Symmetric methylation of cytosine in CpG dinucleotides is one of the widespread modifications in animal genomes. It is associated with “closed” (inactive) chromatin state and, therefore, negative regulation of transcription. To date, this modification has been found both in invertebrates (Drosophila melanogaster [1]) and in chordates (from Ciona intestinalis [2] to mammals).

It is known that DNA methylation plays an important role in so-called “epigenetic” regulation of gene expression—regulation that is not directly dependent on primary structure of DNA, but is maintained by many protein or non-protein factors (like histone modification, chromosome territory, etc., i.e., “epi”-genomic factors).

DNA methylation affects gene expression directly or indirectly. Some transcriptional factors (i.e., Sp1) can interact only with non-methylated DNA sequences, whereas methylation of cytosine abolishes interaction [3]. This in turn leads to less effective transcription of certain genes. On the other hand, there is a different mechanism of action of CpG methylation. So-called MBD (methyl-DNA-binding domain) proteins [4] specifically recognize modified sequences and attract large multiprotein complexes that can change chromatin conformation from “opened” to “closed”.

In the genome of vertebrates, approximately 80% of all CpG-dinucleotides are subject to methylation. Exceptions to this rule are so-called “CpG-islands”—short (1000–1500 bp) regions of DNA with high density of CpGs. Usually these regions are associated with regulatory sequences and do not undergo methylation during either early development or establishment of tissue-specific expression [5].

It is generally accepted that DNA methylation is a unidirectional process. If any sequence acquires CpG methylation then this modification becomes stable and will be inherited after cell division. So, both daughter DNA molecules will have same pattern of methylation. However, CpG methylation is much more dynamic during early embryonic development.

As a result of gametogenesis, DNA in sperm or oocytes become almost fully methylated. Fertilization starts the process of active demethylation of the male genome [6] followed by de novo methylation (Fig. 1). At the same time passive demethylation of the female genome occurs. The normal level of methylation is reached by the time of implantation. Such “failure” in gross methylation of the genome is quite strong in embryogenesis of the house mouse Musculus musculus, much less during the development of Xenopus laevis [7], and was not reported for zebra fish Danio rerio [8].
Although some modifications associated with establishment of tissue-specific expression still happen during later stages of embryogenesis, major changes in DNA methylation level are finished by the time of implantation.

The most popular explanation of heritability of methylation during cell divisions is the suggestion of a key role of maintenance methyltransferase DNMT1 in this process [9]. During replication DNMT1 simply “copies” pattern of methylation modifying cytosines on newly synthesized chain in accordance with modifications on the initial strand. So-called de novo methyltransferases DNMT3a and DNMT3b are responsible for re-methylation of the genome during early embryonic development and changes in overall methylation in gametogenesis [10].

But this theory has some weak points. It has been shown, for example, that CpG-methylation can be maintained even without DNMT1 [11]. Moreover, there is no common opinion concerning what factors direct the de novo methylation process.

Epigenetic regulation of gene expression will be discussed later with examples of genomic imprinting and X-inactivation. Another important function of DNA methylation is transcriptional repression of retrotransposones and other mobile elements. It was shown, for example, that expression of murine retrotransposone element IAP can be increased up to 100-fold in the absence of DNA methylation [12]. This repression is necessary because expression of mobile element can lead either to genetic rearrangements or increment of “transcriptional noise” and as a consequence to the genesis of different disorders.

At the same time, another hypothesis explaining such repression exists. mRNA that arises during transcription of so-called “junk DNA” can degenerate with production of small double-strand RNA molecules. This leads to siRNA-dependent methylation of retrotransposones and other mobile elements [13]. There is a possibility that this effect is the first step in the process of de novo methylation of DNA.

It is worth mentioning that a small part of CpG-islands undergo methylation in a normal organism. These CpG-islands belong to imprinted genes or genes on inactivated X-chromosome. Promotors associated with such CpG-islands remain silent during the whole life of the organism. But if methylation of CpG-islands of X-like genes occurs during early embryonic development [14], methylation status of imprinted genes remains invariable from gametogenesis and is not subject to passive or active demethylation.

Genomic imprinting. There are a number of genes whose expression depends on what allele they are located, paternal or maternal. Two alleles of imprinted genes differ in such features as DNA methylation, chromatin structure (histone modifications, nuclease hypersensitivity), and time of replication [15]. The important characteristics of imprinting are heritability in cell divisions and reversibility in gametogenesis. Usually imprinted genes organized in big clusters on the chromosome. Expression of several genes in one cluster can be ruled by a common regulation element.

One of best known models used for investigation of imprinting is the system of H19/IGF2 genes. H19 and IGF2 are two genes located approximately 90 kb apart. H19-gene is transcribed only when it is located on the maternal chromosome. Vice versa, IGF2-gene is expressed only from the paternal chromosome. A key role in regulation of these genes is played by a 2 kb DNA fragment that is located immediately before the promotor of H19 [16]. This fragment is called a Differentially Methylated Region (DMR) because it is heavily methylated on the paternal but not methylated on the maternal chromosome.

There is a common model explaining maintenance of imprinting in the H19/IGF2 locus. Although the mechanism providing imprinting of the H19 gene is not exactly clear and is rather autonomous (i.e., does not need any external factor except maintenance DNA-methyltransferase DNMT1 and, possibly, methyl-DNA-binding proteins), expression of IGF2 depends on a methylation-sensitive insulator between its promotor and tissue-specific enhancers (Fig. 2). A crucial role in insulation is played...