INTRODUCTION

As human beings we are influenced by our surrounding environment in every way. How we live and commute, what we eat and drink influence us more than hitherto realized and understood. Importantly, we live together with trillions of bacteria and viruses. The constituents of the microbiota (i.e., bacteria, viruses, and eukaryotes) have been shown to interact with one another and with the host immune system in ways that influence the development of disease [1]. The human host has coevolved with normal bacteria over thousands of years and developed complex mechanisms that monitor, and control this ecosystem (Fig. 32.1).

Likely no other place is the interaction between human cells and infective agents as important as in the gastrointestinal tract (Fig. 32.2) where microbes may influence on both physiological and pathogenetic processes through various molecularly regulated mechanisms [1,2]. Such cellular mechanisms have homeostatic roles beyond the traditional concept of defense against potential pathogens, suggesting these pathways contribute directly to the well-being of the gut [2]. For example, the microbes of the large bowel provide us with genetic and metabolic attributes we have not been required to evolve on our own (Fig. 32.2), including the ability to harvest otherwise inaccessible nutrients [3].

However, microbiota may influence the epigenetic makeup at an even earlier stage, even before birth. Indeed, the microbiota changes during the course of a lifetime, and affects several aspects of health and disease, and may even influence the process of aging.

This chapter will review some associations between both commensal and pathogenic infective agents (bacteria and viruses), and their (known or potential) influence on human health and disease through direct, and epigenetic mechanisms. Since the previous volume of this chapter [4], several developments have occurred and
inclusion of all aspects, associations and mechanisms of this emerging field is beyond the scope of this chapter. Several mechanisms are reviewed in other chapters of this book. Thus, recent and updated reviews on the related topics are referred to, wherever applicable. The microbiome and its (epi)genetics is explored through a vast field of potential anatomic locations (from immune system to parenchymal organs) and types of diseases (from autoimmune disease to neoplasia). Thus, the chapter will include some examples from other aspects that may serve as educational points into the link between microbiota, epigenetics, and human health and disease.

EARLY MICROBIOME EXPOSURE AND EPIGENETIC INFLUENCE

It is now generally understood that exposure to microbes influences the epigenome and may in part explain disease patterns and disease development [5]. Evidence from epidemiological studies has highlighted links between changes in life-style factors, exposures to microflora and the subsequent risk for developing disease. Of the many diseases whose risks are tied to such exposure, a few examples are autoimmune diseases (including allergies and asthma), obesity, and cancer. Alongside with the improvement in socioeconomic living and advances in technology (e.g., refrigerators), the birth rate has dropped, and that of cesarean birth increased, meaning that babies have fewer siblings and less exposure to germs (Fig. 32.3A). This has subsequently been accompanied by an increase in autoimmune diseases in society [6,7]. Likewise, changes in diet and caloric food intake may influence the microbiome, which again influences the epigenetics, and risk of various diseases, including cancer [8].

Early exposure to microbiota during fetal life appears to have important roles for development, and some suggest that even exposure to maternal microbiota long before fetal life may be influential on later disease risk [6,9].
Previously, it was believed that the fetus resides within a sterile environment and that the newborn attains its microbiota only during, and after birth. Numerous studies in humans as well as other species contradict this dogma [9]. While the child is exposed to oral, vaginal and gut microflora during, and after birth, there appears to be important and substantial links between mother, the microbiome and the fetus [6,9]. Indeed, exposure to essential microbiota during vaginal delivery establishes the type of gut intestinal flora (and, thus, influences conditions for health and disease). This knowledge has led to concerns about the rates and consequences of cesarean deliveries (which results in no exposure for the baby to the vaginal canal and are performed in a sterile surgical environment) and has raised the question of whether a vaginal swab to introduce maternal vaginal microbes into cesarean-sectioned infants’ gastrointestinal tracts may be beneficial. On the other side of the age spectrum...
FIGURE 32.3  Lifetime microbial exposure and risk/benefit in health and disease. (A) There is a temporal dissociation between the major changes in microbial exposure to children in westernized countries, which occurred during the first-half of the century, and the onset of the accelerating increase in allergic diseases that happened during the second-half of the century. (B) Epigenetic changes of the promoter region of immunoregulatory genes important for allergy development could explain how the effect of microbial exposure to the mother during or even before pregnancy is transferred to the next generation. (C) Development of the microbiota. The gastrointestinal tract of the fetus is sterile until birth, after which the newborn is initially colonized. Depending on delivery mode, the initial communities tend toward a skin-like (cesarean section) or a vaginal-like (vaginal delivery) configuration. During the first weeks of life, there is a reduced activity of toll-like receptor (TLRs), potentially allowing the necessary formation of a stable bacterial community in the gut. As the infant grows, and with the introduction of solid foods, the microbiota diversity increases, and the community converges toward an adult-like state. At the same time, the immune system “learns” to differentiate between commensal and pathogenic bacteria. By adulthood, a relatively stable community composition (but varying between different individuals) is achieved, dominated mostly by Bacteroidetes and Firmicutes. Different diseases are characterized by significant changes in the microbiota and associated changes in the production of cytokines. Source: Parts A and B, Reprinted from Abrahamsson TR, Wu RY, Jenmalm MC. Gut microbiota and allergy: the importance of the pregnancy period. Pediatr Res 2015;77(1–2):214–9 [6], with permission from Macmillan Publishers Ltd., Copyright 2015 by Elsevier. Part C, Reprinted from Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell 2012;148(6):1258–70 [1], with permission. Copyright 2012 by Elsevier.
is the knowledge that the gut microbiota may change with age (Fig. 32.3) and even speed up some of the processes known to be associated with biological aging [10,11], such as cognitive function.

Advances in understanding and techniques of microbial studies have enabled progress in characterizing the taxonomic composition, metabolic capacity, and immunomodulatory activity of the human microbiota, further establishing its role in human health and disease [2,3,12]. Fact is, the microbiota has established multiple mechanisms to influence the eukaryotic host, generally in a beneficial fashion, and maintain their stable niche [2,3,13]. As their prokaryotic genomes allow a number of metabolic processes beyond the capabilities of the host genome, the microbes are seen as an essential part of normal physiology in humans (Fig. 32.4).

Gaining a fuller understanding of both partners in the normal gut–microbiota interaction may thus shed light on how the relationship can go awry and contribute to a number of immune, inflammatory, and metabolic disorders. Further, increased understanding may reveal mechanisms through which these relationships could be manipulated toward therapeutic ends [2,3,15].

THE HUMAN GUT MICROFLORA

Humans live in direct and continuous interaction with a complex microbial environment. The total number of bacteria, viruses and archaea normally inhabiting our mucosal surfaces exceeds the quantity of cells (∼10^{13}) in our bodies. Bacteria, which alone constitute more than 99% in volume and genome of the microbiota inhabiting the human host, were until very recently thought to outnumber our cells in a 10:1 ratio. This ratio is nowadays estimated to be more likely 1:3:1 [16]. Perhaps the greatest habitat for microbes is the GI tract, with increasing recorded concentrations of bacteria from the stomach (∼10^3–10^4 cells/g or mL) to the small (∼10^8–10^9 cells/g or mL) and large bowel (10^{10}–10^{12} cells/g or mL) [17,18].
Interestingly, it was suggested that a single defecation event could momentarily reverse the order of bacterial-to-human cells magnitude [16].

Gut microbiota concentration and composition is largely influenced by diet [19], type of birth (as mentioned earlier), age [15], and geographical location [20]. Molecular analysis has demonstrated different communities of bacteria from individual to individual [21–23]. The intestinal habitat of an individual contains on average 300–500 different species of bacteria [12]. In a study on 124 European individuals, between 1000 and 1150 different species of bacteria were discovered inhabiting the gastrointestinal tract, with each individual harboring at least 160 species, with a great overlap [24]. The stomach and small intestine contain only a few species of bacteria. Contrasting this, the large intestine contains a complex and dynamic microbial ecosystem with high densities of living bacteria, which may achieve concentrations of $>10^{11}$ cells/g of luminal contents [17,18]. In fact, about 60% of fecal solids consist of bacteria.

The great bioreactor that the gut microbiota represents in the human body produces a plethora of low-molecular, biologically active molecules as a result of both direct production and food digestion. These include, but are not limited to neurotransmitters (serotonin, dopamine, etc.), short-chain fatty acids (SCFAs) (butyrate, propionate, etc.), chemical moieties (acetyl-, methyl-, etc.), and simple gases (hydrogen sulfide, H$_2$S; nitrous oxide, NO; carbon mono- and dioxide, CO/CO$_2$; ammonia, NH$_3$; methane, CH$_4$ etc.). Such molecules are able to signal in auto-, para-, and endocrine fashion, exerting well-established functions as digestion, inflammation [14,25], neuroendocrine signaling [26,27], and epigenetic [28,29] mediators. Bacteria are able to influence the host epigenome directly by producing metabolites that affect it, or indirectly by activating signaling cascades that lead to epigenetic regulation of genes. Similar to viruses, bacteria provoke histone modifications and chromatin remodeling in infected cells, thereby altering the host’s transcriptional program and, in most cases, dampening the host innate immune response. Still, our current knowledge is likely to represent just the “tip of the iceberg” of the full spectrum of microbiota influence on the human body.

Furthermore, microorganisms inhabiting us do not only yield molecules as by-product of the digestion of food we ingest; they also produce a great deal of their own. The microbiome is defined as the collection of over 3 million genes (~150 times the number of human genes, estimates vary) [24] expressed by the totality of microbes inhabiting the human body.

Scattered amidst the commensal microflora are potential pathogens—viruses, bacteria, or parasites—intrinsically capable of producing symptomatic infectious disease [2,3,12,13,30]. The long coexistence of bacterial pathogens with their eukaryotic hosts, and their coevolution, have provided pathogens with an amazing capacity to exploit host cell functions for survival, replication inside or outside cells, and escape from early innate immune responses [31]. The fact that bacteria are so well adapted to their host has been of great benefit for cell biologists who are increasingly using them to study fundamental cell processes.

Several hundred grams of bacteria living within the colonic lumen have thus a great influence on the host’s homoeostatic processes, with important health benefits. When the fine-tuned symbiosis that we and our “forgotten organ” evolved into is thrown out of balance, though, the dysbiotic endogenous microflora can negatively affect our health.

### THE MICROBIOME, EPIGENETICS, AND EFFECT ON METABOLISM

The influence of the gut microbiota state in human health and disease has been increasingly recognized and investigated in recent years.

In humans, differences in microbiota composition, functional genes, and metabolic activities are observed between obese and lean individuals, suggesting a contribution of the gut microbiota to these phenotypes [32–35] (Fig. 32.5).

The use of germ-free animals and microbiota transplant showed that the gut microbiota composition might play a causal role in the development of obesity and associated metabolic disorders, and led to identification of several mechanisms.

In the human gut, the dominant bacterial phylae are *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*. Their relative proportion and overall host health are believed to be mutually influenced according to diet, metabolic state, and weight, particularly obesity and as such, the microbiota is considered a potential tool in therapy and diagnostics [19,33,36–39]. Obese individuals exhibit increased proportions of *Firmicutes* and decreased proportions of *Bacteroidetes* in the gut [40]. Variations in the composition of gut microbiota are indeed found in obese mice and humans [41], and whole-genome methylation analysis significantly correlated high levels of the phylum with aberrant methylation pattern in genes associated with metabolism, obesity, and obesity-related diseases, in a recent pilot study [42]. There is, however, still debate over the link between a particular phylum and energy absorption from the diet.

SCFA, such as butyrate, acetate, and propionate are end by-product of the fermentation of intestinal bacteria in the presence of complex carbohydrates or other indigestible foods (reviewed in Ref. [35]). These fatty acids...
are believed to influence lipogenesis, glucose homeostasis and insulin sensitivity, and are found at varying concentrations in obese and lean individuals.

Different microbiota composition was shown to affect epigenetic regulation of free fatty acid receptors (FFARs) by decreased promoter methylation in obese and diabetes type 2 patients [43]. Promoter methylation increased in obese patients following nutritional counseling and decrease in body mass index. The molecular mechanisms of the microbiota–host gene methylation observation remain elusive, but it is known that bacteria are important contributors of methyl group donor molecules, such as folate and cofactors needed in the DNA methylation process, and imbalances in the gut microbiota composition can lead to hypo- or hypermethylation in the host [8, 28].

Low levels of diffused inflammation in the gut are also associated with both obesity and type 2 diabetes. Butyrate, of which Firmicutes are the main contributors in the human gut, is thought to have a protective effect towards low-grade inflammation and therefore obesity. This is believed to happen through inhibition of histone deacetylases (HDAC) and consequent upregulation of key genes that prevent immune cells infiltration [25, 29].

The epigenome is influenced by environmental factors throughout life [8]. For example, nutritional factors can have profound effects on the expression of specific genes via epigenetic modification, and these may be passed on to subsequent generations with potentially detrimental effects.

Many cancers are associated with altered epigenetic profiles, leading to perturbations in expression of the genes involved in cell growth, differentiation, or DNA damage repair [44]. Autoimmune and neoplastic diseases increase in frequency with increasing age, alongside with epigenetic dysregulation [10]. Studies in monozygotic twins revealed increasing epigenetic differences with age. Differences in methylation status of CpG sites, monoallelic silencing, and other epigenetic regulatory mechanisms have been observed in key inflammatory response genes.

GUT MICROBIOTA, INFLAMMATION, AND COLORECTAL CARCINOGENESIS

The thought that dietary habits contribute to colorectal cancer (CRC) development through modulation of the gut microbiota is not new and CRC development has been related to infections of viruses, bacteria, and parasites [45]. Nevertheless, accumulating evidence points to a dual role of the gut microbiota, both promoting and protective, in the context of colorectal carcinogenesis. Bacteria produce active metabolites with a varied range of effects as the result of food fermentation. Hydrogen sulfide, for example, a secondary metabolite produced by digestion of red meat by sulfate-reducing bacteria, is a known oncometabolite [46, 47].

On the other side, bacterial fermentation is known to produce a multifaceted tumor-suppressive effect.
via production of metabolites, such as equol, urolithins, and more importantly SCFAs, such as butyrate, from digestion of dietary fibers and plants. A thorough review of the protective and detrimental metabolites of bacterial fermentation is given by Hullar et al. [48].

As of the Warburg effect that tumor cells endure to survive in anaerobic conditions, butyrate is no longer efficiently metabolized in mitochondria. Consequently, it is free to accumulate in the nucleus, where it actively inhibits HDACs, inhibiting proliferation and inducing apoptosis of cancer cells in an epigenetic fashion [49]. The protective effect of dietary fiber and butyrate on CRC was recently confirmed in a gnotobiotic mouse model [50].

One of the most-studied contexts in which the mediator role of the microbiota is recognized is the inflammatory process. Several mechanisms of tumor promotion have been suggested, as reviewed by Irrazabal et al. [47], including the ability of the microbial flora of the colon to produce a state of chronic low-grade inflammation. The genotoxic stress that results may contribute to colon carcinogenesis and also influence genetic stability [47,51] (Figs. 32.6 and 32.7).

In a cellular model, it was demonstrated that macrophage cyclooxygenase-2 (COX-2) was induced by superoxide from Enterococcus faecalis and promoted chromosomal instability in mammalian cells through diffusible factors [52].

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**FIGURE 32.6** Role of dysbiosis and immune dysfunctions in colon carcinogenesis. (A) Dysbiosis, as a result of inflammasome deficiencies, could promote tumorigenesis. Inflammasome-derived interleukine (IL)-18 is necessary for tissue repair, protection against tumors, and the maintenance of the microbial ecology equilibrium. In turn, this phenotype associated with the lack of IL-18 could be exacerbated by a dysbiotic microbiota that could lead to chronic inflammation, increased IL-6 signaling, and tumorigenesis. In intestinal epithelial cells, IL-6 activates STAT3 signaling, protecting normal and premalignant cells from apoptosis. (B) Dysbiosis and immune dysfunctions may allow increased bacterial translocation due to altered barrier function. Microorganism-associated molecular patterns (MAMPs) are detected by Toll-like receptors (TLRs) present in epithelial cells, macrophages, and myofibroblasts, leading to the activation of different pathways that promote cancer development. Epiregulin (EREG) and amphiregulin (AREG) are epidermal growth factor receptor (EGFR) ligands and therefore induce proliferation through MAPK/ERK pathway activation. Th17 cytokines mark the early stages of colorectal cancer (CRC) by induction of STAT3. Source: Reprinted from Irrazábal T, Belcheva A, Girardin SE, Martin A, Philpott DJ. The multifaceted role of the intestinal microbiota in colon cancer. Mol Cell 2014;54(2):309–20, with permission [47]. Copyright 2014 by Elsevier.
Conversely, the SCFAs butyrate and propionate produced by microbial fermentation was repeatedly shown in mice to activate Foxp3 expression in naïve CD4+ T-lymphocytes, which results in expansion of the Th17 Tregs cells, thus playing an immunosuppressive role [46,53,54].

Inflammatory bowel disease (IBD) results from a dysregulated immunologic response to commensal microbial flora residing in the intestinal lumen [55,56]. Although this response is probably due at least in part to a genetic predisposition, patients with IBD have also been reported to house an abnormal intestinal microflora. Whether this altered flora is the cause or result of the associated chronic inflammation remains unclear. What appears important is the role of tumor necrosis factor (TNF-α) in the role of IBD development, as it may alter the microbial composition, enhance virulence, and increase adherence and invasion [57].

While bacteria may cause instability at the chromosomal level in colonic epithelium directly, via both inflammation and production of oncometabolites, they are also known to produce a marked protective effect, suggesting that the final outcome depends on phylogenetic diversity (in turn influenced by dietary factors) and host endogenous mechanisms.
PATHOGENIC INFECTIONS AND CANCER

Of notice, several viral and bacterial infections have been linked to different types of cancer, of which CRC is one of the best documented [45]. Infective agents are thought to be responsible for almost one-fifth of all cancers, with an estimated total of infection-attributable cancer at almost 2 million cases of the global cancer burden [58]. The principal agents involved have been estimated to be (in decreasing order): the bacterium Helicobacter pylori (5.5% of all cancer), the human papilloma viruses [HPV (5.2%)], the hepatitis B and C viruses (4.9%), Epstein–Barr virus [EBV (1%)], human immunodeficiency virus (HIV) together with the human herpes virus 8 (0.9%) [58]. Relatively less important causes of cancer are the schistosomes (0.1%), human T-cell lymphotropic virus type I (0.03%), and the liver flukes (0.02%). Estimates by Parkin suggest there would be about 26% fewer cancers in developing countries (1.5 million cases per year) and almost 8% in developed countries (390,000 cases) if these infectious diseases were prevented. The fraction of infectious-induced neoplasia at the specific sites varies from 100% of cervix cancers attributable to the HPV to a tiny proportion (0.4%) of hepatocellular carcinomas caused by liver flukes on a global scale [58].

Nonetheless, certain bacterial strains and the metabolites resulting from their fermentation are believed to have a protective effect towards carcinogenesis.

PATHOGENIC INFECTIONS AND EPIGENETIC MODIFICATIONS

The genomes of certain viruses and the proviral genomes of retroviruses are regularly targeted by epigenetic regulatory mechanisms (DNA methylation, histone modifications, binding of regulatory proteins) in infected cells [59]. In parallel, proteins encoded by viral genomes may affect the activity of a set of cellular promoters by interacting with the very same epigenetic regulatory machinery [60]. This may result in epigenetic dysregulation and subsequent cellular dysfunctions that may manifest in or contribute to the development of pathological changes (e.g., carcinogenesis or immunodeficiency). Bacteria infecting mammals may cause diseases in a similar manner, by causing hypermethylation of key cellular promoters at CpG dinucleotides (promoter silencing, e.g., by Campylobacter rectus in the placenta or by H. pylori in gastric mucosa).

Bacterial Infections

Bacterial pathogens have evolved various strategies to avoid immune surveillance, depending on their in vivo “lifestyle” [61,62]. The identification of few bacterial effectors capable to enter the nucleus and modifying chromatin structure in host raises the questions of how pathogens modulate chromatin structure and why. Chromatin is a dynamic structure that maintains the stability and accessibility of the host DNA genome to the transcription machinery. Arbibe [61] review the various strategies used by pathogens to interface with host chromatin. In some cases, chromatin injury can be a strategy to take control of major cellular functions, such as the cell cycle. In other cases, manipulation of chromatin structure at specific genomic locations by modulating epigenetic information provides a way for the pathogen to impose its own transcriptional signature onto host cells. A well-studied example are the eukaryote-like SET domain proteins, histone-methylating proteins encoded by bacteria, such as Legionella and Chlamydia that are able to modify chromatin structure in the host [63].

Knowledge in how bacteria may influence the host environment through genetic and epigenetic manipulation is increasing [22,63–65]. Clearly, upon infection, pathogens may reprogram host gene expression. In eukaryotic cells, genetic reprogramming is induced by the concerted activation/repression of transcription factors and various histone modifications that control DNA accessibility in chromatin [66]. One study exploring the microbe-host interaction during infection reported that the bacterial pathogen Listeria monocytogenes induced a dramatic dephosphorylation of histone H3 as well as a deacetylation of histone H4 during early phases of infection. This effect was mediated by the major listerial toxin listeriolysin O in a pore-forming-independent manner. A similar effect was also observed with other toxins of the same family, such as Clostridium perfringens perfringolysin and Streptococcus pneumoniae pneumolysin. The decreased levels of histone modifications correlate with a reduced transcriptional activity of a subset of host genes, including key immunity genes. In their findings, control of epigenetic regulation emerged as an unsuspected function shared by several bacterial toxins, highlighting a common strategy used by intracellular and extracellular pathogens to modulate the host response early during infection [66].

Innate immunity is the first line of defense against a bacterial infection, and most organisms are able to mount an efficient early, nonspecific response leading to the recruitment of cellular effectors and inflammation. Microbial components that elicit an inflammatory response have been called microbial-associated molecular patterns (MAMPs) and include LPS, bacterial flagellin, lipoteichoic acid, peptidoglycan, and nucleic acids [2,31]. Host cells recognize MAMPs through pattern recognition receptors (PRRs) present either at the cell surface and/or on endosomes, for Toll-like receptors (TLRs), or in the cytoplasm, for nucleotide-binding
oligomerization domain proteins (NODs) and NOD-like receptors (NLRs). These receptors activate signaling cascades leading to transcriptional activation of immunity genes, such as cytokine genes.

**Helicobacter pylori Infection and the Stomach**

The decline of gastric cancer in the western world over the past decades has been attributed (among others) to the socioeconomic improvements, better hygiene, and thus less exposure to *H. pylori*, for one. On the contrary, the incidence of CRC is still on the rise, and it is tempting to speculate that what we eat and new ways of processing food has allowed for new ways for different types, and strains of bacteria to enter the gut epithelium [67,68], alter the bacterial milieu [69], and thus potentially induce carcinogenic effects at the molecular level [70]. Notably, alteration of gut microbiota homeostasis is linked to extra-colonic diseases as well, such as in breast and liver [71–73]. *H. pylori* are bacteria that colonize the stomach persistently in over half of the world’s population. *H. pylori* is linked to various diseases of the stomach, such as peptic ulcer disease, gastric lymphoma, and gastric cancer [64]. In addition, *H. pylori* may also play a role in IBD, irritable bowel disease, and potentially also in CRC.

Aberrant expression of cell cycle control proteins has been demonstrated in *H. pylori* infected gastric epithelial cells, suggesting that perturbation of the cell cycle plays a role in the pathogenesis of various *H. pylori* associated diseases. Downregulation of E-cadherin (an adhesion molecule involved in tumor invasion and metastasis) in *H. pylori* associated gastric cancer has been known for more than a decade [74,75], and is caused by silencing of E-cadherin by promoter CpG methylation [75]. Further, in a study of the modulation of the cell cycle control protein p21(WAF1) by *H. pylori* in a gastric carcinoma cell line and in primary gastric cells derived from healthy tissue [76], the investigators observed an upregulation of p21(WAF1) in both gastric cancer cells and primary cells. Analysis revealed that the increased expression of p21(WAF1) induced by *H. pylori* is associated with the release of HDAC-1 from the p21(WAF1) promoter and hyperacetylation of histone H4 [76].

Recently, it was demonstrated that antibiotic eradication of *H. pylori* infected patients, reversed the methylation pattern of important tumor promoters in the gastric mucosa [77]. Knowing that aberrant epigenetic changes may be induced by *H. pylori* infection, such as hypermethylation and silencing of hMLH1 [77], the same effects may occur in the colorectal epithelium, either by *H. pylori*, or tentatively by other unrecognized bacterial species. Several bacterial toxins interfere with cellular signaling mechanisms in a way that is characteristic of tumor promoters [78]. Such toxins could play a direct, yet unappreciated, role in cancer causation and progression.

**Viral Infections**

Viruses are capable of influencing the human genome as well, with proposed mechanisms for disease development. EBVs is a human herpesvirus hiding in a latent form in memory B cells in the majority of the world population. Although, primary EBV infection is asymptomatic or causes a self-limiting disease, infectious mononucleosis, the virus is associated with a wide variety of neoplasms developing in immunosuppressed or immunodeficient individuals, but also in patients with an apparently intact immune system [79]. In memory B cells, tumor cells, and lymphoblastoid cell lines (LCLs, transformed by EBV in vitro) the expression of the viral genes is highly restricted. There is no virus production (lytic viral replication associated with the expression of all viral genes) in tight latency. The expression of latent viral oncogenes and RNAs is under a strict epigenetic control via DNA methylation and histone modifications that results either in a complete silencing of the EBV genome in memory B cells, or in a cell-type dependent usage of latent promoters in tumor cells, germinal center B cells, and LCLs. Both the latent and lytic EBV proteins are potent immunogens, and elicit vigorous B- and T-cell responses. In immunosuppressed and immunodeficient patients, or in individuals with a functional defect of EBV-specific T cells, lytic EBV replication is regularly activated and an increased viral load can be detected in the blood. Enhanced lytic replication results in new infection events and EBV-associated transformation events, and seems to be a risk factor both for malignant transformation and the development of autoimmune diseases [79]. As reviewed by Niller et al., current speculation includes the idea that an increased load or altered presentation of a limited set of lytic or latent EBV proteins that cross-react with cellular antigens triggers and perpetuates the pathogenic processes that results in multiple sclerosis, systemic lupus erythematosus (SLE), and rheumatoid arthritis. In addition, in SLE patients’ EBV may cause defects of B-cell tolerance checkpoints because latent membrane protein 1, an EBV-encoded viral oncoprotein can induce B-cell activating factor (BAFF), that rescues self-reactive B cells and induces a lupus-like autoimmune disease in transgenic mice [79].

**VIRUSES AND EPIGENETIC ROLE IN CANCER DEVELOPMENT**

Herpesviruses, papillomaviruses, and retroviruses are the three most important groups of infectious (viral) carcinogens [80,81]. In females, HPV infections on a global scale account for more than 50% of infection-linked cancers, in males for barely 5% [80]. Vaccines against the high-risk HPV types 16 and 18 represent...
the first preventive vaccines directly developed to protect against a major human cancer (cervical carcinoma), and its precursor steps (cervical intraepithelial neoplasia (CIN)) [82–84].

EBV, a human herpesvirus, is associated with a wide variety of malignant tumors [85,86]. The expression of the latent viral RNAs is under strict, host-cell dependent transcriptional control [87]. This results in an almost complete transcriptional silencing of the EBV genome in memory B cells. In tumor cells, germinal center B cells and lymphoblastoid cells, distinct viral latency promoters are active. Epigenetic mechanisms contribute to this strict control. In EBV-infected cells, epigenetic mechanisms also alter the expression of cellular genes, including tumor suppressor genes. In immortalized oral keratinocytes, for example, EBV infection produced genome-wide gene methylation and recapitulated the methylation patterns seen in EBV-associated neoplasia [88]. In nasopharyngeal carcinoma, the hypermethylation of certain cellular promoters is attributed to the upregulation of DNA methyltransferases by the viral oncoprotein latent membrane protein 1 (LMP1) via JNK/AP1-signaling.

The proposed role of a number of other viral latency products in the epigenetic dysregulation of the cellular genome (reviewed in Ref. [87]) remains to be completely understood. Analysis of epigenetic alterations in EBV-associated neoplasms may result in a better understanding of their pathogenesis and may facilitate the development of new therapies [85].

Proliferation is necessary for premalignant cells to accumulate genetic alterations and to acquire a transformed phenotype [89]. However, each cell division is associated with a progressive shortening of the telomeres, which can suppress tumor development by initiating senescence and irreversible cell cycle arrest. Therefore, the ability of virus-infected cells to circumvent the senescence program is essential for the long-term survival and proliferation of infected cells and the likelihood of transformation. Consequently, multiple strategies are being used by human DNA and RNA tumor viruses to subvert telomerase functions during cellular transformation, and carcinogenesis [90]. EBV, Kaposi sarcoma-associated herpesvirus, HPV, hepatitis B virus, hepatitis C virus, and human T-cell leukemia virus-1 each can increase transcription of the telomerase reverse transcriptase. Several viruses appear to mediate cis-activation or enhance epigenetic activation of telomerase transcription [90,91]. EBV and HPV have each developed posttranscriptional mechanisms to regulate the telomerase protein. Finally, some tumor virus proteins can also negatively regulate telomerase transcription or activity. It is likely that, as future studies further expose the strategies used by viruses to deregulate telomerase activity and control of telomere length, novel mechanisms will emerge and underscore the importance of increased telomerase activity in sustaining virus-infected cells, and its potential in therapeutic targeting.

Endogenous retrovirus-like elements, or ERVs, are an abundant component of all eukaryotic genomes. Their transcriptional and retrotranspositional activities have great potential for deleterious effects on gene expression. Consequences of such activity may include germline mutagenesis and cell transformation. As a result, mammalian genomes have evolved means of counteracting ERV transcription and mobilization. In a review by Mak-sakova et al. [92], they discuss epigenetic mechanisms of ERV and LTR retrotransposon control during mouse development, focusing on involvement of DNA methylation, histone modifications, small RNAs and their interaction with one another, and the relevance of research performed in the mouse system may be relevant for humans [92].

Boland et al. [30] have demonstrated a potential role for virus-induced carcinogenesis in CRC, in that most CRCs contain the DNA of JCV that encodes an onco- genic T-antigen, which is capable of interacting with key growth regulatory pathways (i.e., APC, p53, Wnt-signaling) in the colon, and has the potential to induce CIN. Thus, this suggests that JCV infection may be involved in the initiation of colorectal neoplasia. Apparently, JCV infection is ubiquitous and remains subclinical throughout the life of most individuals, but can cause disease when activated. Activation of the virus in the colon may lead to induction of the adenoma formation, CIN, and eventually CRC [93].

Fernandez et al. [59] investigated the complete DNA methylomes of the HPV16, HPV18, and HBV viruses and the DNA methylation analyzes all the transcription start sites of EBV obtained by bisulfite genomic sequencing of multiple clones. The dynamic changes in the viral DNA methylome and their functional relevance in the natural history of the disease were investigated. The researchers found that the DNA methylome of these viruses evolve from an unmethylated to a highly methylated genome in association with the progression of the disease, from asymptomatic healthy carriers, through tissues with chronic infection and premalignant lesions, to the full-blown invasive cancers [59]. One interpretation of this finding given by the authors is the possibility that DNA methylation might be a device to camouflage the virus from the human immune system. Further, the investigators suggest that the DNA methylomes found in the study could be used for further research in order to understand how the viral proteins themselves are able to use the human DNMTs to favor the establishment of persistent infection [59]. Also, the potential clinical applications of these findings include the non-invasive detection of methylated viral genomes in biological fluids, serum, and blood [59].

VII. EPIGENETIC EPIDEMIOLOGY
BACTERIAL INFLUENCE ON THE CELL CYCLE

The mammalian cell cycle is involved in many processes and, thus, it is not surprising that many bacterial pathogens manipulate the host cell cycle with respect to these functions. Cyclomodulins are a growing family of bacterial toxins and effectors that interfere with the eukaryotic cell cycle, and include cytolethal distending toxins (CDTs), vacuolating cytotoxin, the polyketide-derived macrolide mycolactone, cycle-inhibiting factor, cytotoxic necrotizing factors, dermonecrotic toxin, Pasteurella multocida toxin and cytoxin-associated antigen A.

Of particular interest are the CDTs [94]. These toxins are known to influence the control system of eukaryotic cells, with mechanisms depending on the cell type involved. For example, CDTs may initiate a eukaryotic cell cycle block at the G2 stage prior to mitosis—and effect which is produced by a number of bacterial pathogens [94,95]. The functional CDT is composed of three proteins; CdtA, B, and C. CdtB potentiates a cascade leading to cell cycle block, and CdtA and CdtC function as dimeric subunits, which bind CdtB and delivers it to the mammalian cell interior. Once inside the cell, CdtB enters the nucleus and exhibits a DNase I-like activity that results in DNA double-strand breaks. The eukaryotic cell responds to the DNA double-strand breaks by initiating a regulatory cascade that results in cell cycle arrest, cellular distension, and cell death. The result of CDT activity can differ somewhat depending on the eukaryotic cell types affected, but epithelial cells are arrested in the cell cycle at the G2/M boundary. The affected cells enlarge until they finally undergo programmed cell death. Of notice, an enlarged, cigar-shaped, elongated cell-type within colorectal adenomatous epithelium has been recognized by histopathologists for decades, and has more recently been described by morphometry and linked to an increased long-term risk for developing CRC [96], together with several alterations in cell-cycle and apoptosis regulating proteins in the same adenomas [97,98]. While speculative at this stage, it should be of interest to further pursue a potential connection between any given bacterial infection with altered cell morphology, changes in intracellular signaling, and the development of neoplasia.

Several issues remain to be elucidated regarding CDT biology, including a molecular understanding of how CDT interacts with DNA [99]. Of notice, other mechanisms, such as the cycle inhibiting factor (Cif) act in a strikingly similar fashion as the CDTs [100]. However, while CDTs inhibit the G2/M transition by activating the DNA-damage checkpoint pathway, Cif does not cause phosphorylation of histone H2AX, which is associated with DNA double-stranded breaks—thus, Cif works through a DNA damage-independent signaling pathway. Furthermore, is has been demonstrated that toxin capabilities may be transferred between bacteria [101].

CONCLUSIVE REMARKS

Modulation of host transcription by microbial pathogens is now a well-accepted concept. For one, the fact that histones can be modified at specific promoters during infection starts to shed light on some of these important issues. There is a need to further determine the molecular mechanisms involved in epigenetic modifications induced by bacteria and viruses. Whether epigenetic changes are specifically induced by the microbiota to subvert normal host responses or are the normal host responses to this pathogen will have to be further investigated for specific diseases and conditions. Future work will determine how these epigenetic phenomena develop and influence disease processes, with potential new therapeutic implications evolving from this research field.


Escherichia coli


