Nilanjana Maulik · Tom Karagiannis
*Editors*

# Molecular Mechanisms and Physiology of Disease

## Implications for Epigenetics and Health

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*This book is dedicated to our beloved grandmother (didibhai) for being there always for us*

Nilanjana Maulik, Ph.D., F.A.H.A.

# Preface

Although coined in the 1940s by Conrad Waddington and now representing an intense field of biomedical research, the precise definition of Epigenetics remains controversial. In its simplest form epigenetics refers to heritable changes that are not due to changes in the underlying DNA sequence. Whereas DNA methylation is a well-known and relatively well-characterized epigenetic mechanism, it is still debatable as to whether other processes such as histone posttranslational modifications represent epigenetics per se, given their transient nature. In this volume, we discuss DNA methylation, histone posttranslational modifications and their alteration by chromatin-modifying compounds, and regulation of gene expression by noncoding RNA and mi-RNA as part of the epigenetic umbrella. Indeed, this volume is quite broad consisting of an array of topics including epigenetic effects in various diseases such as autoimmune conditions, cardiovascular diseases, and asthma. The second part of this volume is dedicated to cancer and highlights epigenetic dysregulation in malignancy as well as a number of chapters related to emerging cancer therapeutics.

In Chap. 1, Hussain discusses epigenetic mechanisms associated with childhood diseases. Hussain provides an excellent overview of epigenetic processes in health and disease. This chapter provides a detailed overview of various childhood conditions with an epigenetic association, including various imprinting disorders, childhood malignancy, and diabetes. This is a very extensive chapter, and it highlights the broad spectrum of childhood diseases that can be linked with epigenetic perturbations. Detailed molecular and epigenetic aspects are further explored in Chap. 2 by Torano et al. in the context of neuronal differentiation. In this chapter, Torano et al. describe epigenetic processes including DNA methylation, histone posttranslational modifications, chromatin remodeling and regulation by noncoding RNA in great detail in the context of maintenance of pluripotency. They further explore how aberrant epigenetic mechanisms are associated with a myriad of neurological diseases and malignancy. Further, a detailed overview of epigenetics is provided by Westerland in Chap. 3. This chapter provides a thorough outline of the major epigenetic mechanisms and potential interventions using DNA methyltransferase inhibitors (DNMTs) and histone deacetylase inhibitors (HDACi), compounds which

are further explored in the following chapters. In a different light, regulation by modifying the histone tails by proteolytic processing is described in Chap. 4 by Mandal et al. Although not as well characterized as the other processes such as histone acetylation or methylation, as described by Mandal et al., proteolytic processing of the core histones is emerging as an important form of regulation of chromatin organization.

In Chap. 5, Turker et al. describe health implications associated with probiotics and their metabolites. This chapter focuses on anti-inflammatory effects of major probiotic metabolites which include the well-known short chain fatty acid HDACi, butyrate. In Chap. 6, Coppede and Migliore provide an excellent outline and overview of the epigenetic mechanisms associated with autoimmune diseases. Indeed, in Chap. 6 epigenetic phenomena, particularly aberrant mi-RNA processes and histone posttranslational modifications, related to major autoimmune conditions including systemic lupus erythematosus and rheumatoid arthritis are identified and described. Emerging evidence for deregulated epigenetic mechanisms associated with other autoimmune diseases such as Sjrogren's disease, psoriasis, and multiple sclerosis is also discussed in Chap. 6. Chapter 7 continues the disease-specific consideration with a discussion by Whayne, of epigenetic processes associated with cardiovascular disease. This also describes aspects of nutrition, including the well-known methyl-donor folate and tobacco use that are associated with disease. Nutritional aspects are further expanded in Chap. 8 by Burgio and Migliore, in the context of obesity and diabetes. This chapter details genetic and epigenetic mechanisms associated with metabolic syndrome, diabetes, and cardiovascular disease.

Both Chaps. 9 and 10 by Tortorella describe genetic and epigenetic mechanisms and emerging therapies associated with asthma. Traditionally, asthma has been viewed as a disease with a heritable genetic component which is exacerbated by various environmental exposures in early childhood or later in life. It is also becoming apparent that prenatal exposure can influence the risk of developing asthma in accordance with the Barker hypothesis of fetal programming (i.e., perturbations in nutritional or environmental conditions in utero lead to altered developmental programming of organs, influencing the propensity to develop the disease later in life). This idea has been investigated predominantly by exploring the use of tobacco and increased risk of developing various lung pathologies, including asthma later in life. In Chap. 9, Tortorella overviews epigenetic effects associated with prenatal and postnatal environmental exposures. Potential therapeutic avenues in the form of trefoil factor 2 (Chap. 10) and emerging nanotechnologies (Chap. 10) for managing asthma are also explored.

Although mechanisms in cancer formed parts of some of the preceding chapters, the remaining chapters are focussed entirely on the aspects of malignancy including carcinogenesis, cancer metabolism, and emerging cancer therapeutics. For example, in Chap. 11, Masih et al. describe the epigenetic effects of one-carbon metabolism and aberrant DNA methylation in cancer. In this chapter, Masih et al. provide a thorough overview of nutrients involved in one-carbon metabolism including folate, vitamins B6 and B12, choline, and betaine. This chapter also provides a

comprehensive review of the effects of these nutrients in ten common malignancies of the gastrointestinal and reproductive systems. A different aspect of aberrant metabolism, namely the Warburg effect, in cancer is the subject of Chaps. 12 and 13 by Molino et al. and Balding et al., respectively. The Warburg effect which postulates that cancer cells predominantly utilize aerobic glycolysis rather than mitochondrial respiration was first described in the 1920s. Although various small groups continued the work sporadically, it was not until the past few years that this topic is reemerging and has been recognized as a critical component of cancer biology. Although the epigenetic component of the Warburg effect is still not well characterized, there is emerging evidence for important links. Indeed, the epigenetic component of all aspects of carcinogenesis is now widely recognized, and epigenetic lesions are now not only being considered as the hallmarks of disease but also being incorporated into the multi-hit model. This is described and characterized by Migheli and Migliore in Chap. 14. Keeping on the topic of epigenetics, nutrition, and metabolism in Chap. 15, Pan et al. describe the effects of nutrition and energy intake in colon cancer. In this chapter, the key colon cancer-associated oncogenes and tumor suppressor genes are discussed in the context of tumor progression, and the cancer cell growth inhibitory effects of nutritional factors are described. Importantly, the potential prophylactic role of an anti-inflammatory diet in colon cancer is outlined.

The final three chapters in this volume deal with potential cancer therapeutics. In Chap. 16, Mazarakis describes the potential protective and therapeutic effects of dietary antioxidants and chromatin-modifying compounds in cancer. This chapter focuses on phenolic compounds from olive and HDACi from a variety of foods. Similarly, the potential of HDACi in combinatorial therapies, in this case phototherapy, is discussed in Chap. 17 by Sung. HDACi have emerged as an important new class of anticancer therapeutics with two compounds, suberoylanilide hydroxamic acid (Zolinza) and depsipeptide (Romidepsin), being approved by the FDA for the treatment of cutaneous T-cell lymphoma and more recently depsipeptide for peripheral T-cell lymphoma. It is widely accepted that the clinical utility of HDACi will predominantly involve combination with other anticancer therapeutics. In Chap. 17, the potential anticancer effects of combinations of HDACi with ultraviolet phototherapy are considered. Anticancer therapy is also the subject of Chap. 18 by Mah et al. in which, nanoparticle formulations for targeted drug delivery are described. There is much excitement regarding the potential of nanoparticles to deliver cytotoxic agents including epigenome-modifying siRNA to selectively induce apoptosis and cell-death in cancer cells. This chapter outlines varying approaches for appropriate nanoparticle preparations with potential clinical applicability.

Overall, this volume encompasses a wide range of topics related to epigenetic mechanisms in health and disease. The scope of the volume spans from descriptions of fundamental epigenetic processes to potential epigenetic interventions for preventing or treating various diseases. Epigenetic phenomena associated with numerous conditions including autoimmune diseases, cardiovascular disease,

asthma, and a variety of malignancies are detailed. Given the scope, this volume would be of appeal to a wide readership including those with interests in epigenetics and chromatin biology, disease-specific epigenetic aberrations, and emerging epigenetic-based cancer therapies.

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I am eternally grateful to my friends, students, and colleagues in the Epigenomic Medicine Laboratory at the Baker IDI Heart and Diabetes Institute for their help and support. You have all made my life a bit easier. This volume is dedicated to all of you.

Tom Karagiannis, Ph.D.

# Contents

# Chapter 1
# Epigenetics in Childhood Health and Disease

**Naveed Hussain**

**Abstract** The part that epigenetic modifications play in the development of childhood health and disease is being established by ongoing research and discoveries in this field. Right after the establishment of the genetic blueprint at the time of fertilization and zygote formation, the human organism is subject to complex and necessary series of epigenetic modifications of this genetic code to bring about differentiation and development. There are well-recognized stages during this process where the epigenetic changes have the most lasting and profound effects and these are considered critical periods of vulnerability. Depending on the timing of insult within the critical time periods in the human life cycle where epigenetic modifications occur, the effect on health and disease could be transient or may persist across many generations. In this chapter classification of human conditions based on the timing and etiology of epigenetic change has been attempted. Beginning with the time of fertilization of the egg with the sperm and subsequent fetal development and continuing from birth to the attainment of puberty, adulthood, and the generation of gametes for the next generation, the list of conditions where epigenetics has been found to play a key role have been listed and described. The role of epigenetics in certain special circumstances such as assisted reproductive technologies, developmental origins of adult disease, and in the brain and behavioral disorders are also discussed. Understanding the critical period of causation of epigenetic effects may yield important clues in prognostication and in designing therapeutic approaches for these conditions.

**Keywords** Infant • Newborn • Epigenetics • Imprinted genes • Development • DNA methylation • Histone modification • microRNA • X-inactivation

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## 1.1 Introduction

If genetics and the transmission of the DNA code could be considered the indelible ink that writes on the parchment of inheritance, epigenetics could be thought of as the annotations made by pencil to this document; not surprisingly, erasures and alterations to this penciled code could leave smudges and marks that could alter the reading of this manuscript (Gosden and Feinberg 2007). If genetics is the link of transmission of DNA code from generation to generation, epigenetics is the interpretation of this code in individual cells and tissues of the organism within a particular generation based on its unique environment. Therefore it is not surprising that a single-celled zygote with one set of genetic instructions can differentiate into tissues as varied as bone and brain and that identical twins can differ significantly in their normal phenotypes and susceptibility to diseases.

## 1.2 Waddington and Historical Background

Waddington in 1952 described the term epigenetics—"the science concerned with the causal analysis of development and the regulative capacities of the early embryo" (Waddington 1952). It was subsequently recognized as a "science of developmental processes in general" by Huxley (1956). Recently, the field of epigenetics has helped provide important clues to determinants of health and disease in animal models and humans. The use of epigenetics is also being made in drug development and design (Marx 2012). Various processes have been found to be responsible for inducing epigenetic effects with the most well known being DNA methylation, histone modifications, and microRNA (miRNA) modulation of transcription.

The discovery of genetic imprinting led to the identification of DNA methylation as the first biological process linked with epigenetic modifications. Genetic imprinting, which distinctly involves the maternal or paternal genome, was first described using gamete transfer experiments with mice in 1984 (McGrath and Solter 1984; Surani et al. 1984); the first imprinted gene *igf2r* was identified soon thereafter in 1991 (Barlow et al. 1991; Bartolomei et al. 1991; DeChiara et al. 1991). Correlation of genetic imprinting with human disease was first reported for Prader–Willi syndrome (PWS) in 1989 (Nicholls et al. 1989); although it was later in 1993 that DNA methylation was identified as the process responsible for genetic imprinting (Li et al. 1993).

Other mechanisms for epigenetic modifications were soon discovered pertaining to histone modifications. The state of nuclear chromatin whether tightly compacted (heterochromatin) or loose (euchromatin) has long been known to be associated with silencing or activation of DNA function and thus important in regulation of cell function (Brown 1966). However, it was in late 1990s that the role of histone proteins in folding of chromatin and altering availability of active regions of genes to transcriptional factor regulation was first suspected (Jones et al. 1998; Nan et al. 1998);

followed soon after by a more definitive review implicating histone modifications in epigenetic regulation (Jenuwein and Allis 2001). Of all the ways histone proteins could be modified (methylation, acetylation), acetylation is perhaps the most important. Histone acetyl transferases (HATs) and histone deacetylases (HDACs) are key enzymes in regulating the balance of lysine acetylation of histones.

In early 2000s, another mechanism of epigenetic regulation was found to be the microRNA-induced modulation of mRNA transcription. microRNAs are evolutionary conserved noncoding nucleotides with 19–24 bases that regulate the translation and degradation of specific mRNA targets based on base pairing to complementary sites mainly in the 3′-untranslated region of the target mRNAs (Ambros 1989; Lagos-Quintana et al. 2001; Lau et al. 2001; Lee and Ambros 2001; He and Hannon 2004). miRNAs are uniquely organized within the genome and can be transcribed either from distinct intergenic loci or as a by-product of a host gene (Chen and Meister 2005; Bartel 2009). With the completion of the ENCODE project involving the "silent" non-gene-linked areas of the human DNA, it is being increasingly recognized that other noncoding RNAs may also play an important role in epigenetic regulation (Maher 2012).

There appears to be a close interaction between various mechanisms of epigenetic regulation. Some of the genes regulated by DNA methylation are for long noncoding RNAs (lncRNA) such as *H19* involved with *IGF2* regulation. DNA methylation can initiate chromatin condensation and histone modifications through methyl-DNA binding (MDB) proteins. Moreover imprinted domains transcribe numerous small noncoding RNAs (sncRNAs defined as <105 base pairs) which include a number of microRNAs (<24 bp). The imprinted domains in humans have the highest density of microRNAs with almost 7 % of microRNAs in humans encoded in this region (Labialle and Cavaille 2011; Girardot et al. 2012). Therefore the strict categorization of epigenetic processes is probably an oversimplification of a complexly interrelated process; but for the purpose of description and classification these processes will be dealt with separately.

## 1.3 Scope of the Chapter

Mammalian life cycle is characterized by an initial near complete reset of genetic potential (establishing totipotency) at the stages of primordial germ cells (PGCs) and early preimplantation embryo (pre-IE); with later epigenomic reprogramming in post-implantation embryo (post-IE); followed by extended phases of cellular differentiation, unidirectional specific development, and effective lineage-restriction modulated through a complex network of transcription factors (Hackett and Surani 2013). The epigenome is represented by a combination of systems of DNA methylation, histone modifications, and small RNA influences regulating gene expression through mitosis and meiosis of cells in the developing organism. The epigenome plays a significant role in gene–environment interactions and it is likely that there are critical periods in early development where the normal process of establishment

of the epigenome puts the organism at higher risk. In this report we plan to use the "critical period" approach in categorizing the epigenetic basis of disease and altered development. Most of the discussion in this chapter is based on DNA methylation changes and effects related to other epigenetic mechanisms such as histone modifications and microRNA will be mentioned briefly as appropriate. A detailed discussion of changes related to histones and microRNA is beyond the scope of this chapter.

## 1.4 Concept of "Critical Period"

The genetic blueprint of an organism is constantly influenced by its environment for the ultimate expression of the phenotype. The capability of the organism in responding to the environmental influences indicates its "developmental plasticity" (Burggren and Reyna 2011). Developmental plasticity connotes not only a manifestation of genotype–phenotype interaction during development but also defines a predictable series of reaction norms that persist at the individual, population, or species level (Hutchings 2011; Symonds et al. 2009). If the molecular basis of this developmental plasticity is transmittable across generations, it is probably a result of epigenetic modifications of the genome. Critical period of hormonal activity and its epigenetic actions have been well documented for sex differences in the brain (Nugent and McCarthy 2011) and also been implicated in the "Developmental Origins of Health and Disease (DOHaD)" hypothesis (Martin-Gronert and Ozanne 2012). Certain developmental transcription factors that have been shown to be epigenetically programmed by the early environment are PPARα, PDX-1, and HNF4α (Park et al. 2008; Martin-Gronert and Ozanne 2012; Lillycrop et al. 2005; Sandovici et al. 2011). The importance of the timing of insult causing the epigenetic changes is also highlighted in the Dutch Famine Cohort where it was noted that certain genes are affected by insults around the perinatal period (*IL10*, *INISIGF*, *LEP*, *ABCA1,* and *MEG3*) but in other genes such as *GNASAS* epigenetic changes were associated with exposure to famine later in gestation (Lumey et al. 2007).

The effect of a stressor (both in dosage and duration) in modifying genotypic expression varies based on the innate biological processes that are ongoing in that particular organism's development. There are certain periods where complex innate developmental changes provide a window of vulnerability that may be termed "critical periods." During this critical period, the dose and/or duration of the stressor may move the organism to a different trajectory of development. A certain threshold may determine if the change is permanent or temporary. The site of the change, whether epigenetic or non-epigenetic will determine if the change may persist in one or many generations (Burggren and Reyna 2011). Thus there may be a biological basis for the truism attributed to Friedrich Nietzsche: "That which does not kill us makes us stronger"; maybe not quite "stronger" but certainly "more adaptable."

The difference in timing of generation of male and female gametes and the differences that exist in the hormonal milieu in the two sexes during periods of

development may be part of the reason that sexual dimorphism is an important consideration in determining the critical period of epigenetic vulnerability (Vige et al. 2008). This is also an important consideration in designing experimental and evaluating epidemiologic studies (Vige et al. 2008).

Another important consideration is the relationship of a particular genetic or epigenetic modification to other interactions within the organism to what is being increasingly referred to as the "interactome" (Venkatesan et al. 2009). Using network-based approaches to understanding systems biology, it is getting increasingly recognized that genes and proteins constitute nodes that link to a network with central and peripheral hubs in which the central hubs are associated with vitally essential elements the abnormalities of which are embryo-lethal and hence do not manifest disease; the peripheral hubs of relatively nonessential elements being compatible with survival are responsible for disease (Barabasi et al. 2011; Barabasi and Oltvai 2004). Since each cell within an individual organism has a unique, time-varying epigenome, an integrated molecular pathological epidemiology (MPE) approach may be needed to fully understand and classify these processes (Ogino et al. 2013). To some extent these above mentioned approaches have been used in this chapter to classify conditions that have an epigenetic basis.

## 1.5 DNA Methylation Dynamics During Life Cycle

Of the various mechanisms of epigenetic regulation, DNA methylation dynamics during the mammalian life cycle are most well studied (Nafee et al. 2008) (Fig. 1.1). Similar life cycle patterns for histone modifications and noncoding RNA regulation are still under intense investigation. DNA methylation occurs at 5′ of cytosine (5mC) within a CpG dinucleotide sequence and is critical for embryonic development in mammals. DNA methylation plays an important role not only in genomic imprinting but also in gene repression, X chromosome inactivation, and transposon silencing among other cellular processes (Bird 2002). The most common sites within the genome for DNA methylation is the intergenic region and in repetitive sequences (such as satellite repeats, and long and short interspersed nuclear elements—LINEs and SINEs). However, the promoter sequences of genes which are mostly GC-rich are usually unmethylated (Weichenhan and Plass 2013). This may be due to the fact that 5mC in DNA is inherently unstable and tends to deaminate to thymidine thus depleting the bulk of genome of CpG motifs except where they are clustered in regions known as CpG islands (CGIs). The CGIs are usually associated with promoters of genes. Generally most CpG sites within the matured genome are by default methylated irrespective of the genetic context but the CGIs remain unmethylated during development (Meissner et al. 2008; Suzuki and Bird 2008; Hackett and Surani 2013).

The mechanism by which methylated DNA (methylated cytosine—5mC) controls gene expression is by the attraction and binding of MDB proteins and the subsequent chromatin condensation into a transcriptional repressive configuration

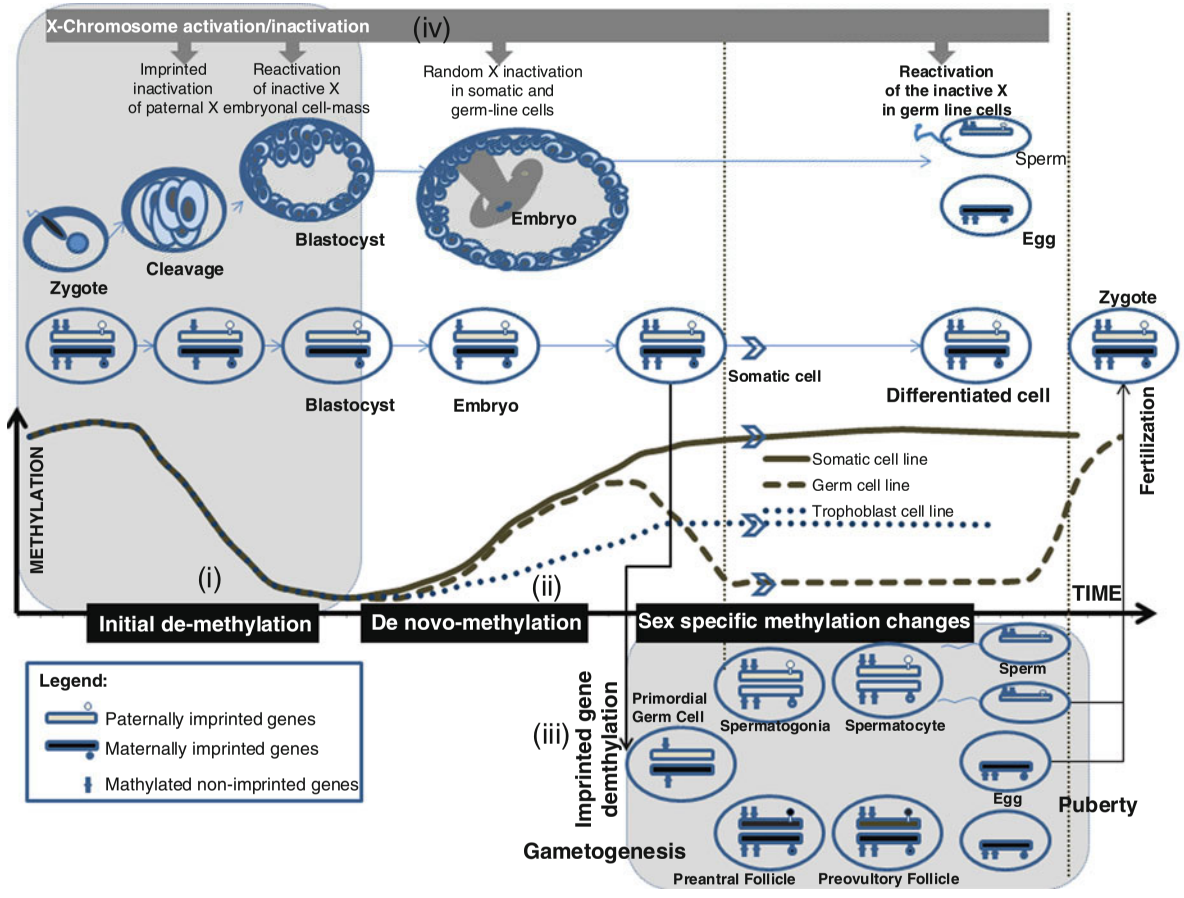



**Fig. 1.1** Periods of epigenetic changes human development [adapted from (Nafee et al. 2008)]. The change is methylation status, i.e.,—(i) initial demethylation, (ii) de novo methylation, for somatic, trophoblastic, and (iii) sex-specific methylation changes in germ cell line, are depicted on an arbitrary time line. Also superimposed on the same timeline are timings of (iv) X chromosome activation and inactivation to illustrate how critical periods of vulnerability are concentrated in the early stages of human development [*reprinted with permission from ARS*]

(Bird 1992; Newell-Price et al. 2000). Four mammalian MDB proteins identified so far are MeCP2, Mdb1, Mdb2, and Mdb4 (Lan et al. 2010).

During mammalian development, immediately after fertilization, DNA derived from both gametes undergoes dynamic remodeling to give rise to a globally demethylated state or a metaphoric "clean slate" on which subsequently, a progressively lineage-specific methylation pattern (methylome) is established for the uniquely new individual and then maintained through subsequent mitosis (Hackett and Surani 2013; Wossidlo et al. 2011). The zygote with subsequent stages of cell division and implantation continues with extensive demethylation giving rise to the pluripotent inner-cell mass which has the capability to form embryonic stem (ES) cells in vitro. Embryonic lineage specification then occurs simultaneously with de novo methylation as the organism locks-in more specific cellular identity. At a future point (corresponding to E6.5 in mice and post-conception day 13–15 in humans), the inner cell mass continues to progress with differentiation and cellular specialization of somatic tissue; the cells destined to form PGC undergo another demethylation phase (E12.5 in mice) that lasts until gamete-specific methylome is developed and imprinting of maternal and paternal genes is completed (Hackett and Surani 2013; Horsthemke 2010). Interestingly, in the germline processes remethylation of the genome completes in the male germline before birth (at approximately embryonic day 18.5 in mice) (Davis et al. 2000); but in the female, remethylation of the oocyte does not occur until after birth and puberty when it is initiated every time a crop of follicles is recruited (Reik and Walter 2001). It is also worth noting that during the initial phase of demethylation after fertilization, the imprinted genes of the germline and the gametes do not get demethylated and preserve their methylation imprint. These gametes when they come together at fertilization from different parents to form the zygote start the cycle of demethylation once again. Importantly the erasure and reestablishment of parent of origin-specific imprints is vital for creating totipotency in the zygote (Hajkova et al. 2002).

Throughout cell division, the maintenance or preservation of methylation of DNA is done by the ubiquitously expressed DNA methyltransferase 1 (DNMT1) which methylates the hemimethylated CpG dinucleotides in the nascent DNA after replication (Hermann et al. 2004). Another important factor in maintenance of methylation is the KRAB zinc-finger protein ZFP57 (Li et al. 2008). When needed, for establishment of new methylation patterns, de novo DNA methyltransferases 3A and 3B (DNMT3A and DNMT3B) are utilized and DNMT3L may act as their cofactor (Hata et al. 2002). Demethylation was previously thought to occur passively in the absence of DNMT1 but recently active demethylation systems have been identified. Active demethylation is mediated by Tet-methylcytosine dioxygenases (Tet 1 and Tet 3) that convert 5-methylcytosine by oxidation to 5-hydroxymethylcytosine and then to 5-formylcytosine (5fC). Subsequently, base excision repair pathway and thymine-DNA glycosylase (TDG) may be involved in removal of these oxidation products (He et al. 2011; Tahiliani et al. 2009; Rivera and Ross 2013; Seisenberger et al. 2013).

Given the complexity of DNA methylation processes that occur at various times in the life cycle, it is not surprising that there may be many critical periods of vulnerability where external or inherited factors may alter the methylome and

depending on the timing of these alterations, the effect may be seen in the zygote or the inner cell mass or the developing germline or the fully differentiated somatic cell; consequently the ultimate expression of the change may be seen in the whole organism within its lifetime or through the gametes manifest the change in a future generation. A good example of the transgenerational effect is the development of cancer seen in female offspring of women who were exposed to diethylstilbestrol (DES) during the germline development of their fetus in early pregnancy (Li et al. 2003; Walker and Gore 2011).

## 1.6 Classification of Epigentics Related Disorders in Childhood

Based on our understanding of DNA methylation dynamics during the human life cycle, it is possible to classify disorders of DNA methylation and other epigenetic mechanisms by the critical time period in which the susceptible cells are affected, starting from the PGCs developing in the genital ridge of the embryo to the transmission of these epigenetic marks to the subsequent generation at the point of fertilization (Fig. 1.2). Long-term effects of the epigenetic change would probably depend on the timing of the change and to the potential generation of cells and tissue that particular cell can produce. In the case of PGCs, the effect of epigenetic imprints may last up to the F3 generation of the organism (Skinner et al. 2013); but an epigenetic change in a mature somatic cell will probably be restricted to that cell's specific progeny in the tissue (Teschendorff et al. 2013). The former could result in significant organism-wide changes involving multiple tissue lines and the latter could significantly impact a single tissue as in the development of malignancy. For the purpose of this chapter, mostly DNA methylation-related epigenetic changes are described and changes related to histone modifications and microRNAs will only be mentioned as appropriate.

A comprehensive classification of all disorders associated with epigenetic changes is provided and most of these disorders have an effect on childhood health and disease. Broadly the disorders that result in epigenetic changes may be classified into two main categories:

1. Primary epigenetic disorders—(Table 1.1) where the changes primarily affect the epigenome without any accompanying or predisposing genetic changes. The inheritance of these disorders is variable and the ability for therapeutic manipulation is more robust because the underlying functional proteins involved are not defective but only their regulation is altered.
2. Secondary epigenetic disorders based on primary genetic conditions—(Table 1.2) where epigenetic changes occur because of primary changes in the genome and its gene product. The inheritance of these disorders follows classical Mendelian or chromosomal inheritance patterns. Since a defect in functional protein may be responsible for the changes seen, the ability for therapeutic manipulation is much more limited.

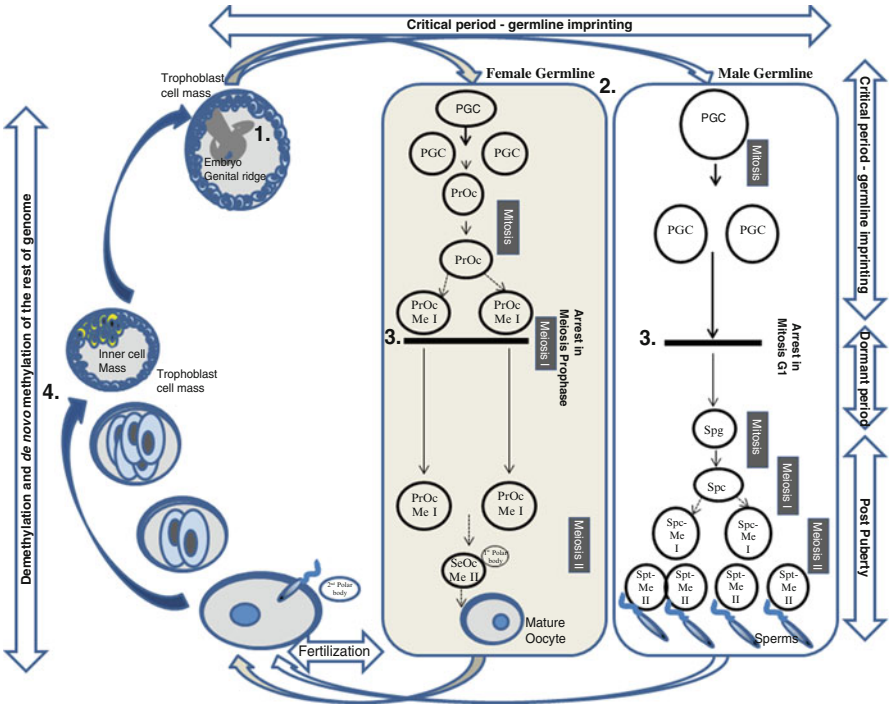



**Fig. 1.2** Germline methylation cycle. *1*. Primordial germ cells originate in epiblast cells arising in the posterior primitive streak at E7.5 in mice (corresponding to post-conception days 15–18 in humans). They then migrate, starting to the genital ridge around E8.5 in mice (post-conception day 19 in humans) and complete their migration around E11.5 in mice (post-conception days 32–33 in humans). *2*. It is soon after the migration to the genital ridge that imprinted genes are demethylated and start the process of reestablishment of the imprint with remethylation. This period of DNA demethylation is between E11.5 and E12.5 in mice and corresponds approximately to post-conception days 33–40 in humans. Following that the genomes of the developing gametes are de novo methylated and acquire imprints and this process continues until at least E18.5 in male mice corresponding to post-conceptional day 49–52 in human males. *3*. In the fetal period, there is an arrest of germline development which in males is in G1 phase of mitosis after completion of remethylation but in females the arrest occurs after initiation of meiosis in the prophase of Meiosis I. In females however, the remethylation can occur until the oocyte matures before ovulation; thus going into puberty and beyond. In fact, female secondary oocytes do not complete their meiosis until right at fertilization with the formation of the second polar body. *4*. After fertilization of male and female gametes to form the zygote, the germline imprinted genes do not participate in the genome-wide demethylation and de novo methylation that is critical in establishing of the embryo and trophoblastic cell lines and their further differentiation. With formation of the epiblasts in the embryo the cycle of germline imprinting starts again. Abbreviations used in the figure: *PGC* primordial germ cells, *PrOc* primary oocyte, *PrOc MeI* primary oocyte undergoing Meiosis I, *SeOc MeII* secondary oocyte undergoing Meiosis II, *Spg* spermatogonia, *Spc* spermatocyte, *Spc-MeI* spermatocyte undergoing Meiosis I, *Spt-MeII* spermatids undergoing Meiosis II

**Table 1.1** Primary epigenetic disorders

| Conditions | Chr | Genes |
|---|---|---|
| I.A. Effects at the primordial germ cell stage of development (disorders of imprinting)[a] | | |
| I.A.1. BWS and SRS syndromes (chromosome 11p15) | | |
| I.A.1.1. Beckwith–Weidemann synd. (BWS) | 11p15pat | *IGF2*, *H19*, *CDKN1C*, *KVLQT1*, *KCNQ10T1* (*LIT1*) |
| I.A.1.2. Silver–Russell synd. (SRS) | 11p15mat; 7q13 | *IGF2*, *H19*, *MEST*, *PEG1*, *CPA4*, *COPG2*, *MESTIT*, *CIT2*/*COPG2IT1* |
| I.A.2. PWS and Angelman (chromosome 15q11) | | |
| I.A.2.1. Prader–Willi syndrome (PWS) | 15q11-13pat | *SNURF-SNRPN*, *NDN*, *MKRN3* *MAGEL2* |
| I.A.2.2. Angelman syndrome (AS) | 15q11-13mat | *UBE3A* |
| I.A.3. Albright hereditary osteodystrophy-like syndromes | | |
| I.A.3.1. PHP-Ia | 20q13.3mat | *GNAS1* |
| I.A.3.2. PPHP | 20q13.3pat | *GNAS1* |
| I.A.4. Transient neonatal diabetes mellitus type I | 6q24 | *PLAG1*, *HYMA1* |
| I.A.5. UPD 14 syndromes | | |
| I.A.5.1. Wang syndrome or UPD(14)pat | 14q32.2pat | *DLK1*, *RTL1*, *MEG3* (*GTL2*), *MEG8* |
| I.A.5.2. Temple syndrome or UPD(14)mat | 14q32.2mat | *DLK1*, *RTL1*, *MEG3* (*GTL2*) |
| I.A.6. Multilocus hypomethylation defects (MHD) | | *PLAG1*, *ZAC1*, *ZFP57*, *NLRP2*, *CTCF*, *MBD3*, *SNRPN*, *PEG3*, *NESPAS*, *H19* |
| I.A.7. Maternal UPD(16) | UPD(16) | |
| I.A.8. Germline epimutations associated with neoplasia | | |
| I.A.8.1. Lynch syndrome | ? | *MLH1*, *MSH2* |
| I.A.8.2. Familial paragangliomas | 11q23 | *SDHD* |
| I.A.8.3. NOEY2 gene-related cancers | ? | *NOEY2* |
| I.B. Effects from conception to completion of embryogenesis including first trimester | | |
| I.B.1. Turner syndrome and selective $X^m$ or $X^p$ effects | Xp | *Xlr* |
| I.B.2. Epigenetic influences on trophoblast and placenta | UPD(14); 6q24.2 | *RTL1*, *PEG11*, *DLK1*, *DIO3*, *NLRP7*, *PLAG1* |
| I.B.3. Epigenetic effects on somatic cells—early development | | |
| I.B.3.1. Twin growth discordance | Many | *H19*, *IGF2* |
| I.B.3.2. Prematurity | Many | *NFIX*, *RAPGEEF2*, *MSRB3* |
| I.B.3.3. Infantile biliary atresia | ? | *ITGAL*(*CD11A*), *RASSF1A*, *p16*, *CDH1*, *TFPI2*, *NPTX2*, *APC* |
| I.B.3.4. Wilms' tumor | 5; 11p15 | *H16*, *IGF2*, *WT1*, *CTNNB1*, *WTX*, *TP53* |
| I.B.3.5. Hepatoblastoma | 11p15 | *H19* |
| I.B.3.6. Retinoblastoma | | *RB1* |
| I.B.3.7. Others | | *H19*, *Igfr2*, *U2af-rs1* |
| I.C. Effects from mid-late gestation and continuing to postnatal development until adulthood | | |
| I.C.1. Postnatal stress and epigenetics | ? | *NR3C1* |

(continued)

**Table 1.1** (continued)

| Conditions | Chr | Genes |
|---|---|---|
| I.C.2. Epigenetic basis of adult onset disease | Many | *IL10*, *GNASAS*, *INSIGF*, *LEP*, *ABCA1*, *MEG3*, *PPARα*, *PDX1*, *HNF4α* |
| I.C.3. Malignancy and epigenetics | Many | |

By convention human genes are represented in capitalized italics and mouse genes in lower case italics

[a]A number of disorders of imprinting are common with chromosomal rearrangements and uniparental disomy, however, purely epigenetic changes can potentially case the same condition

*Abbreviations*: *Chr*. chromosomes, $X^m$ maternally derived X chromosome, $X^p$ paternally derived X chromosome, *synd*. syndrome

**Table 1.2** Secondary epigenetic disorders due to disorder of genetic DNA

| Conditions | Chr | Genes |
|---|---|---|
| II.A. Gene abnormalities with DNA methylation effects | | |
| II.A.1. ICF (type 1) syndrome | 1, 9, 16 & X | *ZBTB244*, *SYK*, *SH3BP5* |
| II.A.2. Rett syndrome—MeCP2 gene | | *MECP2*, *MKX*, *CKB*, *FYN* |
| II.A.3. Fragile X syndrome | Xq27.3 | *FMR1* |
| II.A.4. X-linked alpha-thalassemia/mental retardation syndrome (ATX-R) | Xq13 | *ATRX* |
| II.A.5. Fascioscapulohumeral dystrophy | 4q35 | *Multiple genes* |
| II.A.6. Hereditary sensory and autonomic neuropathy type I (HSAN1) | 19p13.2 | *Multiple genes* |
| II.A.7. Autosomal dominant cerebellar ataxia, deafness, and narcolepsy | | |
| II.B. Genetic syndromes causing histone modifications | | |
| II.B.1. Rubinstein–Taybi syndrome | 16p13.3 | *CREBBP*, *EP300* |
| II.B.2. Genitopatellar syndrome (GPS) | | *KAT6B* |
| II.B.3. Say-Barber-Biesecker-Young-Simpson syndrome (SBBYS) | | *KAT6B* |
| II.B.4. Coffin–Lowry syndrome | Xp22.2 | *RPS6KA3* |
| II.B.5. Sotos syndrome | 5q35.2 | *NSD1* |
| II.B.6. Weaver syndrome | | *EZH2*, *NSD1* |
| II.B.7. Brachydactyly-mental retardation syndrome (BDMR) | | |
| II.B.8. Kleefstra syndrome | 9q34.3 | *EHMT1* |
| II.B.9. Kabuki syndrome | X | *MLL2*, *KDM6A* |
| II.B.10. Siderius X-linked mental retardation syndrome (MRXSSD) | X | *PHF8* |
| II.B.11. Claes–Jensen X-linked mental retardation syndrome | X | *JARID1C* (*SMCX*) |
| II.C. Genetic mutations that affect noncoding RNAs | | |
| II.C.1. Amyotrophic lateral sclerosis | | *TARDBP* |
| II.C.2. DiGeorge syndrome | | *DGCR8* |
| II.C.3. Goiter, multinodular1, with or without Sertoli–Leydig cell tumors | 22q11. | *DICER1* |
| II.D. Chromosome deletion and rearrangements—epigenetic changes: | | |
| II.D.1. 9q subteloric deletion syndrome: | 9q | *EHMT1* |
| II.D.2. 46XY inversion(10)(q11.1;q21.3) | X, 10q | *TRIP8* |
| II.D.3. Wolf–Hirschhorn syndrome | 4p16.3 | *WHSC1L1* |

By convention human genes represented in capitalized italic and mouse genes in lower case italics

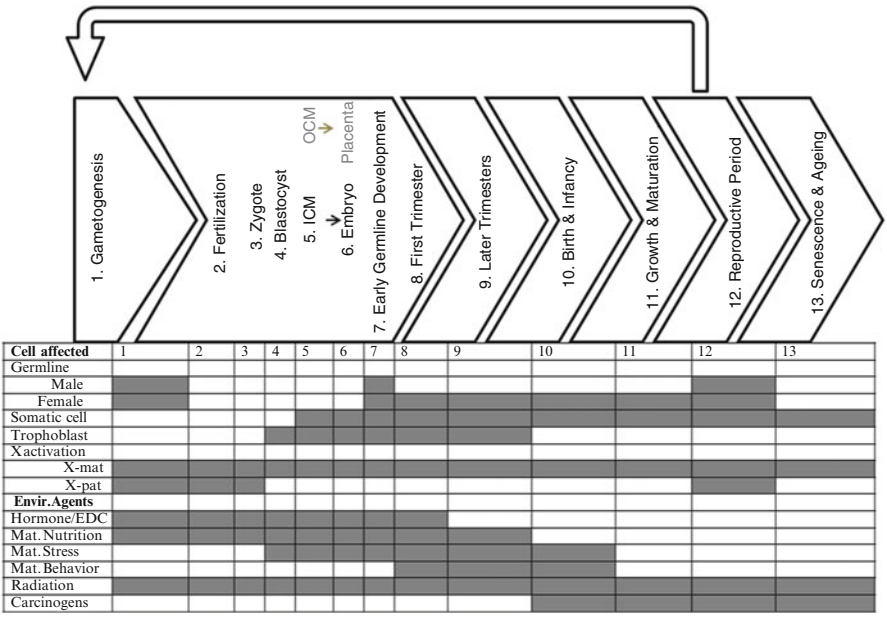
*Abbreviations*: *Chr* chromosomes

## 1.7 Primary Epigentic Disorders

The primary disorders of epigenetic regulation may be further classified into three groups based on the cell populations affected at critical periods of epigenetic changes that occur within an individual's lifetime (Table 1.1; Fig. 1.3).

### *1.7.1 Germline*

*Effects at the primordial germ cell stage of development* (*disorders of imprinting*): Most of the disorders in this category may also be caused by chromosomal rearrangements and uniparental disomy (UPD) but purely epigenetic changes can potentially cause a similar condition. Formation of gametes occurs from PGCs and "imprinted genes" are established that do not get erased during the rest of the life in that generation. However, these imprints can be transmitted through subsequent




| Cell affected | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Germline | | | | | | | | | | | | | |
| Male | ■ | | | | | | ■ | | | | | ■ | |
| Female | ■ | | | | | | ■ | ■ | ■ | ■ | ■ | ■ | |
| Somatic cell | | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| Trophoblast | | | | ■ | ■ | ■ | ■ | ■ | ■ | | | | |
| Xactivation | | | | | | | | | | | | | |
| X-mat | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| X-pat | ■ | ■ | ■ | | | | | | | | | ■ | |
| **Envir.Agents** | | | | | | | | | | | | | |
| Hormone/EDC | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | | | |
| Mat. Nutrition | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | | |
| Mat. Stress | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | |
| Mat. Behavior | | | | | | | | ■ | ■ | ■ | | | |
| Radiation | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| Carcinogens | | | | | | | | | | ■ | ■ | ■ | ■ |

**Fig. 1.3** Critical periods of vulnerability: *shaded* areas of the table corresponding to the numbered stages of human development indicate critical periods of vulnerability of different cellular populations affected at different stages of a lifetime. Putative environmental agents implicated in bringing about the epigenetic changes are also listed with similar *shaded* areas indicating presumed time of their maximum effect. Abbreviations: *ICM* inner cell mass, *OCM* outer cell mass, *X-Mat* maternally derived X chromosome, *X-pat* paternally derived X chromosome, *EDC* endocrine disrupting chemicals such as vinclozolin and bisphenol A

generations. The timing of this starts in the later stages of early embryogenesis, corresponding to E11.5–E12.5 in mice (post-conception days 33–40 in humans) and ends by E18.5 in male mice (corresponding to post-conception days 49–52 in humans). In females of both mice and humans on the other hand, this stage may not be completed through puberty and adulthood.

### *1.7.2 Somatic and Trophoblastic Cells Including X Chromosome Inactivation*

*Effects from conception to completion of embryogenesis including first trimester*: Fertilization of the gametes and formation of the early embryo from conception to most of the first trimester of gestation involves a period of demethylation and de novo methylation of the genome. Most purely epigenetic disorders are part of this group because this is the stage where most of the methylation markers are removed and reestablished (except the imprinted genes which do not change); also the inactivation of the extra X chromosome occurs in females.

### *1.7.3 Later Somatic Cell*

*Effects from mid-late gestation and continuing to postnatal development until adulthood*: Conditions listed under this category have the most potential for modifications to promote health and prevent or treat disease. The field of study of "Developmental Origins of Health and Disease (DoHaD)" and research related to the origins or cancers and neuropsychiatric disorders are directly influenced by the epigenetic changes that may occur during this stage.

The subsequent part of this chapter will detail the processes involved and the conditions that occur due to abnormalities in the normal process of epigenetic regulation and differentiation.

## 1.8 Effects at the Primordial Germ Cell Stage of Development (Disorders of Imprinting) (*Potential Multigenerational Effects*)

Human embryo develops sexual identity by about 8 weeks after conception. Determination of gonadal sex is by the differentiation of the bipotential embryonic gonad into male testis or female ovary. Subsequently the action of testicular hormones are involved in the development of male external genitalia and regression of female internal reproductive organs; by default the lack of action of testicular hormones leads

to the development of the female reproductive organs (Ludbrook and Harley 2004). PGCs originate in epiblast cells arising in the posterior primitive streak at E7.5 in mice (corresponding to post-conception days 15–18 in humans) (Morgan et al. 2005; O'Rahilly 1979). They then migrate to the genital ridge around E8.5 in mice (post-conception day 19 in humans) and complete their migration around E11.5 in mouse (post-conception days 32–33 in humans). It is soon after the migration to the genital ridge that imprinted genes are demethylated and start the process of reestablishment of the imprint with remethylation. This period of DNA demethylation is between E11.5 and E12.5 in mice and corresponds approximately to post-conception days 33–40 in humans (Morgan et al. 2005; O'Rahilly 1979). Following that the genomes of the developing gametes are de novo methylated and acquire imprints and this process continues until at least E18.5 in male mice corresponding to post-conceptional days 49–52 in human males. In females of both species however, the remethylation can occur until the oocyte matures before ovulation; thus going into puberty and beyond (Morgan et al. 2005; O'Rahilly 1979). Changes occurring in the germline at this stage have the potential to have manifestations into the next two generation of offspring (Manikkam et al. 2012; Skinner et al. 2013).

### *1.8.1 Mechanism of Imprinting*

In contrast to the biallelic expression of most genes, expression of genes subject to genomic imprinting is monoallelic and based on the sex of the transmitting parent. The mechanism of imprinting was discovered from a series of nuclear transplantation experiments in the early 1980s in which it was shown that gene function could differ based on whether a particular set of genes were inherited from the mother or the father (McGrath and Solter 1984; Surani et al. 1984; Cattanach and Kirk 1985). The correlation of these observations to human disease was initially made with the finding of maternal heterodisomy with altered gene imprinting in PWS (Nicholls et al. 1989). Soon other human diseases related to imprinting were identified and currently there is a growing list of conditions that could be classified as imprinting disorders (Eggermann et al. 2013). More and more conditions are being identified because there are at least 150 imprintable genes in the mouse and at least 60 have been identified as human imprintable genes (http://igc.otago.ac.nz/home.html) (Harwell 2013; Horsthemke 2010). Genomic imprinting could be understood as a series of steps where imprints are reset at generation. Imprints are erased in PGCs, established in the germline according to the sex of the embryo and maintained throughout life until the development of PGCs of the next generation. Imprinting marks are inherited from paternal gametes and maintained unchanged during developmental reprogramming and demethylation in early embryogenesis and persist during mitosis in somatic cells through the lifetime. However, during later embryogenesis when PGCs are in the process of migrating to the genital ridge in the development of the new gonad, the imprints are erased and reestablished for the next

generation according to the sex of the contributing parent (Eggermann et al. 2013). The process of imprinting is mostly regulated by imprinting centers (ICs) on the same gene on which it has its effect (*cis*-acting). Imprinted genes and ICs seem to occur in clusters and thus far they have been found mostly on chromosomes 6, 7, 11, 14, 15, and 20 in humans. Imprinted genes are typically associated with genes regulating growth, development, and evolutionary survival and are characterized by the presence of differentially methylated regions (DMRs). Imprinted gene DMRs represent parental allele-specific methylation profiles which could be maintained across many generations (Murphy et al. 2012; Park et al. 2012). Epigenetic transitions during gametogenesis are important in their impact on development and disease in infants (Kota and Feil 2010). Most imprint control regions (ICRs) have DNA imprints conferred during oogenesis and could be termed "maternal ICRs"—e.g., *KCNQ1* on chromosome 11, *GNAS* on chromosome 20, and *PWS* on chromosome 15. Conversely, only two could be termed "paternal ICRs"—viz. *IGF2-H19* imprinted domain on chromosome 11 and *DLK1-DIO3* on chromosome 14 (Girardot et al. 2012).

### *1.8.2 Molecular Changes Involved in Imprinting Disorders*

There are four common ways by which imprinting disorders originate and only one of them involves abnormal methylation of specific DNA regions termed epimutation (in contrast to genetic mutations involving DNA sequence). The other three mechanisms involve alteration of genetic material: (a) UPD where both chromosomal homologs are inherited from one parent, (b) microdeletions of specific regions or duplications of a particular DNA segment, and (c) point mutations involving DNA sequences in the imprinted genes. For the purpose of the following discussion on imprinting, no distinction will be made regarding the various mechanisms involved but rather the focus will be on the effect of the change of imprint.

### *1.8.3 Sex Differences in Imprinting*

The process of genetic imprinting though largely similar has important differences in males and females (Fig. 1.2). As mentioned above, the process of imprint erasure from germline cells occurs at the same time in both males and females in early embryogenesis (E11.5–E12.5 in mice corresponding to post-conception days 33–40 in humans). However, the process of gamete formation and thus the process of reestablishment of the methylation imprint differs greatly between male and female embryos. Male germline cells enter into a state of mitotic arrest at this stage (approximately around post-conception days 40–42 in humans) and will reinitiate mitosis and the two phases of meiosis (Meiosis I and Meiosis II) to generate spermatozoa