Published in final edited form as:

Int J Parasitol. 2008 January ; 38(1): 57–64. doi:10.1016/j.ijpara.2007.06.007.

Acetylation of the *Entamoeba* histone H4 N-terminal domain is influenced by short chain fatty acids that enter trophozoites in a pH-dependent manner

Jennifer Byers^{a,1} and Daniel Eichinger^{a,*}

^aDepartment of Medical Parasitology, New York University School of Medicine, New York, NY USA

Abstract

Treatment of higher eukaryotic cells with short chain fatty acids (SCFA) such as butyrate causes decreased levels of histone deacetylase (HDAC) activity and hyperacetylation of histones, and thereby affects gene expression, cell growth and differentiation. *Entamoeba* parasites encounter high levels of SCFA in the host colon, and in vitro these compounds allow trophozoite stage parasites to multiply but prevent their differentiation into infectious cysts. The *Entamoeba invadens* IP-1 histone H4 protein has an unusual number of lysines in its N-terminus, and these become hyperacetylated in trophozoites exposed to the HDAC inhibitors trichostatin A (TSA) or HC-toxin, but not in trophozoites exposed to butyrate. We have now found that several other commonly studied isolates of *Entamoeba* parasites also have an extended set of histone H4 acetylation sites that become hyperacetylated in response to TSA, but hypoacetylated in response to butyrate, suggesting an unusual sensitivity of this parasite's histone modifying enzymes to SCFA. Butyrate was found to enter trophozoites in a pH-dependent manner consistent with diffusive entry of the un-ionized form of the fatty acid into the amoebae. Transit of the *Entamoeba* organism through areas of the host intestine with distinct pH and SCFA concentrations would therefore result in very different levels of SCFA within the parasite. *Entamoeba* appears to have acquired unique alterations of its histone acetylation mechanism that may allow for its growth in the presence of varying amounts of the bacterial fermentation products.

Keywords

Entamoeba histolytica; *invadens*; *dispar*; Histone; Acetylation; Short-chain fatty acids; Colon

1. Introduction

After their ingestion as encysted forms, newly excysted *Entamoeba* trophozoites must establish themselves amongst the large number of resident colonic bacteria. Distinct subpopulations of these bacteria metabolize nutrients from host dietary and tissue sources, and release varying concentrations of fermentation products into the intestinal lumen (Cummings and MacFarlane, 1997; Pryde et al., 2002). Members of a major class of such products, the short chain fatty acids

*Corresponding author. Department of Medical Parasitology, New York University School of Medicine, 341 East, 25th Street, New York, NY 10010 USA, Tel.: +1-212-263-8171; fax: +1-212-263-8116. E-mail address: eichid01@med.nyu.edu.

¹Current address: National Academy of Sciences, Washington, D.C. USA

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

(SCFA) acetate, propionate and butyrate, serve directly as an energy source for the host and other bacteria, (Cummings et al., 1987; Topping and Clifton, 2001), and indirectly define microbial habitation boundaries along the length of the intestine. For example, the relatively small amounts of SCFA present in the distal small intestine can up-regulate virulence genes in pathogenic species of *Salmonella* and *Pseudomonas*, allowing them to colonize that region of the intestine (Levinson, 1973; Durant et al., 2000). The greater amounts of SCFA found in the large intestine, however, do not up-regulate the virulence genes and instead inhibit the growth of these bacteria, thereby preventing their establishment in the colon. *Entamoeba* parasites reside primarily in the host's colon and we recently determined that the amounts of SCFA typically found in the colon do not inhibit growth of the trophozoite stage of *Entamoeba* parasites but do inhibit the process by which the trophozoite stage differentiates into the infectious cyst stage (Byers et al., 2005).

In a variety of eukaryotic cell types butyrate induces changes in cell cycle progression, differentiation and gene expression (Kruh, 1982; Katula et al., 2002). In these butyrate-treated cells the gene expression changes correlate with increased levels of histone acetylation that result from the inhibition of Class I/II histone deacetylase (HDAC) enzymes (Davie, 2003). The core histone H4 contains four highly conserved lysine residues in its N-terminal tail that are substrates for acetyl group attachment and removal reactions carried out by the counteracting histone acetyltransferase (HAT) and HDAC enzymes (Galasinski et al., 2002). Several adaptations of *Entamoeba* parasites to colonic anaerobic growth conditions have been described, including a physically and functionally reduced mitochondrion (Clark and Roger, 1995). It might also therefore be expected that the parasite would display adaptations that allow for growth in the presence of SCFA that normally inhibit the HDAC activity in other types of eukaryotic cells. Suggestively, in addition to the four highly conserved lysines, the N-terminal domain of *Entamoeba* histone H4 contains three additional lysines that are not found in the H4 histones of higher eukaryotes or in other protistan parasites that occupy non-colonic sites in their hosts (Ramakrishnan et al., 2004; Sullivan et al., 2006) (Fig. 1). All seven of the histone H4 lysines appear to be sites of acetylation in axenically grown trophozoites of *Entamoeba invadens*, a pathogen of reptiles (Byers et al., 2005). Exposure of *E. invadens* trophozoites to the HDAC inhibitors trichostatin A (TSA) or HC-toxin causes an increased level of acetylation of the bulk histone H4 population (Byers et al., 2005), whereas exposure to SCFA does not result in an increase in the extent of histone H4 acetylation as is seen in other eukaryotes (Thiagalingam et al., 2003). Both TSA and SCFA, however, inhibit *E. invadens* IP-1 encystation in concentration-dependent manners (Byers et al., 2005). Whether the human parasite *Entamoeba histolytica* is also responsive to these compounds had not previously been determined since axenically grown cultures of the one strain tested, *E. histolytica* strain HM-1, did not contain detectable levels of histone H4 acetylation (J. Byers, unpublished data).

We report here that a number of commonly studied isolates of *E. histolytica*, *Entamoeba dispar* and *E. invadens* also displayed the extended range of histone H4 acetylation density that was previously described for *E. invadens* strain IP-1. Trophozoites of *E. histolytica* isolate HM-1, however, did not contain histone H4 proteins with detectable levels of acetylation unless the cells were treated with high amounts of TSA. Growth in the presence of SCFA induced a hypoacetylated histone H4 pattern in most trophozoite strains. Trophozoites of *E. invadens* simultaneously treated with SCFA and TSA displayed discrete hypo- and hyperacetylated subpopulations of H4 histones. SCFA uptake by trophozoites was pH-dependent, suggesting that SCFA enter trophozoites by diffusion through the membrane as un-ionized molecules.

2. Materials and methods

2.1. Parasites

Trophozoites of *E. histolytica* strains HM-1:IMSS and 200:NIH, and *E. invadens* strains IP-1 and VK-1:NS were obtained from the American Type Culture Collection. Trophozoites of *E. histolytica* strain Rahman and *E. dispar* clone SAW760 were obtained from Dr. Upinder Singh, Stanford University School of Medicine. All cultures were maintained as trophozoites by growth at the appropriate temperatures in low glucose (LG) medium (Sanchez et al., 1994), which is TYI-S-33 medium (Diamond et al., 1978) made without glucose. Per liter, LG medium contains 1.0 g K₂HPO₄, 0.6 g K H₂PO₄, 2.0 g NaCl, 13 g Bacto tryptone, 17 g Bacto yeast extract, 1.0 g cysteine, 0.2 g ascorbic acid, 22.8 mg ferric ammonium citrate. Unless noted, all compounds were obtained from Sigma. Following autoclaving the pH of the solution was between 6.3 and 6.5, and this was not adjusted except as described below. Adult bovine serum (ABS) was purchased from Gemini BioProducts, West Sacramento, CA., and added to LG at 10% v/v. The bacteria accompanying the *E. dispar* culture were determined by the Tisch Hospital Clinical Laboratory, New York University School of Medicine, to be a monoculture of *Pseudomonas aeruginosa*.

2.2. Media

When testing for an effect of sodium concentration on growth and encystation, LG medium was made without NaCl, and the final sodium concentrations in control media that did not contain SCFA were adjusted with NaCl to match the amount of sodium added with the SCFAs. Media with defined pHs of 5.0, 6.0 and 7.0 were made by changing the ratio of the mono- and di-potassium phosphate salts of LG, maintaining a final 10 mM K concentration, and when necessary adjusting the pH of the final medium with NaOH or HCl.

2.3. Isolation and analysis of amoeba histones

Trophozoites grown in the various media with or without SCFA or TSA were harvested in mid to late log phase and processed for nuclear proteins as previously described (Byers et al., 2005). Acid-soluble nuclear proteins were fractionated on acid-urea-triton (AUT) polyacrylamide gels (Kijima et al., 1993), which were either stained with Coomassie blue or blotted to nitrocellulose for antibody probing. To reveal proteins containing acetylated lysines, Western blots were incubated with anti-pan-acetyllysine antibody (Santa Cruz) as previously described (Byers et al., 2005). Core histones from chicken erythrocytes (Upstate) were used as markers of protein migration.

2.4. Uptake of ¹⁴C-butyrate

Log-phase *E. invadens* IP-1 trophozoites grown in complete LG were pelleted, washed once in cold LG without ABS, and suspended in LG of defined pH without ABS at a density of 10⁵ cells/ml. N-butyric acid-carboxy-[C¹⁴] (16 mCi/mmol, Amersham) was added and cells were incubated at room temp for 5 min. Following incubation cells were washed three times in PBS, the final pellet was suspended in a scintillation cocktail and radioactivity was measured in a Beckman Coulter LS6500 scintillation counter. All experimental variables were tested in triplicate and each experiment carried out at least twice.

2.5. Encystation

Encystation of *E. invadens* trophozoites was carried out using 47% LG as previously described (Sanchez et al., 1994). The effect of pH on encystation was examined using LG with the pH adjusted as described above. The effect of pH on the inhibition of encystation by SCFA used the same pH-adjusted media plus a pool of SCFA, yielding final concentrations of 70 mM sodium acetate, 20 mM sodium propionate and 10 mM sodium butyrate. This concentration

was considered 1 X with respect to average in vivo caecal concentrations, which was previously found to completely inhibit encystment. For intermediate levels of inhibition, amounts of the pool were added to the 47% LG encystment medium that yielded 0.2 X and 0.56 X concentrations. Trophozoites were collected from LG medium, resuspended in 47% LG without or with the SCFA pool, incubated for 24 h at 25°C and assessed for percent encystation (Sanchez et al., 1994).

3. Results

3.1. Effect of sodium chloride on cell growth

All of the SCFAs were purchased as sodium salts. To determine if the additional Na added with the SCFAs to various media had any effect on the rates of trophozoite growth, LG-based media made with and without the 34.2 mM amounts of NaCl specified for TYI-S-33 medium (Diamond et al., 1978) were supplemented with either 70 mM sodium acetate, 20 mM sodium propionate, 10 mM sodium butyrate or a pool of all three SCFAs at the same concentrations. Cell growth rates were slightly reduced, only in the cultures that contained all three SCFAs in addition to the 34.2 mM amount of NaCl in LG, (total [Na] greater than 130 mM) (data not shown). All subsequent experiments used final NaCl concentrations that did not inhibit the trophozoite growth rate.

3.2. Acetylation of histone H4

The unique presence of seven lysines in the N-terminal tails of *Entamoeba* H4 histones (Fig. 1) suggests that these parasites have a greater potential range of sensitivity to varying ratios of HAT and HDAC activities than other eukaryotes. The response of the amoebae to SCFAs appeared to differ from those of other eukaryotes, however, since butyrate did not cause an obvious increase in hyperacetylated forms of *E. invadens* IP-1 histone H4 as it does to other eukaryotic cell types. Instead, visual analysis of multiple independent AUT gels used to analyze H4 histones from SCFA-treated *E. invadens* IP-1 trophozoites suggested that SCFAs, or butyrate alone, induced decreased levels of acetylation of at least part of the histone H4 population. In the simplest case, this could result from SCFAs acting to either increase HDAC activity or to decrease HAT activity. *Entamoeba invadens* trophozoites treated with TSA, (to inhibit HDAC activity), were therefore simultaneously treated with SCFAs, to see if the latter compounds had an effect on HAT activity. The histone H4 pattern from these cells now showed two distinct populations, one hyperacetylated and one hypoacetylated (Fig. 2A), suggesting that a discrete subpopulation of the *E. invadens* H4 histones is either normally acetylated by a HAT which is inhibited by SCFA, or is deacetylated by a HDAC which is still active in the presence of TSA and is activated by SCFA.

The histone H4 sequences of *E. histolytica*, *E. dispar* and *E. invadens* are each predicted to contain seven lysines in the N-terminal tail domain, and previous results showed the presence of what appeared to be the full range of multiply-acetylated forms of histone H4 in *E. invadens* IP-1 (Byers et al., 2005). To explore the generality of this feature of *Entamoeba* we examined H4 histones from other frequently studied *Entamoeba* strains. Another isolate of *E. invadens*, VK-1:NZ, which efficiently encysts in vitro, had a histone H4 acetylation profile identical to that of LG-grown IP-1 (data not shown). A monoxenic culture of the human parasite *E. dispar* SAW760 grown in LG also displayed a ladder of acetylated bands in the histone H4 region of AUT gels (Fig. 2B, lane 3). This entire ladder pattern migrated more slowly relative to that of *E. invadens* for undetermined reasons. Trophozoites of *E. dispar* SAW760 displayed a hyperacetylated histone H4 pattern when exposed to TSA (Fig. 3A, lane 5), and a near complete reduction in the intensity of all acetylated histone H4 bands when grown in the presence of SCFA (lane 4). No acid-soluble proteins from the *P. aeruginosa* cells that

accompanied the *E. dispar* trophozoites were recognized by the anti-acetyllysine antibody (data not shown).

An axenic *E. histolytica* Rahman isolate grown in LG also displayed a hyperacetylated ladder pattern of acetylated histone H4 when grown in the presence of TSA (Fig. 3B, lane 5). Again, this pattern essentially disappeared when the cells were grown in the presence of SCFA (lane 4). *Entamoeba histolytica* strain HM-1:IMSS grown in LG did not express any detectable acetylated histone H4 (Fig. 3C, lane 3). This result did not change when HM-1 trophozoites were cultured either with SCFA or with the *P. aeruginosa* cells from the *E. dispar* culture (data not shown). Only when HM-1 trophozoites were treated with 300 nM TSA was any form of acetylated histone H4 detected (Fig. 3D, lane 5). The TSA-induced acetylation of *E. histolytica* HM-1 histone H4 did generate a ladder pattern, but one that did not show the same extreme shift to a hyperacetylated population as was typical for TSA-treated *E. dispar* or *E. invadens*. Also, the 300 nM amounts of TSA needed to induce detectable histone H4 acetylation appeared to be toxic to the *E. histolytica* HM-1 trophozoites, as 24 h exposure to the compound resulted in swollen, non-adherent cells (not shown).

In contrast to strain HM-1, another axenically maintained *E. histolytica* isolate with pathogenic potential, strain 200:NIH, did display a readily detectable acetylated histone H4 population when it was grown in LG (Fig. 3D, lane 3). This histone H4 population was hyperacetylated in cells treated with TSA (lane 5). The 300 nM amounts of TSA did not appear to be toxic to the 200:NIH trophozoites at 24 h (data not shown). Similar to the response of *E. invadens*, when the 200:NIH trophozoites were grown in the presence of SCFA the histone H4 ladder displayed a hypoacetylated pattern (lane 4).

3.3. Effect of pH on SCFA uptake and encystation

How SCFAs might get into *Entamoeba* trophozoites is suggested by the physical properties of the fatty acids. Un-ionized SCFA molecules are assumed to enter cells by diffusion through the plasma membrane (Sellin, 1999), whereas ionized forms require active transport (Garcia et al., 1994; Hadjiogiannou et al., 2000). The pK_a's of acetate, propionate and butyrate are approximately 4.6 at 0.1 mol/l ionic strength. As the contents of the intestine are normally above pH 4.6, less than 50% of these three SCFAs will be in un-ionized forms in the lumen of the distal small intestine (pH 6.8) or in the colon (pH 5.2-6.8). Approximately 30% will be in an un-ionized form at pH 5.2 (caecum) and this amount decreases to less than 2% at pH 6.8 (distal colon). Up to this point all of our studies of *E. invadens* IP-1 encystation had been carried out using versions of LG media with a pH of approximately 6.3. The effect of pH on SCFA entry into live trophozoites was therefore tested by adding [¹⁴C]-butyrate to *E. invadens* trophozoites suspended in LG that was adjusted to pH 5.0, 6.0 or 7.0. Following incubation and washing, total cell-associated radioactivity was measured. Greater amounts of [¹⁴C]-butyrate were associated with cells in pH 5.0 medium, and incrementally lesser amounts in cells in the pH 6.0 or pH 7.0 media (Fig. 4A). The rate of uptake of [¹⁴C]-butyrate was linear with respect to input concentration and non-saturable over a 2 log concentration range, (Fig. 4B), suggestive of diffusive rather than transporter-mediated entry.

To determine if the ability of SCFAs to inhibit encystation was influenced by their increased entry into cells at low pH, encystation of *E. invadens* IP-1 was induced in 47% LG media made with the pH adjusted to 5.0, 6.0 or 7.0. In the absence of SCFAs there was a slight effect of pH itself on encystation, with lower pH supporting greater yields of cysts (Fig. 4C). When SCFAs were added to each type of medium, encystation was inhibited to the greatest extent at the lower pH (Fig. 4D), and to the least extent at the high pH. For example, medium at pH 7.0 containing a 1:5 dilution of the 70:20:10 mM acetate:propionate:butyrate pool did not inhibit encystation, whereas at pH 5.0 the same dilution of SCFAs reduced encystation by 75%.

4. Discussion

Based on the small number of amoeba isolates examined here, the presence of histone H4 molecules with more than four acetyllysine groups appears to be typical of axenic and monoxenic cultures of *Entamoeba*. Our cultured strain of HM-1 was the one exception, as it showed no acetylation of histone H4 unless it was treated with high amounts of TSA. This isolate also appeared to be uniquely sensitive to TSA exposure, suggesting that it expresses forms or levels of histone-directed deacetylase activity that are somehow different from all the other isolates. Why the HM-1 isolate displayed different levels of baseline histone acetylation is not clear. Historically, each *Entamoeba* parasite isolate analyzed here was obtained from an infected individual and adapted to culture in the absence of the SCFAs the parasites would normally be exposed to in vivo. It is possible, then, that the culture-adaptation process selected for slightly different perturbations of the histone modification mechanism in each isolate. Whether the lack of acetylation of histones in cultured HM-1 cells has an effect on their methylation has not been directly examined by us. The addition of those methyl groups that might first require the removal of acetyl groups would seemingly be allowed to occur without the need for acetyl group removal. How this may affect methylation-dependent control of other cellular functions, including gene expression, is not clear, but it seems likely that the HM-1 cells would display features in this regard that are distinct from all the other amoeba isolates.

In all the *Entamoeba* isolates other than HM-1 examined here, trophozoites grown without SCFAs contained histone H4 populations displaying what appeared to be a range of one to seven acetylated lysines. The amounts of the hyperacetylated forms of histone H4, (those with greater than four acetyllysines), were reduced when SCFAs were present. This hypoacetylation effect of SCFAs on amoebas is opposite to the effect butyrate has on the acetylation of histones in other eukaryotes, including those of *Dictyostelium discoideum* (Boto et al., 1986; D.Eichinger, unpublished data). Butyrate treatment of most eukaryotic cell types causes decreased HDAC activity levels, allowing for HAT-mediated hyperacetylation of histones and the resulting changes in expression of those genes that are sensitive to high levels of histone acetylation. *Entamoeba invadens* trophozoites treated with TSA contained only hyperacetylated H4, showing that the amoeba HDAC activity that acts on H4 is TSA-sensitive, and that this HDAC activity normally acts in opposition to an H4-directed HAT activity. SCFAs appear to selectively affect an amoeba HAT activity, since cells treated with both TSA and SCFAs contained a hypoacetylated subpopulation in addition to the hyperacetylated forms. Trophozoites of 200:NIH exposed to TSA also displayed a hyperacetylated bulk H4 population, whereas those grown with only SCFAs had a hypoacetylated bulk H4 pattern, showing that the H4-directed HDAC activity of the human parasite is also TSA sensitive, and that either its HAT activity is inhibited by SCFA or its HDAC activity is stimulated by SCFAs. Again, either possibility would be different from the responses of higher eukaryotic cells when they are treated with butyrate. Due to technical difficulties that make resolution of the other amoeba core histones difficult, we have not yet been able to examine SCFA-induced changes in the acetylation status of histones other than H4.

The pH of the luminal contents varies along the length of the gastrointestinal (GI) tract (Genz et al., 1999). In the transitional region encompassing the distal small intestine, caecum and proximal large intestine, the pH abruptly changes from near neutral to acidic, reflecting the increase in the density of the resident caecal bacteria and the amounts of SCFAs they produce. When newly excysted *Entamoeba* trophozoites pass from the small intestine into the caecum, they will experience an acute drop in pH from approximately 7.0 to 5.5, and a change in luminal SCFA (acetate, propionate and butyrate) concentration from approximately 10 mM to > 100 mM. Diffusive entry of the un-ionised fraction of the SCFAs in each of these settings would allow for a potential 150-fold range of concentrations of SCFAs inside the trophozoites, from 0.1 mM in trophozoites located in the small intestine, to 15 mM in trophozoites in the caecum.

The 15 mM potential values would gradually decrease as the amoebae are carried from the caecum toward the more distal areas of the colon where the pH rises and the luminal SCFA concentration is diminished due to absorption by the colonic epithelium. Whether the trophozoites contribute to production of the un-ionised SCFA fraction by local release of H⁺ via Na⁺-H⁺ exchange, as colonic epithelial cells do (Genz et al., 1999; Schroder et al., 2000), has not yet been determined.

The *Entamoeba* encystation process is accompanied by changes in gene expression (Sanchez et al., 1994; Coppi and Eichinger, 1999; Ehrenkaufer et al., 2007a). However, the unusual hypoacetylation response of the amoebas to butyrate leaves unanswered the question of whether SCFAs inhibit encystation via a mechanism that directly alters levels of histone and/or non-histone protein acetylation with resulting changes in gene expression. In a separate, collaborative study, we found very little difference in gene expression in 200:NIH trophozoites grown with versus without SCFAs (Ehrenkaufer et al., 2007b). In contrast, TSA induced hundreds of gene expression changes, many of which overlapped with the gene expression pattern previously seen in cultures containing encysting cells (Ehrenkaufer et al., 2007a). This shows that hyperacetylation of histones can affect gene expression (and possibly differentiation) in *Entamoeba*. It also suggests that the state of relative histone hyperacetylation found in *Entamoeba* isolates selected to grow (as trophozoites) in the absence of SCFAs falls below the threshold required to trigger these gene expression changes. The natural sensitivity of *Entamoeba* to SCFAs will therefore likely be best examined using recent clinical isolates that are maintained in culture in the presence of SCFAs.

Acknowledgments

Support for J.B. was provided in part by training grant AI 07180, and for D.E. by grant AI 44893, both from the NIH.

References

- Boto L, Cano A, Pestana A. Biochemical and morphological effects of sodium butyrate on *Dictyostelium discoideum* development. *Molecular and Cellular Biochemistry* 1986;74:137–147. [PubMed: 3037305]
- Byers J, Faigle W, Eichinger D. Colonic short-chain fatty acids inhibit encystation of *Entamoeba invadens*. *Cellular Microbiology* 2005;7:269–279. [PubMed: 15659070]
- Clark CG, Roger AJ. Direct evidence for secondary loss of mitochondria in *Entamoeba histolytica*. *Proceedings of the National Academy of Science USA* 1995;92:6518–6521.
- Coppi A, Eichinger D. Regulation of *Entamoeba invadens* encystation and gene expression with galactose and N-acetylglucosamine. *Molecular and Biochemical Parasitology* 1999;102:67–77. [PubMed: 10477177]
- Cummings JH, Pomare EW, Branch WJ, Naylor CPE, MacFarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 1987;28:1221–1227. [PubMed: 3678950]
- Cummings JH, MacFarlane GT. Role of intestinal bacteria in nutrient metabolism. *Clinical Nutrition* 1997;16:3–11. [PubMed: 16844615]
- Davie JR. Inhibition of histone deacetylase activity by butyrate. *Journal of Nutrition* 2003;133:2485S–2493S. [PubMed: 12840228]
- Diamond LS, Harlow DR, Cunnick CC. A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1978;72:431–432. [PubMed: 212851]
- Durant JA, Corrier DE, Ricke SC. Short-chain volatile fatty acids modulate the expression of the hila and invF genes of *Salmonella typhimurium*. *Journal of Food Protection* 2000;63:573–578. [PubMed: 10826713]
- Ehrenkaufer GM, Haque R, Hackney JA, Eichinger DJ, Singh U. Identification of developmentally regulated genes in *Entamoeba histolytica*: insights into mechanisms of stage conversion in a

- protozoan parasite. *Cellular Microbiology*. 2007a(Online Articles) doi.10.1111/j.1462-5822.2006.00882.x
- Ehrenkaufer GM, Eichinger DJ, Singh U. Transcriptional activation of the encystation pathway by Trichostatin A in the protozoan parasite *Entamoeba histolytica*. *BMC Genomics*. 2007bin press
- Galasinski SC, Louie DF, Gloor KK, Resing KA, Ahn NG. Global regulation of post-translational modifications on core histones. *Journal Of Biological Chemistry* 2002;277:2579–2588. [PubMed: 11709551]
- Garcia CK, Goldstein JL, Pathak RK, Anderson RGW, Brown MS. Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle. *Cell* 1994;76:865–873. [PubMed: 8124722]
- Genz AK, von Engelhardt W, Bushe R. Maintenance and regulation of the pH microclimate at the luminal surface of the distal colon of guinea-pig. *Journal of Physiology* 1999;517:507–519. [PubMed: 10332098]
- Hadjioagiu C, Schmidt L, Dudeja PK, Layden TJ, Ramaswamy K. Mechanism(s) of butyrate transport in Caco-2 cells: role of monocarboxylate transporter 1. *American Journal of Physiology (Gastrointestinal and Liver Physiology)* 2000;279:G775–G780. [PubMed: 11005765]
- Katula KS, Fields A, Apple P, Rotruck T. Cell cycle specific changes in the human cyclin B1 gene regulatory region as revealed by response to trichostatin A. *Archives of Biochemistry and Biophysics* 2002;401:271–276. [PubMed: 12054478]
- Kijima M, Yoshida M, Sugita K, Horinouchi S, Beppu T. Trapoxin, an antitumor cyclic tetrapeptide, is an irreversible inhibitor of mammalian histone deacetylase. *Journal of Biological Chemistry* 1993;268:22429–22435. [PubMed: 8226751]
- Kruh J. Effects of sodium butyrate, a new pharmacological agent, on cells in culture. *Molecular and Cellular Biochemistry* 1982;42:65–82. [PubMed: 6174854]
- Levinson ME. Effect of colon flora and short-chain fatty acids on growth in vitro of *Pseudomonas aeruginosa* and *Enterobacteriaceae*. *Infection and Immunity* 1973;8:30–35. [PubMed: 4198102]
- Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. The microbiology of butyrate formation in the human colon. *FEMS Microbiology Letters* 2002;217:133–139. [PubMed: 12480096]
- Ramakrishnan G, Gilchrist CA, Musa H, Torok MS, Grant PA, Mann BJ, Petri WAPJ. Histone acetyltransferases and deacetylases in *Entamoeba histolytica*. *Molecular and Biochemical Parasitology* 2004;138:205–216. [PubMed: 15555732]
- Sanchez LB, Enea V, Eichinger D. Identification of a developmentally regulated transcript expressed during encystation of *Entamoeba invadens*. *Molecular and Biochemical Parasitology* 1994;67:125–135. [PubMed: 7838173]
- Schroder O, Opritz J, Stein J. Substrate and inhibitor specificity of butyrate uptake in apical membrane vesicles of the rat distal colon. *Digestion* 2000;62:152–158. [PubMed: 11025362]
- Sellin JH. SCFAs: the enigma of weak electrolyte transport in the colon. *News in Physiological Science* 1999;14:58–64.
- Sullivan WJ, Naguleswaran A, Angel SO. Histones and histone modifications in protozoan parasites. *Cellular Microbiology* 2006;8:1850–1861. [PubMed: 17026479]
- Thiagalingam S, Cheng KH, Lee HJ, Mineva N, Thiagalingam A, Ponte JF. Histone deacetylases: unique players in shaping the epigenetic histone code. *Annals of the New York Academy of Science* 2003;983:84–100.
- Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews* 2001;81:1031–1064. [PubMed: 11427691]

		5	8	12	16	20	
		↓	↓	↓	↓	↓	
Gl_H4	----	MSG K GKG-KG----	YGKS-----	-----	KRHS-K-EKDTLLLITKPAIRR..		
Eh_H4	MATDTGSGRGKGGKGV	T-LGKGS K GA K AS K GGKRI	RTKTQQD	ALKGITKPAIRR..			
Mm_H4	----	MSGRGKGGKG----	LGKGG-----	-----	AKRHR-KVLRDNIQGITKPAIRR..		
Pp_H4	----	MSGRGKGGKG----	LGKGG-----	-----	AKRHR-KVLRDNIQGITKPAIRR..		
Pf_H4	----	MSGRGKGGKG----	LGKGG-----	-----	AKRHR-KILRDNIQGITKPAIRR..		
Mb_H4	----	MTGRGKGGKVL	SVGGKGG K GA-----	-----	KRHR-KVLRDNIQGITKPAIRR..		
Ac_H4	---	MSHVGRGKGGK---	GLGKGG K GL---	GGAKRHR-KLLRDNIQGITKPAIRR..			
Tb_H4	-----	MAKG K KSGE--	AKG-----	SQKRQ K -KVLRENVRGITRGSIRR..			
Dd_H4	MSSAQSRGGKTGGK---	VGGKVG-----	-----	AKRHR K KTQKEHINGITKPAIRR..			
Tv_H4	MSGRGKGGKG----	GKGG-----	-----	AKRHR-KVMRENIQGITKPAIRR..			

Gl, *Giardia lamblia*Mm, *Mus musculus*Pf, *Plasmodium falciparum*Ac, *Acanthamoeba castellanii*Dd, *Dictyostelium discoideum*Eh, *Entamoeba histolytica*Pp, *Physarum polycephalum*Mb, *Mastigamoeba balamuthi*Tb, *Trypanosoma brucei*Tv, *Trichomonas vaginalis***Fig. 1.**

Alignment of the N-terminal domain of H4 histones from the indicated organisms. Positions of the conserved lysines (K 5, 8, 12, 16, 20) are indicated with arrows. Additional lysines are shown in bold. Sequences were aligned with ClustalW.

Fig. 2. Western blot analysis of histone acetylation. Histones were separated in acid-urea-triton gels, transferred to nitrocellulose, and probed with anti-acetyllysine antibody. A) Marker core histones (chicken), lane 1; histones from *Entamoeba invadens* grown in low glucose medium (LG)/short chain fatty acids (SCFA), lane 2; grown in LG/trichostatin A (TSA), lane 3; or grown in LG/TSA/SCFA, lane 4. B) Marker core histones, lane 1; histones from *E. invadens* grown in LG, lane 2; histones from *Entamoeba dispar* SAW760 grown in LG, lane 3.

Fig. 3.

Western blot analysis of H4 histone acetylation. Each panel contains core histone markers in lane 1 and histones from *Entamoeba invadens* trophozoites grown in low glucose medium (LG) in lane 2. Lanes 3-5 contain histones from the experimental *Entamoeba* species (indicated below each panel) that were grown in LG, lane 3; grown in LG/short chain fatty acids, lane 4; or grown in LG/trichostatin A, lane 5. A, B, D and E) Western blots probed with anti-acetyllysine antibody. C) Coomassie-stained version of the samples contained in the Western blot in D, to demonstrate the amounts of protein loaded in each lane.

Fig. 4.

Uptake and inhibitory effects of butyrate. A) Uptake of [^{14}C]-butyrate by *Entamoeba invadens* trophozoites in medium made with the indicated pH. B) Concentration-dependent uptake of [^{14}C]-butyrate by *E. invadens* trophozoites. C) Encystation of *E. invadens* in 47% low glucose (LG) medium made with the indicated pH. D) Effect of pH on the ability of short chain fatty acids (SCFA) to inhibit *E. invadens* encystation. Trophozoites were suspended in 47% LG medium made with the indicated pH, without or with the indicated fractional amounts of a 70:20:10 mM pool of acetate:propionate:butyrate. The 70:20:10 mM SCFA pool was considered 1 X; the indicated fractional inputs of 0.20 X and 0.56 X yielded final concentrations of SCFA of 14:4:2 and 39.2:11.2:5.6 mM acetate:propionate:butyrate, respectively.