The Genome of the Kinetoplastid Parasite, *Leishmania major*

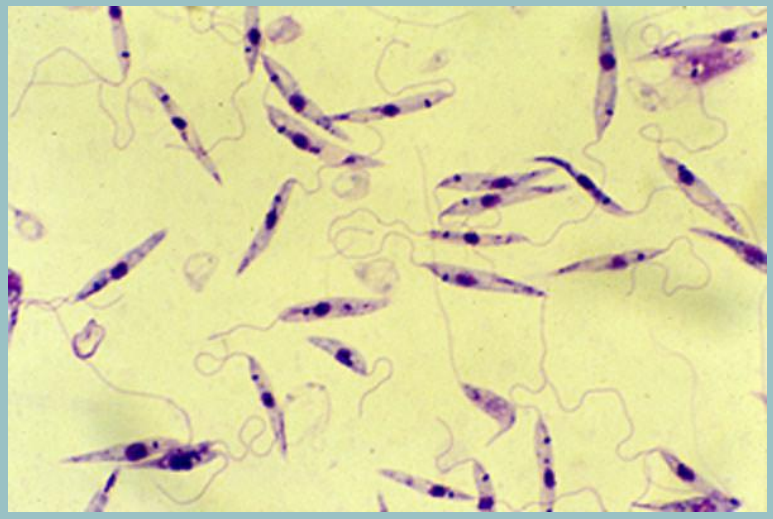
A Journal Club Presentation by
Lena Hunt, Viktoria Kuehn, Kevin McGee, & Gabriel Leis

Biological Databases
Loyola Marymount University
November 12th, 2013
Doolan 219

Alasdair C. Ivens, Christopher S. Peacock, Elizabeth A. Worthey, Lee Murphy, Gautam Aggarwa, Matthew Berriman, Ellen Sisk, Marie-Adele Rajandream, Ellen Adlem, Rita Aert, Atashi Anupama, Zina Apostolou, Philip Attipoe, Nathalie Bason, Christopher Bauser, Alfred Beck, Stephen M. Beverley, Gabriella Bianchettin, Katja Borzym, Gordana Bothe, Carlo V. Bruschi, Matt Collins, Eithon Cadag, Laura Ciarloni, Christine Clayton, Richard M. R. Coulson, Ann Cronin, Angela K. Cruz, Robert M. Davies, Javier De Gaudenzi, Deborah E. Dobson, Andreas Duesterhoeft, Gholam Fazelina, Nigel Fosker, Alberto Carlos Frasch, Audrey Fraser, Monika Fuchs, Claudia Gabel, Arlette Goble, André Goffeau, David Harris, Christiane Hertz-Fowler, Helmut Hilbert, David Horn, Yiting Huang, Sven Klages, Andrew Knights, Michael Kube, Natasha Larke, Lyudmila Litvin, Angela Lord, Tin Louie, Marco Marra, David Masuy, Keith Matthews, Shulamit Michaeli, Jeremy C. Mottram, Silke Müller-Auer, Heather Munden, Siri Nelson, Halina Norbertczak, Karen Oliver, Susan O'Neil, Martin Pentony, Thomas M. Poh, Claire Price, Bénédicte Purnelle, Michael A. Quail, Ester Rabbinowitsch, Richard Reinhardt, Michael Rieger, Joel Rinta, Johan Robben, Laura Robertson, Jeronimo C. Ruiz, Simon Rutter, David Saunders, Melanie Schäfer, Jacquie Schein, David C. Schwartz, Kathy Seeger, Amber Seyler, Sarah Sharp, Heesun Shin, Dhileep Sivam, Rob Squares, Steve Squares, Valentina Tosato, Christy Vogt, Guido Volckaert, Rolf Wambutt, Tim Warren, Holger Wedler, John Woodward, Shiguo Zhou, Wolfgang Zimmermann, Deborah F. Smith, Jenefer M. Blackwell, Kenneth D. Stuart, Bart Barrel, Peter J. Myler (2005) The Genome of the Kinetoplastid Parasite, Leishmania major. Science;309(5733): 436-42.

Outline

- Why Study Leishmania
- Research Methods
- Genome Structure and Contents
- Significant Genetic Findings



http://www.uni-tuebingen.de/modeling/Mod_Leish_Intro_en.html

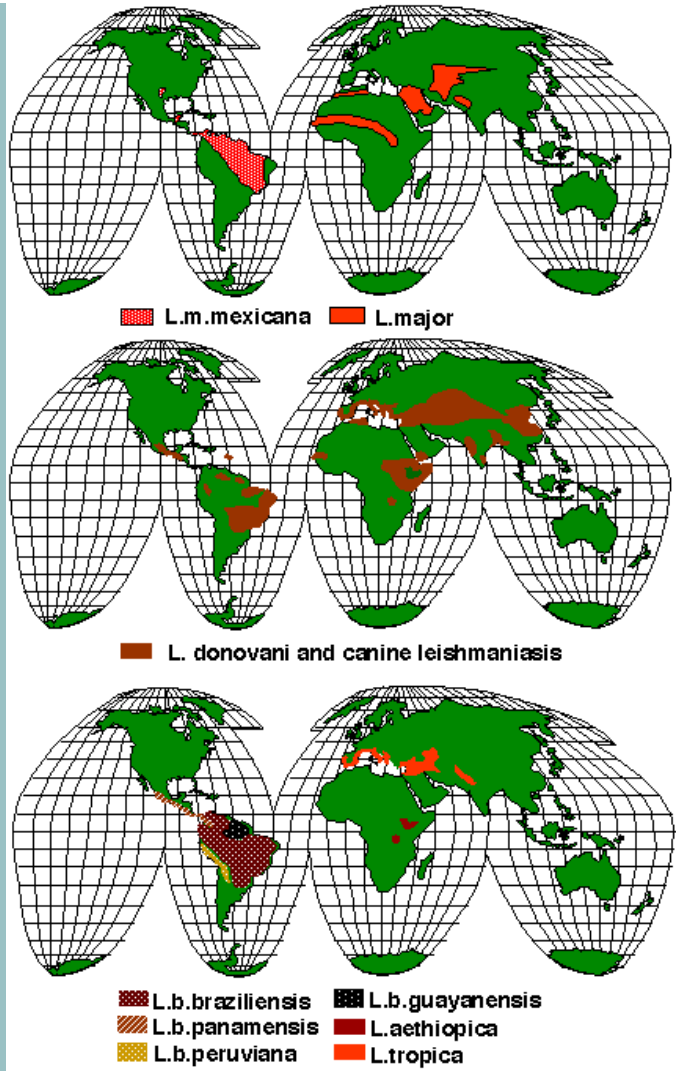
Why Study Leishmania

- Major pathogenic parasite
- 2 million infections in 88 countries annually
- spectrum of diseases: “leishmaniases”

http://www.icp.ucl.ac.be/~opperd/parasites/images/promast.jpg

Leishmania is uniquely successful parasite

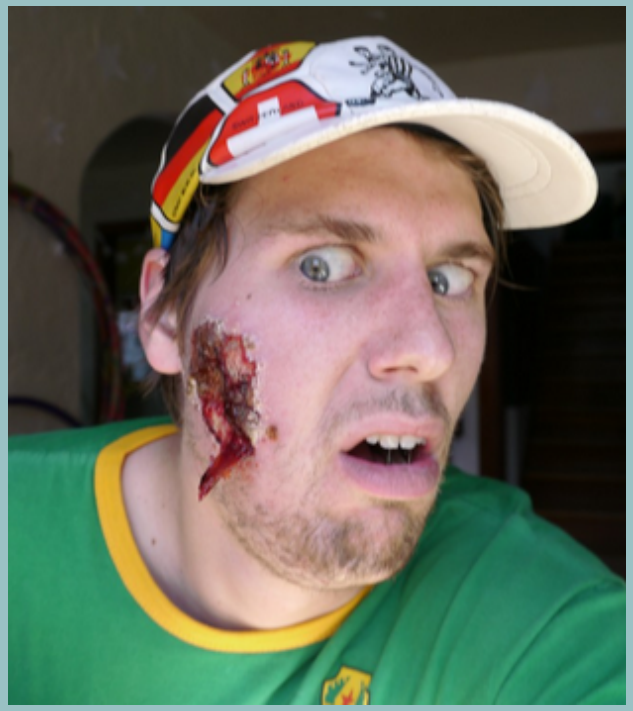
- Tropic/sub-tropic
- Uniquely adapted to avoid host-destruction
- Thrive and proliferate due to unique gycoconjugates on outside of cells



Symptoms of the Leishmaniases

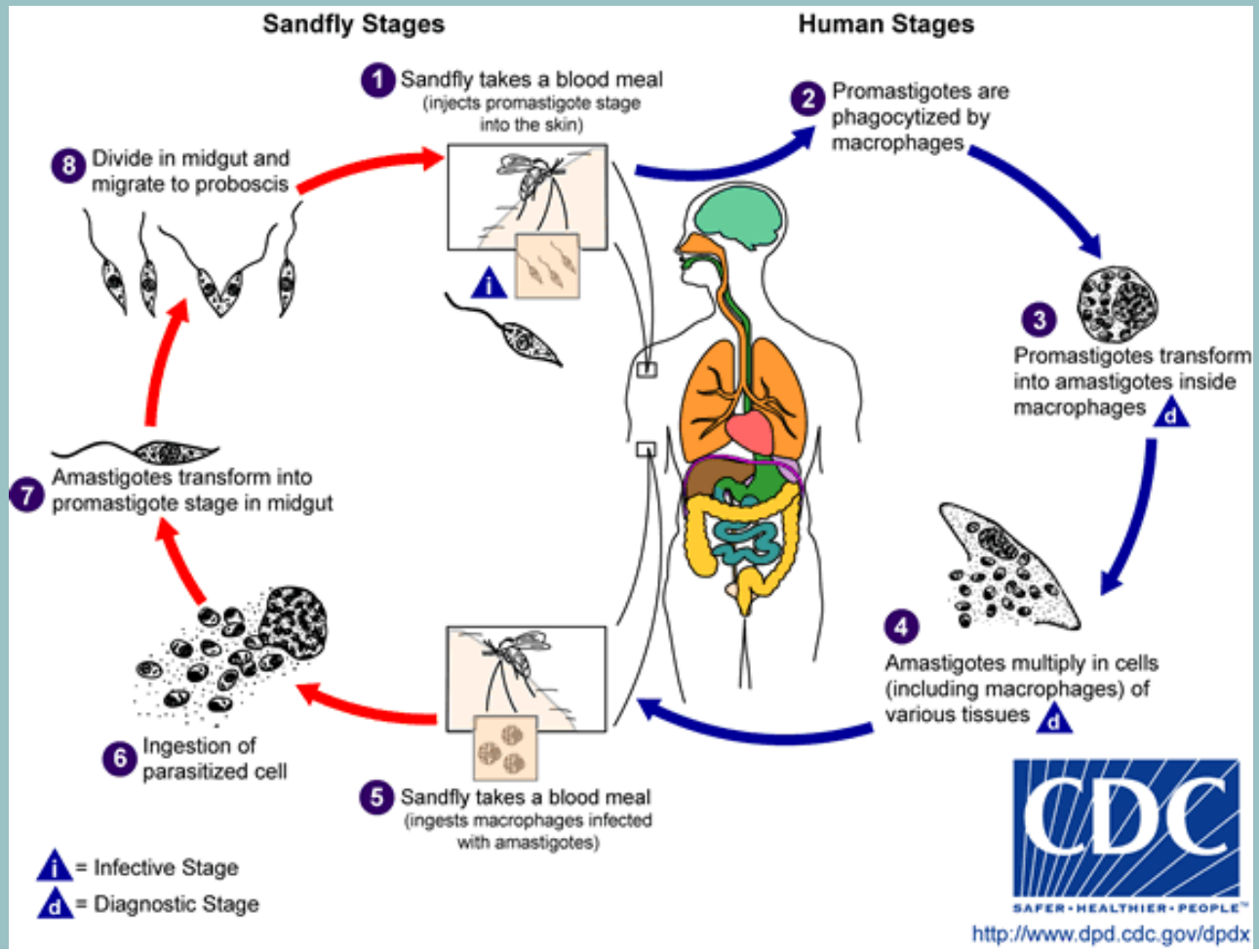
- Skin sores and ulcers
- Difficulty breathing/swallowing
- Eroding away in mouth, tougue, gums, lips, and nose

http://anthropology.net/2009/09/10/ancient-leishmaniasis-from-coyo-oriente-cemetery-in-chile/



http://adamspencerphotography.com/leishman/

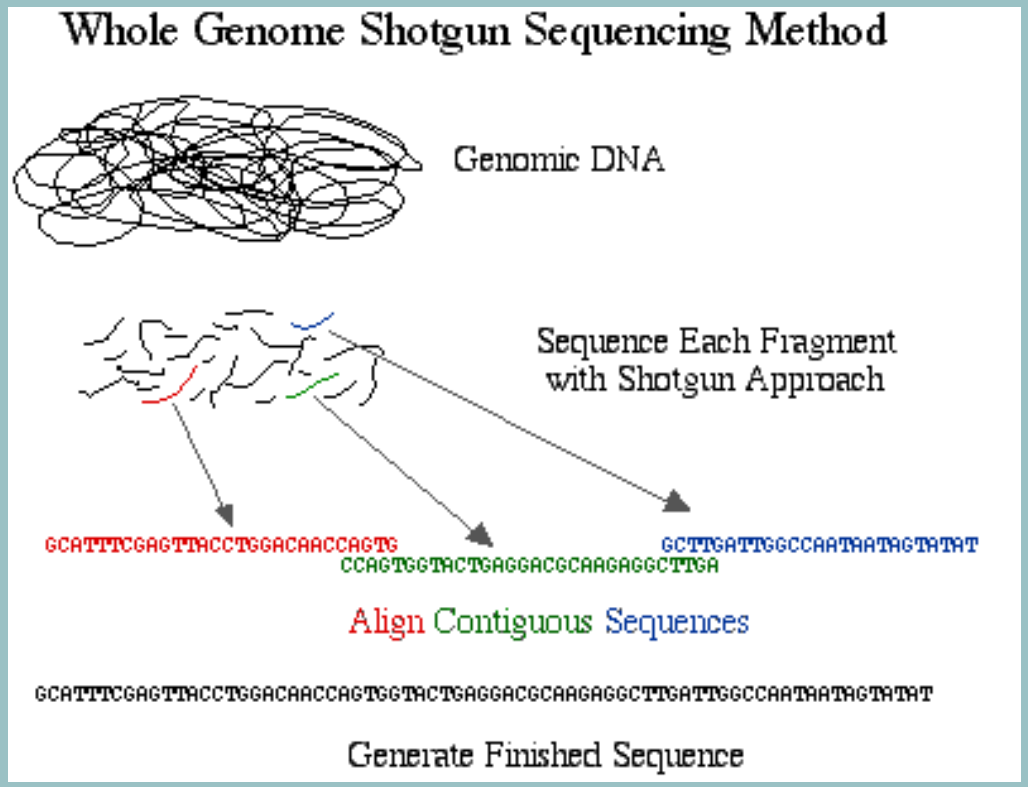
Life cycle of Leishmania



Shotgun Sequencing

- DNA broken up into many segments
- Sequenced to obtain *reads*
- overlapping *reads* assembled to a continuous sequence

→ Sequences compared between 3 trypanosomatids, “tritryps”



Leishmania major genome structure and content

- 32,816,678 base pairs
- 36 chromosomes
- Accuracy was compared to an optical map
- Smaller gene families
- Larger gene families

Parameter	Number
The genome	
Size (bp)	32,816,678
G+C content (%)	59.7
Chromosomes	36
Sequence contigs	36
Percent coding	47.9
Protein-coding genes	
Genes	8272
Pseudogenes	39
Mean CDS length (bp)	1901
Median CDS length (bp)	1407
G+C content (%)	62.5
Gene density (genes per Mb)	252
Intergenic regions *	
Mean length (bp)	2045
G+C content (%)	57.3
RNA genes	
tRNA	83
rRNA†	63
slRNA†	63
snRNA	6
snoRNA	695
srpRNA	1

* Region between protein-coding CDS.

† The exact number cannot be determined because of misassembly.

Table 1: Summary of L. major genome

Some genes are unique to L. major when compared to those in T. brucei and T. cruzi

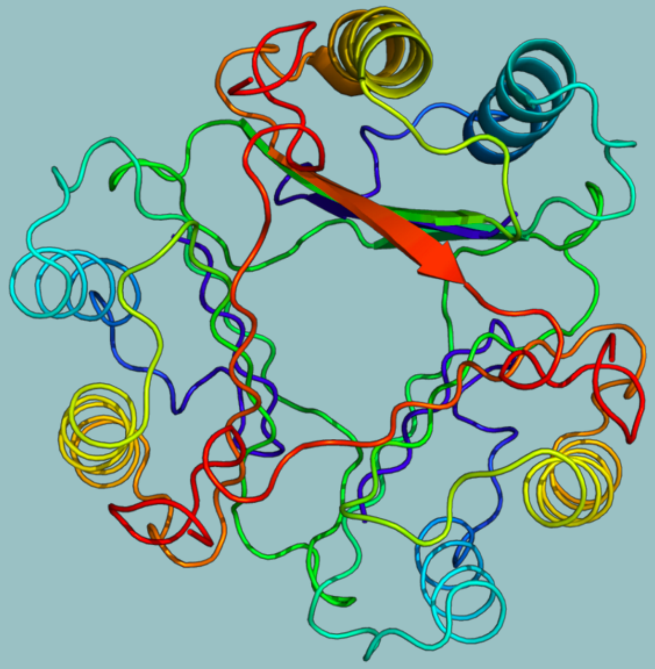
- L. major telomeres are distinct heterogeneous in structure
- 910 L. major genes have no orthologs in other 2 Tritryp genomes
- “Leishmania restricted genes”
  - Randomly distributed in genome
  - Some responsible for metabolic differences
  - 68% have unknown functions

Family size*	Gene product(s)	*L. major*–specific	Organization†	Chromosome(s)
491	Hypothetical proteins (several annotations)	Some	D	Multiple
189	Kinesins/hypothetical proteins	Some	T+D	Multiple
60	Protein kinases (several groups)	Some	T+D	Multiple
46	Amastins	Most	T+TI+D	8, 31, 34, 36
32	Protein kinases (CMGC group)	One	D	Multiple
32	PSA-2 (GP46)	All	T+D	12, 21, 31, 35
29	RNA helicases/eIF-4a	None	T+D	Multiple
27	ATPase/serine peptidases	None	D	Multiple
29	Hypothetical proteins (kinesin-like)	One	D	Multiple
25	Protein phosphatases	None	T+D	Multiple
25	Tuzins	Some	TI+D	8, 34, 36
24	Protein kinases (STE group)	Some	D	Multiple
23	Amino acid permeases	Some	T+D	Multiple
19	HSP83	None	T+D	29, 33
18	DNA helicases	Some	D	Multiple
18	β-tubulins	None	T+D	8, 21, 33
17	Hypothetical proteins (LACK)	One	D	Multiple
17	Hypothetical proteins	Some	T+D	11, 13, 21, 29, 31, 36
15	Calpain-like cysteine peptidases	Some	T+D	4, 20, 25, 31, 36
14	HSP70 and related proteins	None	T+D	1, 18, 26, 28, 30, 35
14	Phosphoglycan β 1,3 galactosyltransferases	Some	T+D	2, 7, 14, 21, 25, 31, 35, 36
14	Dynein heavy chain	One	D	Multiple
14	RNA helicases	None	D	Multiple
14	α,γ,ε-tubulins	Bone	T+D	13, 21, 25
13	Hypothetical proteins (PIPK-like protein)	One	D	Multiple
13	Pteridine transporters	Some	T+D	4, 6, 10, 19, 35

Table 2: L. major Friedlin protein coding gene families

Two closely related genes of interest: LmjF33.1740 and LmjF33.1750

- Macrophage migration inhibition factor (MIF)
- MIF genes in humans encode a lymphokine protein mediator involved in innate immunity
- L. major MIFs vs. host MIFs
- May modulate host macrophage response to promote parasite survival



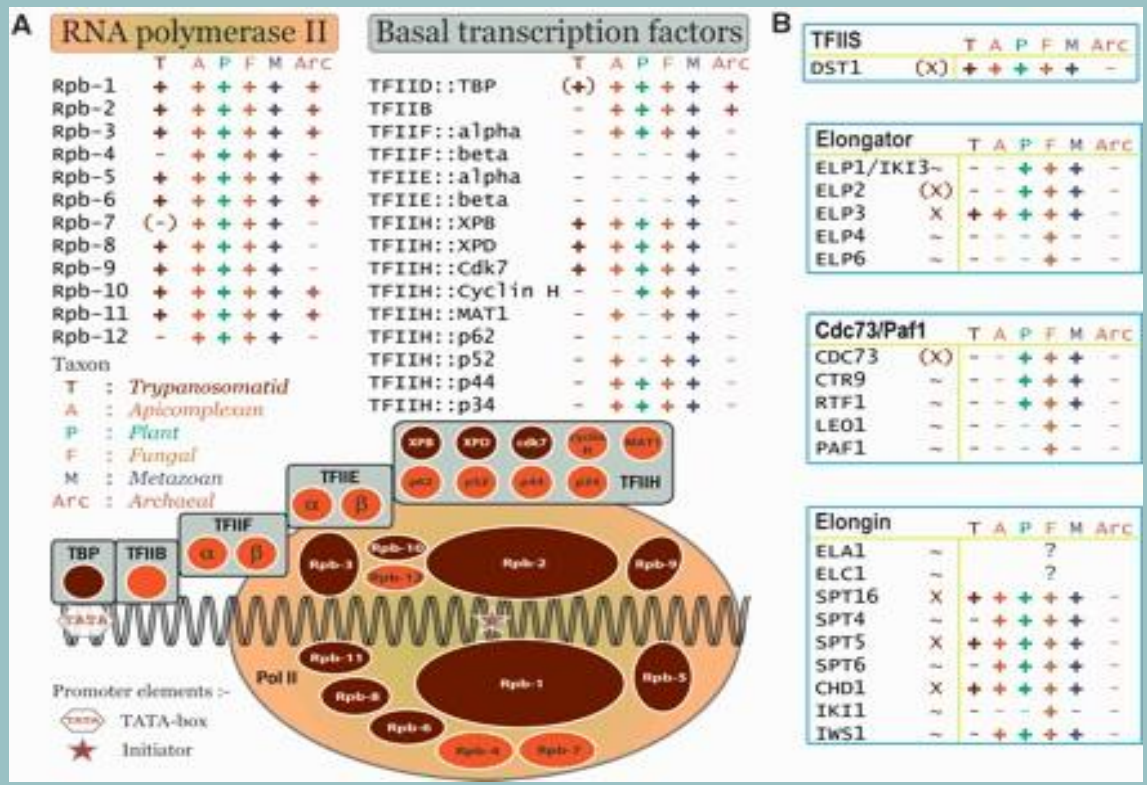
Structure of MIF protein
http://en.wikipedia.org/wiki/Macrophage_migration_inhibitory_factor

Significant Findings- Transcription Machinery of Leishmania Compared to Other Eukaryotes

Figure 1

RNA Polymerase II

- Protein Families
- Subunits
- Transcription Factors



Significant Findings- Protein Families Involved in the Regulation of Gene Expression in Model Eukaryotes

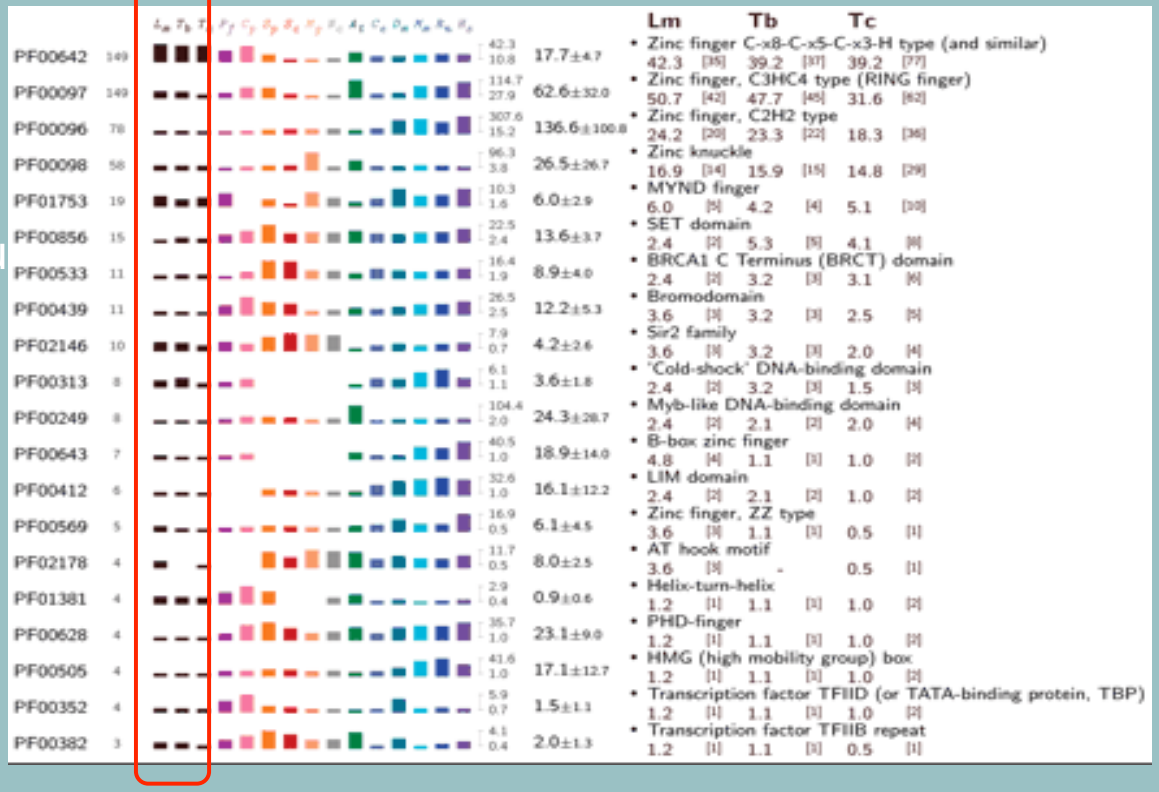
Regulation of Gene Expression

Figure 2-

Gene Expression of Variou

Protein Domains

by Species



Significant Findings- Proteins Involved in Post Translational Modification are Potential Drug Targets

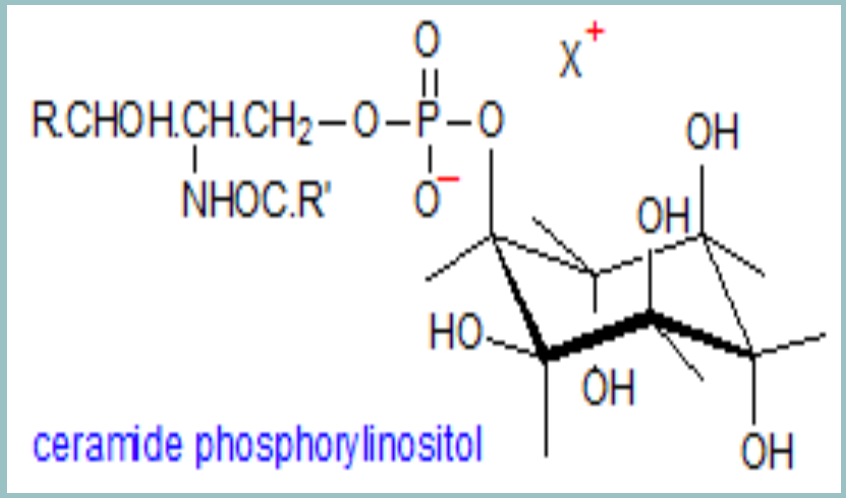
Post Translational Modification

- Protein modification typical of a eukaryote
    - Typical number of genes code for most protein modifications
- High number of genes code for myristoylation and prenylation
- These enzymes could be drug targets due to high number of substrates

Significant Findings- Surface Molecules Unique to Leishmania are Potential Drug Targets

Surface molecules

- Surface molecules critical for pathogenic species
- Primary sphingolipid expressed is Inositol Phosphorylceramide (IPC)
- Not produced in mammals



http://lipidlibrary.aocs.org/Lipids/glyP_ino/index.htm

- Enzymes that construct IPC are excellent drug targets given that this important surface molecule is not produced in humans

Implications: Tritryp genomes help show unique biology of Leishmania and give insight to Eukaryote evolution

- Differences from other eukaryotes: post-translational modification
  - polycistronic gene clusters
  - mRNA trans-splicing coupled with polyadenylation

- Leishmania branched off from other eukaryotes very early on
  - Differences arose after branching off of Leishmania

Full genome provides crucial information for new therapies of Leishmaniasis

- analysis of virulence factors
- enzymes in metabolic pathways
- potential vaccine candidates

http://www.dailymail.co.uk/health/article-1191257/Ben-Fogle-I-nearly-lost-half-face-flesh-eating-bug--The-cure-injections-poison.html

Conclusion

- Leishmania: sub-tropical parasite that causes Leishmaniasis, an infection affecting 2 million people annually
- 36 chromosomes sequenced
  - compared to Tritryp organisms and humans to understand biology and function of Leishmania genes
- Drug targets were found in both post translational modification enzymes and in surface molecules
  - Full genome allows for analysis and development of therapies

Acknowledgments

Dr. Dionisio

Dr. Dahlquist

Librarian Glenn Johnson-Grau

Seaver College of Science and Engineering

References

http://health.nytimes.com/health/guides/disease/leishmaniasis/

Rosengren E, Bucala R, Aman P, Jacobsson L, Odh G, Metz CN, Rorsman H (January 1996). "The immunoregulatory mediator macrophage migration inhibitory factor (MIF) catalyzes a tautomerization reaction". *Mol. Med.* **2** (1): 143–9. PMC 2230029.PMID 8900542.

Alasdair C. Ivens, Christopher S. Peacock, Elizabeth A. Worthey, Lee Murphy, Gautam Aggarwa, Matthew Berriman, Ellen Sisk, Marie-Adele Rajandream, Ellen Adlem, Rita Aert, Atashi Anupama, Zina Apostolou, Philip Attipoe, Nathalie Bason, Christopher Bauser, Alfred Beck, Stephen M. Beverley, Gabriella Bianchettin, Katja Borzym, Gordana Bothe, Carlo V. Bruschi, Matt Collins, Eithon Cadag, Laura Ciarloni, Christine Clayton, Richard M. R. Coulson, Ann Cronin, Angela K. Cruz, Robert M. Davies, Javier De Gaudenzi, Deborah E. Dobson, Andreas Duesterhoeft, Gholam Fazelina, Nigel Fosker, Alberto Carlos Frasch, Audrey Fraser, Monika Fuchs, Claudia Gabel, Arlette Goble, André Goffeau, David Harris, Christiane Hertz-Fowler, Helmut Hilbert, David Horn, Yiting Huang, Sven Klages, Andrew Knights, Michael Kube, Natasha Larke, Lyudmila Litvin, Angela Lord, Tin Louie, Marco Marra, David Masuy, Keith Matthews, Shulamit Michaeli, Jeremy C. Mottram, Silke Müller-Auer, Heather Munden, Siri Nelson, Halina Norbertczak, Karen Oliver, Susan O'Neil, Martin Pentony, Thomas M. Poh, Claire Price, Bénédicte Purnelle, Michael A. Quail, Ester Rabbinowitsch, Richard Reinhardt, Michael Rieger, Joel Rinta, Johan Robben, Laura Robertson, Jeronimo C. Ruiz, Simon Rutter, David Saunders, Melanie Schäfer, Jacquie Schein, David C. Schwartz, Kathy Seeger, Amber Seyler, Sarah Sharp, Heesun Shin, Dhileep Sivam, Rob Squares, Steve Squares, Valentina Tosato, Christy Vogt, Guido