Review

Epigenetic responses to environmental change and their evolutionary implications

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Chromatin is a complex of DNA, RNA, histones and non-histone proteins and provides the platform on which the transcriptional machinery operates in eukaryotes. The structure and configuration of chromatin are manipulated by families of enzymes, some catalysing the dynamic addition and removal of chemical ligands to selected protein amino acids and some directly altering or displacing the basic structural units. The activities of many of these enzymes are sensitive to environmental and metabolic agents and can thereby serve as sensors through which environmental agents can alter gene expression. Such changes can, in turn, precipitate either local or cell-wide changes as the initial effect spreads through multiple interactive networks. This review discusses the increasingly well-understood mechanisms through which these enzymes alter chromatin function. In some cases at least, it seems that the effects on gene expression may persist even after the removal of the inducing agent, and can be passed on, through mitosis, to subsequent cell generations, constituting a heritable, epigenetic change. If such changes occur in germ cells or their precursors, then they may be passed on to subsequent generations. Mechanisms are now known to exist through which an epigenetic change might give rise to a localized change in DNA sequence exerting the same functional effect, thereby converting an epigenetic to a genetic change. If the induced genetic change has phenotypic effects on which selection can act, then this hypothetical chain of events constitutes a potential route through which the environment might directly influence evolution.

Keywords: chromatin; epigenetics; gene expression; environment; evolution

1. INTRODUCTION

Single-celled organisms must sense and respond to their environment, often by detecting changes in nutrient levels and up- or downregulating expression of selected genes. Regulation of the three genes that make up the *Lac* operon in *Escherichia coli* provides a classic example, the fundamentals of which were worked out almost 50 years ago and revealed concepts that underpin the basics of gene regulation across the living world (Jacob & Monod 1961; Vilar *et al.* 2003). Two proteins, the *Lac* Operator and Repressor, bind to defined sequences at the 5' end of the *Lac* operon and, respectively, enhance or repress the coordinated transcription of the three *Lac* genes. The *Lac* Repressor protein is able to bind lactose and its derivatives. The bound form is inactive as a repressor, thus allowing transcription of genes encoding lactose metabolizing enzymes in an environment rich in this particular sugar. Single-celled eukaryotes operate in a similar way. The yeast *Saccharomyces cerevisiae* switches the expression of a defined family of genes depending on whether its primary nutrient source is glucose or galactose (Holstege *et al.* 1998; Bennett *et al.* 2008).

The switch involves increasingly well-defined signalling pathways that detect the environmental change and transmit an appropriate signal to selected regions of the genome.

It seems likely that most, if not all, cells in multicellular organisms have retained the ability to respond to environmental changes with altered programmes of gene expression. In some higher eukaryotes, environmental changes can drive major changes in phenotype. For example sex determination in some fish is temperature sensitive (Fernandino *et al.* 2008; Marshall Graves 2008; Ospina-Alvarez & Piferrer 2008), while appropriately timed flowering in some plants requires exposure to a cold period, a process known as vernalization (Finnegan *et al.* 2004; Shindo *et al.* 2006). Nonetheless, for most cells in higher eukaryotes, the environment in which they exist is determined by the physiology and metabolism of the organism and of the cells in their immediate neighbourhood. Signals from this local environment, either cell–cell contacts or soluble factors, prompt the cell to put in place programmes of gene expression appropriate for replication, differentiation, quiescence or apoptotic death, depending on the cell type and developmental context. Contact with the external environment is filtered by these surroundings, but may still be influential. For example, although the mammalian embryo is protected from the physical

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and chemical dangers of the outside world by the uterine environment, elements of the maternal diet, drugs or accidentally ingested toxins can find their way into the embryo via the maternal circulation. Such environmental effects can be dramatic, as evidenced by the morphological effects of drugs such as valproic acid (VPA), a simple short-chain fatty acid and an effective anti-epileptic (Duncan 2007; Eadie 2008), or more subtle, as in the complex effects on foetal development associated with high maternal alcohol consumption (Chiaffarino *et al.* 2006; Disney *et al.* 2008).

Establishing the extent to which particular environmental factors influence genome function is an essential first step in defining their potential effects on long-term viability of the target organism. If an environmental factor can induce an epigenetic change that is heritable through mitosis, and that persists even in the absence of the original factor, then there is the potential for significant phenotypic effects long after the initiating factor has disappeared. Further, if such mitotically heritable changes are induced in germ cells, then there is the potential for transmission through meiosis to succeeding generations (figure 1). It is well known, through many years of work on imprinted genes, that epigenetic effects (i.e. whether a gene is expressed or not) can be transmitted through the germ line (Jaenisch & Bird 2003; Reik *et al.* 2003).

In this review, I will explore mechanisms by which environmental factors impact on the functioning of the genome in higher eukaryotes. I will focus on molecular mechanisms that operate through families of enzymes that modify chromatin-associated proteins and will use these mechanisms to describe how an environmental agent might induce a change in gene expression that is heritable through mitosis (effectively an epigenetic mutation) or even through the germ line to subsequent generations. Such a picture is necessarily incomplete and the increasingly important families of regulatory RNAs, reviewed recently (Carthew & Sontheimer 2009), will not be dealt with in any detail; their actions are likely to complement the mechanisms discussed here. I will argue that known mechanisms provide the potential for environmental agents to exert a direct influence on evolutionary change, perhaps by accelerating incremental, Darwinian processes.

2. CONTROL OF GENE EXPRESSION IN HIGHER EUKARYOTES; PROBLEMS POSED BY LARGER GENOMES

The operon model in which activator and repressor proteins bind to specific DNA sequences in ways that are sensitive to the concentrations of metabolites and environmental nutrients is still the paradigm on which our understanding of gene regulation is based. Transcription factors (TFs) (i.e. operators, repressors, etc.), metabolic sensing and positive and negative feedback loops can act together as sophisticated control networks regulating families of genes. This model works well for small genomes, but encounters problems when confronted with the large genomes common in multicellular eukaryotes. It is a surprising fact that, as organisms have increased in complexity

through evolutionary time, genome size has increased out of all proportion to the relatively modest increase in gene number. For example, while *E. coli* has 4200 genes in 4.5×10^6 bp of DNA (approx. 1.1 kb per gene), *Homo sapiens* has approximately 24 000 genes in 3.3×10^9 bp of DNA (approx. 140 kb per gene). The reasons for this remain a matter of conjecture, but it is clear that this excess of non-coding DNA makes mechanisms of gene regulation based solely on TF binding untenable in higher eukaryotes. For example, a typical 6 bp consensus TF-binding sequence will occur by chance once every 4096 (4^6) base pairs, or about 700 000 times in the human genome. Even a relatively large binding sequence of 10 bp, as used by the *Lac* repressor, would still occur about 3000 times by chance alone. As most TFs bind to several sequences similar to the consensus sequence, the number of potential binding sites will be even higher. One way round this numerical problem would be for higher eukaryotes to have evolved much larger TF-binding sequences, but in fact, most binding sequences have remained relatively short at around 6–8 bp (Wray *et al.* 2003). What distinguishes those sites that bind TFs in a functionally meaningful way and the vast majority that do not? Mechanisms by which the TF-binding site problem might be resolved include the selective packaging of DNA as chromatin, a structure unique to eukaryotes, and the enzyme-catalysed, post-translational modification of chromatin and TFs themselves. Both these processes are generally classed as 'epigenetic' and are considered in more detail below.

3. THE NUCLEOSOME AND ITS MODIFICATIONS; A POSSIBLE RESPONSE TO GENOME ENLARGEMENT

Of the various protein–DNA, and RNA–DNA interactions that mediate genome function, that between the histones and DNA occupies a special place, owing to both the abundance of the histones and the intimacy of their association with DNA. The basic unit of DNA packaging is an octamer of histones (two each of H2A, H2B, H3 and H4) around which are wrapped 146 bp of DNA in $1\frac{3}{4}$ superhelical turns (Luger *et al.* 1997). This structure, the nucleosome, is found in virtually all eukaryotes and is the first in a complex series of folding steps that, in higher eukaryotes, package approximately 2 m of genomic DNA into a cell nucleus of approximately 10 μ m diameter. Packaging DNA in this way inevitably influences its ability to bind TFs and other DNA-binding proteins. Experiments over many years have shown that placing a nucleosome over a TF binding site can, in itself, block factor binding (Fragoso *et al.* 1995), and directed nucleosome positioning is a potentially important control mechanism (see below). But the nucleosome can also influence genomic functions in more subtle ways. The four core histones are subject to over 100 different post-translational modifications to defined amino acids, including acetylation of lysine, methylation of lysine and arginine, phosphorylation of serine and threonine and attachment of the short peptide ubiquitin (reviewed in Turner 2005; Kouzarides 2007). All are put in place by specific

Figure 1. An overview of the interacting networks through which environmentally induced changes in gene expression influence cell behaviour and potential. Gene expression is regulated through an interlinked complex of DNA, histones, non-histone proteins (including TFs) and RNA, collectively referred to as chromatin. The functional properties of chromatin are manipulated by families of enzymes; some modify histones and TFs while others directly alter DNA packaging. Environmental agents influence gene expression by regulating or subverting the activities of these enzymes. Some exert a direct effect by inhibiting or activating the enzymes themselves while others act more distantly through cellular signalling pathways that then alter regulatory proteins or metabolites. Subsequent events are characterized by an inevitable proliferation of network interactions. Transcription itself alters chromatin structure, sometimes with functional consequences, while the transcriptome contains many regulatory RNAs, some directly involved in gene silencing. The proteome contains both metabolic enzymes whose activities regulate the levels of key metabolites required for chromatin modification (as illustrated in figure 2) and all the proteins involved in chromatin assembly and manipulation, including chromatin-modifying enzymes. The environmentally induced change in gene expression can occur in either a somatic cell or a cell within the male or female germ line. If the induced change is not passed on, through mitosis, to the progeny of the original cell, then its effects will be restricted to that cell and will be of minimal importance to the organism. If the change is mitotically heritable, then the effects may be far reaching. In a somatic cell, a heritable change can generate a dysfunctional clone of cells with phenotypic consequences (e.g. a tumour). In a germ-line cell, a heritable change may be transmitted to the germ cells themselves (sperm or ova) and potentially, depending on the nature of the change, to the next generation. Mechanisms are available through which an environmentally induced epigenetic change might trigger a targeted change in DNA sequence, leading to a *genetically* heritable mutation exerting an effect on the phenotype of subsequent generations (as illustrated in figure 3).

enzyme families, and removed by others (figure 2). The most extensively studied modifications are found along the N-terminal histone tails, regions that contain little secondary structure and are exposed on the surface of the nucleosome (Luger *et al.* 1997). The histone tails, or their modifications, have little direct effect on nucleosome structure, though they may influence internucleosome interaction and hence higher-order chromatin structure (Luger & Richmond 1998). However, recent analyses, often by mass spectrometry, have shown that numerous amino acids within the globular histone domains inside the nucleosome are subject to modifications exactly analogous to those on the histone tails (Cosgrove 2007). The functional significance of these internal modifications remains to be defined, but they are likely to exert a direct effect on nucleosome structure, and it has been plausibly suggested that they influence nucleosome mobility and perhaps positioning (Cosgrove 2007).

The enzymes known to be involved in setting and removing histone modifications are increasingly numerous; for example, there are 18 histone deacetylases (HDACs) in humans and mice (Gregoret *et al.* 2004; Frye *et al.* 2007) and 28 different methyltransferases known to act on histones, at least *in vitro*, and no

doubt more remain to be discovered (Allis *et al.* 2007). Some histone modifications (e.g. lysine acetylation) reduce the net positive charge of the histone tails and thereby reduce histone–DNA binding, perhaps with functional consequences. Alternatively, specific modified residues, or combinations thereof, can form binding sites for non-histone proteins, which in turn influence chromatin structure and function. This concept was first proposed over 15 years ago (Turner 1993), but only recently has the true diversity of the range of binding options available, and their functional outcomes, become apparent (de la Cruz *et al.* 2005; Taverna *et al.* 2007; Turner 2007). For the present discussion, two points are particularly important. The first is that the steady-state level of each modification represents a dynamic balance between the effects of the modifying and de-modifying enzymes, with turnover likely to vary from one part of the genome to another and between cell types. The second is that many, if not all, of the enzymes are dependent upon, or influenced by, metabolites or components present in the intra- or extra-cellular environment (figure 2). Thus, the nucleosome, through the array of histone modifications it carries and the enzymes that put them in place, can be seen as

Figure 2. Metabolic and environmental influences on histone-modifying enzymes. The N-terminal tail domains of the core histones are acted upon by a variety of enzyme families, many of whose members require common metabolites as substrates or cofactors. The level of modification at any particular amino acid is determined by the dynamic equilibrium between the activities of modifying and de-modifying enzymes. The figure shows just two types of modification: lysine acetylation, controlled by the balanced activities of lysine acetyltransferases and deacetylases, and lysine methylation, controlled by lysine methyltransferases and demethylases. Acetyl- and methyltransferases use acetyl-CoA and S-adenosyl methionine as acetyl and methyl donors, respectively, while demethylases require either flavine adenine dinucleotide (FAD) or α -ketoglutarate and Fe^{++} , depending on whether they act on mono- and di-methyl lysine (e.g. KDM1/LSD1) or tri-methyl lysine (e.g. jmj-domain enzymes). Class III deacetylases (the sirtuins) require NAD as a cofactor. Most deacetylases are inhibited by salts of various short-chain fatty acids, such as sodium butyrate, at concentrations commonly found in the large intestine, and by fungal hydroxamic acids such as TSA. Some demethylases can be inhibited by polyamine derivatives. See text for details and references.

a finely tuned sensor of the metabolic state of the cell and the composition of its environment. It provides a potential platform through which environmental variables can influence genomic function.

As might be expected of a system in which combinations of modifications determine the specificity of binding of effector proteins, there is interaction (also called cross-talk) between modifications that helps determine the pattern of modifications to be generated. For example, treatment of cells with a variety of HDAC inhibitors (figure 2) not only leads, as expected, to global hyperacetylation of core histones, but also generates hypermethylation of H3 lysine 4 (Nightingale *et al.* 2007). Methylation at other lysines is unaffected. The explanation for this may lie in the properties of the methyltransferase catalytic domain. The catalytic (SET) domain of the enzyme responsible for H3 lysine 4 methylation, MLL1, shows enhanced methylation activity against highly acetylated histone tail substrates (Nightingale *et al.* 2007). Other factors may also play a role (Lee *et al.* 2006), but the important point is that an inhibitor can generate changes in histone modification beyond those expected from the catalytic activity of the enzyme it initially inhibits.

4. POST-TRANSLATIONAL MODIFICATION OF TRANSCRIPTION FACTORS

Chromatin-associated non-histone proteins, including many TFs, are subject to a similar variety of enzyme-catalysed post-translational modifications to those on

the nucleosome. For example, androgen receptor (AR), cMYC and p53 can all be phosphorylated at specific serines and threonines, acetylated at specific lysines and ubiquitinated, also at specific lysines (Glozak *et al.* 2005; Vervoorts *et al.* 2006; Popov *et al.* 2007; Vousden & Lane 2007). The modifications are put in place and removed by the same enzyme families that are involved in histone modifications, often with several different enzymes acting on the same factor; e.g. the acetyltransferases GCN5, TIP60 and CBP/p300 all act on cMYC and have been associated with distinct functional outcomes (Popov *et al.* 2007).

Specific modifications have selective effects on TF function. For example, acetylation of the AR enhances its ligand-dependent activation of a subset of target genes, leading to enhanced cell growth, but so far has been found to have little or no effect on its repressive or apoptosis-inducing activities (Fu *et al.* 2004; Popov *et al.* 2007). Acetylation of p53 is essential for its role as a mediator of the stress response, though other functional effects seem less acetylation dependent (Tang *et al.* 2008). As with histones, modifications of TFs are interdependent and interactive. At the simplest level, acetylation of the ϵ -amino group of a lysine blocks ubiquitinylation of that same lysine. Attachment of a single ubiquitin molecule can enhance TF function, while a ubiquitin polymer at the same residue can target the protein to the proteasome for degradation (Vervoorts *et al.* 2006). This is an important mechanism for downregulating the transcriptional response to TFs. Interactions can be more subtle. For example

cMYC can be phosphorylated at threonine 58, but only if serine 62 is phosphorylated first (Yeh *et al.* 2004; Arnold & Sears 2006). Ubiquitinylation of cMYC at K48 by the SCF-FBW7 complex requires phosphorylation of threonine 58, but occurs only if serine 62 is first dephosphorylated by the PP2A phosphatase (Vervoorts *et al.* 2006).

5. CHROMATIN-MODIFYING ENZYMES AS SENSORS OF ENVIRONMENTAL AND METABOLIC CHANGE

Chromatin-modifying enzymes are susceptible to environmental agents and metabolite fluctuations. For example, a combination of genetic and biochemical experiments has shown that vernalization in flowering plants requires methylation of specific histone arginine and lysine residues (Finnegan & Dennis 2007; Schmitz *et al.* 2008), revealing a link between temperature and chromatin state. Chromatin-modifying enzymes are also susceptible to the concentrations of various metabolites. The kinases, acetylases and methylases that act on histones and TFs are all dependent on high-energy cosubstrates (figure 2), the levels of which can affect their activities. However, for one enzyme family at least, specific mechanisms are in place that allow a more subtle response to metabolic change. The NAD-dependent class III deacetylase SIRT1 has been shown to act on both histones and TFs such as P53 (Vaziri *et al.* 2001) and AR (Fu *et al.* 2006) and provides an intriguing link with the metabolic state of the cell (Rodgers *et al.* 2005). A high NAD/NADH ratio enhances SIRT1 activity, with deacetylation of AR and diminution of its growth-promoting activity. Conversely, low levels of NAD, or high levels of the inhibitor (and SIRT1 product) nicotinamide, suppress SIRT1 activity and hence can enhance the acetylation-dependent activities of AR. SIRT1 may act as a sensor of the redox state of the cell (Fulco *et al.* 2003). This is particularly significant in light of the long-standing observation that many cancers, including AR-dependent prostate cancer, show enhanced glycolysis, even under aerobic conditions, with a consequent diminution in NAD and enhanced lactate levels (Altenberg & Greulich 2004; Baron *et al.* 2004).

Intriguingly, a second product of the SIRT1-catalysed deacetylation reaction, the NAD metabolite *O*-acetyl-ADP ribose (OAADPR), binds selectively to the macro-domain of the histone variant macroH2A, a marker of heterochromatin (Borra *et al.* 2004; Kustatscher *et al.* 2005; Tong *et al.* 2009). OAADPR binding induces only a subtle structural change and the functional outcomes remain uncertain. However, the finding that OAADPR is bound by the splice variant macroH2A1.1 and not by a second variant, macroH2A1.2, suggests a high level of binding specificity (Kustatscher *et al.* 2005). The catalytic mechanism of SIRT1 is far more complex than necessary to deacetylate proteins; the 11 class I and class II deacetylases are straightforward hydrolases without obligatory cosubstrates (Marmorstein & Trievel 2008). However, it is interesting that when these deacetylases are assayed against native histone substrates (rather than

the commonly used synthetic peptides), their activity is enhanced by ATP and chaperone proteins such as the stress response protein HSP70 (Johnson *et al.* 2002). Collectively, these findings suggest that the mechanisms of action of at least some protein deacetylases have evolved to provide a link between intermediary metabolism, or environmental components, and gene function.

6. CHROMATIN AND TRANSCRIPTION FACTOR BINDING

For some TFs, there is evidence to suggest that histone-modification state is somehow linked to their selective binding. cMYC is a member of the MYC/MAX/MAD family and forms a heterodimeric complex with MAX to activate the expression of a diverse range of genes. Deregulated expression of c-MYC has been documented in a wide range of human malignancies (Vita & Henriksson 2006). Like many TFs, MYC has the potential to target a large proportion (11%) of all genes in the human genome (Fernandez *et al.* 2003), but the set of genes to which it actually binds in any particular cell is much more restricted and regulated by a variety of factors, including interacting proteins. For example, the MAD family of transcriptional repressors are, like MYC, MAX-binding proteins and antagonize the activity of MYC by competing for MAX binding at E-box sequences in target gene promoters, actively repressing transcription of MYC target genes (Adhikary & Eilers 2005). The specificity and affinity of MYC binding may also be influenced by the configuration of the chromatin packaging at potential binding sites, and particularly by patterns of histone modification (Guccione *et al.* 2006). MYC was found to bind E-boxes in regions enriched for several histone modifications including acetylated H3 (specifically H3 acetylated at lysines 9, 14 and/or 18), but showed the strongest association with H3 tri-methylated at lysine 4 (H3K4me3). All these modifications are generally associated with relatively 'open' euchromatin. Reciprocally, MYC binding was inversely correlated with the repressive polycomb group mark H3K27me3 (Guccione *et al.* 2006). Whether these correlations reflect a specific underlying mechanism or are simply due to overall differences in chromatin compaction, and hence accessibility, remains to be seen.

A second example is provided by the pioneer factor FoxA1, a factor central to certain oestrogen receptor (ER α) functions (Carroll *et al.* 2005; Laganier *et al.* 2005). FoxA1 binds with a high specificity to a genomic consensus sequence, but, as with other factors, only a small proportion of possible sites (3.7%) are actually occupied and chromatin immunoprecipitation (ChIP) analyses show that these occupied sites are significantly enriched in H3K4me1 and H3K4me2 (Lupien *et al.* 2008). Knock-down of FoxA1 does not alter the levels of these modifications, indicating they are present prior to FoxA1 binding, presumably to facilitate preferential recruitment. Significantly, over-expression of the histone demethylating enzyme KDM1/LSD1 decreased levels of H3K4me2 and significantly inhibited FoxA1 binding to chromatin (Carroll *et al.* 2005; Lupien *et al.* 2008).

Recent evidence indicates that information encoded in the DNA itself, beyond the consensus-binding sequences, also plays a role in determining TF binding. Odom and colleagues mapped the binding sites of selected TFs across human chromosome 21 (chr.21), first when it was present in wild-type human fibroblasts and then when it was the only human chromosome in mouse × human hybrid fibroblasts (Wilson *et al.* 2008). In the latter case, TFs and epigenetic control elements originated almost exclusively from the mouse. Surprisingly, the distribution of TFs across chr.21 in a mouse background closely resembled that seen in exclusively human cells, and differed from the distribution across extensive regions of the mouse genome homologous to chr.21 (Wilson *et al.* 2008). The authors conclude that information encoded in DNA, beyond the genetic code itself, is a major determinant of TF positioning. The code(s) involved remain to be deciphered.

The simplest way in which the nucleosome can influence TF binding is by being positioned such that the recognition sequence is tightly associated with the histone core and inaccessible to the TF. It is known that nucleosomes can occupy defined positions at the promoters and control regions of certain genes (Mellor 2005; Montecino *et al.* 2007; Tirosh *et al.* 2007; Jiang & Pugh 2009) and that certain DNA sequences strongly favour the placement of a nucleosome while others discourage it, probably because they are less 'bendable' (Tirosh *et al.* 2007). Nucleosome positioning is determined by multiple factors, but there seems no doubt that DNA sequence plays an important role, though the sequences involved are complex and the formulation of rules is challenging (Segal *et al.* 2006; Kaplan *et al.* 2009). Thus, at least for those TF-binding sites, and indeed those gene promoter regions, whose accessibility is regulated by nucleosome positioning, it is likely to be the DNA sequence that is the primary determinant of nucleosome placement. The histone octamer itself is likely to play a functional role that is modulated, in some instances at least, by post-translational modifications. DNA sequence, histones and chromatin-modifying enzymes are all involved in producing the functional end result.

TF function, and thus correctly regulated gene expression, seems to require an integrated network of genetic and epigenetic components, comprising a triumvirate of DNA, TFs and chromatin, with none acting in isolation or having priority over the other two (figure 1). Thus, while it is common to distinguish between genetic and epigenetic processes, the former being based directly on information encoded in the DNA sequence and the latter being those necessary for the interpretation of this information (Holliday 2006; Ptashne 2007), the two processes are often so closely interlinked and interdependent that attempts to tease them apart are problematic and potentially misleading.

7. HERITABILITY OF EPIGENETIC CHANGE AND CELL MEMORY

Epigenetic effects are often heritable, in the sense that they are passed on from one cell generation to the next.

Proof of principle comes from X-inactivation, the process by which most genes on just one of the two X chromosomes in female cells are inactivated early in development (Heard *et al.* 1997). The X to be inactivated is chosen at random in each of the 20 or so cells of the (mouse) inner cell mass (cells that will go on to form the complete embryo). Once chosen, the X undergoes a series of epigenetic changes, including various changes in histone modification and composition and increased DNA methylation at selected regions. Once in place, these changes persist, in all the progeny of the original cell, though the lifetime of the organism. Under normal circumstances, reactivation occurs only in the primordial germ cells. Thus, epigenetic silencing, particularly if it involves multiple layers of epigenetic changes, can be both heritable and stable.

X inactivation can be seen as the answer to a very specific situation (i.e. a chromosome imbalance between males and females), but all cells must have access to mechanisms by which epigenetic properties can be passed on to daughter cells, if only as a means of retaining cell identity. It seems reasonable to assume that cells are defined by the tissue-specific genes that they express and, if a cell is to retain its identity through DNA replication and mitosis, then this characteristic pattern of gene expression must be maintained. This is sometimes called 'cellular memory'. Many characteristics distinguish active from inactive genes, and it is not unreasonable to propose that transcriptional states are faithfully transmitted, by default, with genes that are on staying on and genes that are off staying off, unless (differentiation) signals tell them to do otherwise. Formulated in this way, the issue of cell memory becomes rather simple. Unfortunately, this analysis avoids some complicating facts.

A cell can usefully be seen as a robust, dynamic system in which the concentration of each component (protein, RNA transcript metabolite, etc.) will vary, through time, within set limits. These limits are determined by the homeostatic checks and balances that operate across the system, as well as by stochastic variations in gene expression levels, sometimes called transcriptional 'noise' (Raj *et al.* 2006; Maamar *et al.* 2007). Thus, there is not one state that defines, for example, a fibroblast, but a vast number of different states in which the components vary, but within the limits that operate for the (fibroblast) system as a whole. This situation was illustrated some years ago by microarray expression analysis to quantify RNA transcript levels in yeast. Transcript levels for individual genes varied over a wide range and, remarkably, 80 per cent of genes classified as 'active' in the culture as a whole, had transcript levels between 1 and 0.1 on a per cell basis (Holstege *et al.* 1998; Holland 2002). PCR analysis shows that many transcripts are present at even lower levels (Holstege *et al.* 1998; Holland 2002). This could be due to the extremely low stability of some mRNAs, with their protein products being rather more stable, but it also raises the possibility that many transcripts are present in some cells, but not in others. On this interpretation, some active genes have no transcript in most cells because the gene is simply not being transcribed in these cells

because, in turn, the gene product is already present in sufficient quantity.

This interpretation of low transcript levels suggests that there should be a wide *variation* in levels of individual transcripts (or their products) from one cell to another. Such analyses are experimentally challenging, but recent results show that cell–cell variability can be enormous for both RNA transcripts and protein products (Raj *et al.* 2006). For example, flow cytometry has been used to measure levels of individual GFP-tagged proteins in yeast on a cell by cell basis (Newman *et al.* 2006). There were dramatic differences, from one gene to another, in the extent to which their protein products varied from cell to cell. This variability (noise) was closely correlated with both the mode of transcription and the function of the protein product. Proteins that respond to environmental changes were particularly ‘noisy’.

The enormous variability in transcript levels from one cell to another can be interpreted in two general ways. The first is that gene transcription is inherently variable (noisy), governed by chance and perhaps occurring in temporally discrete bursts (Raj *et al.* 2006; Raj & van Oudenaarden 2008). While this seems at first an unlikely strategy, it can, by generating a variable population, provide a colony of cells with a cost-effective way of protecting itself against the vagaries of environmental change (see below for an example). On the other hand, cell–cell variability could be the inevitable result of a carefully controlled process of cellular homeostasis. Under this interpretation, many, perhaps most, genes are transcribed only when their product (RNA or the protein translated from it or a dependent metabolite) has fallen below a set minimum level. Transcription continues until the maximum level is reached, when the gene is switched off again. Depending on a variety of factors, such as the stability of the mRNA, protein product or metabolite, the on–off cycle may happen several times in a single cell cycle, or once in several cell cycles. The latter presents a situation where the overall (population-wide) transcript level may be less than one per cell.

Whether gene expression is essentially stochastic or carefully regulated through maintenance of cellular homeostasis, or a combination of both, the issue of ‘cell memory’ changes its character when viewed from this systems biology perspective. Heritability must be seen as a property of the whole system, with the transcription of each gene being determined by the requirements of the system, perhaps over a period of several cell cycles. It may be that genes that are classed as active when the whole cell population is examined, are transcriptionally silent in many individual cells, perhaps for one or more complete cell cycles. What is inherited cannot be the transcriptional state itself, but perhaps the ability to respond appropriately to signals that reflect the level of specific components of the system. The components and signals involved inevitably vary from one gene to another and there may be no unifying mechanism.

8. ENVIRONMENTALLY INDUCED EPIGENETIC CHANGE

Cells are programmed to respond to specific environmental signals. Patterns of gene expression in

single-celled eukaryotes change in a specified way depending on available nutrients (Holstege *et al.* 1998; Bennett *et al.* 2008) while the cells of higher eukaryotes progress down pathways of differentiation in response to specific signals, often from their neighbours. Once a cell is primed to respond, then even a simple chemical such as retinoic acid can initiate a dramatic change in gene expression patterns and cell phenotype (Muller 2007). Conversely, cells seem to be remarkably resistant to change when exposed to agents to which they have not been primed, even when these agents generate what appears to be a major epigenetic change. As an example of this, I will explore how cells respond to growth in the presence of HDAC inhibitors such as sodium butyrate, reagents that cause global hyperacetylation of all four core histones (figure 2). These reagents are not just of experimental interest. The salts of various short-chain fatty acids, including sodium butyrate, are present at millimolar concentrations in the large intestine in humans and rodents, largely produced by endogenous bacteria (Pryde *et al.* 2002; Louis & Flint 2009). There is evidence to suggest that their intra-intestinal concentrations are influenced by diet and they have been implicated in protection against colon cancer (Dashwood & Ho 2007; Waldecker *et al.* 2008). The branched chain analogue VPA, also an HDAC inhibitor, is an effective and widely used anti-epileptic, well tolerated by healthy adults, but a known teratogen (Phiel *et al.* 2001).

Increased levels of histone acetylation are characteristic of actively transcribed genes and one might expect HDAC inhibitors to cause a major upregulation of gene expression and serious disruption of the properties of the cell. In fact, a variety of studies have shown that only a small proportion of genes show altered transcription in response to HDAC inhibitors, and among the genes that do change, downregulation is as common as upregulation (Peart *et al.* 2005). Consistent with this, effects on cell behaviour are usually modest, with slowed cell cycle progression and, on prolonged exposure, increased frequency of apoptotic cell death being common responses (Phiel *et al.* 2001).

A possible explanation for this limited response to environmentally induced histone hyperacetylation comes from the analysis of histone-modification levels at individual gene promoters by ChIP. In a variety of cell types, exposure to HDAC inhibitors caused no increase in histone acetylation across the great majority of genes tested, even those that showed increased expression (VerMilyea *et al.* 2009; M. D. VerMilyea 2008, unpublished data). It seems that the global histone hyperacetylation detected by western blotting of bulk histones is confined largely to non-genic regions, with the majority of genes remaining unaffected. The reasons for this remain to be defined, but may reflect differences in the turnover of histone acetates from one genomic region to another. The results illustrate the ability of adult and embryonic cells to retain their characteristic phenotypes even in the face of what seems to be a major, environmentally induced epigenetic change.

However, in specific cell systems, the physiological response to HDAC inhibitors can be more dramatic. Studies of epidermal stem cells have provided an intriguing illustration of the relationship between a TF, chromatin modifications and adult stem cell differentiation (Frye *et al.* 2007). Quiescent stem cells are induced to leave their niche in the interfollicular epidermis and hair follicle bulge by activation of cMYC, an oncogene and TF. The process is accompanied by globally increased H4 acetylation and di-methylation of H3K9 and H4K20. Remarkably, induction of histone hyperacetylation by treatment with the HDAC inhibitor Trichostatin A (TSA), either amplified the differentiation promoting effects of cMYC, or substituted for it in inducing epidermal differentiation. As noted earlier, if a cell is primed to respond to a defined and specific signal, then a generalized environmentally induced change might also trigger that response.

It may be that only a subpopulation of cells in any group of cells are 'primed' to respond in a particular way, possibly as a result of cell–cell variation or transcriptional noise. While this is pure speculation in higher eukaryotes, there is evidence for just such an effect in *Bacillus subtilis*. When a culture of *B. subtilis* moves into quiescence, usually through nutrient depletion, a proportion of cells become able, through expression of several specific genes, to take up foreign DNA from the surroundings, a state described as 'competence'. The switch to competence is controlled by a master regulator protein, ComK. Expression of *ComK* varies widely from one cell to another, i.e. its transcription is noisy. As the culture moves into quiescence, cells that happen, by chance, to be expressing relatively high levels of the *ComK* competence regulator can move into a state where *ComK* enhances its own transcription through a positive feedback loop. This leads to a rapid increase in ComK levels, which pushes the cell into a stable 'competent' state. The opportunity for cells in the culture to switch to competence persists for approximately 2 h, by which time approximately 15 per cent of cells have become competent. Thereafter, these cells remain competent, but no further switching occurs (Maamar *et al.* 2007). Thus, noisy transcription had provided a subpopulation of 'primed cells' that could respond to a generalized environmental stimulus by differentiating into an altered state.

9. HERITABILITY OF INDUCED EPIGENETIC CHANGE THROUGH MITOSIS

Recent work has shown that *Hox* genes, specifically the mouse *Hoxb* cluster, are unusual in that, in both embryonic stem (ES) cells (Chambeyron & Bickmore 2004) and the pre-implantation embryo itself (VerMilyea *et al.* 2009), they show increased histone acetylation following treatment with HDAC inhibitors, including VPA. The increased acetylation is not accompanied by any immediate increase in transcription, which remains undetectable in both embryos and ES cells. Histone hyperacetylation is clearly not sufficient to override mechanisms responsible for *Hox* gene silencing in the early embryo, but

it is interesting that, despite their silent state, histone acetates across the *Hoxb* loci are turning over rapidly. Given the crucial importance of the *Hox* genes as determinants of positional and temporal gene expression in the embryo, and their ability to induce a major morphological change when their function is subverted, the finding that their chromatin is unusually susceptible to environmentally induced change is of some interest. Remarkably, when mouse embryos were cultured from the 8-cell stage to morula in the presence of VPA, and then further cultured, in the absence of inhibitor, to the blastocyst stage, acetylation at *Hox* gene promoters was always higher in blastocysts derived from VPA-treated morulae than in their untreated counterparts. Thus, the environmentally induced change in histone acetylation has been passed on, through mitosis, to a later developmental stage in the absence of any change in transcription. Whether this change affects the timing or location of *Hox* gene expression later in development (i.e. at stages when *Hox* genes are normally induced) remains to be seen and requires reimplantation of the cultured embryos. However, the observation shows that mechanisms for the inheritance, through mitosis, of induced histone modification are present in the early embryo and are not transcription dependent. In contrast to the situation in the pre-implantation embryo, experiments in the author's laboratory have so far shown that the VPA-induced hyperacetylation of *Hox* gene promoters in cultured ES cells, derived from the inner cell mass of the blastocyst, is not heritable (H. Stower & B. M. Turner 2008, unpublished data). Thus, as with the transcriptional responses to HDAC inhibitors described earlier, heritability seems to be dependent on both cell type and/or developmental stage.

The ability of HDAC inhibitor to induce a mitotically heritable change in histone acetylation and gene expression was first demonstrated in the yeast *Schizosaccharomyces pombe* (Ekwall *et al.* 1997). Growth for several cell cycles in the presence of the HDAC inhibitor TSA induced hyperacetylation and transcription in normally silent test genes inserted into centric heterochromatin. The active, hyperacetylated state, though spontaneously reversible at low frequency, was retained through many cell cycles in the absence of inhibitor. However, because acetylation and transcription remained closely linked throughout these experiments, it was not possible to determine which of these two factors was the primary determinant of heritability. More recently, nuclear transplantation in *Xenopus* has been used to show that some genes (e.g. the endodermal gene *edd*) can retain a memory of an active gene state, even in an inappropriate (e.g. non-endodermal) cell lineage; the memory can be transmitted through up to 24-cell generations from zygote to tadpole (Ng & Gurdon 2008). What makes this particularly significant is that through the first 12 cleavage divisions of the *Xenopus* embryo, there is no genomic transcription, so the memory mechanism involved does not require active transcription. Chromatin seems to have a role in this memory in that the variant histone H3.3 and specifically its methylatable lysine 4 residue, seems to be necessary

for re-expression (memory) of the active state after progression through the early cleavage cycles. H3.3 associates preferentially with active genes (Ahmad & Henikoff 2002; McKittrick *et al.* 2004) and may play a role in the maintenance of an active state, even in the absence of ongoing transcription.

10. EPIGENETIC HERITABILITY THROUGH THE GERM LINE

If mitotically heritable changes are induced in germ cells, then there is the potential for transmission through meiosis to succeeding generations (figure 1). It is well known, through many years of work on imprinted genes (i.e. genes that are differentially expressed in offspring depending on whether they were transmitted through the maternal or paternal germ line) that epigenetic effects can be transmitted through the germ line, though the mechanisms remain mysterious (Jaenisch & Bird 2003; Reik *et al.* 2003; Santos & Dean 2004). DNA methylation is likely to be involved, but seems not to provide a complete explanation.

Attempts to demonstrate experimentally the germline inheritance of induced phenotypic changes are fraught with difficulty. It has been claimed that exposure to the fungicide and endocrine disruptor vinclozolin at specific stages of embryonic development can trigger changes in male fertility and reproductive behaviour that are heritable, over several generations, through the male germ line (Anway *et al.* 2005, 2008). Perhaps inevitably, the interpretation of these difficult experiments remains controversial (Schneider *et al.* 2008). In a different approach to the same issue, statistical analysis of disease susceptibilities in an isolated human population in northern Norway revealed an intriguing correlation between age of death from specified diseases and the nutritional status (i.e. success or otherwise of the harvest) of the grandparental generation (Kaati *et al.* 2007).

Transmission through the male germ line presents additional problems for epigenetic inheritance. Sperm DNA is in a particularly condensed state, with the great majority of histones replaced by protamines. Within minutes of fertilization, sperm DNA is repackaged with maternal histones, followed by an overall loss of methylated cytosine. However, it is likely that some regions (imprinted genes perhaps) are protected from demethylation (Santos & Dean 2004), while careful analyses have shown retention of a small amount of histone in sperm chromatin (Gatewood *et al.* 1987, 1990), with enrichment of selected variants, such as H3.3 and H2AZ (Ooi & Henikoff 2007). Further, H3.3 in sperm is rich in modifications associated with transcriptionally active chromatin, such as methylation of lysine 4 (Ooi & Henikoff 2007). Sperm histones may be associated with specific genes, perhaps those that need to be expressed very early in zygotic development, but this remains to be definitively shown.

While acknowledging the complexity and experimental challenges posed by work on epigenetic inheritance, there is now no reason to dismiss it because (potential) mechanisms do not exist. If

environmental agents can induce a heritable change in, for example, histone modification in somatic cells, then it is likely that it can also happen in germ cell precursors and be transmitted to the germ cells themselves and thence to the zygote. Effects on the developing embryo and the adult organism will depend on the genes involved, but in the case of the *Hox* genes could be far reaching. It has been suggested that the teratogenic effects of VPA might be mediated, in part at least, through disruption of *Hox* gene expression (Faiella *et al.* 2000; Duncan 2007). While the VPA effects studied so far are exerted in the (early) embryo itself, it is conceivable that a heritable change induced in the germ cells of either parent and transmitted to the zygote could exert an effect. If the epigenetic change were to persist, through multiple mitoses, in the germ cell of the next generation, then one has true epigenetic inheritance.

11. CAN EPIGENETIC CHANGE ALTER DNA SEQUENCE?

It has been shown that induced epigenetic changes can be inherited through mitosis, and plausible mechanisms exist by which epigenetic changes could be inherited through either the male or female germ lines. However, if environmental changes are to lead to heritable changes that persist over many generations, and perhaps even influence evolutionary change, then they must surely, at some stage, lead to changes in the DNA sequence itself that mimic, functionally, the initiating epigenetic change. Are there any mechanisms by which a conversion from epigenetic to genetic information might occur? One possibility is presented by the enzyme-catalysed methylation of cytosines in DNA.

Methylation of cytosine at carbon 5 of the pyrimidine ring (5'meC) is a relatively frequent modification of DNA in many, though not all, higher eukaryotes. It is put in place by well-characterized DNA methyltransferases and is generally a stable modification, though rapid demethylation occurs in specific physiological situations. For example, in the mouse zygote the paternal genome is demethylated shortly after fertilization (Morgan *et al.* 2005). Demethylation is problematic owing to the very high energy required to split the C–C bond and the mechanism remains controversial. It may involve complete removal of methylated cytosine and replacement by the unmethylated base using enzymes of the DNA repair system (Ooi & Bestor 2008; Gehring *et al.* 2009). In mammals, cytosine methylation occurs almost exclusively at CpG dinucleotides, reflecting the specificities of the enzymes involved. In addition, some DNA methyltransferases (e.g. Dnmt1 in mice) preferentially methylate the cytosine of a CpG dinucleotide if the cytosine on the complementary DNA strand is already methylated. They are referred to as maintenance methylases. This catalytic preference is complemented by proteins that bring the enzymes to hemi-methylated sites (Sharif *et al.* 2007) and by interactions between different methyltransferases (Liang *et al.* 2002), collectively ensuring that patterns of

DNA methylation are retained through DNA replication. Thus, a mechanism for the inheritance of DNA methylation through the cell cycle is built into the enzymology of the system.

In invertebrates, DNA methylation is confined to a small fraction of the genome or cannot be detected at all. In contrast, in vertebrates, DNA methylation is distributed throughout the genome and is generally associated with regions in which transcription is suppressed (Bird 1993; Bird & Tweedie 1995). It has been suggested that DNA methylation has evolved to allow the increased efficiency of gene silencing demanded by larger genomes (Bird 1995) and, at least in its role as a silencing mechanism, it seems to have evolved rather later than histone modification (Bird 1995; Weber *et al.* 2007; Mohn & Schubeler 2009). The mechanisms by which DNA methylation leads to transcriptional silencing remain to be clarified, and it is clear that an increased level of DNA methylation across a region does not, in itself, guarantee that the genes within that region will be silenced (Mohn & Schubeler 2009). However, the correlation between elevated CpG methylation, particularly at promoter regions, and transcriptional silencing remains strong overall. A family of methyl-DNA-binding proteins recognize and bind to methylated CpGs and thereby, often through attracting other proteins, alter the conformation and functional state of the DNA (Dhasarathy & Wade 2008). Thus, like histone modifications, DNA methylation is often likely to exert its functional effects indirectly through the actions of binding proteins.

Methyl cytosine can be regarded as a fifth base in DNA and constitutes a powerful, intrinsically heritable, epigenetic mark. It can also, over evolutionary time, influence DNA sequence. Spontaneous hydrolytic deamination of cytosine, yielding uracil, is an inevitable and frequent mutation. When confronted with a G=U mismatch, repair enzymes recognize the uracil as inappropriate and replace it. On the other hand, deamination of 5'meC yields thymidine and the resulting G=T mismatch is less easy to resolve correctly and will, on occasion, result in the replacement of the G with an A, leaving an A=T base pair in place of the original G=C. This process will lead not only to new mutations, but also to loss of cytosines from CpG dinucleotides. It is likely to explain the unexpectedly low frequency of CpG dinucleotides across vertebrate genomes (Mohn & Schubeler 2009). Remarkably, this low frequency is not genome wide, with selected regions retaining the expected CpG frequency. These regions, referred to as CpG islands (Bird 1986), often incorporate gene promoters and are generally free of CpG methylation (Mohn & Schubeler 2009). It seems probable that the lack of CpG methylation has protected CpG islands from cytosine depletion through deamination, but what has prevented their methylation in the first place? It may be that the evolutionarily more ancient histone-modification system is involved and there is biochemical evidence to suggest that the high levels of H3K4me3 present in many CpG islands (Weber *et al.* 2007) protect these regions from DNA methylation by blocking

the action of DNA methyltransferases (Ooi *et al.* 2007).

Work in model systems has established strong links between DNA methylation and histone modification, specifically methylation of H3 lysine 9. This was first noted in the filamentous fungus *Neurospora crassa* where mutation of a gene encoding a histone methyltransferase abolished DNA methylation (Tamaru & Selker 2001). Later work showed that DNA methyltransferase was brought to chromatin in which H3 was tri-methylated at lysine 9 (H3K9me3) by the heterochromatin protein HP1, which binds selectively to H3K9me3 through its chromodomain (Tamaru *et al.* 2003; Freitag *et al.* 2004). In the flowering plant *Arabidopsis*, DNA methyltransferases are also targeted by chromatin, but in this case, the mechanism seems to be more direct with the methyltransferase itself binding to H3 tails methylated at lysines 9 and/or 27 (Lindroth *et al.* 2004). In both these organisms, DNA methylation is not CpG based, nor is it genome wide, but it seems that a similar link between H3 lysine 9 methylation and DNA methylation exists in the mouse and probably involves HP1, though the details remain to be worked out (Lehnertz *et al.* 2003; de la Cruz *et al.* 2007). In many human cancers, silencing of key regular genes has been linked to hypermethylation of CpG island promoters. Whether DNA methylation, in any particular circumstance, is a cause or consequence of transcriptional changes remains uncertain, but it remains an intriguing possibility that specific histone modifications are determinants of DNA methylation levels (Ohm *et al.* 2007). In all these systems, the interaction between the histone H3 tail and the methylating enzyme is likely to be mediated by other modifications to the local chromatin, and other histone-modifying enzymes, including deacetylases, have been implicated (Lawrence *et al.* 2004; Probst *et al.* 2004; Smith *et al.* 2008).

The complexity of the situation in mammals is exemplified by recent work on silencing of the master regulatory gene *Oct4* in mouse ES cells. The histone methyltransferase G9a methylates H3K9 in ES cells, leading to regional heterochromatin formation (involving binding of the HP1 protein) and silencing of early embryonic genes, including *Oct4* (Feldman *et al.* 2006). There is subsequent DNA methylation at these genes and long-term silencing. It was originally thought likely that, as in *Neurospora*, the DNA methyltransferase was brought to the gene promoter by association with HP1, which bound H3K9me3. However, a catalytically inactive G9a mutant (with a point mutation in its SET domain) did not allow heterochromatinization, but did attract Dnmt3a/3b (de novo DNA methyltransferases) via its ANK domain and did trigger increased DNA methylation and long-term silencing (Epsztejn-Litman *et al.* 2008). This more detailed analysis shows that while G9a is essential for DNA methylation and long-term silencing, its catalytic activity is not. Perhaps the methylation of H3K9 by G9a was originally the sole mechanism of *Oct4* silencing, but was superseded, later in evolution, by the advent of DNA methylation.

Figure 3. A possible chain of epigenetic events through which an environmental agent might trigger a change in DNA sequence. The chain of events shown is speculative but the individual elements are all based on established biochemical mechanisms. The process starts with inhibition, by an environmental agent, of an enzyme demethylating H3 trimethyl lysine 9 (H3K9me3) in chromatin. This results in an increase in H3K9me3 levels, which may be global or local depending on the distribution of the enzyme. In some regions of the genome, determined perhaps by their particular chromatin constitution, H3K9me3 can trigger gene silencing, in part through well-characterized mechanisms involving direct binding of the protein HP1. There is evidence that H3K9me3 can also attract and activate DNA methyltransferases (Dnmt), leading to increased DNA methylation (exclusively at CpG dinucleotides in mammals). DNA methylation strengthens or maintains the local transcriptionally silent state. Transcriptionally inactive chromatin may be more susceptible to further increases in H3K9me3 and DNA methylation, thus further strengthening silencing. As outlined in the text, deamination of 5' methyl cytosine (meC) forms thymidine (T), resulting in a G=T base mismatch, repair of which could involve replacement of either base. Replacement of the G with an A results in an altered DNA sequence on both strands, in which the original meC is replaced with T. Such a change could exert phenotypic effects, even if it does not occur in a coding region or TF-binding site, as both nucleosome positioning and binding of DNA methyltransferases are known to be dependent on DNA sequence, though the sequences involved are complex. Over evolutionary time, localized changes in DNA sequence, perhaps through their effects on nucleosome positioning and Dnmt binding, might result, eventually, in a region of silencing determined genetically by DNA sequence, rather than epigenetically, as originally.

12. A SPECULATIVE CHAIN OF EVENTS LINKING ENVIRONMENTAL FACTORS TO GENOMIC CHANGE

Whatever the mechanisms involved, it seems that chromatin and histone modifications can influence the targeting of DNA methylation, and that DNA methylation itself can influence DNA sequence by facilitating C to T substitutions. Thus, it is possible to construct a chain of events, based on experimentally verified biochemical mechanisms, through which an environmentally induced change in the activity of chromatin-modifying enzymes can lead to a change in DNA sequence. If the change occurs in somatic cells, then the resulting mutations might be significant in triggering abnormal cell behaviours and disease states, such as cancer. If the change occurs in germ cells, then the resulting DNA mutations will be passed on to subsequent generations where they might exert a selectable phenotypic change. If the environmental agent that precipitates this chain of events were to be persistent over many generations (e.g. through a period of progressive warming or accumulation of an environmental toxin), then in each generation, the same chain of epigenetic events would be triggered, leading to progressive DNA change in selected regions of the genome. It has been appreciated for many years that environmentally driven, epigenetic processes are a potentially significant force in evolution (Jablonka & Lamb 1995) and the recent characterization of enzyme-catalysed processes regulating chromatin and gene expression is providing molecular mechanisms by which this potential may be realized.

Figure 3 shows the chain of interconnected events whereby an environmental inhibitor of an H3K9 demethylase (e.g. a member of the KDM4 family) might lead to a change in DNA sequence in germ

cell precursors. The chain itself is entirely speculative, though the individual links are all well-established mechanisms. Thus, inhibition of KDM4 increases local levels of H3K9me3, leading both to targeted gene silencing (through HP1 recruitment and chromatin condensation) and to DNA methylation, which will itself further enhance silencing. Once started, the sequence of events is likely to be self-supporting, with transcriptionally silent chromatin regions being more susceptible to further DNA methylation. As discussed above, the rare but inevitable hydrolytic deamination of 5' methyl cytosine generates thymidine and a G–T mismatch that is not always correctly repaired. Thus, there is an enhanced rate of DNA mutation at a genomic region targeted by an environmental agent. These mutations might, in turn, exert an effect on DNA methylation levels by enhancing or suppressing binding of DNA methyl transferases (Dnmt) or on gene silencing or activation by altering nucleosome positioning or TF binding. Again, these are potentially self-supporting cycles. Crucially, over evolutionary time, it may be that the altered DNA sequence itself becomes sufficient to drive, or at least support, the gene silencing that was originally a purely epigenetic event.

It is interesting to note that changes in nucleosome positioning, dependent on the altered DNA sequence, have been shown to accompany the constitutive activation or silencing of sets of genes during evolution of yeast species (Field *et al.* 2009). Thus, in an aerobic yeast such as *Candida albicans*, the DNA sequence of the promoters of normally active respiration genes is such as to inhibit nucleosome assembly, keeping the promoter region accessible for binding of TFs and the transcriptional machinery. In contrast, in anaerobic (fermenting) species such as *S. cerevisiae*,

the DNA of the promoter regions of orthologous respiration genes favours nucleosome assembly, rendering these promoters less accessible and transcriptionally less active (Field *et al.* 2009). These exciting results establish the potential importance of sequence-dependent nucleosome positioning in evolution. Unfortunately for the hypothetical pathway presented in figure 3, there is no DNA methylation in yeast, so an alternative mechanism would have to be postulated to link epigenetic and genetic changes during evolution of this organism.

The pathway outlined in figure 3 does not require meiotic epigenetic inheritance, though inheritance of the induced epigenetic changes through the germ line, via positioned histones or DNA methylation, would increase the time over which localized DNA methylation could be converted into changes in DNA sequence. Nor does the pathway necessarily support 'inheritance of acquired characteristics', at least as conventionally defined. However, if a phenotypic change (an acquired characteristic) were itself to precipitate an epigenetic change of the sort outlined, then a similar series of molecular events could conceivably lead to a targeted change in DNA sequence. The molecular events shown in figure 3 are driven by environmental changes, and provide a molecular adaptation to such change. They provide a possible mechanism by which environmental factors can bring about a targeted (potentially heritable) epigenetic change that can generate an altered DNA sequence. If this triggers, in turn, a phenotypic change, then the usual processes of Darwinian selection will operate. Such environmentally driven epigenetic–genetic changes might generate variation across a relatively narrow range of phenotypes, more or less adapted to cope with a particular environment. Pathways such as the hypothetical example in figure 3 should reduce the number of generations required to fix an adaptive phenotypic change within a population and their existence could, in itself, offer a selective advantage.

Despite the irrefutable power of Darwinian natural selection, it is generally accepted that there are aspects of evolutionary change that are not easily explained by the progressive accumulation of small genetic and phenotypic changes (Muller 2007; Stevens 2009). Chance alone ('genetic drift') or dramatic environmental events triggering periods of rapid change may also play a role. The importance of gene control elements as drivers of evolutionary change, and particularly how they might operate during embryonic development, has been emphasized (Muller 2007; Carroll 2008; Stevens 2009). A recent review considers various ways in which an epigenetic change might exert evolutionary effects, with an emphasis on how spreading of suppressive chromatin might generate quantitative changes in gene expression (Zuckerandl & Cavalli 2007). Perhaps the main value of speculation on evolutionary processes is that it can sometimes suggest experimentally testable mechanisms, in the present case, those by which environmental factors can influence epigenetic/genetic processes leading to a heritable change. Such mechanisms not only have long-term implications for evolutionary change itself, but are of immediate relevance to human and animal health.

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REFERENCES

- Adhikary, S. & Eilers, M. 2005 Transcriptional regulation and transformation by Myc proteins. *Nat. Rev. Mol. Cell Biol.* **6**, 635–645. (doi:10.1038/nrm1703)
- Ahmad, K. & Henikoff, S. 2002 The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol. Cell* **9**, 1191–1200. (doi:10.1016/S1097-2765(02)00542-7)
- Allis, C. D. *et al.* 2007 New nomenclature for chromatin-modifying enzymes. *Cell* **131**, 633–636. (doi:10.1016/j.cell.2007.10.039)
- Altenberg, B. & Greulich, K. O. 2004 Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. *Genomics* **84**, 1014–1020. (doi:10.1016/j.ygeno.2004.08.010)
- Anway, M. D., Cupp, A. S., Uzumcu, M. & Skinner, M. K. 2005 Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* **308**, 1466–1469. (doi:10.1126/science.1108190)
- Anway, M. D., Rekow, S. S. & Skinner, M. K. 2008 Transgenerational epigenetic programming of the embryonic testis transcriptome. *Genomics* **91**, 30–40. (doi:10.1016/j.ygeno.2007.10.002)
- Arnold, H. K. & Sears, R. C. 2006 Protein phosphatase 2A regulatory subunit B56alpha associates with *c-myc* and negatively regulates *c-myc* accumulation. *Mol. Cell Biol.* **26**, 2832–2844. (doi:10.1128/MCB.26.7.2832-2844.2006)
- Baron, A., Migita, T., Tang, D. & Loda, M. 2004 Fatty acid synthase: a metabolic oncogene in prostate cancer? *J. Cell. Biochem.* **91**, 47–53. (doi:10.1002/jcb.10708)
- Bennett, M. R., Pang, W. L., Ostroff, N. A., Baumgartner, B. L., Nayak, S., Tsimring, L. S. & Hasty, J. 2008 Metabolic gene regulation in a dynamically changing environment. *Nature* **454**, 1119–1122. (doi:10.1038/nature07211)
- Bird, A. P. 1986 CpG-rich islands and the function of DNA methylation. *Nature* **321**, 209–213. (doi:10.1038/321209a0)
- Bird, A. P. 1993 Functions for DNA methylation in vertebrates. *Cold Spring Harb. Symp. Quant. Biol.* **58**, 281–285.
- Bird, A. P. 1995 Gene number, noise reduction and biological complexity. *Trends Genet.* **11**, 94–100. (doi:10.1016/S0168-9525(00)89009-5)
- Bird, A. & Tweedie, S. 1995 Transcriptional noise and the evolution of gene number. *Phil. Trans. R. Soc. Lond. B* **349**, 249–253. (doi:10.1098/rstb.1995.0109)
- Borra, M. T., Langer, M. R., Slama, J. T. & Denu, J. M. 2004 Substrate specificity and kinetic mechanism of the Sir2 family of NAD⁺-dependent histone/protein deacetylases. *Biochemistry* **43**, 9877–9887. (doi:10.1021/bi049592e)
- Carroll, S. B. 2008 Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell* **134**, 25–36. (doi:10.1016/j.cell.2008.06.030)
- Carroll, J. S. *et al.* 2005 Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. *Cell* **122**, 33–43. (doi:10.1016/j.cell.2005.05.008)
- Carthew, R. W. & Sontheimer, E. J. 2009 Origins and mechanisms of miRNAs and siRNAs. *Cell* **136**, 642–655. (doi:10.1016/j.cell.2009.01.035)
- Chambeyron, S. & Bickmore, W. A. 2004 Chromatin decondensation and nuclear reorganization of the HoxB

- locus upon induction of transcription. *Genes Dev.* **18**, 1119–1130. (doi:10.1101/gad.292104)
- Chiaffarino, F., Parazzini, F., Chatenoud, L., Ricci, E., Sandretti, F., Cipriani, S., Caserta, D. & Fedele, L. 2006 Alcohol drinking and risk of small for gestational age birth. *Eur. J. Clin. Nutr.* **60**, 1062–1066. (doi:10.1038/sj.ejcn.1602419)
- Cosgrove, M. S. 2007 Histone proteomics and the epigenetic regulation of nucleosome mobility. *Expert Rev. Proteomics* **4**, 465–478. (doi:10.1586/14789450.4.4.465)
- Dashwood, R. H. & Ho, E. 2007 Dietary histone deacetylase inhibitors: from cells to mice to man. *Semin. Cancer Biol.* **17**, 363–369. (doi:10.1016/j.semcancer.2007.04.001)
- de la Cruz, X., Lois, S., Sanchez-Molina, S. & Martinez-Balbas, M. A. 2005 Do protein motifs read the histone code? *Bioessays* **27**, 164–175. (doi:10.1002/bies.20176)
- de la Cruz, C. C., Kirmizis, A., Simon, M. D., Isono, K., Koseki, H. & Panning, B. 2007 The polycomb group protein SUZ12 regulates histone H3 lysine 9 methylation and HP1 alpha distribution. *Chromosome Res.* **15**, 299–314.
- Dhasarathy, A. & Wade, P. A. 2008 The MBD protein family-reading an epigenetic mark? *Mutat. Res.* **647**, 39–43. (doi:10.1016/j.mrfmmm.2008.07.007)
- Disney, E. R., Iacono, W., McGue, M., Tully, E. & Legrand, L. 2008 Strengthening the case: prenatal alcohol exposure is associated with increased risk for conduct disorder. *Pediatrics* **122**, e1225–e1230. (doi:10.1542/peds.2008-1380)
- Duncan, S. 2007 Teratogenesis of sodium valproate. *Curr. Opin. Neurol.* **20**, 175–180. (doi:10.1097/WCO.0b013e32805866fb)
- Eadie, M. J. 2008 Antiepileptic drugs as human teratogens. *Expert Opin. Drug Saf.* **7**, 195–209. (doi:10.1517/14740338.7.2.195)
- Ekwall, K., Olsson, T., Turner, B. M., Cranston, G. & Allshire, R. C. 1997 Transient inhibition of histone deacetylation alters the structural and functional imprint at fission yeast centromeres. *Cell* **91**, 1021–1032. (doi:10.1016/S0092-8674(00)80492-4)
- Epsztejn-Litman, S. *et al.* 2008 De novo DNA methylation promoted by G9a prevents reprogramming of embryonically silenced genes. *Nat. Struct. Mol. Biol.* **15**, 1176–1183. (doi:10.1038/nsmb.1476)
- Faiella, A., Wernig, M., Consalez, G. G., Hostick, U., Hofmann, C., Hustert, E., Boncinelli, E., Balling, R. & Nadeau, J. H. 2000 A mouse model for valproate teratogenicity: parental effects, homeotic transformations, and altered HOX expression. *Hum. Mol. Genet.* **9**, 227–236. (doi:10.1093/hmg/9.2.227)
- Feldman, N., Gerson, A., Fang, J., Li, E., Zhang, Y., Shinkai, Y., Cedar, H. & Bergman, Y. 2006 G9a-mediated irreversible epigenetic inactivation of Oct-3/4 during early embryogenesis. *Nat. Cell Biol.* **8**, 188–194. (doi:10.1038/ncb1353)
- Fernandez, P. C., Frank, S. R., Wang, L., Schroeder, M., Liu, S., Greene, J., Cocito, A. & Amati, B. 2003 Genomic targets of the human c-Myc protein. *Genes Dev.* **17**, 1115–1129. (doi:10.1101/gad.1067003)
- Fernandino, J. I., Hattori, R. S., Kimura, H., Strussmann, C. A. & Somoza, G. M. 2008 Expression profile and estrogenic regulation of anti-Müllerian hormone during gonadal development in pejerrey *Odontesthes bonariensis*, a teleost fish with strong temperature-dependent sex determination. *Dev. Dyn.* **237**, 3192–3199. (doi:10.1002/dvdy.21731)
- Field, Y., Fondufe-Mittendorf, Y., Moore, I. K., Mieczkowski, P., Kaplan, N., Lubling, Y., Lieb, J. D., Widom, J. & Segal, E. 2009 Gene expression divergence in yeast is coupled to evolution of DNA-encoded nucleosome organization. *Nat. Genet.* **41**, 438–445. (doi:10.1038/ng.324)
- Finnegan, E. J. & Dennis, E. S. 2007 Vernalization-induced trimethylation of histone H3 lysine 27 at FLC is not maintained in mitotically quiescent cells. *Curr. Biol.* **17**, 1978–1983. (doi:10.1016/j.cub.2007.10.026)
- Finnegan, E. J., Sheldon, C. C., Jardinaud, F., Peacock, W. J. & Dennis, E. S. 2004 A cluster of *Arabidopsis* genes with a coordinate response to an environmental stimulus. *Curr. Biol.* **14**, 911–916. (doi:10.1016/j.cub.2004.04.045)
- Fragoso, G., John, S., Roberts, M. S. & Hager, G. L. 1995 Nucleosome positioning on the MMTV LTR results from the frequency-biased occupancy of multiple frames. *Genes Dev.* **9**, 1933–1947. (doi:10.1101/gad.9.15.1933)
- Freitag, M., Lee, D. W., Kothe, G. O., Pratt, R. J., Aramayo, R. & Selker, E. U. 2004 DNA methylation is independent of RNA interference in *Neurospora*. *Science* **304**. (doi:10.1126/science.1099709)
- Frye, M., Fisher, A. G. & Watt, F. M. 2007 Epidermal stem cells are defined by global histone modifications that are altered by Myc-induced differentiation. *PLoS One* **2**, e763. (doi:10.1371/journal.pone.0000763)
- Fu, M., Wang, C., Zhang, X. & Pestell, R. G. 2004 Acetylation of nuclear receptors in cellular growth and apoptosis. *Biochem. Pharmacol.* **68**, 1199–1208. (doi:10.1016/j.bcp.2004.05.037)
- Fu, M. *et al.* 2006 Hormonal control of androgen receptor function through SIRT1. *Mol. Cell Biol.* **26**, 8122–8135. (doi:10.1128/MCB.00289-06)
- Fulco, M., Schiltz, R. L., Iezzi, S., King, M. T., Zhao, P., Kashiwaya, Y., Hoffman, E., Veech, R. L. & Sartorelli, V. 2003 Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state. *Mol. Cell* **12**, 51–62. (doi:10.1016/S1097-2765(03)00226-0)
- Gatewood, J. M., Cook, G. R., Balhorn, R., Bradbury, E. M. & Schmid, C. W. 1987 Sequence-specific packaging of DNA in human sperm chromatin. *Science* **236**, 962–964. (doi:10.1126/science.3576213)
- Gatewood, J. M., Cook, G. R., Balhorn, R., Schmid, C. W. & Bradbury, E. M. 1990 Isolation of four core histones from human sperm chromatin representing a minor subset of somatic histones. *J. Biol. Chem.* **265**, 20 662–20 666.
- Gehring, M., Reik, W. & Henikoff, S. 2009 DNA demethylation by DNA repair. *Trends Genet.* **25**, 82–90. (doi:10.1016/j.tig.2008.12.001)
- Glozak, M. A., Sengupta, N., Zhang, X. & Seto, E. 2005 Acetylation and deacetylation of non-histone proteins. *Gene* **363**, 15–23. (doi:10.1016/j.gene.2005.09.010)
- Gregoret, I. V., Lee, Y. M. & Goodson, H. V. 2004 Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. *J. Mol. Biol.* **338**, 17–31. (doi:10.1016/j.jmb.2004.02.006)
- Guccione, E. *et al.* 2006 Myc-binding-site recognition in the human genome is determined by chromatin context. *Nat. Cell Biol.* **8**, 764–770. (doi:10.1038/ncb1434)
- Heard, E., Clerc, P. & Avner, P. 1997 X-chromosome inactivation in mammals. *Annu. Rev. Genet.* **31**, 571–610. (doi:10.1146/annurev.genet.31.1.571)
- Holland, M. J. 2002 Transcript abundance in yeast varies over six orders of magnitude. *J. Biol. Chem.* **277**, 14 363–14 366. (doi:10.1074/jbc.C200101200)
- Holliday, R. 2006 Epigenetics: a historical overview. *Epigenetics* **1**, 76–80.
- Holstege, F. C., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S. & Young, R. A. 1998 Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* **95**, 717–728. (doi:10.1016/S0092-8674(00)81641-4)

- Jablonka, E. & Lamb, M. J. 1995 *Epigenetic inheritance and evolution*. New York, NY: Oxford University Press.
- Jacob, F. & Monod, J. 1961 Genetic regulatory mechanisms in the synthesis of proteins. *J. Mol. Biol.* **3**, 318–356.
- Jaenisch, R. & Bird, A. 2003 Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.* **33**, 245–254. (doi:10.1038/ng1089)
- Jiang, C. & Pugh, B. F. 2009 Nucleosome positioning and gene regulation: advances through genomics. *Nat. Rev. Genet.* **10**, 161–172. (doi:10.1038/nrg2522)
- Johnson, C. A., White, D. A., Lavender, J. S., O'Neill, L. P. & Turner, B. M. 2002 Human class I histone deacetylase complexes show enhanced catalytic activity in the presence of ATP and co-immunoprecipitate with the ATP-dependent chaperone protein Hsp70. *J. Biol. Chem.* **277**, 9590–9597. (doi:10.1074/jbc.M107942200)
- Kaati, G., Bygren, L. O., Pembrey, M. & Sjöström, M. 2007 Transgenerational response to nutrition, early life circumstances and longevity. *Eur. J. Hum. Genet.* **15**, 784–790. (doi:10.1038/sj.ejhg.5201832)
- Kaplan, N. *et al.* 2009 The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature* **458**, 362–366. (doi:10.1038/nature07667)
- Kouzarides, T. 2007 Chromatin modifications and their function. *Cell* **128**, 693–705. (doi:10.1016/j.cell.2007.02.005)
- Kustatscher, G., Hothorn, M., Pugieux, C., Scheffzek, K. & Ladurner, A. G. 2005 Splicing regulates NAD metabolite binding to histone macroH2A. *Nat. Struct. Mol. Biol.* **12**, 624–625. (doi:10.1038/nsmb956)
- Laganière, J., Deblois, G., Lefebvre, C., Bataille, A. R., Robert, F. & Giguère, V. 2005 From the cover: location analysis of estrogen receptor alpha target promoters reveals that FOXA1 defines a domain of the estrogen response. *Proc. Natl Acad. Sci. USA* **102**, 11 651–11 656. (doi:10.1073/pnas.0505575102)
- Lawrence, R. J., Earley, K., Pontes, O., Silva, M., Chen, Z. J., Neves, N., Viegas, W. & Pikaard, C. S. 2004 A concerted DNA methylation/histone methylation switch regulates rRNA gene dosage control and nucleolar dominance. *Mol. Cell* **13**, 599–609. (doi:10.1016/S1097-2765(04)00064-4)
- Lee, M. G., Wynder, C., Bochar, D. A., Hakimi, M. A., Cooch, N. & Shiekhattar, R. 2006 Functional interplay between histone demethylase and deacetylase enzymes. *Mol. Cell Biol.* **26**, 6395–6402. (doi:10.1128/MCB.00723-06)
- Lehnertz, B. *et al.* 2003 Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr. Biol.* **13**, 1192–1200. (doi:10.1016/S0960-9822(03)00432-9)
- Liang, G., Chan, M. F., Tomigahara, Y., Tsai, Y. C., Gonzales, F. A., Li, E., Laird, P. W. & Jones, P. A. 2002 Cooperativity between DNA methyltransferases in the maintenance methylation of repetitive elements. *Mol. Cell Biol.* **22**, 480–491. (doi:10.1128/MCB.22.2.480-491.2002)
- Lindroth, A. M. *et al.* 2004 Dual histone H3 methylation marks at lysines 9 and 27 required for interaction with CHROMOMETHYLASE3. *EMBO J.* **23**, 4286–4296. (doi:10.1038/sj.emboj.7600430)
- Louis, P. & Flint, H. J. 2009 Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol. Lett.* **294**, 1–8.
- Luger, K. & Richmond, T. J. 1998 The histone tails of the nucleosome. *Curr. Opin. Genet. Dev.* **8**, 140–146. (doi:10.1016/S0959-437X(98)80134-2)
- Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. 1997 Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251–260. (doi:10.1038/38444)
- Lupien, M., Eeckhoutte, J., Meyer, C. A., Wang, Q., Zhang, Y., Li, W., Carroll, J. S., Liu, X. S. & Brown, M. 2008 FoxA1 translates epigenetic signatures into enhancer-driven lineage-specific transcription. *Cell* **132**, 958–970. (doi:10.1016/j.cell.2008.01.018)
- Maamar, H., Raj, A. & Dubnau, D. 2007 Noise in gene expression determines cell fate in *Bacillus subtilis*. *Science* **317**, 526–529. (doi:10.1126/science.1140818)
- Marmorstein, R. & Trievel, R. C. 2009 Histone modifying enzymes: structures, mechanisms, and specificities. *Biochim. Biophys. Acta* **1789**, 58–68.
- Marshall Graves, J. A. 2008 Weird animal genomes and the evolution of vertebrate sex and sex chromosomes. *Annu. Rev. Genet.* **42**, 565–586. (doi:10.1146/annurev.genet.42.110807.091714)
- McKittrick, E., Gafken, P. R., Ahmad, K. & Henikoff, S. 2004 Histone H3.3 is enriched in covalent modifications associated with active chromatin. *Proc. Natl Acad. Sci. USA* **101**, 1525–1530. (doi:10.1073/pnas.0308092100)
- Mellor, J. 2005 The dynamics of chromatin remodeling at promoters. *Mol. Cell* **19**, 147–157. (doi:10.1016/j.molcel.2005.06.023)
- Mohn, F. & Schubeler, D. 2009 Genetics and epigenetics: stability and plasticity during cellular differentiation. *Trends Genet.* **25**, 129–136.
- Montecino, M. *et al.* 2007 Nucleosome organization and targeting of SWI/SNF chromatin-remodeling complexes: contributions of the DNA sequence. *Biochem. Cell Biol.* **85**, 419–425. (doi:10.1139/O07-070)
- Morgan, H. D., Santos, F., Green, K., Dean, W. & Reik, W. 2005 Epigenetic reprogramming in mammals. *Hum. Mol. Genet.* **14**, R47–R58. (doi:10.1093/hmg/ddi114)
- Muller, G. B. 2007 Evo-devo: extending the evolutionary synthesis. *Nat. Rev. Genet.* **8**, 943–949. (doi:10.1038/nrg2219)
- Newman, J. R., Ghaemmaghami, S., Ihmels, J., Breslow, D. K., Noble, M., DeRisi, J. L. & Weissman, J. S. 2006 Single-cell proteomic analysis of *S. cerevisiae* reveals the architecture of biological noise. *Nature* **441**, 840–846. (doi:10.1038/nature04785)
- Ng, R. K. & Gurdon, J. B. 2008 Epigenetic memory of an active gene state depends on histone H3.3 incorporation into chromatin in the absence of transcription. *Nat. Cell Biol.* **10**, 102–109. (doi:10.1038/ncb1674)
- Nightingale, K. P., Gendreizig, S., White, D. A., Bradbury, C., Hollfelder, F. & Turner, B. M. 2007 Cross-talk between histone modifications in response to histone deacetylase inhibitors: MLL4 links histone H3 acetylation and histone H3K4 methylation. *J. Biol. Chem.* **282**, 4408–4416. (doi:10.1074/jbc.M606773200)
- Ohm, J. E. *et al.* 2007 A stem cell-like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing. *Nat. Genet.* **39**, 237–242. (doi:10.1038/ng1972)
- Ooi, S. K. & Bestor, T. H. 2008 The colorful history of active DNA demethylation. *Cell* **133**, 1145–1148. (doi:10.1016/j.cell.2008.06.009)
- Ooi, S. L. & Henikoff, S. 2007 Germline histone dynamics and epigenetics. *Curr. Opin. Cell Biol.* **19**, 257–265. (doi:10.1016/j.ceb.2007.04.015)
- Ooi, S. K. *et al.* 2007 DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* **448**, 714–717. (doi:10.1038/nature05987)

- Ospina-Alvarez, N. & Piferrer, F. 2008 Temperature-dependent sex determination in fish revisited: prevalence, a single sex ratio response pattern, and possible effects of climate change. *PLoS One* **3**, e2837. (doi:10.1371/journal.pone.0002837)
- Peart, M. J., Smyth, G. K., van Laar, R. K., Bowtell, D. D., Richon, V. M., Marks, P. A., Holloway, A. J. & Johnstone, R. W. 2005 Identification and functional significance of genes regulated by structurally different histone deacetylase inhibitors. *Proc. Natl Acad. Sci. USA* **102**, 3697–3702. (doi:10.1073/pnas.0500369102)
- Phiel, C. J., Zhang, F., Huang, E. Y., Guenther, M. G., Lazar, M. A. & Klein, P. S. 2001 Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. *J. Biol. Chem.* **276**, 36 734–36 741. (doi:10.1074/jbc.M101287200)
- Popov, V. M., Wang, C., Shirley, L. A., Rosenberg, A., Li, S., Nevalainen, M., Fu, M. & Pestell, R. G. 2007 The functional significance of nuclear receptor acetylation. *Steroids* **72**, 221–230. (doi:10.1016/j.steroids.2006.12.001)
- Probst, A. V. *et al.* 2004 Arabidopsis histone deacetylase HDA6 is required for maintenance of transcriptional gene silencing and determines nuclear organization of rDNA repeats. *Plant Cell* **16**, 1021–1034. (doi:10.1105/tpc.018754)
- Pryde, S. E., Duncan, S. H., Hold, G. L., Stewart, C. S. & Flint, H. J. 2002 The microbiology of butyrate formation in the human colon. *FEMS Microbiol. Lett.* **217**, 133–139. (doi:10.1111/j.1574-6968.2002.tb11467.x)
- Ptashne, M. 2007 On the use of the word 'epigenetic'. *Curr. Biol.* **17**, R233–R236. (doi:10.1016/j.cub.2007.02.030)
- Raj, A. & van Oudenaarden, A. 2008 Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell* **135**, 216–226. (doi:10.1016/j.cell.2008.09.050)
- Raj, A., Peskin, C. S., Tranchina, D., Vargas, D. Y. & Tyagi, S. 2006 Stochastic mRNA synthesis in mammalian cells. *PLoS Biol.* **4**, e309. (doi:10.1371/journal.pbio.0040309)
- Reik, W., Santos, F., Mitsuya, K., Morgan, H. & Dean, W. 2003 Epigenetic asymmetry in the mammalian zygote and early embryo: relationship to lineage commitment? *Phil. Trans. R. Soc. Lond. B* **358**, 1403–1409. (doi:10.1098/rstb.2003.1326)
- Rodgers, J. T., Lerin, C., Haas, W., Gygi, S. P., Spiegelman, B. M. & Puigserver, P. 2005 Nutrient control of glucose homeostasis through a complex of PGC-1 α and SIRT1. *Nature* **434**, 113–118. (doi:10.1038/nature03354)
- Santos, F. & Dean, W. 2004 Epigenetic reprogramming during early development in mammals. *Reproduction* **127**, 643–651. (doi:10.1530/rep.1.00221)
- Schmitz, R. J., Sung, S. & Amasino, R. M. 2008 Histone arginine methylation is required for vernalization-induced epigenetic silencing of FLC in winter-annual *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* **105**, 411–416. (doi:10.1073/pnas.0710423104)
- Schneider, S., Kaufmann, W., Buesen, R. & van Ravenzwaay, B. 2008 Vinclozolin—the lack of a transgenerational effect after oral maternal exposure during organogenesis. *Reprod. Toxicol.* **25**, 352–360. (doi:10.1016/j.reprotox.2008.04.001)
- Segal, E., Fondufe-Mittendorf, Y., Chen, L., Thastrom, A., Field, Y., Moore, I. K., Wang, J. P. & Widom, J. 2006 A genomic code for nucleosome positioning. *Nature* **442**, 772–778. (doi:10.1038/nature04979)
- Sharif, J. *et al.* 2007 The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* **450**, 908–912. (doi:10.1038/nature06397)
- Shindo, C., Lister, C., Crevillen, P., Nordborg, M. & Dean, C. 2006 Variation in the epigenetic silencing of FLC contributes to natural variation in *Arabidopsis* vernalization response. *Genes Dev.* **20**, 3079–3083. (doi:10.1101/gad.405306)
- Smith, K. M., Kothe, G. O., Matsen, C. B., Khlafallah, T. K., Adhvaryu, K. K., Hemphill, M., Freitag, M., Motamedi, M. R. & Selker, E. U. 2008 The fungus *Neurospora crassa* displays telomeric silencing mediated by multiple sirtuins and by methylation of histone H3 lysine 9. *Epigenetics Chromatin* **1**, 5. (doi:10.1186/1756-8935-1-5)
- Stevens, C. F. 2009 Darwin and Huxley revisited: the origin of allometry. *J. Biol.* **8**, 14. (doi:10.1186/jbiol119)
- Tamaru, H. & Selker, E. U. 2001 A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. *Nature* **414**, 277–283. (doi:10.1038/35104508)
- Tamaru, H., Zhang, X., McMillen, D., Singh, P. B., Nakayama, J., Grewal, S. I., Allis, C. D., Cheng, X. & Selker, E. U. 2003 Trimethylated lysine 9 of histone H3 is a mark for DNA methylation in *Neurospora crassa*. *Nat. Genet.* **34**, 75–79. (doi:10.1038/ng1143)
- Tang, Y., Zhao, W., Chen, Y., Zhao, Y. & Gu, W. 2008 Acetylation is indispensable for p53 activation. *Cell* **133**, 612–626. (doi:10.1016/j.cell.2008.03.025)
- Taverna, S. D., Li, H., Ruthenburg, A. J., Allis, C. D. & Patel, D. J. 2007 How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nat. Struct. Mol. Biol.* **14**, 1025–1040. (doi:10.1038/nsmb1338)
- Tirosh, I., Berman, J. & Barkai, N. 2007 The pattern and evolution of yeast promoter bendability. *Trends Genet.* **23**, 318–321. (doi:10.1016/j.tig.2007.03.015)
- Tong, L., Lee, S. & Denu, J. M. 2009 Hydrolase regulates NAD metabolites and modulates cellular REDOX. *J. Biol. Chem.* **284**, 11 256–11 266.
- Turner, B. M. 1993 Decoding the nucleosome. *Cell* **75**, 5–8.
- Turner, B. M. 2005 Reading signals on the nucleosome with a new nomenclature for modified histones. *Nat. Struct. Mol. Biol.* **12**, 110–112. (doi:10.1038/nsmb0205-110)
- Turner, B. M. 2007 Defining an epigenetic code. *Nat. Cell Biol.* **9**, 2–6. (doi:10.1038/ncb0107-2)
- Vaziri, H., Dessain, S. K., Ng Eaton, E., Imai, S. I., Frye, R. A., Pandita, T. K., Guarente, L. & Weinberg, R. A. 2001 hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell* **107**, 149–159. (doi:10.1016/S0092-8674(01)00527-X)
- VerMilyea, M. D., O'Neill, L. P. & Turner, B. M. 2009 Transcription-independent heritability of induced histone modifications in the mouse preimplantation embryo. *PLoS One* **4**, e6086.
- Vervoorts, J., Luscher-Firzlauff, J. & Luscher, B. 2006 The ins and outs of MYC regulation by posttranslational mechanisms. *J. Biol. Chem.* **281**, 34 725–34 729. (doi:10.1074/jbc.R600017200)
- Vilar, J. M., Guet, C. C. & Leibler, S. 2003 Modeling network dynamics: the *lac* operon, a case study. *J. Cell Biol.* **161**, 471–476. (doi:10.1083/jcb.200301125)
- Vita, M. & Henriksson, M. 2006 The Myc oncoprotein as a therapeutic target for human cancer. *Semin. Cancer Biol.* **16**, 318–330. (doi:10.1016/j.semcancer.2006.07.015)
- Vousden, K. H. & Lane, D. P. 2007 p53 in health and disease. *Nat. Rev. Mol. Cell Biol.* **8**, 275–283. (doi:10.1038/nrm2147)
- Waldecker, M., Kautenburger, T., Daumann, H., Busch, C. & Schrenk, D. 2008 Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. *J. Nutr. Biochem.* **19**, 587–593. (doi:10.1016/j.jnutbio.2007.08.002)
- Weber, M., Hellmann, I., Stadler, M. B., Ramos, L., Paabo, S., Rebhan, M. & Schubeler, D. 2007 Distribution, silencing potential and evolutionary impact of

- promoter DNA methylation in the human genome. *Nat. Genet.* **39**, 457–466. (doi:10.1038/ng1990)
- Wilson, M. D., Barbosa-Morais, N. L., Schmidt, D., Conboy, C. M., Vanes, L., Tybulewicz, V. L., Fisher, E. M., Tavaré, S. & Odom, D. T. 2008 Species-specific transcription in mice carrying human chromosome 21. *Science* **322**, 434–438. (doi:10.1126/science.1160930)
- Wray, G. A., Hahn, M. W., Abouheif, E., Balhoff, J. P., Pizer, M., Rockman, M. V. & Romano, L. A. 2003 The evolution of transcriptional regulation in eukaryotes. *Mol. Biol. Evol.* **20**, 1377–1419. (doi:10.1093/molbev/msg140)
- Yeh, E. *et al.* 2004 A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. *Nat. Cell Biol.* **6**, 308–318. (doi:10.1038/ncb1110)
- Zuckerandl, E. & Cavalli, G. 2007 Combinatorial epigenetics, ‘junk DNA’, and the evolution of complex organisms. *Gene* **390**, 232–242. (doi:10.1016/j.gene.2006.12.001)