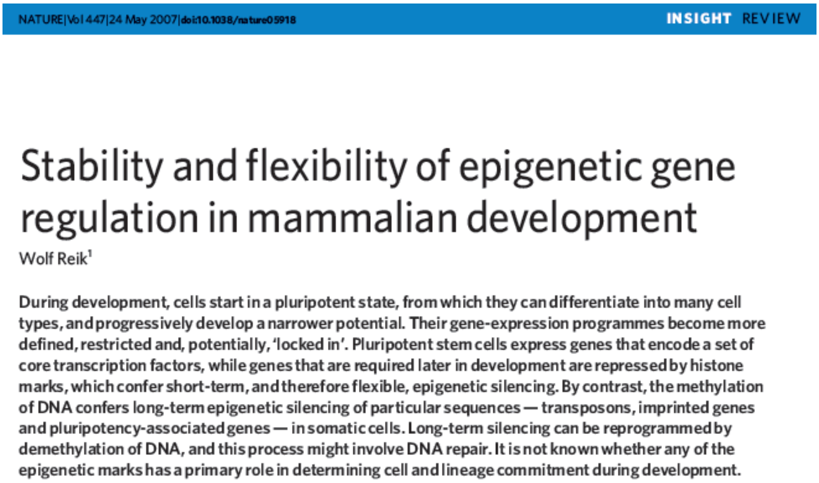
Last week we have examined some further properties of chromatin, by examining the mechanisms for establishment, maintenance and spreading of heterochromatin as well as boundaries (insulators) that limits specific chromosome “domains”.

This allowed us to join back to the very first lesson on genomes, when we considered the spatial organization of euchromatin and heterochromatin in cell nuclei, cell-type dependency and put forward hypothesis on how this organization may reflect the overall spatial relationship of the cell and the perceptive signals coming from the environment.

Today, we will consider in depth another property of mammalian chromatin, i.e. methylation of DNA, trying to discover relationship with type of genes and promoters, transcription and development. This will bring us to close another loop, reaching again the problem of gene regulation by signalling pathways, that you studied in the first module of the course.



NATURE|Vol 447|24 May 2007|doi:10.1038/nature05918 **INSIGHT** REVIEW

Stability and flexibility of epigenetic gene regulation in mammalian development

Wolf Reik[1]

During development, cells start in a pluripotent state, from which they can differentiate into many cell types, and progressively develop a narrower potential. Their gene-expression programmes become more defined, restricted and, potentially, ‘locked in’. Pluripotent stem cells express genes that encode a set of core transcription factors, while genes that are required later in development are repressed by histone marks, which confer short-term, and therefore flexible, epigenetic silencing. By contrast, the methylation of DNA confers long-term epigenetic silencing of particular sequences — transposons, imprinted genes and pluripotency-associated genes — in somatic cells. Long-term silencing can be reprogrammed by demethylation of DNA, and this process might involve DNA repair. It is not known whether any of the epigenetic marks has a primary role in determining cell and lineage commitment during development.

This review is available as accompanying Lesson 9

DNA methylation was measured gene-by-gene in the **past**, using bisulfite conversion, followed by PCR, cloning and sequencing

DNA methylation can **today** be assayed on a genome-wide level, by essentially two methods:

1) methyl-C-DNA immunoprecipitation, followed by promoter arrays, tiling arrays or deep-sequencing

2) bisulfite conversion followed by direct re-sequencing

Articolo di Supporto

nature genetics

Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome

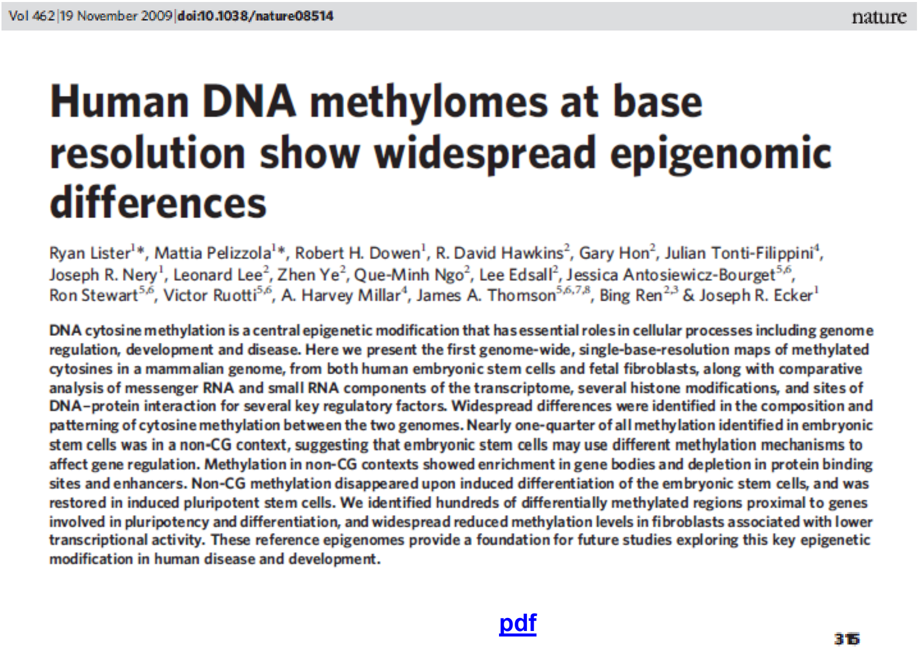
Michael Weber[1], Ines Hellmann[2,3], Michael B Stadler[1], Liliana Ramos[4], Svante Pääbo[2], Michael Rebhan[1] & Dirk Schübeler[1]

To gain insight into the function of DNA methylation at *cis*-regulatory regions and its impact on gene expression, we measured methylation, RNA polymerase occupancy and histone modifications at 16,000 promoters in primary human somatic and germline cells. We find CpG-poor promoters hypermethylated in somatic cells, which does not preclude their activity. This methylation is present in male gametes and results in evolutionary loss of CpG dinucleotides, as measured by divergence between humans and primates. In contrast, strong CpG island promoters are mostly unmethylated, even when inactive. Weak CpG island promoters are distinct, as they are preferential targets for *de novo* methylation in somatic cells. Notably, most germline-specific genes are methylated in somatic cells, suggesting additional functional selection. These results show that promoter sequence and gene function are major predictors of promoter methylation states. Moreover, we observe that inactive unmethylated CpG island promoters show elevated levels of dimethylation of Lys4 of histone H3, suggesting that this chromatin mark may protect DNA from methylation.

Bias to promoters (known promoters)

Measurements not quantitative

low resolution



Vol 462|19 November 2009|doi:10.1038/nature08514 nature

Human DNA methylomes at base resolution show widespread epigenomic differences

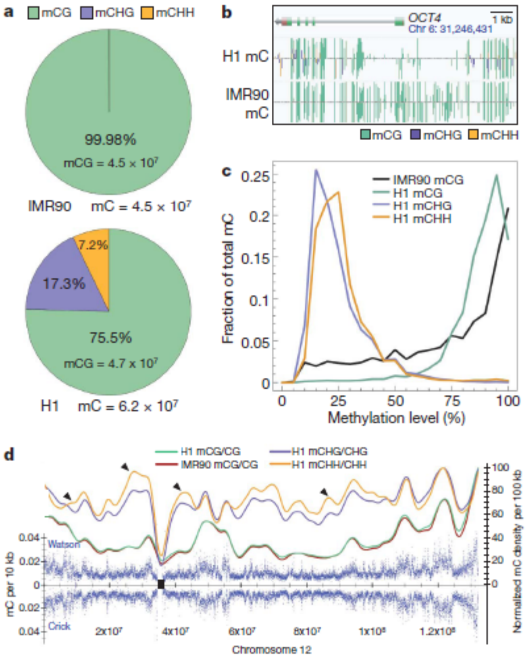
Ryan Lister[1]*, Mattia Pelizzola[1]*, Robert H. Dowen[1], R. David Hawkins[2], Gary Hon[2], Julian Tonti-Filippini[4], Joseph R. Nery[1], Leonard Lee[2], Zhen Ye[2], Que-Minh Ngo[2], Lee Edsall[2], Jessica Antosiewicz-Bourget[5,6], Ron Stewart[5,6], Victor Ruotti[5,6], A. Harvey Millar[4], James A. Thomson[5,6,7,8], Bing Ren[2,3] & Joseph R. Ecker[1]

DNA cytosine methylation is a central epigenetic modification that has essential roles in cellular processes including genome regulation, development and disease. Here we present the first genome-wide, single-base-resolution maps of methylated cytosines in a mammalian genome, from both human embryonic stem cells and fetal fibroblasts, along with comparative analysis of messenger RNA and small RNA components of the transcriptome, several histone modifications, and sites of DNA–protein interaction for several key regulatory factors. Widespread differences were identified in the composition and patterning of cytosine methylation between the two genomes. Nearly one-quarter of all methylation identified in embryonic stem cells was in a non-CG context, suggesting that embryonic stem cells may use different methylation mechanisms to affect gene regulation. Methylation in non-CG contexts showed enrichment in gene bodies and depletion in protein binding sites and enhancers. Non-CG methylation disappeared upon induced differentiation of the embryonic stem cells, and was restored in induced pluripotent stem cells. We identified hundreds of differentially methylated regions proximal to genes involved in pluripotency and differentiation, and widespread reduced methylation levels in fibroblasts associated with lower transcriptional activity. These reference epigenomes provide a foundation for future studies exploring this key epigenetic modification in human disease and development.

pdf

H1 are human embryonic stem cells

IMR90 are fetal lung fibroblasts



NEWS & VIEWS

NATURE|Vol 462|19 November 2009

EPIGENOMICS

Methylation matters

Dirk Schübeler

Genome-wide maps of methylated cytosine bases at single-base-pair resolution in human cells reveal distinct differences between cell types. These maps provide a starting point to decode the function of this enigmatic mark.

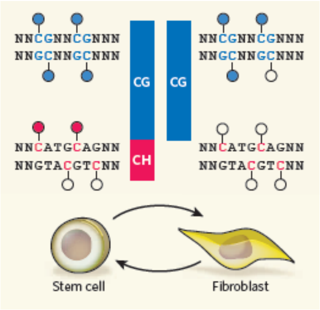


Figure 1 | DNA methylation patterns differ between stem cells and differentiated cells[1]. In stem cells, regions of DNA with CpG methylation (blue) are mostly uniformly methylated, whereas this modification is more heterogeneous in fibroblasts. Non-CpG methylation (red), which occurs primarily at CA nucleotides, is detected only in stem cells, yet is asymmetric and more scarce and patchy than CpG methylation. If fibroblasts are converted to induced pluripotent stem cells they regain non-CpG methylation. Filled circles, methylated cytosines; unfilled circles, unmethylated cytosines. H stands for A, C or T; N stands for any nucleotide.