INTRODUCTION: EPIGENETIC INHERITANCE

1.1 INTRODUCTION

In addition to its genetic resources, the structural and functional attributes of an organism are determined by the epigenetic makeup of its genome. Recent work has suggested that gene activity state acquired in one generation of an organism can be carried forward to the progeny in a non-genetic way by means of epigenetic marks like DNA methylation or histone modification. The epigenetic changes can be categorised into context dependent epigenetic change or heritable epigenetic modification. The context dependent changes occurs mitotically in the somatic cells within a particular generation (Kierszenbaum 2002). Transgenerational Epigenetic Inheritance (TEI) involves the transmission of certain characters or phenotypes from one generation to the next epigenetically and involves the germline cells (Anway et al. 2005; Crews et al. 2007; Daxinger and Whitelaw 2010; Jablonka and Raz 2009; Lang-Mladek et al. 2010; Mathieu et al. 2007; V. Rakyan and Whitelaw 2003; V. K. Rakyan et al. 2003; Xing et al. 2007; Youngson and Whitelaw 2008). It is thought that these kind of epigenetic changes in response to the environmental trigger or due to unfavourable condition endow the organism to cope up better and survive in extreme conditions (Dickins and Rahman 2012; Scoville et al. 2011). Epigenetic modifications like DNA methylation, histone modification or small RNA bring about a change in the epigenetic memory of the cell and can alter the expression levels of a gene from one generation to another and hence play a vital role in bringing about transgenerational inheritance.

1.2 ENVIRONMENT AND EPIGENETIC INHERITANCE

Although all the cells in an organism have the same DNA sequence yet their morphology and function varies. This is due to the constant exposure of the cells to extracellular factors. This has been noted for cells belonging to organisms from yeast to plants to

humans and is documented by studies on the transmission of diseases brought about by environmental stress (Choi and Sano 2007; Iwasaki and Paszkowski 2014; Luo et al. 2012; Su et al. 2015). Of late, a growing understanding of this interaction of environment and cells has brought to fore the role of epigenetics in this non genetic mode of diseased state inheritance.

Inheritance of altered epigenetic state (epimutation) has been observed by several groups. This inheritance could be across one generation in the presence of a specific environmental cue (intergenerational inheritance) (Ashe et al. 2012; Kelly 2014; Szyf 2014) or across several generation after exposure to environmental cue/insult only in the first generation (transgenerational) (Geoghegan and Spencer 2013; Lim and Brunet 2013; Morgan and Whitelaw 2008; Skinner and Guerrero-Bosagna 2009; Vickers 2014). The continuous multigenerational exposure of the organism to the factor which gives rise to the phenotype is known as intergenerational inheritance or extrinsic transgenerational inheritance. An example of this is the good maternal behaviour like postnatal pup licking in rodents (Meaney and Szyf 2005). This behaviour can program the same good maternal behaviour in the next generation by epigenetic modifications in the brain and this behaviour is lost when the mother doesn't exhibit good maternal behaviour in one generation. There has been studies wherein it was shown that mental or physical stress given early during the lifespan of mice can lead to the traumatic effect on their progenies.

The transgenerational epigenetic inheritance requires one time exposure of the organism to the factor which brings about the effect. The concept of direct and indirect exposure becomes clear if we consider the case of a gestating F_0 female. If such female is exposed to a factor then the F_0 female, F_1 embryo and the germline of F_1 which gives rise to the F_2 generation are said to be directly exposed to the particular factor. Inheritance of

transgenerational non-genetic factors are transgenerational only if they are observed from the F_3 generation onwards. In case of the intrinsic transgenerational inheritance there is the absence of direct exposure continuously but still the phenotype persists and keeps transmitting through multiple generations (C. M. Guerrero-Bosagna and Skinner 2009; Sgaramella 2013; Toth 2015). This makes it critical to study the phenotype in multiple generations in order to be sure that the effect we are observing is intrinsic or extrinsic transgenerational inheritance.

1.3 Non-genetic inheritance: Role of epigenetic modifications

1.3.1 DNA METHYLATION

DNA methylation is one of the best known epigenetic modification that has been found to be important in the regulation of gene expression during both embryonic development and adult life. Its role is best exemplified by genomic imprinting and X-chromosome inactivation (Burgers et al. 2002; Lichtenstein and Kisseljova 2001; Razin and Shemer 1995; Yoder and Bestor 1996). In mammals DNA methylation predominantly takes place at the fifth position of the cytosine in the context of CpG dinucleotides in mammals. Specific DNA methyltransferases are responsible for the transfer of a methyl group from the donor S-adenosyl methionine (SAM) to the fifth position in the cytosine of the CpG dinucleotide (Cardoso and Leonhardt 1999; del Mazo et al. 1994). Methylated DNA is usually associated with inactive state of chromatin and its presence within gene promoters inhibits transcription (Fransz and de Jong 2002; Hall et al. 2002; Urnov 2002; Zhimulev and Beliaeva 2003). It can do so by recruiting the methyl binding protein or the histone deacetylases or it can prevent the various transcription factors from binding to the DNA thereby preventing the transcription from the DNA (Deplus et al. 2002; Im et al. 2002; Maehara et al. 2002).

The mammalian methyltransferases have been broadly classified into maintenance methyltransferase and the de novo DNA methyltransferases (Aapola et al. 2000; Majumder et al. 2002; Mizuno et al. 2001; Reik et al. 2001). The first category includes *DNMT1* which is responsible for maintaining the methylation pattern from parental to newly synthesized strand by using hemi-methylated DNA as a substrate. It is known to be recruited to the replication foci and has been implicated in the process of embryonic development, imprinting and X-inactivation (Beard et al., 1995; Li et al., 1992, 1993). Knockout DNMT1 mouse showed reduced methylcytosine levels and delayed development (Li et al., 1992). The *de novo* DNA methyltransferase consists of *DNMT3A*, *DNMT3B* and the catalytically inactive regulatory factor DNA methyltransferse 3-like (*DNMT3L*) (Aapola et al. 2002; Mizuno et al. 2001; Razin and Shemer 1995). These brings about the methylation of the DNA following the embryonic implantation prior to which most of the methylation marks in the genome are erased. These methyltransferases bring about methylation by using unmethylated DNA as their substrate.

There are waves of methylation and demethylation that take place during mammalian development (Furuhashi and Kelly 2010; Molaro et al. 2014; O'Doherty and McGettigan 2014; Tian et al. 2009). During the formation of germ cells from the primordial germ cells (PGCs) around E10.5-12.5 there is genome wide demethylation that erases most of the parental marks. The new methylation marks are then established around E15.5 by the *de novo* DNA methyltransferases DNMT3A and DNMT3B (Nasonkin et al. 2011; L. W. Zheng et al. 2014). But immediately after fertilization both the inherited haploid genomes undergo waves of demethylation (Marchal et al. 2004; O'Doherty and McGettigan 2014). In case of the male pronucleus active demethylation sets in within four hours of fertilization even before the DNA replication sets in while the female pronucleus

undergoes passive demethylation after the first cell cycle takes place (Razin and Shemer 1995; Reik et al. 2001; Trewick et al. 2005).

Few examples of transgenerational epigenetic inheritance mediated by DNA methylation are given below-

- The oldest examples of DNA methylation mediated epigenetic inheritance is the change in flower symmetry from bilateral to radial in *Linaria vulgaris*. The change in the symmetry of the flower was found to be due to change in the degree of DNA methylation of the *Lcyc* gene which was responsible for the active or silent state of the gene and was responsible for the bilateral or radial symmetry of the flower respectively. Occasionally due to hypomethylation there was reversion to wild type flower in certain branch. This silent state of the *Lcyc* gene is maintained across the generation at this locus (Cubas et.al, 1999).
- Parent allele-specific imprinting- In case of imprinted genes, either of the maternal or paternal allele is methylated to ensure mono-allelic expression of that particular gene. This pattern of methylation has been found to be maintained by epigenetic inheritance of methylation mark across the progenies.

1.3.2 HISTONE MODIFICATIONS

Histones, the small basic protein that binds to DNA in the nucleus to form nucleosomes are the structural and functional constituents of chromatin in eukaryotes. In addition to packaging the DNA into nucleus, chromatin provides for transcriptional regulation as the tails of histone can be variously modified by epigenetic modifications. Various epigenetic modifications like methylation, acetylation, ubiquitination etc. can alter chromatin conformation of a gene and the final transcriptional output is determined by the sum total

of all these modifications. While acetylation of histones is usually associated with active euchromatin, deacetylation is associated with repression of transcription (Chen et al. 2002; J. Y. Fang and Lu 2002; Gray and Teh 2001; Howe et al. 2001; Im et al. 2002; K. Zhang et al. 2002). Methylation of lysine residues within histone proteins is well characterized and its role in epigenetic transcriptional regulation can be varying. While the methylation of lysine residues at position 4 and 36 are associated with active chromatin the methylation at positions 9 and 27 are associated with the inactive chromatin (Bannister et al. 2005; Briggs et al. 2001; Jacobs and Khorasanizadeh 2002; Liang et al. 2004; Liu et al. 2004; Manzur et al. 2003; Mathieu et al. 2005; Noma and Grewal 2002; Tachibana et al. 2002). The methylation of the histones can also take place at the arginine residues of the histone tail (Herrmann et al. 2005; S. Huang et al. 2005; C. H. Lin et al. 2002).

Histone from the parent cell contribute towards the epigenome of the daughter cell as nearly half of the histones in the newly formed daughter cell are from the parent cell and the remaining half is the nascent histones. As many of the histone marks like H3K27me3, H3K4me2 and H3K4me3 are important for regulation of genes, histone modifications can provide epigenetic memory across mitotic generations. During spermatogenesis, the histones are replaced by arginine rich protamines in the sperm for tighter packaging of the chromatin (Collins et al. 2002; Govin et al. 2004; Kasinsky et al. 2011). However in some parts of the chromatin the parental histones remain even in the sperm. How exactly the histone modifications are transmitted across the generation is not well understood but several studies have shown inheritance of histone marks across generations. A few examples of these are listed below.

- In *C.elegans* histone marks like H3K27me3 were found to be passed on to the daughter cell after replication (Gaydos et al. 2012).
- H3K4 methylation was shown to be and involved in inherited longevity and sterility in C.elegans (Benayoun and Brunet 2012; Greer et al. 2011).
- In another study, it was shown that if the H3K4me2 mark was not brought back to the normal level for certain genes in the progenitor germ cells, a progressive transgenerational sterility was observed in the worms (Katz et al. 2009; Pinskaya and Morillon 2009). The deficiency in the H3K4me3 was due to deficiency in COMPASS complex and linked to the alteration in the H3K36me3 marks (Acquaviva et al. 2013; Tenney and Shilatifard 2005).
- In the mice fed on low protein diet, the levels of the repressive histone modification mark H3K27me3 was found to be low in certain loci. It has been predicted that a decrease in the levels of H3K4me3 brings about a decrease in the levels of the MES-4 or H3K36me3 at certain gene loci in the germline cells which might be involved in the epigenetic transmission of sterility of longevity in the offspring.
- *Drosophila* subjected to environmental stress showed disruption in the heterochromatin state over several generations. This was found to be mediated by *Drosophila* activation of transcription factor-2 (dATF-2) (H. Siomi and Siomi 2011).

All these data supports the notion that a change in the parent's genome can have bearing on the progenies. This means that any environmental trigger which can modify the RNA or the chromatin state of the organism can modify its genome or the epigenome and such changes can be passed on to the progenies by the process of transgenerational epigenetic inheritance.

1.3.3 SMALL RNAs

Small RNAs like siRNA, piRNA and miRNA are considered important components of the mechanism by which epigenetic modifications are inherited from one generation to another (Alcazar et al. 2008; Ashe et al. 2012; W. Fang et al. 2012; Grentzinger et al. 2012).

1.3.3a miRNAs

Micro RNAs (miRNAs) are small RNAs which brings about post transcriptional repression by pairing with mRNAs during the translation process of a particular gene (Fabbri et al. 2007; Marcon et al. 2008; Nishida and Siomi 2006; Shah et al. 2010). miRNA are present in the nucleus of the germ cells and can bring about epigenetic silencing of the certain genes in the zygote and hence are involved in the transgenerational inheritance. Kit locus in mice is an example of such a regulation (Yagi et al. 2007). When a cross was set up between two Kit heterozygous mice, representation of the wild type phenotype in the progenies was much lower than expected. Most progenies showed white tail tip and white feet, characteristics for the heterozygous kit mice, but these characteristics were also seen in wild type offspring. This was due to a reduction in the levels of the Kit mRNA levels mediated by the miRNAs miR-221 and miR-222. When wild type mice were injected with miRNA for miR-221 and miR-222, they too showed white tail and feet phenotype indicating the involvement of these miRNAs in the silencing of the Kit allele. This confirmed that the miRNAs were able to pass from the gametes of the heterozygous Kit mice into the zygote of the wild type progenies that led to the aberrant phenotype.

1.3.3b piRNAs and PIWI protein

When a cross was set up between wild type male *Drosophila* from the wild environment and the female *Drosophila* from the laboratory strain it was found that there were no progenies formed due to a defect in the gametogenesis. But when the cross was set up

such that the female was taken from wild environment and the male was taken from the laboratory strain then there was no defect in gametogenesis and progenies were formed. This phenomenon is known as hybrid dysgenesis (Brennecke et al. 2008; Chambeyron et al. 2008; Grentzinger et al. 2012; Saito 2013; Simmons et al. 2014). This was a result of mobilization in dysgenic progeny of P-element or I-element transposons which were absent in laboratory strain and present in the wild environment strain. Hence there is the involvement of cytoplamically inherited determinants of the phenotype which depends on the parents of origin of the transposon. The maternally inherited piRNA is important for triggering a silencing response and the lack of maternal piRNA (Horwich et al. 2007; Kim 2006; H. Lin 2007; Nishida and Siomi 2006; Peters and Meister 2007; Yin and Lin 2007). The animal germ cells can be the host for a variety of transposons which can get propagated and is deleterious for the organism.

The PIWI (P-element induced whimpy testis) proteins are a group of germline specific proteins belonging to the Argonaute family of RNAi specific effector proteins. The PIWI proteins include the Aubergine (Aub), AGO3 and Piwi proteins (Baumberger and Baulcombe 2005; Janowski et al. 2006; Kataoka et al. 2001; Kavi et al. 2006; Yuan et al. 2005; X. Zheng et al. 2007). They can bring about euchromatic histone modification and can co-operate with the piRNAs to bring about repression of the transposable elements in the germ cell. PIWI interacting RNAs (piRNAs) are small 24-30 nucleotide long RNA which are produced from piRNA clusters in the genome which lies within the euchromatin heterochromatin boundary in *Drosophila* (Aravin et al. 2006; Brower-Toland et al. 2007; Kim 2006; Lau et al. 2006; Parker and Barford 2006; Peters and Meister 2007; Saito et al. 2007; Yin and Lin 2007). Hence the piRNA are longer than the miRNAs and siRNA and each of the piRNA clusters can produce thousands of piRNAs out of a total estimated

50,000 different species of piRNAs. Comparative genomics has shown that during evolution the sequence of the piRNA locus remains conserved and the sequence of the piRNAs varies. The abundance of a particular piRNA from a particular locus determines the true function of a piRNA. Their expression levels are particularly high in the germ cell including the spermatocyte. They are involved in silencing of the retrotransposons in the germ cells and known to regulate gene expression of certain genes and also protecting the host against pathogens like viruses.

The piRNA biogenesis involves two kinds of processing. The primary pathway occurs both in the somatic cells as well as in the germline cells. The secondary processing takes place by ping-pong cycle and occurs only in the germline (Grivna et al. 2006; Kim 2006; Kirino and Mourelatos 2007; Liao et al. 2010; Nagao et al. 2010; O'Donnell and Boeke 2007; Senti and Brennecke 2010). The defective heterochromatic transposable elements (TE) produce the antisense primary piRNA which guides the PIWI proteins to the complimentary sense transcript of the functional euchromatic TE. The PIWI endonuclease then cleaves those TEs and produces the sense secondary piRNAs which can guide the cutting of heterochromatic transcripts to produce the antisense secondary piRNAs subsequently. This mechanism is called the ping pong cycle as it involves the interplay between the defective TE and functional TEs. Hence the cooperation between the heterochromatic and euchromatic copies of a TE is very necessary for the piRNA to mediate silencing efficiently. In *Drosophila* the maternal transmission of piRNA is known to play a role in fertility of the organism mediated via the process of hybrid dysgenesis which is basically the silencing of the transposable elements mediated by the piRNAs (Brennecke et al. 2008; Hodl and Basler 2009; Nishida et al. 2007; M. C. Siomi et al. 2010). These molecules are slowly being recognised as means of carrying genetic information

from one generation to the next and the maternally deposited piRNA in the offspring can trigger the production of piRNA which brings about silencing of TEs. In *C.elegans* it was reported that the small RNAs in the nucleus is able to do stable inheritance when it is mediated by the piRNAs (Ashe et al. 2012). Now we still don't know if these signals can move from the germline cells into the somatic cells after the transmission of such small RNA across the generation through the germ cells. The loss of function of Piwi leads to defects in the germ cell and meiotic defects while leads to the increase in transposon activity in the cells (Girard et al. 2006; Yin and Lin 2007).

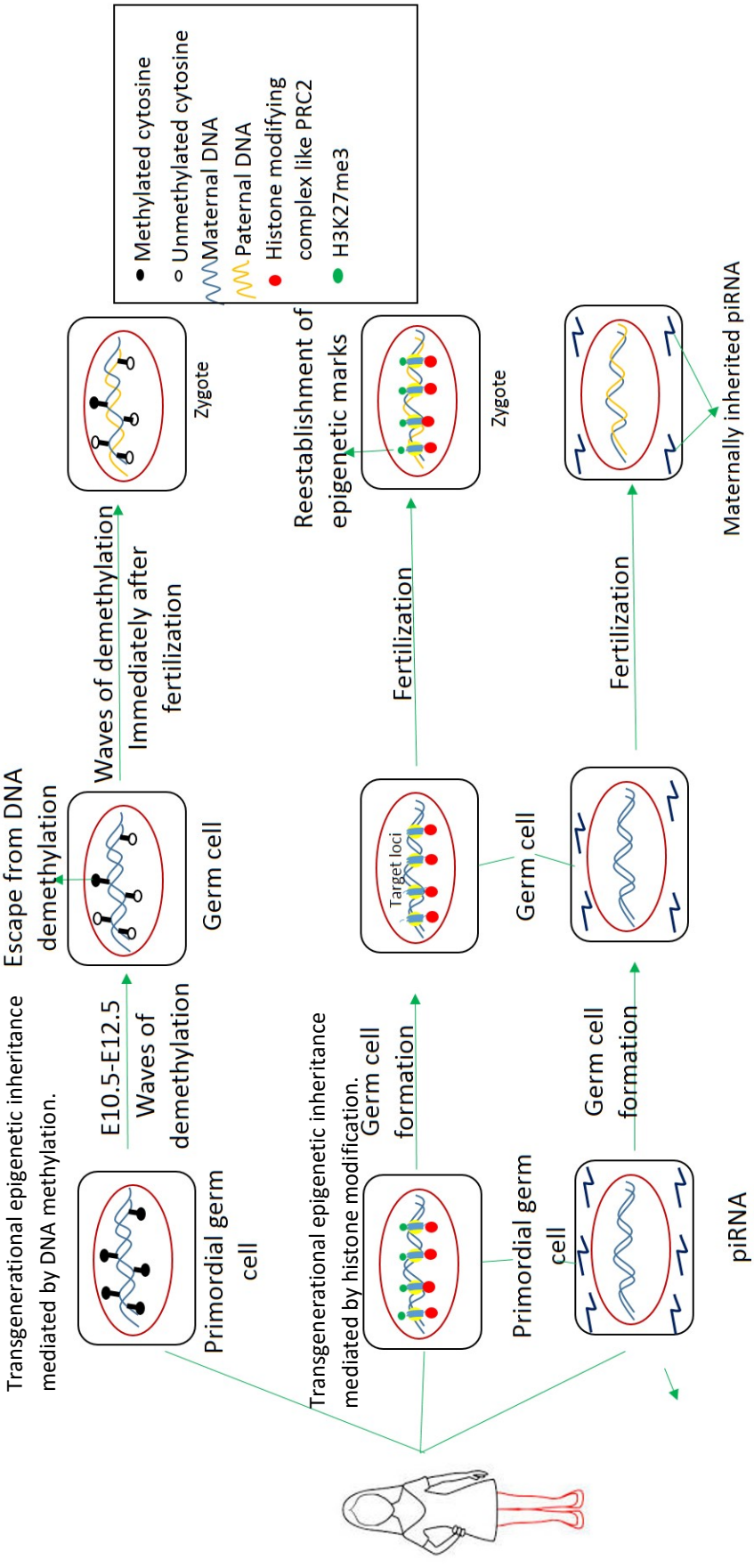

Fig. 1.1: Various modes of transmission of Epigenetic Inheritance

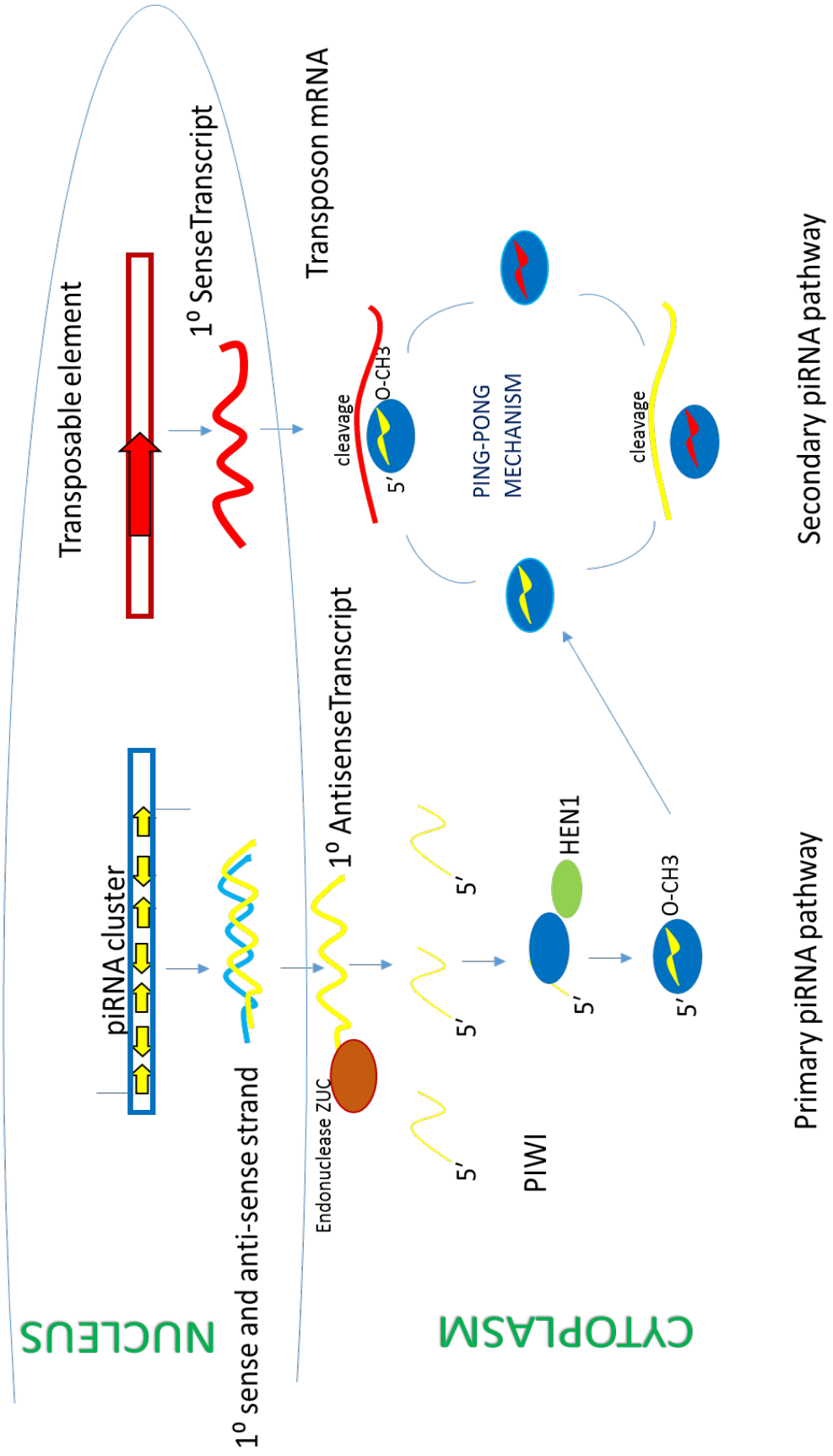

Fig. 1.2: **piRNA biogenesis pathway in Drosophila**

Organism	Environmental cue	Phenotype/Effect
Mice	Good maternal behaviour	postnatal pup licking in rodents (Champagne et al., 2008)
Rat	metabolic changes in parents.	Impaired insulin metabolism and pancreatic cell gene expression is observed in the female F1 progenies when the father of such progeny is exposed to a chronic fat diet (Weaver et al., 2004).
Drosophila	environmental stress	When there is a defective heterochromatin state, it can get inherited over several generations when such organism is subjected to stress (Seong et al., 2011).
C. elegans	olfactory cue	Olfactory imprinting behaviour. Olfactory stimulus is the process in which the exposure of an organism to any olfactory cue effects the behaviour of an organism towards that substance responsible for the olfactory cue during it's adult stage (Remy et al., 2010).

Table 1.1: Example of Epigenetic Inheritance mediated by environment

Organism	Epigenetic inheritance	Phenotype/Effect
Tomatoes (Solanum lycopersicum)	Insect dependent resistance	Transgenerational induced resistance to herbivory due to the siRNAs which are able to move across the cells as well as the vasculature in plants and carry the acquired resistance traits (Luna et al., 2012)
C. elegans	RNAs derived from a viral sequence	These worms produced a large number of small interfering RNAs derived from the virus which are involved in the silencing of the viral genome. The silencing function gets passed on to the progenies of such worms as well due to the inheritance of the viRNAs in them (Guo etal., 2012).
Mice	Phenotype caused by genomic locus disruption.	Mice with an insertion of LacZ into the Kit gene gave rise to genetically wild-type offspring that still exhibited the tail and paw color phenotype characteristic of Kit mutants for at least two generations (Rassoulzadegan et al., 2006).

Table 1.2: Example of Epigenetic Inheritance mediated by small RNAs

Organism	Epigenetic inheritance	Phenotype/Effect
Toadflax (Linaria vulgaris)	DNA methylation pattern	Common Toadflax and Peloric Toadflax are identical in every way except for the shape of the flower. There are two variants of the same plant with a difference in one gene. It is an epigenetic difference. Peloric toadflax can pass on this epimutation to it's progenies (Cubas et al.,1999).
Homo sapien	Diseases due to malnutrition in grand parents	Germany had blocked the supply of food as well as fuel to western Germany during the winter months. As a results a large number of people died and starved due to the lack of food materials. The pregnant women also gave birth to children who had several diseases like schizophrenia, heart disease, diabetes etc. Even the grandchildren of the effected individuals were susceptible to the diseases (Heijmans et al., 2008).

Table 1.3: Example of Epigenetic Inheritance mediated by DNA methylation

Organism	Epigenetic inheritance	Phenotype/Effect
C.elegans	inheritance of fertility and longevity.	When mutation was done in the spr-5 gene which is the worm homolog of the KDM1 gene sterility stated setting in from the 20th generation onwards. This gene is involved in bringing about H3K4 demethylation. It has been recently shown that H3K4me2 actually increases throughout the entire germline of spr-5 mutants. Together, these results suggest that failure to reset H3K4me2 marks at select germline genes in the PGCs may result in progressive transgenerational sterility (Greer et al., 2011).

Table 1.4: Example of Epigenetic Inheritance mediated by Histone modification

1.4 Genomic Imprinting: An example of epigenetic inheritance

Genomic imprinting, the phenomenon involving parent-of-origin-specific expression of certain (imprinted) genes. It is dependent on epigenetic inheritance of DNA methylation and histone modification state, from one generation to the next. In this phenomenon, differential DNA methylation based on the sex of parent is established in the germ cells and inherited to the progeny where it resists any further reprogramming. Thus the epigenetic states are maintained and transferred from one generation to another in exactly the same pattern.

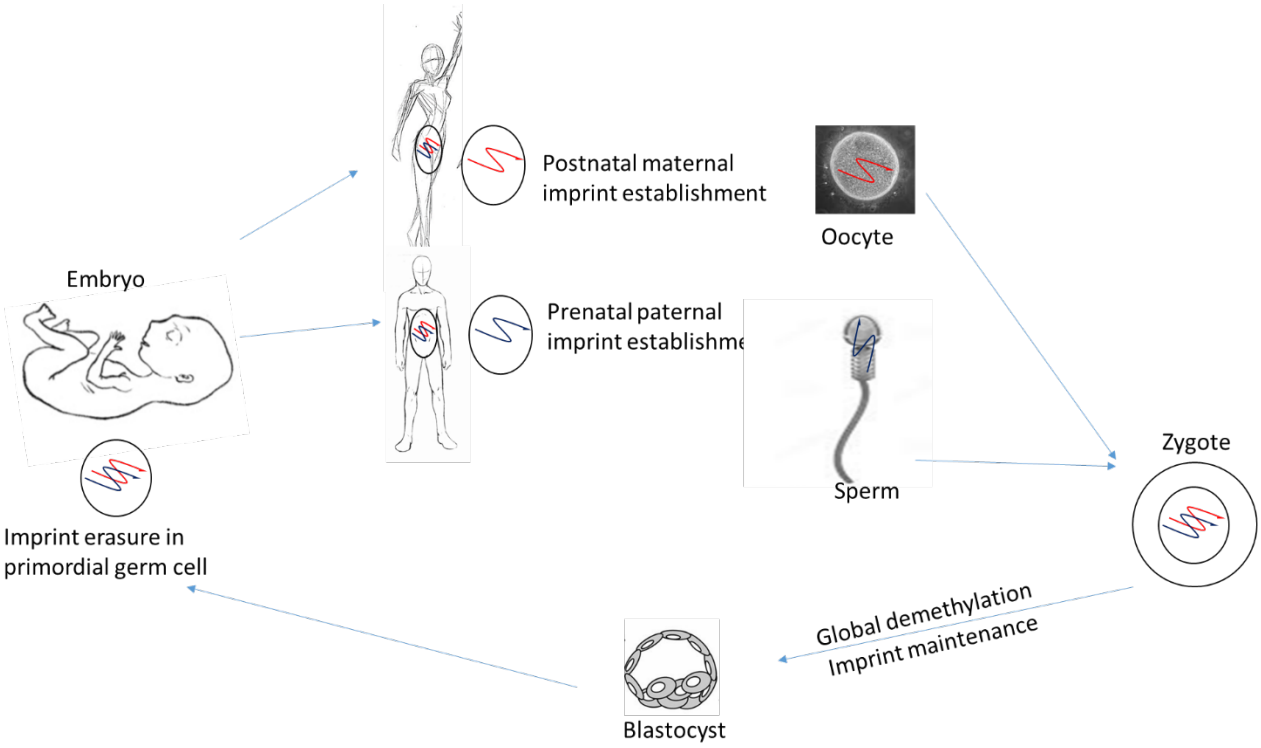

Fig. 1.3 Life cycle of Imprinted genes

1.5 Role of DNMT3L in genomic imprinting

More than 100 imprinted genes have been reported so far. But the exact mechanism as to how these genes escape gain of parent-of-origin–specific marks and then reprogramming is not fully understood. Amongst the various cues available, the role of the catalytically inactive DNA methyltransferase 3-like (DNMT3L) in bringing about methylation of the maternally methylated genes in oocytes is well established.

The sequence of amino acid of catalytically inactive methyltransferase DNMT3L in the cysteine rich zinc finger binding domain is very similar to the DNMT3A and DNMT3B but it lacks the key amino acid residues in the C terminal domain which is responsible for giving the catalytic activity to the protein (Aapola et al. 2000; Aapola et al. 2001). The C terminal of both DNMT3A as well as DNMT3B can interact with C terminal domain of DNMT3L (Gowher et al. 2005). The binding of DNMT3L with DNMT3A strongly enhances the methyltransferase activity of the DNMT3A protein (Suetake et al. 2004). DNMT3L is known to co-operate with the *de novo* DNA methyltransferase DNMT3A to bring about methylation of maternally imprinted genes. It was also found that the Dnmt3l is essential for the methylation of maternally imprinted Imprint Control Loci (ICRs) like *SNRPN* and Igf2r (Bourc'his et al. 2001; Hata et al. 2002). The embryos which didn't had the maternal DNMT3L showed lack of maternal methylation imprint and as a result showed abnormal expression of the maternally imprinted genes. No effect was observed for the paternally methylated gene expression. This indicated that DNMT3L is involved only in the methylation of the maternally imprinted loci during the process of oogenesis.

1.6 DNMT3L as a special epigenetic regulator

DNMT3L is catalytically inactive methyltransferse which can cooperate with the other *de novo* methyltransferase and increase their activity. DNMT3L is highly expressed in testis and to a lesser levels in ovary, thymus and foetal tissue. The mouse Dnmt3l is 74% similar to the human DNMT3L and is known to bring about transcriptional repression by binding to the histone deacetylases like HDAC protein via its PHD like Zn domain which mediates the silencing in the absence of methylation (Aapola et al. 2002; Deplus et al. 2002). In mice, the Dnmt3l expression is regulated by methylation of its own promoter. (Aapola et al. 2004). It has also been found that alpha thalassemia/mental retardation syndrome X-linked homologue (A*TRX*)–DNMT3–DNMT3L (ADD) domain of DNMT3L interacts specifically with the fourth lysine residue of histone H3 when it is unmethylated (H3K4me0) (Guo et al. 2015; Hashimoto et al. 2010). Any methylation in the lysine residue of histone H3 is inhibitory for the binding of DNMT3L. For the interaction between histone H3 and DNMT3L, the first seven amino acid in N terminal of Histone H3 is necessary (Henckel et al. 2009; Hu et al. 2009; Jia et al. 2007; Ooi et al. 2007). Latest studies indicates that the ADD domain reads the combined methylation state of lysine 4 and lysine 9 of Histone H3 (Dhayalan et al. 2011).

Gene knockout study has shown that homozygous *Dnmt3l* knockout mice have normal phenotype and viable but are sterile (Neri et al. 2013; Takashima et al. 2009). The homozygous female knockout mice had normal oogenesis and heterozygote mice born from such homozygous knockout female developed normally till E8.5, but showed reduced size at E9.5 and died till they reached E10.5. Also such progenies showed defect in the neural tube and extraembryonic tissue abnormalities. The male progenies born from homozygous *Dnmt3l* knockout developed hypogonadism and suffered from

azoospermia. Thus the whole process of spermatogenesis gets disregulated and no differentiation occurs from spermatogonia to spermatocytes.

DNMT3L exhibits dual binding function of binding to unmethylated histone H3 as well as triggering the activity of the de novo DNA methyltransferases DNMT3A and DNMT3B. The interacting DNMT3A and DNMT3L can further dimerize to form a tetrameric complex having two active sites. These active sites are separated from each other by around one DNA helical turn. This kind of oligomerization leads to a periodicity of 8-10 bp between the CpG which can then get methylated (Glass et al. 2009; Jurkowska et al. 2008). This type of periodicity in the arrangement of the CpGs has also been observed in case of the Differentially Methylated Regions (DMRs) of 12 maternally imprinted genes in mice.

Recently it was found that in mouse ES cells Dnmt3l functions as positive regulator of methylation in the gene body while in the bivalent promoter region of genes it function as negative regulator of methylation (Neri et al. 2013). There is competition between Dnmt3l and other *de novo* methyltransferases like Dnmt3a and Dnmt3b for binding with the Polycomb group of proteins (PRC2) like EZH2 and Suz12 in the MES cells and this helps in maintaining low methylation level in regions which are enriched for the H3K27me3 repressive mark. This observation very well explains as to why Dnmt3l is highly expressed in MES cells even though the promoter of most genes are hypomethylated. During the process of differentiation the promoters with H3K27me3 mark acquires DNA methylation as the level of Dnmt3l goes down while the levels of Dnmt3a as well as Dnmt3b goes up. Also DNMT3L is very essential for the differentiation of *ESC*s into Primordial Germinal Cells (PGCs) as the levels of markers for PGCs was negligible in *Dnmt3l* silenced cells after 7 days from differentiation of ES cells into embryoid bodies (EB). It was found that loss of DNA methylation was observed at the *DNMT3L* promoter

in cervical and ocular cancer samples (Gokul et al. 2007). An increase in the levels of DNMT3L can have serious consequences. It was found that HeLa cells were reprogrammed on overexpression of DNMT3L in their late passage (around passage 20) and the process mimicked carcinogenesis (Gokul et al. 2009).

1.7 AIM of the thesis

The functional consequence of DNMT3L action is mediated by its interactions with *de novo* methyltransferases DNMT3A and DNMT3B and histone H3. Previous work in our laboratory had shown the loss of DNA methylation for a CpG island spanning the human DNMT3L promoter/exon1 region (promoter for DNMT3L variant 2 and first exon in case of DNMT3L variant 1) for cervical and ocular cancer samples (Gokul et.al, 2009).

Since genome-wide changes in DNA methylation are correlated with carcinogenesis, the loss of DNA methylation observed in the CpG Island around the DNMT3L promoter could either be coincidental to the process of carcinogenesis or has a role to play during carcinogenesis indicative of a role for this region in the regulation of the DNMT3L expression. Therefore the first objective of this thesis was to examine the functional role of this region in regulating DNMT3L transcription.

A second observation from the laboratory was regarding DNMT3L function. Overexpression of DNMT3L in mammalian cell lines caused nuclear reprogramming. Importantly, the DNMT3L dependent reprogramming was gradual and the morphological phenotypic changes were observed only 20 passages after transfection (Gokul et. Al, 2009). The second objective of our study was to examine the reason for this gradual and progressive nuclear reprogramming and determine the mechanism by which DNMT3L achieves it. In particular, we wanted to probe whether the interactions of DNMT3L with DNMT3A/3B on one hand and histone H3 on the other were redundantly influencing a

common subset of genes or whether each of these interactions influenced a different subset of genes. The interaction of DNMT3L with histone H3 in the absence of de novo DNA methyltransferases could either be examined in mammals (mice or cell lines) deleted for DNMT3A and 3B or in an organism that normally lacks these proteins. Deletion of DNMT3A and 3B causes lethality in mice and cell lines are prone to epigenetic instability. In this thesis I have examined the functional significance of DNMT3L action.