Imprint stability and plasticity during development

Sarah-Jayne Mackin, Avinash Thakur*, and Colum P Walsh

Genomic Medicine Research Group, School of Biomedical Sciences, Ulster University, Northern Ireland, UK

Correspondence should be addressed to C P Walsh; Email: cp.walsh@ulster.ac.uk

*(A Thakur is currently at Terry Fox Laboratory, BC Cancer Research Centre, 675 West 10th Avenue, Room 13-112, Vancouver, BC V5Z 1L3, Canada)

Abstract

There have been a number of recent insights in the area of genomic imprinting, the phenomenon whereby one of two autosomal alleles is selected for expression based on the parent of origin. This is due in part to a proliferation of new techniques for interrogating the genome that are leading researchers working on organisms other than mouse and human, where imprinting has been most studied, to become interested in looking for potential imprinting effects. Here, we recap what is known about the importance of imprints for growth and body size, as well as the main types of locus control. Interestingly, work from a number of labs has now shown that maintenance of the imprint post implantation appears to be a more crucial step than previously appreciated. We ask whether imprints can be reprogrammed somatically, how many loci there are and how conserved imprinted regions are in other species. Finally, we survey some of the methods available for examining DNA methylation genome-wide and look to the future of this burgeoning field.

Reproduction (2018) **156** R43–R55

Introduction

Genomic imprinting is a classic epigenetic phenomenon, and after X-inactivation, one of the best understood. In its simplest form, imprinting refers to genes showing mono-allelic expression depending on their parent of origin (Bartolomei & Ferguson-Smith 2011, Barlow & Bartolomei 2014). There is however considerable variation seen in where in the body and when the imprinted expression is seen. Imprints need to be set up when the alleles are separated during germ line development and then the imprints need to be interpreted by the transcriptional machinery in the target tissues. Recent work has advanced our understanding of (1) the processes behind establishment of imprints as well as (2) how the imprinting mark is maintained and interpreted. For the former, there is an excellent review by Kelsey (Hanna & Kelsey 2014). Here, we wish to focus on the latter process of maintenance, as well as ask what we have learned from species other than mouse, and touch on the technical difficulties, which arise when working in organisms other than mouse and human, where the most resources are available.

Importance of known imprints for body mass and feeding

Effects on growth and body size

One of the most enduring explanations for why imprinting may have arisen is the parental conflict

hypothesis (Haig & Graham 1991, Moore & Haig 1991). Many imprinting phenomena involve effects on growth, nutrition or metabolic balance, and this theory was partly based on observations from uniparental conceptuses where the paternal contribution seemed particularly important for the embryo, while the maternal genome was important for placental development (Barton *et al.* 1984). This turned out to be partly due to the paternal expression of the major growth factor insulin-like growth factor-2 (IGF2), and the maternal expression of an antagonistic binding partner insulin-like growth factor-2 receptor (IGF2R), one of the first observations leading to the idea of parental conflict in imprinting. Several imprinted genes were also shown to be dysregulated in the placentas of these mouse conceptuses (Walsh *et al.* 1994), including genes specifically imprinted only in placenta such as *Mash2* (Guillemot *et al.* 1995). Indeed, the placenta is generally considered to have a higher number of imprinted genes than in other tissues (Court *et al.* 2014); though in the mouse and human, many of these are maternally expressed. An alternative to the parental conflict hypothesis, called coadaptation theory, also stresses the importance of interactions between offspring and parents for nutrient provision and acquisition (Keverne & Curley 2008).

Much of the support for these theories has been gleaned in mouse systems. Interestingly however, a single nucleotide substitution in a non-coding region of *IGF2* underlies a major QTL in pigs that affects muscle growth, heart size and fat deposition (Jeon *et al.* 1999,

Van Laere *et al.* 2003). The mutation disrupts the binding of a recently evolved nuclear protein ZBED6 that appears to be acting as a repressor of *IGF2* transcription, not only in pigs but potentially in all placental mammals, where it is highly conserved (Markljung *et al.* 2009, Younis *et al.* 2018). A more general defect called large offspring syndrome (LOS) in livestock (namely sheep and cattle) also involves perturbed imprinted gene expression, thought to be due to preimplantation disturbances. LOS is associated with an increase in gestational length, 50–80% increase in birth weight, hypoglycaemia and an enlarged tongue (Chen *et al.* 2013). The phenotypic characteristics of LOS are reminiscent of those exhibited by Beckwith–Wiedemann syndrome in humans, which can be driven by defects at the *IGF2* imprint control region (ICR) or the nearby *KvDMR* (Weksberg *et al.* 2010). Rivera and colleagues found however that LOS was not characterised by loss of imprinting at any one locus, but seemed instead to be accompanied by misregulation of multiple imprinted genes, with greater dysregulation seen in the largest offspring (Chen *et al.* 2015). The requirement for delivery of large offspring by caesarean section, the frequent post-natal mortality, and other complications, mean that LOS restricts the full use of modern reproductive strategies in ruminants.

The callipyge muscle hypertrophy phenotype observed in sheep is also caused by a SNP, in this case located intergenically between known imprinted genes, the paternally expressed *DLK1* and maternally expressed *MEG3* long non-coding RNA (lncRNA) within the telomeric region of chromosome 18. This region is known to share a high degree of homology with other mammals including cattle and humans (Freking *et al.* 2002a,b). Callipyge syndrome can cause adverse changes to normal muscle development, body shape and even changes to meat quality (Freking *et al.* 2002a) and the composition of their carcass (Bidwell *et al.* 2014). Interestingly, the expression of this unique phenotype is the only distinctive example of polar overdominance in mammals (Freking *et al.* 2002a). Heterozygous callipyge sheep have a normal maternal allele while carrying the callipyge SNP on the paternal allele (Bidwell *et al.* 2014).

Neurological effects involving feeding and weight gain

Another general feature of imprinted genes is that many are transcribed primarily in the neurons and in some cases only show imprinted expression there. While initially this posed difficulties for the parental conflict hypothesis, which was originally proposed on the basis of imprinting affecting placentation and embryonic growth, with the discovery that some of the genes controlled feeding behaviour or weight gain later in life, these results could be reconciled with the hypothesis. Thus, Prader–Willi syndrome in humans (OMIM176270), where transcription of the neuronal genes at the *SNRPN* cluster are affected, involves

excessive eating (hyperphagia), while the *Peg3* gene in mouse is crucial for feeding behaviour (Curley *et al.* 2005). Likewise, loss of expression of the *Gnas* and *Gnasxl* genes in mice can give hypo- and hyperphagia respectively, while a number of other imprinted genes also have effects on fat/body mass ratios (Peters 2014).

Furthermore, the aforementioned LOS or large calf syndrome can often cause a reluctance to suckle in young cattle. This may be due to the dysregulation of a number of imprinted genes reported in these animals (Zhang *et al.* 2004, Chen *et al.* 2015). However apart from these few studies, little work appears to have been done to date investigating imprinted genes and feeding behaviours outside of the mouse and human systems.

Imprinting disorders in hybrid species: the case of the hinny

A classic example of parent-of-origin effects in livestock is the horse–donkey cross: when the horse is the sire, the resultant offspring is a mule; if the donkey is the sire, then a hinny results, and these are quite distinguishable animals even though their genomic complements are the same. Initially, it was argued that this may be due to differences in uterine environment and the placental–uterine exchange. Early investigation identified that serum concentrations of the placental hormone equine chorionic gonadotrophin were considerably higher in mule than in hinny pregnancies, suggestive of paternal genome-specific expression (Allen 1969). Recent use of RNA sequencing of trophoblast tissue from embryos derived from each reciprocal cross of horse and donkey (Wang *et al.* 2013) identified 15 ‘core’ imprinted genes that are conserved in equids, mouse and human, including genes such as *SNRPN*, *H19* and *PEG3*. Interestingly, paternal expression seems favoured, as 10/15 core imprints – and all of the larger group of 78 genes identified in that study – showed paternal bias, but with many only imprinted in placenta. Additionally, some of the genes in the second, larger group showed incomplete or variable silencing. The authors speculate that this variability may reflect the flexibility of the structure and function of the placenta, which varies widely between mammals. Interestingly, *XIST* does not seem to be one of the imprinted genes, and X-inactivation in the placenta is random in both the horse parent and the hybrids (Wang *et al.* 2012).

Establishment of parental-specific methylation at imprints

It was recognised already from early studies that imprints would most likely be established in the germ line, where each allele of the diploid pair is separate and therefore can be separately marked. While studies on germ cell development were long hampered by the

difficulty of access to the early stages of development of this migratory cell population, and the small numbers of cells involved, recent advances in cell labelling and separation, coupled with the refinement of sequencing techniques and single-cell approaches (see below) have meant that we now have a much deeper understanding of the process of imprint establishment. This area has been dealt with very expertly by others in a recent review (Hanna & Kelsey 2014). We will concentrate here on insights gained in some studies published since then, confining ourselves first to a brief recap of the varied imprinting mechanisms.

The main types of imprinted loci and their structures

Current studies suggest that there are three main types of imprinted locus, controlled by different mechanisms. This has implications for where the imprint controllers are located and what they look like, as well as how the locus might respond to different perturbations.

Insulator

The archetype here is the cluster of genes around the *H19* locus (Bartolomei & Ferguson-Smith 2011): these are controlled by an intergenically located ICR that is not a CpG island (CGI) (Bird 1987) but is relatively CG rich, making it sensitive to DNA methylation. The ICR contains several repeats of a sequence that binds the insulator protein CTCF. Methylation blocks CTCF binding on the paternal allele at the locus (Bell & Felsenfeld 2000, Hark *et al.* 2000), preventing *H19* transcription but facilitating expression of *Igf2* and, in mouse, the *Insulin 2* gene expressed in the yolk sac. The mark on the ICR that is set up in the germ cells is called a primary differentially methylated region (1° DMR) or gametic DMR (gDMR). Methylation that occurs post implantation, for example, on the *H19* promoter after it has already been silenced, is known as a secondary (2°) DMR or somatic DMR (sDMR). There are a number of other imprinted loci with CTCF-binding sites, in some cases lacking CGI, where this protein is thought to play an important role (Prickett *et al.* 2013).

Long non-coding RNA promoter

Here the index locus is *Igf2r* that, unlike *H19*, is an orphan imprinted gene. The DMR at the *Igf2r* promoter was, against expectation, the secondary DMR, and the primary DMR and functional ICR were found to be located at an intragenic island. This turned out to control transcription of an antisense lncRNA, whose transcription blocked the sense transcript in cis (Sleutels *et al.* 2002). This type of arrangement is also seen at the *Kcnq1*, *Grb10* and *Dlk1* loci among others (Barlow & Bartolomei 2014) and as more lncRNA are uncovered, this is becoming the largest group.

Promoter

For some imprinted loci such as *Snrpn*, *Plagl1* and *Grb10*, transcription appears to be directly regulated by methylation of the promoter. Here, a promoter CGI is methylated to block transcription rather than any indirect mechanism. Although there is no antisense transcript, the *Snrpn* promoter does drive the production of a long RNA called *Snurf/Snrpn* that extends far downstream and contains multiple small RNA species that are processed from it (Buiting 2010), so it could be argued that it falls into the lncRNA category. However, for other genes in this group such as *Grb10*, no associated lncRNA have so far been found.

Recent insights into imprint establishment

Seminal work from the Bestor lab (Ooi *et al.* 2007) showed that the methyltransferase cofactor DNMT3L, which is only required in germline, bound to CGI but was blocked by methylation of the fourth lysine on the histone 3 tail (H3K4me3), and further that imprinting was disrupted in the knockout (KO) mice. This link seemed strengthened by the finding that KDM1B, a histone demethylase, was required to establish DNA methylation at imprints (Ciccone *et al.* 2009). However, the idea that DNMT3L might be specific to imprints was weakened by studies charting the establishment of methylation in oocytes, which showed that not only did many thousands of non-imprinted CGI become hypermethylated during oocyte maturation in a DNMT3L-dependent fashion, but DNMT3L was also required for methylation of many other non-CGI sequences (Kato *et al.* 2007, Smallwood *et al.* 2011, Kobayashi *et al.* 2012), suggesting that DNMT3L was in fact more of a general cofactor for the *de novo* methyltransferases, required primarily in the germline. What appeared instead to distinguish imprinted loci from other genes was that they could *maintain* their DMR in the face of the two main waves of remodelling in the early embryo, namely active and passive demethylation in the first few cell divisions and then the widespread *de novo* methylation seen post implantation. While few imprints are established in the male germ line, indications are that here too it is the maintenance of these DMR in the face of remodelling that separates them from other loci (Wang *et al.* 2014).

In effect, this marks a real shift in thinking with regards to imprinting, moving the emphasis away from the mechanisms that might target regions to become imprinted and towards identifying factors that are responsible for maintaining methylation differences at specific loci in the face of extensive epigenomic remodelling. Since this is a crucial new insight, we wish to expand on these findings below and begin by looking in more detail at the protein factors that may be involved prior to implantation, and then look at events post implantation.

Maintenance of imprints pre implantation

Methylation marks are applied in a sex-specific manner during gametogenesis at the gDMRs and several proteins play important roles at various stages in this process. In particular, *Pgc7* (also referred to as *Stella*) is a critical component required in early maternal development and encodes a protein that has a SAP-like domain and a splicing factor-like domain (Aravind & Koonin 2000). It has an important role in protecting the methylation status of imprinted genes by limiting demethylation in early embryogenesis (Nakamura *et al.* 2007) and as such is crucial for normal development. PGC7 has been experimentally shown to be actively expressed in primordial germ cells (PGCs) in mouse from E7.25 to E15.5 (Sato *et al.* 2002) but more importantly in the immature ovaries of the neonate females (Payer *et al.* 2003). Through depletion of the protein in the oocytes, Payer *et al.* (2003) found there was a reduction in the number of blastocysts, successful implantations and a reduction in the number of viable offspring. Nakamura *et al.* (2007) initially showed that PGC7 was required to maintain methylation on most imprinted loci in the preimplantation embryo, and later went on to show that PGC7 bound to H3K9me2 at both paternally and maternally marked loci protects against active demethylation by TET3, a finding confirmed by others (Nakamura *et al.* 2012, Szabó & Pfeifer 2012).

Another factor necessary for DNA methylation maintenance at ICRs in both mouse and human is ZFP57 (Krüppel-like zinc finger protein). Removal of the protein from the mouse zygote by use of both maternal and zygotic deletions caused embryonic death mid-gestation with a loss of all maternally methylated imprints (Li *et al.* 2008). In humans too, mutations in *ZFP57* causes hypomethylation at multiple imprinted loci (Mackay *et al.* 2008). ZFP57 binds to a target hexanucleotide found at imprinted gDMRs, but only when methylated, and recruits DNMT1 and its cofactor UHRF1 to maintain methylation on the marked alleles (Quenneville *et al.* 2011). Additionally, through KAP1, it recruits SETDB1 that modifies the chromatin by adding H3K9me3. Indeed, loss of KAP1 also causes a failure to maintain imprints in the preimplantation embryo (Messerschmidt *et al.* 2012). Following this protective step in early development, DNA methylation is thought to be maintained along with this chromatin mark throughout the offspring's life at the respective ICRs (Proudhon *et al.* 2012).

Post-implantation changes at transient and stable imprints

The small number of known imprinted loci characteristically show (1) methylation differences between gametes (gDMRs), (2) dependence on

DNMT3L (Kato *et al.* 2007) and (3) an ability to maintain differential methylation during preimplantation development (Hanna & Kelsey 2014). However, the genome-wide studies of the methylation landscape in germ cells mentioned earlier found that a relatively large number of loci (>1000) were methylated in a DNMT3L-dependent fashion in oocytes, but not sperm, and that many of them maintained high levels of methylation on the maternal allele until at least the blastocyst stage (Borgel *et al.* 2010, Smallwood *et al.* 2011, Shirane *et al.* 2013). Initial work by Michael Weber and colleagues (Borgel *et al.* 2010), suggested that not only imprinted loci, but also genes that were specifically expressed in the germline showed these features. Work from our own lab confirmed this (Rutledge *et al.* 2014) and further uncovered a class of genes expressed in brain showing these characteristics, suggesting three classes of genes with DNMT3L-programmed gDMRs: imprinted, germline and brain-specific genes (Fig. 1A). However, in stark contrast to the canonical imprinted loci, most of the brain and germline genes examined lost their differential methylation in adult tissues tested due to *gains* in methylation on the paternal, unmethylated allele. However, it was possible that among these latter two classes there were some true imprinted genes, which would show allele-specific expression in a tissue- or stage-specific fashion.

To identify such genes, Ferguson-Smith and colleagues used ZFP57 binding as an extra criterion in addition to the three indicated above (Strogantsev *et al.* 2015). Using this approach, they could confirm recently identified imprints such as *Cdh15* and *Gpr1*, and also uncovered evidence for imprinting at the *Fkbp6* gene. *Fkbp6* showed methylation in oocytes but not sperm in their study (Strogantsev *et al.* 2015), and maternal methylation was maintained in placenta at e16.5. In keeping with this, there was also predominant expression from the paternal allele as assayed using a transcribed SNP in interspecific mouse crosses, and this allele also carried H3K4me3 marks. Notably, in brain, both transcription and H3K4me3 continued to be associated with the paternal allele, even though the DNA methylation was assayed at 80%, rather than the expected 50%. This suggests that (1) either a subset of cells in brain continues to maintain the DNA methylation difference (in keeping with the low levels of transcription in this tissue); (2) some of the methylation being assayed may in fact be 5-hydroxymethylation (5hmC- see below), which is more prominent in brain and may cloud the picture, or most intriguingly (3) the DNA methylation difference is not as important as the histone marks. In addition to this complexity, *Fkbp6* may show differences in imprinting due to genetic variation, since sperm methylation varies between mouse strains (Rutledge *et al.* 2014), and there have been reports of polymorphic imprinting in humans (Hanna *et al.* 2016). ZFP57 is also capable of mediating such strain-specific effects (Strogantsev *et al.* 2015).

Figure 1 Some experimentally observed dependencies for imprint and imprint-like genes in mouse. Three classes of genes have gametic Differentially Methylated Regions (gDMR): testis, brain and imprinted genes (see key at bottom right: filled bar, 100% methylation). Genes that acquire methylation post implantation (somatic or sDMR) are also represented. (A) Germline: All three classes of gDMRs lose methylation in oocytes lacking DNMT3L. (B) Blastocyst/ESC: The gDMRs retain their methylation aided by DNMT1 and by DNMT3A/B, with other possible contributors indicated. Loss of methylation on imprints in *Dnmt1* knockout (1KO) cells cannot be rescued with *Dnmt1* cDNA (1Ko+1). In contrast, imprints in *Dnmt3a/b* KO (3abKO) can recover following rescue with *Dnmt3a2* (3abKO+a2). (C) Adult: Unlike true imprints, the testis and brain gDMRs cannot maintain the unmethylated allele in the face of the wave of *de novo* methylation after implantation. Likewise, they are more sensitive to inhibitors such as 5'-aza-2'-deoxycytidine (Aza).

Using one of the other three criteria above, namely dependence on DNMT3L, the Bourc'h's lab also identified novel imprinted gDMRs including *Cdh15*, *AK008011*, *Zfp777* and *Zfp787* (Proudhon *et al.* 2012). The *Cdh15* gDMR was found to control transcription of an allele-specific RNA in the hypothalamus, but there was no evidence for imprinted expression at the other three loci. All four gDMRs again lost their parental mark through *gains* in methylation in most adult tissues, highlighting the crucial role of protection from *de novo* methylation for imprint stability. They proposed that such genes, which would include *Fkbp6*, *Cdh15*, *Gpr1* and others, should be referred to as *transient imprints*. The subtlety of regulation of these loci led to the question of how functionally important this was. In a subsequent paper, they showed however that for the *Gpr1* locus, DNA methylation did indeed play an important role in fine-tuning transcription levels (Duffie *et al.* 2014). At this locus, interestingly, the histone marks seem to play a secondary role.

In contrast to these transient imprints, the canonical imprinted loci maintain methylation differences at the gDMR even in tissues that do not express the associated gene (Woodfine *et al.* 2011, Court *et al.* 2014, Wang *et al.* 2014), highlighting the existence of mechanisms to (1) prevent demethylation of the methylated (usually

maternal) allele and (2) protect the unmethylated (usually paternal) allele from gaining methylation. Studies comparing the sizes of DMR concluded that a certain degree of shrinkage occurs from the gametic state as the embryo matures (Court *et al.* 2014) but that the boundaries do not markedly shift upstream or downstream, consistent with a mechanism centred on the sequence determinants in the gDMRs.

Possible role of other cytosine modifications at imprinted loci

The dynamic changes in DNA methylation seen during early development are now known to be driven both by passive dilution through replication in the absence of maintenance activity, as well as active demethylation via the action of the three ten-eleven translocation (Tet) enzymes (Guo *et al.* 2014, Wang *et al.* 2014). The Tets have been shown to catalyse the oxidation of normal 5-methylcytosine (5mC), first to 5-hydroxymethylation (5hmC), then to 5-formylcytosine (5fC) and 5-carboxycytosine (5caC), at which stage the modified nucleotide is excised from DNA by the base excision repair enzyme thymidine DNA glycosylase (TDG) or possibly removed through an as-yet uncharacterised

decarboxylase activity (reviewed in [Hahn et al. 2014](#)). Active demethylation explains the rapid loss of 5mC in the pronuclei of the fertilised egg in the absence of replication, particularly notable for the male pronucleus, and lies behind much of the swift demethylation seen at some other time points such as germ cell specification and preimplantation development (reviewed in [Hill et al. 2014](#)). Blocking the action of the Tets thus prevents the demethylation at ICR in the germ cells required to reset the imprints, and it is only through the protective action of PGC7 ([Nakamura et al. 2012](#), [Szabó & Pfeifer 2012](#)) that imprints can be maintained pre implantation when Tet activity drives rapid demethylation at other methylated regions ([Guo et al. 2014](#), [Wang et al. 2014](#)).

In addition to being an intermediate in the active demethylation pathway, however, there is some evidence that 5hmC may also act as a stable epigenetic mark in itself ([Hahn et al. 2014](#)). Support for this derives from the observations that 5hmC levels in brain are too high to be consistent with being an intermediate in active demethylation in the non-replicating neurons and that proteins with high affinities for 5hmC, which may represent specific readers of this modification, appear to exist ([Iurlaro et al. 2013](#), [Spruijt et al. 2013](#)). Thus, 5hmC is frequently detected at high levels in neural tissues in the absence of 5fC and 5caC and may play an instructive role. Since it cannot be distinguished from 5mC by standard bisulfite modification and sequencing, it may contribute a substantial proportion of the DNA methylation reported at some loci. Examination of imprinted genes using methods that detect 5hmC have indicated however that the majority of methylation seen at ICR is indeed 5mC in adult tissues, though 5hmC is relatively enriched at imprinted loci compared to the levels elsewhere in the genome ([Hernandez Mora et al. 2005](#)). The 5hmC was present on the same allele as the 5mC, suggesting to the authors that it might be a stable mark here and not a sign of active demethylation. However, it is possible that this might reflect a dynamic turnover of methyl groups here, since Zhang and colleagues recently showed that G9a/GLP was required to recruit *de novo* methyltransferases to counteract TET activity at imprinted ICR ([Zhang et al. 2016](#)). However, levels of oxidised methyl cytosine products at ICR were not measured directly in the latter experiments. Much of the 5hmC that was detected by Hernandez Mora and colleagues was in transcribed regions of imprinted genes, not ICR, in keeping with an earlier report for *H19* ([Nestor et al. 2012](#)). Intriguingly, an association has been reported between 5hmC levels in *H19* and small-for-gestational-age babies ([Piyasena et al. 2015](#)).

Thus, while there is good evidence for high levels of 5hmC and other modified cytosines being seen at imprints during reprogramming, levels appear to be much lower in adult tissues except brain, and may be more related to transcription in the latter, since found in the gene bodies.

Can somatic reprogramming of imprints occur?

One of the key features of imprinted regions is their programming in the germ line, when the parental alleles are separated. Early work showed that in *Dnmt1*^{-/-} ES cells (1KO – [Fig. 1B](#)), imprints lost DNA methylation and could not recover it even when DNMT1 was restored using a cDNA (1KO+1), unlike the rest of the genome. However, when the rescued ES cells were used to make mice, the imprints were restored following passage through the germline ([Tucker et al. 1996](#)). This is thought to be due to the imperviousness of imprints to the normal reprogramming events seen in the soma. Indeed, the inability to reprogram in somatic tissues is a key difference between the imprinted gDMRs and the other two classes of gDMR, namely those found in germline and brain genes ([Borgel et al. 2010](#), [Rutledge et al. 2014](#)), whose methylation can be restored in 1KO+1 cells ([Fig. 1B](#)). We and others have confirmed these results both in ES cells ([Chen et al. 2003](#), [Thakur et al. 2016](#)) and in adult cell lines ([Chen et al. 2003](#), [Wernig et al. 2007](#)) ([Fig. 1B](#) and [C](#)). Interestingly, imprints appear to be resistant to demethylation as well as remethylation: treatment of cells with 5'Aza-2'deoxytidine (Aza) results in robust demethylation of most targets, but not imprints ([O'Neill et al. 2018](#)).

However, recently two ES cell systems have been described in which imprints can apparently be restored, breaking one of the main rules of imprinting. Wong and colleagues, working with UHRF1-deficient ES cells ([Qi et al. 2015](#)), found that most imprinted gDMRs lost methylation, as expected, since UHRF1 appears to be a vital cofactor for DNMT1 in somatic cells ([Bostick et al. 2007](#), [Sharif et al. 2007](#)). Surprisingly however, they found that when they rescued these cells using a UHRF1 cDNA that not only was methylation restored on bulk DNA, but also is a subset of the imprinted loci showed recovery. In particular, the *H19*, *Nnat* and *Dlk1* gDMRs showed some recovery of methylation, though not to WT levels, and ZFP57 binding was restored at 2/3 loci ([Qi et al. 2015](#)). While it would be reasonable to assume that an underlying chromatin mark might be retained, allowing restoration of methylation, there was no clear correlation between recovery and chromatin marks at the loci ([Qi et al. 2015](#)).

Using a different ES cell system, we too found somatic recovery of imprints could occur under some conditions. In cells lacking DNMT3A and DNMT3B (3abKO), imprinted gDMRs lost their parental allele-specific methylation to almost the same extent as in DNMT1-deficient cells. However, in contrast to rescue experiments in 1KO cells, when DNMT3A2 was added back to the 3abKO cells, we found that DNA methylation was restored at most gDMRs examined (11/14), with many achieving levels similar to the WT cells ([Fig. 1B](#)). Results were confirmed using up to three different techniques ([Thakur et al. 2016](#)).

Taken together, these two sets of results independently confirm that in some ES cell types, methylation can be restored on imprinted gDMRs outside of the germline opening the way for further exploration of the factors and signals that may be involved.

Implications for reproductive biology

How many imprinted loci are there?

In the index species mouse, where most data are available and there have been some dedicated searches, the number of verified imprinted loci remains relatively steady at around 150. Despite a few high-profile studies suggesting more may exist, the number of genes with confirmed uniparental expression has not increased greatly. Many of the known imprinted genes in mouse (<http://www.mousebook.org/imprinting-gene-list> (accessed 24/1/2018)) are also imprinted in human (<http://igc.otago.ac.nz/1601summarytable.pdf> (accessed 24/1/2018)). A recent study by Wang *et al.* (2014) identified a small number of new germline DMR, for which uniparental methylation has been verified by ourselves or others (Thakur *et al.* 2016), but the effects on transcription are yet to be confirmed. Likewise a study in human by Court *et al.* (2014) using a combination of arrays and whole-genome bisulfite sequencing (WGBS – see below) added some new DMR. These were largely confined to known imprinted loci, although some were novel placenta-specific DMR. One caveat with the latter is that the differential methylation appears to have been established postnatally, which would require a radical rethink of mechanisms. However, some evidence exists to suggest that this may also occur in mouse (Wagschal & Feil 2006).

In addition to the ‘true’ imprints, there are a number of ‘transient’ imprinted genes that show widespread imprinting in the early embryo that later becomes confined to one tissue (see ‘Post-implantation changes’ section above). This larger group of neuronal and germline genes have gDMRs in the early embryo that become erased post-implantation, largely through the *de novo* methylation of the unmethylated paternal allele (Borgel *et al.* 2010, Proudhon *et al.* 2012, Rutledge *et al.* 2014). These loci have not all been tested for uniparental expression in early development, but may contribute to parent-of-origin effects in early embryogenesis.

How conserved are imprints in other species?

Initial reports of imprinting in mouse were quickly followed by the investigation of corresponding human loci, and the subsequent discovery that at least one of the index loci *IGF2R* was not imprinted in all humans, but may instead show polymorphic imprinting (Xu *et al.* 1993). As the number of well-characterised imprinted loci in mouse grew, the general trend was for

the homologous locus in human to be imprinted, but with a number of notable exceptions (Hanna & Kelsey 2014, Peters 2014). In general, though, the emerging complexity of imprint locus control has tended to discourage attempts to verify in detail in the second species, with an assumption of similarity being adopted on the whole unless forced to be re-evaluated due to clinical or experimental evidence.

Some early studies also tried to map the index imprinted loci in marsupials in order to test parental conflict theories as well as to investigate evolutionary mechanisms. This met with some success, despite the difficulty of the exercise. There is now support for an origin for imprinting at the time of divergence of marsupials (metatheria) and egg-laying mammals (montremes), which lack imprinting (Renfree *et al.* 2013). The oldest imprinted loci with a conserved DMR are *H19* and *PEG10*, though a number of other imprinted genes such as *IGF2R* and *PEG1* are also imprinted, but lack a conserved DMR. Many other loci appear to have acquired imprinting in eutheria (Renfree *et al.* 2013). *PEG10* is a retroposon-derived gene, and it has long been suggested that there is a link between methylation at newly acquired retrotransposons and imprinting (Yoder *et al.* 1997). It is interesting in this context that rodentia are notable for having the highest number of imprinted loci and also a unique methyltransferase locus *Dnmt3c*, initially thought to be a pseudogene based on an early draft of the rat genome (Lees-Murdock *et al.* 2004) but now shown to produce a functional enzyme confined to the male rodent germline that appears dedicated to retroposon methylation (Barau *et al.* 2016).

ART and imprints

In livestock species, there has also been a lively interest in imprinted loci (O’Doherty *et al.* 2012), partly due to the widespread use of artificial reproduction techniques (ART), which are thought to be particularly associated with perturbed methylation and development including syndromes such as the aforementioned LOS (see above), enlarged placenta and perinatal death. These have been tied to changes in methylation at imprinted loci in a number of studies in cattle (O’Doherty *et al.* 2012, 2014). While studies in mouse have found substantial support for ART-induced alterations in imprints (Rivera *et al.* 2008, Denomme & Mann 2012), in humans, any link between methylation changes at imprinted loci and standard IVF has been controversial, with most reviews of the area not able to fully exclude an effect on imprints or other epigenetically regulated loci (Grafodatskaya *et al.* 2013). However, the addition of large enough concentrations of inhibitors such as aphidicolin have conclusively been shown to alter the programmed demethylation of the genome (Guo *et al.* 2014) at the pronuclear stage, so

Table 1 Technologies for genome-wide methylation analysis in animals.

Type ^a	Acronym	Technology	Spp ^b	Cost ^c	Coverage ^d	meC ^e	Input ^f	Res ^g	Notes ^h	Reference
Array	450K/EPIC	450K/850K probe array (Illumina)	Hu	L	Exome, CGI, regulatory	L	M	L	No TEs	Bibikova <i>et al.</i> (2011)
	EDMA	EmbryoGENE DNA methylation analysis	Bo	L	Exome, CGI, promoter	L	M	L	Qualitative, no TEs	Shojaei Saadi <i>et al.</i> (2014)
Reseq	WGBS	Whole-genome bisulfite sequencing	Any	H	Total	H	H	H	Bioinformatic analysis-heavy	Cokus <i>et al.</i> (2008), Lister <i>et al.</i> (2008), Meissner <i>et al.</i> (2008)
IP	RRBS	Reduced representation bisulfite sequencing	Any	M	Fraction	M	M	H	Partial CGI cover	Meissner <i>et al.</i> (2005)
	PBAT	Post-bisulfite adaptor tagging	Any	M	Total	H	L	H	Amplification-free	Miura <i>et al.</i> (2012)
	MeDIP-seq	Methylated DNA immunoprecipitation	Any	M	Fraction	M	M	H	Favours sparse CpG	Weber <i>et al.</i> (2005)
	MBD-CAP	Methyl binding domain CAPture	Any	M	Fraction	M	M	H	Favours CGI	Brinkman <i>et al.</i> (2010), Serre <i>et al.</i> (2010)
uCGI	MRE-seq	Methylation-sensitive restriction enzyme	Any	M	Fraction	M	M	L	uCGI-specific	Maunakea <i>et al.</i> (2010)
SC	CFP1-seq	CFP1 protein IP	Any	M	Fraction	M	M	H	uCGI-specific	Thomson <i>et al.</i> (2010)
	scWGBS	Single-cell WGBS	Any	H	Total	H	L	H	Bioinfo-heavy	Farlik <i>et al.</i> (2015)
modC	scRRBS	Single-cell RRBS	Any	M	Fraction	M	L	H	Bioinfo-heavy	Guo <i>et al.</i> (2013)
	scPBAT	Single-cell PBAT	Any	H	Total	H	L	H	Bioinfo-heavy	Smallwood <i>et al.</i> (2014)
	SMART	Single molecule real time (PacBio)	Any	H	Total	H	H	H	All modifications	Flusberg <i>et al.</i> (2010)
	hMeDIP-Seq	5hmC antibody IP	Any	M	Fraction	M	M	H	5hmC-specific	Jin <i>et al.</i> (2011)
TAB-Seq	oxBS-seq	Oxidative bisulfite sequencing (CEGX)	Any	M	Total	H	H	H	5hmC and 5mC	Booth <i>et al.</i> (2012)
	TAB-Seq	TET-assisted bisulfite sequencing	Any	M	Total	H	H	H	5hmC and 5mC	Yu <i>et al.</i> (2012, 2018)
	fCAB-seq	JBP1 5hmC-binding protein IP	Any	M	Total	H	H	H	5fC-specific	Song <i>et al.</i> (2013)
	JBP1-seq	JBP1 5hmC-binding protein IP	Any	M	Fraction	M	M	H	5hmC-specific	Cui <i>et al.</i> (2014)

^aType: Array, microarray; Reseq, resequencing; IP, immunoprecipitation; uCGI, targets unmethylated CGI; SC, single-cell; modC, targets modified 5mC. ^bSpp: species; Hu, human-specific; Bo, bovine-specific; any, can in theory be applied to any. ^cCost: L, Low; M, Medium; H, High. ^dCoverage: genome-wide coverage: Exome, exons; CGI, CpG islands; Regulatory, promoters and enhancers; Total, sequences whole genome; Fraction, subsets the genome but includes all types of sequence. ^emeC: fraction of all meC covered: Low, Medium, High. ^fInput: amount and purity of DNA: Low, lower quality and amount needed; Medium, better quality and more needed; High, highly pure DNA at higher amounts. ^gRes: resolution at target, i.e. are all 5mC covered in target areas (e.g. RRBS) or only a fraction at each location (arrays): Low, Medium, High. ^hNotes: TE, transposable elements; 5hmC, 5-hydroxymethylcytosine detection only; 5fC, 5-formylcytosine only.

environmental perturbations of imprinting remain at least theoretically plausible.

Use of sequencing technologies to investigate known and potential imprinted regions

There have been a number of approaches taken to identify potentially imprinted genes and to more closely define the size of the imprint, partly dictated by the tools and approaches available in the differing species. In humans for example, microarrays have made assessing methylation easier, but low heterozygosity levels and incomplete data make assignment of parental allele and gametic imprints – even with resequencing approaches – problematic, whereas in mice, no methylation arrays are available but interspecific crosses can be generated to maximise heterozygosity. Ruminants on the other hand have a long reproductive cycle and inter-strain hybrids are not commonly used, and with some exceptions, there are no arrays here either. For these reasons, resequencing approaches offer the most widely applicable tool (Table 1). While sequencing can be restrictive in cost, it represents a powerful discovery platform.

Wang and colleagues for example used WGBS to investigate the methylation status of the 54 imprinted DMR in the mouse genome (Wang *et al.* 2014), and in particular to sort unclassified DMR into gametic or somatic categories. They compared oocyte, sperm and early embryo, and began by confirming they could correctly sort all the DMR whose status was known (29) into gametic vs somatic types. They then used the same criteria to classify 25 imprinted DMRs whose status was unknown into gametic or somatic DMR, which is essential for determining where primary control of each locus is being exerted. At least four of these newly classified DMRs could subsequently be independently confirmed using a pyrosequencing-based assay (Thakur *et al.* 2016).

In humans, microarray technologies and in particular the Illumina EPIC array, have become so reliable in terms of assessing methylation levels quantitatively that confirmation by a second technology has become almost redundant. The low relative cost of the array makes it an attractive approach for initial screening and a comparison of uniparental disomies could correctly identify all 30 DMRs for which the array had probes (one DMR at *MEG3/DLK1* is not covered). They additionally identified 21 novel putative imprints, 15 specific to placenta, albeit a number of them were at known imprinted loci already (Court *et al.* 2014). However, the low relative resolution of the array meant that they needed to use WGBS to accurately delineate the DMR.

Reliable standardised methylation microarrays are largely confined to humans, though the success of the 450K and EPIC platforms has meant that Illumina appears to be considering a similar BeadChip array

for mouse. An exception is cattle, where the EDMA array has been developed for assessing genome-wide methylation (Shojaei Saadi *et al.* 2014), though this is based on cutting with methylation-sensitive restriction enzymes rather than oligos. While the array gives good internal consistency and technical reproducibility and could correctly identify methylation status at a number of imprinted regions in this species, it gives relative rather than absolute levels of methylation and is therefore difficult to correlate with outputs from many other assays (Desmet *et al.* 2016), though not all (Ispada *et al.* 2018). Whole-genome sequencing represents the gold standard for exploratory work and can definitively assess methylation across all sites. Confirming parental origin of the methylation mark requires heterozygous SNPs

Figure 2 Schematic comparison of reduced representation bisulfite sequencing (RRBS) vs post-bisulfite adaptor tagging (PBAT). In RRBS (left), the DNA is initially fragmented by a methylation-insensitive restriction endonuclease such as *MspI* to an optimal fragment size of between 400 and 600bp, and this fraction isolated following gel electrophoresis or similar. Adaptors are ligated to these DNA fragments that are subsequently treated through bisulfite conversion to convert unmethylated cytosines to thymines for the differential readout. Amplification is carried out using primers specific to the adaptor sequences, but there is substantial loss of sample at this point due to strand breakage (flashes) between adaptors caused by bisulfite. PBAT (right) skips the initial enzyme digestion and instead takes advantage of the bisulfite-induced breakage to fragment the DNA here. Instead of PCR, the adaptors are used for two rounds of random primer extension, which serves to produce numerous reads from a small amount of input genomic DNA.

however. While Liu and colleagues could find sufficient variation in an inter-strain cross for mice (Wang *et al.* 2014), generating cross-bred cattle is not trivial given cost implications and slow reproductive times and most reported work in the area does not feature such animals.

An alternative, more widely applicable and cost-effective approach for assessing methylation is to subset the genome and do targeted resequencing. Reduced representation bisulfite sequencing has been the most extensively used method in this category so far (Meissner *et al.* 2005), but is being superseded by post-bisulfite adaptor tagging (PBAT) (Miura *et al.* 2012), which appears to offer improvements in yield and efficiency, particularly when starting with small amounts of material such as oocytes (Smallwood *et al.* 2014) (Fig. 2). Apart from cost considerations, a significant bottleneck for resequencing-based analyses is the extensive bioinformatic analysis required, with concomitant need for higher-specification computing hardware, local network clusters and data warehousing, all adding substantial capital and human resource costs to the equation (Lewitter *et al.* 2009). While this has resulted in the majority of such work being undertaken at large central institutes that have developed the required infrastructure, often with national support, there remains a niche for smaller players out-sourcing the sequencing to larger centres with surplus capacity and who may in future also be able to avail of cloud-based storage and analysis capabilities (Liu *et al.* 2014).

Another point to consider here is that some species that may be of interest for biologists wishing to understand the origins or phylogenetic reach of imprinting may not be sequenced yet. In cases where a reference genome framework is unavailable, assembling sequence reads and defining variation become considerably more challenging (Cahais *et al.* 2012).

Finally, the impact of third-generation sequencing technologies is yet to be fully felt, given that this market is currently in flux and no clear front-runner appears yet to have emerged. Current technologies require a separate sequence library for each type of cytosine modification for example (meC, 5hmC, 5fC, 5caC) (Yu *et al.* 2012), whereas certain new approaches (Table 1) such as PacBio's technology have the capability to also call methylated bases while sequencing, obviating the need for multiple sequence sets (Lister & Ecker 2009).

Conclusions and future prospects

Imprinting clearly plays an important role in body size determination and nutrient transfer to the young, making assessment of methylation at key loci a useful tool for reproductive biologists and breeders alike. Despite the improvement in technologies for assessing and delineating imprinting, the number of verified imprinted loci has not increased very significantly. However, our understanding of the mechanisms by which these regions

are established and maintained in early life, as well as the contexts in which some plasticity in imprinting may occur, have greatly increased. We can look forward to further insights from comparative genomic approaches based on the increasing application of sequencing technologies to an ever-expanding range of organisms, including the so far rather surprisingly neglected livestock species, which play such an important role in human food production and welfare.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

The authors' work was supported in part by the Medical Research Council (grant number MR/J007773/1) and a grant jointly funded by the ESRC/BBSRC (grant number ES/N000323/1).

Acknowledgements

The authors thank members of the Walsh lab for helpful comments and apologize to colleagues whose work could not be cited owing to length limitations.

References

- Allen WR 1969 Factors influencing pregnant mare serum gonadotrophin production. *Nature* **223** 64–65. (<https://doi.org/10.1038/223064a0>)
- Aravind L & Koonin EV 2000 SAP – a putative DNA-binding motif involved in chromosomal organization. *Trends in Biochemical Sciences* **25** 112–114. ([https://doi.org/10.1016/S0968-0004\(99\)01537-6](https://doi.org/10.1016/S0968-0004(99)01537-6))
- Barau J, Teissandier A, Zamudio N, Roy S, Nalesso V, Herault Y, Guillou F & Bourc'his D 2016 The DNA methyltransferase DNMT3C protects male germ cells from transposon activity. *Science* **354** 909–912. (<https://doi.org/10.1126/science.aah5143>)
- Barlow DP & Bartolomei MS 2014 Genomic imprinting in mammals. *Cold Spring Harbor Perspectives in Biology* **6** (<https://doi.org/10.1101/cshperspect.a018382>)
- Bartolomei MS & Ferguson-Smith AC 2011 Mammalian genomic imprinting. *Cold Spring Harbor Perspectives in Biology* **3** a018382. (<https://doi.org/10.1101/cshperspect.a002592>)
- Barton SC, Surani MA & Norris ML 1984 Role of paternal and maternal genomes in mouse development. *Nature* **311** 374–376. (<https://doi.org/10.1038/311374a0>)
- Bell AC & Felsenfeld G 2000 Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. *Nature* **405** 482–485. (<https://doi.org/10.1038/35013100>)
- Bibikova M, Barnes B, Tsan C, Ho V, Klotzle B, Le JM, Delano D, Zhang L, Schroth GP, Gunderson KL *et al.* 2011 High density DNA methylation array with single CpG site resolution. *Genomics* **98** 288–295. (<https://doi.org/10.1016/j.ygeno.2011.07.007>)
- Bidwell CA, Waddell JN, Taxis TM, Yu H, Tellam RL, Neary MK & Cockett NE 2014 New insights into polar overdominance in callipyge sheep. *Animal Genetics* **45** (Supplement 1) 51–61. (<https://doi.org/10.1111/age.12132>)
- Bird AP 1987 CpG islands as gene markers in the vertebrate nucleus. *Trends in Genetics* **3** 342–347. ([https://doi.org/10.1016/0168-9525\(87\)90294-0](https://doi.org/10.1016/0168-9525(87)90294-0))
- Booth MJ, Branco MR, Ficiz G, Oxley D, Krueger F, Reik W & Balasubramanian S 2012 Quantitative sequencing of 5-methylcytosine and 5-hydroxymethylcytosine at single-base resolution. *Science* **336** 934–937. (<https://doi.org/10.1126/science.1220671>)

- Borgel J, Guibert S, Li Y, Chiba H, Schubeler D, Sasaki H, Forne T & Weber M 2010 Targets and dynamics of promoter DNA methylation during early mouse development. *Nature Genetics* **42** 1093–1100. (<https://doi.org/10.1038/ng.708>)
- Bostick M, Kyong Kim J, Esteve P, Clark A & Pradhan S 2007 UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* **317** 1760–1764. (<https://doi.org/10.1126/science.1147939>)
- Brinkman AB, Simmer F, Ma K, Kaan A, Zhu J & Stunnenberg HG 2010 Whole-genome DNA methylation profiling using MethylCap-seq. *Methods* **52** 232–236. (<https://doi.org/10.1016/j.ymeth.2010.06.012>)
- Buiting K 2010 Prader-Willi syndrome and Angelman syndrome. *American Journal of Medical Genetics: Part C, Seminars in Medical Genetics* **154C** 365–376. (<https://doi.org/10.1002/ajmg.c.30273>)
- Cahais V, Gayral P, Tsakogeorga G, Melo-Ferreira J, Ballenghien M, Weinert L, Chiari Y, Belkhir K, Ranwez V & Galtier N 2012 Reference-free transcriptome assembly in non-model animals from next-generation sequencing data. *Molecular Ecology Resources* **12** 834–845. (<https://doi.org/10.1111/j.1755-0998.2012.03148.x>)
- Chen T, Ueda Y, Dodge JE, Wang Z & Li E 2003 Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b. *Molecular and Cellular Biology* **23** 5594–5605. (<https://doi.org/10.1128/MCB.23.16.5594-5605.2003>)
- Chen Z, Robbins KM, Wells KD & Rivera RM 2013 Large offspring syndrome: a bovine model for the human loss-of-imprinting overgrowth syndrome Beckwith-Wiedemann. *Epigenetics* **8** 591–601. (<https://doi.org/10.4161/epi.24655>)
- Chen Z, Hagen DE, Elsik CG, Ji T, Morris CJ, Moon LE & Rivera RM 2015 Characterization of global loss of imprinting in fetal overgrowth syndrome induced by assisted reproduction. *PNAS* **112** 4618–4623. (<https://doi.org/10.1073/pnas.1422088112>)
- Ciccone DN, Su H, Hevi S, Gay F, Lei H, Bajko J, Xu G, Li E & Chen T 2009 KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. *Nature* **461** 415–418. (<https://doi.org/10.1038/nature08315>)
- Cokus SJ, Feng S, Zhang X, Chen Z, Merriman B, Haudenschild CD, Pradhan S, Nelson SF, Pellegrini M & Jacobsen SE 2008 Shotgun bisulphite sequencing of the Arabidopsis genome reveals DNA methylation patterning. *Nature* **452** 215–219. (<https://doi.org/10.1038/nature06745>)
- Court F, Tayama C, Romanelli V, Martin-Trujillo A, Iglesias-Platas I, Okamura K, Sugahara N, Simon C, Moore H, Harness JV *et al.* 2014 Genome-wide parent-of-origin DNA methylation analysis reveals the intricacies of human imprinting and suggests a germline methylation-independent mechanism of establishment. *Genome Research* **24** 554–569. (<https://doi.org/10.1101/gr.164913.113>)
- Cui L, Chung TH, Tan D, Sun X & Jia XY 2014 JBP1-seq: a fast and efficient method for genome-wide profiling of 5hmC. *Genomics* **104** 368–375. (<https://doi.org/10.1016/j.ygeno.2014.08.023>)
- Curley JP, Pinnock SB, Dickson SL, Thresher R, Miyoshi N, Surani MA & Keverne EB 2005 Increased body fat in mice with a targeted mutation of the paternally expressed imprinted gene Peg3. *FASEB Journal* **19** 1302–1304. (<https://doi.org/10.1096/fj.04-3216fje>)
- Denomme MM & Mann MR 2012 Genomic imprints as a model for the analysis of epigenetic stability during assisted reproductive technologies. *Reproduction* **144** 393–409. (<https://doi.org/10.1530/REP-12-0237>)
- Desmet KL, Van Hoeck V, Gagne D, Fournier E, Thakur A, O'Doherty AM, Walsh CP, Sirard MA, Bols PE & Leroy JL 2016 Exposure of bovine oocytes and embryos to elevated non-esterified fatty acid concentrations: integration of epigenetic and transcriptomic signatures in resultant blastocysts. *BMC Genomics* **17** 1004–1022. (<https://doi.org/10.1186/s12864-016-3366-y>)
- Duffie R, Ajjan S, Greenberg MV, Zamudio N, Escamilla del Arenal M, Irazzo J, Okamoto I, Barbaux S, Fauque P & Bourc'his D 2014 The Gpr1/Zdbf2 locus provides new paradigms for transient and dynamic genomic imprinting in mammals. *Genes and Development* **28** 463–478.
- Farlik M, Sheffield NC, Nuzzo A, Datlinger P, Schonegger A, Klughammer J & Bock C 2015 Single-cell DNA methylome sequencing and bioinformatic inference of epigenomic cell-state dynamics. *Cell Reports* **10** 1386–1397. (<https://doi.org/10.1016/j.celrep.2015.02.001>)
- Flusberg BA, Webster DR, Lee JH, Travers KJ, Olivares EC, Clark TA, Korlach J & Turner SW 2010 Direct detection of DNA methylation during single-molecule, real-time sequencing. *Nature Methods* **7** 461–465. (<https://doi.org/10.1038/nmeth.1459>)
- Freking BA, Keele JW, Nielsen MK, Leymaster KA 2002a Evaluation of the ovine callipyge locus: II. Genotypic effects on growth, slaughter, and carcass traits. *Journal of Animal Science* **76** 2549–2559. (<https://doi.org/10.2527/1998.76102549x>)
- Freking BA, Murphy SK, Wylie AA, Rhodes SJ, Keele JW, Leymaster KA, Jirtle RL & Smith TP 2002b Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar overdominance in mammals. *Genome Research* **12** 1496–1506. (<https://doi.org/10.1101/gr.571002>)
- Grafodatskaya D, Cytrynbaum C & Weksberg R 2013 The health risks of ART. *EMBO Reports* **14** 129–135. (<https://doi.org/10.1038/embor.2012.222>)
- Guillemot F, Caspary T, Tilghman SM, Copeland NG, Gilbert DJ, Jenkins NA, Anderson DJ, Joyner AL, Rossant J & Nagy A 1995 Genomic imprinting of Mash2, a mouse gene required for trophoblast development. *Nature Genetics* **9** 235–242. (<https://doi.org/10.1038/ng0395-235>)
- Guo H, Zhu P, Wu X, Li X, Wen L & Tang F 2013 Single-cell methylome landscapes of mouse embryonic stem cells and early embryos analyzed using reduced representation bisulfite sequencing. *Genome Research* **23** 2126–2135. (<https://doi.org/10.1101/gr.161679.113>)
- Guo F, Li X, Liang D, Li T, Zhu P, Guo H, Wu X, Wen L, Gu TP, Hu B *et al.* 2014 Active and passive demethylation of male and female pronuclear DNA in the mammalian zygote. *Cell Stem Cell* **15** 447–459. (<https://doi.org/10.1016/j.stem.2014.08.003>)
- Hahn MA, Szabo PE & Pfeifer GP 2014 5-Hydroxymethylcytosine: a stable or transient DNA modification? *Genomics* **104** 314–323. (<https://doi.org/10.1016/j.ygeno.2014.08.015>)
- Haig D & Graham C 1991 Genomic imprinting and the strange case of the insulin-like growth factor II receptor. *Cell* **64** 1045–1046. ([https://doi.org/10.1016/0092-8674\(91\)90256-X](https://doi.org/10.1016/0092-8674(91)90256-X))
- Hanna CW & Kelsey G 2014 The specification of imprints in mammals. *Heredity* **113** 176–183. (<https://doi.org/10.1038/hdy.2014.54>)
- Hanna CW, Penaherrera MS, Saadeh H, Andrews S, McFadden DE, Kelsey G & Robinson WP 2016 Pervasive polymorphic imprinted methylation in the human placenta. *Genome Research* **26** 756–767. (<https://doi.org/10.1101/gr.196139.115>)
- Hark AT, Schoenherr CJ, Katz DJ, Ingram RS, LeVorse JM & Tilghman SM 2000 CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. *Nature* **405** 486–489. (<https://doi.org/10.1038/35013106>)
- Hernandez Mora JR, Sanchez-Delgado M, Petazzi P, Moran S, Esteller M, Iglesias-Platas I & Monk D 2005 Profiling of oxBS-450K 5-hydroxymethylcytosine in human placenta and brain reveals enrichment at imprinted loci. *Epigenetics* **13** 182–191. (<https://doi.org/10.1080/15592294.2017.1344803>)
- Hill PW, Amouroux R & Hajkova P 2014 DNA demethylation, Tet proteins and 5-hydroxymethylcytosine in epigenetic reprogramming: an emerging complex story. *Genomics* **104** 324–333. (<https://doi.org/10.1016/j.ygeno.2014.08.012>)
- Ispada J, de Lima CB, Sirard MA, Fontes PK, Nogueira MFG, Annes K & Milazzotto MP 2018 Genome-wide screening of DNA methylation in bovine blastocysts with different kinetics of development. *Epigenetics and Chromatin* **11** 1. (<https://doi.org/10.1186/s13072-017-0171-z>)
- Iurlaro M, Ficiz G, Oxley D, Raiber E, Bachman M, Booth MJ, Andrews S, Balasubramanian S & Reik W 2013 A screen for hydroxymethylcytosine and formyl cytosine binding proteins suggests functions in transcription and chromatin regulation. *Genome Biology* **14** R119. (<https://doi.org/10.1186/gb-2013-14-10-r119>)
- Jeon JT, Carlborg O, Tornsten A, Giuffra E, Amarger V, Chardon P, Andersson-Eklund L, Andersson K, Hansson I, Lundstrom K *et al.* 1999 A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. *Nature Genetics* **21** 157–158. (<https://doi.org/10.1038/5938>)
- Jin SG, Wu X, Li AX & Pfeifer GP 2011 Genomic mapping of 5-hydroxymethylcytosine in the human brain. *Nucleic Acids Research* **39** 5015–5024. (<https://doi.org/10.1093/nar/gkr120>)
- Kato Y, Kaneda M, Hata K, Kumaki K, Hisano M, Kohara Y, Okano M, Li E, Nozaki M & Sasaki H 2007 Role of the Dnmt3 family in de novo methylation of imprinted and repetitive sequences during male germ cell

- development in the mouse. *Human Molecular Genetics* **16** 2272–2280. (<https://doi.org/10.1093/hmg/ddm179>)
- Keverne EB & Curley JP 2008 Epigenetics, brain evolution and behaviour. *Frontiers in Neuroendocrinology* **29** 398–412. (<https://doi.org/10.1016/j.yfrne.2008.03.001>)
- Kobayashi H, Sakurai T, Imai M, Takahashi N, Fukuda A, Yayoi O, Sato S, Nakabayashi K, Hata K, Sotomaru Y *et al.* 2012 Contribution of intragenic DNA methylation in mouse gametic DNA methylomes to establish oocyte-specific heritable marks. *PLoS Genetics* **8** e1002440. (<https://doi.org/10.1371/journal.pgen.1002440>)
- Lees-Murdock DJ, McLoughlin GA, McDaid JR, Quinn LM, O'Doherty A, Hiripi L, Hack CJ & Walsh CP 2004 Identification of 11 pseudogenes in the DNA methyltransferase gene family in rodents and humans and implications for the functional loci. *Genomics* **84** 193–204. (<https://doi.org/10.1016/j.ygeno.2004.02.004>)
- Lewitter F, Rebhan M, Richter B & Sexton D 2009 The need for centralization of computational biology resources. *PLoS Computational Biology* **5** e1000372. (<https://doi.org/10.1371/journal.pcbi.1000372>)
- Li X, Ito M, Zhou F, Youngson N, Zuo X, Leder P & Ferguson-Smith AC 2008 A maternal-zygotic effect gene, *Zfp57*, maintains both maternal and paternal imprints. *Developmental Cell* **15** 547–557. (<https://doi.org/10.1016/j.devcel.2008.08.014>)
- Lister R & Ecker JR 2009 Finding the fifth base: genome-wide sequencing of cytosine methylation. *Genome Research* **19** 959–966. (<https://doi.org/10.1101/gr.083451.108>)
- Lister R, O'Malley RC, Tonti-Filippini J, Gregory BD, Berry CC, Millar AH & Ecker JR 2008 Highly integrated single-base resolution maps of the epigenome in Arabidopsis. *Cell* **133** 523–536. (<https://doi.org/10.1016/j.cell.2008.03.029>)
- Liu B, Madduri RK, Sotomayor B, Chard K, Lacinski L, Dave UJ, Li J, Liu C & Foster IT 2014 Cloud-based bioinformatics workflow platform for large-scale next-generation sequencing analyses. *Journal of Biomedical Informatics* **49** 119–133. (<https://doi.org/10.1016/j.jbi.2014.01.005>)
- Mackay DJ, Callaway JL, Marks SM, White HE, Acerini CL, Boonen SE, Dayanikli P, Firth HV, Goodship JA, Haemers AP *et al.* 2008 Hypomethylation of multiple imprinted loci in individuals with transient neonatal diabetes is associated with mutations in *ZFP57*. *Nature Genetics* **40** 949–951. (<https://doi.org/10.1038/ng.187>)
- Markljung E, Jiang L, Jaffe JD, Mikkelsen TS, Wallerman O, Larhammar M, Zhang X, Wang L, Saenz-Vash V, Gnirke A *et al.* 2009 ZBED6, a novel transcription factor derived from a domesticated DNA transposon regulates IGF2 expression and muscle growth. *PLoS Biology* **7** e1000256. (<https://doi.org/10.1371/journal.pbio.1000256>)
- Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, Johnson BE, Hong C, Nielsen C, Zhao Y *et al.* 2010 Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature* **466** 253–257. (<https://doi.org/10.1038/nature09165>)
- Meissner A, Gnirke A, Bell GW, Ramsahoye B, Lander ES & Jaenisch R 2005 Reduced representation bisulfite sequencing for comparative high-resolution DNA methylation analysis. *Nucleic Acids Research* **33** 5868–5877. (<https://doi.org/10.1093/nar/gki901>)
- Meissner A, Mikkelsen TS, Gu H, Wernig M, Hanna J, Sivachenko A, Zhang X, Bernstein BE, Nussbaum C, Jaffe DB *et al.* 2008 Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* **454** 766–770. (<https://doi.org/10.1038/nature07107>)
- Messerschmidt DM, de Vries W, Ito M, Solter D, Ferguson-Smith A & Knowles BB 2012 Trim28 is required for epigenetic stability during mouse oocyte to embryo transition. *Science* **335** 1499–1502. (<https://doi.org/10.1126/science.1216154>)
- Miura F, Enomoto Y, Dairiki R & Ito T 2012 Amplification-free whole-genome bisulfite sequencing by post-bisulfite adaptor tagging. *Nucleic Acids Research* **40** e136. (<https://doi.org/10.1093/nar/gks454>)
- Moore T & Haig D 1991 Genomic imprinting in mammalian development: a parental tug-of-war. *Trends in Genetics* **7** 45–49. ([https://doi.org/10.1016/0168-9525\(91\)90230-N](https://doi.org/10.1016/0168-9525(91)90230-N))
- Nakamura T, Arai Y, Umehara H, Masuhara M, Kimura T, Taniguchi H, Sekimoto T, Ikawa M, Yoneda Y, Okabe M *et al.* 2007 PGC7/Stella protects against DNA demethylation in early embryogenesis. *Nature Cell Biology* **9** 64–71. (<https://doi.org/10.1038/ncb1519>)
- Nakamura T, Liu YJ, Nakashima H, Umehara H, Inoue K, Matoba S, Tachibana M, Ogura A, Shinkai Y & Nakano T 2012 PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. *Nature* **486** 415–419. (<https://doi.org/10.1038/nature11093>)
- Nestor CE, Ottaviano R, Reddington J, Sproul D, Reinhardt D, Dunican D, Katz E, Dixon JM, Harrison DJ & Meehan RD 2012 Tissue type is a major modifier of the 5'-hydroxymethylcytosine content of human genes. *Genome Research* **22** 467–477. (<https://doi.org/10.1101/gr.126417.111>)
- O'Doherty A, O'Shea LC & Fair T 2012 Bovine DNA methylation imprints are established in an oocyte size-specific manner, which are coordinated with the expression of the DNMT3 family proteins. *Biology of Reproduction* **86** 1–10. (<https://doi.org/10.1095/biolreprod.111.094946>)
- O'Doherty A, O'Gorman A, al Naib A, Brennan L, Daly E, Duffy P & Fair T 2014 Negative energy balance affects imprint stability in oocytes recovered from postpartum dairy cows. *Genomics* **104** 177–185. (<https://doi.org/10.1016/j.ygeno.2014.07.006>)
- O'Neill KM, Irwin RE, Mackin SJ, Thursby SJ, Thakur A, Bertens C, Masala L, Loughery JEP, McArt DG & Walsh CP 2018 Depletion of DNMT1 in differentiated human cells highlights key classes of sensitive genes and an interplay with polycomb repression. *Epigenetics and Chromatin* **11** 12. (<https://doi.org/10.1186/s13072-018-0182-4>)
- Ooi SK, Qiu C, Bernstein E, Li K, Jia D, Yang Z, Erdjument-Bromage H, Tempst P, Lin SP, Allis CD *et al.* 2007 DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* **448** 714–717. (<https://doi.org/10.1038/nature05987>)
- Payer B, Saitou M, Barton SC, Thresher R, Dixon JPC, Zahn WH, Carlton MBL, Nakano T & Surani MA 2003 Stella is a maternal effect gene required for normal early development in mice. *Current Biology* **13** 2110–2117. (<https://doi.org/10.1016/j.cub.2003.11.026>)
- Peters J 2014 The role of genomic imprinting in biology and disease: an expanding view. *Nature Reviews Genetics* **15** 517–530. (<https://doi.org/10.1038/nrg3766>)
- Piyasena C, Reynolds RM, Khulan B, Seckl JR, Menon G & Drake AJ 2015 Placental 5-methylcytosine and 5-hydroxymethylcytosine patterns associate with size at birth. *Epigenetics* **10** 692–697. (<https://doi.org/10.1080/15592294.2015.1062963>)
- Prickett AR, Barkas N, McCole RB, Hughes S, Amante SM, Schulz R & Oakey RJ 2013 Genome-wide and parental allele-specific analysis of CTCF and cohesin DNA binding in mouse brain reveals a tissue-specific binding pattern and an association with imprinted differentially methylated regions. *Genome Research* **23** 1624–1635. (<https://doi.org/10.1101/gr.150136.112>)
- Proudhon C, Duffie R, Ajjan S, Cowley M, Iranzo J, Carbajosa G, Saadeh H, Holland ML, Oakey RJ, Rakan VK *et al.* 2012 Protection against de novo methylation is instrumental in maintaining parent-of-origin methylation inherited from the gametes. *Molecular Cell* **47** 909–920. (<https://doi.org/10.1016/j.molcel.2012.07.010>)
- Qi S, Wang Z, Li P, Wu Q, Shi T, Li J & Wong J 2015 Non-germ line restoration of genomic imprinting for a small subset of imprinted genes in ubiquitin-like PHD and RING finger domain-containing 1 (Uhrf1) null mouse embryonic stem cells. *Journal of Biological Chemistry* **290** 14181–14191. (<https://doi.org/10.1074/jbc.M114.626697>)
- Quenneville S, Verde G, Corsinotti A, Kapopoulou A, Jakobsson J, Offner S, Baglivo I, Pedone PV, Grimaldi G, Riccio A *et al.* 2011 In embryonic stem cells, ZFP57/KAP1 recognize a methylated hexanucleotide to affect chromatin and DNA methylation of imprinting control regions. *Molecular Cell* **44** 361–372. (<https://doi.org/10.1016/j.molcel.2011.08.032>)
- Renfree MB, Suzuki S & Kaneko-Ishino T 2013 The origin and evolution of genomic imprinting and viviparity in mammals. *Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences* **368** 20120151. (<https://doi.org/10.1098/rstb.2012.0151>)
- Rivera RM, Stein P, Weaver JR, Mager J, Schultz RM & Bartolomei MS 2008 Manipulations of mouse embryos prior to implantation result in aberrant expression of imprinted genes on day 9.5 of development. *Human Molecular Genetics* **17** 1–14. (<https://doi.org/10.1093/hmg/ddm280>)
- Rutledge CE, Thakur A, O'Neill KM, Irwin RE, Sato S, Hata K & Walsh CP 2014 Ontogeny, conservation and functional significance of maternally inherited DNA methylation at two classes of non-imprinted genes. *Development* **141** 1313–1323. (<https://doi.org/10.1242/dev.104646>)
- Sato M, Kimura T, Kurokawa K, Fujita Y, Abe K, Masuhara M, Yasunaga T, Ryo A, Yamamoto M & Nakano T 2002 Identification of PGC7, a new gene expressed specifically in preimplantation embryos and germ cells.

- Mechanisms of Development* **113** 91–94. ([https://doi.org/10.1016/S0925-4773\(02\)00002-3](https://doi.org/10.1016/S0925-4773(02)00002-3))
- Serre D, Lee BH & Ting AH 2010 MBD-isolated genome sequencing provides a high-throughput and comprehensive survey of DNA methylation in the human genome. *Nucleic Acids Research* **38** 391–399. (<https://doi.org/10.1093/nar/gkp992>)
- Sharif J, Muto M, Takebayashi S, Suetake I, Iwamatsu A, Endo TA, Shinga J, Mizutani-Koseki Y, Toyoda T, Okamura K *et al.* 2007 The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* **450** 908–912. (<https://doi.org/10.1038/nature06397>)
- Shirane K, Toh H, Kobayashi H, Miura F, Chiba H, Ito T, Kono T & Sasaki H 2013 Mouse oocyte methylomes at base resolution reveal genome-wide accumulation of non-CpG methylation and role of DNA methyltransferases. *PLoS Genetics* **9** e1003439. (<https://doi.org/10.1371/journal.pgen.1003439>)
- Shojaei Saadi HA, O'Doherty AM, Gagne D, Fournier E, Grant JR, Sirard MA & Robert C 2014 An integrated platform for bovine DNA methylome analysis suitable for small samples. *BMC Genomics* **15** 451–468. (<https://doi.org/10.1186/1471-2164-15-451>)
- Sleutels F, Zwart R & Barlow DP 2002 The non-coding Air RNA is required for silencing autosomal imprinted genes. *Nature* **415** 810–813. (<https://doi.org/10.1038/415810a>)
- Smallwood SA, Tomizawa S, Krueger F, Ruf N, Carli N, Segonds-Pichon A, Sato S, Hata K, Andrews SR & Kelsey G 2011 Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. *Nature Genetics* **43** 811–814. (<https://doi.org/10.1038/ng.864>)
- Smallwood SA, Lee HJ, Angermueller C, Krueger F, Saadeh H, Peat J, Andrews SR, Stegle O, Reik W & Kelsey G 2014 Single-cell genome-wide bisulfite sequencing for assessing epigenetic heterogeneity. *Nature Methods* **11** 817–820. (<https://doi.org/10.1038/nmeth.3035>)
- Song CX, Szulwach KE, Dai Q, Fu Y, Mao SQ, Lin L, Street C, Li Y, Poidevin M, Wu HC 2013 Genome-wide profiling of 5-formylcytosine reveals its roles in epigenetic priming. *Cell* **153** 678–691. (<https://doi.org/10.1016/j.cell.2013.04.001>)
- Spruijt CG, Gnerlich F, Smits AH, Pfaffeneder T, Jansen PW, Bauer C, Munzel M, Wagner M, Muller M, Khan F *et al.* 2013 Dynamic readers for 5-(hydroxy)methylcytosine and its oxidized derivatives. *Cell* **152** 1146–1159. (<https://doi.org/10.1016/j.cell.2013.02.004>)
- Strogantsev R, Krueger F, Yamazawa K, Shi H, Gould P, Goldman-Roberts M, McEwen K, Sun B, Pedersen R & Ferguson-Smith AC 2015 Allele-specific binding of ZFP57 in the epigenetic regulation of imprinted and non-imprinted monoallelic expression. *Genome Biology* **16** 112–119. (<https://doi.org/10.1186/s13059-015-0672-7>)
- Szabó PE & Pfeifer GP 2012 H3K9me2 attracts PGC7 in the zygote to prevent Tet3-mediated oxidation of 5-methylcytosine. *Journal of Molecular Cell Biology* **4** 427–429. (<https://doi.org/10.1093/jmcb/mjs038>)
- Thakur A, Mackin SJ, Irwin RE, O'Neill KM, Pollin G & Walsh C 2016 Widespread recovery of methylation at gametic imprints in hypomethylated mouse stem cells following rescue with DNMT3A2. *Epigenetics and Chromatin* **9** 53–67. (<https://doi.org/10.1186/s13072-016-0104-2>)
- Thomson JP, Skene PJ, Selfridge J, Clouaire T, Guy J, Webb S, Kerr AR, Deaton A, Andrews R, James KD *et al.* 2010 CpG islands influence chromatin structure via the CpG-binding protein Cfp1. *Nature* **464** 1082–1086. (<https://doi.org/10.1038/nature08924>)
- Tucker KL, Beard C, Dausmann J, Jackson-Grusby L, Laird PW, Lei H, Li E & Jaenisch R 1996 Germ-line passage is required for establishment of methylation and expression patterns of imprinted but not of nonimprinted genes. *Genes and Development* **10** 1008–1020. (<https://doi.org/10.1101/gad.10.8.10080>)
- Van Laere AS, Nguyen M, Braunschweig M, Nezer C, Collette C, Moreau L, Archibald AL, Haley CS, Buys N, Tally M *et al.* 2003 A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. *Nature* **425** 832–836. (<https://doi.org/10.1038/nature02064>)
- Wagschal A & Feil R 2006 Genomic imprinting in the placenta. *Cytogenetic and Genome Research* **113** 90–98. (<https://doi.org/10.1159/000090819>)
- Walsh C, Glaser A, Fundele R, Ferguson-Smith A, Barton S, Surani MA & Ohlsson R 1994 The non-viability of uniparental mouse conceptuses correlates with the loss of the products of imprinted genes. *Mechanisms of Development* **46** 55–62. ([https://doi.org/10.1016/0925-4773\(94\)90037-X](https://doi.org/10.1016/0925-4773(94)90037-X))
- Wang X, Miller DC, Clark AG & Antczak DF 2012 Random X inactivation in the mule and horse placenta. *Genome Research* **22** 1855–1863. (<https://doi.org/10.1101/gr.138487.112>)
- Wang X, Miller DC, Harman R, Antczak DF & Clark AG 2013 Paternally expressed genes predominate in the placenta. *PNAS* **110** 10705–10710. (<https://doi.org/10.1073/pnas.1308998110>)
- Wang L, Zhang J, Duan J, Gao X, Zhu W, Lu X, Yang L, Zhang J, Li G, Ci W *et al.* 2014 Programming and inheritance of parental DNA methylomes in mammals. *Cell* **157** 979–991. (<https://doi.org/10.1016/j.cell.2014.04.017>)
- Weber M, Davies JJ, Wittig D, Oakeley EJ, Haase M, Lam WL & Schubeler D 2005 Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nature Genetics* **37** 853–862. (<https://doi.org/10.1038/ng1598>)
- Weksberg R, Shuman C & Beckwith JB 2010 Beckwith-Wiedemann syndrome. *European Journal of Human Genetics* **18** 8–14. (<https://doi.org/10.1038/ejhg.2009.106>)
- Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE & Jaenisch R 2007 In-vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* **448** 318–324. (<https://doi.org/10.1038/nature05944>)
- Woodfine K, Huddleston JE & Murrell A 2011 Quantitative analysis of DNA methylation at all human imprinted regions reveals preservation of epigenetic stability in adult somatic tissue. *Epigenetics and Chromatin* **4** 1. (<https://doi.org/10.1186/1756-8935-4-1>)
- Xu Y, Goodyer CG, Deal C & Polychronakos C 1993 Functional polymorphism in the parental imprinting of the human IGF2R gene. *Biochemical and Biophysical Research Communications* **197** 747–754. (<https://doi.org/10.1006/bbrc.1993.2542>)
- Yoder JA, Walsh CP & Bestor TH 1997 Cytosine methylation and the ecology of intragenomic parasites. *Trends in Genetics* **13** 335–340. ([https://doi.org/10.1016/S0168-9525\(97\)01181-5](https://doi.org/10.1016/S0168-9525(97)01181-5))
- Younis S, Schonke M, Massart J, Hjorteborg R, Sundstrom E, Gustafson U, Bjornholm M, Krook A, Frystyk J, Zierath JR *et al.* 2018 The ZBED6-IGF2 axis has a major effect on growth of skeletal muscle and internal organs in placental mammals. *PNAS* **115** 2048–2057. (<https://doi.org/10.1073/pnas.1719278115>)
- Yu M, Hon GC, Szulwach KE, Song CX, Zhang L, Kim A, Li X, Dai Q, Shen Y, Park B *et al.* 2012 Base-resolution analysis of 5-hydroxymethylcytosine in the mammalian genome. *Cell* **149** 1368–1380. (<https://doi.org/10.1016/j.cell.2012.04.027>)
- Yu M, Han D, Hon GC & He C 2018 Tet-assisted bisulfite sequencing (TAB-seq). *Methods in Molecular Biology* **1708** 645–663. (https://doi.org/10.1007/978-1-4939-7481-8_33)
- Zhang S, Kubota C, Yang L, Zhang Y, Page R, O'Neill M, Yang X & Tian XC 2004 Genomic imprinting of H19 in naturally reproduced and cloned cattle. *Biology of Reproduction* **71** 1540–1544. (<https://doi.org/10.1095/biolreprod.104.031807>)
- Zhang T, Termanis A, Ozkan B, Bao XX, Culley J, de Lima Alves F, Rappsilber J, Ramsahoye B & Stancheva I 2016 G9a/GLP complex maintains imprinted DNA methylation in embryonic stem cells. *Cell Reports* **15** 77–85. (<https://doi.org/10.1016/j.celrep.2016.03.007>)

Received 26January2018

First decision 5March2018

Revised manuscript received 5April2018

Accepted 8May2018