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Review Article

A model for the aberrant DNA methylomes in aging cells and cancer cells

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DNA methylation at the fifth position of cytosine is a major epigenetic mark conserved in plants and mammals. Genome-wide DNA methylation patterns are dynamically controlled by integrated activities of establishment, maintenance, and removal. In both plants and mammals, a pattern of global DNA hypomethylation coupled with increased methylation levels at some specific genomic regions arises at specific developmental stages and in certain abnormal cells, such as mammalian aging cells and cancer cells as well as some plant epigenetic mutants. Here we provide an overview of this distinct DNA methylation pattern in mammals and plants, and propose that a methylstat, which is a *cis*-element responsive to both DNA methylation and active demethylation activities and controlling the transcriptional activity of a key DNA methylation regulator, can help to explain the enigmatic DNA methylation patterns in aging cells and cancer cells.

Introduction

DNA methylation at the fifth position of cytosine is a major epigenetic mark present in plants and mammals. Through regulation of transcription activities and genome stability, 5-mC DNA methylation is involved in many important biological processes, such as fruit ripening in plants and aging in humans [1,2]. Both plants and mammals use *S*-adenosyl-methionine as the methyl donor for DNA methylation that is catalyzed by DNA methyltransferases with conserved catalytic domains. The landscape of DNA methylome is dynamically shaped by integrated activities of establishment, maintenance, and removal [1,2]. In both plants and mammals, alterations in genomic DNA methylation patterns can be triggered by intrinsic developmental programs and by certain environmental factors. For instance, aging in mammals is accompanied by a general loss of DNA methylation particularly at repeat sequences and transposable elements on the autosomal chromosomes [3], while a recent study reported a trend of acceleration in Y-chromosomal DNA methylation with increasing age [4]. Besides, locus-specific DNA hypermethylation and the concomitant gene silencing are being increasingly recognized as potential biomarkers for diseases such as cancer [5,6].

DNA methylation in plants and mammals

In mammals, DNMT3 (DNA methyltransferase 3) family members catalyze *de novo* DNA methylation [7]. Mammalian piRNAs, which are small RNAs of 25–30 nucleotides, are thought to pair with nascent transcripts and recruit *de novo* DNA methyltransferases [8]. In another proposed model, DNMT3A is activated or recruited to its target loci via protein interaction with DNMT3L, a noncatalytic DNMT3 paralog that binds unmethylated histone 3 lysine 4 (H3K4) [9,10]. Once established, mammalian DNA methylation is maintained by DNMT1, which restores hemimethylated DNA to a fully methylated status during DNA replication [7].

Plant DNA methylation occurs in all cytosine contexts and can be established through the RNA-directed DNA methylation (RdDM) pathway, in which complementary pairing between 24-nt siRNAs and nascent scaffold RNAs, together with protein–protein interactions, recruits the protein machinery for DNA methylation [1,11]. Once established, DNA methylation in the CG context is maintained by the DNMT1 ortholog MET1 (Methyltransferase 1), while CHG (H represents A, T, or C) methylation is maintained by CMT2 (Chromomethylase 2) and CMT3 through a reinforcing loop that involves KYP4-mediated histone H3K9 methylation [12]. The asymmetric CHH methylation is maintained through persistent *de novo* methylation catalyzed either by DRM2 (Domains Rearranged Methylase 2) through the RdDM pathway or by CMT2 that requires the chromatin remodeling protein DDM1 (Decreased DNA Methylation 1) [13].

Active DNA demethylation is accomplished through a base excision repair pathway. In contrast with mammals, which initiate active DNA demethylation with oxidation and/or deamination of 5-mC, plants directly excise the 5-mC base utilizing 5-mC DNA glycosylases [14–16].

DNA methylome in aging cells and cancer cells

In some mammals including humans, the DNA methylome undergoes global alterations at certain developmental stages. After fertilization, the zygote experiences genome-wide demethylation in the paternal pronuclei, followed by a replication-dependent passive DNA demethylation in the maternal genome [16–18]. At the stage of blastocyst formation, *de novo* DNA methyltransferases reestablish genomic DNA methylation in both parental origins [16,19]. Global demethylation also occurs in primordial germ cells and is followed by extensive chromatin rearrangement, presumably allowing for epigenetic reprogramming toward totipotency [20]. Incomplete and aberrant DNA methylation reprogramming has been postulated as a cause of the high frequency of cloning failure and of developmental abnormalities in cloned animals [14,21,22].

In mammalian cells, aging is characterized by a general loss of DNA methylation and repressive histone marks in heterochromatic regions [23]. In addition to the global hypomethylation, locus-specific DNA hypermethylation also occurs during the process of aging. The age-dependent hypermethylation often exists in actively transcribed genomic regions, including regulatory genes for differentiation, apoptosis, and transcription [23,24]. Aging-associated genome hypomethylation may result from the gradual reduction in the levels of the DNA methyltransferase DNMT1 [25]. Aging-associated DNA methylome is also subject to the impacts of certain environmental factors such as dietary folate and oxidative stress. Aging with dietary deficiency of folate, which is a key source of the methyl group for DNA methylation, causes not only promoter hypomethylation of a couple of proto-oncogenes but also promoter hypermethylation of some tumor suppressor genes including p53 [26]. Oxidative stress due to mitochondria dysfunction occurs during aging and the stress can be enhanced by environmental toxins such as heavy metals and ozone [27,28]. During oxidative stress, accumulation of reactive oxygen species (ROS) can either directly cause DNA lesion or disrupt cellular metabolism and thereby affect TET-mediated DNA demethylation, leading to alterations in epigenomic landscapes [27,29,30]. The locus-specificity and the reproducibility of aging-associated DNA methylation changes indicate that DNA methylation may play a regulatory role in certain biological processes during aging, instead of simply being a stochastic consequence of cellular deterioration. The gradual changes in DNA methylation during aging have been proposed to be an 'epigenetic clock' [31,32]. Indeed, in certain cases such as human blood DNA, changes in DNA methylation levels are clearly associated with the aging process, and are so highly reproducible that they can be used for age prediction [33].

Besides aging, cancer cells also generally display not only global hypomethylation at heterochromatic regions, but also loci-specific DNA hypermethylation [34]. Increased methylation of tumor suppressor genes is an early event in many tumors, suggesting that altered DNA methylation patterns could be one of the first detectable neoplastic changes associated with tumorigenesis [35,36]. ctDNA bearing cancer-specific methylation patterns have been investigated as feasible biomarkers in cancers [37]. In both aging cells and cancer cells, DNA hypermethylation commonly occurs at loci that are targeted by Polycomb-group (PcG) proteins [38–40]. Because PcG-mediated transcriptional silencing can be antagonized by active histone modifications such as H3K4me3, additional DNA methylation at PcG-targeted loci seems to serve a double-locking role to secure transcriptional silencing. In the *min⁻* mouse model of cancer that displays tumor at ages of 3–5 months, it was shown that the appearance of cancer can be prevented by consecutive chemical inhibition of DNA methylation starting from birth, but not by treatments that started from 3 months of age [41]. These observations suggest that DNA methylation, possibly through developmental accumulation, contributes to tumorigenesis. In addition, DNA methylation abnormalities at gene promoters in elderly cells phenocopy cancer cells; particularly,

aging-associated DNA hypermethylation can occur at the promoter regions of some tumor suppressor genes [24,42–44]. Thus cancer formation appears to be tightly correlated with the changes in DNA methylation during the aging process.

Dynamic regulation of DNA methylome in plants

Similar to mammals, plants also go through massive alterations in their DNA methylome at specific developmental stages. In *Arabidopsis thaliana*, both the male and female gamete companion cells undergo DME (DEMETER)-mediated global demethylation, resulting in genome-wide DNA hypomethylation with reinforced CHH methylation at transposons in the endosperm [1]. During ripening, tomato fruits display DML2 (DEMETER Like 2)-mediated progressive DNA demethylation at hundreds of gene loci, many of which are known to regulate fruit ripening [45,46]. Chemical inhibition of DNA methylation causes premature ripening of tomato fruits, whereas a loss of DML2 function results in non-ripening fruits [45–47]. Thus the changes in DNA methylome during fruit ripening serve a crucial function.

Genetic disruption of key methylation regulators leads to abnormal DNA methylome in plants. Notably, dysfunction of *Arabidopsis* MET1 or one of the RdDM core factors results in not only global hypomethylation but also hypermethylation at specific loci across the genome [48,49]. The locus-specific increase in DNA methylation is attributed to the activity of the remaining DNA methyltransferases combined with the reduced gene expression of the DNA demethylase ROS1 (Repressor of Silencing 1), whose promoter region harbors a methylation monitoring sequence (MEMS) [50]. DNA methylation at MEMS positively regulates ROS1 gene transcription through a transcription activation complex that contains the SUVH (Suppressor of Variegation 3–9 Homolog) DNA methylation readers [51,52]. Similar to the observations that changes in DNA methylation levels are highly correlated with age changes [33], DNA methylation level of the MEMS is quantitatively associated with the extent of binding of the SUVHs [51]. ROS1 antagonizes MET1 and RdDM activities at several thousands of genomic regions [49], thereby providing a coordination between DNA methylation and demethylation. Similar to the observations that DNA hypermethylation in aging cells and cancer cells preferentially occurs at regions with H3K27me3, ROS1-targeted regions are enriched with this repressive histone modification [49]. Because the methylation level of MEMS is co-regulated by MET1, the RdDM pathway, and ROS1, this unique element is considered as a methylstat that maintains homeostasis of ROS1-dependent DNA methylation across the genome of *Arabidopsis thaliana* [1,50]. Methylation-sensitive regulation of demethylase gene expression has also been observed in rice, maize, and *A. lyrata* [53–55]. Thus, the methylstat may be a conserved mechanism for regulating DNA methylation dynamics in plants.

A model for the altered DNA methylome in aging cells and cancer cells

Methylstats may also exist in mammals and contribute to shaping the DNA methylome. In such a scenario, the increased DNA methylation at specific loci in aging cells and cancer cells could result from DNA hypomethylation of a methylstat, which controls the expression of a key regulator of DNA methylation or demethylation reactions. In the methylstat model (Figure 1), DNA hypomethylation in aging cells and cancer cells leads to transcriptional activation of the methylstat-controlled DNA establishment or maintenance enzyme or its regulator; alternatively, DNA hypomethylation in aging cells and cancer cells may lead to transcriptional repression of the methylstat-controlled DNA demethylation enzyme or its regulator.

Unlike the autosomal chromosomes that show a general hypomethylation pattern during aging, the Y chromosome shows age-dependent increases in CG methylation [4]. It is possible that the Y chromosome is intensively targeted either by methylstat-mediated demethylation that is anti-correlated with aging, or by methylstat-mediated methylation that is positively correlated with aging. While the methylstat does not necessarily locate in the promoter region of the methylation/demethylation factor that it controls, its methylation levels should be quantitatively correlated with the transcription levels of the methylation/demethyaltion factor. This expectation may help with the searching for the methylstat, whose methylation levels decline in an age-dependent manner, as well as for the methylation/demethylation factor that it controls. In addition, abnormal gene expression levels of the methylation/demethylation factor would be common in the aging-related diseases that are associated with aberrant DNA methylation patterns, as well as in the individuals that show abnormal
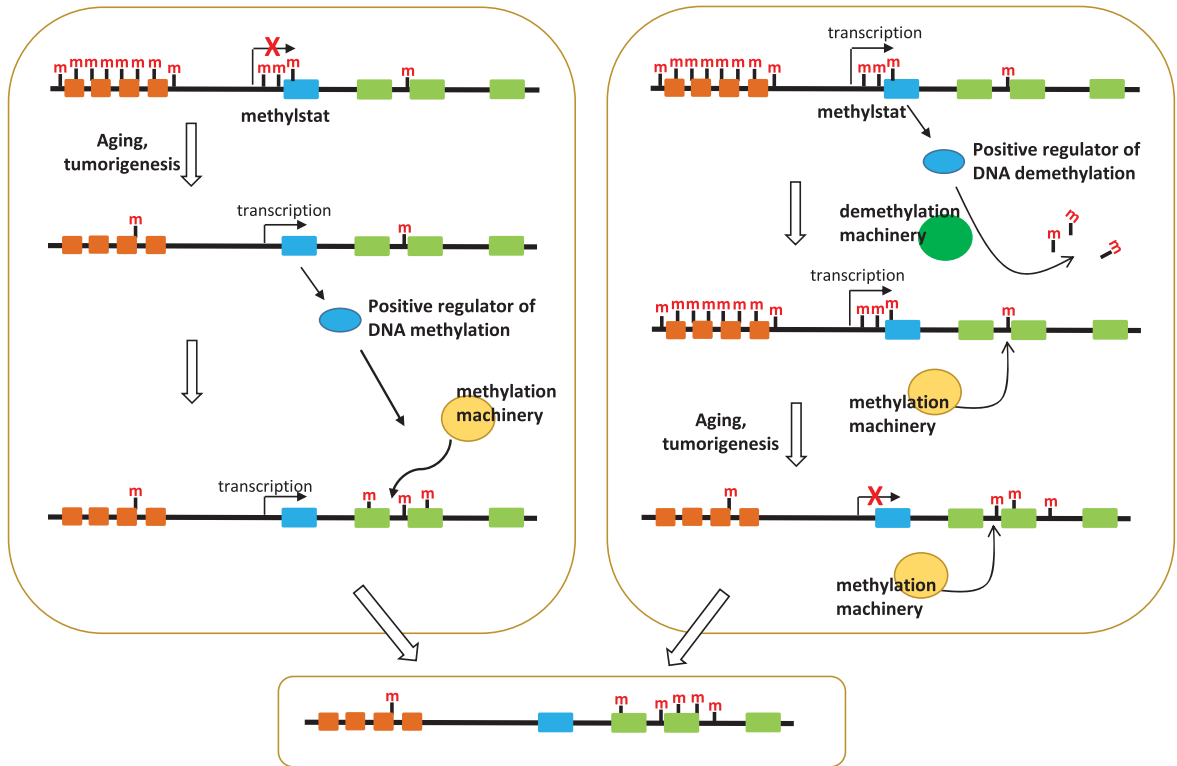
Figure 1. A model for the altered DNA methylome in aging cells and cancer cells.

Regulation of the DNA methylome in mammalian cells may involve a methylstat, which responds to DNA methylation levels and regulates gene expression in *cis*. The methylstat may contribute to the formation of DNA methylation patterns in aging cells and cancer cells in two possible ways. Left upper panel, aging or tumorigenesis causes DNA hypomethylation and consequently transcription activation of the methylstat. The methylstat controls a positive regulator of DNA methylation, the expression of which results in DNA hypermethylation. Right upper panel, DNA methylation in *cis* is required for transcriptional activation of the methylstat. The methylstat controls a positive regulator of DNA demethylation, whose expression results in the active removal of DNA methylation. Aging or tumorigenesis causes DNA hypomethylation and consequently transcriptional repression of the methylstat-controlled demethylation regulator. As a result, DNA methylation accumulates in regions that are normally pruned by the demethylation machinery. Methylation at the fifth position of cytosine is denoted by a red letter m.

rates of aging. DNA hypermethylation in both aging cells and cancer cells preferentially occurs at PcG-targeted loci [38–40]. It would be interesting to determine whether DNA methylation at these loci is pruned by active demethylation, and if so, whether the corresponding demethylation factors themselves are subject to methylation-dependent transcription regulation. An EZH2 (Enhancer of Zeste Homolog 2)-containing PcG complex was reported to recruit the methyltransferases for DNA methylation in cancer cells [56]. Thus it would be also interesting to examine whether DNA methylation negatively regulates the PcG-recruited DNA methylation machinery including the methyltransferases.

Summary
- Importance of the field: DNA methylation confers epigenetic regulation on many important biological processes. The genome-wide hypomethylation coupled with locus-specific hypermethylation is a hallmark of aging and tumorigenesis. Understanding the dynamic regulation of DNA methylome may help us understand and eventually manage aging and tumorigenesis.

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- Summary of current thinking: Because the regulation of DNA methylation shares conserved features in plants and mammals, we propose that, similar to plants, mammalian cells also dynamically control genome-wide DNA methylation through a methylstat-dependent mechanism, and that this dynamic regulation contributes to the seemingly contradictory changes in DNA methylome in aging cells and cancer cells. This methylstat-based model implies that the locus-specific methylation increases observed in aging cells are not due to random epigenetic drift. Importantly, targeted intervention of the DNA methylation of the methylstat may slow down or prevent the DNA methylation increases in genes important for senescence and other old-age disorders and thus avert aging.

- Comments on future directions: While the methylstat model is enticing, the putative methylstats in mammalian cells have yet to be identified. Future investigations on this methylstat model may be aided by clues from the specific features of euchromatic regions where abnormal DNA methylation occurs.

Abbreviations

MEMS, methylation monitoring sequence; ROS, reactive oxygen species; ROS1, Repressor of Silencing 1; RdDM, RNA-directed DNA methylation; SUVH, Suppressor of Variegation 3–9 Homologue.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

1 Zhang, H., Lang, Z. and Zhu, J.K. (2018) Dynamics and function of DNA methylation in plants. *Nat. Rev. Mol. Cell Biol.* **19**, 489–506 https://doi.org/10.1038/s41580-018-0016-z

2 Luo, C., Hajkova, P. and Ecker, J.R. (2018) Dynamic DNA methylation: in the right place at the right time. *Science* **361**, 1336–1340 https://doi.org/10.1126/science.aat6806

3 Wilson, A.S., Power, B.E. and Molloy, P.L. (2007) DNA hypomethylation and human diseases. *Biochim. Biophys. Acta, Rev Cancer* **1775**, 138–162 https://doi.org/10.1016/j.bbcan.2006.08.007

4 Lund, J.B., Li, S., Christensen, K., Mengel-From, J., Soerensen, M., Marioni, R.E. et al. (2019) Age-dependent DNA methylation patterns on the Y chromosome in elderly males. *Aging Cell* **21**, e12907 https://doi.org/10.1111/acel.12907

5 Morgan, A.E., Davies, T.J. and Mc Auley, M.T. (2018) The role of DNA methylation in ageing and cancer. *Proc. Nutr. Soc.* **77**, 412–422 https://doi.org/10.1017/S0029665118000150

6 Xie, W., Baylin, S.B. and Easwaran, H. (2019) DNA methylation in senescence, aging and cancer. *Oncoscience* **6**, 291–293 https://doi.org/10.18632/oncoscience.476

7 Goll, M.G. and Bestor, T.H. (2005) Eukaryotic cytosine methyltransferases. *Annu. Rev. Biochem.* **74**, 481–514 https://doi.org/10.1146/annurev.biochem.74.010904.153721

8 Watanabe, T., Tomizawa, S., Mitsuya, K., Totoki, Y., Yamamoto, Y., Kuramochi-Miyagawa, S. et al. (2011) Role for piRNAs and noncoding RNA in *de novo* DNA methylation of the imprinted mouse Rasgrf1 locus. *Science* **332**, 848–852 https://doi.org/10.1126/science.1203919

9 Ooi, S.K., Qiu, C., Bernstein, E., Li, K., Jia, D., Yang, Z. et al. (2007) DNMT3 l connects unmethylated lysine 4 of histone H3 to *de novo* methylation of DNA. *Nature* **448**, 714–717 https://doi.org/10.1038/nature05987

10 Cheng, X. and Blumenthal, R.M. (2008) Mammalian DNA methyltransferases: a structural perspective. *Structure* **16**, 341–350 https://doi.org/10.1016/j.str.2008.01.004

11 Matzke, M.A. and Mosher, R.A. (2014) RNA- directed DNA methylation: an epigenetic pathway of increasing complexity. *Nat. Rev. Genet.* **15**, 394–408 https://doi.org/10.1038/nrg3683

12 Du, J.M., Johnson, L.M., Jacobsen, S.E. and Patel, D.J. (2015) DNA methylation pathways and their crosstalk with histone methylation. *Nat. Rev. Mol. Cell Biol.* **16**, 519–532 https://doi.org/10.1038/nrm4043

13 Zemach, A., Kim, M.Y., Hsieh, P.H., Coleman-Derr, D., Eshed-Williams, L., Thao, K. et al. (2013) The *Arabidopsis* nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. *Cell* **153**, 193–205 https://doi.org/10.1016/j.cell.2013.02.033

14 Zhu, J.K. (2009) Active DNA demethylation mediated by DNA glycosylases. *Annu. Rev. Genet.* **43**, 143–166 https://doi.org/10.1146/annurev-genet-102108-134205

15 Wu, X. and Zhang, Y. (2017) TET-mediated active DNA demethylation: mechanism, function and beyond. *Nat. Rev. Genet.* **18**, 517–534 https://doi.org/10.1038/nrg.2017.33

16 Zhang, H. and Zhu, J.K. (2012) Active DNA demethylation in plants and animals. *Cold Spring Harb. Symp. Quant. Biol.* **77**, 161–173 https://doi.org/10.1101/sqb.2012.77.014936

17 Oswald, J., Engemann, S., Lane, N., Mayer, W., Olek, A., Fundele, R. et al. (2000) Active demethylation of the paternal genome in the mouse zygote. *Curr. Biol.* **10**, 475–478 https://doi.org/10.1016/S0960-9822(00)00448-6

18 Mayer, W., Niveleau, A., Walter, J., Fundele, R. and Haaf, T. (2000) Demethylation of the zygotic paternal genome. *Nature* **403**, 501–502 https://doi.org/10.1038/35000656

19 Wu, S.C. and Zhang, Y. (2010) Active DNA demethylation: many roads lead to Rome. *Nat. Rev. Mol. Cell Biol.* **11**, 607–620 https://doi.org/10.1038/nrm2950

20 Hajkova, P., Ancelin, K., Waldmann, T., Lacoste, N., Lange, U.C., Cesari, F. et al. (2008) Chromatin dynamics during epigenetic reprogramming in the mouse germ line. *Nature* **452**, 877–881 https://doi.org/10.1038/nature06714

21 Morgan, H.D., Santos, F., Green, K., Dean, W. and Reik, W. (2005) Epigenetic reprogramming in mammals. *Hum. Mol. Genet.* **14**, R47–R58 https://doi.org/10.1093/hmg/ddi114

22 Yang, X., Smith, S.L., Tian, X.C., Lewin, H.A., Renard, J.P. and Wakayama, T. (2007) Nuclear reprogramming of cloned embryos and its implications for therapeutic cloning. *Nat. Genet.* **39**, 295–302 https://doi.org/10.1038/ng1973

23 Booth, L.N. and Brunet, A. (2016) The aging epigenome. *Mol. Cell* **62**, 728–744 https://doi.org/10.1016/j.molcel.2016.05.013

24 Maegawa, S., Hinkal, G., Kim, H.S., Shen, L., Zhang, L., Zhang, J. et al. (2010) Widespread and tissue specific age-related DNA methylation changes in mice. *Genome Res.* **20**, 332–340 https://doi.org/10.1101/gr.096826.109

25 Jung, M. and Pfeifer, G.P. (2015) Aging and DNA methylation. *BMC Biol.* **13**, 7 https://doi.org/10.1186/s12915-015-0118-4

26 Najar, R.A., Wani, N.A., Bhat, J.A., Dar, N.J., Rahat, B., Gupta, A.P. et al. (2018) Modulation of dietary folate with age confers selective hepatocellular epigenetic imprints through DNA methylation. *J. Nutr. Biochem.* **53**, 121–132 https://doi.org/10.1016/j.jnutbio.2017.10.007

27 Chia, N., Wang, L., Lu, X., Senut, M.C., Brenner, C. and Ruden, D.M. (2011) Hypothesis: environmental regulation of 5-hydroxymethylcytosine by oxidative stress. *Epigenetics* **6**, 853–856 https://doi.org/10.4161/epi.6.7.16461

28 Kudryavtseva, A.V., Krasnov, G.S., Dmitriev, A.A., Alekseev, B.Y., Kardymon, O.L., Sadritdinova, A.F. et al. (2016) Mitochondrial dysfunction and oxidative stress in aging and cancer. *Oncotarget* **7**, 44879–44905 https://doi.org/10.18632/oncotarget.9821

29 Lewandowska, J. and Bartoszek, A. (2011) DNA methylation in cancer development, diagnosis and therapy–multiple opportunities for genotoxic agents to act as methylome disruptors or remediators. *Mutagenesis* **26**, 475–487 https://doi.org/10.1093/mutage/ger019

30 Guillaumet-Adkins, A., Yañez, Y., Peris-Diaz, M.D., Calabria, I., Palanca-Ballester, C. and Sandoval, J. (2017) Epigenetics and oxidative stress in aging. *Oxid. Med. Cell Longev.* **2017**, 9175806 https://doi.org/10.1155/2017/9175806

31 Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S. et al. (2013) Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol. Cell* **49**, 359–367 https://doi.org/10.1016/j.molcel.2012.10.016

32 Field, A.E., Robertson, N.A., Wang, T., Havas, A., Ideker, T. and Adams, P.D. (2018) DNA methylation clocks in aging: categories, causes, and consequences. *Mol. Cell* **71**, 882–895 https://doi.org/10.1016/j.molcel.2018.08.008

33 Weidner, C.I., Lin, Q., Koch, C.M., Eisele, L., Beier, F., Ziegler, P. et al. (2014) Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biol.* **15**, R24 https://doi.org/10.1186/gb-2014-15-2-r24

34 Kulis, M., Merkel, A., Heath, S., Queirós, A.C., Schuyler, R.P., Castellano, G. et al. (2015) Whole-genome fingerprint of the DNA methylome during human B cell differentiation. *Nat. Genet.* **47**, 746–756 https://doi.org/10.1038/ng.3291

35 Baylin, S.B. and Jones, P.A. (2016) Epigenetic determinants of cancer. *Cold Spring Harb. Perspect. Biol.* **8**, a019505 https://doi.org/10.1101/cshperspect.a019505

36 Hao, X., Luo, H., Krawczyk, M., Wei, W., Wang, W., Wang, J. et al. (2017) DNA methylation markers for diagnosis and prognosis of common cancers. *Proc. Natl Acad. Sci. U.S.A.* **114**, 7414–7419 https://doi.org/10.1073/pnas.1703577114

37 Xu, R.H., Wei, W., Krawczyk, M., Wang, W., Luo, H., Flagg, K. et al. (2017) Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. *Nat. Mater.* **11**, 1155–1161 https://doi.org/10.1038/nmat4997

38 Schlesinger, Y., Straussman, R., Keshet, I., Farkash, S., Hecht, M., Zimmerman, J. et al. (2007) Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for *de novo* methylation in cancer. *Nat. Genet.* **39**, 232–236 https://doi.org/10.1038/ng1950

39 Teschendorff, A.E., Menon, U., Gentry-Maharaj, A., Ramus, S.J., Weisenberger, D.J., Shen, H. et al. (2010) Age-dependent DNA methylation of genes that are suppressed in stem cells is a hallmark of cancer. *Genome Res.* **20**, 440–446 https://doi.org/10.1101/gr.103606.109

40 Beerman, I., Bock, C., Garrison, B.S., Smith, Z.D., Gu, H., Meissner, A. et al. (2013) Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. *Cell Stem Cell* **12**, 413–425 https://doi.org/10.1016/j.stem.2013.01.017

41 Laird, P.W., Jackson-Grusby, L., Fazeli, A., Dickinson, S.L., Jung, W.E., Li, E. et al. (1995) Suppression of intestinal neoplasia by DNA hypomethylation. *Cell* **81**, 197–205 https://doi.org/10.1016/0092-8674(95)90329-1

42 Gautrey, H.E., van Otterdijk, S.D., Cordell, H.J.; Newcastle 85+ Study Core Team, Mathers, J.C. and Strathdee, G. (2014) DNA methylation abnormalities at gene promoters are extensive and variable in the elderly and phenocopy cancer cells. *FASEB J.* **28**, 3261–3272 https://doi.org/10.1096/fj.13-246173

43 Belshaw, N.J., Pal, N., Tapp, H.S., Dainty, J.R., Lewis, M.P., Williams, M.R. et al. (2010) Patterns of DNA methylation in individual colonic crypts reveal aging and cancer-related field defects in the morphologically normal mucosa. *Carcinogenesis* **31**, 1158–1163 https://doi.org/10.1093/carcin/bgq077

44 Tamura, G. (2004) Promoter methylation status of tumor suppressor and tumor-related genes in neoplastic and non-neoplastic gastric epithelia. *Histol. Histopathol.* **19**, 221–228 https://doi.org/10.14670/HH-19.221

45 Liu, R., How-Kit, A., Stammitti, L., Teyssier, E., Rolin, D., Mortain-Bertrand, A. et al. (2015) A DEMETER- like DNA demethylase governs tomato fruit ripening. *Proc. Natl Acad. Sci. U.S.A.* **112**, 10804–10809 https://doi.org/10.1073/pnas.1503362112

46 Lang, Z., Wang, Y., Tang, K., Tang, D., Datsenka, T., Cheng, J. et al. (2017) Critical roles of DNA demethylation in the activation of ripening- induced genes and inhibition of ripening- repressed genes in tomato fruit. *Proc. Natl Acad. Sci. U.S.A.* **114**, E4511–E4519 https://doi.org/10.1073/pnas.1705233114

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47 Zhong, S., Fei, Z., Chen, Y.R., Zheng, Y., Huang, M., Vrebalov, J. et al. (2013) Single- base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. *Nat. Biotechnol.* **31**, 154–159 https://doi.org/10.1038/nbt.2462

48 Mathieu, O., Reinders, J., Čaikovski, M., Smathajitt, C. and Paszkowski, J. (2007) Transgenerational stability of the *Arabidopsis* epigenome is coordinated by CG methylation. *Cell* **130**, 851–862 https://doi.org/10.1016/j.cell.2007.07.007

49 Tang, K., Lang, Z., Zhang, H. and Zhu, J.K. (2016) The DNA demethylase ROS1 targets genomic regions with distinct chromatin modifications. *Nat. Plants* **2**, 16169 https://doi.org/10.1038/nplants.2016.169

50 Lei, M.G., Zhang, H., Julian, R., Tang, K., Xie, S. and Zhu, J.K. (2015) Regulatory link between DNA methylation and active demethylation in *Arabidopsis*. *Proc. Natl Acad. Sci. U.S.A.* **112**, 3553–3557 https://doi.org/10.1073/pnas.1502279112

51 Xiao, X., Zhang, J., Li, T., Fu, X., Satheesh, V., Niu, Q., et al. (2019) A group of SUVH methyl-DNA binding proteins regulate expression of the DNA demethylase ROS1 in *Arabidopsis*. *J. Integr. Plant Biol.* **61**, 110–119 https://doi.org/10.1111/jipb.12768

52 Zhao, Q.Q., Lin, R.N., Li, L., Chen, S. and He, X.J. (2019) A methylated-DNA-binding complex required for plant development mediates transcriptional activation of promoter methylated genes. *J. Integr. Plant Biol.* **61**, 120–139 https://doi.org/10.1111/jipb.12767

53 Erhard, K.F., Talbot, J.E., Deans, N.C., McClish, A.E. and Hollick, J.B. (2015) Nascent transcription affected by RNA polymerase IV in *Zea mays*. *Genetics* **199**, 1107–1125 https://doi.org/10.1534/genetics.115.174714

54 Hu, L., Li, N., Xu, C., Zhong, S., Lin, X., Yang, J. et al. (2014) Mutation of a major CG methylase in rice causes genome- wide hypomethylation, dysregulated genome expression, and seedling lethality. *Proc. Natl Acad. Sci. U.S.A.* **111**, 10642–10647 https://doi.org/10.1073/pnas.1410761111

55 Williams, B.P., Pignatta, D., Henikoff, S. and Gehring, M. (2015) Methylation- sensitive expression of a DNA demethylase gene serves as an epigenetic rheostat. *PLoS Genet.* **11**, e1005142 https://doi.org/10.1371/journal.pgen.1005142

56 Viré, E., Brenner, C., Deplus, R., Blanchon, L., Fraga, M., Didelot, C. et al. (2006) The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* **439**, 871–874 https://doi.org/10.1038/nature04431