The N-terminal domain of TET1 promotes the formation of dense chromatin regions refractory to transcription

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Abstract
TET (ten-eleven translocation) enzymes initiate active cytosine demethylation via the oxidation of 5-methylcytosine. TET1 is composed of a C-terminal domain, which bears the catalytic activity of the enzyme, and a N-terminal region that is less well characterized except for the CXXC domain responsible for the targeting to CpG islands. While cytosine demethylation induced by TET1 promotes transcription, this protein also interacts with chromatin-regulating factors that rather silence this process, the coordination between these two opposite functions of TET1 being unclear. In the present work, we uncover a new function of the N-terminal part of the TET1 protein in the regulation of the chromatin architecture. This domain of the protein promotes the establishment of a compact chromatin architecture displaying reduced exchange rate of core histones and partial dissociation of the histone linker. This chromatin reorganization process, which does not rely on the CXXC domain, is associated with a global shutdown of transcription and an increase in heterochromatin-associated histone epigenetic marks. Based on these findings, we propose that the dense chromatin organization generated by the N-terminal domain of TET1 could contribute to restraining the transcription enhancement induced by the DNA demethylation activity of this enzyme.

Keywords Ten-eleven translocation enzyme · DNA methylation · DNA hydroxymethylation · Chromatin · Transcription · Fluorescence microscopy

Introduction
In the nucleus of eukaryotic cells, DNA is wrapped around histones to form the chromatin fiber which, itself, displays a hierarchy of folding steps. The complex organization of chromatin is essential to ensure that the entire genetic material of the cell can be contained within the nucleus, where it occupies 20 to 50% of the nuclear volume (Ou et al. 2017).

Despite this high level of packaging, the chromatin structure is not fixed and constantly evolves in relation to multiple cellular processes which require access to DNA such as transcription, replication, or repair (Fierz and Poirier 2019). Key actors in the regulation of chromatin folding are epigenetic marks such as post-transcriptional modifications found on histones and base modifications found in DNA. These dynamic marks attract or repel effectors of DNA transactions and control chromatin organization either directly, by modulating for example nucleosome stability, or indirectly, by recruiting remodelers or chromatin scaffolding proteins (Talbert and Henikoff 2017).

While epigenetic marks found on histones are extremely diverse, only a handful of modifications are found at the DNA level, the main one being 5-methylcytosine (5-mC) (Rausch et al. 2019). This mark, mainly found at CpG dinucleotides, has a repressive impact on transcription, in particular through the recruitment of repressor complexes and by regulating the binding of transcription factors to DNA at regulatory regions (Watt and Molloy 1988; Iguchi-Ariga and Schaffner 1989). 5-mC is written by several DNA
methyltransferase enzymes and its removal arises either by passive dilution due to DNA replication or by active DNA demethylation (Bhutani et al. 2011; Mulholland et al. 2020). The main active demethylation pathway is based on the successive oxidation of 5-mC into 5-hmC (5-hydroxymethylcytosine), 5-fC (5-formylcytosine), and finally 5-caC (5-carboxylcytosine), which are carried out by members of the TET (ten-eleven translocation) enzyme family (Ito et al. 2011). While the 5-fC and 5-caC forms are rapidly excised by the DNA repair machinery to restore an unmethylated cytosine (Maiti and Drohat 2011), 5-hmC has a relatively long lifetime and could thus constitute a fully fledged epigenetic mark (Münzel et al., 2011).

The TET family is composed of three members showing distinct expression patterns hinting at different cellular functions (Lorsbach et al., 2003). All members share a catalytic domain responsible for 5-mC oxidation that is localized at the C-terminus of the proteins (Tahiliani et al. 2009; Hu et al. 2013). Furthermore, TET1 and TET3, but not TET2, bear a CXXC domain that binds to unmethylated CpGs and is thought to be responsible for the localization of these proteins at CpG-rich genomic sites like CpG islands, protecting them from aberrant methylation (Tahiliani et al., 2009). By controlling the balance of 5-mC/5-hmC levels within the genome, the TET enzymes are key regulators of transcriptional processes associated with cell differentiation, tumorigenesis, and neurodegeneration (Rasmussen and Helin 2016; Jin et al. 2016). However, the role of TET enzymes is not limited to their catalytic activity. Indeed, they have been shown to serve as a binding platform for effectors that regulate transcription such as the histone methyltransferase complex PRC2 (Wu et al. 2011), the histone acetyltransferase hMOF (Zhong et al., 2017), or the O-linked N-acetylglucosamine transferase OGT (Shi et al. 2013; Bauer et al. 2015). Importantly, the existence of a shorter isoform of TET1 (TET1short) has been recently reported. While the long isoform of TET1 (TET1long) is expressed mainly in undifferentiated cells, TET1short levels seem to increase upon differentiation as well as during tumorigenesis (Zhang et al. 2016; Good et al. 2017). Despite lacking the N-terminal domain of TET1long, including the CXXC domain, TET1short is still able to demethylate CpG islands (Zhang et al. 2016). Nevertheless, TET1short cannot fully complement the longer isoform, suggesting that the N-terminal domain of the full-length protein fulfills specific cellular functions that remain to be clarified.

In the present work, we demonstrate that the N-terminal region of the TET1 protein, which is lacking in TET1short, is able to induce a global reorganization of chromatin in three different human cell lines (U2OS, HeLa, and MCF-7). This restructuring process is seen at the larger chromatin folding scales but also at the molecular level since histone exchange rates are impacted by the TET1 N-terminal domain. We also show that the chromatin reorganization is associated with a global shutdown of transcription. Altogether, our findings uncover a previously unidentified function of TET1 in the regulation of the chromatin structure with potential consequences regarding its roles in the regulation of transcription.

Methods

Plasmids

Plasmids coding for the full-length TET1, TET2, and TET3 tagged with GFP on their N-terminal end are gifts from Heinrich Leonhardt and Cristina Cardoso (Frauer et al. 2011). The plasmids coding for the C-terminal and N-terminal fragments of TET1, both tagged with GFP on their C-terminal end, as well as the TET1 mutant lacking the CXXC domain, tagged with GFP on its N-terminal end, were provided by Wei Xie (Zhang et al. 2016). The catalytically inactive mutant of full-length TET1 fused to GFP on its N-terminal end was obtained by site-directed mutagenesis on GFP-tagged TET1 with primers 5′-gttgtgcatgttgtgaatg-3′ and 5′-ggtgtgtgcatgtgtgaatg-3′ using Phusion polymerase (Thermo Scientific). Haico Van Attikum (Rother et al. 2020) provided the plasmid coding for GFP fused to a NLS (nuclear localisation sequence), which promotes GFP accumulation in the nucleus. The H2B-mCherry was a gift from Jan Ellenberg (Euroscarf P30632 (Neumann et al. 2010)). mEGFP was cut from H1-mEGFP (gift from Gyula Timin-szky) and replaced with mCherry using the restriction sites BshTI and Bsp1407I (Thermo Scientific), to generate the H1-mCherry construct.

Cell culture

The experiments were performed on three different human cell lines: osteosarcoma U2OS cells, cervical carcinoma HeLa cells, and breast cancer MCF-7 cells. All cell lines were cultured in DMEM (4.5 g/L glucose, Sigma) supplemented with 10% fetal bovine serum (Life Technologies), 2 mM glutamine (Sigma), 100 µg/mL penicillin, and 100 U/mL streptomycin (Sigma) in a humidified incubator at 37 °C with 5% CO2. For transient expression of fluorescent constructs, cells were transfected 5 h after seeding into glass-bottom chambers (Thermo Scientific and Zell Contact) with XtremeGENE HP (Roche) or Xfect (Takara Bio) according to the manufacturer’s instructions. U2OS stably expressing H2B-mCherry were generated by transfection of the H2B-mCherry plasmid followed by a 2-week selection in growing medium supplemented with puromycin (0.25 µg/mL, Invivogen). Immediately prior to imaging live cells,
The overexpression of GFP-TET1 leads to chromatin reorganization. a Images of Hoechst-stained U2OS cells expressing either unfused GFP, or GFP-tagged TET1, TET2, or TET3. Cells were imaged 24 h after transfection. More than 30 cells were imaged per condition and a typical image is shown for each of them. b Time-lapse image sequence of live U2OS cells stably expressing H2B-mCherry and transiently expressing GFP-TET1. The images were pseudocolored according to the look-up table displayed below the images. The timelapse acquisition started 6 h after transfection. c Images from b were quantified to monitor the changes in GFP-TET1 expression levels (black curve), assessed by GFP fluorescence intensity, in parallel to the chromatin spatial distribution, assessed by the fluorescence contrast estimated from the images of the H2B-mCherry signal (red curve). The curves show a mean ± SD of 17 cells. Before averaging, the individual curves for the chromatin contrast and the expression level were aligned in time using as a reference the timepoint at which the normalized expression level equaled 0.5. The time was then estimated relative to this reference timepoint. d Images of Hoechst-stained U2OS cells expressing either unfused GFP, or full-length TET1 (GFP-FL), a catalytically inactive mutant of TET1 (GFP-FLmut), or a TET1 construct lacking the CXXC domain (GFP-ΔCXXC), all tagged with GFP. Cells were imaged 24 h after transfection. For panels a, b, and d, scale bars = 10 µm.
Fig. 2. The N-terminal domain of TET1 promotes chromatin reorganization. a Schematic representation of the GFP-tagged TET1 protein domains. Cys-rich corresponds to a cysteine-rich region and DSBH stands for double-stranded β-helix. b Images of Hoechst-labeled U2OS cells expressing either unfused GFP, full-length (GFP-FL), N-terminal domain (NTER-GFP), or C-terminal domain (CTER-GFP) of TET1, all tagged with GFP. More than 100 cells were imaged per condition and a typical image is shown for each. c Images of Hoechst-labeled HeLa and MCF-7 cells expressing unfused GFP or the N-terminal of TET1 tagged with GFP. More than 30 cells were imaged per condition and a typical image is shown for each. d Image sequences of the fluorescence redistribution after photobleaching a circular area of the nucleus (dotted red circles) of U2OS cells expressing either GFP fused to a nuclear localisation sequence (GFP-NLS), full-length TET1 (GFP-FL), the N-terminal domain of the protein (NTER-GFP), or its C-terminal domain (CTER-GFP), all tagged with GFP. e Images from d were quantified to assess the fluorescence recovery within the bleached region. The curves show a mean ± SD of 15 to 20 cells for each condition. f Characteristic recovery times estimated from the fitting of the curves shown on panel e. For all the data shown on this figure, cells were imaged 24 h after transfection. For panels b to d, scale bars = 10 μm.

Confocal imaging and fluorescence recovery after photobleaching

Fluorescence imaging of live and fixed cells was on a LSM880 point-scanner confocal microscope (Zeiss) using a 60×/1.4 N.A. oil-immersion objective lens. The pinhole was set to one Airy unit and fluorescence detection was performed on a GaAsP detector array. The fluorescence of the Hoechst was excited at 405 nm and detected within a window ranging from 415 to 470 nm. The fluorescence of the GFP was excited at 488 nm and detected within a window ranging from 500 to 550 nm. The fluorescence of the mCherry and Alexa Fluor 555 was excited at 561 nm and detected within a window ranging from 580 to 650 nm. The fluorescence of the Alexa Fluor 594 was excited at 594 nm and detected within a window ranging from 605 to 695 nm. The pixel size was set to 70 nm. Laser intensities and detector gains were chosen to avoid detector saturation and the acquisition settings were kept constant within the course of a given experiment. FRAP of H2B-mCherry was performed on the same setup. Photobleaching was achieved by 30 iterations of scanning half the nucleus at 100% power of the 561 nm laser. Then, cells were imaged for 11 h at 1 image per hour to monitor the progressive recovery within the bleached area. FRAP of H1-mCherry as well as the full-length or truncated forms of TET1 tagged with GFP was completed on a Ti-E inverted microscope from Nikon equipped with a CSU-X1 spinning-disk head from Yokogawa, a Plan APO 60×/1.4 N.A. oil-immersion objective lens, and a sCMOS ORCA Flash 4.0 camera. A 4 μm-diameter circle within the nucleus was bleached by 40 consecutive scans at 100% of 561 or 488 nm and subsequent fluorescence recovery was monitored by imaging the cells for 25 to 50 s at 4 frames/second. For all live-cell experiments, cells were maintained at 37 °C with a heating chamber.

Image analysis

Image analysis was performed using the ImageJ software. For the quantification of fluorescence signals, the mean intensity within manually defined region delineating the nucleus was measured and subtracted from background. Colocalization between two channels was performed by calculating the Pearson coefficient using the JACoP plugin (Bolte and Cordelieres 2006). To assess chromatin compaction, we estimated the fluorescence contrast on images of nuclei expressing H2B-mCherry or stained with Hoechst, using the plugin GLCM_Texture written by Julio E. Cabrera. The contrast is one of the Haralick features (Haralick et al. 1973) and measures the mean-squared intensity difference between pixels separated by a given distance d. This distance

Immunofluorescence staining

Cells were fixed using 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 at room temperature. For 5-hmC staining, chromatin was denatured by adding hydrochloric acid at 2 M for 30 min. Cells were blocked with blocking buffer (BSA 3% Tween 0.05% in PBS) before incubation overnight at 4 °C with primary antibodies recognizing double-strand DNA (Abcam, ab27156), 5-hmC (Active Motif, ref 39769), or the trimethylation of histone H3 at lysine 27 (Upstate, ref 07–449), diluted in blocking buffer (BSA 3% Tween 0.05% in PBS) before incubation overnight at 4 °C with primary antibodies recognizing double-strand DNA (Abcam, ab27156), 5-hmC (Active Motif, ref 39769), or the trimethylation of histone H3 at lysine 27 (Upstate, ref 07–449), diluted in blocking buffer. The secondary antibodies labeled with Alexa Fluor 555 (Thermo Scientific, ref A21428) diluted in blocking buffer were added for 1 h at room temperature. DNA was stained for 15 min with Hoechst 33342 at 1 μg/mL (Thermo Scientific).

Nascent RNA staining by 5-ethynyl uridine

For nascent RNA staining, cells were incubated with 5-ethynyl uridine (EU) at 1 mM in culture medium for 1 h before being fixed with 4% paraformaldehyde. Cells were permeabilized with 0.1% Triton X-100 for 15 min before RNA was labeled using the Click-iT® RNA Alexa Fluor 594 Imaging Kit according to the manufacturer’s instructions (Thermo Scientific).
$d$ was set to 10 pixels, which corresponded to the characteristic size of the dense chromatin domains generated by TET1 overexpression. Since we found that the measured contrast was independent of the cardinal directions, this parameter was only calculated along the east direction. The contrast estimated for each nucleus was the average of the values measured for three regions of 20*20 pixels randomly chosen within this nucleus, taking care that these regions do not overlap with the nucleus border or the nucleoli. For the timelapse acquisitions shown on Figs. 1b and S1b, the changes in fluorescence contrast were measured from the images of the H2B-mCherry patterns and the levels of expression of unfused GFP or GFP-TET1 were assessed by the mean intensity in the GFP channel. The expression level was then rescaled to range between 0 and 1. Before averaging, the individual curves for the chromatin contrast and the expression level were aligned in time using as a reference the timepoint at which the normalized expression level equalled 0.5. The time was then estimated relative to this reference timepoint.

To analyze the FRAP acquisitions, regions of interest were defined manually to measure the fluorescence intensities within the bleached area ($I_{\text{bleach}}$), the whole nucleus ($I_{\text{nuc}}$), and outside the cell ($I_{\text{bg}}$), along the entire image sequence. Based on these intensity measurements, the intensity within the bleached area was corrected from background and from the loss of total nuclear intensity due to both local photobleaching and timelapse imaging (Bancaud et al., 2010), using the following expression:

$$I_{\text{frac}} = \frac{I_{\text{nuc}} - I_{\text{bg}}}{I_{\text{nuc}} - I_{\text{bg}}}$$

After subtracting the signal estimated immediately after the bleach, the intensity was normalized to the pre-bleach signal. To estimate the characteristic recovery time, the post-bleach curves were fitted with the following single-exponential model:

$$I_{\text{rec}}(t) = 1 - \exp\left(-t/\tau\right)$$

with $t$ the time after bleaching and $\tau$ the fitted characteristic recovery time.

Flow cytometry

For staining with the Click-iT® RNA reagent (Thermo Scientific), U2OS cells were seeded into 6-well plates and transfected with the corresponding GFP-tagged TET1 expression plasmid. After 16 h, cells were treated for 1 h at 37 °C with 1 mM of 5-ethynyl uridine diluted in DMEM medium. Then, cells were harvested by trypsinization and fixed with 4% paraformaldehyde for 20 min at room temperature. After permeabilization for 15 min using the Click-iT® saponin-based permeabilization reagent, the cells were stained with the click-chemistry cocktail according to the manufacturer’s instructions for 30 min at room temperature and protected from light. For H3K27me3 staining, U2OS cells were seeded into 6-well plates and transfected with the corresponding GFP-tagged TET1 expression plasmid. After 16 h, cells were fixed with 4% paraformaldehyde, permeabilized with saponin-based permeabilization reagent, and incubated with rabbit polyclonal antibody against H3K27me3 (Sigma-Aldrich, 07–449) for 1 h at 1/100 dilution in the permeabilization reagent. After three cycles of washes/centrifugation with the permeabilization buffer, the pellet was resuspended and stained for 30 min using a 1/1000 dilution of Alexa Fluor 647 secondary antibody (Thermo Scientific, A315573). Finally, the samples were washed/centrifuged three times with PBS and the pellets were resuspended with PBS for cytometry analysis.

All the conditions were analyzed on a LSRFortessa X-20 (BD Bioscience) using BD FACSDiva Software v8.0.1. GFP was excited at 488 nm laser and detected within 30 nm window centered on 530 nm. For the Click-iT® staining using Alexa Fluor 594, fluorescence was excited with a 575 nm laser and detected within a 30 nm window centered on 620 nm. Alexa Fluor 647 was excited at 640 nm laser and detected within a 30 nm window centered on 670 nm. At least 10^5 events were collected for each condition. Quantifications were performed with the FACSDiva software (BD Biosciences).

Statistics

All the experiments were performed in at least three replicates with reproducible results for each replicate. When a single experiment is shown, a typical replicate was chosen. For the curves displaying time courses, means ± SD are shown. Boxplots were generated using a web tool developed by the Tyers and Rappsilber labs (http://boxplot.tyerslab.com/). The box limits correspond to the 25th and 75th percentiles and the bold line indicates the median value. The whiskers extend 1.5 times the interquartile range and outliers are shown by dots. The numbers in parentheses below each condition refer to the number of cells, for the imaging data, or to the number of experiments, for the cytometry data. $p$ values were calculated using either two-sided unpaired Student’s $t$-test assuming unequal variances for data showing normal distributions, or unpaired two-sample Wilcoxon test. On the boxplots, * refers to $p < 0.05$, ** to $p < 0.01$, *** to $p < 0.001$, **** to $p < 0.0001$, and n.s. to non significant.
Fig. 3 The N-terminal domain of TET1 affects the association of histones H2B and H1 with chromatin. a Image sequences of the fluorescence recovery after photobleaching of the mCherry signal within half of the nucleus (dotted red outlines) of U2OS cells expressing H2B-mCherry together with either unfused GFP or the N-terminal domain of TET1 (NTER-GFP) tagged with GFP. b Images from a were quantified to assess the fluorescence recovery within the bleached region (left). The curves show a mean ± SD of 15 and 17 cells for the unfused GFP control and the NTER-GFP condition, respectively. The FRAP curves were then fitted with a single-exponential recovery model to extract the characteristic recovery times (right). c Image sequences of the fluorescence recovery after photobleaching of the mCherry signal within a circular area of the nucleus (dotted red circles) of U2OS cells expressing H1-mCherry together with either unfused GFP or the N-terminal domain of the protein (NTER-GFP) tagged with GFP. d Images from c were quantified to assess the fluorescence recovery within the bleached region (left). The curves show a mean ± SD of 18 and 17 cells for the unfused GFP control and the NTER-GFP condition, respectively. The FRAP curves were then fitted with a single-exponential recovery model to extract the characteristic recovery times (right). For all the data shown on this figure, cells were imaged 24 h after transfection. For panels a and c, scale bars = 10 µm.
between the timing of the chromatin reorganization and the fluorescence contrast of H2B-mCherry was observed upon cells expressing GFP (Fig. S1b, c), a rapid increase of the no significant change in the chromatin pattern was observed out of the chromatin compaction state in living cells. While rescence contrast of the chromatin pattern, providing a read- GFP fluorescence levels in parallel with changes in the fluo-

To better understand how chromatin reorganization arises on the impact of TET1 expression on chromatin structure. This phenotype was more pronounced for TET1, we focused leaving some nuclear areas largely devoid of staining. Since GFP-TET2 led to chromatin condensation into large bundles, space relatively evenly, the expression of GFP-TET1 and GFP-TET3, led to a global reorganization of the chromatin within overexpression of GFP-TET1 and GFP-TET2, but not GFP- TET3, led to a global reorganization of the chromatin within the nucleus of human osteosarcoma U2OS cells as seen both by Hoechst and anti-DNA staining (Fig. 1a and S1a). While the chromatin in control cells occupied the entire nuclear space relatively evenly, the expression of GFP-TET1 and GFP-TET2 led to chromatin condensation into large bundles, leaving some nuclear areas largely devoid of staining. Since this phenotype was more pronounced for TET1, we focused on the impact of TET1 expression on chromatin structure. To better understand how chromatin reorganization arises upon TET1 overexpression, we transiently transfected GFP- tagged TET1 or free GFP in U2OS cells stably expressing H2B-mCherry and monitored the progressive increase in the GFP fluorescence levels in parallel with changes in the fluorescence contrast of the chromatin pattern, providing a read- out of the chromatin compaction state in living cells. While no significant change in the chromatin pattern was observed in cells expressing GFP (Fig. S1b, c), a rapid increase of the fluorescence contrast of H2B-mCherry was observed upon expression of GFP-TET1 (Fig. 1b, c). The tight correlation between the timing of the chromatin reorganization and the rising of TET1 levels within the nucleus suggests that the

The N-terminal domain of TET1 is sufficient to induce chromatin reorganization

To better delineate the domains of TET1 responsible for chromatin reorganization, we analyzed the impact of the expression of two truncated forms of the full-length protein: a C-terminal fragment (CTER) bearing the catalytic domain shared between the different TET proteins, and a N-terminal fragment (NTER) which remains poorly characterized except for the CXXC domain (Fig. 2a). Importantly, the CTER is reminiscent of the recently identified short isoform of TET1 found expressed in differentiated cells. While the CTER is still able to oxidize 5-mC into 5-hmC (Fig S2a, b), in line with what is reported for TET-1short (Good et al. 2017), its expression in U2OS cells did not lead to visible changes in the chromatin organization (Fig. 2b). In contrast, expression of the NTER fragment, while not affecting 5-hmC levels within the nucleus (Fig S2a, b), led to a global reorganization of the chromatin resembling the one observed when overexpressing the full-length protein (Fig. 2b). The amount of chromatin reorganization, assessed by the fluorescence contrast of the Hoechst staining, correlated with the levels of expression of the NTER fragment of TET1, similar to what was observed for the full-length protein (Fig S2c). Importantly, this chromatin restructuring phenotype upon expression of the NTER fragment was also observed in human HeLa and MCF-7 cells, demonstrating that it is not specific to U2OS cells (Fig. 2c).

We also assessed the affinity of these two truncated TET1 constructs for chromatin by monitoring the kinetics of fluorescence recovery after photobleaching (FRAP) for each GFP-tagged construct. The NTER fragment displayed a much slower recovery than the CTER construct (Fig. 2d–f), in agreement with previous findings showing that it is principally the NTER region that controls TET1 binding to chromatin (Zhang et al. 2016). Nevertheless, the NTER fragment appeared less bound to chromatin than the full-length TET1, which was also confirmed by a reduced colocalization of

Results

Overexpression of TET1 leads to a global reorganization of the chromatin structure

By using confocal fluorescence imaging, we observed that overexpression of GFP-TET1 and GFP-TET2, but not GFP- TET3, led to a global reorganization of the chromatin within the nucleus of human osteosarcoma U2OS cells as seen both by Hoechst and anti-DNA staining (Fig. 1a and S1a). While the chromatin in control cells occupied the entire nuclear space relatively evenly, the expression of GFP-TET1 and GFP-TET2 led to chromatin condensation into large bundles, leaving some nuclear areas largely devoid of staining. Since this phenotype was more pronounced for TET1, we focused on the impact of TET1 expression on chromatin structure. To better understand how chromatin reorganization arises upon TET1 overexpression, we transiently transfected GFP- tagged TET1 or free GFP in U2OS cells stably expressing H2B-mCherry and monitored the progressive increase in the GFP fluorescence levels in parallel with changes in the fluorescence contrast of the chromatin pattern, providing a read- out of the chromatin compaction state in living cells. While no significant change in the chromatin pattern was observed in cells expressing GFP (Fig. S1b, c), a rapid increase of the fluorescence contrast of H2B-mCherry was observed upon expression of GFP-TET1 (Fig. 1b, c). The tight correlation between the timing of the chromatin reorganization and the rising of TET1 levels within the nucleus suggests that the change in the chromatin pattern is directly linked to the presence of TET1 rather than a secondary consequence of cellular mechanisms controlled by this enzyme.

TET1 is responsible for 5-mC oxidation to allow its later replacement by unmethylated cytosine (Tahiliani et al. 2009). However, when expressing a catalytically inactive form of TET1 we observed the same chromatin pattern as with the active enzyme (Fig. 1d). Furthermore, this reorganization phenotype also did not rely on the CXXC domain of TET1 (Fig. 1d). Therefore, these data show that the change in chromatin structure promoted by TET1 is not the consequence of 5-mC oxidation processes or further DNA demethylation steps.
the former with Hoechst (Fig S2d). Altogether, these results
demonstrate that the dynamic association of the N-terminal
domain of TET1 with chromatin, which displays charac-
teristic exchange times of about 10 s, alters higher-order
chromatin states, as shown by light microscopy, ultimately
leading to a spatial redistribution of the genomic material
within the nucleus.

**Large-scale chromatin reorganization promoted by the N-terminal domain of TET1 is associated with changes in histone association with chromatin**

We next assessed whether the changes in chromatin fold-
ing induced by the N-terminal domain of TET1 were also
associated with altered dynamics of its molecular con-
stituents. Histones are key chromatin scaffolding proteins
regulating the spatial organization of the genome within
the nucleus, and their exchange rates correlate with the
chromatin compaction state (Misteli et al. 2000; Bancaud et al. 2009). Therefore, we assessed the impact of the
N-terminal domain of TET1 on the exchange rates of
two different histones: the core histone H2B as well
as the linker histone H1. Using FRAP, we observed that
expression of the N-terminal domain of TET1 decreased
H2B exchange rate (Fig. 3a, b), in line with the fact that
this domain seems to promote the local condensation of
chromatin. Conversely, the linker histone H1 displayed
reduced association with chromatin in cells expressing the
N-terminal domain of TET1 compared to controls (Fig. 3c,
d). Therefore, the dense chromatin domains generated by
the expression of the N-terminal domain of TET1 differ
from transcriptionally silent heterochromatic areas in
which H1 displays increased binding (Misteli et al. 2000;
Bancaud et al. 2009).

**Expression of the N-terminal domain of TET1 leads to a global shutdown of transcription**

The results obtained so far demonstrate that the N-terminal
domain of TET1 regulates the architecture of chromatin
at multiple scales. Since this enzyme was shown to play
key roles in the regulation of transcription, we next tested
whether the changes in chromatin organization induced by
the N-terminal domain of TET1 were also associated with
a modulation of the transcriptional state. By quantifying
5-ethyluridine (EU) staining both by confocal imaging
and by flow cytometry to assess the level of nascent RNA in
cells expressing the N-terminal domain of TET1, we found
that the presence of this domain of TET1 led to a partial
inhibition of transcription, similarly to the overexpression
of the full-length protein (Fig. 4a–c and S3b). Furthermore,
the negative colocalization between the EU and Hoe-
chst staining in cells expressing the N-terminal domain of
TET1 suggests that transcription is confined at the interface
between the dense chromatin domains and the nucleoplasm
(Fig. S3a). Concomitant to the reduction in RNA synthesis
rates, immunofluorescence staining also showed that the
expression of the N-terminal domain of TET1 increased
the levels of trimethylation of the lysine 27 of histone H3
(H3K27me3) (Fig. 4d, e), an epigenetic mark written by
the PRC2 complex and found within facultative hetero-
chromatin (Cao et al. 2002). These results were confirmed
by flow cytometry, which also revealed a positive correla-
tion between the amount the N-terminal domain of TET1
expressed in the cells and the levels of H3K27me3 mark
(Fig. 4f and S3b). Altogether, these findings demonstrate
that the N-terminal domain of TET1 is able to shutdown
transcription by establishing a chromatin environment dis-
playing some of the characteristics found in heterochromatin
regions.

**Discussion**

Our data show that overexpression of TET1 promotes
chromatin remodeling in three different human cell lines
(U2OS, MCF-7, and HeLa), which leads to the establish-
ment of a condensed chromatin architecture refractory to
transcription. These dense chromatin domains observed
in the presence of TET1 share some features found in
heterochromatin, such as increased levels of H3K27me3
(Wiles and Selker 2017), but nevertheless differ from
these compact regions. Indeed, while the histone linker
H1 was shown to be crucial for the condensation of the
chromatin fiber (Willcockson et al. 2021), we observed
reduced association of this histone in the compact chro-
matin conformation promoted by TET1. Interestingly,
senescence-associated heterochromatin foci, which
arise from heterochromatic areas detaching from the
nuclear lamina, also show reduced H1 binding due to
its replacement by the high mobility group protein A2
(HMGA2) (Funayama et al. 2006). It would be useful
to assess whether TET1 overexpression could promote
cell senescence.

The TET1-mediated chromatin condensation process
reported here is independent of both the catalytic activity
of this enzyme and its targeting to CpG islands via the
CXXC domain. The N-terminal region of the protein, which
triggers this condensation, displays a strong affinity for chro-
matin. Therefore, it could serve as a platform to recruit factors
modulating chromatin conformation, as it was recently pro-
posed for OGT (Zhang et al. 2016). TET1 has been shown
to interact with the histone-deacetylases Sin3A and HDAC1
as well as the chromatin remodeler CHD4, three actors
known to promote chromatin compaction (Shi et al. 2013).
Future experiments would be needed to assess whether the
recruitment of these proteins to chromatin through their binding to TET1 could contribute to the establishment of the compact genome organization observed in cells expressing the N-terminal domain of TET1. Furthermore, the increase in H3K27me3 staining in these dense domains may be promoted by the association between TET1 and the PRC2 complex (Neri et al. 2015), contributing to a global silencing of transcription.

In this work, we uncovered a new role of the N-terminal region of TET1 in the regulation of the chromatin organization and transcription inhibition. The inhibitory impact of this region of the protein might be needed to balance the positive influence of the catalytic domain on transcription, where unrestrained activity of the recently identified short isoform of TET1 is associated with tumorigenesis (Good et al. 2017). Further work will be needed to better delineate the characteristics of this coordination mechanism between the two domains of TET1.

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Author contribution A.L., S.Z., S.H., and R.S. completed the experiments within the manuscript. C.C. generated cell lines and DNA constructs. A.L., S.Z., S.H., and O.D.A. performed data analysis. G.S. and S.H. conceived and supervised this study. S.H. wrote the manuscript. All authors read and commented on the manuscript.

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Data availability The raw datasets used during the current study are available from the corresponding authors on reasonable request.

Code availability Not applicable

Declarations

Ethics approval and consent to participate Not applicable

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Competing interests The authors declare no competing interests.

References

Bancaud A, Huet S, Daigle N et al (2009) Molecular crowding affects diffusion and binding of nuclear proteins in heterochromatin and reveals the fractal organization of chromatin. EMBO J 28:3785–3798. https://doi.org/10.1038/emboj.2009.340

Bancaud A, Huet S, Rabut G, Ellenberg J (2010) Fluorescence perturbation techniques to study mobility and molecular dynamics of proteins in live cells: FRAP, photoactivation, photoconversion, and FLIP. Cold Spring Harb Protoc 2010:pdb.top90. https://doi.org/10.1101/pdb.top90

Bauer C, Göbel K, Nagaraj N et al (2015) Phosphorylation of TET proteins is regulated via O-GlcNAcylation by the O-Linked N-acetyl-glucosamine transferase (OGT). J Biol Chem 290:4801–4812. https://doi.org/10.1074/jbc.M114.605881

Bhutani N, Burns DM, Blau HM (2011) DNA demethylation dynamics. Cell 146:866–872. https://doi.org/10.1016/j.cell.2011.08.042

Bolte S, Cordelieres FP (2006) A guided tour into subcellular colocalization analysis in light microscopy. J Microsc 224:213–232. https://doi.org/10.1111/j.1365-2818.2006.01706.x

Cao R, Wang L, Wang H et al (2002) Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 298:1039–1043. https://doi.org/10.1126/science.1076997

Fierz B, Poirier MG (2019) Biophysics of chromatin dynamics. Annu Rev Biophys 48:321–345. https://doi.org/10.1146/annurev-biophys-070317-032847

Frauer C, Rottach A, Meilinger D et al (2011) Different binding properties and function of CXXC zinc finger domains in Dnmt1 and Tet1. PLoS ONE 6:e16627. https://doi.org/10.1371/journal.pone.0016627

Funayama R, Saito M, Tanobe H, Ishikawa F (2006) Loss of linker histone H3 lysine 27 methylation in Polycomb-group silencing. Science 298:1039–1043. https://doi.org/10.1126/science.1076997

Gao X, Wang L, Wang H et al (2002) Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 298:1039–1043. https://doi.org/10.1126/science.1076997

Iguchi-Ariga SM, Schaffner W (1989) CpG methylation of the cAMP-dependent protein kinase regulatory subunit. Cell 59:1016–1026. https://doi.org/10.1016/0092-8674(89)90002-7

Misteli T, Gunjan A, Hock R et al (2000) Dynamic binding of histone 

H1 to chromatin in living cells. Nature 408:877–881. https://doi.org/10.1038/35048610
Mulholland CB, Nishiyama A, Ryan J et al (2020) Recent evolution of a TET-controlled and DPPA3/STELLA-driven pathway of passive DNA demethylation in mammals. Nat Commun 11:5972. https://doi.org/10.1038/s41467-020-19603-1

Münzel M, Globisch D, Carell T (2011) 5-Hydroxymethylcytosine, the sixth base of the genome. Angew Chem Int Ed Engl 50:6460–6468. https://doi.org/10.1002/anie.201101547

Neri F, Incarnato D, Krepelova A et al (2015) TET1 is controlled by pluripotency-associated factors in ESCs and downmodulated by PRC2 in differentiated cells and tissues. Nucleic Acids Res 43:6814–6826. https://doi.org/10.1093/nar/gkv392

Rasmusson KD, Helin K (2016) Role of TET enzymes in DNA methylation, development, and cancer. Genes Dev 30:733–750. https://doi.org/10.1101/gad.276568.115

Rother MB, Pellegrino S, Smith R et al (2020) CHD7 and 53BP1 regulate distinct pathways for the re-ligation of DNA double-strand breaks. Nat Commun 11:5775. https://doi.org/10.1038/s41467-020-19502-5

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