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Entamoeba histolytica adaptation to glucose starvation: a matter of life and death

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Parasites are often challenged by constant changes of the glucose concentration in their different hosts and/or within the different biotopes in the same host. During its life cycle, *Entamoeba histolytica*, the causative protozoan parasite of human amoebiasis, is exposed to both a glucose-poor environment in the colon and a glucose-rich environment in the liver. High-throughput 'omics' technologies are now widely used to characterize the cell's global response to various stresses and these technologies can survey *E. histolytica*'s global response to fluctuations in glucose concentration in its environment. In this review, we discuss the phenotypic and metabolic responses of *E. histolytica* to glucose challenges, and compare these responses to those of other protozoan parasites.

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Introduction

Amoebiasis is a parasitic infection of the human intestine caused by the single-celled protozoan parasite, *Entamoeba histolytica*. Of all the environmental stresses, metabolic stresses, such as fluctuations in glucose concentration, are very challenging for the parasite. In the colon, the parasite's environment is deprived of glucose because of the efficient absorption of dietary glucose by transporters in the membranes of epithelial cells that line the small intestine [1]. In contrast, the parasite is exposed to a rich glucose environment in the liver [2]. As an anaerobic organism, *E. histolytica* depends on glucose fermentation to obtain energy [3,4•]. Accordingly, *E. histolytica* has many glucose transporters (permeases) in its membrane in order to ensure an uninterrupted supply of glucose and survive [5•,6]. Glucose starvation is a nutrient stress in *E. histolytica* and has been studied in the parasite using high-throughput omics technologies [7••,8••,9••] which are now widely used to characterize the parasite's global response to various stresses. The purpose of this review is to inform on the global response of *E. histolytica* to changes in their environmental glucose concentration and to compare this response to those of other protozoan parasites and to other stresses.

Is the response of *E. histolytica* to glucose starvation similar to that of other protozoan parasites?

Nutritional response to glucose starvation

During glucose starvation, *E. histolytica* and *Entamoeba invadens* down-regulate the expression of glycolysis-related genes and mainly use amino acids as their energy source [7••,10•,11••]. Among the enzymes which are involved in amino acid catabolism, methionine γ-lyase (MGL1), which is involved in the catabolism of sulfur-containing amino acids, is of particular interest. Its expression is up-regulated in *E. histolytica* trophozoites which are glucose-starved [7••], exposed to oxidative and nitrosative stresses (Table 1) [12], and in trophozoites which were isolated from the colon of *E. histolytica*-infected mice [13]. Such findings suggest that MGL1 is involved in the cell's response to stress. Amino acid catabolism has also been reported in other protozoan parasites, such as *Trypanosoma brucei*, *Leishmania donovani*, *Trichomonas vaginalis*, and *Toxoplasma gondii* when they are present in glucose-poor environments in their respective hosts (Figure 1, Table 2) [14•,15•,16•,17•,18]. Another common response of many cells to nutrient starvation often includes the use of stored nutrients [19]. Glycogen granules in *E. histolytica*'s cytoplasm are a central energy reserve in the parasite [3,20,21], and α-amylase and β-amylase, which degrade glycogen, amylose, and amylopectin, are up-regulated in glucose-starved *E. histolytica* [7••,21]. For many micro-organisms, glycogen is crucial for their survival under adverse conditions, including nutrient starvation [22] and it will be interesting to determine whether the same applies to *E. histolytica*. Glycogen is not the sole nutrient reserve used by parasites. For example, β1-2 mannan oligosaccharides are stored by the infective promastigotes and amastigotes of *Leishmania mexicana* instead of glycogen. Interestingly, β1-2 mannan oligosaccharides help the parasite to survive in the poor glucose environment of macrophages [23•,24]. In contrast, *Trypanosoma cruzi* does not have any polysaccharide reserves, but stores proteins inside specific organelles, termed reservosomes [25].

Table 1

High-throughput 'omics' technologies that have been used to characterize *E. histolytica*'s global response to various stresses

Type of stress	'Omics' technology	Ref.	Key findings
Long term glucose starvation	Transcriptomics	[7••]	• Up-regulation of virulence factors (e.g. Gal/GalNAc lectins) • Up-regulation of DPD • Up-regulation of enzymes involved in amino acid catabolism (e.g. MGL1) • Up-regulation of amylases • Down-regulation of glycolysis-related genes
Short term glucose starvation	Proteomics	[8••]	• Up-regulation of virulence factors (e.g. Gal/GalNAc lectins) • Up-regulation of DPD
Heat shock	Transcriptomics	[44]	• Up-regulation of heat shock proteins • Differential expression of virulence genes (e.g. Gal/GalNAc lectins)
Oxidative stress	Transcriptomics	[12]	• Up-regulation of enzymes involved in DNA repair mechanisms • Up-regulation of heat shock proteins • Up-regulation of signaling and regulatory proteins • Up-regulation of MGL1 • Up-regulation of anti-oxidative enzymes (mainly iron-sulfur flavoproteins)
Nitrosative stress	Transcriptomics and Proteomics	[12,37,67,71]	• Decreased virulence (inhibition of Gal/GalNAc lectins) • Up-regulation of enzymes involved in DNA repair mechanisms • Up-regulation of heat shock proteins • Up-regulation of signaling and regulatory proteins • Up-regulation of MGL1 • Up-regulation of glycolysis-related genes • Modulating the expression of anti-oxidative enzymes
Cysteine deprivation	Transcriptomics and metabolomics	[72,73]	• Up-regulation of iron-sulfur flavoprotein anti-oxidative enzymes • Up-regulation of membrane transporter proteins • Down-regulation of glycolysis-related genes • Modulating amino acid and phospholipid concentrations

Because of a limited supply of fermentable carbohydrates in the colon, colonic mucin oligosaccharides are reported to be an important source of carbohydrates for many human intestinal bacteria [26,27]. As a colonic inhabitant, it has been proposed that *E. histolytica* also uses mucin oligosaccharides as a carbon source. It is also posited that during invasive disease, the parasite secretes glycosidases, which then release mucus oligosaccharides, which are then broken down by β-amylases so that they could be used as simple sugars for energy production [28,29••]. Fat often supplies cell's energy during prolonged starvation [30]. When living inside macrophages, *Leishmania* amastigotes use the beta oxidation of fatty acids as their energy source [31]. Since *E. histolytica* lacks any functional peroxisomes, lipid metabolism and in particular the beta oxidation of fatty acids cannot be used by the parasite as an energy source [3,4•]. Recently, it was discovered that dihydropyrimidine dehydrogenase (DPD), an enzyme that is involved in the degradation of pyrimidines, is essential for the adaptation of *E. histolytica* to a low glucose environment. This observation is supported by the results of previous studies which found that DPD's expression is up-regulated *in vitro* during long-term and short-term glucose starvation (Table 1) [7••,8••] and *in vivo* in trophozoites which were isolated from the colon of *E. histolytica*-infected mice [13]. Interestingly, DPD's expression was reported to be also up-regulated in other low glucose environments, such as in human colorectal cancer cells and inside macrophages [32,33]. Although the exact role of DPD in *E. histolytica* cell's survival during glucose starvation is still not understood, in mammals, the end products of pyrimidine degradation, β-alanine and β-aminoisobutyric acid, are transported into the mitochondria where they are catabolized into malonic semialdehyde and methylmalonic semialdehyde, respectively. These compounds are then further metabolized to acetyl-CoA and propionyl-CoA in order to generate energy [34–36]. Although the existence of this pathway in *E. histolytica* has not yet been established, it is possible that the induction of DPD expression during glucose starvation leads to energy production through the degradation of pyrimidines. At the moment, there is no other report about DPD induction by glucose starvation in other parasites which suggests that this response is unique to *E. histolytica*.

Phenotypic changes associated with glucose starvation

High-throughput 'omics' technologies have provided an overall view of *E. histolytica*'s response to various environmental stresses when the parasite is inside its host (Table 1). Modulation of *E. histolytica*'s virulence is detected in trophozoites that are exposed to various stresses. For some stresses, such as nitrosative stress [37], the parasite's virulence is impaired, whereas, in glucose-starved trophozoites the virulence is boosted [7••,8••]. This increase in virulence has been attributed to the

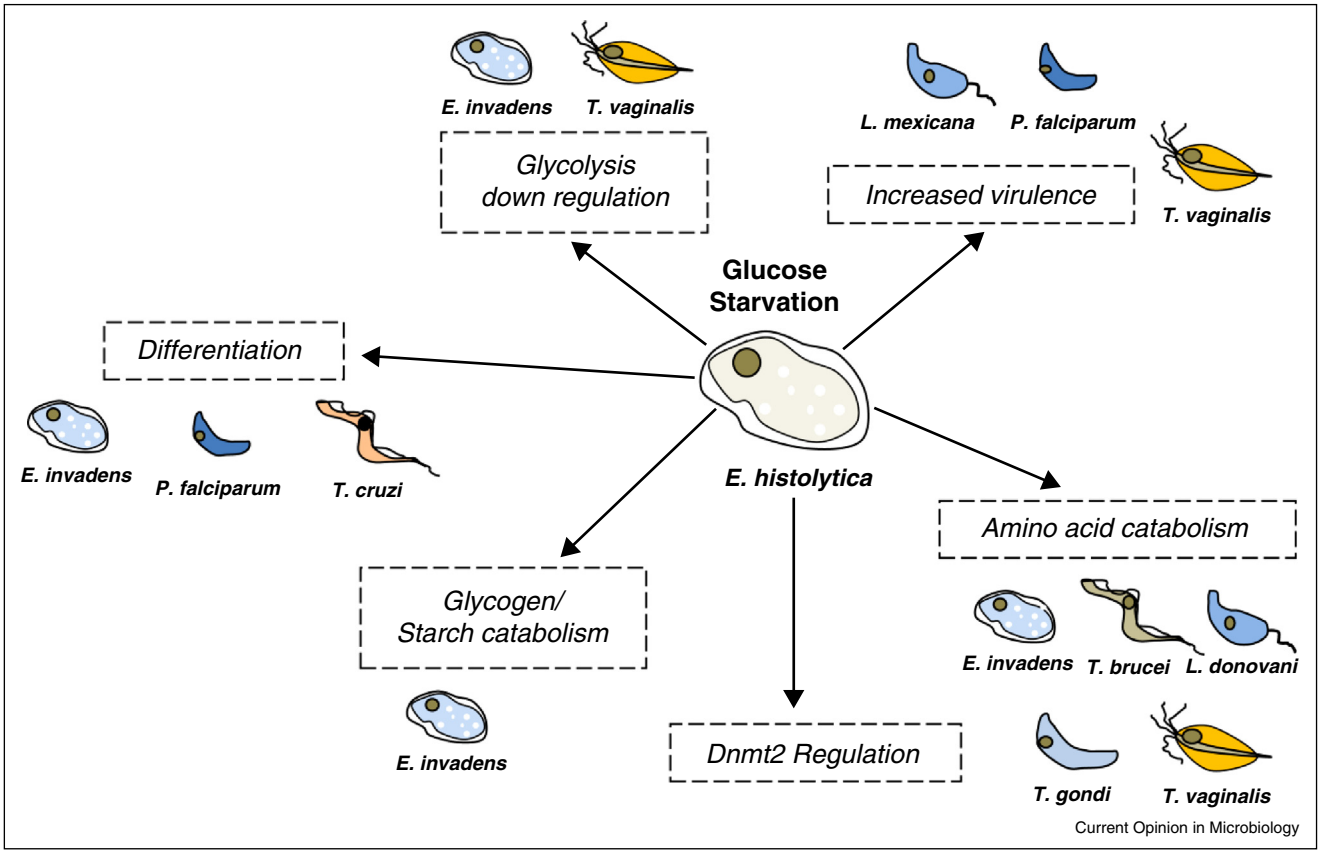
Table 2

High-throughput 'omics' technologies that have been used to characterize the global response of protozoan parasites to glucose starvation

Parasite	Low glucose environments	'Omics' technologies	Key findings	Ref.
E. histolytica	• Human colon	Proteomics and transcriptomics	• Cyst-like structure formation • Up-regulation of virulence factors • Up-regulation of DPD • Increased use of amino acids for energy production • Up-regulation of amylases • Down-regulation of glycolysis-related genes	[7••,8••,9••,11••,48]
E. invadens	• Reptile colon	Metabolomics and transcriptomics	• Encystation • Down-regulation of glycolysis-related genes • Up-regulation of phospholipase D • Induction of γ-aminobutyric acid • Increased use of amino acids for energy production • A decrease in nucleotides pool • Increased number of biogenic amines	[10•,50•,74]
P. falciparum	• Anopheles mosquito's hemolymph	Transcriptomics	• Up-regulation of virulence factors (var genes) • Up-regulation of factors associated with gametocytogenesis • Up-regulation of phospholipase C • Modulation in transcription of ribosomal RNA genes	[45•,75,76]
T. brucei	• Tsetse fly's digestive tract		• Use of proline for energy production	[15•]
T. cruzi	• Triatomine bug's digestive tract		• Flagellum extending in epimastigote as part of metacyclogenesis • Use of protein reserve in reservosomes	[25,51•]
L. mexicana/ donovani	• Sand fly's digestive tract. • Macrophages		• Use of proline for energy production • Beta oxidation of fatty acids for energy production • Use of β1-2 mannan oligosaccharides for energy production	[18,23•,24,31]
T. gondii	• Feline intestine • Macrophages		• Use of glutamine for energy production	[14•,17•]
T. vaginalis	• Human vagina	Transcriptomics	• Down-regulation of glycolysis-related genes • Up-regulation of genes that are involved in oxidative stress resistance • Up-regulation of aminotransferases and of genes involved in amino acid catabolism • Induction of autophagy	[16•]

increased expression of various virulence factors, such as (a) the cysteine protease, EhCP-A4, an enzyme which facilitates parasitic invasion in the host by disrupting the intracellular matrix [7••,38], (b) the surface antigen, ARIEL-1 [7••,39,40], (c) the pore-forming peptide, amoebapore A [7••,41], (d) lysine-rich protein 1, KRiP1 [8••,40], (e) and the Gal/GalNAc lectins, which mediate the adhesion of the parasite to colonic epithelial cells [7••,8••,42,43]. Interestingly, the modulation of Gal/GalNAc lectin expression or activity is triggered by many stresses, which include glucose starvation [7••,8••], nitrosative stress [37], and heat shock [44]. These findings suggest that the Gal/GalNAc lectins act as a stress sensor in the parasite (Table 1). When glucose is restored to glucose-starved *E. histolytica* trophozoites, their virulence is decreased significantly. This finding suggests that glucose strongly modulates *E. histolytica's* virulence [7••,8••]. At a first glance, these results seem contradictory because those trophozoites, which migrate from the colon to the liver, can cause abscesses in the liver which is a rich glucose environment. However, the results of transcriptome analysis indicate that the parasite's behavior in the liver is different from its behavior when glucose is given to glucose-starved trophozoites. For example, the expression of lysine and glutamic acid-rich protein, KERP1, is strongly up-regulated in trophozoites that were isolated from hamster liver abscesses but not during restoration of glucose. This finding suggests that glucose is not the sole factor that regulates *E. histolytica*'s virulence in the liver [7••,40]. Interestingly, glucose starvation regulates the transcription of those genes which are involved in the survival of other parasites inside their hosts (Figure 1, Table 2). For example, the expression of *var* genes that are involved in antigenic variation is up-regulated in *Plasmodium falciparum* [45•,46], and the expression of superoxide dismutase and thioredoxin peroxidase, two enzymes that are involved in oxidative stress resistance, is up-regulated in *T. vaginalis* [16•]. During their life cycle,

Figure 1



The global response to glucose starvation of protozoan parasites. Strategies shared between *E. histolytica* and other parasites are indicated by black arrows.

many protozoan parasites undergo stage conversion which is thought to improve their chances of survival both outside and inside of their hosts. Evidences are accumulating that glucose starvation triggers the stage conversion of *E. histolytica*, *E. invadens*, and *Acanthamoelba castellanii* to the environmentally *resistant* cyst stage (Figure 1, Table 2) [47•,48,49•,50•]. Glucose starvation is also a signal for *T. cruzi* epimastigotes to extend their flagellum [51•] and gametocytogenesis in *P. falciparum* [45•]. One of the cell's response to glucose starvation is a massive reduction in cellular energy levels [19]. Glycolysis-related genes are down-regulated during glucose starvation in *E. histolytica* and *E. invadens*, and such findings are consistent with the well-known decrease in cellular ATP and the substantial diminution in total cellular energy content [7••,10•]. Cellular signals, that are activated as a result of changes in AMP:ATP ratio, are a common strategy of cells to sense changes in cellular glucose concentration [52]. For example, a decrease of cellular ATP levels is a signal for the initiation of sporulation in *Bacillus subtilis* in response to nutrient deprivation [53]. Although the glucose signals that trigger stage conversion in *Entamoeba* parasites still require elaboration and elucidation, changes in the AMP:ATP ratio during glucose starvation may be one such signal.

Epigenetics

RNA methylation is an abundant and dynamically regulated epigenetic phenomenon [54,55]. Several environmental stresses have been reported to alter transfer RNAs (tRNA) modifications, such as that detected during iron limitation in *Escherichia coli* [56], during heat-shock stress in several *Archaea* spp. [57], and during the exposure of *Saccharomyces cerevisiae* cells to various chemical toxicants [58•]. In S. *cerevisiae*, stress-specific signature reprogramming of tRNA modifications appears to induce the selective translation of stress response proteins [58•]. In *E. histolytica*, very little is known about tRNA modifications except that cytosine-38 tRNAasp methylation is catalyzed by Ehmeth, a Dnmt2-type methyltransferase [9••,59,60,61••]. Members of the Dnmt2 family have weak or no DNA methyltransferase (MTase) activity [62–65] and robust methylation activity on tRNAasp [66•]. Recent findings indicate that the activity of human Dnmt2 and Ehmeth is regulated by enolase, a glycolytic enzyme that interacts with the catalytic domain of these proteins [9••].

Interestingly, it was shown that enolase accumulates in the nucleus and interacts with Ehmeth in glucose-starved *E. histolytica* trophozoites. The formation of the enolase-Ehmeth complex ultimately results in the inhibition of Ehmeth activity, a decrease in tRNAasp methylation [9••], and to an increased susceptibility of glucose-starved *E. histolytica* trophozoites to oxidative stress [8••]. Moreover, trophozoites which overexpress Ehmeth exhibit high levels of tRNAasp methylation [67] and are resistant to oxidative [68] and nitrosative [67] stresses. Although the mechanism that links tRNAasp methylation and stress resistance is still not completely understood, we have reported recently that Ehmeth-mediated tRNA methylation exerts a positive effect on protein synthesis in general and on stress response-related proteins in particular [67]. Moreover, it has been also reported that *Drosophila* Dnmt2 is associated with resistance to oxidative stress and heat shock [69,70•] and protects *Drosophila* tRNAasp from being cleaved by the ribonuclease, angiogenin [70•]. Presently, we do not know if Ehmeth mediated tRNAasp methylation prevents *E. histolytica* tRNAasp from being cleaved by stress-induced ribonucleases. In the absence of an obvious angiogenin homolog in *E. histolytica*, we are currently trying to establish whether other ribonucleases may have the same action as angiogenin.

Conclusions

During the last few years, the use of 'omics' technologies have contributed greatly to our present understanding of *E. histolytica*'s response to various stresses that the parasite encounters in its host. Notable advances include the unexpected role of DPD and β-amylase in the survival of the parasite to low glucose environments and the role of tRNA modifications in the stress response. However, many challenges still lie ahead, like the development of better tools for the integration of data coming from various *Entamoeba* omics analysis studies. These tools will be useful for identifying new factors that are essential for the parasite's adaptation to the host environment and for developing new anti-parasitic drugs.

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