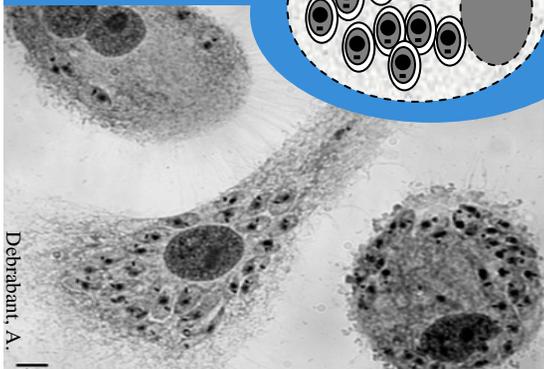
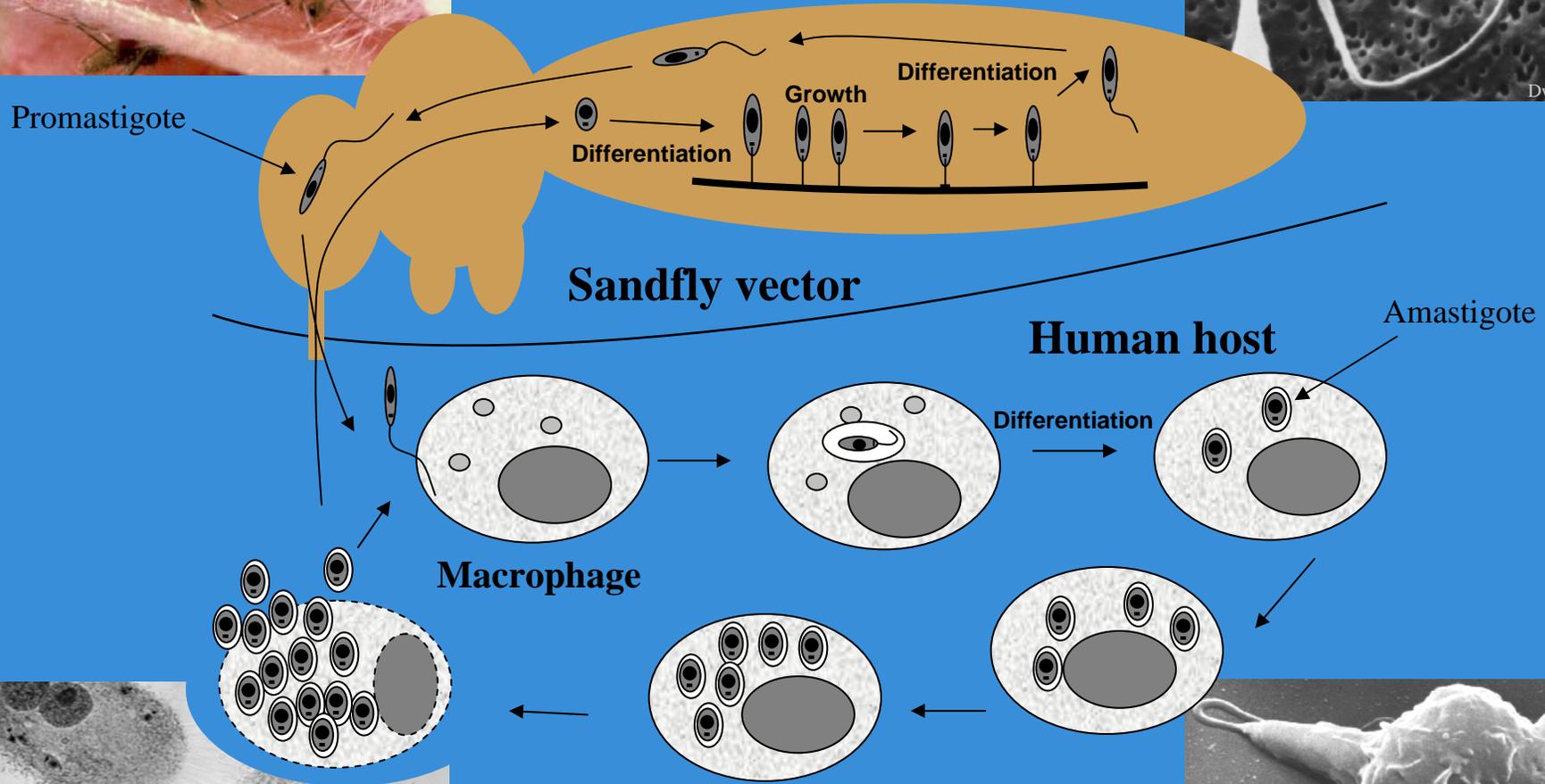


The Programmed Cell Death Pathway of Trypanosomatid Parasites

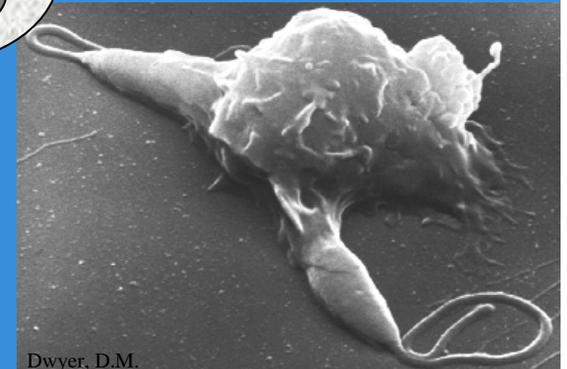
Alain Debrabant, Ph.D.

**Laboratory of Bacterial Parasitic and Unconventional Agents
Division of Emerging and Transfusion Transmitted Diseases
OBRR/CBER / FDA**

Life cycle of *Leishmania*



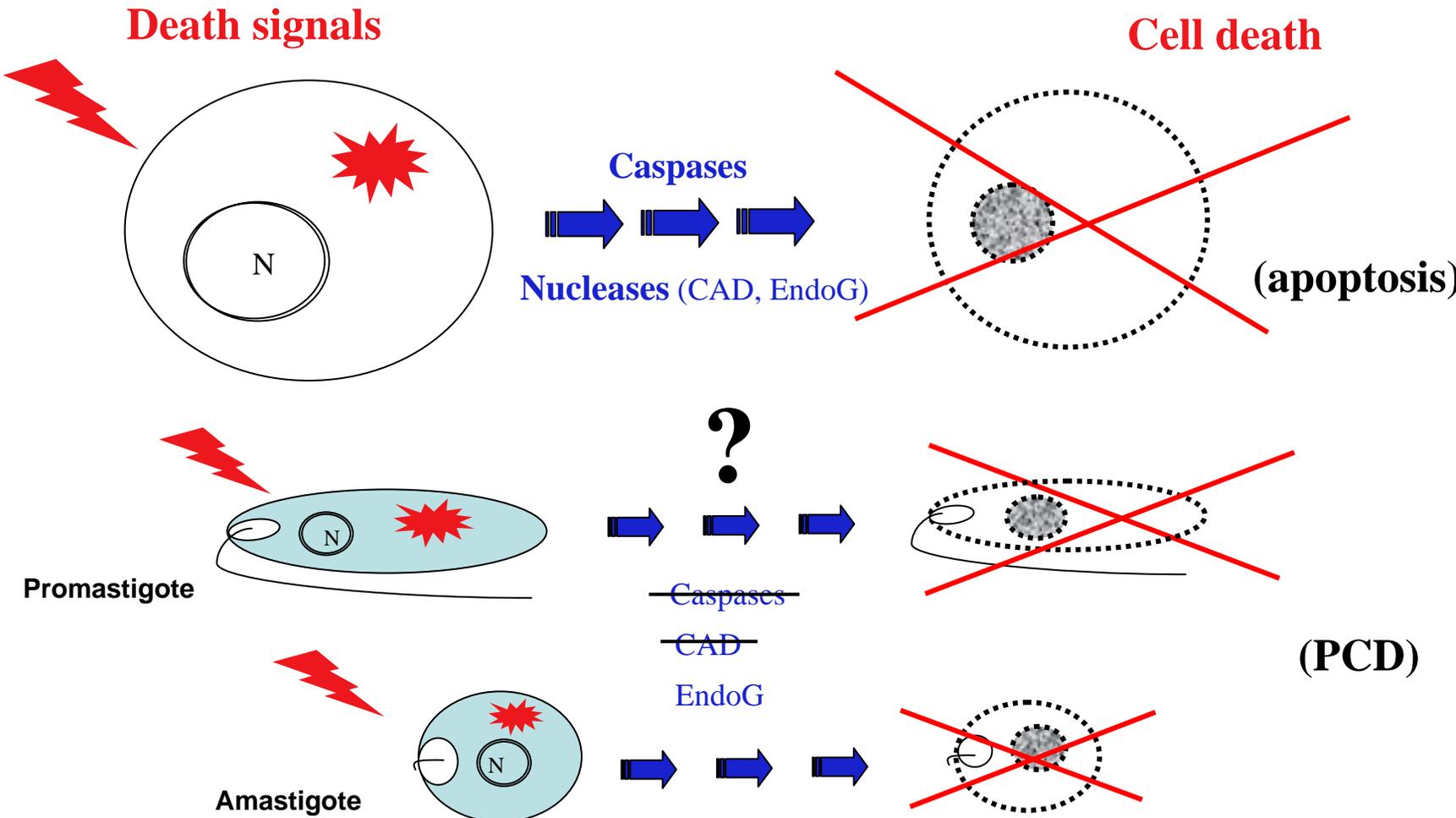
Debrabant, A.



Dwyer, D.M.

Adapted from Paulo Pimenta

Programmed Cell Death (PCD)



Objectives

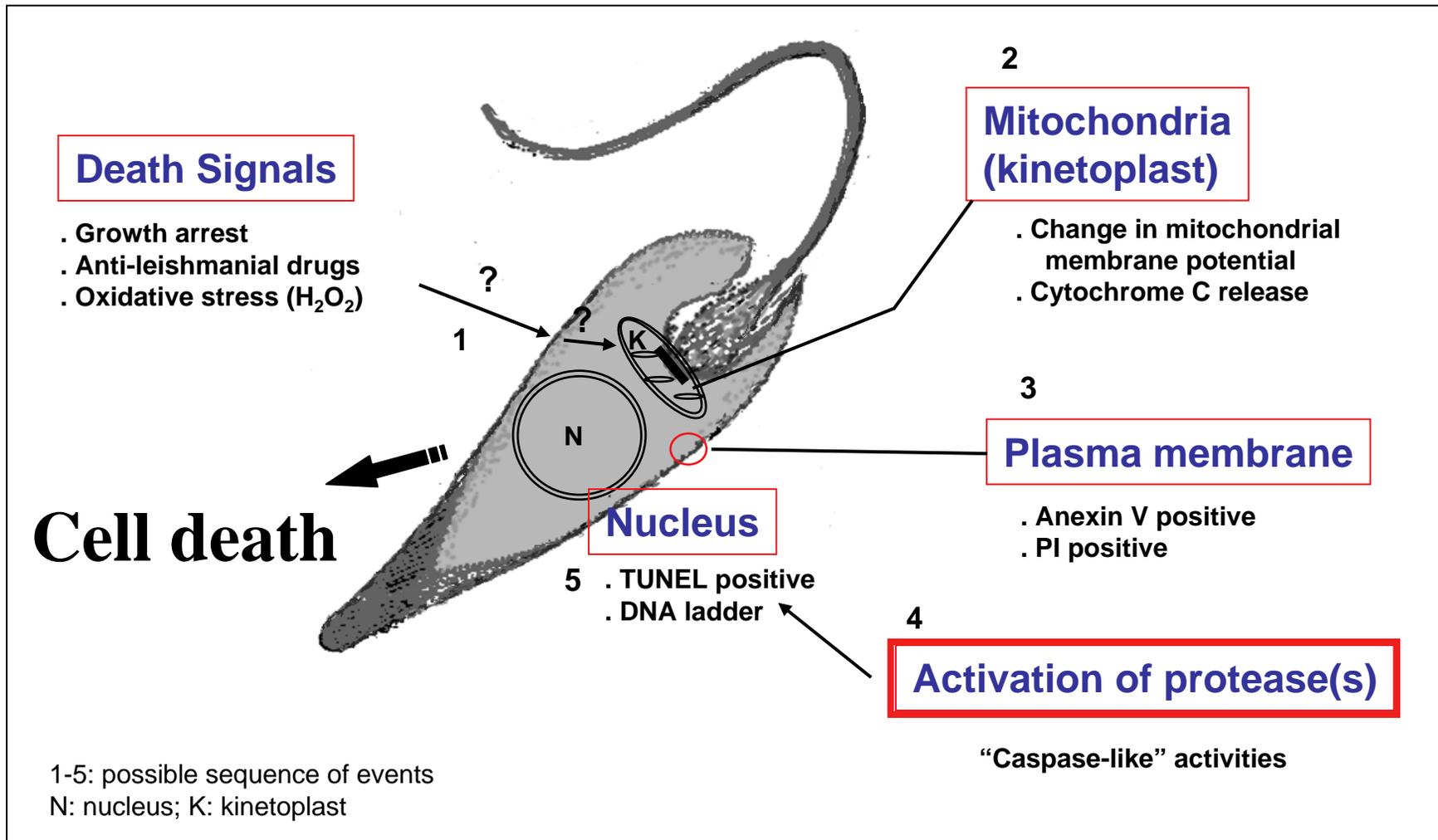
Identify the effector molecules of the *Leishmania* programmed cell death pathway

Outcomes

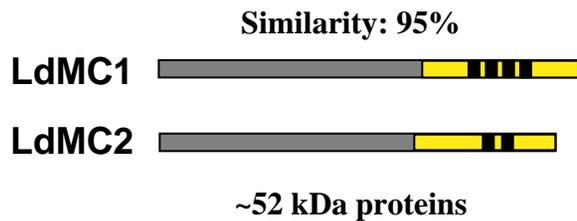
Better understanding of parasite growth and pathogenesis

Effector molecules of trypanosomatid PCD pathways are potential new targets for drugs that would selectively trigger parasite death

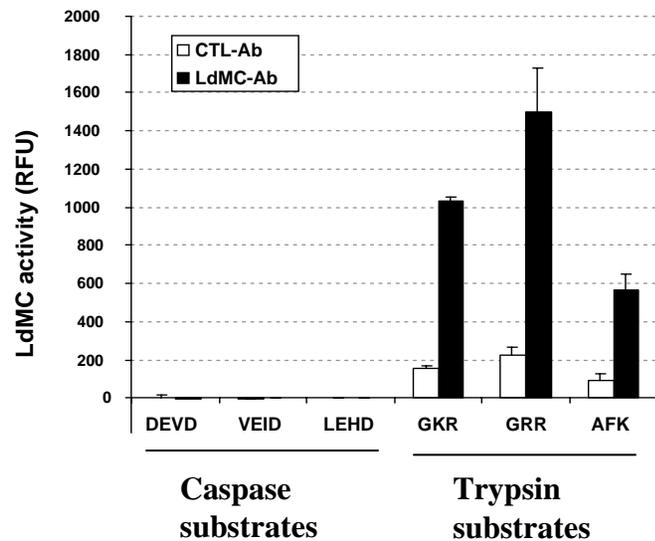
Programmed Cell Death Pathway in *Leishmania*



Leishmania donovani metacaspases



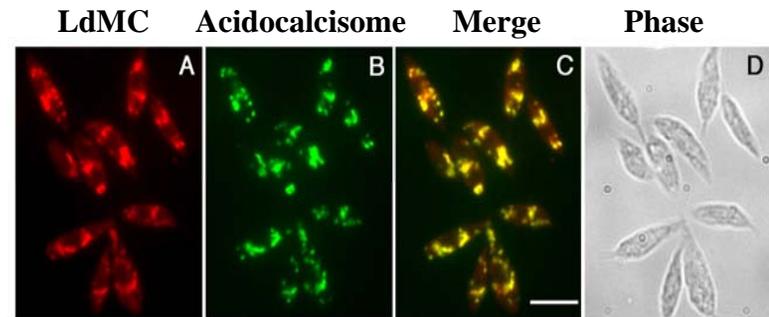
LdMC enzymatic activity



- Insensitive to caspase inhibitors (e.g. Z-VAD-fmk)
- Inhibited by serine/cysteine protease inhibitors (e.g. leupeptin, antipain, TLCK)

Metacaspase have “trypsin-like” activity

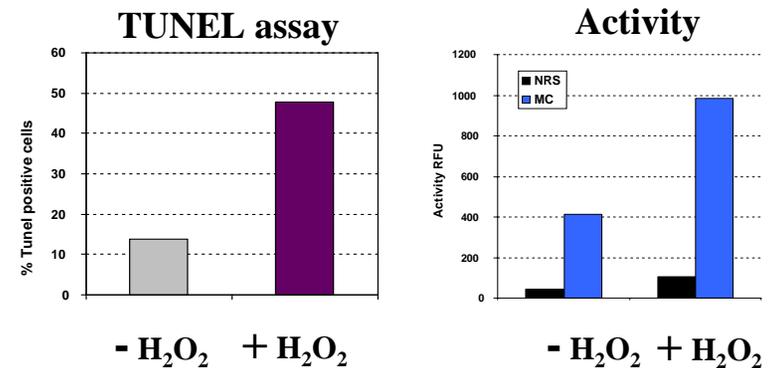
Localization of LdMCs



Anti-LdMC Anti-TcPPase

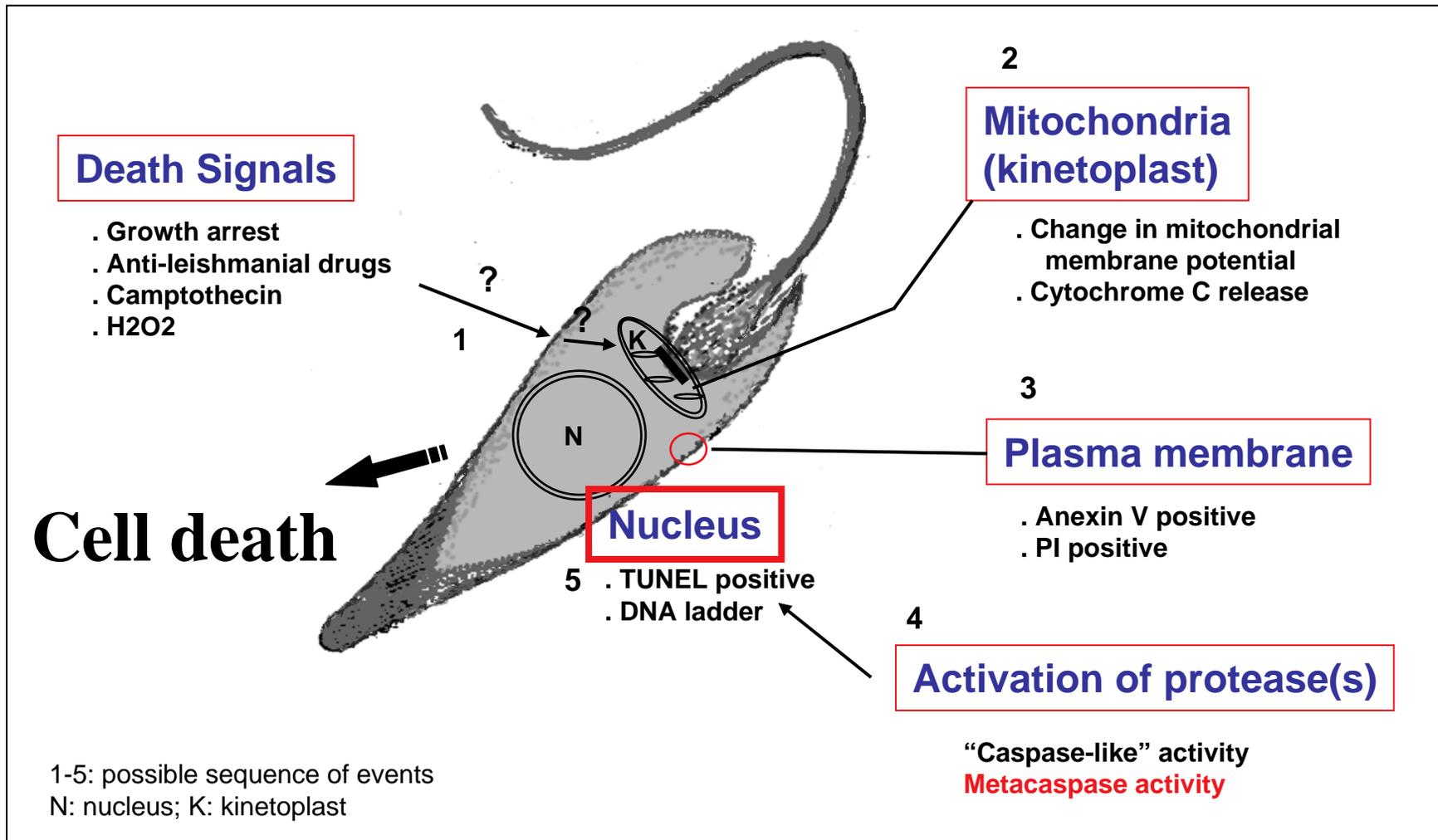
Metacaspases are stored in acidocalcisomes

Metacaspases involved in *Leishmania* PCD ?



LdMC are probably involved in *Leishmania* PCD

Programmed Cell Death Pathway in *Leishmania*



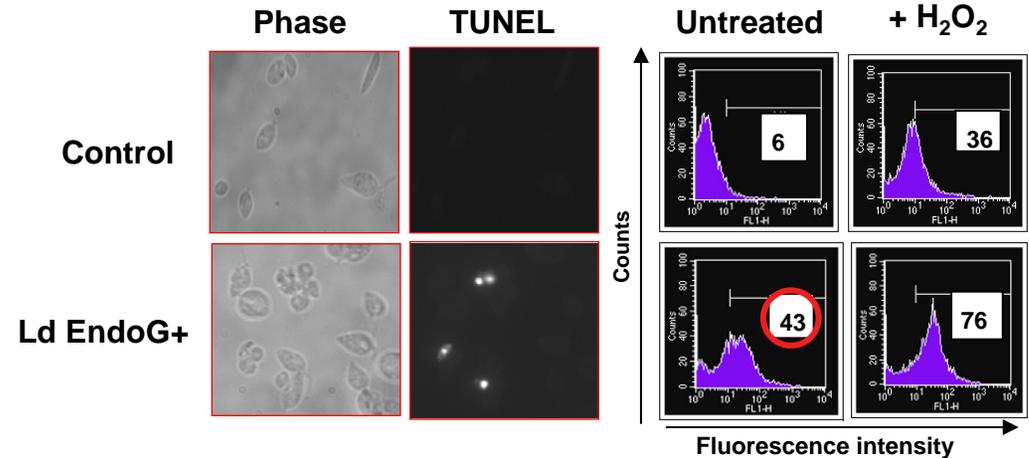
Leishmania Endonuclease G

Leishmania EndoG

- Single copy gene
- 54 kDa protein
- 29% similarity with human EndoG
- Constitutively transcribed in both parasite stages (RT-PCR)

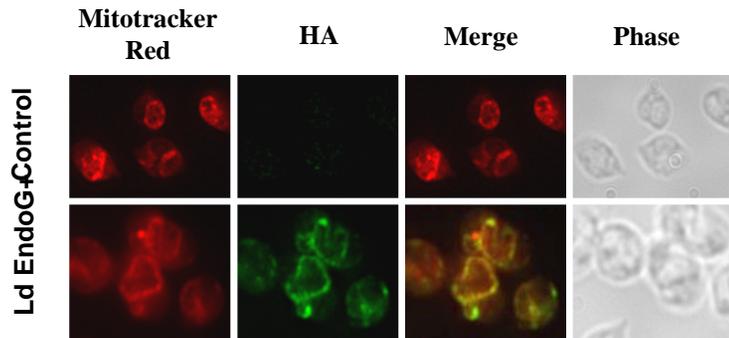


Overexpression of EndoG in Leishmania



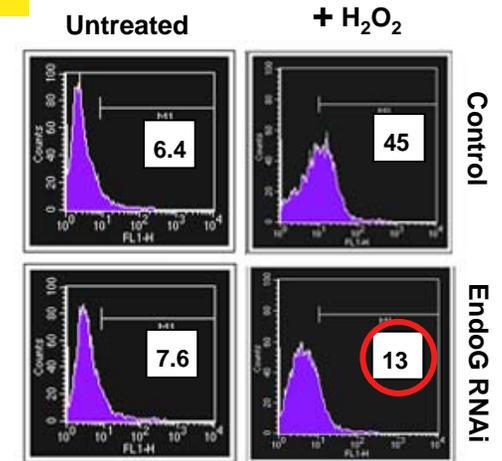
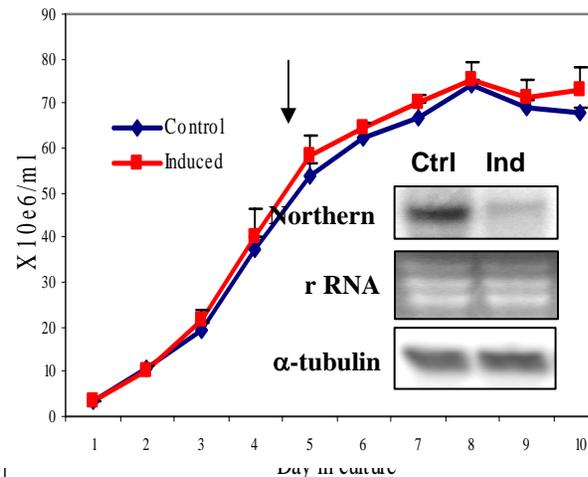
Parasites overexpressing EndoG spontaneously undergo PCD

Localization of Leishmania endoG



EndoG-HA is associated with Leishmania mitochondria

EndoG RNAi in Trypanosoma brucei



Parasites with reduced expression of EndoG are more resistant to H₂O₂ induced PCD

Summary

Goal:

Identify effector molecules of *Leishmania* PCD pathway

Results:

We identified and characterized two effector molecules of the *Leishmania* PCD pathway (metacaspases and endonuclease-G)

Outcomes:

1. Better understanding of *Leishmania* growth and pathogenesis
2. Effector molecules of PCD represent new drug targets

Acknowledgments

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Thank You