Introduction

Current research into epigenetic mechanisms aims at an understanding how the genome is regulated in development and disease. Applying findings to a clinical setting is not straightforward and will require the development of new conceptual frameworks. Even for basic questions the investigation of the establishment of epigenetic patterns in development has been difficult. If epigenetic pathways are blocked, often many developmental aberrations arise resulting in a complex phenotype. The difficulty in interpreting such experiments hinders the development of a coherent view of epigenetic regulation in cell differentiation. Model systems in which epigenetic regulation can be studied independently of developmental anomalies provide an alternative and facilitate the understanding of some basic questions. Such a system is X chromosome inactivation. Here we describe the features that characterize the cellular permissivity to initiation of X inactivation and note that these can also occur in cancer cells and in specific haematopoietic progenitors. We propose that embryonic pathways for epigenetic regulation are re-established in adult progenitor cells and tumour cells. Understanding their reactivation will deepen our understanding of tumourigenesis and may be exploited for cancer therapy.

How is epigenetic regulation relevant for disease?

Deoxyribonucleic acid (DNA) methyltransferases, Polycomb group (PcG) proteins and chromatin remodelling complexes have all been linked to human disease in a number of studies (Table I). DNA cytosine methylation has been examined in different types of cancer (Laird & Jaenisch, 1996). Promoters of tumour suppressor genes can become methylated when they are transcriptionally repressed (Gal-Yam et al, 2008; Laird & Jaenisch, 1996). In addition, the global DNA cytosine methylation level of tumour cell genomes is often reduced when compared to normal cells and hypomethylation has been associated with genomic instability (Eden et al, 2003). Frequently, loss of imprinted methylation and expression is correlated with tumourigenesis (reviewed in Iacobuzio-Donahue, 2009). Defects in the DNA methylation system have also been linked to developmental diseases. Mutations in the DNA de novo methyltransferase DNMT3B gene are associated
with immunodeficiency, centromeric region instability, facial anomalies (ICF) syndrome (Hansen et al, 1999). A mutation in the methyl-cytosine DNA binding protein methyl CpG binding protein 2 (MeCP2) has been linked to RETT syndrome (Amir et al, 1999). Interestingly, restoring MeCP2 function in a mouse model of RETT syndrome can ameliorate the symptoms of the disease (Guy et al, 2007). While technically challenging, restoring MeCP2 expression could therefore be considered for therapeutic approaches.

Polycomb group proteins form complexes that modify chromatin and regulate gene expression (reviewed in Ringrose & Paro, 2007; Schuettengruber et al, 2007; Schwartz & Pirrotta, 2008). PcG proteins are also important players in human diseases. Examples are provided by the observations that elevated expression levels of PcG proteins are associated with brain tumour development (Leung et al, 2004) and prostate cancer progression (van Leenders et al, 2007; Varambally et al, 2002). Significantly, the PcG protein B lymphoma Mo-MLV insertion region 1 oncogene (BMI1) has been shown to collaborate with Harvey rat sarcoma oncogene (H-RAS) to induce an aggressive and metastatic phenotype in a breast cancer model (Hoenerhoff et al, 2009). Recent work also shows that PcG regulation in leukaemia could have important implications for treatment outcomes (Boukarabila et al, 2009). In addition to PcG genes, other components of chromatin remodelling complexes have been associated with diseases such as X-linked alpha-thalassemia with mental retardation (ATR-X syndrome) which is caused by mutations in the SWI/SNF (Switch/Sucrose NonFermentable) homologue 2 (SNF2) chromatin remodelling protein Alpha thalassemia/mental retardation syndrome X-linked (ATRX) (Gibbons et al, 1995). These examples illustrate that understanding the basic principles of chromatin biology is of immediate relevance for a variety of human diseases.

Epigenetic processes also influence human diseases through their normal function in development. Such processes include, but are not limited to, X inactivation and genomic imprinting (Table I). One of the two X chromosomes in female cells becomes inactivated in a random manner to compensate the gene doses for X-linked genes between the sexes. X inactivation leads to a genetic mosaic of cells expressing either the maternal or the paternal X chromosome. Normally about half the cells are expected to express either one of the X chromosomes but deviations from this ratio can be observed. Non-random patterns of X inactivation lead to phenotypic variability in a number of human diseases associated with mutations on one of the two X chromosomes in female patients. The severity of symptoms arising from mutations of the X-linked MeCP2 gene in female RETT patients has been observed to correlate with the pattern of X inactivation (reviewed in Chahrour & Zoghbi, 2007). In Incontinentia pigmenti, which is caused by mutations in the X-linked NF kappa B essential modulator (NEMO) gene, the patches of X inactivation result in a characteristic whorled pattern of skin hyperpigmentation (reviewed in Sun & Tsao, 2008). Similarly, mutations in the X-linked ectodysplasin A gene lead to anhidrotic ectodermal dysplasia with an absence of sweat glands in affected tissue patches (Lexner et al, 2008). Non-random patterns of X inactivation have also been linked to the development of female-predominant autoimmune disease (reviewed in Invernizzi et al, 2008). Non-randomness in X inactivation is often associated with mutations or structural changes of one of the X chromosomes, but its exact cause is not known (Morleo & Franco, 2008). It is likely that mutations on the X chromosome can influence the X inactivation pattern either by cell selection during development or via direct influence on the choice of which X chromosome to inactivate. In general, mutations that affect non-randomness do not appear to necessarily overlap with the disease causing mutation.
Genomic imprinting is a related mechanism that may also contribute to disease development (reviewed in Charalambous et al., 2007). A small number of genes are expressed in a monoallelic fashion dependent upon which parent they were inherited. As a result, mutation of the expressed copy of an imprinted gene has phenotypic consequences. Prader–Willi syndrome is caused by maternal inheritance of deletions on the chromosome 11 that affect the HBII-85 C/D box small nucleolar ribonucleic acid (RNA) cluster and is associated with hypotonia, short stature, obesity, small hands and feet, hypogonadism and mild mental retardation (Sahoo et al., 2008). Other examples are Angelman syndrome, a neurogenetic disease caused by

| Epigenetics related disease | Type of defect | Gene involved | References |
|-----------------------------|----------------|---------------|------------|
| Mutations in chromatin regulators | Neurological disease | MeCP2 gene mutation | Amir et al (1999) |
| RETT syndrome | Chromosome instability and immunological defect | DNMT3b mutation | Hansen et al (1999) |
| ICF syndrome | Neurological disease and alpha-thalassemia | ATRX gene mutation | Gibbons et al (1995) |
| ATR-X syndrome | Malignancy | BMI1 overexpression | Leung et al (2004) |
| Brain tumour | Tumour stem cell proliferation | BMI1 regulates proliferative activity | Lessard and Sauvageau (2003) |
| Leukaemia | Tumour initiation | myeloid/lymphoid or mixed-lineage leukemia (MLL) translocations | Liedtke and Cleary (2009) |
| Leukaemia | Retinoic acid resistant Promyelocytic leukemia zinc finger/retinoic acid receptor, alpha (PLZF/RARA) translocation | BMI1, ring finger protein 1B (RING1B) are essential for transformation | Boukarabila et al (2009) |
| Prostate cancer | Cancer progression; associated with poor clinical outcome | Enhancer of zeste homologue 2 (EZH2), BMI1, RING1 overexpression | van Leenders et al (2007), Varambally et al (2002) |
| Breast cancer | Aggressiveness and metastasis | BMI1 overexpression cooperates with H-RAS | Hoenerhoff et al (2009) |
| Diseases involving imprinted genes | Hypotonia, short stature, obesity, small hands and feet, mild mental retardation | Paternal deficiency for the HBII-85 C/D box snRNA cluster | Nicholls et al (1989), Sahoo et al (2008) |
| Prader–Willi syndrome | Neurogenetic disorder | Maternal deficiency for the UBE3A gene | Kishino et al (1997), Malcolm et al (1991), Matsuura et al (1997) |
| Angelman syndrome | Overgrowth, increase in cancer risk | IGF2 overexpression | Ohlsson et al (1993) |
| Beckwith–Wiedemann syndrome | Disease severity | X-linked MeCP2 mutation | Archer et al (2007), Huppke et al (2006) |
| Haemophilia | Severe disease in heterozygous female patients | X-linked factor VIII mutation | Favier et al (2000) |
| Autism | Potential association with non-random X inactivation | Unknown X-linked gene | Talebizadeh et al (2005) |
| X-linked hypohidrotic ectodermal dysplasia | Severe disease in heterozygous female patients | X-linked ectodysplasin A (EAD) gene mutation | Lexner et al (2008) |
| Chronic granulomatous disease | Severe disease in heterozygous female carriers | X-linked gp91-phox mutation | Anderson-Cohen et al (2003) |
| Epigenetic gene silencing contributing to disease | Neurological disease onset and severity | CGG repeat expansion causes silencing of fragile X mental retardation 1 (FMR1) | Bassell and Warren (2008) |
| Fragile X syndrome | Cancer progression | Silencing of tumour suppressor genes | Gal-Yam et al (2008), Laird and Jaenisch (1996) |

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maternal deficiency of the UBE3A gene (Kishino et al, 1997; Matsuura et al, 1997), and Beckwith–Wiedemann syndrome, an overgrowth syndrome with elevated cancer predisposition associated with over expression of insulin like growth factor 2 (Ohlsson et al, 1993). These examples illustrate how epigenetic processes can contribute to human diseases and modulate the severity of disease symptoms.

A glimpse at the establishment of epigenetic patterns through X inactivation

Understanding X inactivation is also important to address basic questions in epigenetic regulation in mammalian cells. Epigenetic modifications of the genome are thought to prevent illegitimate changes in cell identity and protect an organism from cancer and other diseases. In mammals, the genetic information within each cell’s nucleus contains the information required to specify over 200 distinct cell types. Cells perform specific functions within tissues and must ensure a stable phenotype and maintain their identity, in some cases over lifetime, to support organ function. Many of the cells are replenished by differentiation of stem cells and immature cell types (Fig 1). To allow a phenotypic change from a stem cell to a distinct differentiated cell, cell identity must become unstable at some point in differentiation. This point is of central importance to epigenetic regulation. How epigenetic patterns are established for stable maintenance of cell identity is poorly understood at present.

Dosage compensation systems have been useful to dissect epigenetic mechanisms in flies, worms and mammals (Lucchesi et al, 2005). Chromosome-wide epigenetic changes are applied to balance the different sex chromosome constitution between the sexes (Fig 2). In the case of X inactivation in mammals, an unusual non-coding RNA—X-inactive-specific transcript (Xist) for inactive X specific transcript (Borsani et al, 1991; Brockdorff et al, 1991; Brown et al, 1991)—triggers the silencing of one of the two X chromosomes in female cells. Xist is transcribed from the X inactivation centre (Xic) locus on the X chromosome and then spreads over the entire chromosome. During the differentiation of mouse embryonic stem (ES) cells, the transition from an active X chromosome to an inactive X chromosome (Xi) one can be followed (Rastan & Robertson, 1985). Studying this process continuously during development has shown that X inactivation is separated into an INITIATION and a MAINTENANCE phase (Fig 2B). The accumulation of Xist initiates the repression of genes along the entire X chromosome. Initially, Xist is required for repression (Marahrens et al, 1997; Penny et al, 1996). Using genetic manipulation of Xist expression it has been shown that gene silencing is reversible and that genes will be reactivated once Xist expression is lost (Wutz & Jaenisch, 2000). Upon differentiation of the cell the Xi becomes subject to further modifications (Blewitt et al, 2008; Chaumeil et al, 2002; Keohane et al, 1996). Heritable changes including DNA methylation and histone hypoacetylation keep genes repressed on the Xi without requiring constant expression of Xist (Brown & Willard, 1994; Csankovszki et al, 1999; Wutz & Jaenisch, 2000). The initial trigger—Xist—is not required for maintenance of repression in differentiated cells, and the shift from Xist dependent initiation to stabilization of the inactive state could be regarded as a change of epigenetic context.

Studies of the mechanism of X inactivation have progressed to a point where it is now possible to apply Xist for probing the epigenetic context of cells (Wutz, 2007). Importantly, Xist can initiate gene silencing only in very specific cell types. Naturally, X inactivation is initiated in the cells of the early embryo (Mak et al, 2004; Okamoto et al, 2004; Takagi et al, 1982) but experimental manipulation of Xist expression has shown that also in the adult mouse, cells exist in which Xist can initiate gene silencing. These cells include lineage-restricted progenitors within the haematopoietic system (Savarese et al, 2006). The haematopoietic stem cell (HSC) and mature blood cells, however, do not provide the appropriate cellular context for

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**Figure 1. A schematic view of stem cell differentiation.** Differentiated cells that constitute organs and tissues are generated during development. Their characteristics and identity are faithfully maintained to ensure proper organ function. Throughout life many of these cells are replenished by the differentiation of stem cells. Therefore, adult stem cells have to be maintained stably. Yet, the differentiation of stem cells requires the cell identity to be changed from a stem cell to a differentiated cell with a specialized function. This transition passes through a point when cell identity is not stable (red). Notably, during embryogenesis adult stem cells, themselves, are generated from progenitors (green arrow).
Xist-mediated silencing (Fig 2C). It therefore appears that the function of Xist in initiating chromosome-wide silencing is dependent on specific pathways that exist in the early embryo and are reactivated transiently during the differentiation of adult stem cells of the blood system. Recently, it has been observed that Xist is able to trigger gene silencing also in certain tumour cells (Agrelo et al, 2009). This was shown using a mouse thymic lymphoma model triggered by an oncogenic nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) fusion kinase within the T-cell compartment (Chiarle et al, 2008). In this tumour model, experimental Xist induction did cause ectopic X inactivation and could block tumour development (Agrelo et al, 2009). Hence, lymphoma cells can also display the cellular context that allows initiation of Xist-mediated gene silencing. The special AT-rich binding protein (SATB1) has been identified as an initiation factor for Xist-mediated silencing by comparing expression profiles from Xist-responsive and Xist-resistant tumours (Agrelo et al, 2009). Expression of SATB1 is restricted in development and has been proposed to characterize the cellular context for Xist-mediated silencing in ES cell differentiation and in T lymphocyte development (Agrelo et al, 2009).

SATB1 is a DNA binding protein that interacts with AT-rich DNA sequences and facilitates the organization of chromatin structure (de Belle et al, 1998; Dickinson et al, 1992). In T-cells, SATB1 forms a network which overlaps the base of chromatin loops, suggesting a topological function in gene regulation (Fig 3) (Cai et al, 2006). SATB1 has also been shown to interact with chromatin modifying complexes and to regulate histone modification and nucleosomal positioning over large regions (Yasui et al, 2002). Furthermore, it is subject to posttranslational modifications that could direct its activity (Pavan Kumar et al, 2006; Purbey et al, 2009; Tan et al, 2008). A role for SATB1 in regulating gene expression in a coordinate manner was first observed in T-cell development (Cai et al, 2006). In X inactivation SATB1 could function to organize the chromatin of genes to enable silencing by Xist (Brockdorff, 2009). Such a model is consistent with the observation that Xist initially localizes to the repetitive core of the X chromosome territory (Chaumeil et al, 2006). Genes are positioned in the periphery of the chromosome territory (Chaumeil et al, 2006), where also SATB1 is observed (Agrelo et al, 2009). These observations suggest that SATB1 is a node for signal integration on chromatin consistent with the view that it has a central role in the establishment of epigenetic patterns. Its precise expression control in development and differentiation make it an interesting marker for certain cellular contexts that are associated with epigenetic transitions and potentially, changes in cell identity.
An epigenetic context of progenitors?

The ability of *Xist* to initiate silencing is restricted to very specific cell types indicating that the underlying silencing pathways are under developmental control. In the haematopoietic system the silencing pathway becomes re-established in all haematopoietic lineages at the stage of lineage committed progenitor or precursor cells (Savarese et al, 2006). This has been explicitly shown for pre-B cells and CD4+ CD8+ double positive T-cells. Presently, it is not clear if *Xist* responsive cell types are exclusive to the embryo and the haematopoietic system or if other adult stem cell niches such as the gut or skin are also characterized by re-establishing an appropriate epigenetic context. However, there are indications that the *Xist* silencing pathway is active in a variety of tumour cells. An ectopic human *XIST* transgene has been found to induce chromosome inactivation in human HT-1080 fibrosarcoma cells (Hall et al, 2002). Further, *XIST* expression has been observed in human testicular germ cell tumours, (Kawakami et al, 2003, 2004a; Looijenga et al, 1997). This is unusual as *XIST* is normally not expressed in male cells. However, in these tumours multiple inactive X chromosomes seem to be present suggesting that the context for initiation of X inactivation is associated with the development of certain testicular tumours (Kawakami et al, 2003, 2004a; Looijenga et al, 1997). A recent study by Han and colleagues has further linked SATB1 expression to an aggressive and metastatic phenotype in breast cancer (Han et al, 2008). SATB1 expression conferred metastatic activity to non-aggressive breast cells suggesting that SATB1 reorganizes the genome of cancer cells to become metastatic (Han et al, 2008; Richon, 2008). These observations suggest that the context for *Xist*-mediated silencing might be found in a wider range of tissues and could define the biology of a class of cancer cells. This could provide an opportunity to distinguish certain tumour cells from normal stem cells and differentiated cells and be amenable to the development of new therapies (Fig 4A and B). Consistent with this, links between tumour cells and embryonic cells have been suggested previously. Similarities between gene expression patterns of ES cells and poorly differentiated aggressive forms of cancer have been noted by bioinformatic analysis of expression data for a large set of tumours (Ben-Porath et al, 2008). Although ES cell specific transcription factors do not appear to play a major role in tumourigenesis, certain gene expression signatures are shared between tumour cells and ES cells. Similarities in chromatin composition and organization between tumour cells and ES cells have also been found (Ohm et al, 2007; Tiwari et al, 2008). This supports the idea that certain pathways for chromatin regulation are shared between the epigenetic context of embryonic cells, progenitor cells and tumour cells.

X inactivation and tumourigenesis

A wider link between X inactivation and tumourigenesis has been made in a number of studies observing a gain or loss of X chromosomes in tumour cells (Table II). Testicular germ cell tumours frequently display an increase in the number of X chromosomes as well as chromosome 12 and 17 (reviewed...
in Lind et al, 2007). Although evidence for inactivation of the additional X chromosomes has been obtained, more analysis is needed to fully address this point (Looijenga et al, 1997). Of note, the presence of an active unmethylated XIST promoter has been suggested as a marker for tumour diagnosis (Kawakami et al, 2004a). The presence of an undermethylated XIST promoter is also reported in prostate cancer, albeit XIST was not found expressed in this type of cancer (Laner et al, 2005). In addition, a gain of an X chromosome is also a frequent karyotypic alteration of malignant lymphomas (McDonald et al, 2000).

A correlation between the response to chemotherapy and the level of XIST expression has been observed in ovarian cancer (Huang et al, 2002). In this case, XIST was found down-regulated in recurrent tumours and the level of XIST expression was correlated with the disease-free period of cancer patients with Taxol in the therapeutic regimen (Huang et al, 2002). A likely explanation for the absence of XIST expression in recurrent ovarian cancer could be a loss of the inactive X chromosome in the tumour cells. This is consistent with a frequent Xi loss and amplification of the active X chromosome reported in a range of female tumours such as ovarian, breast and cervical cancer (Kawakami et al, 2004b). Loss of the inactive X chromosome could be a consequence of the genomic instability of cancer cells. Hence, a reduction of XIST expression might be a useful marker for disease progression in certain female tumours.

The gain of active X chromosomes is observed among all classes of breast cancer and is accompanied by a considerable heterogeneity of XIST expression within a tumour sample (Sirchia et al, 2009; Vincent-Salomon et al, 2007). Activation of XIST expression from the active X chromosome has also been observed (Sirchia et al, 2009). Xi also appears to be absent in a number of breast cancer 1 (BRCA1)-deficient breast cancer cells, which has led to the proposal that the breast and ovarian tumour suppressor BRCA1 contributes to XIST localization and X inactivation (Ganesan et al, 2002). However, more detailed analysis has shown that BRCA1 status does not correlate with XIST expression or localization (Pageau et al, 2007; Vincent-Salomon et al, 2007). X chromosome abnormalities have further been linked to the pathogenesis of sporadic basal-like breast cancers, a distinct class of breast cancer with phenotypic similarities to BRCA1-deficient breast tumours (Richardson et al, 2006). Taken together, analyses of XIST expression and X chromosome number might provide opportunities for developing diagnostic markers with strictly tumour type-specific interpretation.

### Conclusions

X inactivation is important to human disease in a variety of ways. Non-random patterns of X inactivation modulate disease onset or the severity of symptoms. The gain and loss of X chromosomes and deregulation of XIST expression has been associated with certain human tumours and holds potential for development of diagnostic markers. There are further aspects of X inactivation that are relevant to human disease without direct clinical application. X inactivation is a model system for addressing basic questions in epigenetic regulation. Recent results suggest that X inactivation studies can facilitate the identification of pathways that are activated in mammals for setting up epigenetic patterns in narrow developmental windows. Detailing the mechanisms behind Xist mediated repression could help defining the cellular context permissive

### Pending issues

- Defining the existence or not of a ‘context for Xist gene silencing’ within adult stem cell systems other than the haematopoietic system.
- Analysing Xist function in a wider range of tumour models to understand how general the silencing context is in tumour formation. Can tumours be classified into Xist-responsive tumours and non-responsive? Does this correlate with specific tumour properties such as metasasis?
- A better understanding of the Xi chromatin structure is needed so that strategies for efficient and selective reactivation of Xi genes can be devised. Note the promising results obtained by reactivating the RETT syndrome associated MeCP2 gene.
- A better understanding of the complex mechanism(s) that regulate(s) random X inactivation, e.g. how do non-random patterns of X inactivation arise?
for epigenetic modifications and highlight differences between tumour and normal cells. While understanding the Xist-mediated gene silencing mechanism provides a potential handle on some of the key mammalian pathways for epigenetic regulation, only a restricted subset of mammalian nuclear pathways can be probed with Xist. X inactivation is specific to placental mammals (Duret et al, 2006). Its recent evolution makes it likely that other components of mammalian dosage compensation already existed before the advent of Xist. These components, which include the Polycomb system or SATB1, are evolutionary older and can be expected to be involved in a wider epigenetic regulation of development. Importantly, the pathways for Xist-mediated gene silencing are limited to very specific cellular contexts. A potential reason for restricting the activity of this silencing pathway might be to safeguard against illegitimate changes of cell identity which could lead to malignant transformation. Studies into this pathway will further our understanding of certain tumour cell contexts and, thus, could help developing new therapies.

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