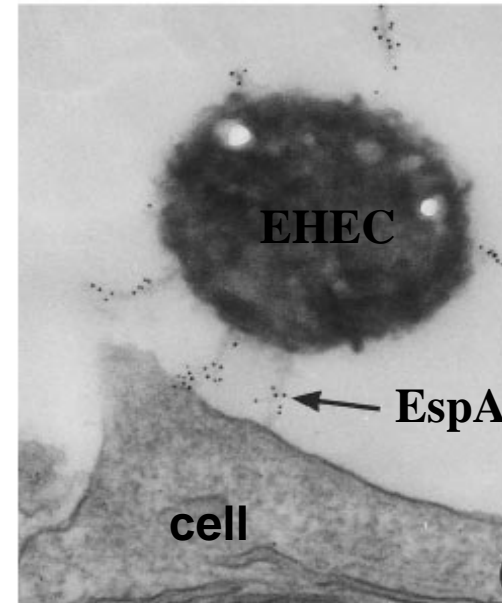
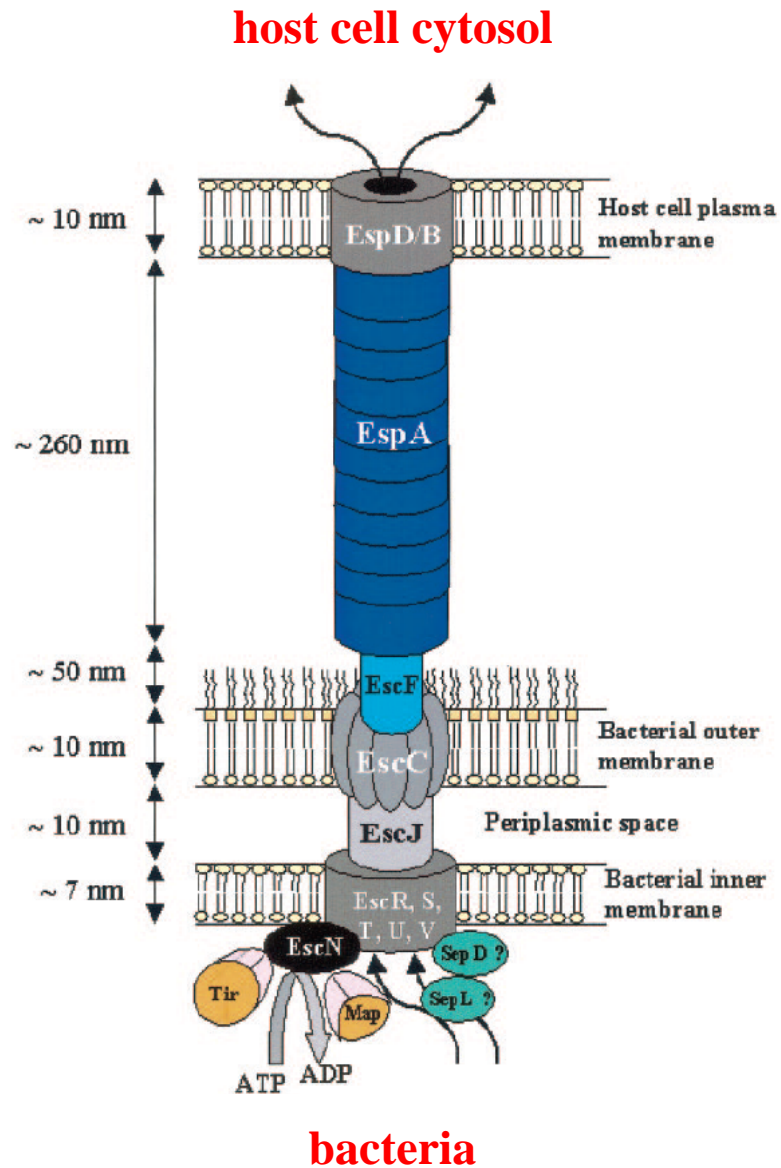
A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens
 (bacterial pathogenesis/epithelial cells/attaching and effacing lesions)

TIMOTHY K. MCDANIEL*†, KAREN G. JARVIS*, MICHAEL S. DONNENBERG†‡§, AND JAMES B. KAPER*†¶

⇒ type III secretion system and effector proteins



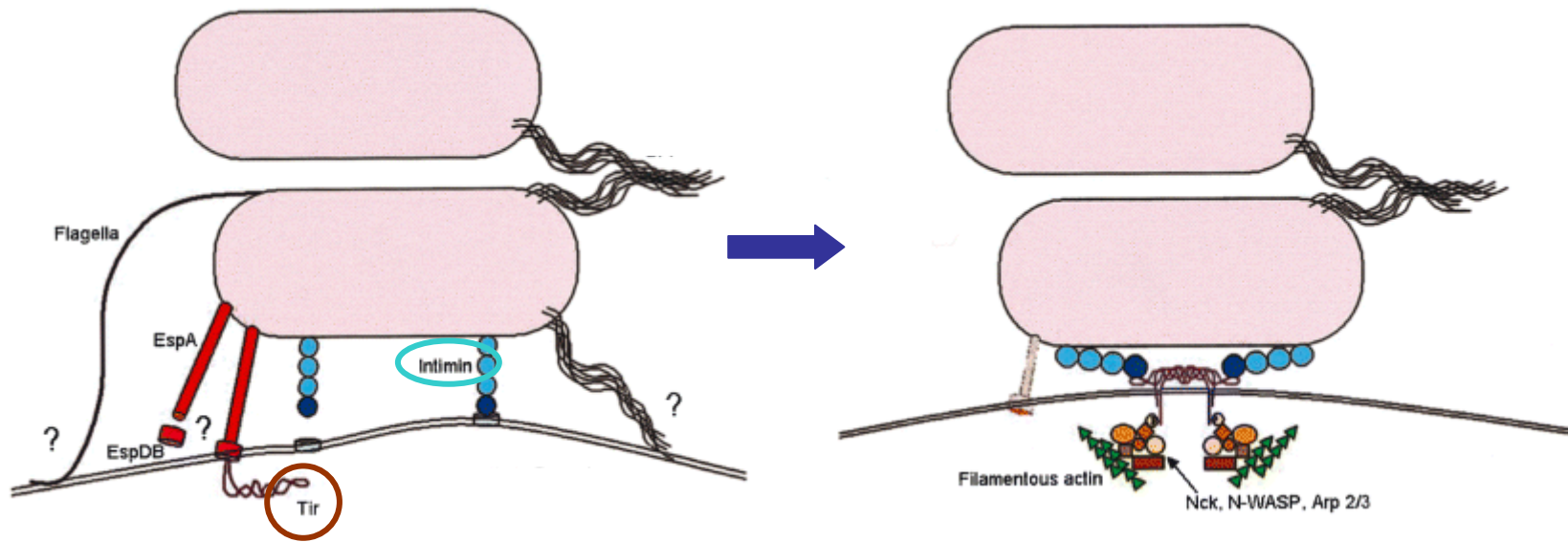
Type III secretion system of EHEC



Type III secretion system of EHEC

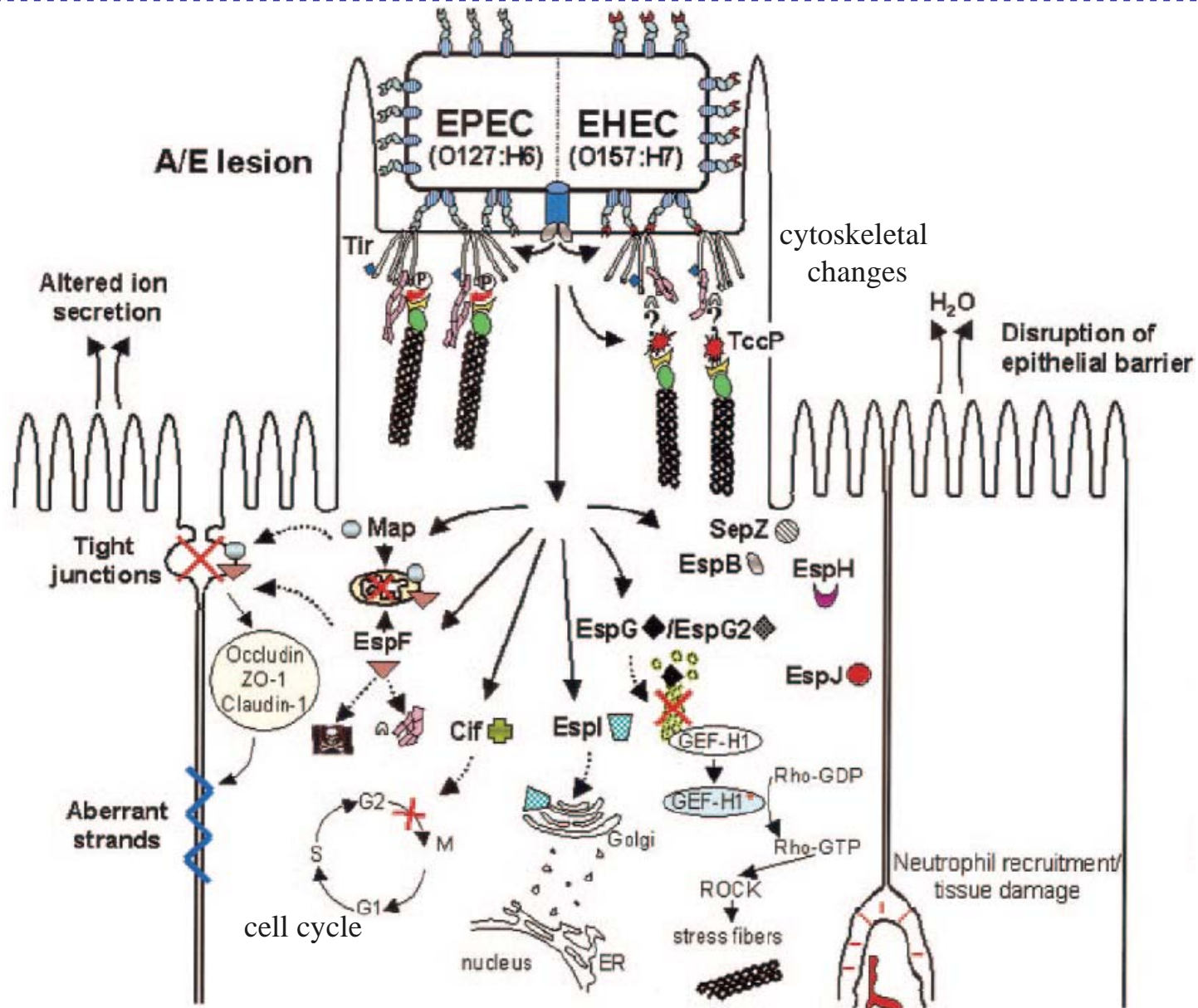
eae gene : intimin protein, bacterial outer membrane

tir gene : Tir protein, secreted, intimin receptor, anchored in the host cell membrane



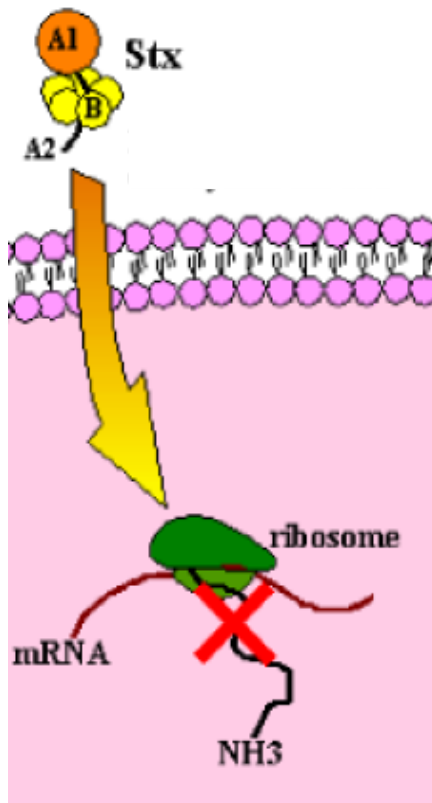
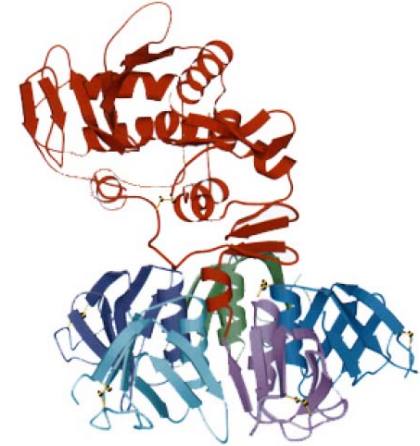
➡ intimate adhesion

Type III secretion system of EHEC



Toxins of EHEC

- most characteristic virulence factors : shiga-toxins
- encoded by **phages**
- **AB₅ toxin**

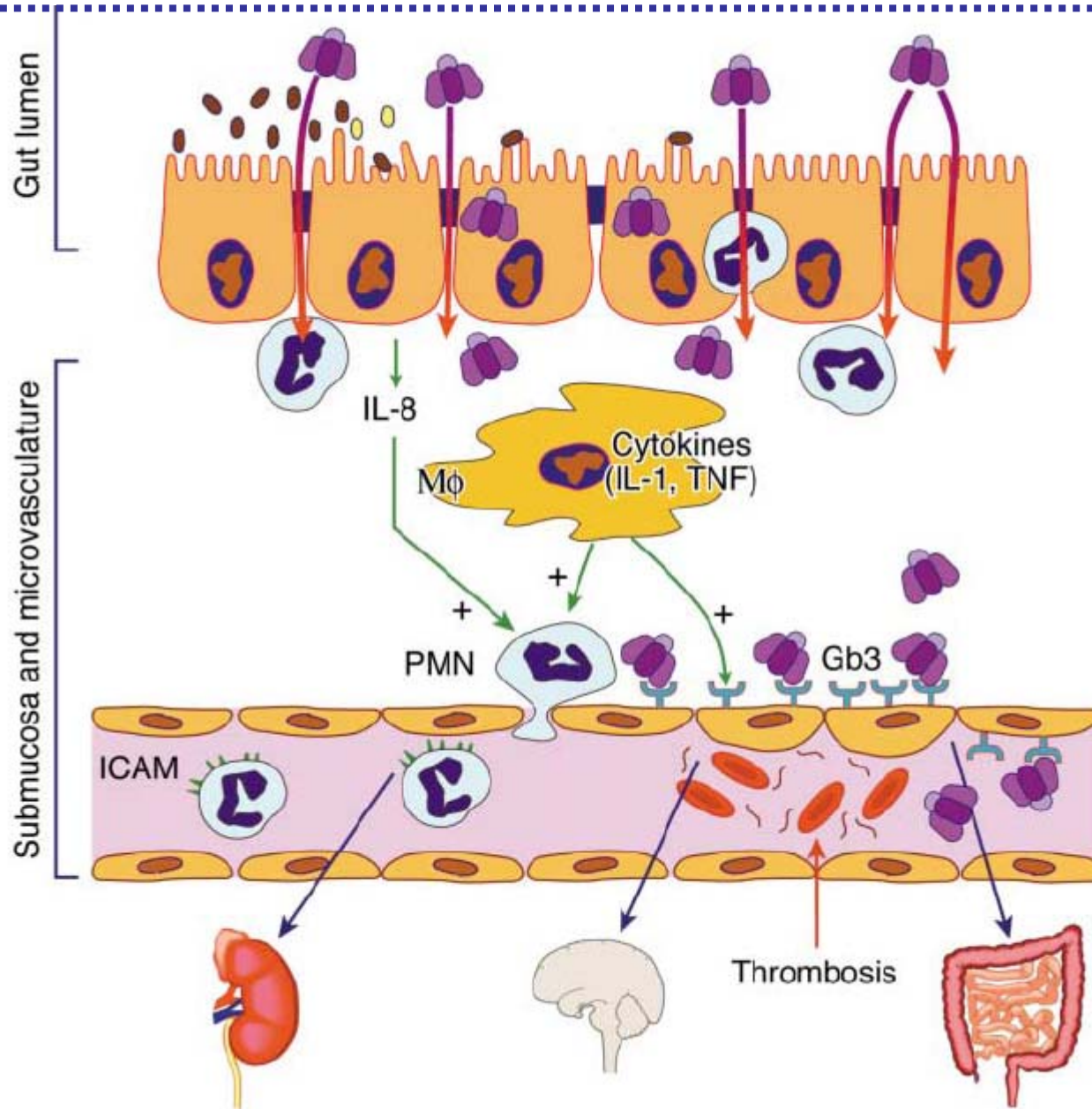


B subunits : binding to specific glycolipids on the host cell
specifically globotriaosylceramide (**Gb3**)

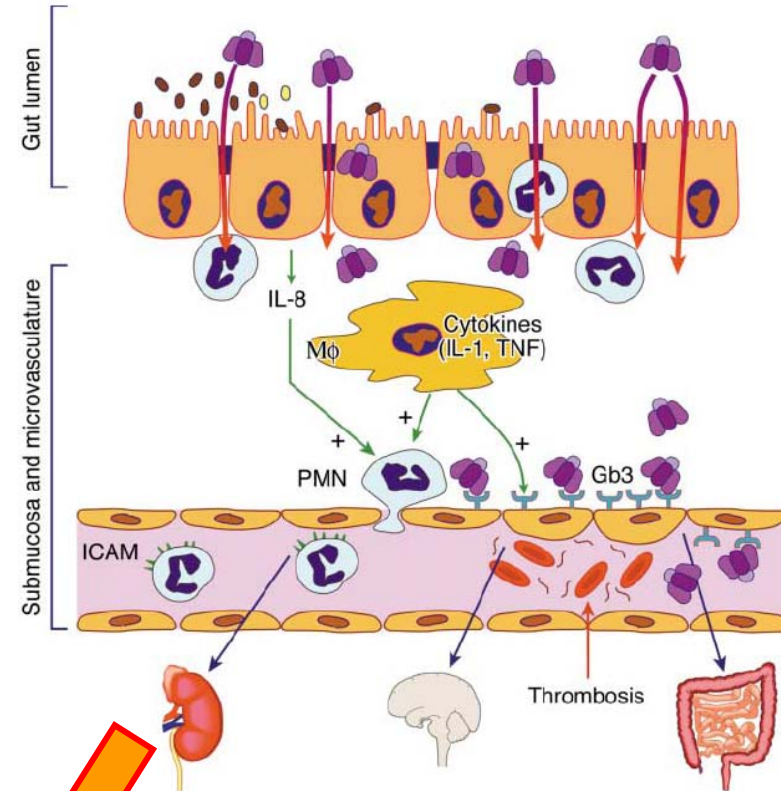
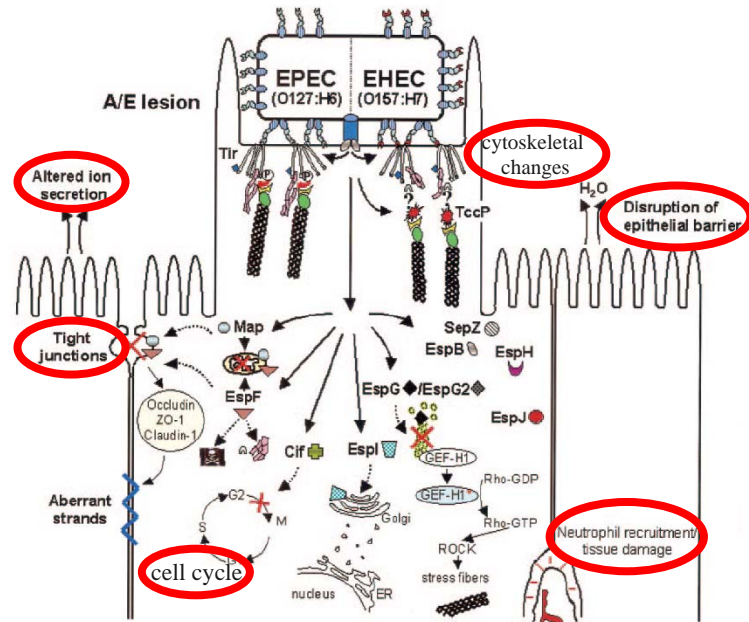
A subunit :

Gb3 receptor : renal epithelial tissues, CNS neurons and endothelium

Toxins of EHEC

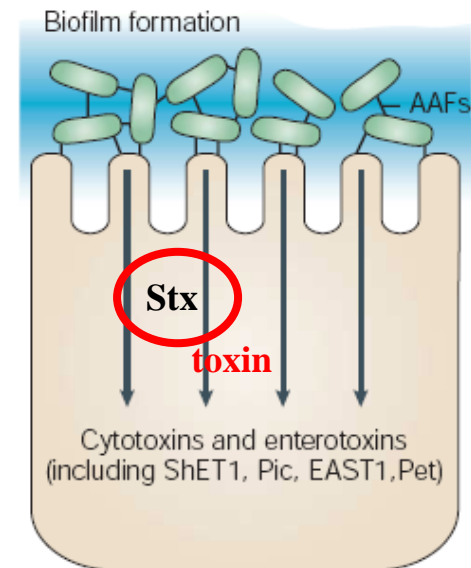
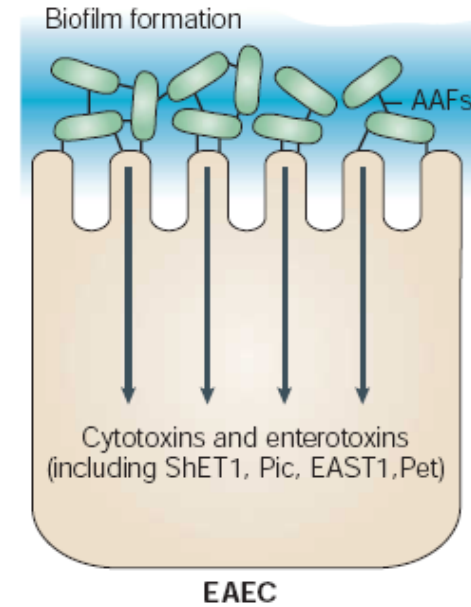
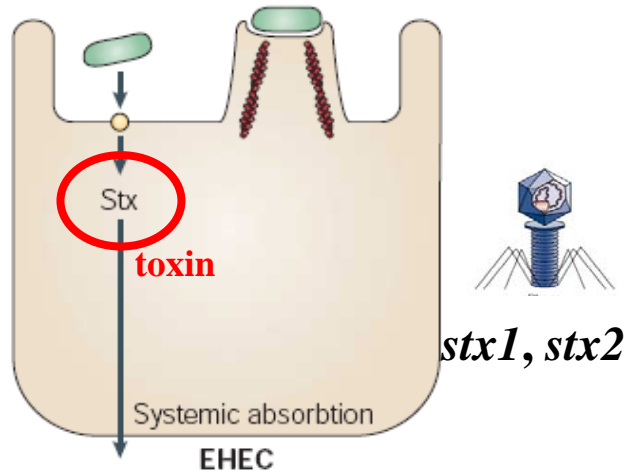


EHEC pathogenesis



bloody diarrhea
thrombosis
kidney failure, HUS

EAHEC : Enteroaggregative haemorrhagic *E. coli*



Enteroaggregative haemorrhagic *E. coli* =

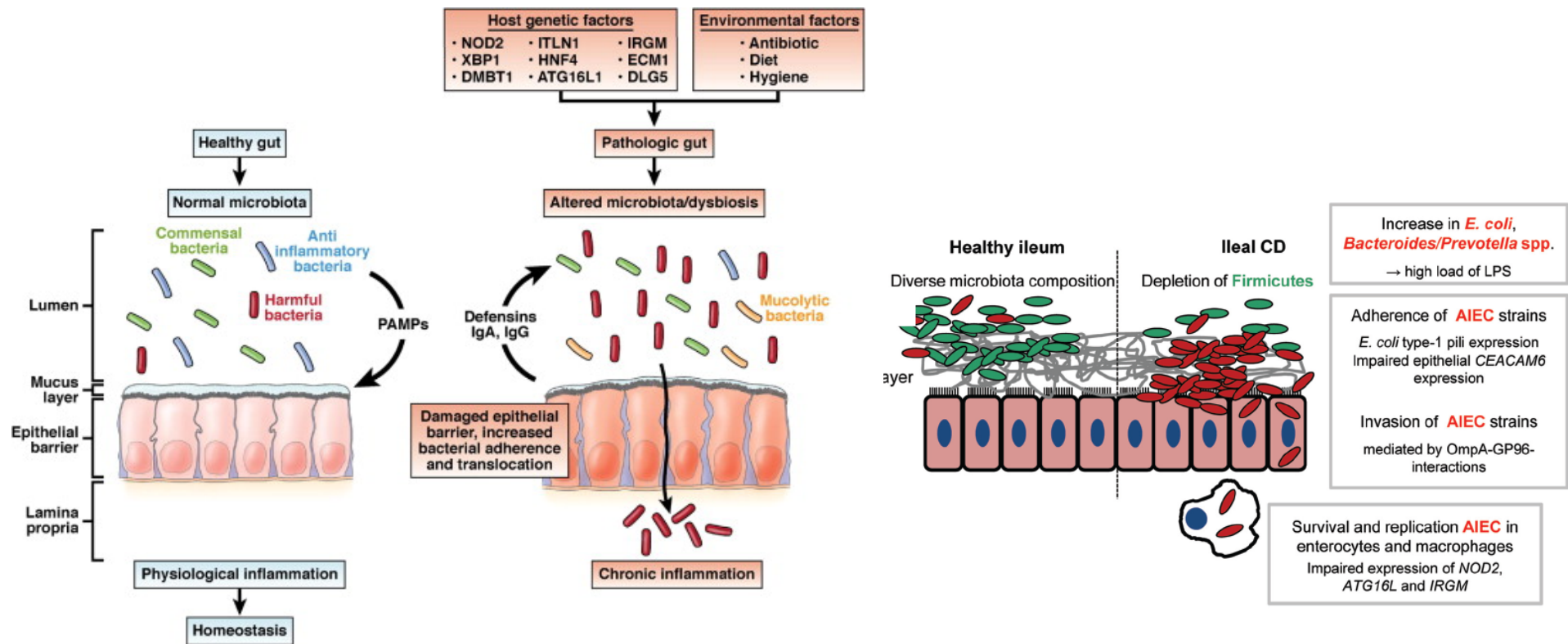


haemolytic uremic syndrome

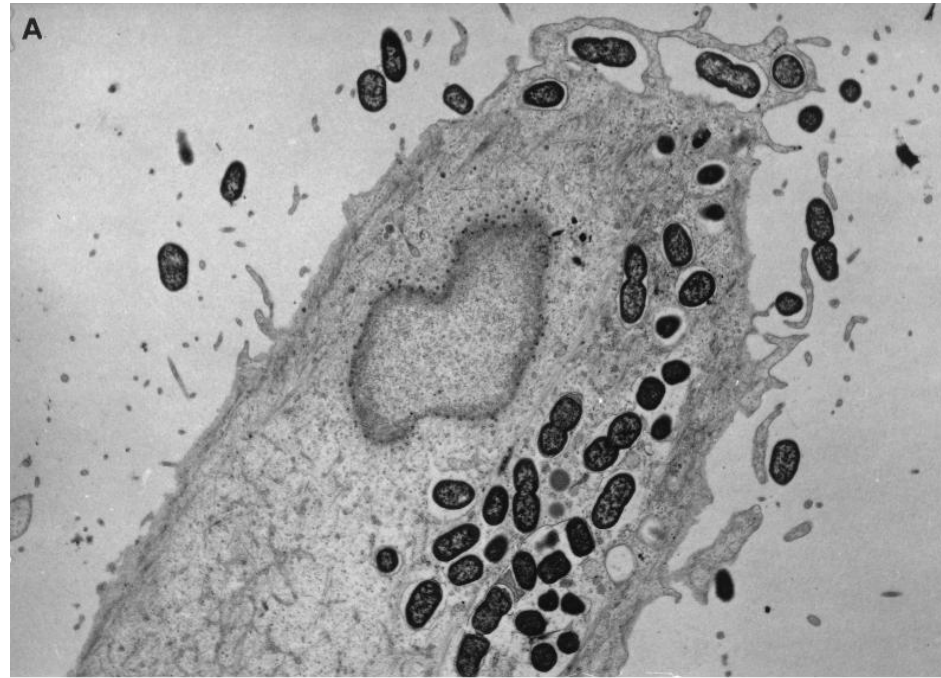
Crohn's disease and AIEC (Adherent-Invasive *E. coli*)

dysbiosis

increased number of mucosa-associated *E. coli* in patients with Crohn's disease



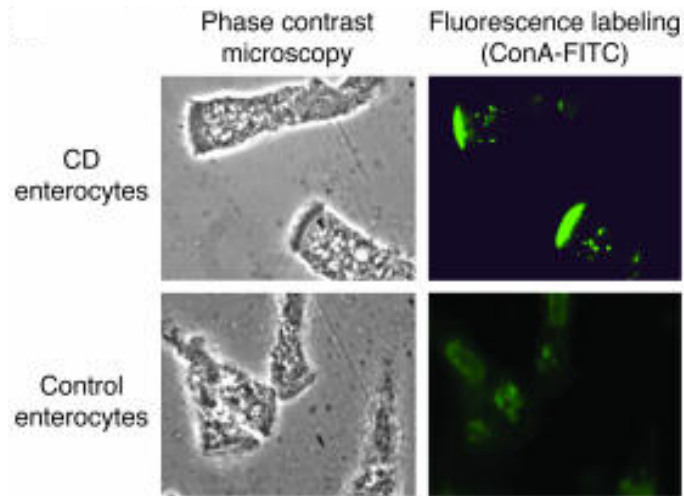
AIEC and intestinal epithelial cells



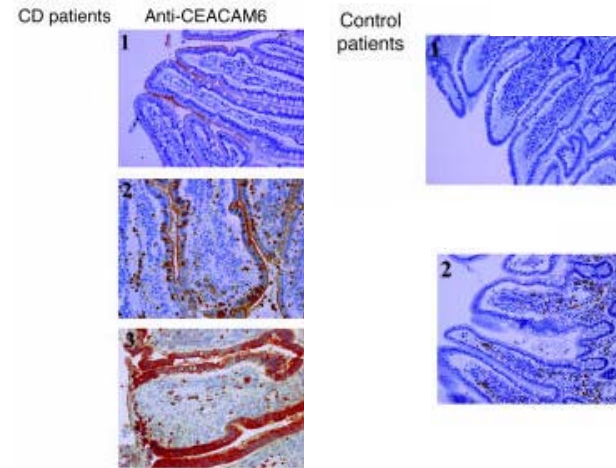
Boudeau *et al.*, I and I, 2001
Darfeuille-Michaud *et al.*, , Gastro. 2004

Attachment of AIEC to host epithelial cells

Labelling of **sugar molecules**



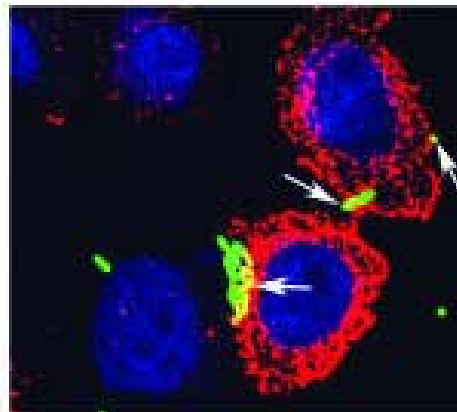
Immunohistochemistry staining (**CEACAM6**)



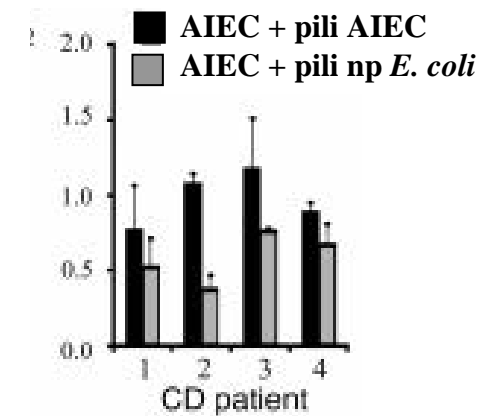
HeLa cells expressing **CEACAM6**, infected with **fluorescent AIEC bacteria**

CD patient : CEACAM6 overexpression
AIEC: pili variant

interaction pili variant - CEACAM6

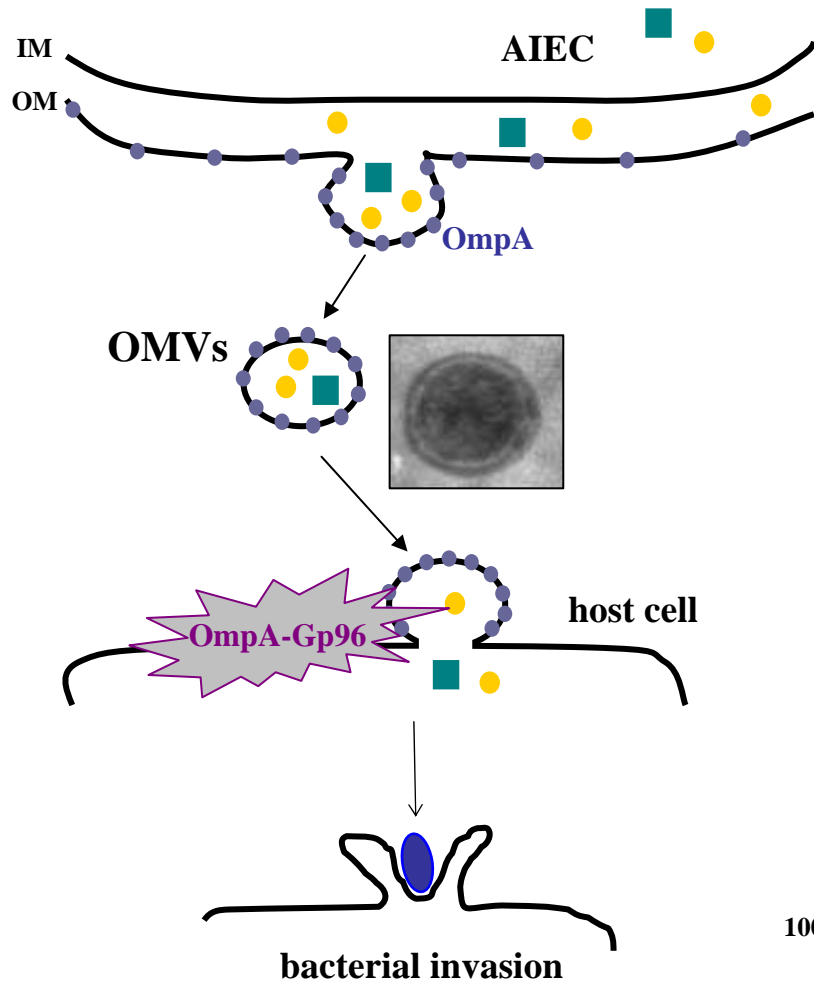


Adhesion index



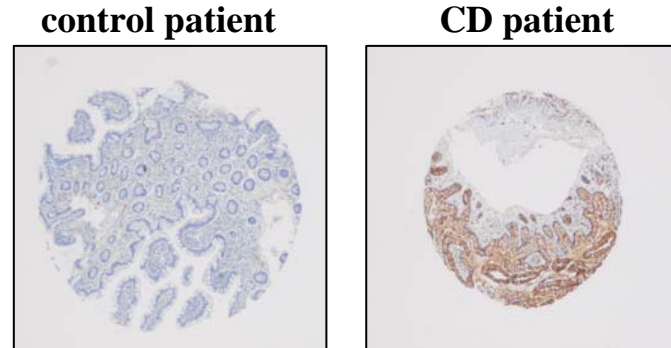
Barnich *et al.*, JCI 2007

Invasion of host epithelial cells



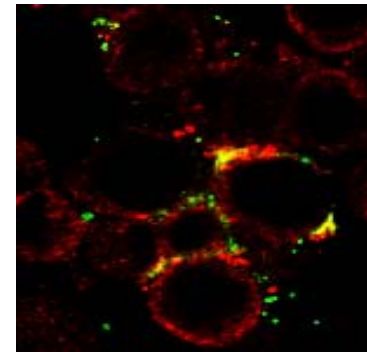
CD patient : Gp96 overexpression
AIEC: OMVs production

Immunohistochemistry staining (Gp96)

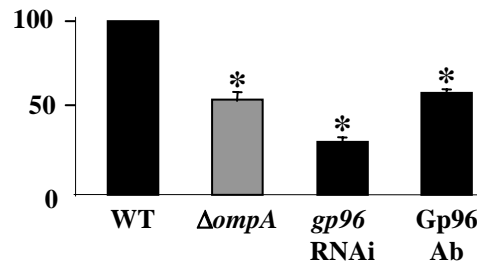


Immunofluorescence :

Gp96 + OmpA



Invasion assay :

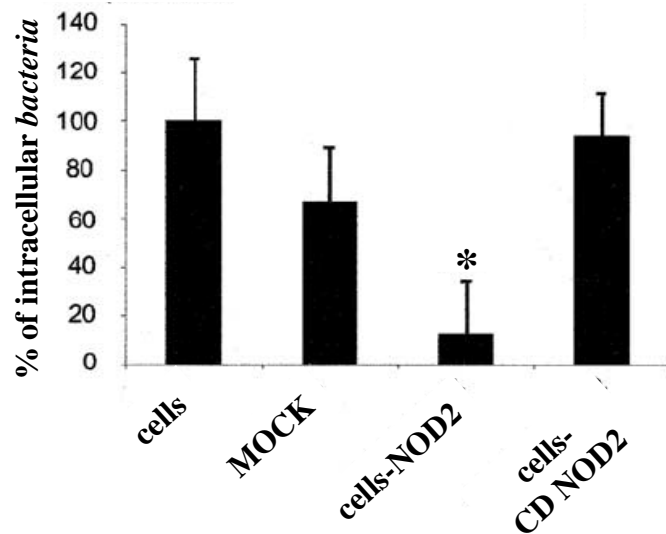


Rolhion *et al.*, Gut 2010
Rolhion *et al.*, Gut Microbes 2011

Survival and replication in host epithelial cells

Disease risk genes:

1) ***NOD2*** (intracellular pattern recognition receptor, bacterial PG), mutated in CD patients

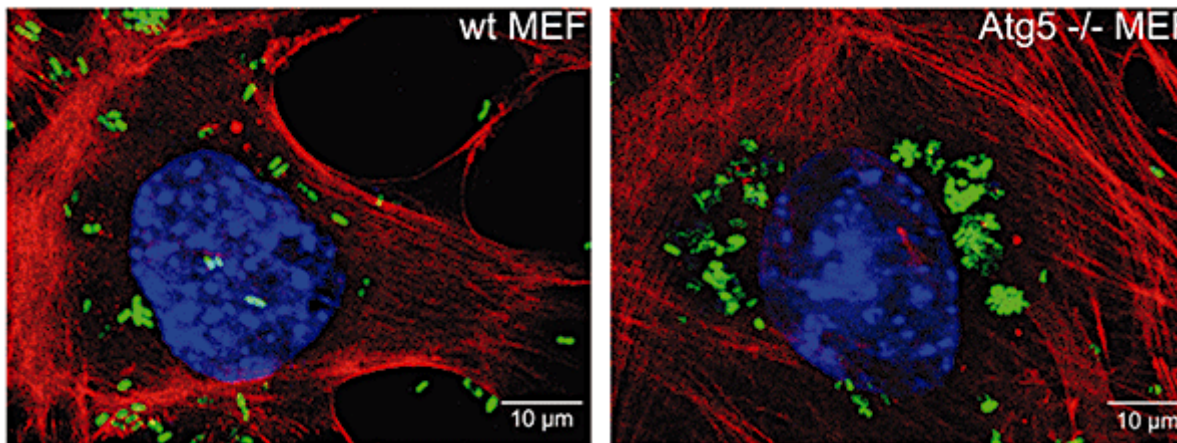


CD patient : *NOD2* mutations,
autophagy-associated genes mutations

AIEC: survival and replication

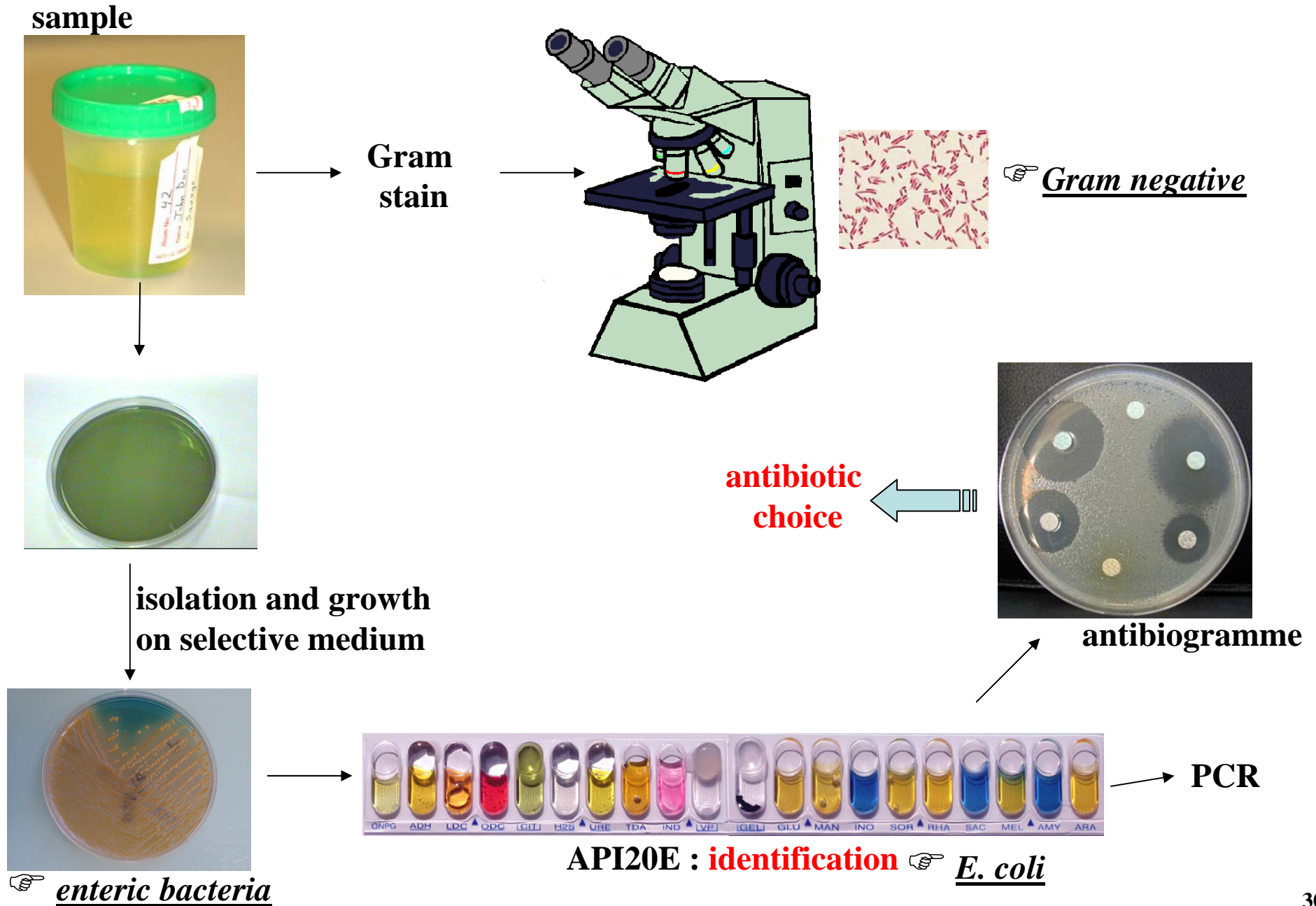
Hisamatsu *et al.*, Gastro 2003

2) autophagy-associated genes (***ATG16L1*** and ***IRGM***)



Lapaquette *et al.*, Cell Micro 2009

Laboratory diagnosis



Antibiotic therapy...

- **antibiotics** : amoxicillin, semi-synthetic penicillins, many cephalosporins, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin....

- **antibiotic resistance : growing problem**

- try to define or to find **news therapeutic targets or news strategies** :

⇒ **targets : structural components of type III secretion system**

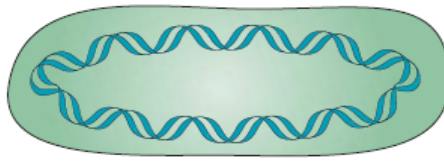
⇒ **strategies : block of bacterial adhesion (antibodies, receptor analogue)**

block of host cell manipulation (inhibition of type III secretion system)

vaccine development (toxins, siderophores...)

CONCLUSION

commensal *E. coli*

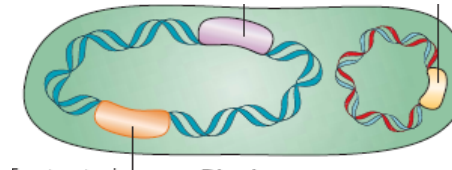


no toxins
no secretion systems/effectors
“no” adhesion factors
no invasion factors
no iron-transport system

no plasmids
smaller genome

- ⇒ **normal intestinal microflora**
- ⇒ **symbiotic relationship**
- ⇒ **harmless bacteria**

pathogenic *E. coli*



toxins
secretion of effectors
adhesion factors = adhesins
invasion factors = invasins
iron-transport system

presence of plasmids
bigger genome (phages, plasmids, PAI...)

- ⇒ **adaptation to new niches**
- ⇒ **host cell manipulation-damage**
- ⇒ **disease**

Selected reading

Kaper J.B *et al.*, Pathogenic *Escherichia coli*. Nature Reviews Microbiology, 2004

Croxen M.A. and Finlay B.B. Molecular mechanisms of *Escherichia coli* pathogenicity. Nature Reviews Microbiology, 2010

Clements A *et al.*, Infection strategies of enteric pathogenic *E. coli*. Gut Microbes, 2012