Molecular studies with Entamoeba histolytica - the causative agent of Human Amoebiasis

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Science as a career

- Explore the unknown mechanisms that govern us.
- The challenge of discovering nature is highly stimulating.
- Service to humanity

  - Imparting knowledge to future generations
  - Producing useful products through technology

- If practiced correctly, science makes you a better human being.
Career options

- Teaching alone
- Research alone
- Teaching + Research
- Technology
  - Medicine
  - Engineering
  - Agriculture
  - Biotechnology
  - Other emerging technology
Pluses and minuses of a science career

**Minuses**
- Many years of study
- Salaries relatively low

**Pluses**
- Job satisfaction
- Flexi time possible
- Held in regard by society
Life cycle of Entamoeba histolytica

Pathogenesis of Invasive Amoebiasis
Movie representing a motile amoeba expressing GFP-CaBP1
Amoebiasis: How to intervene

1. Infection cannot spread
2. Disease cannot progress
Amoebiasis: Intervention

1. Infection cannot spread

Trophozoite → Cyst

2. Disease cannot progress

Trophozoite → Invasive form
*E. histolytica* and *E. dispar* are sibling species. High sequence similarity in all genes studied. Total conservation of ribosomal DNA sequence. Virulence factors (Adhesins, amebapore, cysteine proteinases) are present in both species: although relative abundance of some of them varies.
E. histolytica

Resides in human gut.

Causes amoebiasis by destroying tissues.

Does not invade tissues and is nonpathogenic.

E. dispar

amoebiasis

nonpathogenic
A major difference between *E. histolytica* & *E. dispar*
Transposons
\{mobile genetic elements\}

Code for the enzymes needed for their movement.

e.g. Endonuclease, Reverse transcriptase
What happens when they move?

Into a gene: Gene inactivation

Near a gene: Modify gene expression

Away from gene: DNA rearrangement
Inserting into a new site involves cutting and joining of two DNAs.

- Target DNA
- Target site cut
- Separation of DNA strand
- Transposon
- Transposon joined to target site
- TSD
Target site specificity of *E. histolytica* EN

Non specific

Specific

DNA fragments Of random sizes

DNA fragments Of defined sizes
Nicking profile of EN on the 174 bp substrate

Bottom strand labelled 174bp substrate

Transposon insertion point

Endonuclease nicks at three hot spots

The transposon inserts at hot spot #3
Is the *E. dispar* Endonuclease different from *E. histolytica*?
Sequence Comparison between Eh and Ed endonuclease

- **Eh LINE1 ORF**
  - (64) ME(1)N NRWIMGS(10)N(3)I (1) DL(5)I (1)R(36)H(1)E(10)MG(1)V R-----------------(10)M(3)C(1)YY(5)F(1)G(36)Q(1)A
  - (4)(4)PD(14)D(50)K(2)N(12)E(26)S(3) LEEDDEDE(14)R(2)A(4)T

- **Ed LINE1 ORF**
  - (00) MG(1)V R-----------------(10)M(3)C(1)YY(5)F(1)G(36)Q(1)A
  - (4)(4)PD(14)D(50)A(2)I (12)T(26)V(3)Q--DEDE (14)T(2)T(4)T
Only a subset of Endonuclease-recognition sites are utilized for Transposon insertion.

Estimated Endonuclease nicking sites in:
- Genic regions of *E. histolytica* - 3,902
- Intergenic regions of *E. histolytica* - 1,852

No Transposon yet found in genic regions.
Understanding Transposon Insertion

Computational analysis of DNA sequences flanking the insertion sites, coupled with experimental knowledge of target site specificity of endonuclease can help to:

1. Derive the rules which govern choice of target site
2. Predict future transposition events
HUMAN GUT FLORA

Anatomy of the Large Intestine

- Right Hepatic Flexure
- Transverse Colon
- Left Splenic Flexure
- Ascending Colon
- Descending Colon
- Cecum
- Sigmoid Colon
- Appendix
- Rectum

Microscope image of bacteria.
Human body is composed of $\sim 1 \times 10^{13}$ cells.

Human gut contains $1 \times 10^{14}$ microorganisms!!

About 1,000 different bacterial species reside in the gut.

Gut flora does useful functions e.g.:

- produces vitamins (biotin, vit K)
- ferments unused carbohydrates
- Prevents growth of harmful species
Gut flora influences digestion of fatty acids— which is linked to obesity.

OBESE mice & human – Bacteroidetes
Firmicutes

(reversed in lean)
Gut flora and Amoebiasis.....

Does the gut flora change in Amoebiasis patients?
Gut flora and Amoebiasis.....II

Identification of bacteria by PCR amplification of **16S ribosomal RNA gene**

**Why 16S rRNA gene ?**

- universally present
- highly conserved
- universal primer available
- species-specific point mutations
Identification of bacteria by PCR

16S rRNA gene

- Species specific point mutation
- Universal primer
- 1 to 4- bacterial species
Gut flora and Amoebiasis

Results with patients:

Stool samples screened from:

1. Amoebic liver abscess patients.
2. Asymptomatic individuals (Eh+ve).
3. Asymptomatic individuals (Eh-ve).
Continued....

- Lactobacillus was low in ALA patients and Eh+ve individuals.
- Bifidobacterium, Bacteroides and Clostridium were low in ALA patients, but this was due to Metronidazole antiamoebic drug.
- No change in Peptostreptococcus, Ruminococcus Campylobacteria and Peptococcus.
Bacteria in pus from Amoebic Liver Abscess patients

- So far it was believed that pus is sterile (from microscopy & culturing)
- Out of 35 ALA patients examined, 25 had Peptostreptococcus and 5 had Bacteroides.

Do bacteria aggravate the outcome of ALA?

Can antibiotics specific for these bacteria help patients who respond poorly to metronidazole?