The TAF10-containing TFIID and SAGA transcriptional complexes are dispensable for early somitogenesis in the mouse embryo

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Abstract
During development, tightly regulated gene expression programs control cell fate and patterning. A key regulatory step in eukaryotic transcription is the assembly of the pre-initiation complex (PIC) at promoters. The PIC assembly has mainly been studied in vitro, and little is known about its composition during development. In vitro data suggests that TFIID is the general transcription factor that nucleates PIC formation at promoters. Here we show that TAF10, a subunit of TFIID and of the transcriptional co-activator SAGA, is required for the assembly of these complexes in the mouse embryo. We performed Taf10 conditional deletions during mesoderm development and show that Taf10 loss in the presomitic mesoderm (PSM) does not prevent cyclic gene transcription or PSM segmental patterning, while lateral plate differentiation is profoundly altered. During this period, global mRNA levels are unchanged in the PSM, with only a minor subset of genes dysregulated. Together, our data demonstrate that the TAF10-containing canonical TFIID and SAGA complexes, are dispensable for early paraxial mesoderm development, arguing against the generic role in transcription proposed for these fully assembled holo complexes.
Introduction

In mouse, the posterior part of the paraxial mesoderm, called presomitic mesoderm (PSM), generates a pair of somites every two hours and plays crucial roles during vertebrate elongation (Pourquié, 2011). This rhythmic process is under the control of a clock characterized by periodic waves of transcription of cyclic genes sweeping from the posterior to the anterior PSM (Hubaud and Pourquié, 2014). In the anterior PSM, the clock signal is converted into a stripe of expression of specific segmentation genes that defines the future somite. This periodic transcription initiation associated to the segmentation clock oscillations in the PSM offers a unique paradigm to study transcriptional regulation in development.

During embryogenesis, gene expression is regulated by a combination of extracellular signals triggering intracellular pathways, which converge towards the binding of transcription factors to enhancers and promoters. These interactions lead to the assembly of the transcriptional machinery. In non-plant eukaryotes, there are three RNA polymerases able to transcribe the genome and among them, RNA polymerase II (Pol-II) is responsible for the production of mRNA and some of the non-coding RNAs (Levine et al., 2014).

Transcription initiation requires the assembly of the Pre-Initiation Complex (PIC) that allows the correct positioning of Pol-II on the promoter and the consequent RNA synthesis (Sainsbury et al., 2015). TFIID is the first element of the PIC recruited to active promoters. In its canonical form in higher eukaryotes, it is composed of TATA binding protein (TBP) and 13 TBP-Associated Factors (TAFs) and is involved in the correct positioning of Pol-II on the transcription start site. While TBP is also part of Pol-I and Pol-III transcription complexes, the TFIID-TAFs are specific for Pol-II transcription machinery. Among the metazoan TAFs, TAF9, TAF10 and TAF12 are also shared by Spt-Ada-Gcn5-acetyl transferase (SAGA) complex, a transcriptional co-activator conserved from yeast to human (Spedale et al., 2012). SAGA exhibits HAT activity at the promoters and also deubiquitylates histone H2Bub in gene bodies (Bonnet et al., 2014; Wang and Dent, 2014; Weake et al., 2011).

Several structural TAFs, including TAF10, share a Histone Fold Domain (HFD) involved in their dimerization with specific partners: TAF10 heterodimerizes with TAF3 or TAF8 within TFIID and with SUPT7L/ST65G within SAGA (Leurent et al., 2002; Soutoglou et al., 2005). Nuclear import of TAF10 is absolutely dependent on heterodimerization with its partners since TAF10 does not have a nuclear localization signal (NLS) (Soutoglou et al., 2005).
TAF10 does not exhibit any enzymatic activity but serves as an interface allowing interaction with other TAFs (Bieniossek et al., 2013; Trowitzsch et al., 2015) or transcription factors such as the human estrogen receptor α (Jacq et al., 1994) or mouse GATA1 (Papadopoulos et al., 2015). In HeLa cells, only 50% of the TFIID complexes contain TAF10 (Jacq et al., 1994). A lower proportion of TFIID complexes lacking TAF10 has also been observed in F9 cells (Mohan et al., 2003), but their functionality is unknown. The structure of TFIID in the absence of TAF10 is unclear. Only partial TFIID sub-complexes, not associated with TBP, were detected in undifferentiated and retinoic acid (RA) differentiated Taf10 mutant F9 cells (Mohan et al., 2003), while lack of TFIID was observed in Taf10 mutant liver cells (Tatarakis et al., 2008). SAGA was not investigated in these experiments (Mohan et al., 2003; Tatarakis et al., 2008). Altogether, these data support the idea that TFIID composition can vary, as also suggested by the existence of TAF paralogs and/or tissue specific TAFs (Goodrich and Tjian, 2010; Müller et al., 2010).

The diversity in TFIID's composition may have functional consequences. Whereas TAF10 is crucial for survival and proliferation of F9 cells, it is dispensable for their differentiation into primitive endoderm (Metzger et al., 1999). Taf10 mutation in mouse leads to embryonic lethality shortly after implantation (Mohan et al., 2003). Interestingly, while inner cell mass cells die by apoptosis, trophoectodermal cells survive, although Pol-II transcription is greatly reduced (Mohan et al., 2003). Taf10 conditional deletion in skin or liver has shown that TAF10 is required for transcription in the embryo, but not in the adult (Indra et al., 2005; Tatarakis et al., 2008). Altogether, these data indicate that TAF10 requirement depends on the cellular and developmental context.

In this study, we aimed to closely analyse TAF10 requirement and its role in transcription during mouse development, and to examine the composition of TFIID and SAGA in the absence of TAF10 in embryonic tissues in vivo. We performed immunoprecipitations coupled to mass spectrometry analyses on embryonic lysates. We show that, in the mouse embryo, absence of TAF10 severely impairs TFIID and SAGA assembly. In order to get insights into the functional importance of TAF10 during development, we focused on paraxial mesoderm dynamic differentiation by carrying out a Taf10 conditional deletion in the mesoderm using the T-Cre line (Perantoni, 2005). While loss of Taf10 eventually led to growth arrest and cell death around E10.5, we identified a time window during which the dynamic transcription of cyclic genes is still maintained in the absence of detectable TAF10 protein. Microarray analysis of mutant PSM revealed that Pol-II
transcription is not globally affected in this context, although expression of some genes, such as genes encoding cell cycle inhibitors, is up regulated.

**Results**

**TAF10 is ubiquitously expressed in the nucleus of embryonic cells at E9.5**

Taf10 is ubiquitously expressed in the mouse embryo at E3.5, E5.5 and E7.5 but with more heterogeneity at E12.5 (Mohan et al., 2003). Whole-mount in situ hybridization (WISH) analyses suggest that Taf10 is also ubiquitously expressed at E8.5 and E9.5 (Fig. S1A,B). TAF10 protein is ubiquitously expressed in the posterior part of the embryo (Fig. S1C, Fig. S2) and no heterogeneity was observed between E9.5 and E10.5. Competition with the peptide used to raise the anti-TAF10 antibody (Mohan et al., 2003) confirms that TAF10 localization is specific since the TAF10 signal, but not the Myogenin signal, is lost under these conditions (Fig. S1D,H). Altogether these results indicate that TAF10 protein is ubiquitously expressed in cell nuclei between E8.5 and E10.5.

**Induced ubiquitous deletion of Taf10 leads to growth arrest at E10, but does not impair transcription at E9.5**

In order to analyse the effects of TAF10 absence on development, we performed a Taf10 inducible ubiquitous deletion using the R26CreERT2 line (Ventura et al., 2007). This strategy deletes the exon 2 of Taf10 that encodes for part of the HFD (Mohan et al., 2003). Since the exon 3 is out of frame, this deletion is expected to produce a 92 amino-acids-truncated protein without HFD (Fig. S3D). Since the HFD is required for heterodimerization and integration of TAF10 into TFIID and SAGA (Leurent et al., 2002; Soutoglou et al., 2005), this potential truncated protein cannot be integrated into mature SAGA or TFIID complexes. Tamoxifen was injected intraperitoneally at E7.5 and Cre recombination was followed by the activity of the Cre reporter allele R26R (Soriano, 1999). Complete Cre recombination is observed at E9.5 (Fig. 1A,B). The development of R26CreERT2/+;Taf10floxflox (R26Cre;Taf10) mutant embryos was arrested at E9.5 as embryos do not further develop when recovered at E10.5 and E11.5 (Fig. 1D,F). The normal development of R26R+;Taf10floxflox embryos littermates (Fig. 1C,D) confirmed that tamoxifen injection at E7.5 does not induce secondary defects.

Whereas no TAF10 protein was detected by western blot at E9.5 after tamoxifen injection at E7.5 (Fig. 1G), TAF10 was still present, albeit at lower levels, at E8.5 (Fig. S3E). This observation is in agreement with a previous study in which TAF10 protein was still
detected one day after induction of its depletion (Metzger et al., 1999). Since our goal is to assess TFIID and SAGA composition in absence of TAF10, we performed our analyses at E9.5.

In order to assess transcription initiation in vivo, we used the Luvelu line (Aulehla et al., 2008) that allows visualization of the dynamic waves of Lfng transcription occurring every 2 hours in the posterior PSM. This line contains the promoter and 3'-UTR destabilizing sequences of the cyclic gene Lfng (Cole et al., 2002; Morales et al., 2002), coupled to the coding sequences of a Venus-PEST fusion. Luvelu expression is not affected in the absence of TAF10 at E9.5 (Fig. 1H,I), clearly indicating that transcription initiation still occurs in the R26Cre;Taf10 mutant embryos, at least in the PSM. Altogether, these results show that in mutants in which Taf10 deletion is induced at E7.5, no TAF10 protein is detected in the PSM at E9.5 yet periodic gene transcription in the PSM is not affected.

Analyses of TFIID and SAGA composition in the absence of TAF10 in the mouse embryo

Next, we set out to analyse TFIID and SAGA composition by mass spectrometry in E9.5 mouse embryos, when no TAF10 protein is detected. To purify these complexes, we collected E9.5 embryos from R26<sup>CreERT2/CrERT2</sup>;Taf10<sup>flox/flox</sup>x Taf10<sup>flox/flox</sup> crosses, treated (mutant) or not (control) with tamoxifen at E7.5. Complete Taf10 deletion was assessed by PCR (data not shown) and western blot analysis that confirmed the absence of detectable full-length TAF10 protein (Fig. 2A). Interestingly, in whole cell extracts from mutants, expression of TBP, TAF4A, TAF5 and TAF6 is not affected, whereas expression of TAF8, the main TFIID partner of TAF10, is strongly decreased (Fig. 2A), indicating that the TAF8-TAF10 interaction is required for the stabilization of TAF8. We then compared TFIID and SAGA composition in the presence or absence of TAF10 by performing immunoprecipitations (IPs) of different TFIID and SAGA subunits using anti-TBP, or anti-TAF7 antibodies (for TFIID) and with anti-TRRAP or anti-SUPT3 (for SAGA). Composition of the immuno-precipitated complexes was analysed by mass spectrometry (Table S1). The normalized spectral abundance factor (NSAF) values were calculated to compare between control and Taf10 mutant samples (Zybailov et al., 2006).

In control embryos, the full-length TAF10 protein is represented by 4 peptides (Fig. S4A). In mutant embryo samples, no TAF10 peptides were detected in TBP- and TRRAP-IPs. In contrast, in TAF7- and SUPT3-IPs, we detected significant amounts (albeit reduced compared to control) of the TAF10 N-terminal peptide (peptide #1) (Fig. S4B,C). The
Taf10\textsuperscript{flox} conditional mutation deletes the exon 2 resulting in an out of frame fusion of exon 1 to exon 3 leading to premature truncation of the protein TAF10. This deletion is thus expected to produce a truncated N-terminal fragment of TAF10 containing peptide #1, but not the other peptides (Fig. S4D). The fact that no TAF10 peptides are detected in TBP- and TRRAP-IPs suggests that the truncated N-terminal peptide remaining in the mutant cannot participate to fully assembled TFIID or SAGA complexes. In addition, importantly, no TFIID subunits could be immunoprecipitated from murine R26\textsuperscript{CreERT2}\textsuperscript{R}; Taf10\textsuperscript{flox/flox} ES cells, after 4-hydroxy tamoxifen treatment, with an antibody that recognizes the N terminal part of the TAF10 protein (Fig. S3B) and is able to immunoprecipitate the TFIID complex (Fig. S5A,B), showing that the truncated peptide is not part of a fully assembled TFIID complex. No conclusion could be drawn for the SAGA complex since this anti Nterm-TAF10 antibody is not able to co-immunoprecipitate any of the mouse SAGA subunits even in control conditions (Fig. S5C). These data are consistent with the fact that the mutant truncated protein does not contain the HFD (Soutoglou et al., 2005). Thus, for further analyses and to score only the full-length protein, we took into account peptides #2 to #4 that should be absent from the full-length TAF10 protein after deletion of the genomic sequences (TAF10\textsuperscript{*}, Fig. 2D and Fig. 3C, and Fig. S4A,D) for TAF7-IPs (Fig. 2D) and SUPT3-IPs (Fig. 3C). Data for TAF7- and SUPT3-IPs taking into account all the TAF10 peptides are shown in Fig. S6.

TBP is also part of SL1 and TFIIB complexes, involved in Pol-I and Pol-III transcription, respectively (Vannini and Cramer, 2012). Importantly, TAF10 absence does not perturb the interaction of TBP with its non-TFIID partners, highlighting the lack of non-specific effect (Fig. 2B). In Taf10 mutant embryos, we observed an increased interaction between TBP and the larger SL1 subunits; TAF1A and TAF1C, suggesting that TBP may be redistributed in Pol-I TAF-containing complexes in TAF10 absence. This is consistent with the observation that there is no free TBP in the cells (Timmers and Sharp, 1991). In control TBP- and TAF7-IPs, all the canonical TFIID subunits were detected (Fig. 2C,D).

Interestingly, in Taf10 mutant embryos, TBP-IP reveals that TBP is mostly disengaged from TFIID as only a few TAFs co-immunoprecipitate with TBP in low amounts (Fig. 2C). This TFIID dissociation is also observed in the TAF7-IP in absence of TAF10 (Fig. 2D). Surprisingly however, due to the very efficient TAF7-IP (Table S1), we can still detect residual canonical TFIID complexes (Fig. 2D). It is important to note that, if the anti-TAF7 antibody is able to co-immunoprecipitate most of the TAFs, TAF9B, TAF12 and TAF13 are not detected in the mutant, further supporting the conclusion that TAF10 absence strongly affects TFIID assembly.
In order to assess SAGA composition, we performed IPs against two SAGA subunits: SUPT3 and TRRAP. TRRAP is also a member of the chromatin remodelling complex TIP60/NuA4 (Sapountzi and Côté, 2011). As the interactions between TRRAP and TIP60/NuA4 subunits were not affected (Fig. 3A), we conclude that TAF10 absence does not interfere with the interactions between TRRAP and its non-SAGA partners. In both mutant TRRAP-IP (Fig. 3B) and SUPT3-IP (Fig. 3C), we observed a dramatic reduction in the amount of SAGA subunits co-immunoprecipitated, clearly showing a defect in the assembly of SAGA. Contrary to TAF7-IP, we were not able to detect any residual canonical SAGA complexes in the mutant samples.

Altogether, these results strongly suggest that TAF10 is crucial for the assembly of both TFIID and SAGA in the mouse embryo, since the formation of both complexes is seriously impaired in R26Cre;Taf10 mutant embryos.

**Taf10 conditional deletion in the paraxial mesoderm**

Our next goal was to analyse TAF10 requirement for transcription during development. Somitogenesis is a dynamic developmental process in vertebrate embryos relying on periodic transcriptional waves sweeping from posterior to anterior in the PSM (Hubaud and Pourquié, 2014). As described above, the dynamic expression of the Luvelu cyclic reporter is not affected in the PSM of E9.5 R26Cre;Taf10 mutant embryos (Fig. 1H,I).

We next carried out a Taf10 conditional deletion of in the PSM using the T-Cre line (Perantoni, 2005). This line expresses Cre in the primitive streak under the control of 500-bp T promoter sequences (Clements et al., 1996) leading to efficient recombination in the mesoderm before E7.5, including in paraxial mesoderm progenitors (Perantoni, 2005). Taf10 conditional deletion is embryonic lethal as no T-Cre/++;Taf10floxt/loxt (T-Cre;Taf10) mutants could be recovered at birth (data not shown). At E9.25, control and T-Cre;Taf10 mutant embryos are very similar, except that some mutant embryos show a curved trunk (Fig. 4A,B).

At E10.25, T-Cre;Taf10 mutant embryos exhibit normal anterior development but show an apparent growth arrest of the trunk region, an helicoidal trunk lacking limb buds (Fig. 4C,D) and a degeneration of the allantois and placenta (data not shown). Whereas at E9.25 mutant and control somites were morphologically similar (Fig. 4A,B), E10.25 mutant somites were much smaller compared to the controls (Fig. 4C,D). Similar observations were made using the Hes7-Cre line (data not shown) that has a similar recombination pattern in the mesoderm (Niwa et al., 2007). Lysotracker Red staining indicates that there is no obvious cell death in the mutants at E9.25 (Fig. 4E,F). Recombination in the mesoderm is efficient as shown by the
profile of activation of the Cre reporter allele \( R26^R \) at E8.75 (Fig. 4G,H). The full-length TAF10 protein expression could not be detected in the mesoderm of mutant embryos as early as E8.5 (Fig. S7 and 4I-L), including the PSM at E9.5 (Fig. 4I,J), while it is detected in the ectoderm. TAF10 expression was mosaic in the mutant neural tube (NT) which shares common progenitors with the mesoderm (Gouti et al., 2014; Tzouanacou et al., 2009). Surprisingly, these data show that there is a time window around E9.5 when embryonic development is not affected upon TAF10 depletion, except for the absence of limb buds, prior to an apparent growth arrest and decay at E10.5.

**Absence of TAF10 in the PSM does not affect somitogenesis at E9.5**

To get more insight into somitogenesis, we compared the somite numbers between the different genotypes at E9.5 (Fig. 5A). Although no significant statistical differences could be detected, mutant embryos tend to have half a somite less than the other genotypes. This could be explained by a slowing down of somitogenesis at late E9.5 stage.

We next analysed the expression of specific PSM markers using WISH. Expression of the posterior PSM marker \( Msgn1 \) (Wittler et al., 2007) (Fig. 5B,C), of the segmentation gene \( Mesp2 \) (Saga et al., 1997) (Fig. 5D,E) or of the caudal somite marker \( Unca4.1 \) (Neidhardt et al., 1997) (Fig. 5F,G) is not affected in absence of TAF10. WISH of cyclic genes of the Notch (\( Lfg \) (Forsberg et al., 1998; McGrew et al., 1998) and \( Hes7 \) (Bessho et al., 2003), Fig. 5H,I and S8A,B), Wnt (\( Axin2 \) (Aulehla et al., 2003), Fig. S8C,D) or FGF (\( Snai1 \) (Dale et al., 2006), Fig. S8E,F) pathways revealed that the different phases of expression could be observed in \( T-Cre;Taf10 \) mutant embryos. Altogether, the rhythmic transcription of the cyclic genes in absence of TAF10 suggests that active transcription proceeds normally in the PSM of mutant embryos.

**Absence of TAF10 differentially affects the mesoderm derivatives**

Limb bud outgrowth requires signals such as FGF8 from the Apical Ectodermal Ridge (AER) which controls proliferation of the underlying mesenchyme derived from the lateral plate mesoderm (LPM) (Zeller et al., 2009). On E10.25 transverse sections from control embryos, mesodermal nuclei (including in the LPM) are regularly shaped (Fig. 6A,C,E). However, in \( T-Cre;Taf10 \) mutants (Fig. 6B), the paraxial mesoderm nuclei appear normal (Fig. 6D) while in the LPM (and in the intermediate mesoderm (data not shown)), we observed massive nuclear fragmentation characterized by the presence of pyknotic nuclei (Fig. 6F). Since we did not observe any difference in the efficiency in TAF10 protein
depletion between the paraxial mesoderm and the LPM as early as E8.5 (Fig. S7), these data indicate that the LPM is more sensitive to Taf10 loss than the paraxial mesoderm.

We carried out WISH in order to test whether Taf10 loss differentially affects the expression of specific markers of the different types of mesoderm. Expression of the LPM marker Hand2 (Fernandez-Teran et al., 2000) is clearly down-regulated in the mutants (Fig. 6G,H). Similar observations were made with Prdm1 that is expressed in the growing mesenchyme during limb bud outgrowth (Vincent et al., 2005) (data not shown). The absence of Fgf8 induction in the presumptive AER in E9.5 T-Cre;Taf10 mutant embryos (Fig. 6K,L) indicates that the LPM defect is early and probably precedes the cell death in this tissue since no obvious cell death could be detected at E9.25 (Fig. 4F). The cell death observed later on in the LPM is however not caused by the lack of Fgf8 expression as it is also observed at non-limb levels. In contrast, paraxial mesoderm markers analysis shows that Pax3 expression in the anterior PSM and early somites (Goulding et al., 1991) is normal (Fig. 6I,J). Similarly, Fgf8 expression domains in the rostral and caudal lips of the dermomyotome (Crossley and Martin, 1995) are not affected at E9.5 in the mutant paraxial mesoderm (Fig. 6K,L).

Expression of Pax3 in the dermomyotome (Goulding et al., 1991) and of Myf5 in the myotome (Ott et al., 1991) is however decreased in T-Cre;Taf10 mutants (Fig. 6I,J,M,N). Delayed myotome formation was evidenced by immuno-localization of Myogenin or Myosin Heavy Chains at E9.5 and E10.5 (data not shown). Similar observations were made in Hes7-Cre/+;Taf10<sup>flox/flox</sup> mutant embryos (Fig. S9). Expression of Shh in the notochord is normal (Echelard et al., 1993) indicating that the axial mesoderm is not obviously affected in the T-Cre;Taf10 mutant embryos (Fig. 6O,P). Altogether, these results indicate different requirements for TAF10 depending on the type of mesoderm. However, we cannot rule out that the effect seen in the LPM arises secondarily to a defect in the developing paraxial mesoderm.

**Absence of TAF10 does not affect global steady state mRNA and cyclic transcription in the PSM**

Our next goal was to investigate Pol-II transcription status in mutant embryos. We first compared steady state rRNA (Pol-I) and mRNA (Pol-II) transcript levels by quantifying the absolute expression levels of RNA18S versus classical Pol-II housekeeping genes (Actc1, Gapdh and Rplp0) (Fig. 7A). No significant differences between mutant and control samples were detected when comparing the results obtained with 3 different pairs of RNA 18S primers (Fig. 7B). The results are similar for Gapdh and Rplp0 (Fig. 7B). Expression of the Luvelu
reporter (Aulehla et al., 2008) in T-Cre;Taf10 mutant embryos (Fig. 7C,D) supports the idea that cyclic transcription initiation still occurs in the T-Cre;Taf10 mutant PSM at E9.5. Altogether these results indicate that, around E9.5, absence of detectable TAF10 does not affect global steady state mRNA and PSM-specific cyclic transcription.

**Expression of specific genes is altered in the PSM at E9.5 in the absence of TAF10**

We next performed a transcriptome analysis in order to see whether specific genes were affected in absence of TAF10. We performed microarray analyses from micro-dissected PSMs of E9.5 (17-19 somites) control and T-Cre;Taf10 mutant embryos (Fig. 8A). Analysis by scatter plot shows that TAF10 loss has only very minor impact on gene expression (Fig. 8B). We then performed a statistical analysis using Fold Change Ranking Ordered Statistics (FCROS) (Dembélé and Kastner, 2014). We found 369 differentially expressed genes using a fold change cut-off of 1.5x (218 down-regulated and 151 up-regulated, see Table S2). This analysis identified genes related to the cell cycle, TAFs, signalling pathways, or Hox/parahox genes (see Table 1). We also observed that some genes previously identified as cyclic genes in the PSM such as Egr1, Cyr61, Dkk1, Spry4 and Rps3a (Krol et al., 2011), are also differentially expressed in T-Cre;Taf10 mutant PSMs (Table 1 and Fig. S10A). Interestingly, the highest up-regulated gene (4.8 fold) is Cdkn1a encoding a cyclin-dependent kinase inhibitor involved in G1 arrest (Dulić et al., 1994). We also identified Gas5, a tumor suppressor gene encoding 2 IncRNAs and several small nucleolar RNAs in its introns (Ma et al., 2015) as the highest down-regulated gene (from -2 to -4.9 fold). We confirmed the up-regulation of Cdkn1a, Cdkn1c, Ccn1 and Cdkl3, and the down-regulation of Gas5 by RT-qPCR using control and T-Cre;Taf10 mutant tail tips (Fig. 8D). Up-regulation of Cdkn1a and Cdkn1c could explain the growth arrest that is observed in T-Cre;Taf10 mutant embryos.

Some TFIID-TAFs were also up-regulated: Taf5 (1.5 fold), Taf6 (1.7 fold) and Taf9b (1.6 fold) (Table 1, Table S2). We validated these differential expressions by RT-qPCR and found that the expression of most of the genes encoding the other TAFs were also up-regulated (Fig. S10B). The biological significance of these differences is not clear as no obvious increase in protein levels could be observed for TAF4A, TAF5 and TAF6 (Fig. 2A). Taf10 expression is down-regulated in T-Cre;Taf10 mutant tail tips and expression of Taf8, encoding TAF10’s main TFIID partner is also down-regulated in the mutants. These data suggest that the decreased level of TAF8 protein observed in R26Cre;Taf10 lysates (Fig. 2A) could also be related to transcriptional regulation. No differences could be detected for the SAGA-specific TAF5L and TAF6L (Fig. S10C). Altogether, our data show that gene
expression controlled by Pol-II is not initially globally affected in the absence of TAF10, however, the lack of TAF10 could induce a change in the steady state mRNA levels of specific genes.

**Discussion**

The composition of TFIID and SAGA complexes in the developing mouse embryo has not yet been described. Here, we analysed the composition of these complexes in E9.5 mouse embryos in presence and absence of TAF10. We showed that the absence of TAF10 strongly affects TFIID and SAGA formation. *Taf10* deletion during somitogenesis confirmed the requirement of TAF10 during embryonic development in agreement with previous studies (Indra et al., 2005; Mohan et al., 2003; Tatarakis et al., 2008). However, in contrast to these studies, we identified a time window around E9.5 when no obvious somitogenesis defects are detected, despite the absence of detectable full-length TAF10 protein in mutant embryos. In these mutants, transcription is still broadly functional as shown by the lack of global effect on Pol-II transcription.

**TAF10 is required for TFIID and SAGA assembly during development**

Our data demonstrate a global decrease in TFIID and of SAGA assembly in *Taf10* mutant embryos. In F9 cells, in absence of TAF10, TFIID is minimally affected by the release of TBP from the complex, while interaction between the different TAFs is maintained (Mohan et al., 2003) and in the liver, TFIID assembly is completely abrogated (Tatarakis et al., 2008). These differences could be explained either by cell-type specific differences or by difference in the timing of these analyses following *Taf10* deletion as Tatarakis *et al.* (2008) performed their experiments 8-15 days after *Taf10* deletion. The status of SAGA has not been previously investigated in *Taf10* mutant embryos. Our work demonstrates for the first time that not only TFIID, but also SAGA is affected in *Taf10* mutant embryos. Our new data show that the defect in the assembly of canonical TFIID and SAGA is observed already 2 days after the induction of *Taf10* deletion, a timing that coincides with the disappearance of detectable full length TAF10 protein. On the other hand, we can still detect reduced interactions between TAF7 and most of the TAFs following *Taf10* deletion suggesting that, as observed in HeLa or F9 cells, there could be some TFIID-like complexes that do not contain TAF10, albeit in reduced levels. Our data exclude the existence of similar TAF10-less SAGA-like complexes in the embryo.
TAF10 depletion is very efficient since no TAF10 proteins can be detected by western blot in the mutant embryo lysates. Analysis of the detected peptides strongly suggests that only in the TAF7-IP (TFIID), potential full-length TAF10 proteins are detected, albeit at very low frequency. This suggests that very low levels of canonical TFIID complexes could still be present at this stage. Furthermore, these results, in comparison with the SAGA-IPs, suggest that TAF10 is very stable when incorporated into TFIID, probably because of the lower rate of TFIID turnover compared to that of SAGA.

TFIID is built from sub-modules that assemble in the cytoplasm, at least in vitro (Bieniossek et al., 2013; Trowitzsch et al., 2015), and it is likely that such TFIID sub-modules are immunoprecipitated in our experiments since we performed our analyses using whole cell extracts. The TAF7 paralog; TAF7L, that has been associated with germ cells and adipocytes (Zhou et al., 2013a; Zhou et al., 2013b), is not present in TFIID-IPs, indicating that the majority of TFIID contains TAF7, at least at E9.5. However, other TAF paralogs, such as TAF4A and TAF4B, TAF9 and TAF9B are detected. This potential TFIID diversity could exist inside all the cells or could be cell specific and could explain the developmental differences observed between LPM and paraxial mesoderm. However, novel methods will be required to characterize the composition of TFIID and SAGA complexes in a cell type specific manner in the embryo.

A TAF10 truncated protein can potentially be integrated into TFIID and SAGA submodules

Our strategy conditionally removes exon 2 and theoretically leads to the splicing of exon 1 to exon 3 (Mohan et al., 2003). These exons are not in frame and therefore, the 77 amino-acids coded by exon 1 are followed by 15 extra amino-acids in the mutant (Fig. S4D). This mutant protein has the N-terminal unstructured domain of TAF10 but more importantly, lacks its HFD required for the interaction with TAF3, TAF8 or SUPT7L/ST65G (Soutoglou et al., 2005). HFD-HFD interactions are crucial for nuclear import of TFA10 which does not contain any NLS (Soutoglou et al., 2005). Since no TFIID subunits could be co-immunoprecipitated from whole cell extracts of R26\textsuperscript{CreERT2/R;Taf10\textsuperscript{flox/flox}} ES cells after 4-hydroxy tamoxifen treatment with an antibody that recognizes the N terminal part of TAF10 (Fig. S5), it is very unlikely that this truncated protein can be incorporated into mature SAGA and TFIID complexes that are functional in the nucleus. However, we cannot rule out that this truncated protein could incorporate into rare cytoplasmic sub-modules containing TAF7 or
SUPT3. Nevertheless, because the Taf10 mutant heterozygotes are indistinguishable from control embryos (Fig. 1A), this also argues against a dominant negative effect of this peptide.

Another interesting question is the functionality of these potentially partial TFIID and/or SAGA complexes fully depleted of TAF10 protein or containing the truncated TAF10 protein. From our data, it is obvious that these different partial complexes cannot fully compensate for the loss of wild type complexes, but one cannot rule out a partial activity. Future analyses of the difference between the different types of mesoderm could help to elucidate whether such partial non-canonical TFIID and/or SAGA complexes have activities.

**Differential sensitivity to Taf10 loss in the mesoderm**

Taf10 deletion in the mesoderm or in the whole embryo leads to growth arrest that could be explained by the up-regulation of Cdkn1a and Cdkn1c expression. Similar observations were made in yeast (Kirschner et al., 2002) and in F9 cells (Metzger et al., 1999), following depletion of TAF10. Surprisingly, we also observed the down regulation of the tumour suppressor Gas5 that is associated with increased proliferative and anti-apoptosis effects in cancer cells (Pickard and Williams, 2015). Interestingly, Cdkn1a expression is positively controlled by Gas5 in stomach cancer at the transcript and protein levels (Liu et al., 2015). It is thus possible that TAF10 is required for the correct functioning of the Gas5 regulatory network during development.

The phenotypes of null mutations of TFIID-TAFs coding genes such as Taf7 (Gegonne et al., 2012) or Taf8 (Voss et al., 2000) are very similar to that of the Taf10 mutant phenotype (Mohan et al., 2003). In particular, these mutations are embryonic lethal around implantation stage. Moreover, Taf7 null MEFs stop proliferating, suggesting that the growth arrest observed in our mutants is a direct consequence of the failure to properly build TFIID. We cannot exclude a potential contribution of SAGA loss in our mutants. However, deletion of genes coding for different enzymatic activities of SAGA such as Kat2a;Kat2b or Usp22 are embryonic lethal, but with phenotypes much less severe than Taf10 mutation (Lin et al., 2012; Xu et al., 2000; Yamauchi et al., 2000). Interestingly, axial and paraxial mesoderm formation is affected in Kat2a;Kat2b mutants whereas, extra-embryonic and cardiac mesoderm formation is not (Xu et al., 2000), strongly suggesting that SAGA could also have different functions in different types of mesoderm.

Another striking observation is that, while no TAF10 protein could be detected as early as E8.5 in the mesoderm of T-Cre:Taf10 mutant embryos, we observed a difference of sensitivity to Taf10 loss between the LPM (and the intermediate mesoderm) and the paraxial
mesoderm. Interestingly, we observed a very early defect in the LPM with strong down-
regulation of specific markers and absence of limb bud outgrowth. The absence of limb buds
could be explained by a defect in FGF10 signalling activation in the mesoderm and/or by cell
death in the LPM that occurs earlier than in the paraxial mesoderm of T-Cre;Taf10 mutants.
The relative resistance of the mutant paraxial mesoderm to cell death also suggests a
difference of sensitivity. A similar observation has been made in F9 cells where RA induced
differentiation of F9 cells into primitive endoderm rescued the apoptosis of Taf10 mutant cells
(Metzger et al., 1999). Interestingly, this effect was not observed when F9 cells are
differentiated into parietal endoderm in the presence of RA and cAMP (Metzger et al., 1999).
One interesting possibility could be that, being the principal source of RA (Niederreither et
al., 1997), the paraxial mesoderm is protected from cell death in the mutant embryos via an
autocrine mechanism. Another difference of sensitivity has also been observed in Taf10
mutant blastocysts where inner cell mass dies by apoptosis whereas trophoblast can be
maintained in culture (Mohan et al., 2003). It is interesting to note that trophoblast, primitive
and parietal endoderm are extra-embryonic structures and are not part of the fully-developed
embryo. The difference of sensitivity to the loss of Taf10 in the mesoderm is the first to be
observed in vivo, in an embryonic lineage. Since Taf10 was deleted in paraxial mesoderm and
LPM progenitors, we cannot rule out that the LPM increased sensitivity is indirect and
mediated by the paraxial mesoderm, although we did not observe any obvious change in gene
expression in the PSM at a time when limb bud development is already affected.
Nevertheless, a tempting speculation is that TAF10 could serve as an interface of interaction
with a LPM specific transcription factor as it has been described recently for GATA1 during
erthropoiesis (Papadopoulos et al., 2015).
Material and Methods

Mice
Animal experimentation was carried out according to animal welfare regulations and guidelines of the French Ministry of Agriculture (ethical committee C2EA-17 projects 2012-077, 2012-078, 2015050509092048). All the lines have already been described (see supplementary methods). The day of vaginal plug was scored as embryonic day (E)0.5. Tamoxifen (Sigma) resuspended at 20 mg/ml in 5% EtOH/filtered sunflower seed oil was injected intraperitoneally (150 µl (3 mg) for a 20 g mouse) at E7.5.

Embryos whole cell extracts
E9.5 mouse embryos (16-20 somites) were lysed in 10% glycerol, 20 mM Hepes (pH7), 0.35 M NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.1% Triton X-100 with protease inhibitor cocktail (PIC, Roche) on ice. Lysates were treated 3 times with pestle stroke followed by 3 liquid nitrogen freezing-thaw cycles. Lysates were centrifuged at 20817 rcf for 15 min at 4°C and the supernatants were used directly for IPs or stored at -80°C for western blots.

Immunoprecipitations
Inputs were incubated with Dynabeads coated with antibodies (see supplementary methods and Table S3) overnight at 4°C. Immunoprecipitated proteins were washed twice 5 min with 500mM KCl buffer (25 mM Tris-HCl (pH7), 5 mM MgCl₂, 10% glycerol, 0.1% NP40, 2 mM DTT, 500 mM KCl and PIC (Roche)), then washed twice 5 min with 100 mM KCl buffer (25 mM Tris-HCl (pH7), 5 mM MgCl₂, 10% glycerol, 0.1% NP40, 2 mM DTT, 100 mM KCl and PIC (Roche)) and eluted with 0.1 M glycine (pH2.8) for 5 min three times. Elution fractions were neutralized with 1.5 M Tris-HCl (pH8.8).

Western blots
Immune complexes or 15 µg of embryo lysates were boiled 10 min in 100 mM Tris-pH6.8, 30% glycerol, 4% SDS, 0.2% bromophenol blue, 100 mM DTT, resolved on precast SDS-polyacrylamide gel 4-12% (Novex) and transferred to nitrocellulose membrane (Protran, Amersham). Membranes were blocked in 3% milk in PBS for 30 min and incubated with the primary antibody (Table S3) overnight at 4°C. Membranes were washed three times 5 min with 0.05% Tween20 in PBS. Membranes were incubated with HRP-coupled secondary antibodies for 50 min at RT, followed by ECL detection (ThermoFisher).
**Mass spectrometry analyses**

Samples were analyzed using an Ultimate 3000 nano-RSLC (Thermo Scientific, San Jose, California) coupled in line with a linear trap Quadrupole (LTQ)-Orbitrap ELITE mass spectrometer via a nano-electrospray ionization source (Thermo Scientific). Data were analyzed by calculation of the NSAF$_{bait}$ (see supplementary methods).

**Section and Immunolocalization**

Embryos were fixed in 4% PFA 2 hours at 4°C, rinsed 3 times in PBS, equilibrated in 30% sucrose/PBS and embedded in Cryomatrix (Thermo Scientific) in liquid nitrogen vapours. Twenty µm sections were obtained on a Leica cryosotat. Immunolabelling was performed as described (Vincent et al., 2014). Sections were counterstained with DAPI (4',6-diamidino-2-phenylindole, dihydrochloride, Molecular Probes) and imaged with a LSM 510 laser-scanning microscope (Carl Zeiss MicroImaging) through a 20x Plan APO objective (NA 0.8).

**Luvelu imaging**

Freshly dissected embryos were kept in DMEM without red phenol (Life technologies). Luvelu signal was detected using a SP5 TCS confocal microscope (Leica) through a 20x Plan APO objective (NA 0.7).

**Whole-mount in situ hybridization (WISH), X-gal and Lysotracker Red staining**

WISH were performed as described (Nagy et al.). Axin2, Fgf8, Hand2, Lfng, Msgn1, Myf5, Shh, Snai1 and Uncx4.1 probes have been described (Aulehla and Johnson, 1999; Aulehla et al., 2008; Crossley and Martin, 1995; Dale et al., 2006; Echelard et al., 1993; Mansouri et al., 1997; Ott et al., 1991; Srivastava et al., 1997; Yoon et al., 2000). A minimum of 3 embryos were used for the classical markers and a minimum of 7 were used for the cyclic genes. X-gal and Lysotracker Red (Molecular Probes) stainings were performed as described (Rocancourt et al., 1990; Vincent et al., 2014).

**RT-qPCR and statistical analysis**

Micro-dissected embryo tail tip or trunk tissue (without limb buds for the controls) were lysed in 500 µl TRIzol (Life technologies). RNA was extracted according to the manufacturer's recommendations and resuspended in 20 µl (trunk) or 11 µl (tail tips) of RNase free water (Ambion). Reverse transcription was performed using the QuantiTect Reverse Transcription
Kit (Qiagen) in 12 µl reaction volume and diluted by adding 75 µl of RNase free water. Quantitative PCRs were performed on a Roche LightCycler II 480 using LightCycler 480 SYBR Green I Master (Roche) in 8 µl reaction (0.4 µl cDNA, 0.5 µM primers). Four mutants and four controls with the same somite number were analysed in triplicates. Statistical analysis and primer sequences are described in the supplementary methods and Table S4.

Microarrays and statistical analysis
Posterior PSM of E9.5 embryos were individually microdissected (Dequéant et al., 2006) and lysed in 200µl TRIzol (Life technologies), and yolk sac was used for genotyping. Three PSM of 17-19 somites embryos of the same genotype were pooled for one replicate and analysed on GeneChip® MoGene1.0ST (Affymetrix). Data were normalized using RMA (Bioconductor), filtered and FCROS (Dembélé and Kastner, 2014) was used for the statistical analysis (see supplementary methods).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
PB performed, interpreted the experiments and contributed to the writing of the manuscript. AH contributed to the imaging and to the Luvelu experiments and MF initiated the mass spectrometry analyses. MJ performed the MS analyses. OP and LT funded, contributed to the planning and interpretation of experiments, and contributed to the writing of the manuscript. SDV supervised, planned, performed, interpreted the experiments and wrote the manuscript.

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Data availability
Raw microarray data were deposited in GEO database (GSE82186). Raw mass spectrometry data are available via ProteomeXchange (PXD004688).
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Dev 11, 1827–1839.


Table 1. Selection of differentially expressed genes in the PSM of E9.5 T-Cre;Taf10 mutant embryos. The statistical analysis has been performed using FCROS with a cut-off of 1.5 for the fold change. Difference is considered significant for a f value below 0.025 or above 0.975.

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Fig. 1. Efficient ubiquitous deletion of Taf10 in E9.5 R26Cre;Taf10 mutant embryos. (A-F) Whole-mount Xgal staining of E9.5 R26CreERT2/+;Taf10flox/+ (A) or E10.5 (C) and E11.5 (E) R26CreERT2/+;Taf10flox/flox control embryos and E9.5 (B), E10.5 (D) and E11.5 (F) R26CreERT2/+;Taf10flox/flox mutant embryos after tamoxifen treatment at E7.5 (+tam). (G) Western blot analysis of E9.5 R26Cre;Taf10 whole embryos treated (+) or not (-) with tamoxifen at E7.5 with anti-TAF10 (top) and anti-H3 (bottom) antibodies. (H-I) Confocal z-stack images projection of E9.25 R26CreERT2/+;Taf10flox/+;Luvelu/+ non treated (H) or treated (I) embryos with tamoxifen (+tam). Scale bars in A-F and H-I represent 500 µm, and 100 µm, respectively tam; tamoxifen.

Fig. 2. TFIID assembly defect in R26Cre;Taf10 mutant embryos. (A) Western blot analysis of the expression of TBP, TAF4A, TAF5, TAF6, TAF8 and TAF10 from whole cell extracts of E9.5 R26Cre;Taf10 control (left, untreated) or mutant (right, treated with tamoxifen at E7.5) embryos. (B) TBP NSAFbait values for SL1 complex subunits (TAF1A, TAF1B, TAF1C, TAF1D and TBP) and TF3B-TBP complex. (C-D) NSAFbait values for TFIID subunits of TBP-IP (C) and TAF7-IP (D). Bait proteins are indicated in red. Control and mutant IPs are indicated in white and grey, respectively. TAF10* corresponds to the full-length TAF10 protein. tam; tamoxifen

Fig. 3. SAGA assembly defect in R26Cre;Taf10 mutant embryos. (A) NSAFbait values for TIP60/NuA4 complex subunits of TRRAP-IP from control (white) and mutant (grey) extracts. (B-C) NSAFbait values for SAGA subunits of TRRAP-IP (C) and SUPT3-IP (D) from control (white) and mutant (grey) extracts. Bait proteins are indicated in red. TAF10* corresponds to the full-length TAF10 protein.

Fig. 4. Efficient Taf10 conditional deletion in the paraxial mesoderm. (A-C) Whole-mount right sided view of control (A,C) and T-Cre;Taf10 mutant (B,D) E9.25 embryos (A,B) and E10.25 (C,D). The arrowhead in C,D indicates the position of the forelimb bud that is absent in the mutant, the arrow indicates the somites. (E,F) Cell death assay by LysoTracker Red staining of E9.25 control (E) and T-Cre;Taf10 mutant (F) embryos. (G,H) Whole-mount X gal stained E8.75 T-Cre/+;R26+/+ control (G) and T-Cre/+;R26+/+;Taf10flox/flox mutant (H) embryos showing the efficient early recombination within the paraxial mesoderm. (I-L) DAPI
counterstained TAF10 immunolocalization on sagittal (IJ) and transverse (K,L) sections from E9.5 (IJ) and E9.75 (K,L) control (I,K) and T-Cre;Taf10 mutant (J,L) embryos. so; somites, PSM; presomitic mesoderm, NT; neural tube, Ec; ectoderm, Pm; paraxial mesoderm. The asterisk (*) in (K,L) indicates background due to secondary antibody trapping in the endoderm lumen. Scale bars in A-H and I-L represent 500 µm and 50 µm, respectively.

**Fig. 5. Absence of TAF10 in the PSM does not affect segmentation.** (A) Somites number quantification (one-way ANOVA, ns; non significant). The error bars indicate the s.e.m. and the middle bar indicates the mean. (B-I) Whole-mount in situ hybridization of E9.5 (B,C,F,G,H,I) and E8.75 (D,E) control (B,D,F,H) and T-Cre/+;Taf10<sup>flox/flox</sup> mutant (C,E,G,I) embryos using posterior PSM marker Msx1 (B,C), segmentation gene Mesp2 (D,E), caudal somite marker Uncx4.1 (F,G) and cyclic gene Lfng (H,I) probes. Dorsal tail tip (B-E,H,I) or right-lateral views (F,G) are presented. Scale bars in B-E,H-I and F-G represent 100 µm and 500 µm, respectively.

**Fig. 6. Absence of TAF10 differentially affects the different types of mesoderm.** (A-F) DAPI-stained transversal sections of E10.25 control (A, magnifications in C,E) and T-Cre/+;Taf10<sup>flox/flox</sup> mutant (B, magnifications in D,F) embryos showing nuclear fragmentation in LPM but normal nuclear morphology in the paraxial mesoderm. (G-P) Whole-mount in situ hybridization of E9.5 control (G,I,K,M,O) and T-Cre/+;Taf10<sup>flox/flox</sup> mutant (H,J,L,N,P) embryos using Hand2 (G,H), Pax3 (I,J), Myf5 (K,L), Fgf8 (M,N) and Shh (O,P). The arrow in M,N indicates the Apical Ectodermal Ridge. Pm; paraxial mesoderm, LPM; lateral plate mesoderm, the asterisks (*) in D and E indicate the endoderm. Scale bars in A-F and G-P represent 50 µm and 500 µm, respectively.

**Fig. 7. Global transcription is not affected in absence of TAF10 in the paraxial mesoderm.** (A) Comparison between Pol-II and Pol-I transcription. Trunk axial structures highlighted in blue were dissected from E9.75 control and T-Cre/+;Taf10<sup>flox/flox</sup> mutant embryos and RT-qPCR was performed for Pol-I and Pol-II specific housekeeping genes. (B) Comparison of averaged and normalized expression of Pol-I (blue) and Pol-II (red) specific markers from control (white) and mutant (grey) samples. **; p-value <0.01 (n=4, Aspin Welch corrected Student’s t-test). The error bars indicate s.e.m. (C,D) Confocal z-stack images projection of E9.5 Luvulu/+ control (C) and T-Cre/+;Taf10<sup>flox/flox</sup>;Luvulu/+ mutant (D). Scale bars in C-D represent 100 µm.
Fig. 8. Limited specific effect on RNA polymerase II transcription in absence of TAF10 in the PSM. (A) Strategy used for the microarray analysis from E9.5 microdissected PSM of control (blue) and T-Cre;Taf10 mutant (red) embryos. (B) Scatter plot comparing gene expression between control and T-Cre;Taf10 mutant PSM. Red dots correspond to statistically significant differences for a fold change greater than 1.5 after a t-test. (C) Vulcano plot comparing gene expression between control and T-Cre/++;Taf10flx/flx mutant PSM after FCROS analysis. Red dots correspond to statistically significant differences for a fold change greater than 1.5. (D) RT-qPCR analysis for cell-cycle genes candidates from E9.25 control (white) and TCre;Taf10 mutant (grey) tail tips: -ΔCp are normalized to Gapdh. ns; non significant, *; p-value <0.05, **; p-value <0.01, ***; p-value <0.001 (n=4, Aspin Welch corrected Student's t-test). The error bars indicate s.e.m.
Figure 2
Figure 3
Figure 4
Figure 7
Figure 8

A: Illustration showing control and T-Cre/+ Taf10^floxed/floxed E9.25 samples.

B: Scatter plot showing expression of the Taf10 mutant compared to control, with red dots indicating FC > 1.5x and p-value < 0.05.

C: Scatter plot with -log10(p-value) on the Y-axis and log2(fold change) on the X-axis, highlighting FC > 1.5x (FCROS f.value < 0.025) for Cdkn1b and Cdkn1c.

D: Bar graph showing -ΔΔCp normalized to Gapdh for control and mutant conditions, with significant differences indicated by ***.