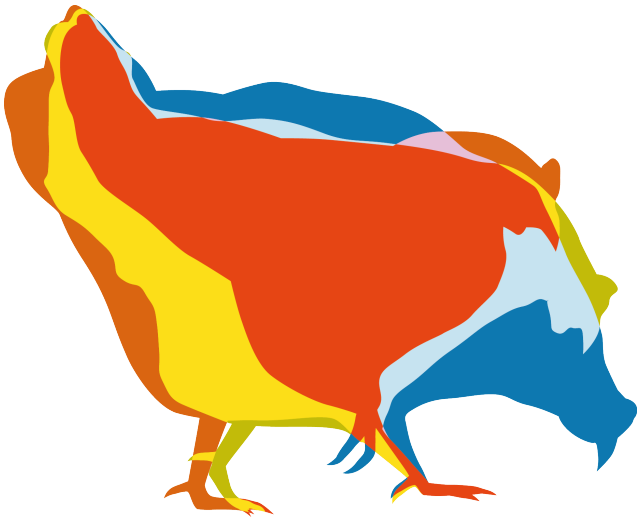
What is epigenetics?

PhD Introduction Essay

by Andrey Höglund



AVIAN

Behavioural Genomics
and Physiology group

What is epigenetics?

Epigenetics

Epigenetics is the study of changes made to gene expression, and ultimately the phenotype, that are not mediated by changes in the DNA sequence. These changes should also be heritable mitotically and/or meiotically (Dupont, Armant, & Brenner, 2009). Coined first by Conrad Waddington in the 1950s the term concerned events that could not be understood with genetic principles (Goldberg, Allis, & Bernstein, 2007), such as the process from which a zygote transformed into a multicellular complex organism (Waddington, 1956). Today the paradigm tells that each somatic cell contains the same DNA composition and merely by altering the gene expression levels are able to differentiate into distinctive tissues. The definition of epigenetics becomes more accurately as the field that studies these stable phenotypic gene function states, how they are inherited, changed and maintained (Berger, Kouzarides, Shiekhattar, & Shilatifard, 2009; Felsenfeld, 2014). These gene expression patterns within a cell are induced by chemical modifications, such as DNA methylation, structural proteins configurations, such as histone and chromatin modifications and even regulatory RNAs with micro-RNA, small-interfering RNA and noncoding RNA able to regulated the quantity of protein produced.

Histone modification and chromatin structure

Histones are proteins that the DNA molecule coils around forming a chromatin structure, reducing the physical size of the DNA molecule and in the process obscuring transcription sites and thus serving as an epigenetic modifier. The major histone proteins are H2A, H2B, H3 and H4, and together they assemble into an octameric structure. The formation of the chromatin structure is induced by addition and/or removal of an acetyl groups ($-COCH_3$), acetylation and deacetylation, respectively (Lewin, 2008). Nan et al., 1998 showed that the methyl-binding protein MeCP2 is attracted by DNA methylation and induced hypoacetylation, by activating HDAC1 a deacetylase, generating a closed heterochromatin structure that prevents transcription factors such as Pol II of interacting with promoters leading to transcriptional silencing (Nan et al., 1998). There appears to be a strong association between DNA methylation, gene silencing and compact chromatin structures.

DNA methylation

DNA methylation is an epigenetic modification were a methyl-group ($-CH_3$) is added to the fifth carbon on a cytosine that is followed by a guanine. These regions are termed CpG-sites and are commonly found in promoter regions (Irizarry, Wu, & Feinberg, 2009; van Eijk et al., 2012) and in transposable elements (Bestor, 1990). Studies have demonstrated a negative correlation between presence of DNA methylation in the promoter and a decreased level of gene expression, most likely by interfering with binding sites of transcription factors (see figure 2) (A. P. Bird & Wolffe, 1999; Docherty et al., 2012). Contrarily, hypermethylation of the genebody has a positive correlation with gene expression. This was established elegantly *in vitro* by ligation of a promoter and the GFP-genebody either fully methylated or unmethylated and measuring the mRNA levels (Lorincz, Dickerson, Schmitt, & Groudine, 2004). The unmethylated plasmid clones showed 40 % less expression compared to the methylated clones.

Besides gene expression levels, a variety of biological processes are affected by DNA methylation such as genomic imprinting, X chromosome inactivation in mammals, cell differentiation, protection against invading DNA molecules and development (A. Bird, 2002). The presence of DNA methylation within the eukaryotic domain varies in an unclear manner. In the nematode *Caenorhabditis elegance* and the yeast *Saccharomyces cervevisiae* (Simpson, Johnson, & Hammen, 1986) no DNA methylations have been found. However, the parasitic nematode *Trichinella spiralis* show evidence of functional DNA methylation (Gao et al., 2012), perhaps due to it having life cycle events within mammalian hosts.

On a micro scale DNA methylation affects gene expression levels locally. On a macro scale effects of global DNA methylation patterns configure and attract histone proteins that transform into tightly packed chromatin structures able to transcriptionally silence long regions of DNA, even able to repress a whole chromosome by X-inactivation.

Transposable elements and DNA methylation

In humans, 35% of the genome is made up of transposable elements (TE). TEs are DNA sequence able to jump within the genome and are originally elements of retroviruses. TEs are regarded as specialized intragenomic parasites capable of inducing homologous recombinations, deletions, insertions and inversions. In mammals, almost all DNA methylation resides in TEs which in a methylated state are silenced. Over time a cytosine is transformed into a thymidine rendering the death of the TE (Kumpatla, Chandrasekharan, Iyer, Guofu, & Hall, 1998; Yoder, Walsh, & Bestor, 1997). Comparing the amino acid sequence of mammalian and several bacterial DNA methyltransferases its evident that homology is at play. The primary role of DNA methylation system might have been to defend against bacteriophage infections (Bestor, 1990), but later evolved a secondary role as gene expression regulator.

Genes associated with maintaining DNA methylation

The DNA methyltransferase (DNMT) family is composed of genes responsible for de novo methylation (DNMT3a and DNMT3b) and maintaining DNA methylation (DNMT1) during DNA replication. Additionally, DNMT2 have been shown to exhibit some yet unclear DNA methyltransferase activity and DNMT3L has some catalytic activity of DNMT3s (Klose & Bird, 2006). On the molecular level DNMT3s convert the cytosine in a CpG-site to 5-methylcytosine and due to CpG arrangement the methylations are sitting diagonally to each other on the opposing DNA strands.

Dosage compensation

Dosage compensation is a phenomenon that occurs in homogametic individuals where transcriptional activity is equalized between the species sexes. In the sex-determination system XY, a process of X-inactivation has been observed where one of the two X-chromosomes are completely transcriptionally inactivated. In mammals a 1Mb region termed *Xic* (X-chromosome-inactivation centre) is responsible for establishing the permanent silent state and occurs early in development (Avner & Heard, 2001). In mice, the locus *DXPas34*, located inside *Xic*, has a uniquely hypermethylated profile in the active X-chromosome, indicating that epigenetic mechanism in place to maintain the transcriptional state (Courtier, Heard, & Avner, 1995).

Epigenetic reprogramming

Epigenetic reprogramming is an event occurring after fertilization where genome-wide demethylation occurs followed by *de novo* methylation unique for specific cell types. This *de novo* methylation is maintained postnatally. Studies have shown that transfer of nucleus into nucleus free eggs yield embryos with high mortality and postnatal foetuses are prone to abnormalities. These phenotypes are linked to epigenetic modifications rather than genetic as cloned animals do produce normal offspring when reproduced sexually (Rideout, Eggan, & Jaenisch, 2001).

Epigenetics and diseases

During carcinogenesis local hypermethylation of CpG-islands (CGI) that usually are hypomethylated has been observed while genome-wide hypomethylation occurs (Feinberg & Vogelstein, 1983). This *de novo* methylation of CGI located in transcriptional regions may spread to neighbouring promoter regions where tumour suppressor genes are located (Jones, 2002). Why this sudden change in the methylome occurs is unclear, but the epigenetic signature is traceable.

Epigenetics and the environment

Homologous recombination creates a palette of genetic variation within a population on which selective pressure can act. However, novel genes are rare and mutations or influx of new alleles into the population are required to increase the phenotypic range. Epigenetic modification can respond to environmental stimuli and alter DNA methylation and chromatin states during a life time but also be stable enough to be propagated to the next generation (Jaenisch & Bird, 2003). Studies done on monozygotic twins highlight effects environmental cues have on the methylome as they show different DNA methylation patterns with aging (Fraga et al., 2005). Being genetically identical, yet showing variation in anthropomorphology and disease susceptibility the phenotypical differences should be explained by epigenetic variations. Additionally, complex phenotypes such as behaviour have been linked to differences in DNA methylation patterns in monozygotic twins (Melka, Castellani, O'Reilly, & Singh, 2015). In *Arabidopsis*, vernalization, flowering after exposure to cold, was correlated with decrease of the *FLF* (flowering locus F) mRNA and genomic hypomethylation indicating a epigenetic modification in response to environmental stimuli (Sheldon, 1999).

The process of aging - senescence, is caused by DNA damage, progressive shortening of the telomeres during mitosis, deterioration of stem cells, genome mutation and other processes (Kirkwood, 2005). Epigenetically, senescence has been correlated with hypomethylation genome-wide and hypermethylation locally, mainly in CGI. This has been observed *in vitro* in cell cultures and in animals (Jaenisch & Bird, 2003; Johnson et al., 2012). How much of the senescence effects are a result of the environment or pre-programmed is yet to be elucidated. One study found that caloric restrictions were correlated with longevity in the mosquito *Aedes aegypti* where both sugar and protein content were reduced (Joy, Arik, Corby-Harris, Johnson, & Riehle, 2010). Another study found three genes (EDARADD, TOM1L1 and NPTX2) at which the promoters' methylation level had a linear correlation with age and explained 73 % of the variance in humans. The population samples came from saliva of 31 males and 29 females at the age of 18 -70 (Bocklandt et al., 2011), a finding that indicates that epigenetic programming continues during all stages of life and raises the question if there is a "epigenetic bio-age".

Another interesting example are the famous *Agouti* mice. The *Agouti* locus codes for coat colour in mice. Researchers have inserted an intracisternal A partical (IAP) retrovirus element into the gene and produced various alleles with colours ranging from yellow to mottled to wild-type. These phenotypes are induced by the methylation states at IAP, with hypermethylation producing the wild phenotype and hypomethylation producing the yellow phenotype, together with short longevity, tumour and obesity. Gene expression in the wild phenotype occurs in the hair follicles, while in the yellow phenotypes gene expression is ectopic and ubiquitous (Duhl, Vrieling, Miller, Wolff, & Barsh, 1994). Additionally, when mothers with *Agouti*^{IAP} genotype where fed a methyl donor rich diet, they produced offspring with the wild phenotype, compared to mothers on standard diet, indicating that environmental stimuli early in development can affect the gene expression levels and the phenotype into adulthood (Wolff, Kodell, Moore, & Cooney, 1998). Furthermore, *Agouti*^{IAP} mothers with the yellow phenotype produced offspring with yellow phenotype, while *Agouti*^{IAP} mothers with the wild phenotype produced offspring with wild phenotype, demonstrating the stability of these epigenetic modifications and their trans-generational effect (Whitelaw, Morgan, Sutherland, & Martin, 1999).

Epigenetics in the chicken

In mammals, genome-wide DNA methylation is unevenly distributed. Hypermethylation usually occurs at heterochromatin, repetitive sequences and transposon regions, while regions next to genes are hypomethylated relative the gene body (Eckhardt et al., 2006; Li et al., 2011). In the majority of cases, DNA methylation patterns in chickens are similar to those in mammals where the majority of CGI are unmethylated, repetitive sequences are hypermethylated (Li et al., 2011), and 80% of all promoters in chickens are methylated (Nätt et al., 2012). The classical pattern of DNA methylation around and within genes observed in mammals is also observed in chickens (see figure 1) (Li et al., 2011), where regions flanking the gene show relatively lower methylation levels than the gene body. The DNMT family is also represented in the chicken genome, where DNMT3b is located on chromosome 20, DNMT3a on chromosome 3 and DNMT1 on one of the microchromosomes (Yu et al., 2008).

Quantitative Trait Locus (QTL) analysis

With QTL analysis it is possible to explain genetic variation of a complex trait (Falconer & Mackay, 1989). The trait of interest should be segregated in two divergent strains and, to establish a genetic basis for the trait, there should be a large average difference between the parental lines when raised in the same environment. Theoretically, any trait that is quantitative can be used, such as gene expression, behaviour and DNA methylation. Recombinant inbred lines, advanced intercrosses, backcrosses and F2 intercrosses are different cross types used to create mapping populations. In a backcross, the F1 generation is mated back to either one or both of the parental strains. In a F2 intercross the F1 generation is mated amongst themselves to produce F2 generation. Thus, the F2 intercross will have three QTL genotypes classes (AA, AB and BB, where A and B represent marker alleles from respective parental lines), while the backcross will only have two (one heterozygote and one homozygote, depending on which parental strain that is backcrossed). Moreover, in the F2 intercross it is possible to estimate the additive and dominance effect between the alleles. Additionally, the interactions between loci (epistasis) can be detected with an F2 intercross.

The advanced intercross lines (AIL) are a continued F2 intercross, where the F2 are randomly intercrossed within the generation to produce a F3 generation. The F4 generation is produced in the same way, by randomly intercrossing the F3 generation, and so on. This can continue for multiple generations resulting in reduced linkage disequilibrium and cause the proportion of recombinants between any linked loci to asymptotically approach 0.5. The many recombination events generate a fine QTL map in a relatively small population compared to and F2 or backcross intercross (Broman & Sen, 2009).

Recombinant inbred lines (RIL), are yet another experimental cross that can be used. They are constructed as an F2, and then mating the F2 siblings are mated in parallel series of repeated sibling mating. This can be done for 10 – 20 generations and generates a mosaic genome of the two initial lines. Eventually the genome will be fixed and the new panel of inbred lines will be immortal. The advantage is they only need to be genotyped once, but are expensive to maintain. A RIL is mostly used by plant biologists were organism also allow selfing (Broman & Sen, 2009). Various genetic markers can be used for genotyping the cross such as single nucleotide polymorphisms (SNPs), transposable elements, micro-satellites (simple sequence repeats, SSR, or short tandem repeats, STR).

A statistical association, linear regression, between phenotype and marker genotype is sought to create a QTL map. When a single marker is used it is not possible to distinguish whether the marker and the phenotype are closely linked to a QTL with a small effect, or loosely linked to a QTL with a big effect. To overcome this, an interval mapping approach can be used, where a genetic linkage map is applied as a framework to locate the QTL. To find the position of the QTL and its effect the maximum likelihood method or regression-based method can be used. The locations of these markers on the chromosomes are referred in terms of genetic distance. Unlike a physical map, which specifies the physical location on the chromosome, genetic map distances are measured by the rate of crossover events during meiosis. The distance between two markers is measured in centiMorgans (cM) where 1 cM is corresponding to 1% chance that the two markers will be separated due to crossover in a single generation (Broman & Sen, 2009).

The effects of DNA methylation

Is it possible to elucidate how much of the DNA methylation variation is due to the environment and/or the underlying DNA sequence? For that a DNA methylation QTL analysis should be conducted, where DNA methylation levels are treated as a phenotype and associated with a dense genetic marker map. The effects of the DNA methylation levels should then be correlated with gene expression levels as a measure of epigenetic control. Additionally, loci with identical sequences, yet associated with gene expression variation (epialleles), should be identified by correlating gene expression levels wth DNA methylation levels without genetic markers involved in identical individuals or inbred individuals. Comparing these two analyses could perhaps explain the mechanism by which DNA methylation controls some parts of the epigenome.

Figures

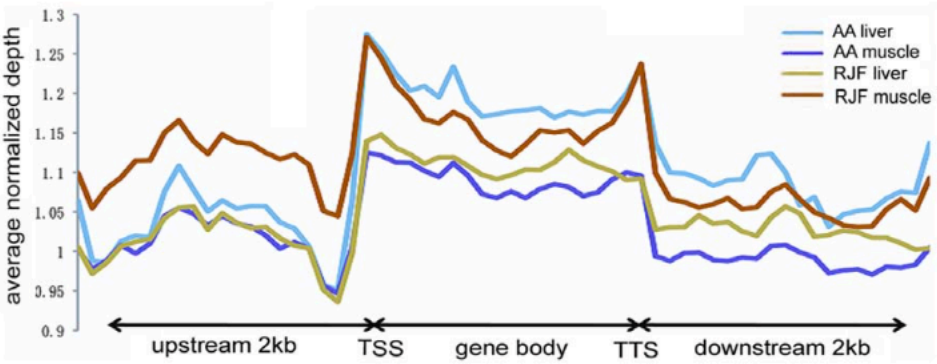


Figure 1 DNA methylation distribution in and around genes in chicken. AA = broiler, RJF = red junglefowl. From (Li et al., 2011)

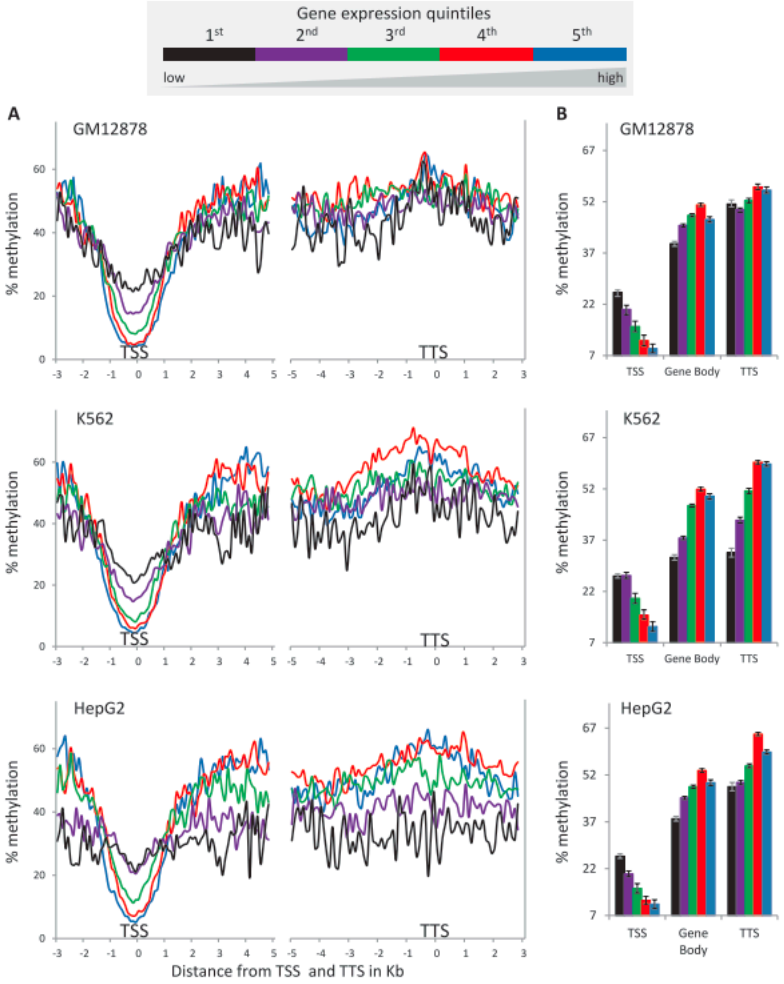


Figure 2 DNA methylation distribution around genes in human cell lines. Different life colours represent the gene expression levels. From (Jjingo, Conley, Yi, Lunyak, & Jordan, 2012)

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