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SUMO-modified Sp3 represses transcription by provoking local heterochromatic gene silencing

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Modification of many transcription factors including Sp3 and steroidogenic factor 1 with the small ubiquitin-like modifier (SUMO) is associated with transcriptional repression. Here, we show that SUMOylation of transcription factors bound to DNA provokes the establishment of compacted repressive chromatin with characteristics of heterochromatin. Chromatin immunoprecipitation experiments revealed SUMO-dependent recruitment of the chromatin remodeller Mi-2, MBT-domain proteins, heterochromatic protein 1, and the histone methyltransferases SETDB1 and SUV4-20H, concomitant with the establishment of histone modifications associated with repressed genes, including H3K9 and H4K20 trimethylation. These results indicate that SUMOylation has a crucial role in regulating gene expression by initiating chromatin structure changes that render DNA inaccessible to the transcription machinery.

Keywords: heterochromatin; SUMO; repression; transcription factor Sp3
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INTRODUCTION

Covalent post-translational modifications with the small ubiquitin-like modifier (SUMO) control various cellular functions, including subcellular transport, genome integrity, DNA repair, stress response and transcription factor activity (Geiss-Friedlander & Melchior, 2007). The largest group of proteins known to be targets of SUMOylation are transcription factors and transcriptional coregulators. Apart from a few exceptions, SUMO modification of transcription factors is linked to transcriptional repression (Gill, 2005; Hay, 2005).

The transcription factor Sp3 is a ubiquitously expressed member of the Sp family of transcription factors (Suske, 1999). It contains a highly conserved DNA-binding domain close to the carboxyl terminus and two glutamine-rich activation domains in the amino-terminal moiety. Depending on the promoter context, Sp3 can activate and repress transcription (Suske, 1999). The repression function of Sp3 is mediated by SUMO modification of lysine residue K551 within the SUMOylation consensus motif ΨKXE (Ross *et al*, 2002; Sapetschnig *et al*, 2002, 2004).

Recently, we identified SUMO-dependent repression components by using a genome-wide RNA-interference (RNAi)-mediated screen in cultured *Drosophila* cells (Stielow *et al*, 2008). Several of the identified genes encode chromatin-associated proteins, including the ATP-dependent chromatin remodeller dMi-2 and the polycomb protein dSfmbt. Both proteins bind to SUMO *in vitro* and are recruited to Sp3-responsive promoters in a SUMOylation-dependent manner (Stielow *et al*, 2008).

Following the mechanistic clues provided by the identification of SUMO-dependent repression components in insect cells, we analysed in detail the chromatin changes established by SUMO-modified transcription factors. Here, we show that promoter-bound SUMO-modified Sp3 led to the establishment of local repressive chromatin with characteristics of compacted heterochromatin. Heterochromatin formation includes recruitment of the chromatin remodeller Mi-2, the MBT-domain proteins L3MBTL1 and L3MBTL2, heterochromatic protein 1 (HP1) and the histone methyltransferases (HMTs) SETDB1 and SUV4-20H together with the establishment of repressive histone modifications such as H3K9 and H4K20 trimethylation. Our studies identify SUMO modification of transcription factors as a novel mechanism for the initiation and formation of localized heterochromatin-like silenced states.

RESULTS

SUMO-dependent silencing of a chromatinized transgene

To study the mechanisms underlying SUMO-dependent transcriptional repression in the context of a chromatin template, we generated human embryonic kidney (HEK) 293 cell lines with a stably integrated luciferase reporter gene driven by five Gal4 binding sites. Eight cell clones were tested for SUMOylation-dependent repression by transfecting SUMOylation-competent Gal4-Sp3WT and a SUMOylation-deficient Gal4-Sp3 mutant in which the K551 SUMO attachment site is destroyed (Gal4-Sp3KEEm; Fig 1A; supplementary Fig S1 online). Clone c1 showed only background luciferase activity that was not affected on transfection with Gal4-Sp3WT, whereas the SUMOylation-deficient

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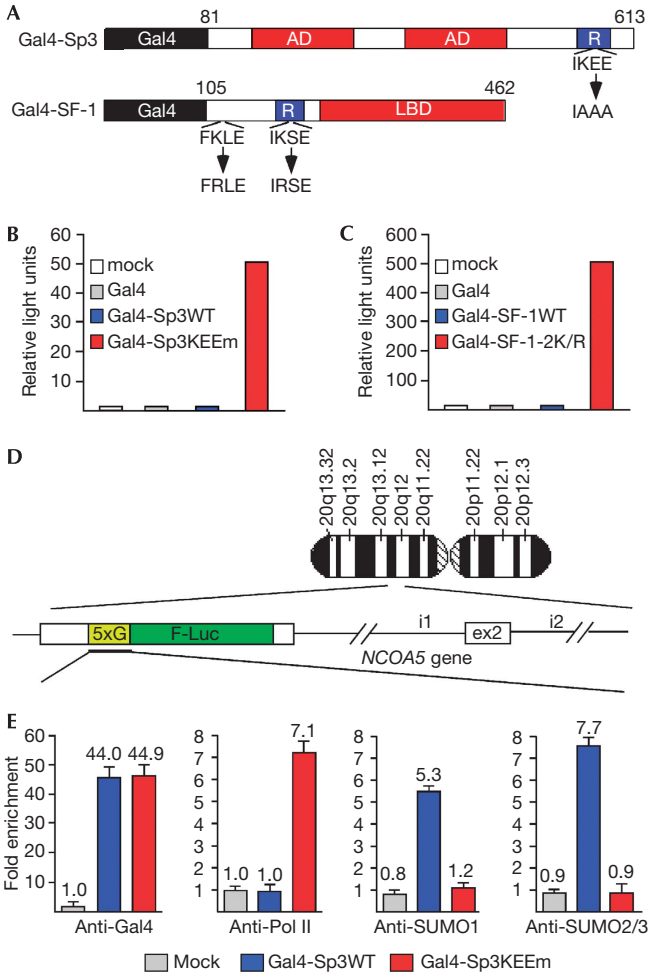

Fig 1 | SUMOylation-dependent gene silencing of a stably integrated transgene. (**A**) Schematic illustration of Gal4-Sp3 and Gal4-SF-1 fusion proteins. The SUMO target sites of Sp3 (IKEE) and SF-1 (FKLE and IKSE), as well as the corresponding sequences in the SUMOylation-deficient mutants, are shown. (**B**) Human embryonic kidney 293 cells with a stably integrated Gal4-driven luciferase reporter were transiently transfected with expression plasmids for the Gal4 DNA binding domain (Gal4), a Gal4-Sp3WT fusion (Gal4-Sp3WT) or a SUMOylation-deficient Gal4-Sp3 mutant (Gal4-Sp3KEEm) along with a β-galactosidase control reporter. At 48 h after transfection, cells were lysed and luciferase activities were determined. (**C**) Transfections were performed as in (**B**) with expression plasmids for Gal4-SF-1 fusions. (**D**) Schematic illustration of the transgene integration site in the first intron of the nuclear receptor coactivator 5 (*NCOA5*) gene on chromosome 20. (**E**) ChIP-qPCR. Crosslinked chromatin was isolated from transfected cells and incubated with the indicated antibodies and control IgGs. Precipitated material was amplified using qPCR with primers specific for the 5xGal4 promoter. Recoveries are expressed as fold enrichment relative to the control antibody (mean ± s.d.). AD, activation domain; ChIP, chromatin immunoprecipitation; LBD, ligand binding domain; qPCR, quantitative PCR; R, repression domain; SF-1, steroidogenic factor 1; SUMO, small ubiquitin-like modifier.

Gal4-Sp3KEEm mutant activated transcription by about 50-fold (Fig 1B; supplementary Fig S1 online). To substantiate the conclusion that SUMOylation represses the activation of the integrated transgene, we transfected Gal4 fusions of the orphan receptor steroidogenic factor 1 (SF-1) that is SUMO-modified at two conserved lysine residues (Fig 1A; Lee *et al*, 2005). Similar to Sp3, Gal4-SF-1WT was repressive, whereas the SUMOylation-deficient Gal4-SF-1-2K/R mutant activated the integrated reporter by up to 500-fold (Fig 1C). Southern blot analysis and subsequent integration site mapping showed that a single copy of the Gal4-luciferase transgene is integrated in the first intron of the nuclear receptor coactivator 5 (*NCOA5*) gene on chromosome 20 (Fig 1D; supplementary Fig 2 online).

Gal4-Sp3WT and the SUMOylation-deficient mutant Gal4-Sp3KEEm were expressed at similar levels on transfection (data not shown). Chromatin immunoprecipitation (ChIP) with Gal4 antibodies and subsequent normal semiquantitative PCR (data not shown) and quantitative PCR (ChIP-qPCR) showed that both proteins are recruited to the integrated 5xGal4 promoter with similar efficiency (Fig 1E); similar results were obtained with Gal4-SF-1 fusions (supplementary Fig 3A online). RNA polymerase II is bound to the promoter in the presence of activating Gal4-Sp3KEEm but not in the absence or presence of repressive Gal4-Sp3WT (Fig 1E). Antibodies to SUMO1, as well as to SUMO2/3, precipitated the 5xGal4 promoter in the presence of Gal4-Sp3WT but not in the presence of the K551 mutant (Fig 1E). This might reflect attachment of both SUMO isoforms to Sp3 or to corepressor components (see below). We consider the latter assumption to be more likely because the SUMO antibodies that we used are insensitive towards Sp3-SUMO1 and Sp3-SUMO2/3, and failed to co-immunoprecipitate Sp3 along with SUMO1 or SUMO2/3 (data not shown).

Recruitment of Mi-2, L3MBTL1 and L3MBTL2

Recently, we identified the nucleosome remodeller Mi-2 and the polycomb protein Sfmbt as SUMO-dependent repression components in *Drosophila melanogaster* cell lines (Stielow *et al*, 2008). Human orthologues of these proteins are Mi-2α/β (Marfella & Imbalzano, 2007) and the MBT-domain-containing proteins L3MBTL1 and L3MBTL2 (Trojer *et al*, 2007) (Fig 2A), respectively, which are expressed in 293 cells (Fig 2B). Comprehensive ChIP-qPCR analyses showed recruitment of all three proteins to the 5xGal4 promoter in the presence of Gal4-Sp3WT, as well as in the presence of Gal4-SF-1WT, but not in the presence of the corresponding SUMOylation-deficient mutants (Fig 2C; supplementary Fig 3B online), indicating that the recruitment of Mi-2, L3MBTL1 and L3MBTL2 is strongly SUMO dependent.

Mi-2 associates with other proteins to form multisubunit complexes known as NuRD (Feng & Zhang, 2003). We have performed ChIPs with antibodies to HDAC1 and p66—two subunits of the NuRD complex. Neither proteins are specifically enriched at the 5xGal4 promoter in the presence of Gal4-Sp3 (data not shown), indicating that Mi-2 might exert its silencing function outside the classical NuRD complex. This result is consistent with the previous observation that Sp3-SUMO-dependent repression in insect cells is independent of histone deacetylase activity, and independent of NuRD subunits other than Mi-2 (Stielow *et al*, 2008).

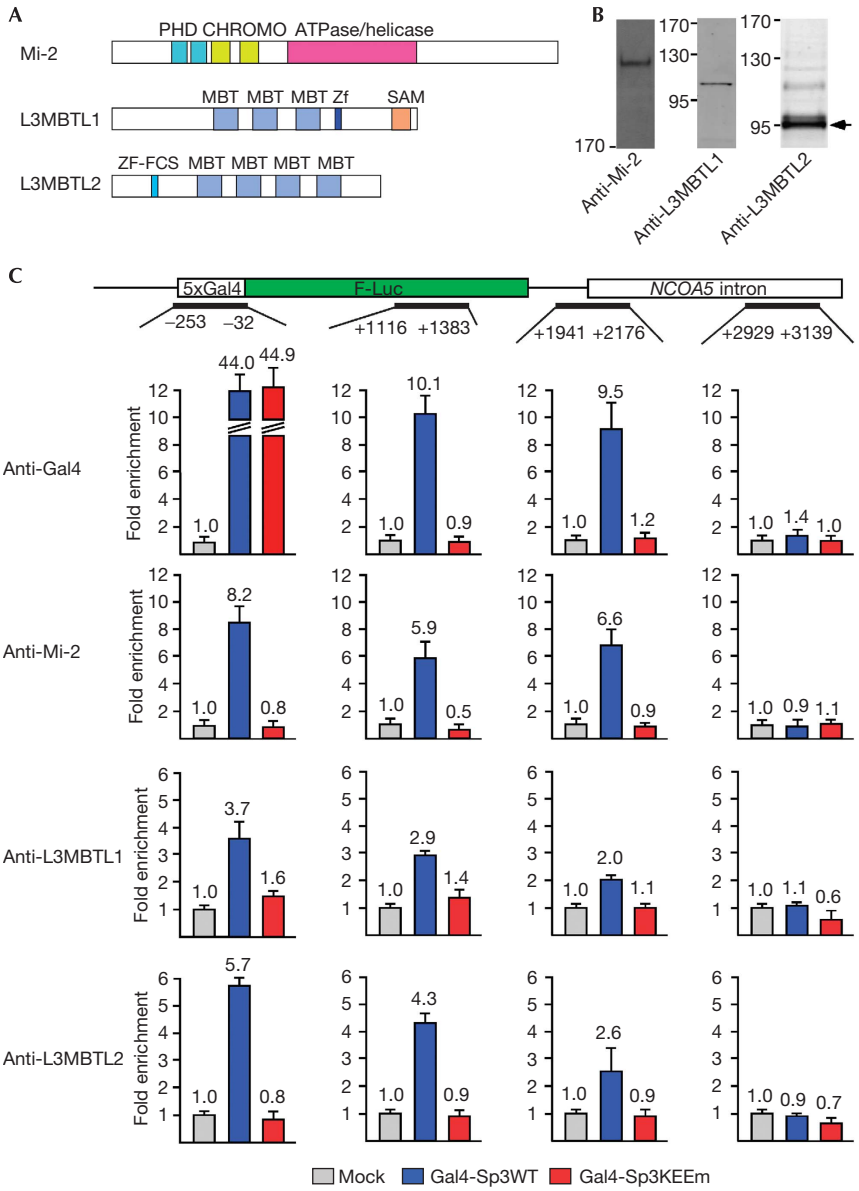

Fig 2 | SUMOylation-dependent recruitment of Gal4-Sp3, Mi-2, L3MBTL1 and L3MBTL2 to the integrated transgene. (**A**) Schematic drawing of Mi-2, L3MBTL1 and L3MBTL2. (**B**) Immunoblot analysis of Mi-2, L3MBTL1 and L3MBTL2 in human embryonic kidney 293 cells used in this study. (**C**) ChIP-qPCR. ChIPs were performed with antibodies to Gal4, Mi-2, L3MBTL1 and L3MBTL2 as indicated. Recoveries are expressed as fold enrichment relative to the control antibody (mean ± s.d.). ChIP, chromatin immunoprecipitation; qPCR, quantitative PCR; SUMO, small ubiquitin-like modifier.

We analysed sequences 1.2, 2 and 3 kb downstream from the transcriptional start site in the coding region of the luciferase gene, at the transgene integration site and within the first intron of the neighbouring *NCOA5* gene, respectively. Mi-2, L3MBTL1, L3MBTL2 and, most strikingly, also Gal4-Sp3WT were associated with regions 1.2 and 2 kb downstream from the 5xGal4 promoter (Fig 2C). Similar results were obtained with the Gal4-SF-1 fusion proteins (supplementary Fig 4 online). We also analysed previous ChIP experiments performed in stably transfected insect cells that contain a GC-box-driven luciferase reporter and express either wild-type Sp3 or the SUMO-deficient mutant (Stielow *et al*, 2008). In this experimental setting, Sp3 is recruited to the promoter by means of its own DNA-binding domain. Similar to the observation with the Gal4 fusion proteins, we found specific association of SUMOylated wild-type Sp3 with the coding region of the luciferase gene (data not shown). The association of promoter-bound SUMO-modified transcription factors with remote regions probably reflects the compaction of chromatin. This conclusion is corroborated by impaired restriction enzyme accessibility to the transgene promoter in the presence of wild-type Sp3

(supplementary Fig 5 online). In compacted chromatin, regions more distant to the promoter would be in close proximity to the promoter-bound transcription factors and are precipitated in ChIP experiments. Such a scenario is consistent with a compaction function of MBT domains (Trojer *et al*, 2007).

SUMO-dependent local heterochromatin formation

Compacted chromatin is a typical characteristic of heterochromatin; therefore, we explored the presence of heterochromatic proteins (HP) and repressive histone marks associated with heterochromatin. HP1α, HP1β and HP1γ are enriched at the 5xGal4 promoter in the presence of Gal4-Sp3WT (Fig 3A). HP1β and HP1γ but not HP1α were also associated with regions 2 and 3 kb downstream from the transgene promoter (supplementary Fig 6 online). The differences between HP1β/γ and HP1α might indicate various modes of recruitment and action of the HP1 isoforms (Hediger & Gasser, 2006).

Next, we analysed various repressive histone modifications, namely H3K9, H3K27 and H4K20 methylation (Fig 3B). H3K9me2 and predominantly H3K9me3 were enriched in the presence of Gal4Sp3WT. H3K27me3 was already present at the 5xGal4 promoter in the absence of transcription factors and did not change markedly in the presence of Gal4-Sp3WT. However, it was approximately threefold reduced in the presence of the transcriptionally active Gal4-Sp3KEEm mutant (Fig 3B). Monomethylated H4K20 was highly enriched at the unoccupied 5xGal4 promoter and slightly reduced in the presence of Gal4-Sp3WT or Gal4Sp3KEEm. H4K20me2 was approximately twofold less abundant in the presence of Gal4-Sp3WT. Most significantly, the repressive H4K20me3 mark was highly enriched in the presence of Gal4-Sp3WT and completely absent in the presence of the SUMOylation-deficient mutant (Fig 3B).

As trimethylation of H3K9 and H4K20 was highly enriched at the 5xGal4 promoter in the presence of Gal4-Sp3WT, we analysed for the presence of HMTs that can catalyse H3K9 and H4K20 trimethylation. Trimethylation enzymes of H3K9 are SUV39H1/2 (KMT1A/B) (Peters *et al*, 2003) and SETDB1/ESET (KMT1E) (Schultz *et al*, 2002; Wang *et al*, 2003). SETDB1 is bound to the 5xGal4 promoter in the presence of Gal4-Sp3WT, whereas SUV39H1 was barely detectable (Fig 3C). The HMTs that trimethylate histone H4K20 are SUV4-20H1 (KMT5B) and SUV4-20H2 (KMT5C) (Schotta *et al*, 2004). ChIPs with antibodies that recognize both proteins showed Gal4-Sp3WT-specific recruitment of SUV4-20H to the 5xGal4 promoter.

Our ChIP data show that promoter-bound SUMO-modified Sp3 led to the establishment of local repressive chromatin with typical characteristics of heterochromatin, including repressive histone tail modifications such as H3K9 and H4K20 trimethylation, and deposition of HP1 proteins (Fig 4). Sp3-SUMO-dependent recruitment of Mi-2, L3MBTL2, HP1 and HMTs, as well as the establishment of repressive methylation marks, is also observed in an alternative cell line (supplementary Fig 7 online), indicating that our finding is not an atypical property of the reporter gene integration site of clone c1.

Heterochromatic marks at the endogenous *Dhfr* promoter

Mi-2 and L3MBTL2 are operative at the endogenous mouse *Dhfr* promoter in wild-type mouse embryonic fibroblasts (MEFs),

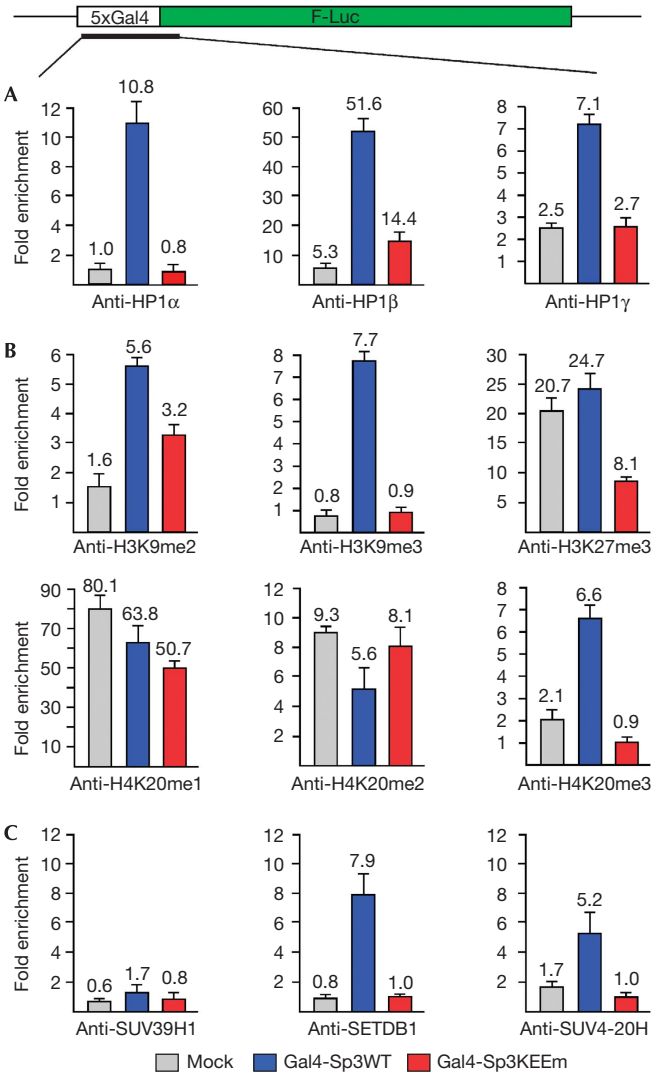

Fig 3 | SUMOylation-dependent local heterochromatin formation. ChIP-qPCR for the presence of heterochromatic marks. (**A**) SUMOylation-dependent recruitment of HP1 isoforms. (**B**) Histone modifications at the 5xGal4 promoter. (**C**) SUMOylation-dependent recruitment of histone methyltransferases. ChIP, chromatin immunoprecipitation; HP1, heterochromatic protein 1; qPCR, quantitative PCR; SUMO, small ubiquitin-like modifier.

but not in *Sp3*$^{-/-}$ MEFs (Stielow *et al*, 2008). Consequently, we analysed the *Dhfr* promoter for the presence of SETDB1, SUV4-20H, HP1 and repressive histone modifications. HP1α, SETDB1 and SUV4-20H, as well as H3K9me3 and H4K20me3 modifications are present at the *Dhfr* promoter in wild-type MEFs but not in Sp3-deficient MEFs (Fig 5). These results indicate that SETDB1 and SUV4-20H are the respective HMTs that catalyse trimethylation of H3K9 and H4K20 at the *Dhfr* promoter. Reduced H3K9me3 after RNAi-mediated knockdown of SETDB1 (supplementary Fig 8 online) and reduced H4K20me3 in SUV4-20H1/2 double knockout MEFs (Benetti *et al*, 2007; supplementary Fig 9 online) corroborate this conclusion.

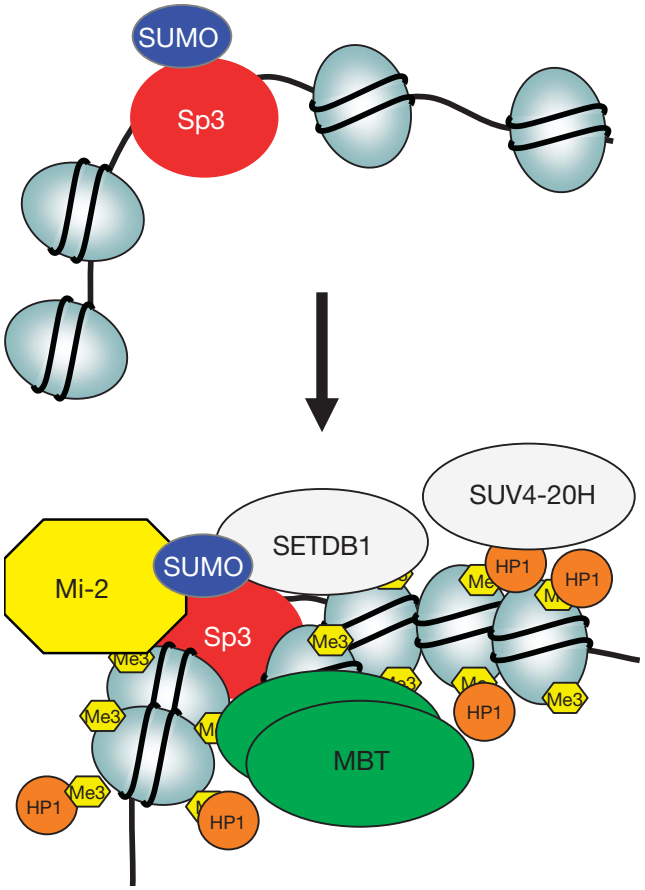

Fig 4 | Model showing the establishment of local repressive chromatin by SUMO-modified transcription factors. SUMO-modified Sp3, as well as other transcription factors such as steroidogenic factor 1, bound to DNA can provoke the establishment of local heterochromatic structures by recruiting the chromatin remodeller Mi-2, MBT-domain proteins (L3MBTL1 and L3MBTL2) involved in chromatin compaction and histone methyltransferases (SETDB1 and SUV4-20H) that catalyse trimethylation of H3K9 and H4K20. The repressive state is manifested by deposition of heterochromatic protein 1 (HP1). SUMO, small ubiquitin-like modifier.

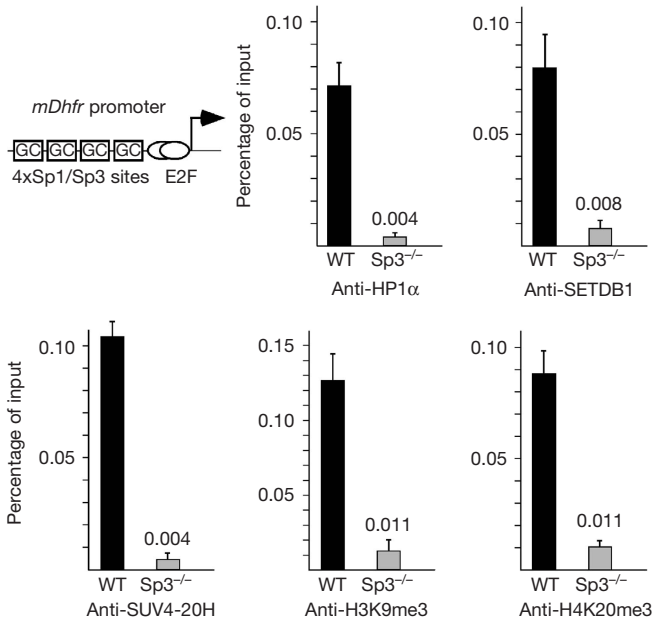

Fig 5 | Sp3-dependent association of HP1, SETDB1, SUV4-20H, H3K9me3 and H4K20me3 at the endogenous mouse *Dhfr* promoter. Wild-type (WT) and Sp3 knockout mouse embryonic fibroblasts ($Sp3^{-/-}$) were subjected to chromatin immunoprecipitation with the indicated antibodies. Precipitates were analysed with primers specific for the mouse *Dhfr* promoter. DNA recoveries are expressed as percentage of input (mean ± s.d.). HP1, heterochromatic protein 1.

To substantiate the conclusion that the SUMO moiety of Sp3 is responsible for the establishment of heterochromatin-like structures at the *Dhfr* promoter, we used $Sp3^{-/-}$ MEFs expressing the small isoforms of Sp3, the long isoform of Sp3, or corresponding SUMOylation-deficient mutants lacking 13 amino acids around the SUMO attachment site (Sapetschnig *et al*, 2002, 2004; Fig 6; supplementary Fig 10 online). All Sp3 variants are expressed at similar levels (Fig 6A; supplementary Fig 10A online) and are bound to the *Dhfr* promoter (Fig 6B; supplementary Fig 10B online). *Dhfr* expression in Sp3 knockout MEFs rescued with the wild-type variants is slightly lower than in MEFs transfected with the SUMOylation-deficient mutants (Fig 6C; supplementary Fig 10C online). The observation that *Dhfr* expression is only weakly affected by SUMOylated Sp3 probably reflects stochastic competition of repressive SUMOylated Sp3 with highly abundant, transcriptionally active Sp1 for binding to the GC-boxes of the *Dhfr* promoter (Fig 6D; supplementary Fig 10B online). Nevertheless, Mi-2, L3MBTL2, HP1, SETDB1, SUV4-20H, as well as H3K9me3 and H4K20me3 modifications are present at the *Dhfr* promoter in Sp3 knockout MEFs rescued with the wild-type isoforms but not in MEFs expressing the SUMOylation-deficient Sp3 mutants (Fig 6D; supplementary Fig 10D online). This result indicates that the SUMO modification of Sp3 is essential for corepressor recruitment and the establishment of heterochromatin-like structures at the endogenous *Dhfr* promoter.

DISCUSSION

Here, we have shown that SUMO-modified transcription factors can provoke the establishment of local heterochromatin-like structures. This process includes recruitment of Mi-2, MBT-domain proteins, HP1 and the HMTs SETDB1 and SUV4-20H, together with the establishment of repressive histone modifications such as H3K9 and H4K20 trimethylation. At this stage, we do not know to what extent the individual proteins contribute to SUMO-mediated silencing. In *Drosophila* cells, RNAi-mediated knockdown of Mi-2 and the MBT-domain protein Sfmbt abrogated transcriptional repression by SUMOylated Sp3 significantly (Stielow *et al*, 2008). However, short interfering RNA-mediated knockdown of mammalian Mi-2α, Mi-2β, L3MBTL1 or L3MBTL2 did not result in significant and reproducible abrogation

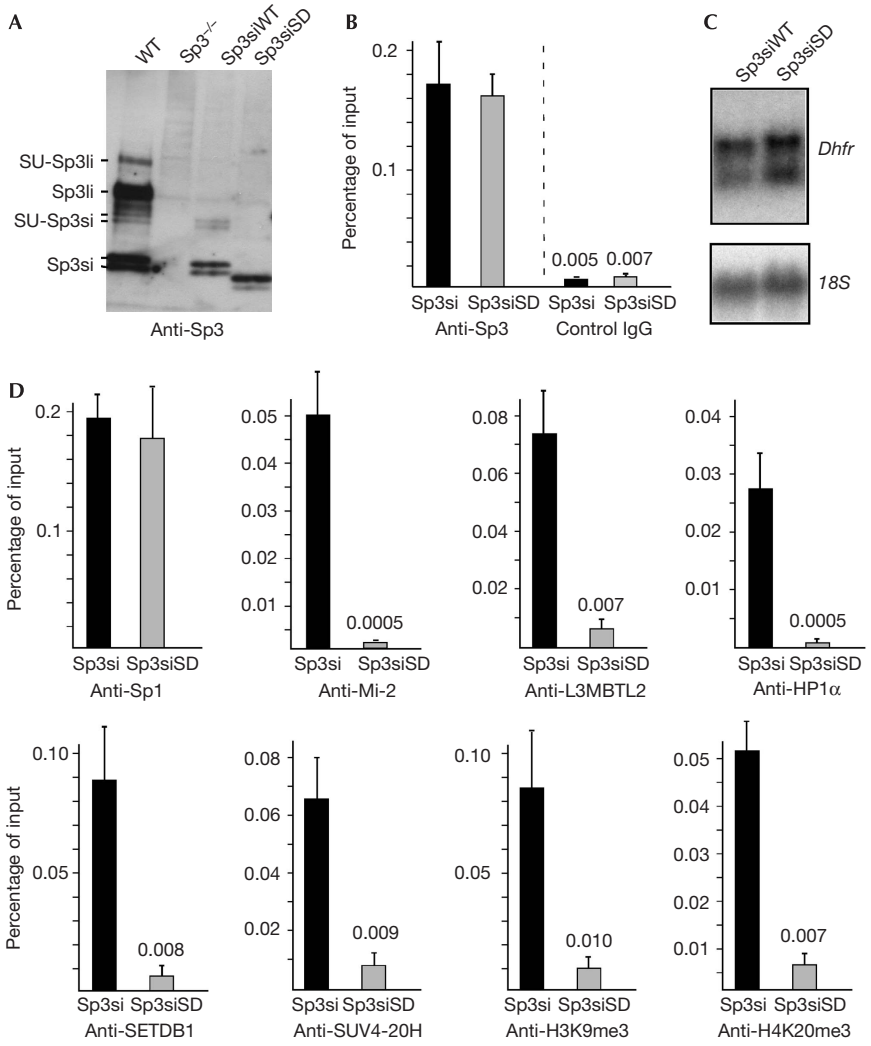

Fig 6 | Repression components and heterochromatic marks at the *Dhfr* promoter in Sp3 knockout mouse embryonic fibroblasts rescued with Sp3si. (**A**) Immunoblot analysis of MEF extracts. (**B,D**) Chromatin immunoprecipitation assays. Immunoprecipitated DNA from *Sp3*$^{-/-}$ MEFs rescued with Sp3siWT or Sp3siSD was amplified by quantitative PCR with primers specific for the *Dhfr* promoter. DNA recoveries are expressed as percentage of input (mean ± s.d.). (**C**) Northern blot analysis of *Dhfr* expression in rescued MEFs. li, long isoform; MEFs, mouse embryonic fibroblasts; SD, small deletion; si, small isoform; SU, sumo; SUMO, small ubiquitin-like modifier; WT, wild type.

of Sp3-SUMO-modified repression (data not shown). This could be due to inefficient depletion of individual proteins, as immunoblot analyses showed significant residual protein after RNAi in all cases. In addition, functional redundancy might obscure activation after knockdown of a single protein. Knockdown of a single protein does not necessarily abrogate the recruitment of other repression components provoked by SUMOylation. For example, RNAi against SETDB1 prevented recruitment of SETDB1 to the *Dhfr* promoter; however, other repression components such as Mi-2, L3MBTL2 and SUV4-20H were still present (supplementary Fig 8 online). Similarly, in SUV4-20H1/2 double knockout MEFs, all other repression components are still associated with the *Dhfr* promoter (supplementary Fig 9 online). These results indicate that at least some of the repression components are recruited independently of each other.

SETDB1 and Mi-2α can interact directly with SUMO1 and SUMO2 through specific SUMO-interacting motifs (Rosendorff *et al*, 2006; Ivanov *et al*, 2007). Moreover, SETDB1 is associated with MCAF1/mAM (MBD1-containing chromatin-associated factor 1/murine ATFa-associated modulator; Ichimura *et al*, 2005; Wang *et al*, 2003) that converts SETDB1 activity from a dimethylase to a trimethylase (Wang *et al*, 2003). MCAF1 is a known SUMO-interacting protein (Uchimura *et al*, 2006) and might thus also be involved directly in Sp3-SUMO-dependent heterochromatin formation. SUMO-interacting motifs are also present in L3MBTL1 and L3MBTL2, and Sfmbt, the *Drosophila* orthologue of L3MBTL2, can bind directly to SUMO and

SUMO-modified Sp3 *in vitro* (Stielow *et al*, 2008). Taken together, it is likely that several direct SUMO contacts of proteins involved in the process of heterochromatin formation are necessary for final gene silencing.

Gene silencing by SUMOylated Sp3 and SF-1 resembles, to some extent, gene silencing mediated by KAP1 (KRAB-associated protein 1), a corepressor for KRAB (Krüppel-associated box) zinc-finger proteins (Ayyanathan *et al*, 2003). SUMOylation of KAP1 mediates recruitment of SETDB1 and Mi-2 to KAP1-responsive promoters (Ivanov *et al*, 2007); moreover, a transgene silenced by KRAB–KAP1 is enriched with H3K9me3 and H4K20me3 (Sripathy *et al*, 2006). We speculate that recognition of SUMO-modified transcriptional regulators by components of repression machineries is a general phenomenon.

METHODS

Plasmids, the generation of stable cell clones and the integration site mapping of the transgene in clone c1 are provided in the supplementary information online.

Antibodies. For immunodetection and ChIPs, the following antibodies were used: anti-Gal4 (Covance Research Products, Emeryville, CA, USA, PRB-255C), anti-SUMO1 (Zymed, San Francisco, CA, USA, 33-2400; Alexis Biochemicals, Lausen, Switzerland, 210-174-R200), anti-Mi-2α/β (Santa Cruz Biotechnology, Santa Cruz, CA, USA, sc-11378), anti-L3MBTL1 (LP-Bio, AR-0160), anti-L3MBTL2 (Lake Placid Biologicals, Lake Placid, NY, USA, AR-0161), anti-HP1α (Upstate Millipore, Temecula, CA, USA, 05-689), anti-HP1β (Abcam, Cambridge, UK, ab49938), anti-HP1γ (Upstate, 05-690), anti-SUV39H1 (Abcam, ab12405), anti-SETDB1 (Upstate, 07-378), anti-SUV4-20H1/2 (Abcam, ab18186), anti-H3K9me2 (Upstate, 07-441), anti-H3K9me3 (Upstate, 07-442), anti-H3K27me3 (Abcam, ab6002), anti-H4K20me1 (Abcam, ab9051), anti-H4K20me2 (Upstate, 07-367) and anti-H4K20me3 (Upstate, 07-463). The SUMO2/3 antibody (Saitoh & Hinchey, 2000) was obtained from Hisato Saitoh; the antibody specific for RNA polymerase II was a gift from Dirk Eick.

Chromatin immunoprecipitation and quantitative PCR. For ChIP experiments, 1×10^6 HEK 293 cells containing the stably integrated 5xGal4-luciferase reporter were transfected with 3 μg of Gal4 fusion expression constructs as indicated in the figures using the Fugene™ transfection reagent (Roche, Mannheim, Germany). At 24 h after transfection, chromatin was prepared by using the Upstate ChIP Assay Kit. Chromatin was sheared with the Diagenode Bioruptor. Preclearing, immunoprecipitation, washing and elution were performed in accordance with the protocol provided by the manufacturer (Upstate). qPCRs were performed in triplicate using the ImmoMix reagent (Bioline, London, UK) including SYBRgreen on the Mx3000P (Stratagene, La Jolla, CA, USA). Results are presented as fold enrichment compared with unrelated antibodies (preimmune IgGs) or as percentage of input calculated by the $2^{-\Delta\Delta C_t}$ method (Livak & Schmittgen, 2001). Primer pairs used for qPCRs are provided in the supplementary information online. ChIPs for the endogenous mouse *Dhfr* promoter in MEFs were performed as described previously (Stielow *et al*, 2008).

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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