Chapter 1
Introduction

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Amebiasis, the result of infection with the protist parasite *Entamoeba histolytica*, is manifested as either commensal or invasive forms of the disease in humans. The majority of infected people do not display any pathology, and the parasite exists as a commensal, continuing to multiply and spread. Only a small fraction of the infected individuals show overt symptoms of amebiasis with invasion in the intestinal tissues or in some extraintestinal sites, such as liver. Therefore, one of the major problems in this field is to understand the mechanisms that make *E. histolytica* invasive in some individuals. *E. histolytica* displays a simple life cycle with two forms, infective cyst and vegetative trophozoites. The cysts are secreted by individuals that harbor the parasite along with fecal material and enter a human through contaminated food and water. Cysts convert into trophozoites in the intestine, and the trophozoites either produce cysts or multiply to produce more trophozoites, or both. Trophozoites can either invade epithelial tissue through the intestinal barrier or enter the circulation by penetrating through the basement membrane, lodging and multiplying in one of the organs, for example, the liver.

A large number of cellular and molecular processes are involved in the survival and pathogenesis of *E. histolytica*. In this book, a number of chapters describe many of these processes and give insight into the mechanisms. The genomes of *E. histolytica* and many other *Entamoeba* species have been recently explored. Moreover, many isolates of *E. histolytica* have been sequenced using "next-generation sequencing" (NGS) platforms. C. Graham Clark and Rune Stensvold describe genetic and genomic variations among different *Entamoeba* species and suggest that continuous future discovery of new divergent *Entamoeba* species will improve our understanding of this important group of anaerobic single-celled eukaryotes (Chap. 2). In the chapter by

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Gareth Weedall (Chap. 3), the results and analysis from these genome projects are summarized and the relationship between genome variations and virulence potential elaborated. They point out the importance of studying genetic polymorphisms at the whole genome level. Over the years a number of methods have been developed that are useful in *E. histolytica* genotyping. After genome sequencing was completed and published, newer methods were developed for *Entamoeba* genotyping taking advantage of sequence information. Carol Gilchrist and Ibne Ali describe two different approaches for genotyping, the multilocus sequence typing system (Chap. 4) and the STR-based genome typing (Chap. 5). These methods are useful in typing large number of isolates and identification of pathogenic genotype.

The availability of NGS technology has not only helped to understand genome organization and genome-wide variations but also provides a powerful technique to qualitatively and quantitatively analyze total mRNA and small RNA transcriptome (RNA-seq). RNA-seq is particularly useful to identify expressed genes, introns, and transcription start sites. If the studies are done under different conditions, changes in gene expression can be measured and correlated with the biology of the organism. These concepts are elaborated in the chapter by Chung-Chau Hon and Nancy Guillen et al., in which the methodology and results from RNA-seq of *E. histolytica* are described (Chap. 7). Their analysis clearly shows the occurrence of a large number of splicings through a stochastic process. Small RNAs, such as microRNAs and siRNAs, have revolutionized our concept of gene regulation and helped to shift the paradigm from protein centric to both RNA- and protein-based regulatory networks. Typical microRNAs are found in higher eukaryotes and may not be present in *E. histolytica* as some of the genes involved in its biogenesis are thought to be missing. However, *E. histolytica* expresses a class of 27 nucleotide novel RNAs. Laura Morf and Upinder Singh summarize current knowledge about generation and functions of these small RNAs and describe the regulatory role of these small RNAs in *E. histolytica* gene expression (Chap. 9). They also show that the machinery for biogenesis of these RNAs can be useful in gene silencing.

Many systems such as *Entamoeba* are not amenable for genetic studies because of their complex life cycle, biology, and the culture conditions used for their cultivation. Therefore, methods are needed for introduction of genes from outside so that alternate techniques, such as RNAi and the dominant-negative approach, can be used for genetic analysis. David Mirelman and Rivka Bracha summarize all the studies that have helped to develop methods for introduction of genes and their use in downregulation of specific gene expression in *E. histolytica* (Chap. 6). These methods have helped in our understanding of some of the molecular processes in this organism. For example, it has been possible to investigate in detail the molecular mechanism of rRNA transcription. The chapter by Abhishek K. Gupta and Sudha Bhattacharya explores this area and gives a glimpse into "how gene expression is regulated in this organism" (Chap. 8). In particular, they point out generation of novel circular RNAs as part of processing intermediates of rRNA precursors. Circular noncoding RNAs have recently been observed in metazoans, and their importance in gene regulation is being highlighted. Vijay Pal Yadav and Sudha Bhattacharya summarize our current understanding of retrotransposable elements

of *E. histolytica* (Chap. 10). These elements constitute a substantial part (about 6 %) of the *Entamoeba* genome and are likely to be important for their biology. This chapter also includes mechanism of transcription and expansion of these elements in the genome. Epigenetic mechanisms are attracting increasing attention as a major regulatory key to different biological processes, such as gene expression and development. In *E. histolytica*, a methyl transferase that modifies tRNA has been identified and studied in relationship to environmental changes (Chap. 11). Preferential modification of a retrotransposable element has also been observed, and a protein that recognizes methylated cytosine has also been identified by Michael Kirschenbaum and Serge Ankri. DNA replication constitutes one of the fundamental processes involved in cell proliferation. A number of enzymes participate in the process so that replication is initiated and terminated at the right time, maintaining fidelity and control. One way to study replication is to understand the participating enzymes. Guillermo Pastor-Palacios and Luis Brieba et al. describe some of the DNA polymerases of *E. histolytica* in their chapter (Chap. 22) and attempt to correlate the structural and biochemical properties with replication machinery.

Cell biology of *E. histolytica* has been extensively studied because many of the cellular processes are intimately related to pathogenesis, such as phagocytosis and movement. *E. histolytica* displays an extensive signaling system made up of a large number of kinases, calcium-binding proteins, and GTP-binding proteins. A combination of these molecules appears to control and co regulate different cellular processes, such as stress response, phagocytosis, and motility. Somlata and Alok Bhattacharya summarize our current understanding about the mechanism of phagocytosis in *E. histolytica*. The molecular process of initiation and formation of phagocytic cups appears to be distinctly different in this organism compared to other eukaryotic systems; however, the subsequent steps involving actin dynamics, pseudopod movement, and scission are likely to be similar (Chap. 12). Direct participation of calcium-binding proteins in actin dynamics has been seen so far only in this organism. Further, Saima Aslam, Shahid Mansuri, and Alok Bhattacharya describe the cellular signaling pathways, highlighting those that are mediated through Ca^{2+} (Chap. 13). They also point out the involvement of other second messengers, such as cyclic AMP in amoebic signaling systems, although not much work has been done to understand the involvement of second messengers other than Ca^{2+}. Protein kinases constitute the major part of the signaling proteome of *E. histolytica*. Different classes of protein kinases make up the kinome, and in this respect the *E. histolytica* kinome is similar to other eukaryotic systems. Transmembrane protein kinases (TMK) represent one of the largest families of the kinome and have been studied more extensively compared to other kinases. TMKs are single-pass membrane proteins containing both extracellular and intracellular domains that can be classified into nine families and are thought to be involved in phagocytosis, proliferation, and virulence. Nathaniel Christy and William A. Petri describe our current understanding on the structure and function of this large group of protein kinases (Chap. 14). Regulated trafficking and secretion of pathogenic factors, such as cysteine proteases, has been extensively studied and is summarized by Kumiko Nakada-Tsukui and Tomoyoshi Nozaki (Chap. 17). During survival in different

niches of the host body, such as the intestinal lumen, liver, lung, and brain, as well as the external environment, *Entamoeba* must acclimate itself to a variety of stresses. Thus, stress-sensing mechanisms are important to study, particularly in relationship to pathogenesis and cell death. Daniela Faust and Nancy Guillen describe a newly characterized global stress-sensing mechanism in *Entamoeba* and elaborate on their importance in amoebic biology (Chap. 15). Regulation of cell cycle and cell division is promiscuous in *Entamoeba*. The unusual mechanism of cell-cycle control generates polynucleated cells. Our current view of the mechanism is described by Jaspreet Singh Grewal and Anuradha Lohia (Chap. 16). *Entamoeba* does not reveal discernible organelles in a form typical for other eukaryotes. The mitochondria have become highly divergent from that of other aerobic eukaryotes and are referred to as mitosomes. Although mitosomes do not have any DNA, they still have unique functional features and have been retained by the cell. Takashi Makiuchi, Fumika Mi-ichi, and Tomoyoshi Nozaki (Chap. 18) describe the mitosomes with respect to the components and their participation in different functions, particularly in protein transport mechanisms.

As a consequence of reductive evolution and secondary loss of aerobic respiration in mitosomes, *Entamoeba* relies solely on glycolysis and fermentation for ATP generation. Thus, understanding glucose metabolism and its regulation is essential for understanding the biology and pathophysiology of *Entamoeba*. This topic is summarized by Erika Pineda and Emma Saavedra et al. (Chap. 20). To comprehensively understand metabolism, combined omics approaches, such as transcriptomics and metabolomics, are becoming essential. Ghulam Jeelani, Dan Sato, and Tomoyoshi Nozaki summarize new key findings on metabolism using these integrated approaches (Chap. 19). Moreover, similar omics-based strategy can also help to identify new pathways involved in differentiation of trophozoites into cysts and the mechanism that initiates and regulates this process. Amino acid metabolism, particularly sulfur-containing amino acid metabolism in *Entamoeba*, is remarkably different from that in the hosts, thus providing a rationale approach for drug discovery. This aspect is clearly evident from the chapter by Isha Raj and Samudrala Gourinath et al. that highlights structural biology-based analyses of the two major enzymes of the cysteine/*S*-methylcysteine biosynthetic pathway and their usefulness in rational drug design (Chap. 21).

It is not clear if the immune system plays any role in initiation and development of invasive amebiasis. This question has been addressed in a number of chapters in which the authors have described roles of different host factors including different arms of the immune system in amebiasis. A large number of parasite and host factors contributes to amoebic pathogenesis, and some of the key questions, such as why only a fraction of infected people have invasive disease and why some people have liver infection, have not yet been satisfactorily answered. Overall pathogenesis in relationship to amebiasis is reviewed by Mineko Shibayama and Victor Tsutsumi (Chap. 23). Innate and acquired immunity against amebiasis and the pathology of amebiasis have been extensively studied. Leanne Mortimer and Kris Chadee describe innate host immunity and defense in the intestine (Chap. 24). Because mucins play a critical role during the entry of parasites from the intestine, the

destruction of the mucin layer can act as an accessory in establishing invasive disease, as highlighted by V. Kissoon-Singh, E. Trusevych, and K. Chadee (Chap. 27). Cysteine proteinases have been implicated in the pathology of a number of infectious diseases, and ever since their discovery in *E. histolytica* cysteine proteases (CP) have been thought to have an important role in invasion, cytolysis, and tissue destruction in amebiasis. *E. histolytica* has a large repertoire of CPs, and their role are described in two chapters by Iris Bruchhaus and Jenny Matthiesen (Chap. 25) and Elena Helk, Hannah Bernin, and Hanna Lotter (Chap. 26).

Infection by *Entamoeba* results in a range of outcomes from drastic symptoms, such as dysentery and liver abscess, to no symptom. Outcome of infections depends in part on the host genotype, which regulates the nature of the host–parasite relationship. So far, only parasite factors have been investigated; efforts have been made recently to also understand host factors. Shannon N. Moonah, Nona M. Jiang, and William A. Petri, Jr. summarize many studies on the role of human genetic polymorphism on susceptibility to amebiasis. They describe various polymorphisms of leptin receptor and their association with disease outcome (Chap. 28). Contributions of HLA polymorphism are highlighted by Cecilia Ximenez et al. (Chap. 29).

Metronidazole has been a drug of choice against amebiasis for decades. Unfortunately, there is no major second-line drug at present, and there is concern that widespread emergence of drug resistance will cause a major public health problem worldwide. Therefore it is important to study metronidazole action, resistance, and new drug development. Michael Duchene summarizes our current view on the mode of action of metronidazole and its relationship to redox regulation affected by the drug in *Entamoeba* (Chap. 30). Development of potential new drugs is covered in a number of chapters, and it appears that it will be possible to develop alternate drugs very soon. Rosa M. Andrade and Sharon Reed describe a new drug target, thioredoxin reductase, identified using a high-throughput technology (Chap. 31). Anjan Debnath summarizes the discovery of a new anti-amebic drug among FDA-approved molecules (Chap. 32), and a description of a range of heterocyclic anti-amebic compounds is given by Amir Azam (Chap. 33).