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Trends in Parasitology

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Review

Going ballistic: *Leishmania* nuclear subversion of host cell plasticity

Hervé Lecoeur [1], Eric Prina [1], Maria Gutiérrez-Sanchez [1,2] and Gerald F. Späth [1,*]

Intracellular parasites have evolved intricate strategies to subvert host cell functions for their own survival. These strategies are particularly damaging to the host if the infection involves immune cells, as illustrated by protozoan parasites of the genus *Leishmania* that thrive inside mononuclear phagocytic cells, causing devastating immunopathologies. While the impact of *Leishmania* infection on host cell phenotype and functions has been well documented, the regulatory mechanisms underlying host cell subversion were only recently investigated. Here we summarize the current knowledge on how *Leishmania* infection affects host nuclear activities and propose thought-provoking new concepts on the reciprocal relationship between epigenetic and transcriptional regulation in host cell phenotypic plasticity, its potential subversion by the intracellular parasite, and its relevance for host-directed therapy.

Highlights

Phenotypic plasticity of macrophages (Mϕs) and dendritic cells (DCs) enables these cells to adapt their immune functions to local tissue microenvironment changes.

Leishmania exploits Mϕ and DC phenotypic plasticity to establish permissive conditions for intracellular survival and chronic infection.

Mϕ polarization and DC maturation result from a complex regulatory relationship between epigenetic and transcriptional gene expression controls.

Leishmania infection changes the host cell epigenetic and transcription factor landscapes, resulting in the expression of a unique, anti-inflammatory host cell phenotype.

Our review provides an overview of *Leishmania* subversion strategies that hijack epigenetic and transcriptional control in infected Mϕs and DCs. New venues for host-directed therapy are proposed to foster host antimicrobial functions.

Macrophage and dendritic cell plasticity: a gateway for *Leishmania* infection

Intracellular parasites of the genus *Leishmania* cause a spectrum of severe immunopathologies termed leishmaniases. These diseases are the direct consequence of the parasites' capacity to colonize and subvert essential immune cells, particularly macrophages and dendritic cells (DCs). *Leishmania* has evolved a multitude of strategies to render these cells permissive to infection by taking advantage of their remarkable **phenotypic plasticity** (see Glossary) (Box 1). Macrophages and DCs adopt various expression profiles and morphologies, often described as states of polarization (macrophages) and differentiation/maturation (DCs) that control highly specialized immune functions, including immune tolerance and response to infection [1]. Recent studies have shed important new light on the regulation of these alternative phenotypes, revealing a highly complex interplay between epigenetic, transcriptional, and post-transcriptional mechanisms involving dynamic changes in **DNA methylation**, histone modification, transcription factor (TF) activity, and the expression of **noncoding RNAs** (Figure 1) [2]. The complexity of this scenario is further increased by the reciprocal regulatory relationship between **epigenetic-regulatory factors** (**EpiRFs**) that control the expression levels of TFs, which vice versa control the expression levels of DNA methylases, histone-modifying enzymes (HMEs) and noncoding RNAs (Figure 1). Thus, in addition to being controlled by individual master regulators, the different cellular states of macrophages and DCs can be considered an **emergent property** of self-organizing, regulatory networks. Intracellular pathogens that infect these immune cells as hosts, such as *Leishmania*, have likely coevolved strategies to tap into these networks and exploit established host cell programs favoring parasite survival and chronic infection. As such, these parasites represent unique biological probes to investigate pathways regulating phenotypic plasticity of macrophages and DCs, which, in turn, informs on immunopathological mechanisms underlying leishmaniases. In this review, we discuss the current knowledge on the regulatory interplay between *Leishmania* and the host cell phenotype, how these parasites may subvert the 'yin–yang' relationship between epigenetic and transcriptional control, and how they may exploit existing host cell developmental/

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Box 1. Plasticity of monocytes, macrophages, and DCs

A hallmark of monocytes, macrophages and DCs is represented by their phenotypic plasticity which (i) drives cellular differentiation, macrophage polarization, and DC maturation, (ii) regulates dynamic, cell-type-specific responses to environmental and molecular cues, and (iii) defines the pleiotropic immune functions of those cells [1]. Monocytes differentiate into different cell type, including macrophages, Langerhans cells, and DCs [65–67].

Macrophages can adopt a spectrum of polarization states whose extremes are represented by classically (M1) and alternatively (M2a, b, c, d) activated macrophages. M1 cells are characterized by (i) the production of energy via glycolysis, (ii) the production of proinflammatory cytokines (TNFα, IL-1β, IL-6, IL-12, and IL-23) and antimicrobial oxidants [reactive oxygen species (ROS) and nitric oxide (NO)], and (iii) the induction of T helper type 1 (Th1) responses that can resolve infection and eliminate tumor cells. In contrast, M2 macrophages are characterized by (i) the use of oxidative phosphorylation/L-arginine metabolism for energy production, (ii) the production of anti-inflammatory cytokines (IL-10, TGFβ), and (iii) the induction of Th2 responses implicated in tissue homeostasis and wound-healing. Some macrophages, such as tumor-associated macrophages (TAMs) display features of both M1 and M2 phenotypes [68,69]. Likewise, *Leishmania*-infected macrophages seem to express a mixed profile and may define a new polarization state that remains to be investigated in detail [70]. These macrophage phenotypes are transient and reversible [71–73] and thus can be exploited for treatment, as illustrated by current efforts to target TAMs for cancer treatment [74].

DC subsets have different and independent ontologies and are divided into myeloid, monocyte-derived, and plasmacytoid DCs. Myeloid DCs give rise to functionally and phenotypically diverse cells implicated in priming adaptive immune responses and generating immune tolerance [75]. They are one of the first subsets expected to encounter *Leishmania* during parasite transmission by blood-feeding sand flies. Conventional DC1 cells (cDC1) have an intrinsic capacity for $CD8^+$ T cell activation, cross-presentation and promoting Th1 responses [76]. Conventional DC2 cells (cDC2A and B) are more efficient for $CD4^+$ T helper polarization, and the induction of Th2, Th17, and regulatory T cell responses [76–78]. Under inflammatory conditions, DCs can also be derived from monocytes (monocyte-derived DCs, MoDCs) that further drive $CD4^+$ T helper responses and $CD8^+$ T cell cross-priming [65].

Plasmacytoid DCs (pDCs) express a unique morphology and are specialized in (i) antiviral defense via IFN-α/β production [79], (ii) antigen presentation, (iii) activation of adaptive immune responses, and (iv) immunoregulation [80]. The phenotypic and functional plasticity allows these DC subsets to elicit tailored pathogen-specific immune responses [81].

Leishmania parasites can exploit the plasticity of these immune cells and subvert macrophage differentiation, polarization, maturation, and the activation of the TLR–NF-κB signaling pathway [26,27], which together defines host sensitivity to infection [70,82].

polarization programs for intracellular survival. We propose the host cell nucleus as a biological target for *Leishmania* immune subversion and a pharmacological target for **host-directed therapy**.

The yin of *Leishmania* nuclear subversion: exploiting host cell TFs to modulate epigenetic regulation

The phenotypic plasticity of macrophages and DCs is regulated by a complex interplay between TFs and epigenetic actors such as microRNAs (Figure 2). Based on regulatory hierarchy and functions, two major classes of TF can be distinguished [2]: first, **lineage-determining transcription factors** (**LDTFs**) are **pioneer TFs** regulating chromatin accessibility by binding to target sequences located in closed, silent chromatin at the nucleosome level. LDTFs enable the subsequent binding of transcriptional and epigenetic regulators to control tissue-specific gene expression [3]. For example, PU.1 and C/EBPβ constitute major LDTFs in macrophages and DCs that induce profound **epigenetic modulations** in a genome-wide manner (see Table S1A in the supplemental information online), in particular by maintaining the H3K4me1 mark at specific enhancers [4,5] and rendering DNA accessible to other TFs [6]. PU.1, in addition, modulates DNA methylation by recruiting the epigenetic modulators TET2 and DNMT3b that respectively induce or repress gene expression (Table S1A). Thus, LDTFs initiate cooperative interactions with nonpioneer TFs and key EpiRFs**,** including chromatin modifiers, histone variants, and repressors [7], which prepares the chromatin landscape (Table S1A) for the binding of the second class of **signal-dependent transcription factors** (**SDTFs**) to the prearranged genome. Consequently SDTFs further regulate stimulus and cell-type-specific transcriptome changes [7] (Table S1B). Interactions between LDTFs,

Glossary

DNA methylation: reversible addition of a methyl group regulated by DNA methyltransferases and demethylases at the 5′ position on the pyrimidine ring of cytosine residues resulting in the generation of 5-methylcytosine in DNA that can repress or induce gene transcription. DNA methylation largely occurs in CpG dinucleotide motifs present in promoter regions but has also been observed inside genes and intergenic regions.
Emergent property: a cellular property that emerges from the interaction of individual pathways and represents more than just the sums of the pathways' functions, for example, life is an emergent property of chemistry.
Epigenetic modulations: an ensemble of mechanisms that regulate gene expression without structural modifications in the DNA sequence by DNA methylation, post-translational histone modifications, nucleosome positioning, chromatin remodeling/accessibility, and expression of noncoding RNAs.
Epigenetic-regulatory factors (EpiRFs): an ensemble of factors involved in epigenetic regulation, including histone- and DNA-modifying enzymes, partners of epigenetic complexes, and noncoding RNAs.
Histone code: reversible post-translational modifications of histone N termini that regulate the chromatin structure to repress or induce gene transcription. They include methylation, acetylation, ubiquitination, phosphorylation, and SUMOylation, and they are introduced or removed by specific histone-modifying enzymes. Most commonly studied marks are acetylation (addition of an acetyl group to lysine residues, such as acetylation of histone H3 at lysine 4, H3K4Ac) and methylation (addition of methyl groups to lysine and/or arginine residues, such as trimethylation of histone H3 on lysine 27, H3K27me3).
Host-directed therapy: a strategy to reduce or eliminate an infectious agent by targeting host-mediated responses to infection rather than acting directly on the pathogen. It is a novel approach to overcome microbial drug resistance.
Immunometabolomics: an evolving field of scientific endeavor that combines immunology and metabolism and investigates the reciprocal relationship between the impact of the immune

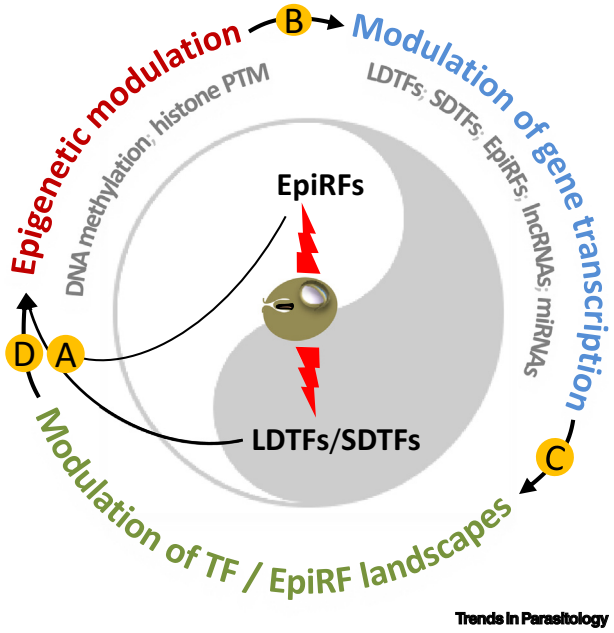


Figure 1. Model of reciprocal interaction between epigenetic and transcriptional regulation. Lineage-determining transcription factors (LDTFs), signal-dependent transcription factors (SDTFs), and epigenetic-regulatory factors (EpiRFs) act in concert to modulate the epigenetic profile (A). The resulting changes in chromatin structure modulate, in turn, gene expression (B) that can further change the transcription factor (TF) and EpiRF landscapes at the protein level (C), and affect the epigenome, thus closing the regulatory cycle (D). As indicated by the flashes, *Leishmania* parasites (at the center) interfere with this complex TF and EpiRF interplay. Abbreviations: lncRNAs, long noncoding RNAs; miRNAs, microRNAs; PTMs, post-translational modifications.

SDTFs, and EpiRFs finally control the transcription of key genes involved in the immune response [cytokines, chemokines, Toll-like receptors (TLRs), NOS2, TFs, and microRNAs] (Tables S1, S2). As detailed in the following, there is good evidence that *Leishmania* acts on these hierarchical, regulatory circuits to either induce or suppress gene expression.

Leishmania subversion of positive regulatory circuits

TFs can promote an open chromatin structure and induce gene expression by recruiting HMEs that deposit activating marks or remove repressive marks (Figure 1A), or by changing the expression levels of HMEs themselves (Figure 1B,C). For example, during M2 polarization of bone-marrow-derived macrophages (BMDMs), the SDTF STAT6 induces the transcription of the demethylase Jumonji domain containing 3 (JMJD3) gene to decrease H3K27me3 suppressive marks at the promoters of various M2 marker genes including ARG1 [8] (Table S1B). Similar regulatory mechanisms operate during M1 polarization via the NF-κB family member v-rel avian reticuloendotheliosis viral oncogene homolog A (RELA) – a key SDTF for the proinflammatory response. RELA phosphorylation triggers recruitment of CREB-binding protein (CBP) and MLL1-2/COMPASS-like complexes that establish active marks on H4 and H3 histones thus promoting NOS2 expression [9] (Table S1B). Again, aside from these direct effects, RELA also modulates the expression of epigenetic regulators, such as the long noncoding RNA lincRNA-Cox2 that, in turn, promotes the expression of late inflammatory-response genes in lipopolysaccharide (LPS)-treated macrophages [10] (Table S1B).

There is good evidence that *Leishmania* acts on both of these TFs, likely establishing a mixed macrophage polarization profile. *Leishmania* survival has been shown to depend on STAT6 expression, which is induced in an miR146a-5p-dependent fashion during infection and enhances the expression of the M2 marker protein ARG1, an arginase that catalyzes the conversion of L-arginine into ornithine, known to promote *Leishmania* replication and survival [11,12]. In contrast, *Leishmania* infection interferes with NF-κB expression and function, and thus the antimicrobial M1 polarization profile at multiple levels (Figure 3), by inducing the formation of

response on cellular metabolisms, and vice versa, the role of metabolic processes and individual metabolites in shaping the function of immune cells.

Lineage-determining transcription factor (LDTF): a pioneer transcription factor that activates or represses tissue-specific genes that determine cellular phenotype and function.

Noncoding RNAs: RNA molecules that are not translated into proteins; they regulate gene expression at the translational and post-transcriptional level. They include two categories: (i) short-chain noncoding RNAs (including miRNAs with a size <200 nucleotides) that mediate transcriptional gene silencing, and (ii) long noncoding RNAs (lncRNAs, size >200 nucleotides) that change gene expression by chromatin remodeling, transcriptional regulation, or post-transcriptional control of mRNA stability, splicing, and distribution.

Phenotypic plasticity: the ability of a cell to display more than one phenotype when exposed to different microenvironments. For example, it allows immune cells to establish phenotypically distinct 'subpopulations' the functions of which are adapted to a given immunological challenge.

Pioneer transcription factor: a transcription factor that regulates chromatin accessibility by binding to target sequences located in silent, tightly packed chromatin and nucleosomes. They can have positive or negative effects on transcription by recruiting chromatin-remodeling enzymes to render DNA accessible to nonpioneer transcription factors.

Signal-dependent transcription factor (SDTF): a transcription factor that modulates gene transcription in response to a stimulus.

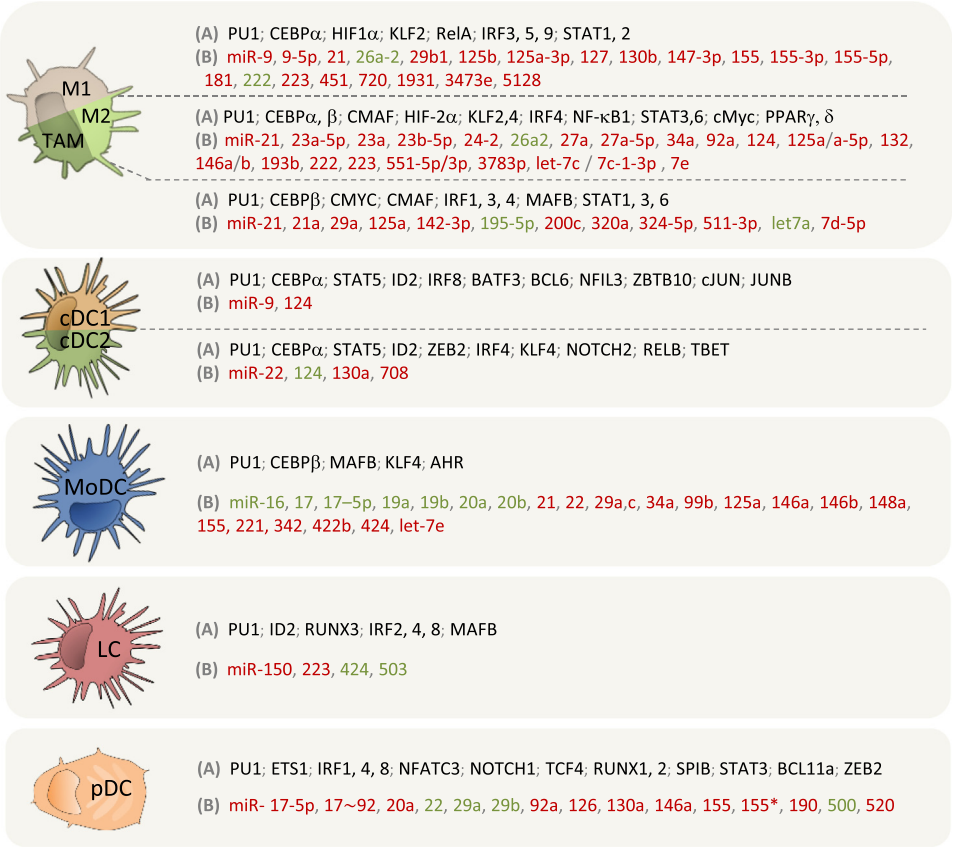


Figure 2. TF/miR landscapes and cell plasticity. Transcription factors (TFs) and microRNAs (miRNAs) constitute dynamic regulators of polarization and plasticity of monocytes, macrophages, and dendritic cells (DCs). The indicated TFs (A) and miRNAs (B) were previously linked to the two canonical subsets of polarized macrophages (M1, M2) and tumor-associated macrophages (TAMs) [6,35,83–90] and the five types of dendritic cell subsets, that is, conventional DC1 and DC2 (cDC1 and cDC2) [91–99], monocyte-derived inflammatory DCs (moDCs) [100–105], skin-resident Langerhans cells (LCs) [106–110], and plasmacytoid DCs (pDCs) [79,91,107,111–116]. An increase or decrease of miRNA abundance in these cells is indicated by, respectively, red and green fonts. Abbreviations: AHR, aryl hydrocarbon receptor; BATF3, basic leucine zipper ATF-like transcription factor 3; BCL6, B cell lymphoma 6; BCL11a, B cell lymphoma 11a; CEBPs, CCAAT enhancer-binding proteins; CMAF, c musculoaponeurotic fibrosarcoma; cJUN, Jun proto-oncogene; cMyc, myelocytomatosis oncogene; ETS1, avian erythroblastosis virus E26 homolog-1; HIF1α, hypoxia-inducible factor 1-alpha; ID2, inhibitor of DNA binding 2; IRFs, interferon regulatory factors; JUNB, JUN protooncogene homolog B; KLFs, Kruppel-like factors; MAFB, V-maf musculoaponeurotic fibrosarcoma oncogene homolog B; NFATC3, nuclear factor of activated T cells, cytoplasmic 3; NFIL3, nuclear factor, interleukin 3 regulated; NF-κB1, nuclear factor NF-kappa-B 1; NOTCH2, neurogenic locus Notch homolog protein 2; PPARγ, peroxisome proliferator-activated receptor gamma; PU1 (purine-rich box-1), a TF encoded by the SPI1 (spleen focus-forming virus proviral integration 1) gene; RELA, rel avian reticuloendotheliosis viral oncogene homolog A; RELB, rel avian reticuloendotheliosis viral oncogene homolog B; RUNXs, *RUNX* family transcription factors; STAT1, signal transducer and activator of transcription 1; SPIB, Spi-B transcription factor; TBET, T-box expressed in T cells; ZBTB10, zinc finger and BTB domain-containing protein 10; ZEB2, zinc finger E-box-binding homeobox 2.

Crel/NF-κB1 or NF-κB1/NF-κB1 dimers (Figure 3A) [13–15], the cleavage of individual NF-κB members (Figure 3B) [16–18], the inhibition of the nuclear translocation of NF-κB dimers (Figure 3C) [19], the specific increase of key inhibitors of TLR signaling such as TNFAIP3, TOLLIP, or IRAKm [20–22], and the inhibition of TRAF3 degradation [23] (Figure 3D). Interestingly, *Leishmania* parasites can also interfere with other TFs than NF-κB, including AP-1 or STAT1 [24,25]. We recently extended this plethora of subversion strategies during early infection by *Leishmania amazonensis* by revealing (i) the reduced expression of RELA, NF-κB1, and

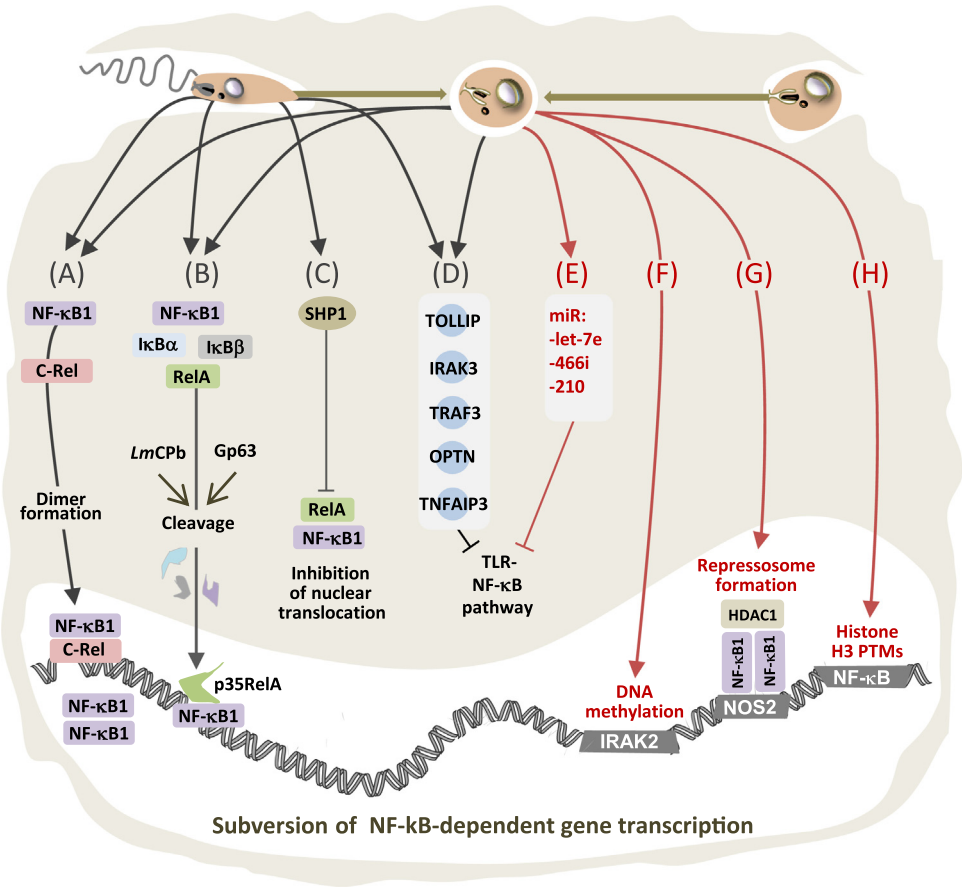


Figure 3. *Leishmania*-driven subversion of NF-κB-mediated gene regulation. At least eight different strategies have been described to date on how *Leishmania* promastigotes and amastigotes subvert NF-κB-dependent gene expression that depend on non-epigenetic (black arrows, A–D) and epigenetic (red arrows, E–H) mechanisms. After 30 min of contact with macrophages, *Leishmania* induces the formation of NF-κB1:c-Rel dimers that were associated with increased IL-10 production and suppression of TLR-induced IL12 production [14,15] (A). In macrophages infected with *Leishmania,* different members of the NF-κB family are cleaved by parasite effectors such as LmCPb and GP63 [17,18]. Promastigotes cleave RELA to release the truncated form p35RelA that binds DNA as a heterodimer with NF-κB1, whereas amastigotes cleave the protein completely [17] (B). *Leishmania* also inhibits the nuclear translocation of NF-κB1:RELA dimers via the activation of the host phosphotyrosine phosphatase SHP1 [19] (C). *Leishmania* infection further inhibits the TLR–NF-κB pathway by inducing degradation of the positive regulator TRAF3 [23] and increasing expression of the negative regulators OPTN, TNFAIP3, TOLLIP, and IRAK3 [20–22,26] (D). Various epigenetic mechanisms interfering with NF-κB-dependent gene expression are described in macrophages harboring intracellular amastigotes (red arrows, E–H), including expression of microRNAs (miRNAs) that target actors of the TLR–NF-κB signaling cascade, in particular miR-466i during infection by antimony-resistant parasites [15,40,41] (E), reduced expression of the positive regulator IRAK2 via DNA methylation [48] (F), formation of the NF-κB1:NF-κB1:HDAC1 repressosome that leads to histone deacetylation of the NOS2 gene promoter [13] (G), and histone H3 hypoacetylation and hypotrimethylation at promoters of NF-κB-related genes that correlates with decreased gene expression [26] (H). Abbreviations: C-REL, proto-oncogene c-rel; GP63, glycoprotein 63; HDAC1, histone deacetylase 1; IκBα, NF-kappa-B inhibitor alpha; IRAK3, interleukin-1 receptor-associated kinase 3; *Lm*CPb: *Leishmania mexicana* cysteine protease b; NOS2, nitric oxide synthase 2; OPTN, optineurin; PTMs, post-translational modifications; RELA, rel avian reticuloendotheliosis viral oncogene homolog A; SHP1, Src homology 2 domain-containing tyrosine phosphatase-1; TNFAIP3, tumor necrosis factor alpha-induced protein 3; TOLLIP, Toll-interacting protein; TRAF3, TNF receptor-associated factor 3.

NF-κB2 in BMDMs [26], and (ii) the increased expression of RELB in DCs, indicating activation of the noncanonical NF-κB pathway required for cross-priming [27]. As discussed further below, these TF expression changes are likely regulated at epigenetic levels.

Leishmania subversion of negative regulatory circuits

Contrary to the factors described above, various TFs trigger the formation of closed heterochromatin and thus inhibit gene expression by directly recruiting HMEs that add repressive or remove active marks (Figure 1A) or by inducing the expression of noncoding RNAs (Figure 1B) that can regulate activity and abundance of key TFs and EpiRFs (Figure 1C). For example, the LDTF PU.1 induces expression of the microRNA mir-424 during monocyte differentiation (Figure 1A,B), leading to the repression of NFI-A transcription [28] (Table S1A). Recent studies linked infection with *Leishmania* to alteration in the miRNA profile [29] (see also section below). During M1 macrophage polarization, PU.1 further forms a complex with the corepressor mSin3A and HDAC1 to reduce the expression of C-MYC, which is a key regulator of M2-like polarization [30]. Conversely, the SDTF STAT6 reduces the amount of the H3K27Ac activation mark leading to a reduced expression of key immune actors such as TLR2 and NLRP3 in IL-4 stimulated M2 macrophages, resulting in decreased responsiveness to proinflammatory stimuli (Table S1B). *Leishmania*-induced STAT6 expression thus further promotes an anti-inflammatory host cell phenotype through such negative regulatory TF circuits. Paradoxically, the proinflammatory SDTF RELA induces the negative feedback regulator MALAT1, a lncRNA that directly interacts with NF-κB1:RELA and interferes with its binding to the proinflammatory target promoters IL-6 and TNF (Table S1B). Enhanced immunity and pathogen clearance in *Leishmania donovani*-infected, MALAT1$^{-/-}$ mice indeed indicates that *Leishmania* may target these endotoxin tolerance pathways to promote its own survival [31]. Together, these examples illustrate how *Leishmania* exploits TFs to subvert host cell epigenetic regulation and to establish an immunometabolomic host cell phenotype conducive to intracellular parasite survival and chronic infection (Figure 1A–D).

The yang of *Leishmania* nuclear subversion: exploiting host cell epigenetic regulation to modulate the TF landscape

Our recent transcriptomic analyses revealed profound changes in TF expression levels in *L. amazonensis*-infected DCs [27] and BMDMs [26] (Figure 4A), including (i) suppression of various proinflammatory NF-κB family members, (ii) induction of the activating transcription factor 3 (ATF3) that negatively regulates inflammatory cytokine gene expression [32], and (iii) reduced expression of the aryl hydrocarbon receptor (AhR) (Figure 4A), a TF linked to macrophage polarization and production of tumor necrosis factor (TNF) and nitric oxide synthase (NOS) in *Leishmania major*-infected macrophages [33]. These examples uncover a recursive regulatory network where the TF landscape itself is under transcriptional control, which may involve different levels of epigenetic regulation. The term 'epigenetics' encompasses several types of inheritable changes in the pattern of gene expression that are linked to structural changes in chromatin without modification of the DNA sequence. Changes include methylation of the DNA itself, expression of variant histones, chromatin remodeling factors, noncoding RNAs, or post-translational modifications of histone proteins. Here we summarize recent evidence that reveals epigenetics as yet another target for *Leishmania* immune subversion (Figure 1).

Noncoding RNAs

MicroRNAs (miRNAs) are small RNAs that interfere with the translation of genes into proteins, each miRNA having thousands of potential transcript targets [34]. They play pivotal roles in innate and acquired immunity, modulating key processes of macrophages and DCs, including differentiation, activation, polarization, response to infection, maturation, tolerance, immune memory, inflammation, and wound repair and regeneration [35,36]. miRNAs can either directly control LDTF and SDTF expression (Figure 4BI, Table S2A) or indirectly affect TF activities, both of which are exploited by *Leishmania*. Given that the changes in miRNA expression during *Leishmania* infection have been extensively reviewed previously [37], we will here only cite key examples that illustrate their role in *Leishmania* subversion of TF functions (Figure 4). For example,

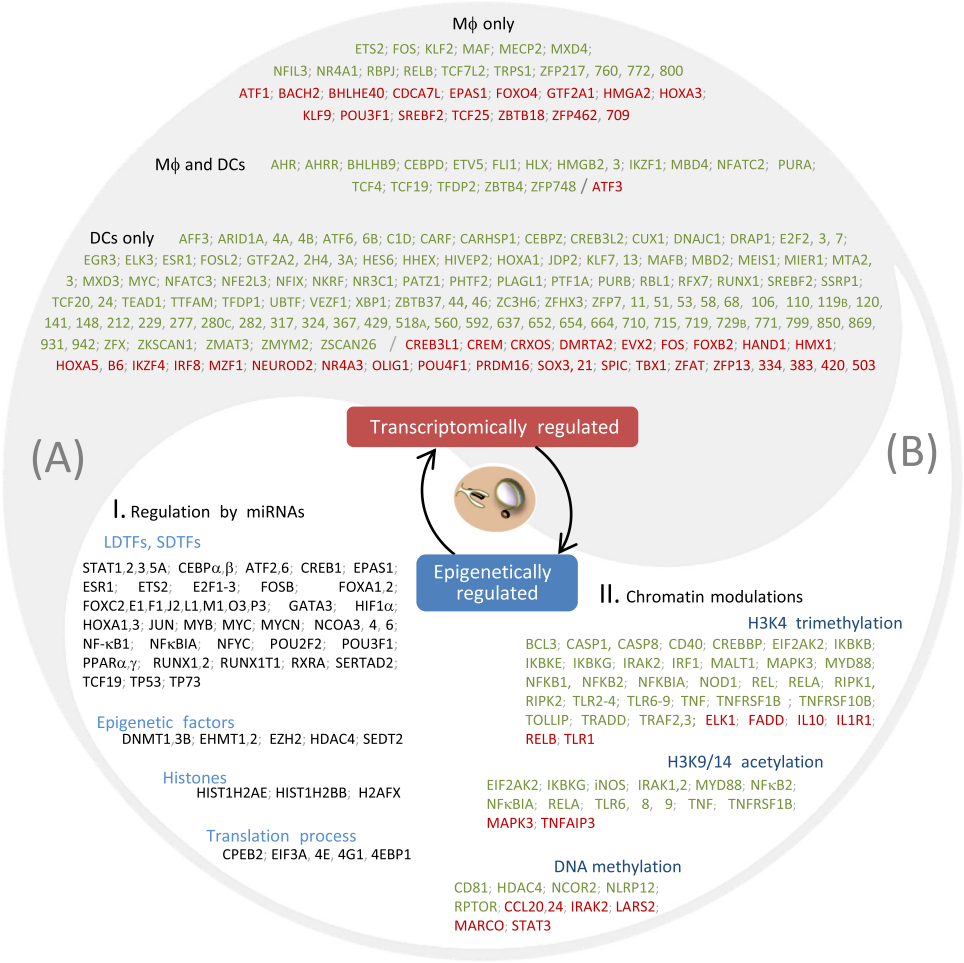


Figure 4. *Leishmania* infection changes transcription factor (TF) and epigenetic-regulatory factor (EpiRF) landscapes in macrophages (Mϕs) and dendritic cells (DCs) by modulating both transcriptional and epigenetic regulation. (A) Changes in transcriptional regulation. Data are derived from two Affymetrix analyses 24 h postinfection with *Leishmania amazonensis* amastigotes in macrophage colony-stimulating factor (M-CSF) differentiated bone-marrow-derived macrophages (BMDMs) [117] and granulocyte-macrophage colony-stimulating factor (GM-CSF)-differentiated DCs [27] (the latter condition generating a cDC2 population including also some monocyte-derived macrophages [118]). Green, reduced expression; red, increased expression. (B) Changes in epigenetic regulation. The following information is shown: (I) Validated targets of miRNAs (TFs, EpiRFs, histones, translation factors) that were modulated in primary human macrophages at 24 h after infection with *Leishmania major* [39]; (II) macrophage gene promoters that show chromatin changes following infection, that is, modification of histone H3 acetylation/methylation (*L. amazonensis*) [13,26] or DNA methylation (*Leishmania donovani*) [48,119].

L. major infection of human macrophages induced the expression of a series of miRNAs known to target TFs involved in macrophage polarization and antimicrobial activity [38], which can interfere with the expression of LDTFs, such as CEBPα and β (hsa-let-7b and hsa-miR-424) [39], and of the SDTFs STAT1, 3, 5, and 6 (hsa-miR-19b, 28-5p, 106b, 146a, 155, let-7a,), NF-κB1 (hsa-miR-22*, 26a, 28-3p, 30c, 146a, 155, 650, let-7a), or MYC (hsa-miR-19b, 22*, 24, 26, 26b, 155, 200c*, let-7b). In addition, various miRNAs affect proinflammatory signaling and thus downstream NF-κB activation by (i) reducing TLR expression (miR-let7e) [40], (ii) reducing MYD88 expression (miR-466i) especially during infection by antimony-resistant *Leishmania* parasites [15], or (iii) interfering with NF-κB1:RELA formation and translocation (miR-210) [41]

(Figure 3E). Together, these parasite-induced miRNAs can affect macrophage differentiation, polarization state, and proinflammatory potential. In contrast, no information is available yet on how *Leishmania* infection affects long noncoding RNAs, another class of regulatory RNAs encoded in intragenic or intergenic regions and exceeding 200 nucleotides in length [42], even though they have emerged as major regulators of TFs, such as C/EBPα (ecCEBPA), NF-κB (lncRNA-Cox2, lncRNA-PACER and lncRNA-Lethe) or HOXA1 (HOTAIRM1) (Table S2B).

DNA methylome

DNA methylation by DNA methyltransferases (DNMTs) generates 5-methylcytosine (5mC) in regulatory CpG (5′-cytosine-phosphate-guanine-3′-dinucleotide) islands of mammalian gene promoters. This modification has been associated with both increased and decreased expression of LDTFs and SDTFs [43]. For example, mCpG dinucleotides can be recognized by methyl-CpG domain-binding proteins that recruit histone deacetylases promoting local chromatin condensation. This change results in reduced gene expression, which has been observed for the promoters of TFs (KLF4, PU.1, PPARγ1), and miR-124, the latter resulting in increased STAT3 expression (Table S2C). Additionally, methylated DNA motifs can also induce gene expression, notably via specific recruitment of TFs such as C/EBPα and KLF4 [43,44]. In contrast, the removal of DNA methylation at *cis*-regulatory sites has been associated with DC differentiation [45] and increased expression of (i) PPARG (peroxisome proliferator-activated receptor gamma), a lipid-activated transcription factor that positively regulates myeloid DC maturation and functions [46], and (ii) AKT1 (v-akt murine thymoma viral oncogene homolog 1) that plays a critical role in proinflammatory-mediated DC survival and maturation [47].

Leishmania infection has been shown to modify the chromatin landscape by regulating the DNA methylome (Figure 4BII). After 24 h of infection with *L. donovani* promastigotes, 443 CpG sites were found to be differentially methylated in THP1 cells, including genes that code for proteins involved in various signaling pathways (JAK/STAT, calcium, MAPK, Notch and mTOR), as well as cell adhesion (integrin beta 1) and changes in host oxidative phosphorylation [48]. Indeed, increased cytosine methylation at the promoter correlated with reduced expression of the interleukin-1 receptor-associated kinase 2 (IRAK2) that is involved in NF-κB activation (Figure 3F). Conversely, CpG methylation was decreased for the nuclear receptor corepressor 2 (NCOR2) and histone deacetylase 4 (HDAC4) genes. Thus, even though no direct effect on TF expression was reported, changes in the DNA methylation pattern seem to synergize with other epigenetic mechanisms to establish permissive conditions for intracellular *Leishmania* survival.

Histone code

The chromatin architecture is regulated by reversible multiple post-translational modifications of histone proteins [49]. These modifications constitute a complex and dynamic '**histone code**' that has a central role in regulating the binding of protein cofactors, the regulation of gene expression, and its dysregulation in pathology, including infection [50]. This code is dynamically regulated by specific HMEs that either add ('writers') or remove ('erasers') a given activating or repressive mark in response to developmental or environmental cues. The expression of macrophage and DC LDTFs (CEBPs, PU.1) and SDTFs (STATs, NF-κB, PPARγ) are themselves regulated at the level of histone modification (Table S2C). Recent evidence suggests that *Leishmania* subverts the histone code to establish the **immunometabolomic** conditions for its intracellular survival. In *L. donovani*- and *L. amazonensis*-infected macrophages, reduced NOS2 expression and production of leishmanicidal NO was linked to induction of histone deacetylase 1 (HDAC1) and the removal of H3K9Ac activation marks, likely via the formation of a repressosome with NF-κB1 homodimers (Figure 3G) [13,51]. We have recently provided direct evidence that *L. amazonensis*-infected macrophages reduced the expression of various

proinflammatory genes that correlated with H3K9/14 hypoacetylation (Figure 3H), including genes of the TLR/cytokine receptor pathway, (e.g., the membrane receptors TLR6, 8, 9, and TNFRSF1b) and genes of the TLR signaling cascade (MYD88, IRAK1/2). Significantly, changes in H3K9/14 acetylation levels were also observed for promoters of the NF-κB family, with hypoacetylation and decreased expression demonstrated for IKKB, NFKBIA, NFKB2, and the TF RELA (Figure 3 and Figure 4BII), while increased H3K9/14ac levels and expression were seen for the promoter of the TNFAIP3 gene that codes for a major inhibitor of the TLR/cytokine receptor–NF-κB pathway [26]. Thus, *Leishmania* causes a dual inhibition of the proinflammatory response by inducing opposite epigenetic marks that reduce proinflammatory but increase anti-inflammatory gene expression and regulatory circuits.

Concluding remarks

The examples discussed above illustrate that *Leishmania* parasites have evolved various strategies to subvert epigenetic and transcriptomic regulation of their host cells. A key challenge for future studies lies in dissociating the precise mechanisms underlying such pathogenic parasite/host cell interactions (see Outstanding questions). Likewise, whether the suppression of the immunological functions of the host cells is a passive process resulting from the metabolic impact of feeding parasites, or an active process governed by the release of parasite proteins, remains to be established. Finally, the phenotype of *Leishmania*-infected macrophages remains only poorly characterized despite its role in promoting the immunopathologies characteristic of leishmaniases. The nuclear subversion strategies described in this review provide new opportunities for the design of novel intervention strategies that are directed towards the host and aim to restore the macrophage or DC immunological and antimicrobial potential [52]. Targeting the host for antimicrobial therapy has been recognized as a new and fertile approach to treat viral, bacterial, and fungal diseases. Targeting the host rather than the pathogen for antimicrobial therapy increases the genetic barrier for drug resistance [53–55], which is a major concern in *Leishmania* clinical infection given the parasite's remarkable genomic plasticity that constantly drives fitness gain, including in response to drug treatment [56,57]. The possibility of host-directed therapy against *Leishmania* is supported by reports on the antileishmanial effects of Imiquimod, which acts as a TLR-7/8 agonist [58], or the compound naloxonazine, which kills intracellular *Leishmania* by targeting host cell vATPases [59]. With respect to our review, pharmacological interference with miRNAs or HMEs represents another fertile, yet underexplored venue for host-directed, antileishmanial therapy. Inhibition of host cell HDAC activity in *L. donovani*-infected THP1 cells has been shown to reduce intracellular parasite burden [51,60] and the antidepressant imipramine was linked to increased HDAC 11 expression and reduced survival of antimony-resistant *L. donovani* in experimental infection [61]. Given that HMEs are key targets for anticancer drug discovery [62], it would be interesting to screen epigenetic compound libraries for antileishmanial activities. Likewise, TFs are prime targets in tumor therapy [63], and available antagonists or agonists may be repurposed to treat leishmaniasis. The remarkable redundancy with which *Leishmania* is suppressing NF-κB-mediated responses defines this SDTF as a key target for the discovery of host-directed drug candidates that can rescue this essential proinflammatory regulator, for example by applying available phenotypic screening protocols [64] on infected macrophages activated with proinflammatory stimuli.

In conclusion, there is ample evidence in the current literature that *Leishmania* instrumentalizes the dynamic interplay between transcriptional and epigenetic regulation to subvert host cell programs linked to differentiation, polarization, and maturation. The major challenge for future studies lies in (i) dissociating the hierarchical relationship between these different layers of gene expression control, (ii) identification of key regulators that are targeted by the parasite and may be exploited for host-directed drug discovery, and (iii) discovery of *Leishmania* effector molecules that are released into the host cell nucleus and directly modify the host cell chromatin landscape.

Outstanding questions

How does *Leishmania* interfere with host cell epigenetic regulation and transcriptional control?

Is *Leishmania* nuclear subversion a passive process resulting from the metabolomic impact of intracellular infection, or an active process governed by the release of parasite proteins (or both)?

Are *Leishmania* effector molecules targeted to the host cell nucleus to modify host cell nuclear regulation?

Are the nuclear subversion strategies dependent on the parasite and host species?

Do virulent/avirulent strains of *Leishmania* display similar subversion strategies?

Do antimony-resistant parasites that cause aggressive pathology rely on similar subversion strategies as susceptible strains?

Do *Leishmania*-infected macrophages express a mixed polarization phenotype and a unique immunometabolomic profile?

Are there common subversion mechanisms between *Leishmania* and other intracellular pathogens known to infect macrophages and dendritic cells?

What are the key nuclear targets in *Leishmania*-infected host cells, and can they be exploited for host-directed, antileishmanial therapy?

Can pharmacoepigenetic intervention restore an antileishmanial phenotype in infected host cells and be applied for host-directed treatment?

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Declaration of interests

No interests are declared.

Supplemental information

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