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A sweet TET-à-tête-synergy of TET proteins and O-GlcNAc transferase in transcription

Daniel Mariappa, Shalini Pathak and Daan MF van Aalten*

MRC Protein Phosphorylation Unit, College of Life Sciences, University of Dundee, Dundee, UK.
*Correspondence to: dmfvanaalten@dundee.ac.uk

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5-hydroxy methyl cytosine (5hmC) is a modification identified in vertebrates several decades ago. More recently, a possible role of 5hmC as an epigenetic modifier and/or transcriptional regulator has started to emerge, with altered levels in early embryonic development, embryonic stem (ES) cell differentiation and tumours (Tahiliani *et al*, 2009; Yang *et al*, 2012). The balance between 5hmC and 5-methyl cytosine (5mC) at gene promoters and CpG islands in the genome appears to be linked to pluripotency and lineage commitment of a cell (Ito *et al*, 2010). However, proteins with 5hmC binding capability have not yet been identified, and it has been proposed that 5hmC may only be a reaction intermediate in the process of demethylation (He *et al*, 2011; Ito *et al*, 2011). Over the last few years, ten-eleven translocation (Tet) family proteins have been shown to be responsible for the conversion of 5mC to 5hmC (Iyer *et al*, 2009; Loenarz and Schofield, 2009; Tahiliani *et al*, 2009). However, how Tet family proteins and 5hmC are linked to transcriptional regulation is currently not clear.

In the quest for finding the functional partners of Tet family proteins, two groups have independently identified N-acetylglucosamine (O-GlcNAc) transferase (OGT) as a strong interactor of TET2 and TET3 (Chen *et al*, 2013; Deplus *et al*, 2013). The role of OGT in catalysing the addition of O-GlcNAc to proteins has been well characterized (Hart *et al*, 2007). Interplay between O-GlcNAcylation and phosphorylation has been proposed to play a role in several metabolic disorders, such as cancer, neurodegeneration and diabetes. Mammalian post-implantation embryonic development is dependent on OGT (Shafi *et al*, 2000). Interestingly, although OGT targets >1000 intracellular proteins for O-GlcNAcylation, it is currently not understood whether OGT recognizes all of its targets via its N-terminal TPR repeats, or if other factors are required for substrate recruitment. Strikingly, both studies do not detect O-GlcNAcylation and/or regulation of TET2/3 activity by OGT. Instead, TET2/3 appear to act as scaffolding proteins, recruiting OGT to chromatin, leading to O-GlcNAcylation of histone 2B (H2B; Chen *et al*, 2013) and host cell factor 1 (HCF1; Deplus *et al*, 2013). This recruitment of OGT to DNA is independent of TET2 catalytic activity, although the catalytic domain of TET2 is required for interaction with OGT. Association of H2B Ser112 GlcNAc with transcriptionally active sites has been previously established (Fujiki *et al*, 2011). Chen *et al* (2013) demonstrate by ChIP sequencing that there is significant overlap of OGT, TET2 and H2B Ser112 GlcNAc target genes. Moreover, transcriptional control of genes that are common targets is via TET2 recruitment of OGT and, consequently, O-GlcNAcylation of H2B. However, ~20% of the target genes are uniquely occupied by H2B Ser112 GlcNAc, and the significance of this is unknown.

Interestingly, ChIP sequencing data from Deplus *et al* (2013), carried out in HEK293T cells instead of ES cells, reveal a lower percentage (42 versus 68% in HEK293T and ES cells, respectively) of common target genes of TET2/3 and OGT in HEK293 cells, suggesting this mechanism of transcriptional control may be more prevalent in ES cells. The transcriptional activation mark, histone3 K4 trimethylation (H3K4me3), is found in almost all of the common TET2/3–OGT targets, and the trimethylation at these sites is TET2- and O-GlcNAcylation-dependent. Deplus *et al* (2013) further identified the direct association and influence of TET2/3–OGT on HCF1, a key component

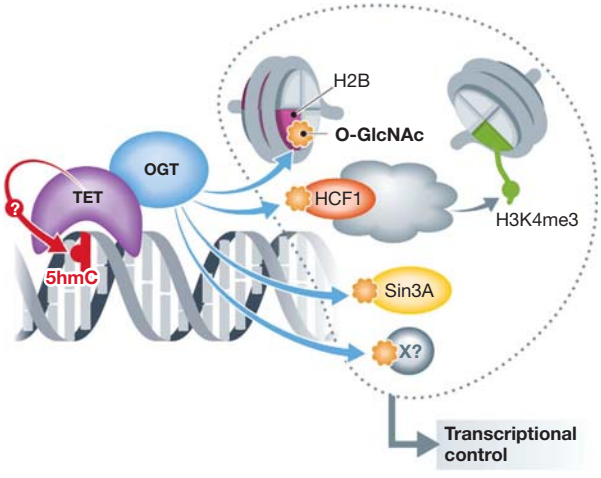

Figure 1 Two recent papers (Chen *et al*, 2013; Deplus *et al*, 2013) demonstrate that TET proteins, independent of their catalytic activity on 5mc, can act as scaffolding proteins recruiting OGT onto chromatin, leading to O-GlcNAc modification of factors associated with transcriptional control.

of the H3K4 methyl transferase SET1/COMPASS complex. HCF1 is found to be O-GlcNAcylated in a TET2/3-dependent manner. O-GlcNAcylation of HCF1 is also associated with formation of the SET1/COMPASS complex, specifically the enrichment of SETD1A (histone methyl transferase) at chromatin. These observations associate O-GlcNAcylation of HCF1 with increased H3K4me3 levels in a TET2/3-dependent manner, leading to transcriptional upregulation of common TET2/3–OGT targets.

These two studies present a very interesting regulation of recruitment of OGT to chromatin. TET proteins appear to be important OGT cofactors and scaffolding proteins, assembling OGT-containing complexes on chromatin (Figure 1). However, the mechanistic detail of how O-GlcNAc on chromatin regulates transcription is still elusive. Furthermore, both studies identify Sin3A, a transcription factor known to be regulated by O-GlcNAc, as an interactor of OGT and TET proteins, but the role of this interaction remains to be explored. A further understanding of the involvement of Sin3A with TET/OGT complexes might give insight into differential transcriptional regulation resulting from epigenetic changes. Finally, among target genes reported for the TET/OGT complexes, it is interesting to note that DNA bound by TET2/3 and OGT lacks either 5hmC or 5mC, and that TET2/3 catalytic activity is not required for this interaction. Given that TET2 binds to cytosine with a lower affinity as compared to 5mC, it would need to be actively recruited to chromatin in a 5mC-independent manner. It is not yet clear how the TET proteins interact with DNA in a non-catalytic manner and achieve recruitment of OGT through their catalytic domain.

Note added in proof: During typesetting of this manuscript another study has appeared that also establishes the interaction of OGT and TET1 to regulate transcriptional control (Vella *et al*, 2013, *Molecular Cell*, in press). In contrast to the two papers reviewed here, Vella *et al* demonstrate O-GlcNAcylation of TET1 and TET2 with a GlcNAc-binding lectin. It is also shown that O-GlcNAcylation negatively affects TET1 activity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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