Role of TIF1α as a modulator of embryonic transcription in the mouse zygote

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The first events of the development of any embryo are under maternal control until the zygotic genome becomes activated. In the mouse embryo, the major wave of transcription activation occurs at the 2-cell stage, but transcription starts already at the zygote (1-cell) stage. Very little is known about the molecules involved in this process. We show that the transcription intermediary factor 1 α (TIF1α) is involved in modulating gene expression during the first wave of transcription activation. At the onset of genome activation, TIF1α translocates from the cytoplasm into the pronuclei to sites of active transcription. These sites are enriched with the chromatin remodelers BRG-1 and SNF2H. When we ablate TIF1α through either RNA interference (RNAi) or microinjection of specific antibodies into zygotes, most of the embryos arrest their development at the 2–4-cell stage transition. The ablation of TIF1α leads to mislocalization of RNA polymerase II and the chromatin remodelers SNF2H and BRG-1. Using a chromatin immunoprecipitation cloning approach, we identify genes that are regulated by TIF1α in the zygote and find that transcription of these genes is misregulated upon TIF1α ablation. We further show that the expression of some of these genes is dependent on SNF2H and that RNAi for SNF2H compromises development, suggesting that TIF1α mediates activation of gene expression in the zygote via SNF2H. These studies indicate that TIF1α is a factor that modulates the expression of a set of genes during the first wave of genome activation in the mouse embryo.

Introduction

After germinal vesicle (GV) breakdown, the fully grown oocyte is transcriptionally silent (Bachvarova, 1985). After fertilization, chromatin remodeling has been proposed to provide a window of opportunity for transcription factors to bind the regulatory sequences of genes that must be activated for development to proceed (Ma et al., 2001; Morgan et al., 2005). Concomitantly, a transcriptionally repressed state would be necessary to prevent promiscuous gene expression as a result of a “general permissiveness” of the genome (for reviews see Thompson et al., 1998; Schultz, 2002). In the mouse, two phases of transcriptional activation lead to the transition from maternal to zygotic control of gene expression (Schultz, 2002). The major and most studied wave of activation is the second one, which begins at the late 2-cell stage. However, less is known about the first wave, which occurs in the pronuclei of the zygote and represents 40% of the transcriptional levels observed at the 2-cell stage (Aoki et al., 1997; Bouniol-Baly et al., 1997; Hamatani et al., 2004).

Transcription intermediary factor (TIF) 1 α (Trim24) was first identified as a transcriptional regulator of nuclear receptors and has been shown to interact with numerous proteins involved in chromatin structure (Le Douarin et al., 1995, 1996; Fraser et al., 1998; Nielsen et al., 1999; Remboutsika et al., 2002; Germain-Desprez et al., 2003). TIF1α is one of four TIFs described in mammals that belong to the tripartite motif superfamily of proteins (Le Douarin et al., 1995, 1996; Venturini et al., 1999; Khetchoumian et al., 2004). TIF1β (Trim28) is required for the proper specification of the anteroposterior axis in the mouse (Cammas et al., 2000). Little is known about the biological function of TIF1α, and its expression pattern is only known at late stages of post-implantation development (Niederreither et al., 1999). Here, we have characterized the role of TIF1α in early mouse embryogenesis. We show that TIF1α acts as a modulator of the transcriptional state of a particular set of genes during the first wave of genome activation and that ablation of TIF1α compromises development.
Results

TIF1α expression is gradually restricted to the inner cells of cleavage stage embryos, and the protein translocates into the pronucleus at the onset of genome activation.

We first analyzed the expression pattern of Tif1α in oocytes and throughout preimplantation development by in situ hybridization and RT-PCR. Tif1α was expressed from the GV stage oocyte to the blastocyst (Fig. 1, a and b). Initially, Tif1α transcripts were present in all blastomeres, but as development progressed, Tif1α transcripts became restricted to the inner cells of the embryo (Fig. 1 a). This became evident at the 16-cell stage, and when the blastocyst formed, Tif1α expression was restricted to the inner cell mass (ICM).

At the GV stage, TIF1α protein was detected in the oocyte cytoplasm (Fig. 1 c). Shortly after fertilization, TIF1α remained predominantly cytoplasmic, but it moved to both pronuclei at the mid and late zygote stages. TIF1α became localized to discrete regions associated with the nuclear-like bodies (NLBs), which are a compact mass of DNA surrounded by a perinucleolar chromatin ring that cause the characteristic pattern of DNA staining visible at these stages (Fig. 1, c and d; Kopecky et al., 1995). This distribution was observed in both male and female pronuclei, although in some cases (11 of 32 zygotes analyzed), TIF1α was only seen in the male pronucleus, most likely reflecting the fact that the male pronucleus undergoes transcriptional activation earlier (Bouniol et al., 1995). TIF1α remained associated with NLBs through the 2-cell stage and, although less prominent, throughout the 4-cell stage. This pattern
of localization in dense spots was specific for TIF1α because TIF1β was uniformly distributed throughout the nucleoplasm of the two pronuclei (Fig. 1 e).

The time when we observed TIF1α translocation into the pronuclei coincides with the time when chromatin remodelers and transcription machinery factors, such as Brahma-related gene 1 (BRG-1; SMARCA4), Brahma (BRM; SMARCA2), and high mobility group box 1 (HMGB-1), translocate into the pronuclei (Bellier et al., 1997; Legouy et al., 1998; Beaujean et al., 2000). This is also associated with the appearance of the hyperphosphorylated (active) form of the RNA polII (Bellier et al., 1997), concomitant with the activation of transcription of the embryonic genome (Bouniol et al., 1995). Thus, the change of TIF1α localization from the cytoplasm to the pronuclei occurs at the time of embryo genome activation.

### Sites enriched with TIF1α colocalize with transcription foci and are enriched with chromatin remodelers

To examine whether TIF1α is associated with regions of active transcription in the embryo, we assayed whether the sites of 5-bromo UTP (BrUTP) incorporation in vivo colocalize with TIF1α in the zygote. BrUTP staining was detected throughout the pronuclear nucleoplasm of the zygote, and sites of higher BrUTP accumulation were observed in the periphery and the proximity of the NLBs (Fig. 2 a, + BrUTP; and Fig. S2, available at http://www.jcb.org/cgi/content/full/jcb.200603146/DC1). Immunostaining for TIF1α in BrUTP-injected zygotes revealed that TIF1α colocalized with some of these sites of greater BrUTP incorporation. Note, however, that not all of the BrUTP sites colocalized with TIF1α (Fig. 2 a). This data indicates that TIF1α is recruited to specific sites of RNA synthesis at the late zygote stage.

Because the fully grown oocyte is transcriptionally silent (Bachvarova, 1985), chromatin remodeling is expected to be required after fertilization to enable embryo genome activation. The TIFs are characterized by the presence of a bromodomain in the C terminus, and it is known that bromodomain-containing proteins can have a role in chromatin remodeling, gene repression, and gene activation (Agalioti et al., 2000; Schultz et al., 2001; Ladurner et al., 2003). This led us to examine whether TIF1α colocalizes with chromatin remodelers. We assayed whether the sites of TIF1α accumulation relate to the localization of the ATPase subunits of the mammalian types switching defective–sucrose nonfermenting (SWI–SNF) and Imitation of Switch (ISWI) remodeling complexes. SNF2H (SMARCA5) is the ATPase subunit of the mammalian ISWI complex (Lazzaro and Picketts, 2001). At the late zygote stage, SNF2H localized to small foci throughout the nucleoplasm of both pronuclei and to larger foci around the NLBs (Fig. 2 b). BRG-1 is the ATPase subunit of the mammalian SWI–SNF complex (Kwon et al., 1994). BRG-1 localized to bigger foci than those of SNF2H and displayed increased accumulation around the NLBs (Fig. 2 b).

A similar distribution has been reported for BRG-1 in early zygotes (Legouy et al., 1998). As expected, TIF1α did not colocalize with SNF2H in early zygotes (Fig. 2 b, top). In contrast at the late zygote stage, we found that sites around the NLBs that were enriched with TIF1α were also enriched with both SNF2H and BRG-1 (Fig. 2 b, middle and bottom). Moreover, similar to the pattern of BrUTP incorporation, not all SNF2H and BRG-1 foci contained TIF1α. Thus, sites of accumulation of TIF1α are also enriched with chromatin remodelers in the late zygote stage.

### RNAi or injection of antibodies against TIF1α compromises early development

We next wished to assess the function of TIF1α at the beginning of development of the mouse embryo. To this end, we used two methods: RNAi and injection of antibodies. For RNAi, zygotes were microinjected with double-stranded RNA (dsRNA) for TIF1α at the fertilization cone stage. Injections of dsRNA
for GFP as well as noninjected embryos were used as negative controls. We found that embryos injected with TIF1α dsRNA proceeded through the first cleavage and reached the 2-cell stage at the same time as the control embryos. However, although the control embryos developed normally to the blastocyst stage (noninjected, 96%; n = 85; dsGFP, 92%; n = 70; five independent experiments), the majority of the embryos injected with dsRNA for TIF1α arrested at the 2–4-cell stage (66%; n = 80; five independent experiments; Fig. 3 a and b). 19% of these embryos arrested at the 2-cell stage, 30% arrested at the 3-cell stage, and 15% developed only to the 4-cell stage (Fig. 3 a). To examine whether the down-regulation of TIF1α upon RNAi in zygotes was efficient, we analyzed embryos that had been injected with dsRNA for TIF1α or for GFP by Western blot, which showed that TIF1α protein was efficiently knocked down (Fig. 3 c). We also verified that injection of dsRNA for TIF1α was specific: it did not result in the reduction of TIF1β, E-cadherin, or β-actin mRNA levels in these embryos, and the protein levels of tubulin were unchanged (Fig. 3 c and Fig. S1 a, available at http://www.jcb.org/cgi/content/full/jcb.200603146/DC1, and see Fig. 5 a).

As an additional approach to test TIF1α function, we performed a similar series of experiments, this time blocking TIF1α protein through injection of antibodies. Although the majority of the Flag antibody–injected (87%; n = 35; four independent experiments) and noninjected control embryos (100%; n = 56; four independent experiments) developed to the blastocyst stage, 86% of the embryos injected with antibodies against TIF1α arrested between the 2- and 4-cell stages (n = 50; four independent experiments; Fig. 3 d). Most embryos (46%) arrested at the 3-cell stage, and 20% of the embryos developed to the 4-cell stage. Thus, injection of antibodies, similarly to RNAi, caused the majority of embryos to arrest at the 2–4-cell stage. Although the arrest was slightly stronger upon antibody injection, this is unsurprising, as the injection of antibodies may result in a more immediate neutralization of TIF1α than RNAi. These results show that reducing the levels of TIF1α by two complementary approaches (either through interference with the message or with the protein) results in a decreased number of embryos that develop to the blastocyst stage.

**Ablation of TIF1α function leads to aberrant localization of SNF2H, BRG-1, and RNA polll**

To further understand the phenotype resulting from ablation of TIF1α, we injected TIF1α antibodies before the onset of genome activation and examined the localization of SNF2H and BRG-1 at the late zygote stage, that is, at the time of genome activation. We also analyzed the localization of the active (Ser5-phosphorylated) form of the RNA polIII in the injected zygotes. We found that ablation of TIF1α resulted in a change in the distribution of the active Ser5-phosphorylated form of RNA polIII (Fig. 4 a). The active RNA polII localized in a patchy and foci-network distribution instead of the homogeneous pattern throughout the nucleoplasm observed in the control embryos (n = 10; Fig. 4 a), suggesting an effect on transcription. Blocking of TIF1α resulted in the mislocalization of BRG-1, which was only barely detected in the pronuclei of the injected zygotes and showed a diffuse staining in the cytoplasm (n = 9; Fig. 4, a and c). The distribution of SNF2H was also affected: the small foci observed throughout the nucleoplasm of the control embryos were no longer visible after ablation of TIF1α. Instead, SNF2H accumulated in few larger foci (n = 10; Fig. 4 a). Given that heterochromatin protein 1 (HP1) recruitment and histone

![Figure 3. Ablation of TIF1α reduces the number of embryos that develop to the blastocyst stage.](image-url)
acetylation increase gradually in the zygote and that both are involved in regulation of gene expression (Adenot et al., 1997; Arney et al., 2002; Hediger and Gasser, 2006), we also examined the effect of TIF1α ablation on HP1 localization and histone acetylation. Moreover, HP1 proteins can associate with both active and silent chromatin (Hediger and Gasser, 2006). Ablation of TIF1α did not affect HP1β (n = 15) or HP1γ (n = 9) localization. Similarly, the acetylation status of lysines 14 and 18 of histone H3 remained unchanged and that of histone H4 was not drastically affected (n = 8; Fig. 4 a). Similar results were observed when the same experiments were performed upon RNAi (unpublished data).

Given that TIF1α ablation provoked a change in the localization of the RNA polIII, we next wished to assess whether ablation of TIF1α elicited a general defect in transcription. To this end, we analyzed the pattern of staining of BrUTP incorporation in the late zygote after ablation of TIF1α. The embryos remained transcriptionally active after interference with TIF1α (Fig. 4 b). Ablation of TIF1α did not appear to result in striking differences in the pattern of BrUTP incorporation in comparison with the control groups. However, an in-depth observation revealed that the general signal of fluorescence was more disperse, and the accumulation of BrUTP around the NLBs seemed slightly enhanced. This observation was confirmed by quantification of the area containing transcription foci, which showed a small and reproducible increase in the area being transcribed in the embryos after ablation of TIF1α compared with the two control groups (Fig. 4 b). Thus, blocking of TIF1α did not abolish transcription, consistent with our observation that TIF1α localizes only to specific sites of active transcription (Fig. 2 a), but induced a significant change in the area of BrUTP incorporation.

Mislocalization of BRG-1 and SNF2H observed by immunofluorescence after TIF1α ablation suggested that TIF1α might be involved in the nuclear localization of these two chromatin remodeling proteins in the late zygote. Given that not all of the BRG-1 colocalized with TIF1α (Fig. 2 b), the reduced signal of BRG-1 staining in the pronuclei resulting from TIF1α ablation suggests that TIF1α may play a role in the nuclearization of BRG-1. Alternatively, the absence of TIF1α could affect the expression of SNF2H and/or BRG-1. We attempted to examine by Western blot whether the protein levels of SNF2H and/or BRG-1 were affected after ablation of TIF1α, but because of technical limitations attributable to the amount of material, we could not draw any conclusion. However, we found that the mRNA levels of both BRG-1 and SNF2H were maintained in the embryos after ablation of TIF1α (Fig. S2 b). We observed a slight decrease of the mRNA levels of SNF2H upon TIF1α ablation, which could correlate with the decreased staining that we observed in our immunofluorescence experiments. Thus, our data indicate that ablation of TIF1α function results in the mislocalization of BRG-1 and SNF2H in the zygote.

**TIF1α modulates transcription of a specific set of genes in the embryo**

The mislocalization of the active form of the RNA polIII, together with the change in the transcriptionally active area resulting from TIF1α loss, could indicate that specific sites within each pronucleus was quantified using Volocity, the same software. The graph shows the mean ± SD of at least six replicates for each group of embryos. *, P = 0.0001; t-test. (c) BRG-1 was localized in the cytoplasm and was barely detected in the pronuclei upon TIF1α ablation. Zygotes were microinjected as in panel a and processed for immunostaining with a BRG-1 antibody in parallel. Shown are representatives of at least 10 zygotes.
of initiation of transcription may be disrupted and/or mislocalized in the zygote after TIF1α ablation. Therefore, we next examined whether TIF1α binds to specific genes in the zygote and whether the expression of these genes would be misregulated as a consequence of TIF1α ablation. To this end, we first used a chromatin immunoprecipitation (ChIP) cloning approach in late zygotes, which we modified to circumvent the constraint of the requirement of large amounts of material (see the supplemental text, available at http://www.jcb.org/cgi/content/full/jcb.200603146/DC1). Our approach allowed us to identify 18 candidate target genes of TIF1α in the late zygote. These encode proteins that perform diverse cellular functions (Table I). Second, to validate these target genes and to explore whether the genes identified by ChIP cloning are indeed regulated by TIF1α, we randomly chose 10 of them and examined their expression in embryos that had been subjected to TIF1α RNAi. We injected dsTIF1α in zygotes before the onset of genome activation (at the fertilization cone stage) and performed RT-PCR at a time when the embryos would have been gone through genome activation. We found that 9 out of the 10 genes that we analyzed were indeed misregulated after TIF1α interference (Fig. 5). The changes elicited in the levels of gene expression varied from complete loss of the corresponding mRNA (Emb and C230093Riken), to partial (Vegfa) or very slight (Pascin3 and Eif5a) down-regulation, to robust up-regulation (Tcf2, Mm.55980, 4732486Riken, and A230103N10Rik). Although the expression pattern between the zygote and the 4-cell stage of most of these genes is not known, Emb expression has been shown to increase its mRNA levels around the zygote stage (Wang et al., 2004), consistent with it being one of the genes that requires TIF1α to be activated at the zygote stage (Fig. 5).

To verify whether the changes in gene expression upon TIF1α RNAi were specific, we analyzed the mRNA levels of three genes as internal control: β-actin, TIF1β, and E-cadherin. None of these three genes showed changes in their mRNA levels (Fig. 5 and Fig. S2). This suggests, in agreement with what we observed for the BrUTP incorporation profile, that down-regulation of TIF1α does not elicit a general defect in transcription but only affects the expression of a specific set of genes. Moreover, TIF1α acts not only as an activator of its target genes but can also prevent the activation of others. Importantly, genes such as Vegfa, Tcf2 (HNF1β), Emb, and Eif5a have documented functions in early embryonic development and/or cell growth (Huang et al., 1993; Barbacci et al., 1999; Coffnier et al., 1999; Miquerol et al., 1999, 2000). Thus, our data indicate that TIF1α is required to determine the transcriptional state (active or repressed) of a set of genes in the late zygote.

Table I. Identification of genes regulated by TIF1α in the mouse zygote

| Gene                | Chromosome |
|---------------------|------------|
| Cytoskeleton/processing | 1          |
| Fi3                 | 1          |
| Pascin3             | 2          |
| Ith2                | 2          |
| Emb                 | 13         |
| Npm-Rar             | 5          |
| Translation         |            |
| Eif5a               | 11         |
| Transcriptional regulators |         |
| Tcf2 (HNF1β)        | 11         |
| Npas3               | 12         |
| Signaling           |            |
| Vegfa               | 17         |
| Ephα6               | 16         |
| Prkca               | 2          |
| Unknown genes       |            |
| 4732486Riken cDNA   | 2          |
| C230093Riken cDNA   | 2          |
| A830008007Riken cDNA| 1          |
| cDNA Mm.55980       | 9          |
| 1700012H17 Riken cDNA| 4          |
| A230103N10Rik cDNA  | 11         |
| LOC384193           | 5          |

A subset of TIF1α target genes is misregulated upon RNAi for SNF2H

After observing the mislocalization of SNF2H and BRG-1 upon TIF1α ablation, we hypothesized that if these chromatin remodelers are relevant for its function in the zygote, the expression of at least some of the TIF1α target genes should be affected when either BRG-1 or SNF2H are knocked down. To test this hypothesis and given that SNF2H can coimmunoprecipitate with TIF1α (Fig. S4, available at http://www.jcb.org/cgi/content/full/jcb.200603146/DC1), we performed RNAi for SNF2H using the same conditions as for TIF1α RNAi. Early zygotes at the fertilization cone stage were microinjected with dsRNA for SNF2H; injections of dsRNA for GFP as well as noninjected embryos were used as negative controls. The embryos subjected to SNF2H RNAi divided to the 2-cell stage normally (n = 52). The control embryos developed normally to the blastocyst stage (noninjected, 95%, n = 23; dsGFP, 91%, n = 23). Although approximately half of the embryos injected with dsRNA for SNF2H developed to the morula and blastocyst stages (54%; n = 52), the other half showed developmental arrest between the 2- and 8-cell stages (46%; n = 52; Fig. 5 c).

We then analyzed the same genes that we analyzed after RNAi for TIF1α. RT-PCR of these genes revealed a subset of genes (Emb, C230093Riken, and Vegfa) that showed a drastic down-regulation in their mRNA levels after injection of dsRNA for SNF2H (Fig. 5 b). These genes corresponded to the genes that were down-regulated upon TIF1α RNAi (Fig. 5 a). Similar to what we observed for TIF1α RNAi, we also observed a slight decrease in the expression of Pascin3 and Eif5a after RNAi for SNF2H. We did not observe any effect on the expression of Tcf2, Mm.55980, 4732486Riken, or A230103N10Riken, which remained not expressed in the embryos after RNAi for SNF2H (Fig. 5 b). This was in contrast to what we observed after RNAi for TIF1α, which resulted in a robust up-regulation of the corresponding mRNA for this second group of genes (Fig. 5, compare a and b). Fig. 5 d shows that SNF2H knockdown was induced efficiently in the embryos. Thus, although the effect on gene regulation elicited upon TIF1α ablation was both up- and down-regulation of target genes, RNAi for SNF2H resulted only in down-regulation of the same target genes that were...
We have investigated the role of TIF1α in the early development of the mouse embryo. We show that at the onset of genome activation, TIF1α translocates into the pronuclei and accumulates in specific regions of RNA synthesis that are enriched with chromatin remodelers. Ablation of TIF1α results in the mislocalization of RNA polII, SNF2H, and BRG-1, and in the misregulation of a particular set of genes. Thus, TIF1α is a maternal factor that functions in the first wave of embryonic genome activation as a modulator of the transcriptional state of a subset of genes.

TIF1α was originally cloned because of its ability to interact with nuclear receptors (Le Douarin et al., 1995). Although we cannot rule out a functional interaction with the nuclear receptors at this stage, the expression of the nuclear receptors known to interact with TIF1α is undetectable in the stages of development that are within the time window of our study (Wang et al., 2004). Our data show that TIF1α plays a role as a modulator of embryonic transcription and suggest that its function in the zygote is required for the proper localization of chromatin remodelers and the RNA polII.

Several reports have documented TIF1α acting as a repressor or as an activator in cultured cells and, therefore, its role as a coactivator appears controversial (Le Douarin et al., 1995, 1996; Venturini et al., 1999; Peng et al., 2002). Although the discrepancies could be explained by the differences in the systems used in those studies, it is also likely that TIF1α plays a dual role in regulating repression versus activation of specific genes. This is supported by the effects on gene expression observed here after TIF1α RNAi in early mouse embryos.

TIF1α function could also be regulated, as it may associate with different chromatin remodeling complexes, ultimately causing changes in the transcription of selected genes. Thus, the proteins TIF1α associates with would determine the specificity and the outcome on transcription (activation versus repression). Indeed, remodeling complexes containing BRG-1 and SNF2H can lead to both activation and repression of gene expression (Pal et al., 2003, 2004; Eberharter and Becker, 2004). Our results suggest that TIF1α regulates activation of a subset of its target genes through SNF2H function. Further, lack of recruitment of BRG-1 may also account for some of the changes in gene expression that we observed after TIF1α ablation. Mislocalization of SNF2H, BRG-1, and RNA polII itself suggests that TIF1α may be involved at least partially in the localization of these remodeling complexes in the zygote. In support, we found that SNF2H can coimmunoprecipitate with TIF1α (Fig. S4). Moreover, TIF1α’s ability to nucleate the formation of a ternary complex with coactivators has recently been documented (Teyssier et al., 2005). Thus, we propose that recruitment of TIF1α to specific sites in the genome would ensure the localization of initiating RNA polII on one hand and of chromatin remodeling complexes on the other, and the “choice” of particular chromatin remodeling complexes will determine the outcome on transcription.

The tripartite motif proteins have been implicated in processes such as cell differentiation, growth, and development.
In *Drosophila melanogaster*, Bonus, a TIF homologue, is essential for cell viability and embryogenesis (Beckstead et al., 2001). Of the four TIF members reported in mammals (Le Douarin et al., 1995, 1996; Venturini et al., 1999; Khetchoumian et al., 2004), TIF1β has been shown to be required for the specification of the anteroposterior axis in the mouse (Cammas et al., 2000). However, in view of the observation that TIF1β is also expressed in early embryos, it remains to be established whether TIF1β also plays a role earlier in development. Although the protein motifs in the TIFs are conserved—a tripartite domain composed of a coiled-coiled, a RING (really interesting new gene) domain, and a B-box, and a bromodomain in the C terminus (Reymond et al., 2001)—some molecular differences have been documented that translate into functional differences among the TIFs: only TIF1β can target histone deacetylase activity, thereby acting as a coexpressor, and it localizes to heterochromatin, the latter via interactions with HP1 (Schultz et al., 2001; Cammas et al., 2002). Additionally, TIF1β has so far not been reported to interact with nuclear receptors, in contrast to TIF1α (Le Douarin et al., 1996). Moreover, TIF1α possesses a kinase activity (Fraser et al., 1998) that has not been documented for the other TIFs. Likewise, the RING domain of TIF1γ acts as a ubiquitinase (Dupont et al., 2005), but this activity has so far not been detected in TIF1α or -β. Interestingly, this RING-like ubiquitinase activity is required for ectoderm induction in *Xenopus laevis* (Dupont et al., 2005). The functional heterogeneity of the TIFs may account for the different roles that so far have been assigned to some of them during embryogenesis.

Our work now documents a role for TIF1α in early development and in regulation of transcription in the mouse zygote. In this context, it is important to note that altogether the group of TIF1α target genes that we have identified cover several cellular processes that are essential for early development, such as translation (Elf5α) and adhesion (Flrt3, Emb, and Itih2; Fleming et al., 2000; Schultz, 2002). In fact, the expression of some translation initiation factors correlates with the maternal-to-zygotic transition in mouse embryos (De Sousa et al., 1998). The target genes under the "unknown" category include a conserved mRNA for a protein containing a highly basic lysine domain of unknown function (4732486Riken), a protein with domains predicted to be involved in RNA processing and transcriptional regulation (C230093Riken), and an mRNA deadenylnase (A230103N10Rik). Although the relevance of each of these genes in early development remains to be investigated, their coordinated expression may be of functional significance in the control of the maternal-to-zygotic transition.

The expression pattern of TIF1α in the preimplantation embryo is reminiscent of that of *Oct4/Pou5f1*, which is expressed initially in all blastomeres but then becomes restricted to the ICM, and whose expression is essential for maintaining the pluripotency of the ICM cells (Nichols et al., 1998). Moreover, TIF1α expression decreases upon differentiation of embryonic stem cells (Remboutsika et al., 1999). It is also noteworthy that TIF1α has been reported to be a direct target gene of Nanog in mouse embryonic stem cells (Loh et al., 2006). Thus, in the future, it will be interesting to determine whether expression of TIF1α contributes to the establishment or the maintenance of the pluripotent capacities of the early mouse embryo. Such a role for TIF1α is supported by the failure of most embryos lacking TIF1α to develop to the blastocyst stage, and by the changes in the localization of SNF2H and BRG-1 resulting from TIF1α ablation, both of which are required for ICM and/or trophoderm survival in the mouse (Bultman et al., 2000; Stopka and Skoultchi, 2003). During the early stages of development, decisions about cell fate determination, pluripotency, and patterning are made. Thus, the chromatin has to be dynamically remodeled for opening and closing specific regions in response to those events. TIF1α could take part in this process by activating or repressing particular sets of genes. Our data suggest that TIF1α is a factor involved in epigenetic mechanisms in early mammalian development.

### Materials and methods

#### Embryo collection and culture

Embryos were collected from F1 (C57BL/6 × CBA/H) 6-wk-old superovulated females as described previously (Hogan et al., 1994). For the RNAi experiments, F1 females were mated with EF-1α MmGFP transgenic males (Zernicka-Goetz and Pines, 2001). All other experiments were performed with F1 × F1 crosses. Zygotes and cleavage stage embryos were collected at the indicated hours after human chorionic gonadotropin (hCG) injection and cultured in KSO medium (Stractly Medica, Inc.) as described previously (Hogan et al., 1994). All animals were handled in accordance to Home Office legislation.

#### In situ hybridization

Freshly collected embryos at various stages were fixed in 4% paraformaldehyde in PBS. In situ hybridizations were performed as described previously (Wilkinson et al., 1990), except that the embryos were not dehydrated and the proteinase K treatment was omitted. The TIF1α probe was prepared and labeled with digoxigenin-UTP using the pSK.TIF1α plasmid (provided by R. Losson, Institut de Génétique et de Biologie Moléculaire et Cellulaire [IGBMC], Strasbourg, France) as a template (Niederreither et al., 1999).

#### Immunostaining and confocal analysis

After removal of the zona pellucida with acid Tyrode’s solution (Sigma-Aldrich), embryos were washed three times in PBS and fixed in 5% paraformaldehyde, 0.04% Triton X-100, 0.3% Tween, and 0.2% sucrose in PBS for 20 min at 37°C. After permeabilization with 0.5% Triton X-100 in PBS for 20 min, the embryos were washed three times in 0.1% Tween in PBS (PBS-T), blocked in 3% BSA in PBS-T, and incubated with the primary antibodies for ~12 h at 4°C. Embryos were then washed twice in PBS-T, blocked for 30 min, and incubated for 2 h at 25°C with the corresponding secondary antibodies. After two washes in PBS-T, the DNA was stained with TO-TO-3 (Invitrogen) and the embryos were mounted in Vectashield (Vector Laboratories) and analyzed using a 60×/1.40 oil objective (Nikon) in an upright confocal laser microscope (Radiance, Bio-Rad Laboratories) using the LaserSharp 2000 software (Bio-Rad Laboratories) at room temperature. The antibodies used in this work are as follows: TIF1α (Santa Cruz Biotechnology, Inc.), KAP1 (TIF1β; Abcam), RNA polym (recognizing the CTD phosphorylated in Ser5; CTD4H8; Upstate Biotechnology), BRG-1 (Santa Cruz Biotechnology, Inc.), HSF2H (provided by P. Varga-Weisz, the Babraham Institute, Cambridge, UK; Bozenek et al., 2002), tubulin (Sigma-Aldrich), HP1β (IGBMC), HP1γ (IGBMC), acetylated histone H4 (Upstate Biotechnology), acetylated K14 histone H3 (provided by B. Turner, University of Birmingham, Birmingham, UK), and acetylated K18 histone H3 (Abcam). Secondary antibodies were coupled with either Alexa Fluor 568 or 488. Images were then prepared or analyzed using Photoshop 7 (Adobe) and Velocity (Improvision), respectively.

#### BrUTP labeling

BrUTP labeling was performed as described previously (Borsuk and Małeszewska, 2002). Embryos were collected 24 h after hCG injection and microinjected using a transjector (model S246; Eppendorf) with 1–2 pl of 100 mM BrUTP (Sigma-Aldrich) in 2 mM Pipes and 140 mM KCl, pH 7.4. Embryos were fixed after 3 h of culture and processed for
immunostaining using an anti-BrdU antibody (Sigma-Aldrich). For quantifi-
cation of BrUTP incorporation in pronuclei after microinjection of anti-
bodies, the embryos were collected at 20 h after hCG injection and
microinjected with BrUTP followed by microinjection of antibodies as de-
scribed (see Microinjection of antibodies). For the analysis after immuno-
staining, the area of the pronucleus of injected zygotes was deﬁned and
cropped using Volocity. The pixels were then selected under a 30% toler-
ance level, and the area displaying BrUTP staining was quantiﬁed using
the measurement tools of the software as recommended by the manufacture.

RNAi and RT-PCR
Zygotes were collected and microinjected at 20 h after hCG injection with
1–2 pl of 1 mg/μl long dsRNA for TIF1α, long dsRNA for SNF2H, or long
dsRNA for GFP (Wianny and Zernicka-Goetz, 2000). The sequence for the
dsRNA targeting TIF1α spans nucleotides 1284–1771, which shares no homol-
ogy with the other members of the family. For the SNF2H RNAi experi-
ments, dsRNA spanning nucleotides 2041–2520 of the cDNA was used.
For RT-PCR analysis, embryos were collected ~42 h after dsRNA in-
jection and processed for RT in pools of ﬁve embryos, each using the Dyna-
beads mRNA direct micro kit (Dynal). Embryos were collected at the same
stages for all the samples to avoid variation resulting from embryos derived
from different stages. Half of the mRNA extracted (10 μl) was used for the
reverse-transcriptase controls and the other half for cDNA synthesis. PCR was
performed with 1/20 of the cDNA [0.5 μl], such that all genes were ana-
yzed in the same sample using 60 cycles for ampliﬁcation, except for E1F5a and
E2F2, in which 35 cycles were used, and Ephad, Snf2h, and Brg-1, in which
50 cycles were used. It was veriﬁed that the cycling conditions were
within the exponential phase of ampliﬁcation for each gene. The products
were transferred onto a Hybond N+ membrane (GE Healthcare), hybridized
against the corresponding probes, and exposed for autoradiography.

Microinjection of antibodies
Antibodies against TIF1α and Flag (Sigma-Aldrich) were microinjected
overnight at 4°C against Tris-EDTA, pH 8.0, and concentrated using a ﬁlter
(Centricron; Amicon) to a final concentration of 215 ng/μl (Bevilacqua et al.,
2000). Zygotes collected at 20 h after hCG injection were microinjected
with ~1–2 pl of antibody solution and cultured. For the immuno-
staining analysis, the embryos were ﬁxed after 7 h of culture.

Western blot analysis
Embryos from ﬁve different experiments were collected 42 h after dsRNA
injection, washed three times in PBS, pooled (155 embryos per group) and subjected to Tris-Glycine PAGE-SDS. Competition assays with the corresponding TIF1α-blocking peptide were performed to en-
sure the speciﬁcity of the antibody (Fig. S3, available at http://www.jcb.
org/cgi/content/full/jcb.200603146/DC1).

ChIP and cloning
We ﬁrst assessed the ability of the antibody to immuno precipitate TIF1α in
pronuclei extracts (Fig. S3). For the ChIP, 413 zygotes were collected in
M2 at 27 h after hCG injection, formaldehyde cross-linked, washed, and
lysed in 5 mM Pipes, pH 8.0, 85 mM KCl, and 0.05% NP-40. The pro-
nuclei were then lysed and sonicated. For the immunoprecipitation, 1 μg
of TIF1α antibody was used after preclariﬁcation of the chromat.”}

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