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Estudos funcionais e estruturais da proteína ortóloga a PinX1 e sua influência
nos telômeros de *Leishmania* spp.

Stephany Cacete de Paiva

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas – Genética da Universidade Estadual Paulista ‘Júlio de Mesquita Filho’, como requisito para obtenção do título de Doutora em Ciências Biológicas(Genética).

Orientadora: Prof^a. Dr^a. Maria Isabel Nogueira Cano

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Dedicatória

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E a todos que enfrentam a leishmaniose, com a esperança de que a ciência possa contribuir para um futuro melhor.

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Epígrafe

"O correr da vida embrulha tudo, a vida é assim: esquenta e esfria, aperta e daí afrouxa, sossega e depois desinquieta. O que ela quer da gente é coragem."

— *João Guimarães Rosa*

Resumo para a sociedade

A leishmaniose é uma doença transmitida pela picada de mosquitinhos-palha infectados com o parasito *Leishmania*. No Brasil, milhares de pessoas são afetadas todos os anos, principalmente nas regiões Norte e Nordeste. A doença pode se manifestar como feridas na pele que não cicatrizam ou como uma forma mais grave que afeta órgãos como fígado e baço, podendo levar à morte. Os medicamentos disponíveis atualmente são antigos, tóxicos e causam efeitos colaterais sérios. Além disso, já existem parasitos resistentes aos tratamentos. Por isso, é urgente identificar novos alvos para desenvolver medicamentos mais eficazes e seguros.

O que este estudo investigou?

Nossa pesquisa estudou uma proteína chamada PinX1 presente no parasito causador da leishmaniose. Em seres humanos e em leveduras que fermentam o pão, vinho e cerveja, essa proteína ajuda a controlar o tamanho dos telômeros - que são como as "capinhas" que protegem as pontas dos cromossomos (estruturas que acondicionam nosso DNA), importantes para manter a proliferação celular e evitar o envelhecimento. Parece que a PinX1 também interliga a maquinaria de montagem dos ribossomos, nossas fábricas de proteínas, à de manutenção dos telômeros. Sua ausência ou diminuição provoca defeitos de crescimento celular e alterações no tamanho telomérico, podendo desencadear envelhecimento celular ou eventos tumorais.

Como foi feita a pesquisa?

Nós "desligamos" parcialmente o gene da PinX1 em *Leishmania* usando uma técnica avançada chamada CRISPR (uma espécie de "tesoura genética"). Depois, observamos o que acontecia com os parasitos. Também produzimos a proteína PinX1 em laboratório para estudar sua estrutura e como ela interage com outras proteínas do parasito.

O que descobrimos?

A proteína é essencial para a sobrevivência do parasito: quando tentamos desligar completamente o gene da PinX1, os parasitos morriam, o que mostra que essa proteína é vital para eles.

Afeta a divisão celular: os parasitos com menos PinX1 cresciam mais devagar, acumulavam células que não finalizavam a divisão.

Interfere na montagem de ribossomos: a PinX1 também afeta a montagem dos ribossomos - estruturas que fabricam proteínas dentro da célula.

Interage diretamente com a telomerase e com a proteína TRF: descobrimos que a PinX1 se liga fisicamente à um componente da enzima telomerase e com os telômeros por sua interação com uma proteína telomérica (TRF). A diminuição de quantidade de PinX1 no parasito leva ao encurtamento dos telômeros. Confirmando sua ação na manutenção dessas estruturas.

Estrutura diferente: a proteína PinX1 da *Leishmania* apresenta regiões desorganizadas (como "partes moles") que permitem que ela interaja com várias outras proteínas, o que explica suas funções em diferentes maquinarias.

Por que isso é importante?

Esta pesquisa revelou que a PinX1 da *Leishmania* é um "faz-tudo" essencial para o parasito: ajuda a manter seus cromossomos estáveis, a montar ribossomos e a se dividir corretamente. Por apresentar diferenças estruturais em relação à proteína humana, ela se torna um alvo promissor para o desenvolvimento de novos medicamentos contra o parasito.

Resumo

As leishmanioses são doenças negligenciadas causadas por parasitos do gênero *Leishmania*, que afetam milhões de pessoas em todo o mundo. A alta toxicidade e resistência aos tratamentos atuais tornam urgente a identificação de novos alvos terapêuticos. Os telômeros, estruturas nucleoproteicas essenciais para a estabilidade genômica, surgem como promissores candidatos. Em eucariotos superiores, a proteína PinX1 é um regulador negativo clássico da telomerase, inibindo sua atividade ao interagir com a subunidade catalítica TERT. No entanto, seu papel em tripanossomatídeos permanecia desconhecido. Nesta tese, realizamos a primeira caracterização funcional e estrutural do ortólogo de PinX1 em *Leishmania* spp. (LmPinX1). Utilizando uma abordagem multifacetada que combinou a superexpressão episomal da proteína marcada com myc, a obtenção da proteína na forma recombinante, a análise de interatoma, a edição gênica por CRISPR-Cas9 em *L. major*, o sequenciamento de DNA de longa leitura (ONT), a análise da expressão relativa, os ensaios de proliferação e de ciclo celular, além da caracterização biofísica e bioquímica da proteína, demonstramos que a LmPinX1 é essencial e multifuncional. Semelhante a outros eucariotos, a LmPinX1 interage com proteínas envolvidas com maturação de RNA ribossomal e pequenos RNAs nucleolares, além de se associar a proteínas de ciclo celular e com os telômeros via a proteína TRF e o componente TERT da telomerase. A análise funcional demonstrou a impossibilidade de gerar um nocaute em ambos os alelos do gene, sugerindo que LmPinX1 é vital para o parasito. Linhagens heterozigóticas (LmPinX1^{+/-}) exibiram fenótipos pleiotrópicos progressivos, incluindo crescimento e divisão celular comprometidos, acúmulo de células na fase S, diminuição da expressão de LSU rRNAs e encurtamento telomérico. O encurtamento progressivo dos telômeros em LmPinX1^{+/-} ao longo das passagens sugere um papel semelhante ao de células tumorais com expressão diminuída de PinX1 e nucleofosmina (proteína envolvida com a biogênese de ribossomos), mas contrasta com leveduras, onde a ausência de PinX1 induz o alongamento dos telômeros. Quanto à diminuição da expressão dos fragmentos rRNA LSU-β e LSU-ε, este fenótipo conecta a LmPinX1 à biogênese de ribossomos, o que foi posteriormente confirmado pela presença de muitos componentes da maquinaria de montagem de ribossomos nos dados de interatoma. Estudos estruturais também revelaram particularidades sobre a LmPinX1. Sua estrutura tridimensional mostra que

ela é intrinsecamente desordenada, apresenta tamanho menor do que seus ortólogos e substituições de resíduos de aminoácidos específicos do gênero. LmPinX1 é capaz de formar monômeros e dímeros, imunoprecipita com a proteína telomérica TRF e interage fisicamente com o domínio N-terminal do componente TERT da telomerase, conforme demonstrado por ensaios de *pull-down*, espectroscopia de fluorescência e *docking* molecular. Coletivamente, nossos resultados revelam que a PinX1 em *Leishmania* pode desempenhar um papel importante na homeostase celular, integrando a manutenção telomérica, o processamento de rRNA e a progressão do ciclo celular. Esta convergência funcional em relação a outros eucariotos destaca a PinX1 como um alvo promissor para o desenvolvimento de novas estratégias quimioterápicas contra as leishmanioses, embora ainda haja muito a ser descoberto sobre esta proteína, especialmente quanto ao seu papel na manutenção dos telômeros.

Palavras-chave: *Leishmania*; PinX1; telômero; telomerase; TERT; TRF; biogênese de ribossomos; proteína intrinsecamente desordenada.

Abstract

Leishmaniasis is a neglected disease caused by parasites of the genus *Leishmania*, affecting millions of people worldwide. The high toxicity and resistance to current treatments make the identification of new therapeutic targets urgent. Telomeres, nucleoprotein structures essential for genomic stability, emerge as promising candidates. In higher eukaryotes, the PinX1 protein is a classic negative regulator of telomerase, inhibiting its activity by interacting with the catalytic subunit TERT. However, its role in trypanosomatids remained unknown. In this thesis, we performed the first functional and structural characterization of the PinX1 ortholog in *Leishmania* spp. (LmPinX1). Using a multifaceted approach that combined episomal overexpression of the myc-tagged protein, recombinant protein production, interactome analysis, CRISPR-Cas9 gene editing in *L. major*, long-reading DNA sequencing (ONT) relative expression analysis, proliferation and cell cycle assays, and biophysical and biochemical characterization of the protein, we demonstrated that LmPinX1 is essential and multifunctional. Similar to other eukaryotes, LmPinX1 interacts with proteins involved in ribosomal RNA maturation and small nucleolar RNAs, in addition to associating with cell cycle proteins and telomeres via the TRF and the telomerase TERT component. Functional analysis demonstrated the impossibility of generating a knockout in both alleles of the gene, suggesting that LmPinX1 is vital for the parasite. Heterozygous lineages (LmPinX1^{+/-}) exhibited progressive pleiotropic phenotypes, including impaired cell growth and division, cell accumulation in the S phase, decreased LSU rRNA expression, and telomere shortening. The progressive telomere shortening in LmPinX1^{+/-} across passages suggests a role similar to that of tumor cells with decreased expression of both PinX1 and nucleophosmin (protein involved in ribosome biogenesis), but contrasts with yeast, where the absence of PinX1 induces telomere elongation. Regarding the decreased expression of LSU-β and LSU-ε rRNA fragments, this phenotype connects LmPinX1 to ribosome biogenesis, which was confirmed by the presence of many components of the ribosome assembly machinery in interactome data. Structural studies also revealed particularities about LmPinX1. Its three-dimensional structure shows that it is intrinsically disordered, smaller in size than its orthologs, and exhibits genus-specific amino acid residue substitutions. LmPinX1 is capable of forming monomers and dimers,

immunoprecipitates with the telomeric protein TRF, and physically interacts with the N-terminal domain of the telomerase TERT component, as demonstrated by pull-down assays, fluorescence spectroscopy, and molecular docking. Collectively, our results reveal that PinX1 in *Leishmania* may play an important role in cellular homeostasis, integrating telomeric maintenance, rRNA processing, and cell cycle progression. This functional convergence with respect to other eukaryotes highlights PinX1 as a promising target for the development of new chemotherapeutic strategies against leishmaniasis, although much remains to be discovered about this protein, particularly regarding its role in telomere maintenance.

Keywords: *Leishmania*; PinX1; telomere; telomerase; TERT; TRF; ribosome biogenesis; intrinsically disordered protein.

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4. Capítulo I

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5. Capítulo II

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Lista de abreviaturas

°C - Grau Celsius

μ - micro

μg - Micrograma

μL - Microlitro

μm - Micrômetro

μM - Micromolar

7-AAD - 7-Aminoactinomycin D (corante para DNA)

Å - Angstrom

a.u. - Unidade arbitrária

ANOVA - Analysis of Variance (Análise de Variância)

ATP - Adenosina Trifosfato

Bis-ANS - 4,4'-dianilino-1,1'-binaphthyl-5,5'-disulfonic acid, dipotassium salt

BLAST - Basic Local Alignment Search Tool

BN-PAGE - Blue Native Polyacrylamide Gel Electrophoresis

Cas9 - CRISPR-associated protein 9

CD - Circular Dichroism (Dicroísmo Circular)

cDNA - DNA complementar

Co-IP - Co-Immunoprecipitation (Co-Imunoprecipitação)

CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats

CT - Cycle Threshold (limiar de ciclo)

CTE - C-Terminal Extension (Extensão C-terminal)

DEAD-box - Motivo de aminoácidos (Asp-Glu-Ala-Asp) em helicases

DIG - Digoxigenina

DKC - Diskerina

DLS - Dynamic Light Scattering (Espalhamento Dinâmico de Luz)

DNA - Deoxyribonucleic Acid (Ácido Desoxirribonucleico)

DNase - Desoxirribonuclease

DTT - Ditionitrito

ECL - Enhanced Chemiluminescence (Quimioluminescência Aprimorada)

EDTA - Ácido Etilenodiamino Tetra-acético

EdU - 5-Etynil-2'-desoxyuridine
et al. - E colaboradores
FASP - Filter-Aided Sample Preparation (Preparo de Amostra Assistido por Filtro)
FDR - False Discovery Rate (Taxa de Falsas Descobertas)
FISH - Fluorescence In Situ Hybridization (Hibridização Fluorescente in situ)
FITC - Fluorescein Isothiocyanate (Isotiocianato de Fluoresceína)
g - Força gravitacional
GAPDH - Glyceraldehyde 3-Phosphate Dehydrogenase (Gliceraldeído-3-fosfato Desidrogenase)
GAR1 - Gar1 ribonucleoprotein
GO - Gene Ontology
GRAMM - Global Range Molecular Matching (software de docking)
GROMACS - GRONingen MACHine for Chemical Simulations (software de dinâmica molecular)
H/ACA - Motivo de sequência em snoRNAs
HEPES - Ácido 4-(2-hidroxietil)-1-piperazinoetanossulfônico
HIFiASM - Haplotype-resolved genome assembly software
HPLC - High-Performance Liquid Chromatography (Cromatografia Líquida de Alta Eficiência)
HRP - Horseradish Peroxidase (Peroxidase de Rábano)
HSP - Heat Shock Protein (Proteína de Choque Térmico)
IDP - Intrinsically Disordered Protein (Proteína Intrinsecamente Desordenada)
IGV - Integrative Genomics Viewer
IP - Immunoprecipitation (Imunoprecipitação)
IPTG - Isopropyl β -D-1-thiogalactopyranoside (Isopropil β -D-1-tiogalactopiranosídeo)
ITS - Internal Transcribed Spacer (Espaçador Interno Transcrito)
kDa - Kilodalton
L - Litro
La - *Leishmania amazonensis*
LACK - *Leishmania* Homologue of Receptors for Activated C Kinase
LaTERT-NT - Proteína recombinante da parte aminoterminal da TERT de *Leishmania amazonensis*

LCTAS - *Leishmania* Conserved Telomere Associated Sequences

LINCS - LINEAR Constraint Solver (algoritmo de restrição)

Lm - *Leishmania major*

LSU - Large Subunit (Subunidade Maior - ribossomal)

M - Molar

MD - Molecular Dynamics (Dinâmica Molecular)

mg - Miligrama

mL - Mililitro

mM - Milimolar

MMseqs2 - Many-against-Many sequence searching (software de busca de sequências)

mRNA - RNA mensageiro

MS - Mass Spectrometry (Espectrometria de Massas)

MSA - Multiple Sequence Alignment (Alinhamento Múltiplo de Sequências)

MW - Molecular Weight (Peso Molecular)

NCBI - National Center for Biotechnology Information

ng - Nanograma

NHP2 - Nhp2 ribonucleoprotein

Ni-NTA - Níquel-Ácido Nitrilotriacético

nm - Nanômetro

NOP - Proteína Nucleolar

NOP10 - Nop10 ribonucleoprotein

NP-40 - Nonidet P-40 (detergente)

NPM - Nucleofosmina

NPT - Ensemble com número de partículas (N), pressão (P) e temperatura (T) constantes

ns - Nanossegundos

NVT - Ensemble com número de partículas (N), volume (V) e temperatura (T) constantes

OD - Optical Density (Densidade Óptica)

OMS - Organização Mundial da Saúde

ONT - Oxford Nanopore Technology

P - P valor

PAGE - Polyacrylamide Gel Electrophoresis (Eletroforese em Gel de Poliacrilamida)

PARN - Poly(A)-specific Ribonuclease

PBS - Phosphate-Buffered Saline (Tampão Fosfato-Salino)

PCR - Polymerase Chain Reaction (Reação em Cadeia da Polimerase)

PDB - Protein Data Bank

pH - Potencial de hidrogênio

pLDDT - predicted Local Distance Difference Test (escore de confiança do AlphaFold)

Plk1 - Polo-like kinase 1

PNK - Polynucleotide Kinase (Polinucleotídeo Quinase)

POT1 - Protection Of Telomeres 1

PVDF - Fluoreto de Polivinilideno

RAP1 - Repressor Activator Protein 1

RBP - RNA-Binding Protein (Proteína de Ligação a RNA)

RIPA - Radioimmunoprecipitation Assay Buffer (tampão de lise)

RMSD - Root Mean Square Deviation (Desvio Médio Quadrático)

RNA - Ribonucleic Acid (Ácido Ribonucleico)

RNase - Ribonuclease

RNP - Ribonucleoprotein (Ribonucleoproteína)

RPA - Replication Protein A (Proteína de Replicação A)

rpm - Rotações por minuto

rRNA - RNA ribossomal

RT - Reverse Transcriptase (Transcriptase Reversa) ou Room Temperature (Temperatura Ambiente)

RT-PCR - Reverse Transcription Polymerase Chain Reaction (Reação de Transcriptase Reversa seguida de Reação em Cadeia da Polimerase)

RT-qPCR - Reverse Transcription quantitative Polymerase Chain Reaction (PCR quantitativa em Tempo Real com Transcrição Reversa)

SAM - Sterile Alpha Motif (domínio proteico)

SDS - Dodecil Sulfato de Sódio

SEC-MALS - Size-Exclusion Chromatography coupled with Multi-Angle Light Scattering (Cromatografia de Exclusão de Tamanho acoplada a Espalhamento de Luz Multiângulo)

SEM - Scanning Electron Microscopy (Microscopia Eletrônica de Varredura)

sgRNA - single-guide RNA (RNA guia simples)

SL - Spliced Leader (sequência líder de trans-splicing)

snoRNA - small nucleolar RNA (pequeno RNA nucleolar)

SSU - Small Subunit (Subunidade Menor - ribossomal)

STRING - Search Tool for the Retrieval of Interacting Genes/Proteins

TBE - Template Boundary Element

TBM - TRF1-Binding Motif (Motivo de Ligação à TRF1)

TCAB1 - Telomerase Cajal body protein 1

TEN - Telomerase Essential N-terminal domain (Domínio N-terminal Essencial da Telomerase)

TER / TERC - Telomerase RNA Component (Componente de RNA da Telomerase)

TERRA - Telomeric Repeat-containing RNA

TERT - Telomerase Reverse Transcriptase (Transcriptase Reversa da Telomerase)

TID - Telomerase Inhibitory Domain (Domínio Inibitório da Telomerase)

TIN2 - TRF1-Interacting Nuclear Factor 2

TPP1 - Proteína do complexo shelterin (TINT1, PTP1, PIP1)

TRAP - Telomeric Repeat Amplification Protocol (Protocolo de Amplificação de Repetição Telomérica) - também refere-se a um motivo na TERT

TRB - Telomerase RNA Binding

TRBD - Telomerase RNA Binding Domain (Domínio de Ligação ao RNA da Telomerase)

TRF - Terminal Restriction Fragment (Fragmento de Restrição Terminal) ou Telomeric Repeat-binding Factor (Fator de Ligação a Repetições Teloméricas)

TRF1 - Telomeric Repeat Factor 1

TRF2 - Telomeric Repeat Factor 2

TRFH - TRF Homology domain (Domínio de Homologia a TRF)

TSM - Trypanosomatid-Specific Motif (Motivo Específico de Tripanossomatídeos)

U - Unidade

UFABC - Universidade Federal do ABC

UNESP - Universidade Estadual Paulista

UniProt - Universal Protein Resource

UV - Ultravioleta

v - Volume

V - Volt

WHO - World Health Organization (Organização Mundial da Saúde)

α - Alfa

β - Beta

γ - Gama

ε - Épsilon

$\emptyset$ - Vazio

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1. Introdução

1.1. Contexto biológico geral

As leishmanioses compreendem um conjunto de doenças causadas por parasitos do gênero *Leishmania* (Neves et al., 2022; Organização Pan-Americana da Saúde, 2021; Secretaria de Vigilância em Saúde e Ambiente, 2025). Estas enfermidades representam um importante problema de saúde pública em regiões das Américas, África e Ásia (WHO, 2020, 2021), sendo classificadas pela Organização Mundial da Saúde (OMS) como “Doenças tropicais negligenciadas” devido ao seu elevado impacto social e econômico nas populações mais pobres de países tropicais e subtropicais (Figura 1-1) ((OMS), 2025). No Brasil, as leishmanioses permanecem endêmicas, com aproximadamente 13 000 casos de leishmaniose tegumentar registrados em 2023, principalmente nas regiões Norte e Nordeste, e cerca de 2 000 casos anuais de leishmaniose visceral, forma mais grave da doença (Secretaria de Vigilância em Saúde e Ambiente, 2025). Esses números evidenciam a persistência da transmissão e a relevância epidemiológica do parasito, reforçando a necessidade de desenvolver novas terapias e estratégias preventivas.



Figura 1-1 Leishmaniose: Uma doença tropical negligenciada. Infográfico sobre a leishmaniose, abordando o vetor transmissor, os reservatórios animais, a distribuição geográfica, as formas clínicas, o tratamento e os aspectos epidemiológicos da doença. Elaborado pela autora, com

aprimoramento visual realizado com auxílio de inteligência artificial generativa (Chatgpt) .¹ Adaptado de dados da Organização Pan-Americana da Saúde (OPAS).

Clinicamente, as leishmanioses podem se apresentar desde formas assintomáticas, a lesões cutâneas autolimitantes, como ocorre na leishmaniose cutânea, ou lesões desfigurantes em regiões mucosas e mucocutâneas; ou ainda, com envolvimento de baço e fígado, podendo levar a óbito, como ocorre na leishmaniose visceral (Neves et al., 2022). A apresentação da doença depende do estado imunológico do hospedeiro e da espécie do parasito. No Brasil, *Leishmania amazonensis* e *L. braziliensis* estão associadas às formas cutânea e mucosa da doença, enquanto *L. infantum* é responsável pela forma visceral (Organização Pan-Americana da Saúde, 2021; Secretaria de Vigilância em Saúde e Ambiente, 2025).

O gênero *Leishmania* pertence à ordem Kinetoplastida e à família Trypanosomatidae. Os parasitos apresentam dimorfismo morfológico ao longo de seu ciclo de vida, alternando entre as formas promastigota e amastigota (**Erro! Fonte de referência não encontrada.**), as quais são adaptadas aos ambientes do inseto vetor e do hospedeiro vertebrado, respectivamente (Bates, 2007; Conceição-Silva; Alves, 2014). A forma promastigota, encontrada no trato digestório do flebotomíneo e mantida em cultura *in vitro*, apresenta morfologia alongada e fusiforme, com comprimento médio variando entre 10 e 20 µm, possuindo um único flagelo emergente da bolsa flagelar, responsável pela motilidade celular (Neves et al., 2022; Real; Mortara, 2012; Wheeler; Gluenz; Gull, 2011a). O núcleo localiza-se aproximadamente na região central da célula, enquanto o cinetoplasto — uma estrutura especializada rica em DNA mitocondrial — encontra-se próximo à extremidade anterior, adjacente à base do flagelo (Neves et al., 2022; Real; Mortara, 2012). Em contraste, a forma amastigota, presente no interior de vacúolos parasitóforos de macrófagos do hospedeiro vertebrado, é arredondada ou oval, medindo cerca de 2 a 5 µm de diâmetro, e não apresenta flagelo externo, embora mantenha a estrutura flagelar curta e interna (Real; Mortara, 2012; Wheeler; Gluenz; Gull, 2011a). Apesar das diferenças morfológicas marcantes entre as duas formas evolutivas, ambas compartilham características celulares típicas dos tripanossomatídeos, como a presença de uma única mitocôndria ramificada, um citoesqueleto organizado por microtúbulos subpeliculares e uma

organização nuclear altamente conservada (Real; Mortara, 2012; Wheeler; Gluenz; Gull, 2011a).

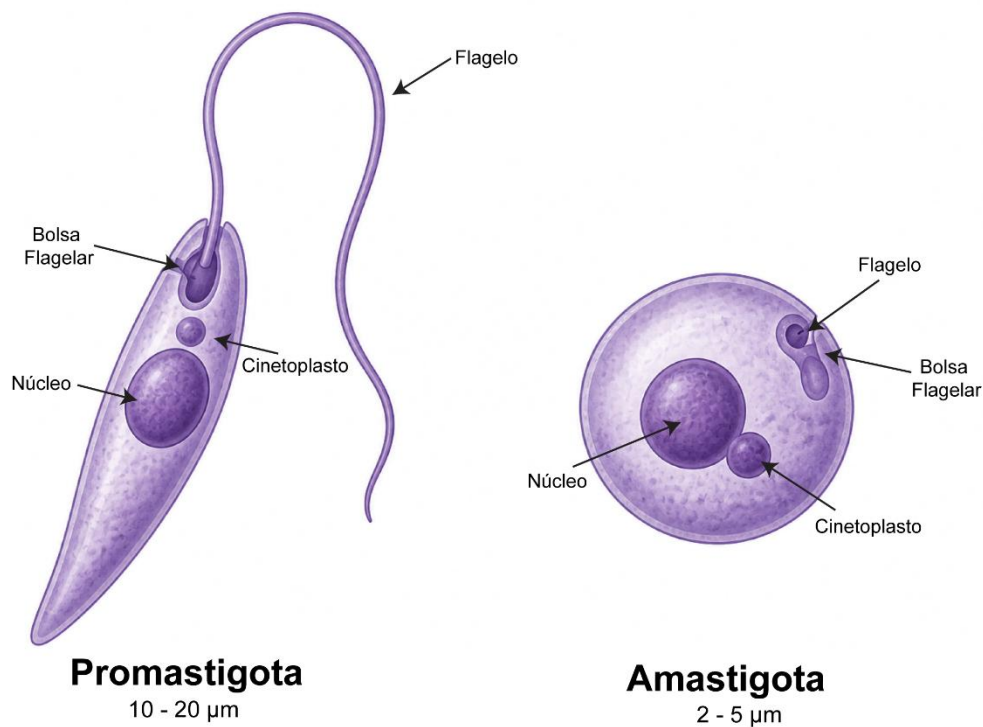


Figura 1-2 Representação esquemática das formas evolutivas de *Leishmania*: promastigota (à esquerda), forma flagelada presente no inseto vetor, e amastigota (à direita), forma arredondada intracelular encontrada em células do hospedeiro vertebrado. Estão indicadas estruturas celulares simplificadas, incluindo núcleo, cinetoplasto, bolsa flagelar e flagelo. Figura elaborada pela autora, com auxílio de inteligência artificial generativa para refinamento visual com base em Neves et al., 2022.

O ciclo de infecção inicia-se quando mosquitos flebotomíneos fêmeas do gênero *Lutzomyia* ingerem formas amastigotas durante o repasto sanguíneo. No intestino do vetor, o parasito se diferencia e se multiplica em promastigotas procíclicas, leptomonadas, retroleptomonadas e promastigotas metacíclicas, esta última forma altamente infectiva. Ao ser inoculada em um hospedeiro vertebrado, a forma metacíclica é fagocitada por neutrófilos e macrófagos, transformando-se novamente em amastigotas no vacúolo parasitóforo. A multiplicação intracelular permite a disseminação da infecção e o surgimento das manifestações clínicas (Neves et al., 2022; Organização Pan-Americana da Saúde, 2021; Serafim et al., 2018) (Figura 1-3).

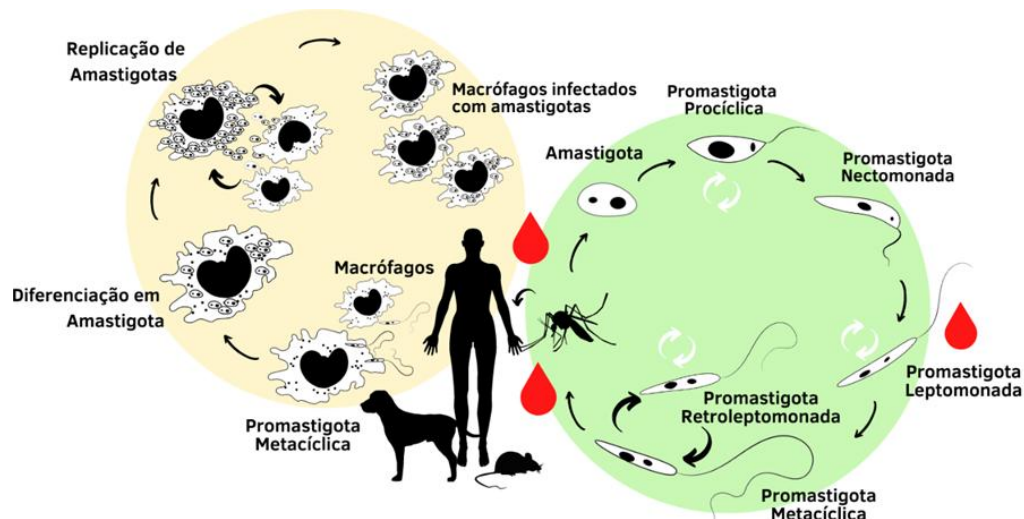


Figura 1-3 Ciclo de desenvolvimento de *Leishmania* sp. O mosquito vetor ingere formas amastigotas durante o repasto sanguíneo de um hospedeiro infectado. No intestino do inseto, o parasito se diferencia em promastigotas procíclicas e leptomonadas, que podem se desdiferenciar em retroleptomonadas após novo repasto. Essas formas se transformam em promastigotas metacíclicas, não replicativas, mas altamente infectivas, que são transmitidas ao hospedeiro vertebrado em um novo repasto sanguíneo. Figura adaptada de Serafim et al., (2018).

Embora existam tratamentos como os antimoniais pentavalentes e a anfotericina B, estes apresentam alta toxicidade e custo elevado, e a resistência dos parasitos a esses fármacos tem aumentado. Além disso, não há vacina disponível, de modo que a prevenção depende do controle do vetor e de melhorias sanitárias e habitacionais (Bethony et al., 2011; Mukherjee et al., 2013; Ponte-Sucre et al., 2017; Taslimi; Zahedifard; Rafati, 2018). Esse cenário reforça a necessidade de identificar novos alvos moleculares para o desenvolvimento de terapias mais eficazes, bem como de ampliar o conhecimento básico sobre a biologia do parasita.

Compreender a biologia celular de *Leishmania* spp., incluindo morfologia, ciclo de vida e dinâmica celular, é fundamental para investigar proteínas nucleares e processos essenciais como a manutenção telomérica. Estes conhecimentos fornecem o contexto necessário para estudar proteínas reguladoras da telomerase, como a PinX1, e seu papel multifuncional no parasito.

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