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Programming of a repressed but committed chromatin structure during early development

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The determination of chromatin for transcription during early development as well as the requirement for *trans*-acting factors during this period has been analysed in *Xenopus*. Basal transcription is repressed both during oogenesis and after the mid-blastula transition (MBT), and transactivators are required to relieve this repression. In contrast, transactivators cannot overcome the generalized transcriptional repression which occurs in embryos before MBT. However, they do bind to promoters leading to a repressed but preset chromatin structure. Experiments involving the pre-binding of TATA binding protein (TBP) or of the strong transactivator GAL4–VP16 further show that there is no limiting factor before the MBT, and that it is the recruitment and stabilization of the basal transcription machinery and not of transactivators which is repressed during early development. This multi-step process in gene activation, with activation of promoters temporally uncoupled from their commitment, may be of importance in the regulation of early embryonic events by providing molecular signposts for future determinations.

Keywords: chromatin/mid-blastula transition/transactivation/transcription/*Xenopus*

Introduction

In eukaryotes, transcriptional regulation occurs on DNA compacted into chromatin. Increasing evidence indicates the importance of this organization in the regulation of eukaryotic transcription (for reviews, see Felsenfeld, 1992; Workman and Buchman, 1993; Paranjape *et al.*, 1994; Wolffe, 1994). In order to be active, regulatory factors must succeed in a dynamic competition with nucleosomes. Two levels at which this competition may act are the assembly of the basal transcription complex (Workman and Roeder, 1987) and the binding of factors which mediate transactivaction (Workman *et al.*, 1991). The notion of determination and differentiation during embryonic development may reflect a remodelling of chromatin driven by such competition, resulting in a multi-step process of gene activation (Wallrath *et al.*, 1994).

During *Xenopus* early development, gene regulation is characterized by sudden transitions in transcriptional activity which affect the whole genome. Active transcription occurs during oogenesis, providing the accumulation of maternal RNAs for the early development. Transcription then ceases at maturation and remains fully repressed until the 13th cell cycle post-fertilization. At this stage, the mid-blastula transition (MBT) occurs relatively abruptly with a generalized reactivation of gene expression. The molecular mechanisms underlying this regulation are not known. Different models have been proposed to explain this first transition during embryonic development. It was initially proposed that the titration of a general maternal repressor stored in excess was responsible for the reactivation of transcription (Newport and Kirschner, 1982). Further experimental data indicated that the unusually large excess of maternal histones stored in the egg, able to assemble 6000–20 000 nuclei (Adamson and Woodland, 1974; Laskey *et al.*, 1977; Woodland and Adamson, 1977), was competing dominantly over transcription complex assembly until the MBT (Prioleau *et al.*, 1994). Transcriptional activation at the MBT was then permitted by the almost complete depletion of free maternal histones by the amount of embryonic DNA synthesized in the embryo at the MBT. However, it was not known if this dynamic competition between histones and transcription factors was acting at the level of the assembly of the basal transcription complex or the binding of *trans*-acting factors.

The role of transactivators during early development remains unclear. In oocytes, transcription of polymerase II genes frequently initiates at incorrect sites (Bendig and Williams, 1984; Steinbesser *et al.*, 1988) and, in some cases, transcriptional elements are dispensable (Mohun *et al.*, 1986; Almouzni and Wolffe, 1993). In contrast, transcription of viral genes such as the herpes virus tk genes (McKnight and Gavis, 1980) and simian virus 40 genes (Green *et al.*, 1987), as well as housekeeping genes (Etkin and Maxson, 1980; Grosschedl and Birnstiel, 1980; Mattaj *et al.*, 1985; Bienz, 1986; Nishikura, 1986) requires specific *cis*-acting sequences. In the early embryo, a generalized repression of transcription occurs before the MBT, although polymerase II basal transcription factors are present and are as active as in oocytes (Prioleau *et al.*, 1994). Recently, Almouzni and Wolffe (1995) proposed that repression before MBT not only reflected the presence of a large pool of histones, as previously shown (Prioleau *et al.*, 1994), but also a deficiency in the activity of transcriptional activators prior to the MBT. In contrast to this observation, it has been shown that several transactivating activities are stored in the oocyte and are present and active in their DNA binding activity in extracts from early embryonic stages (Ovsenek and Heikkila, 1990; Ovsenek *et al.*, 1991, 1992). However, little is known regarding their ability to bind DNA in the context of transcriptional repression by the large excess of histones present in the early embryos.

In this report we analysed the requirement for transactivators both during oogenesis and early development, as well as their interaction with chromatin during these developmental stages. We show that basal transcription is repressed both in oocytes and in post-MBT embryos, and that endogenous or exogenous transactivators are necessary to activate transcription at these stages. In contrast, before MBT, transcription is repressed even when in the presence of a strong *trans*-acting factor like GAL4–VP16. However, footprinting analyses show that transactivating factors have access to their sites on the promoter leading to the developmentally regulated formation of a repressed but nevertheless predetermined chromatin structure before the MBT. Finally, the use of potentiated templates allowed us to show that repression of transcription during early development is not due to the inactivation of *trans*-acting factors, as recently suggested (Almouzni and Wolffe, 1995). In contrast, transactivators do bind to their sites but they cannot recruit the basal transcription complex which remains dominantly repressed by chromatin assembly. The formation of pre-bound transactivator–DNA complexes before injection favours the assembly of the transcription complex on naked DNA and an activated transcription complex stabilized against repression is observed.

Results

The binding and activity of transactivators during oogenesis and early development

To study the role of transactivators on RNA polymerase II transcription, we used microinjection of three reporter plasmids. One contains the promoter of the *Xenopus* c-*myc* gene, whose expression has been well characterized during early development (King *et al.*, 1986; Taylor *et al.*, 1986; Vriz *et al.*, 1989). This promoter contains two important upstream regulatory elements containing conserved Sp1 sites that are necessary and sufficient for its expression (Nishikura, 1986; Modak *et al.*, 1993). The second reporter plasmid used contains only the TATA box of the P2 promoter from the c-*myc* gene (pMyc ΔSp1), and allowed us to analyse if basal transcription can occur in the absence of *cis*-acting sequences. The third reporter plasmid contains the same TATA box linked to five GAL4 binding sites, facilitating an analysis of activated transcription in the presence of exogenous GAL4–VP16 protein. GAL4–VP16 is a strong transactivating protein which contains the DNA binding domain from the yeast transcription factor GAL4 and the acidic activation domain from herpes simplex virus coat protein. The injection of this purified transactivating protein, normally not present in the oocyte, permits a better analysis of transcriptional activation.

We initially examined transcription from the microinjected c-*myc* promoter. Figure 1A shows that deletion of the Sp1 sites abolished c-*myc* transcription in the oocyte, suggesting that transactivation was required to permit activation of the basal transcription machinery. The level of stimulation in the presence of Sp1 sites was at least 20-fold. Figure 1B shows that the promoter region spanning these Sp1 sites was a major DNase I-hypersensitive site in the endogenous chromatin of proliferating *Xenopus* A6 somatic cells, where c-*myc* is transcriptionally active (Taylor *et al.*, 1986, and data not shown). In all six hypersensitive sites which can be discerned (HSS I–VI), a pattern very similar to that previously described for the active c-*myc* promoter in both mouse cells (Mango *et al.*, 1989) and human cells (Dyson and Rabbits, 1985) is found. The major hypersensitive site, HSS V, can be resolved into two overlapping sites (Va and Vb) in the region corresponding to the Sp1 sites absolutely required for c-*myc* transcription (Modak *et al.*, 1993). In the oocyte, a similar DNase I footprint is detected in the chromatin of a microinjected c-*myc* promoter containing 5.4 kb of upstream sequence (Figure 1C). Again the major DNase I-hypersensitive site corresponds to the region encompassing the Sp1 sites. We concluded that endogenous transactivators present in the oocyte have access to this site and permit activation of transcription. These data also confirm that this upstream element was sufficient to obtain transcription in oocytes (Figure 1A and our unpublished data using various c-*myc* constructs).

In early embryos, transcription from the injected plasmid pMyc, containing the c-*myc* promoter and associated Sp1 sites, is initially repressed. Transcription is then strongly reactivated at the MBT (Figure 2A), as for the endogenous promoter. Analysis of the plasmid chromatin structure was performed using corresponding samples from the same experiment, taken at different times after injection, as described in Materials and methods (Figure 2B). The DNase I chromatin profiles obtained show that the promoter region containing the Sp1 elements is emerging as the most prominent site which becomes hypersensitive during the first 4 h after injection, in accordance with the kinetics of chromatin assembly occurring on the injected plasmid (Prioleau *et al.*, 1994; R.S.B., unpublished data). At 30 min, the Sp1 hypersensitive site is already created, although this is somewhat masked due to the incomplete nucleosomal assembly at this time, resulting in an unavoidable background cleavage pattern contributed to by naked DNA. Therefore, despite transcriptional repression, the DNase I-hypersensitive site profile created over this region is similar to the one observed after MBT. As time proceeds, the contribution of cleavage in naked DNA decreases as chromatin matures on the plasmid template, and the Sp1 hypersensitive site becomes prominent long before MBT.

We concluded first that the major hypersensitive site present during early development includes Sp1 elements, and that this site is also present in the endogenous chromatin of *Xenopus* A6 somatic cells and in injected oocytes when c-*myc* is active (Figures 1 and 2). This experiment further shows that endogenous transactivators present before the MBT could compete efficiently with chromatin assembly to access their binding sites without activating transcription. The succession of rapid cell cycles is not responsible for the repression (Prioleau *et al.*, 1994), nor does the repression seems to be caused by a block of elongation, as transcripts were not detected in run-on experiments in the presence of sarkosyl (data not shown).

The presence of GAL4–VP16 is not sufficient to activate transcription before MBT

To confirm the regulation observed with the natural c-*myc* promoter, we used a chimeric promoter containing the TATA box from the c-*myc* P2 promoter linked to GAL4 binding sites. The possibility of co-injecting the exogenous

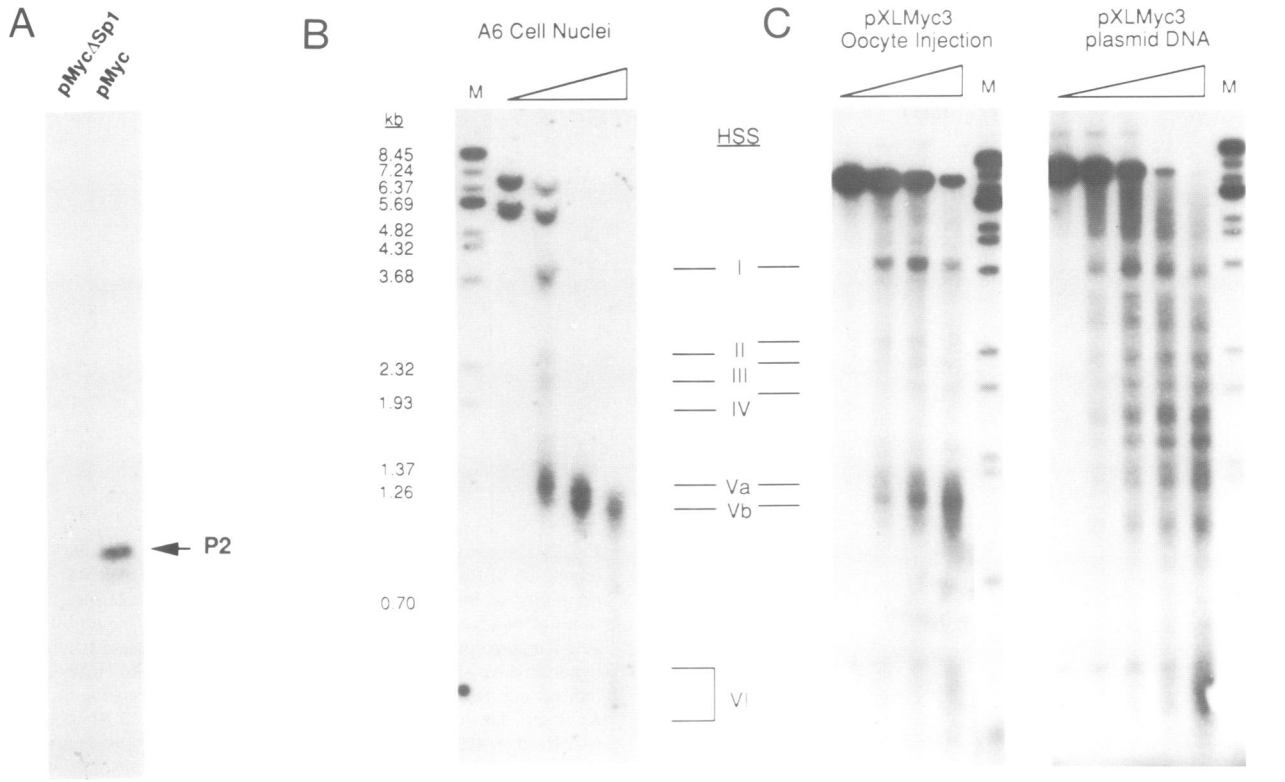


Fig. 1. DNase I-hypersensitive sites in the c-*myc* promoter in somatic cells and in injected oocytes. (**A**) Transcription in oocytes injected with the pMyc plasmid containing Sp1 sites (pMyc) or not (pMycΔSp1) 2 h post-injection. The arrow corresponds to accurately initiated transcription at the P2 promoter of the c-*myc* gene. (**B**) DNase I-hypersensitive site mapping of the endogenous c-*myc* gene in *Xenopus* A6 somatic cells. The mapping was performed by indirect end labelling from an *Eco*RI site in c-*myc* exon 2, as described in Materials and methods. DNase I digestions were performed at 0, 2, 5 and 10 U/ml. The two parental bands of 5.5 and 6.5 kb correspond to the two c-*myc* genes present in *Xenopus* (Taylor *et al.*, 1986). (**C**) DNase I-hypersensitive site mapping in c-*myc* containing minichromomes isolated after injection of pXLMyc3 DNA containing 5.4 kb of the DNA region upstream of the c-*myc* promoter. DNase I digestions were at 0, 2, 5 and 10 U/ml. A control DNase I digest of pure plasmid pXLMyc3 DNA at 0, 0.05, 0.1, 0.2 and 0.4 U/ml DNase I is also shown. *Bst*EII-digested λ DNA was used as a molecular weight marker and the prominent hypersensitive sites (HSS I–VI) are indicated. HSS I corresponds to a previously uncharacterized site ~2.7 kb upstream from promoter P2.

GAL4–VP16 transactivator also permits a more detailed analysis of transcriptional regulation during early development.

Figure 3A shows that the addition of exogenous GAL4–VP16 is absolutely required for transcription of this construct in the oocyte, consistent with the observation with the c-*myc* promoter (Figure 1A). We observed that transactivation was maximal with a 2- to 4-fold molar excess over binding sites (Figure 3A), and that the level of stimulation was at least 30-fold. We concluded that, in oocytes, *cis*-acting sequences and transactivators are required to reach a high level of transcription because basal transcription is strongly repressed.

As the transactivating factor GAL4–VP16 can cooperate with the transcriptional basal machinery in *Xenopus*, it could be a valuable tool to investigate the mechanism of transcriptional inhibition during early development. One explanation for the lack of transcription before MBT might be the absence of transactivation activity, leading to the failure of stable transcription complex assembly in the presence of the excess of histones accumulated in the early embryo.

To address this question, the plasmid carrying five GAL4 binding sites was injected into the fertilized egg without or in presence of GAL4–VP16. Figure 3A shows that no transcription was detected when the reporter plasmid was injected alone, either before or after MBT. Co-injection of GAL4–VP16 was performed with a 4-fold molar excess of GAL4–VP16 over GAL4 binding sites, as this ratio gave the maximum stimulation in the oocyte (Figure 3A), in agreement with the observation that GAL4 is not an abundant protein *in vivo* in yeast (Vashee *et al.*, 1993). These conditions are rather different from those used by Almouzni and Wolffe (1995), where the ratio was ~300. Figure 3B shows that transcription remains repressed during early development, despite the addition of GAL4–VP16. A similar observation was recently reported by Xu *et al.* (1994). However, when MBT is reached, the developmental reactivation of transcription occurs, which is entirely dependent on the presence of GAL4–VP16 protein (Figure 3B). The activation of transcription at the MBT is as efficient as in oocytes and argues against a degradation of the injected GAL4–VP16 during early development. An identical result was obtained using another promoter, the E4 promoter linked to five GAL4 binding sites (data not shown). The reactivation process at the MBT occurs exponentially (Figure 3C), in accordance with the titration of the excess of histones by the exponential increase of genomic DNA synthesized during the succession of cell cycles (Prioleau *et al.*, 1994).

We concluded that both during oogenesis and after the MBT, transcription relies on the presence of *cis*-acting

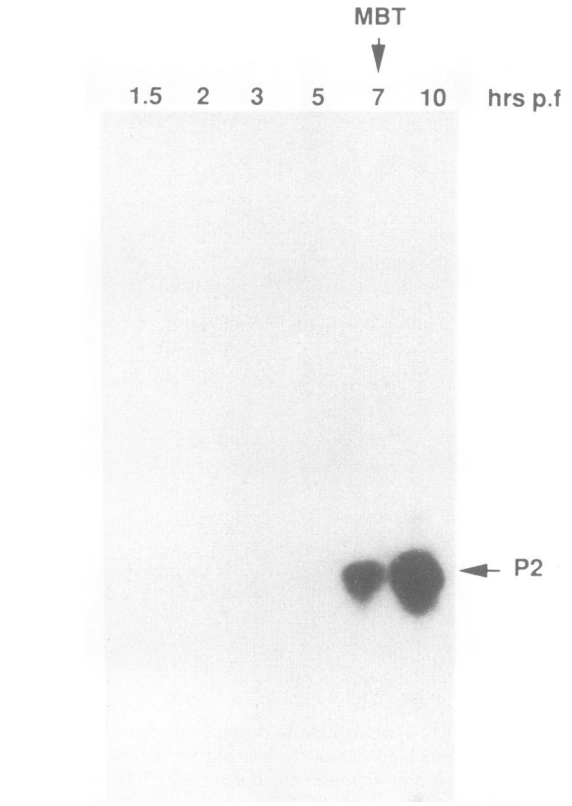
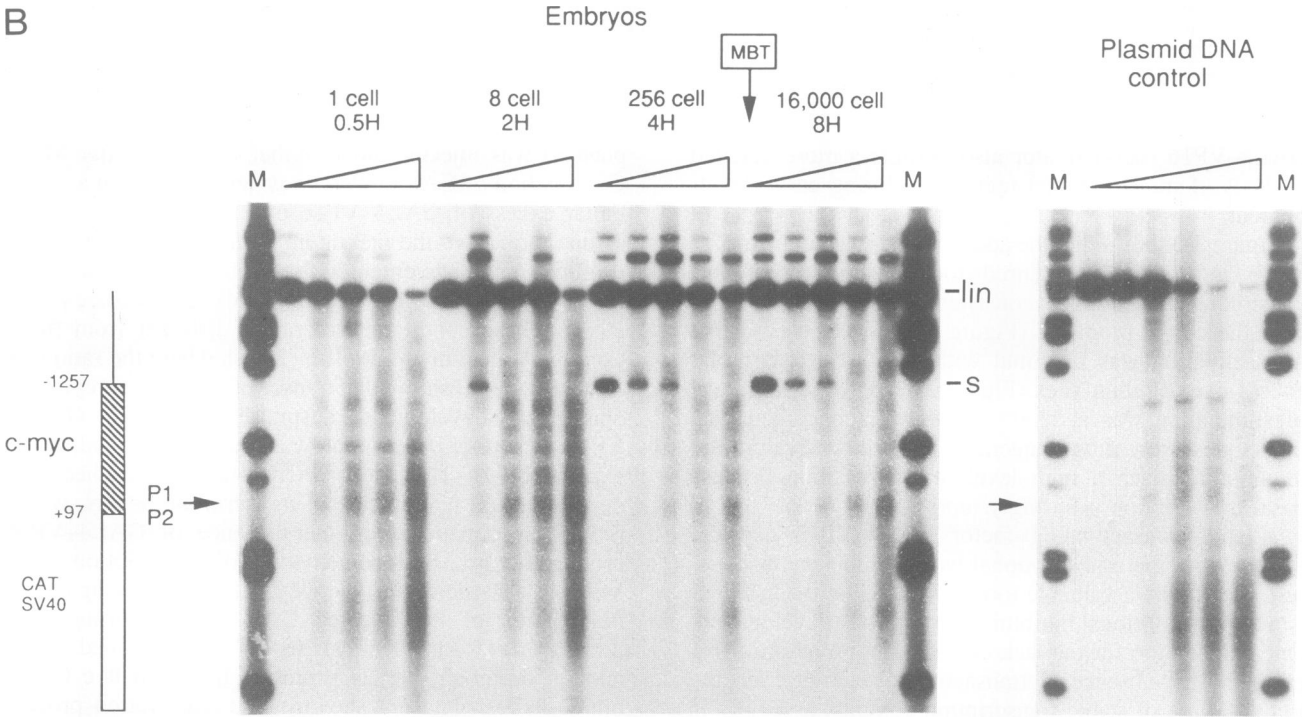


Fig. 2. The c-*myc* promoter is DNase I hypersensitive despite repression of transcription before MBT. (**A**) Kinetics of RNA accumulation in embryos after injection of the pMyc plasmid. The times of incubation after fertilization are indicated at the top of the gel; 7 h corresponds to the MBT. (**B**) Mapping of DNase I-hypersensitive sites in the c-*myc* promoter injected into embryos, by indirect end labelling of DNase I cleavage products relative to a vector *Kpn*I site. The c-*myc* insert is –1257 to +97 relative to P1 promoter (–1310 to + 46 relative to P2). The structure of the c-*myc* promoter is analysed either at 0.5, 2, 4 or 8 h (after MBT) post-injection. Injection was done 1 h after fertilization. The c-*myc* promoter is indicated by the stippled bar and an arrow marks the position of the Sp1 sites. Naked DNA corresponds to the analysis of DNase I cleavage on the naked pMyc plasmid. M is a labelled λ *Bst*EII digest used as a size marker. s and lin correspond respectively to the residual supercoiled and linear forms of the pMyc plasmid.



elements and their corresponding proteins. Before MBT, basal transcription is also repressed but, in contrast to the situation in oocytes or in post-MBT embryos, transactivators cannot relieve this repression.

Predetermination of the promoter by GAL4–VP16 before MBT

Cis-acting proteins may not be sufficient to allow gene expression before MBT because they cannot compete with

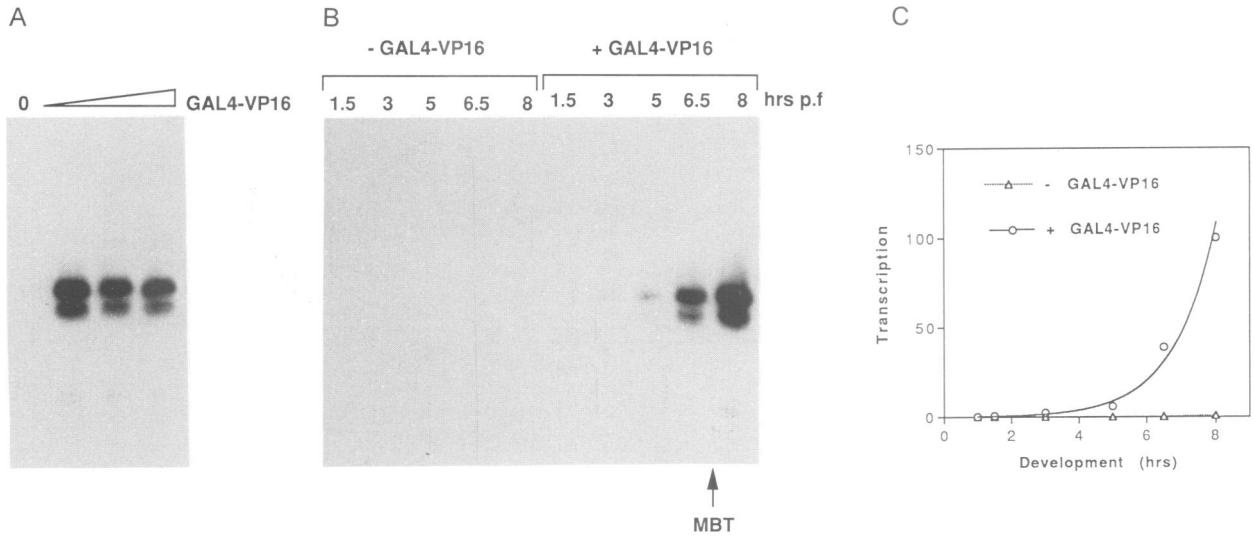


Fig. 3. The strong transactivator GAL4–VP16 is unable to relieve the repression before MBT. (**A**) Transcription in oocytes injected with the pGal-*myc* plasmid without (0) or with increasing amounts of GAL4–VP16 (the molar excess of GAL4–VP16 over binding sites was 2-, 4- and 8-fold) 2 h post-injection. (**B**) Kinetics of RNA accumulation in embryos after injection of the pGal-*myc* plasmid without (–GAL4–VP16) or with GAL4–VP16 (+GAL4–VP16). The GAL4–VP16/GAL4 binding sites molar ratio is 4. The times of incubation after fertilization are indicated at the top of the gel. (**C**) Graphic representation of the kinetics of RNA accumulation observed in Figure 2B. 100% corresponds to the level of RNA found 8 h after fertilization. The yield of transcription products was quantified with a Phosphorimager (Molecular Dynamics).

the unusual excess of maternal histones and gain access to their binding sites. Another possibility is that transactivators can bind to their sequences but that it is the communication with the basal transcription machinery which is the limiting event repressed by the excess histones. This possibility was suggested by the reported observation that derivatives of the GAL4 protein bind to nucleosome cores containing GAL4 binding sites, resulting in the formation of a ternary complex of GAL4, core histones and DNA (Taylor *et al.*, 1991). It is also consistent with the detection of a major hypersensitive site created on Sp1 elements in the c-*myc* promoter before MBT (Figure 2B). We therefore performed comparative footprinting analysis in the promoter region in the oocyte or in post-MBT embryos, when transcription is active, or in pre-MBT embryos during repression.

Analysis of the chromatin structure assembled in the oocyte clearly shows that the GAL4 binding sites are within a protected region bordered by two DNase I-hypersensitive sites (Figure 4A). The analysis at a higher resolution confirms that the footprint is due to the binding of GAL4–VP16 (Figure 4B) and indicates that the majority of the molecules stably interacted with the activator, as found with the endogenous Sp1 factor (Figure 1).

In embryos, analysis of hypersensitive sites on agarose gels and footprinting at the nucleotide resolution level show that the footprint over the GAL4–VP16 site is similar before and after the MBT (Figure 5A and B). Thus, induction of transcription at the MBT does not change the footprint significantly, and the footprint is also similar to that obtained during transcription in the oocyte (Figure 4). The binding of transcription complexes at the TATA box was not observed, possibly because only a part of the DNA population was engaged in transcription. Alternatively, the dynamics of an active transcription complex may not permit detection of such a footprint *in vivo* (Rozek and Pfeifer, 1993). In any case, these *in vivo* observations confirm that GAL4–VP16 is able to access DNA in a context of strong repression by chromatin assembly, and that it occurs at a relative low ratio of GAL4–VP16/DNA. However, this binding is not sufficient to activate transcription before MBT and, therefore, the limiting step in early embryos is not the recruitment of transactivators but their productive interaction with the basal transcription machinery.

Potentiation by GAL4–VP16 induces a stable transactivated transcription during early development, as opposed to an unstable basal transcription induced by potentiation with TBP

To get a better insight into the mechanism involved in transcriptional repression during early development, we pre-assembled DNA and TATA binding protein (TBP) and/or GAL4–VP16 experimentally before their injection into fertilized eggs. Pre-assembly of the promoter with the TBP is sufficient to induce a premature but transient transcription during early development (Prioleau *et al.*, 1994). We confirm this result here, in contrast to a recent report indicating that TBP cannot relieve the repression during early development unless competitor non-specific DNA is also injected (Almouzni and Wolffe, 1995). Figure 6 shows that transcription indeed occurs with a TBP-potentiated template, without co-injection of non-specific DNA, as previously shown (Prioleau *et al.*, 1994). One important difference between Almouzni and Wolffe's protocol and ours is the kinetics of collection of embryos after injection. Almouzni and Wolffe's observations are done 2–3.5 h after injection (3–4.5 h post-fertilization). We have already stressed that the premature transcription induced by TBP occurs in a transient manner, between 30 and 60 min after injection, and that no transcripts are detected from 2 h post-injection (Figures 2, 3 and 7 of

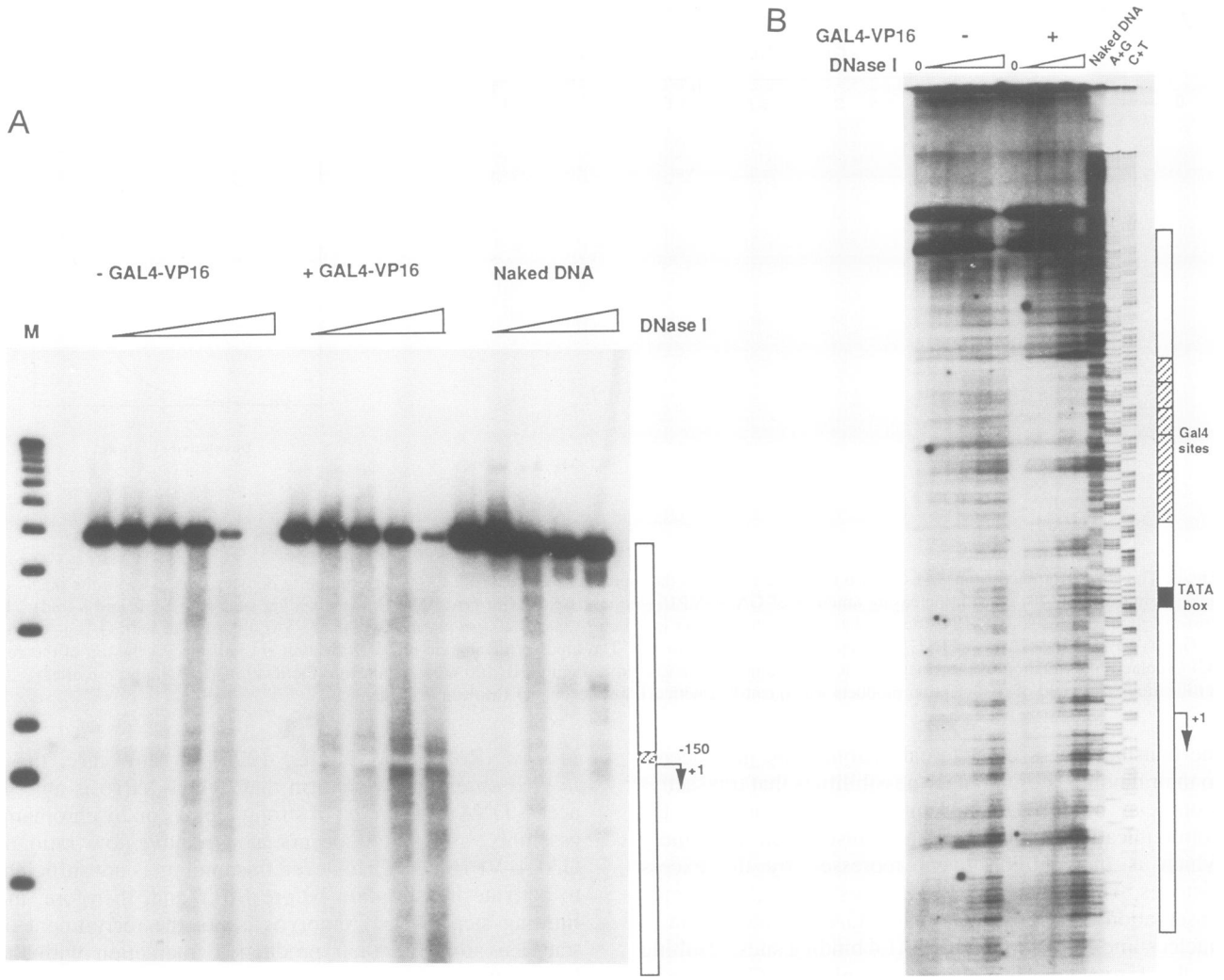


Fig. 4. GAL4–VP16 interacts with the majority of the template in oocytes. (**A**) Mapping of DNase I-hypersensitive sites by indirect end labelling on the pGal-*myc* promoter injected with or without GAL4–VP16 into oocytes. Chromatin analysis was performed 4 h after injection into oocytes. GAL4 binding sites are indicated by hatched bars and the initiation site of transcription by an arrow. M is a 5′ end-labelled 1 kb ladder used as a size marker. (**B**) Primer extension analysis of DNase I cleavages on a 6% sequencing gel of the pGal-*myc* promoter injected with or without GAL4–VP16 into oocytes. GAL4 binding sites and the TATA box are indicated by hatched and solid bars The initiation start site of transcription is indicated by an arrow. A+G and C+T are sequencing reactions.

Prioleau *et al.*, 1994). In addition, we have also shown that co-injection of 25 ng of non-specific DNA stabilizes the premature transcription induced by TBP (Figures 7 and 8 of Prioleau *et al.*, 1994). Therefore, despite the apparent discrepancy raised by the Almouzni and Wolffe (1995) report, it is clear that the data are perfectly concordant.

In addition, we show here (Figure 6) that this premature transcription is not dependent on *cis*-acting sequences, as it does not require Sp1 sites (Figure 6A). Similarly, pre-incubation of the pGAL-*myc* promoter with TBP also induces a transient transcription which does not require GAL4–VP16 (Figure 6B and C). Thus, potentiation of the promoter with TBP nucleates an active but basal transcription complex which is consequently unstable and rapidly repressed by chromatin assembly in the early embryo.

A contrasting result was obtained by injection of the pGAL-*myc* promoter pre-assembled with GAL4–VP16. In this case, GAL4–VP16 is already bound to its sequence before injection and does not have to compete with histones to access its site. A substantial level of transcription is then detected (Figure 6B). While lower than with the TBP-potentiated template, this transcriptional activity remains stable during early development and it is further increased after the MBT (Figure 6B and D). In our experiment, where the excess of GAL4 over GAL4 binding sites is only 4, we observe a premature transcription only if GAL4–VP16 is pre-bound to DNA before injection (Figure 6B). Transcription is not detected before MBT if GAL4–VP16 is not pre-bound to the DNA template (Figure 3B and independent recent result of Xu *et al.*, 1994). These data are consistent with our conclusion that a dynamic competition between chromatin assembly and the assembly of transcription complexes regulates gene expression during early development (Prioleau *et al.*, 1994). The formation of a pre-bound complex before injection overrides this competition. Co-injection of a large excess of GAL4–VP16 over GAL4 sites might also favour the formation of transcription complexes and therefore explain the data

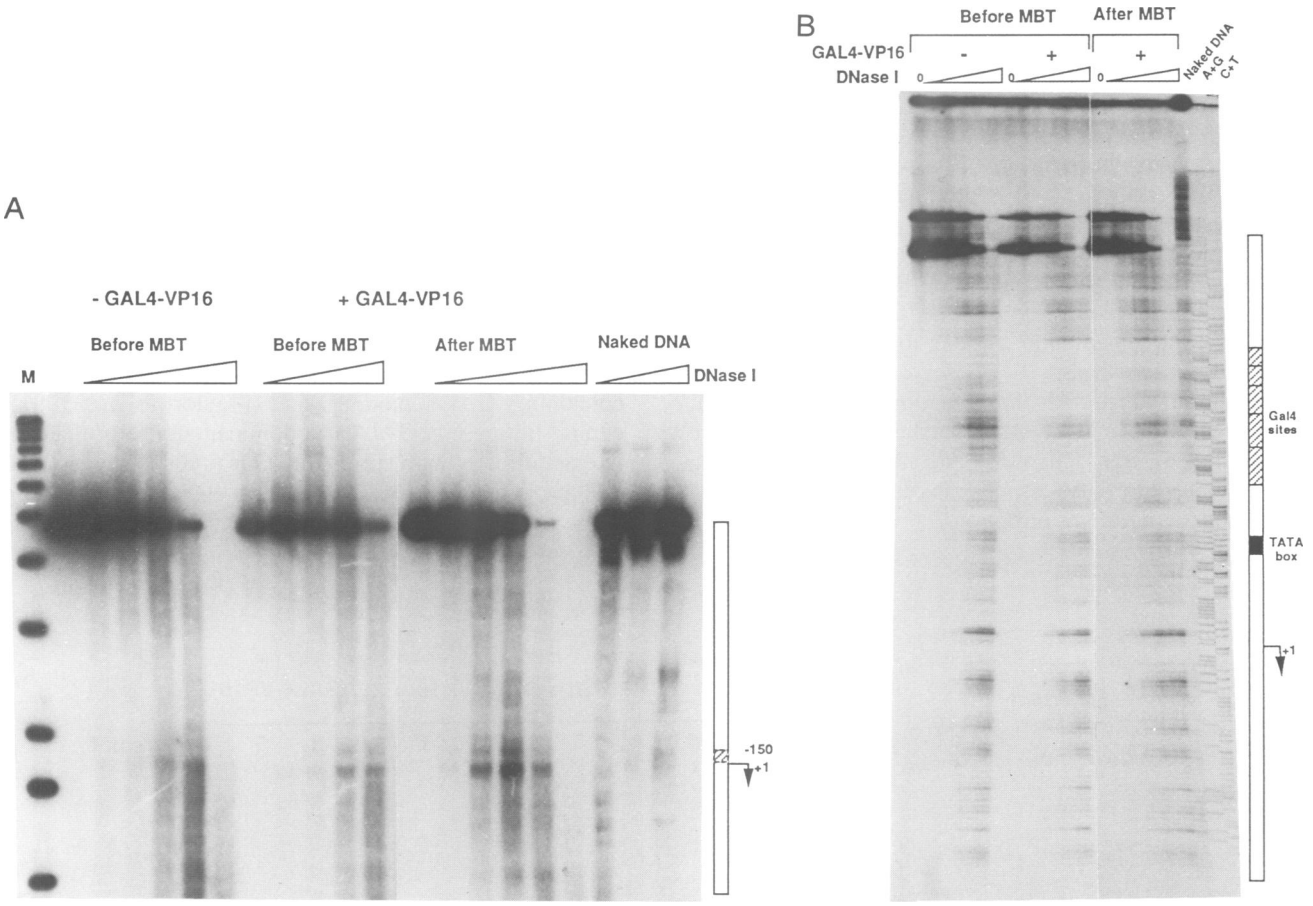


Fig. 5. Despite the absence of transcription before MBT, GAL4–VP16 binds to the pGAL-*myc* promoter. Mapping of DNase I-hypersensitive sites on the pGal-*myc* promoter injected into embryos with or without GAL4–VP16. The analysis was made 4 h post-fertilization (Before MBT) and 8 h post-fertilization (After MBT) either in agarose (**A**) or in polyacrylamide gel (**B**).

observed in Almouzni and Wolffe (1995), where the calculated GAL4–VP16 over GAL4 binding sites ratio is 300. In this condition, transcriptional activation occurs, a result consistent with the displacement of the dynamic equilibrium in favour of transcription complex assembly. The combination of TBP and GAL4–VP16 showed no synergy, as if they acted independently (compare Figure 6C, D and E).

Altogether, these results confirm that the transcriptional step that is repressed during early development is the formation of the basal transcription complex. Pre-incubation with TBP, in the absence of competition with nucleosome assembly, allows the nucleation of a basal complex immediately after injection into the egg (Figure 6F), but this basal complex is further destabilized by the assembly of chromatin. The formation of the transcription complex, which is resistant to nucleosomal repression, requires a template already primed with a transactivator before its introduction into the egg. In this case, the pre-bound activator can recruit the basal machinery at a time when chromatin assembly is not complete, resulting in the assembly of a transactivated transcription complex stabilized against repression (Figure 6F). This last observation also confirms that factors required for the connection between activators and the basal machinery are present and active during early development.

Discussion

In this report, we have investigated the role of *cis*-acting sequences and transactivators in the transitions in gene regulation which occur during the early development of *Xenopus*. We observe that during oogenesis, and after the MBT, transcription of polymerase II genes is dependent on the presence of transactivators. Before MBT, transcription is repressed despite the acquisition of DNase I hypersensitivity at the *cis*-acting elements. Finally, we show that the transcriptional step which is repressed during early development is the recruitment of the basal transcription machinery by the transactivating factors.

Repression of basal transcription during oogenesis and after MBT

Xenopus oocytes carry out the accurate and efficient transcriptional initiation of microinjected genes transcribed by RNA polymerase I and III (for review, see Gurdon and Melton, 1981). However, genes coding for tissue-specific proteins transcribed by RNA polymerase II have been reported to be poorly and non-specifically expressed in oocytes, in contrast with housekeeping genes. We demonstrate here that basal transcription is repressed in oocytes and requires *cis*-acting elements as well as the presence of their activators. The repression

of basal transcription in the oocyte probably reflects the ability of this cell to assemble chromatin efficiently (Wyllie *et al.*, 1978), and is in agreement with the known function of *cis*-acting sequences to relieve chromatin repression. Consistently, Perlmann and Wrange (1991) showed that chromatin assembly in *Xenopus* oocytes was necessary to reproduce the induction of the mouse mammary tumour virus promoter by the glucocorticoid receptor. These observations are consistent with the differential regulation of RNA polymerase II genes in oocytes and suggest that oocytes could be a valuable complementation system to screen for tissue-specific transactivators.

When we examined the situation after the MBT, when transcription is re-established in the embryo, we observed that transactivators and their corresponding binding sites are also strictly necessary to overcome repression of basal transcription.

Repression of both basal and transactivated transcription before MBT

In contrast to gene expression in the oocyte or in post-MBT embryos, repression still occurs in the early embryo in the presence of the strong transactivator GAL4–VP16. Differences in chromatin assembly in an egg or an oocyte (Sealy *et al.*, 1986), as well as the inaccessibility of the cytoplasmic histone pool to nuclear DNA in the oocyte, might contribute to this differential gene expression. However, despite transcriptional repression, we observed that GAL4–VP16 as well as endogenous activators can be bound stably to DNA in pre-MBT embryos. GAL4 proteins have been shown to bind to nucleosome cores containing GAL4 binding sites (Taylor *et al.*, 1991), demonstrating that GAL4 is a dominant activator that can interact directly with chromatin. Our findings suggest that this property is not sufficient to alleviate the strong repression which characterizes the early embryo. This

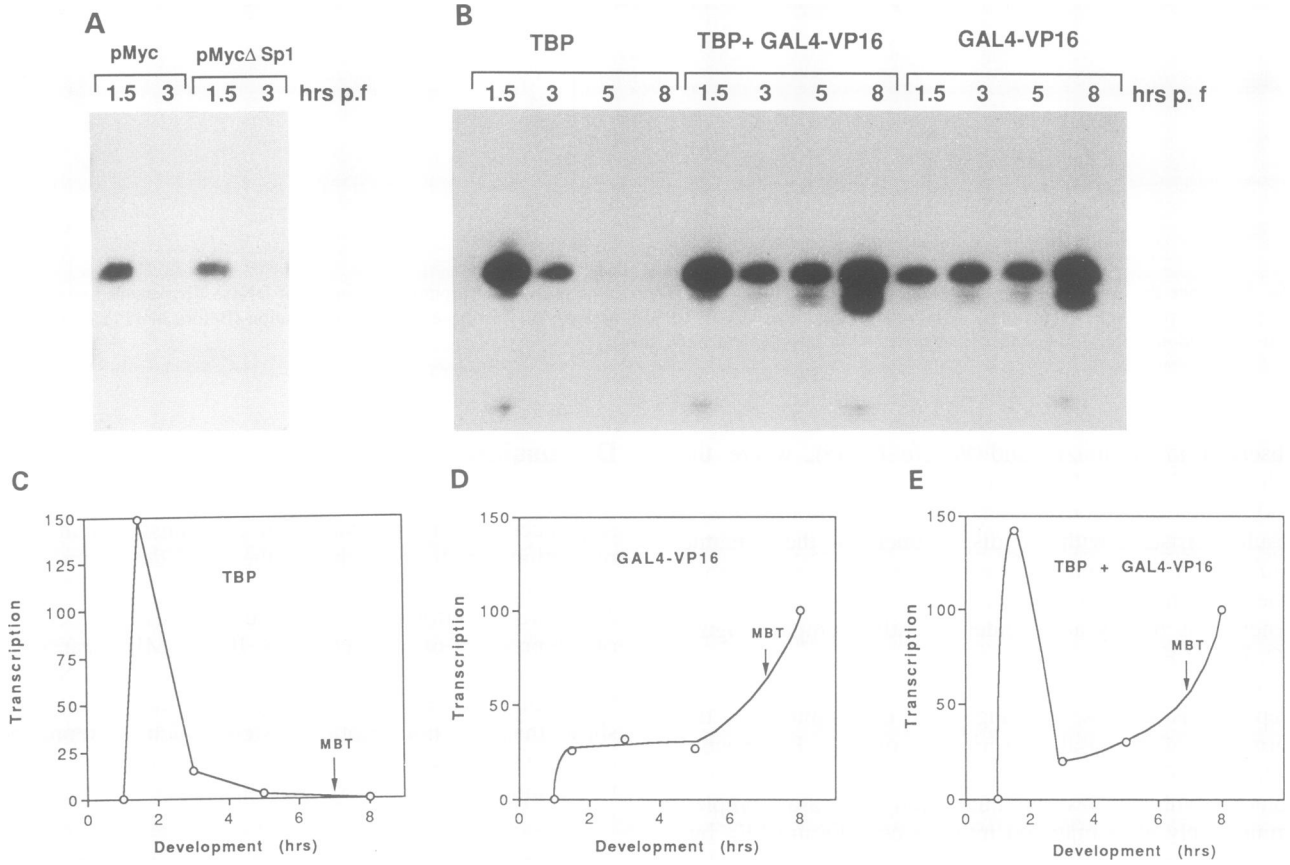


Fig. 6. Potentiation with GAL4–VP16 induces a premature and stable transcription. (**A**) The pMyc plasmid and the pMycΔSp1 plasmid were pre-incubated with TBP, as indicated in Materials and methods, and injected into embryos 60–75 min post-fertilization. RNA was extracted from the embryos at the indicated times post-fertilization. (**B**) The pGal-*myc* plasmid was pre-incubated with TBP, GAL4–VP16 or TBP+GAL4–VP16, as described in Materials and methods, and then injected into embryos 60–75 min post-fertilization. RNA was extracted at the indicated times post-fertilization. (**C**, **D** and **E**) Graphic representations of the kinetics of RNA accumulation observed in Figure 4B and quantified by phosphorimager analysis. 100% corresponds to the level of RNA found 8 h post-fertilization. (**F**) Potentiation by TBP or GAL4–VP16 does not lead to the same transcriptional activity. When TBP is bound to DNA, it permits the recruitment of the endogenous basal machinery immediately after injection, leading to a premature transcription in the embryo. As the basal complex is not stabilized by transactivating proteins, it is further repressed by chromatin assembly. When the template is potentiated with the activating protein GAL4–VP16, a transactivated complex is established by the recruitment of the basal machinery, probably including TFIID. This complex is resistant to chromatin assembly and a stable transcription is established in the embryo. If the pre-binding to DNA is not done before injection, both the activator and the basal complex compete with histones for access to DNA. In these conditions, chromatin assembly is dominant over the assembly of the basal complex but not over the access of the activator to its site.

in vivo observation is in agreement with observations concerning the binding of GAL4 *in vitro* during chromatin assembly. The access of GAL4 to its site is not sufficient for transcriptional stimulation (Workman *et al.*, 1991), and the binding of five GAL4 dimers does not displace the underlying histones, but rather results in the formation of a ternary complex of GAL4, core histones and DNA. While the DNase I-hypersensitive site mapping indicates that the activator can access its binding site efficiently, it does not provide precise structural information allowing the determination of whether a ternary complex is present. It is possible that the difference between oocytes (or post-MBT embryos) and early embryos relies on the absence of nucleosomes or an altered chromatin configuration at the activator binding site in the oocyte, as opposed to a ternary complex in the early embryo.

These observations suggest the following conclusions for transcriptional regulation during early development (Figure 7). Before MBT, the large excess of maternal histones represses transcription by a dynamic competition with the assembly of transcription complexes (Prioleau *et al.*, 1994). We have shown here that it is the assembly of the basal transcription complex and not the assembly of transactivators which is inhibited. *Cis*-acting proteins can have access to their sites in this repressive context, and a footprint is readily detected (Figure 4). However, the recruitment of the basal machinery cannot occur (Figure 2B and Xu *et al.*, 1994). After the MBT, most of the histones in excess have been titrated, and a somatic-like histone/DNA ratio is reached. In this context, pre-fixed endogenous transactivating factors now permit and stimulate the assembly of the basal transcription complex.

F

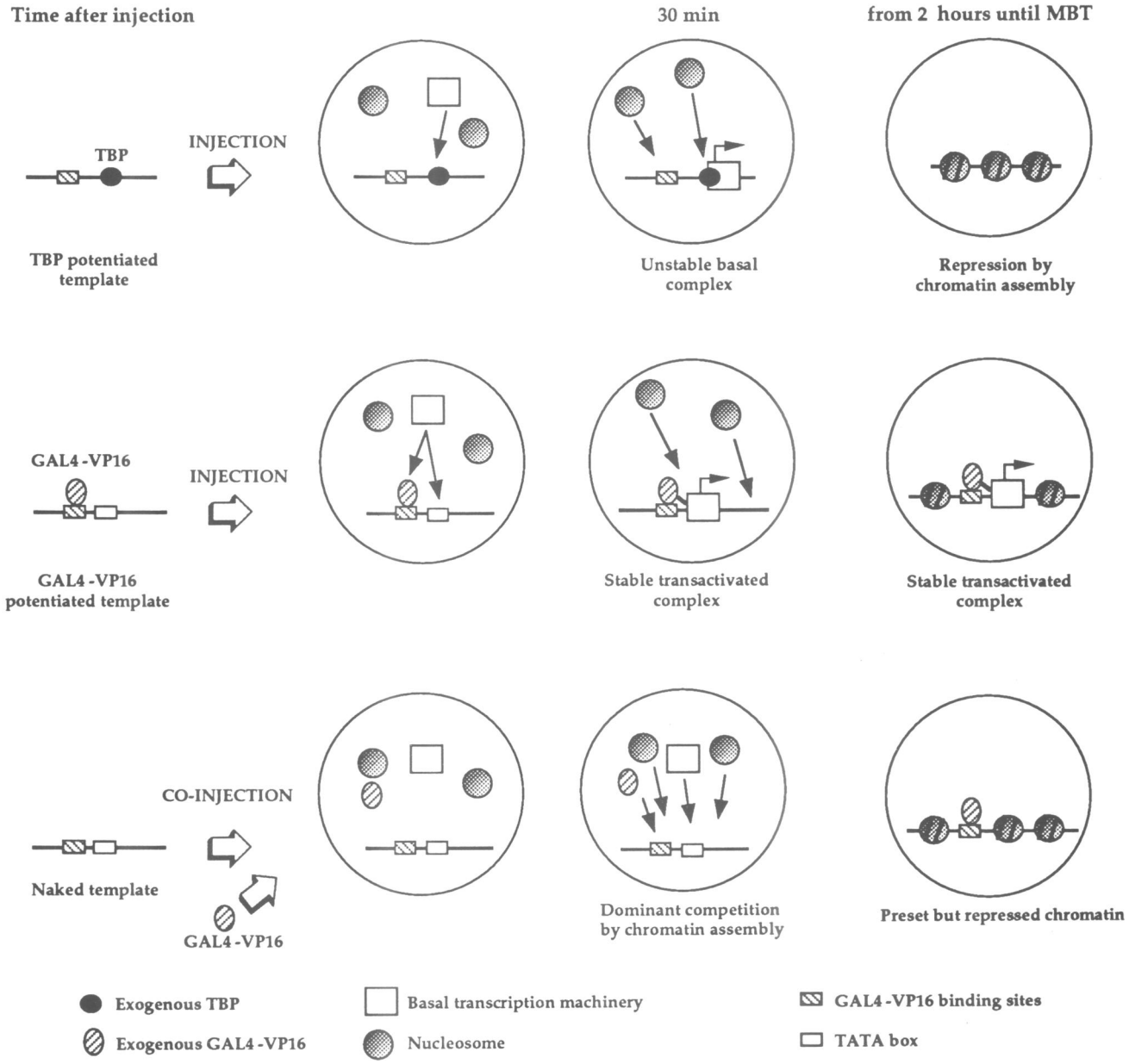


Fig. 6. *Cont.*

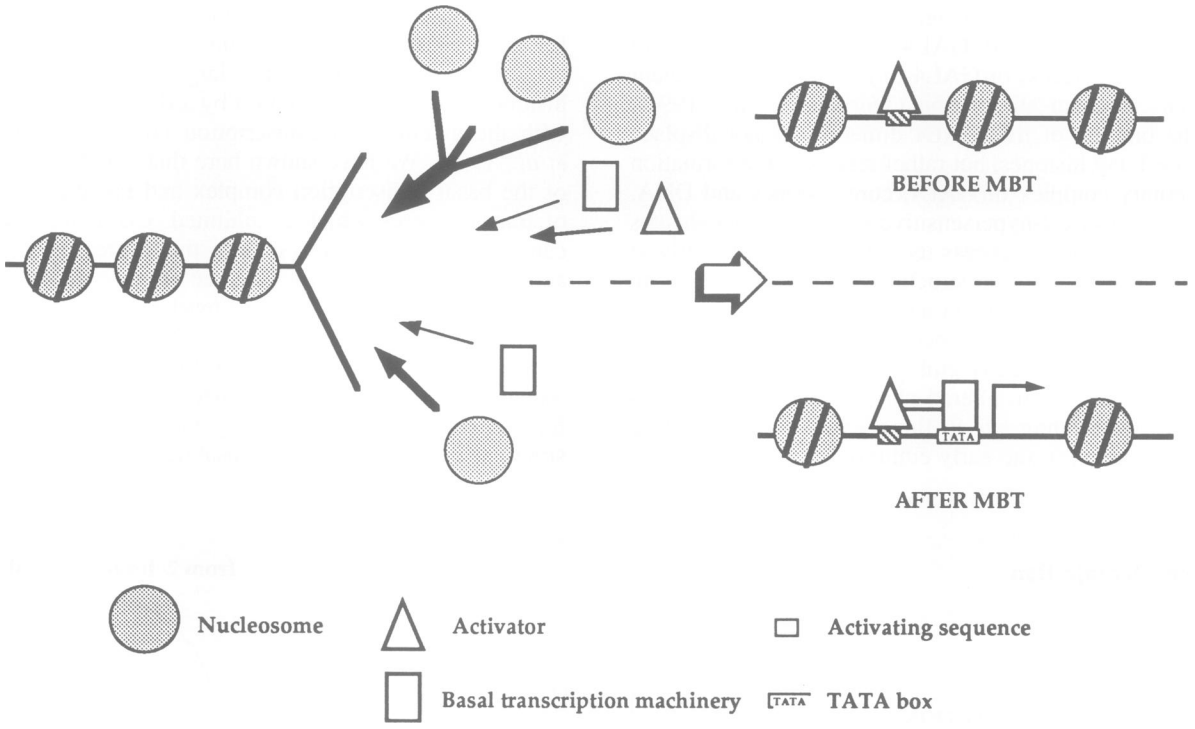


Fig. 7. Regulation of transcription during early development. Before MBT, the large excess of histones stored in the egg prevents the assembly of the basal machinery of transcription, but *cis*-acting proteins can have access to their sites. During early development, the excess of histones is titrated in an exponential fashion by the accumulation of DNA resulting from the embryonic divisions. At the MBT, the histone/DNA ratio becomes close to the normal somatic value and the recruitment of the basal machinery can occur, stabilized by the *cis*-acting proteins. Only transactivated transcription can be established at this stage. According to this model, the selective reactivation of transcription at the MBT will depend on the presence, concentration and strength of interaction of *cis*-acting protein with the promoter considered.

This model is consistent with previous data showing that a mass of competitor DNA able to sequestrate the excess maternal histones can activate transcription before MBT. This required the injection of at least 50 ng of non-specific DNA (the histone pool is 40–120 ng, see also Figure 9 of Prioleau *et al.*, 1994), and not injection of 5 ng as is cited in Almouzni and Wolffe (1995). Five ng of non-specific DNA is the minimum amount of DNA which permits the stabilization of transcription only when TBP has been pre-bound to the template, and is ineffective with naked template DNA (Figures 7, 8 and 9 of Prioleau *et al.*, 1994). Again, these data are consistent with the notion of a dynamic competition and with the advantage given to TBP by its pre-binding to chromatin before its exposure to chromatin assembly.

Our data are also in agreement with the increased instability of nucleosomes in the presence of GAL4–VP16 (Workman and Kingston, 1992). They are also in accordance with the establishment of a stable activated transcription complex when GAL4–VP16 is pre-bound to the DNA before its injection (Figure 6), as the recruitment of the basal complex will then be favoured before nucleosomal repression. The natural *in vivo* situation is likely to be closer to the experiment performed when GAL4–VP16 is not pre-bound to its site and has to compete with chromatin assembly. In this context, we observed the assembly of a programmed chromatin structure which is, like the endogenous chromatin, transcriptionally inactive.

Programming of chromatin before MBT

One important feature of the early embryo is the establishment of cell lineages and their progressive commitment. This determination arises mainly from the accumulation of determinants and their spatial segregation.

The formation of promoter hypersensitive sites by endogenous factors before the MBT, together with the reproduction of this phenomenon with the GAL4–VP16 construct, suggests a multi-step process in the activation of transcription during early development, with activation of promoters temporally uncoupled from their commitment. This predetermination of chromatin is of maternal origin as it is observed at a time when the zygote does not transcribe. Two mechanisms can be envisaged to explain this predetermination. The first one could result from stable inheritance of the oocyte chromatin structure during the initial embryonic cell cycles. A second mechanism could be simply dictated by a maternal inheritance of factors previously accumulated in the oocyte, which will access their regulatory sequences during early development. We cannot discriminate between these mechanisms, but it is clear that, during this stage, competition by chromatin assembly affects the formation of the basal complex but not the access of transactivators to their sites.

The reactivation of gene expression at the MBT has been described as an abrupt phenomenon (Newport and Kirschner, 1982), and gene programming by the pre-binding of transactivators may be necessary to permit this sudden transition. The presence of DNase I-hypersensitive sites in early *Drosophila* embryos (Lowenhaupt *et al.*, 1983), where MBT occurs at the 14th cell cycle, is also in agreement with such a mode of regulation. The triggering signal at the MBT might, therefore, be the depletion of the excess free histone pool which makes possible the recruitment of the basal transcription

machinery on the predetermined chromatin. Although transcription is not active, important determinations occur during early development in specific blastomeres of the embryo (for a general review of this subject, see Gimlich and Gerhart, 1984; Gurdon *et al.*, 1985; Davidson, 1986). Such events, which occur before MBT, could be dictated by asymmetric segregation of maternal activators during the first divisions, whereas housekeeping genes, activated early at the MBT, might be programmed homogeneously in the embryo. This determination is reminiscent of the multi-step process of activation for the vitellogenin (Burch and Weintraub, 1983) and heat shock genes (Rougvie and Lis, 1988; Thomas and Elgin, 1988; Tsukiyama *et al.*, 1994), and could be of general importance in the regulation of early embryonic events by providing molecular signposts for development.

Materials and methods

Recombinant plasmids

The pMyc and pMycΔSp1 plasmids are respectively the pMyc (1–9) CAT and pMyc (8–9) CAT plasmids described in Modak *et al.* (1993). pMyc contains sequences −1310 to +46 and pMycΔSp1 contains sequences −30 to +46 of the *Xenopus* c-*myc* promoter. Neither sequence includes the T2 termination site (Bentley and Groudine, 1988). The pXLMyc3 plasmid contains a 6.5 kb *Eco*RI promoter fragment from −5400 to +1100 of the P2 c-*myc* promoter. The pGAL-*myc* plasmid was constructed from a *Xba*I–*Pst*I fragment of pG5E4T (Lin *et al.*, 1988) inserted into the *Xba*I–*Pst*I-digested pMyc (8–9) CAT plasmid.

Purification of TBP and GAL4–VP16

The recombinant yeast TBP was a generous gift from A.Sentenac. The purification was essentially as described by Burton *et al.* (1991), by DEAE–Sephacel, heparin–Ultragel and heparin–SPW chromatography. GAL4–VP16 was purified as described by Chassman *et al.* (1989). Both proteins were judged to be >90% pure by SDS–PAGE.

Pre-incubations with transcription factors

Template plasmids were pre-incubated at 30 μg/ml with 90 ng of TBP and (or)125 ng of GAL4–VP16 in 10 μl reactions for 1 h at 30°C in 20 mM HEPES (pH 7.7), 6 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 0.5 mM ATP, 250 μg/ml bovine serum albumin (BSA). When TBP or GAL4–VP16 were omitted from the pre-incubations, the corresponding volumes were made up with the TBP or GAL4–VP16 storage buffer. The GAL4–VP16/GAL4 binding site molar ratio was 4.

Embryo and oocyte injections

In all experiments described, 25 nl of reaction medium containing 0.75 ng DNA were injected prior to the first cleavage into at least 15 fertilized *Xenopus* eggs per experimental point. Development of the embryos proceeded in 4% Ficoll, 1× MBSH at 22–24°C. Injections into oocytes were with 25 nl pre-incubation reaction injected into germinal vesicles. Injected oocytes were then incubated in 0.1× MBSH supplemented with streptomycin and penicillin at 10 μg/ml. All the oocytes or embryos used for each experiment are from the same animal and injected with the same calibrated micropipette. The assays were performed the same day in a common experiment and do not derive from pools of oocytes or embryos injected in separate experiments. After different times of incubation, oocytes or embryos were homogenized in 0.2 ml 30 mM EDTA, 1% SDS, 0.5% Triton X-100 and incubated at 42°C for 1 h with 1 mg/ml proteinase K, using protocols already described in Prioleau *et al.* (1994) and previously extensively tested using labelled known RNAs and DNAs for recovery and efficiency. For RNA analysis, the homogenates were extracted once with guanidine and phenol/chloroform, once with chloroform isoamylalcohol (24:1), ethanol precipitated, and further analysed by primer extension.

Mapping of DNase I-hypersensitive sites

DNase I mapping on endogenous chromatin from *Xenopus* A6 somatic cells was performed on isolated nuclei further purified through a 0.8 M sucrose cushion in 10 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.25 mM EDTA, 0.5 mM DTT. Nuclei were pelleted at 4000 *g* for 15 min, washed and resuspended in DNase I digestion buffer [20 mM HEPES, pH 7.6, 85 mM KCl, 3 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.5 mM DTT, 0.5 mM spermidine, 5% sucrose, 0.25 M phenylmethylsulphonyl fluoride (PMSF), 10 μg/ml aprotinin, leupeptin and pepstatin], at 5×10^7 nuclei/ml. Nuclei were digested with DNase I (RQI grade, Pharmacia) for 5 min at 22°C, and reactions were terminated by addition of EDTA to 10 mM final. Samples were digested with 100 μg/ml RNase A for 30 min at 37°C, and then overnight at 37°C after addition of SDS and proteinase K to 0.2% and 200 μg/ml respectively. Genomic DNA was purified by phenol/chloroform extraction and ethanol precipitation, and then digested to completion with *Eco*RI. Purified DNA was resolved on 1.2% agarose gels, prior to Southern blotting onto Hybond N+ (Amersham). Indirect end labelling was performed by hybridizing the blot with a labelled *Eco*RI–*Bst*XI probe from c-*myc* exon 2 (+1081 to + 818 from P2), overnight at 42°C in the presence of 50% formamide. Filters were washed to high stringency and autoradiographed at −80°C.

Analysis on injected oocytes or embryos was performed with at least 20 embryos or oocytes per experimental point, homogenized in 180 μl of 10 mM HEPES (pH 7.6), 85 mM KCl, 5.5% sucrose, 0.5 mM spermidine, 0.5 mM DTT, 0.5% NP-40, 2 mM ATP, and centrifuged at 14 000 *g* for 10 s to pellet the yolk. The supernatant was adjusted to 3 mM $MgCl_2$, 0.5 mM $CaCl_2$ and digested for 15 min at 20°C with increasing amounts of DNase I (0, 2, 4, 10 and 20 U/ml). Purified DNA was digested to completion with *Kpn*I, which cuts in the vector at +1720 from P2, separated on a 1.2% agarose gel, and transferred to nylon membranes (Hybond N+, Amersham). The membranes were hybridized with a ^{32}P-labelled *Kpn*I (+1720)–*Sty*I (+1160) fragment.

The analysis at the nucleotide resolution was done by primer extension. DNA was digested by DNase I, as described above, and linearized by an *Eco*RI–*Hin*dIII double digestion. A ^{32}P-labelled primer (−100 to −80) was extended with VentR (exo-) DNA polymerase (Biolabs) for 5 cycles. Samples were ethanol precipitated and analysed by 6% denaturing PAGE.

Primer extension

For transcription analysis, primer extension was performed with a 20mer oligonucleotide primer specific for the CAT coding sequence (Modak *et al.*, 1993). Primers were ^{32}P-labelled with T4 polynucleotide kinase. Hybridizations were performed for 1 h at 40°C, in 10 μl final volume containing 400 mM NaCl. Extension reactions were in 50 mM Tris–HCl pH 8.3, 50 mM KCl, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM spermidine, 1 mM each dNTP and 20 U of AMV reverse transcriptase (Promega) at 42°C for 90 min. Samples were then ethanol precipitated and analysed by 6% denaturing PAGE. The yield of transcription products was quantified with a phosphorImager (Molecular Dynamics).

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References

Adamson,E.D. and Woodland,H.R. (1974) Histone synthesis in early amphibian development: histone and DNA synthesis are not co-ordinated. *J. Mol. Biol.*, **88**, 263–285.

Almouzni,G. and Wolffe,A.P. (1993) Replication coupled chromatin assembly is required for the repression of basal transcription *in vivo*. *Genes Dev.*, **7**, 2033–2047.

Almouzni,G. and Wolffe,A.P. (1995) Constraints on transcriptional activator function contribute to transcriptional quiescence during early *Xenopus* embryogenesis. *EMBO J.*, **14**, 1752–1765.

Bending,M.M. and Williams,J.G. (1984) Fidelity of transcription of *Xenopus laevis* globin genes injected into *Xenopus* laevis oocytes and unfertilized eggs. *Mol. Cell. Biol.*, **4**, 2109–2119.

Bentley,D.L. and Groudine,M. (1988) Sequence requirements for premature termination of transcription in the human c-*myc* gene. *Cell*, **53**, 245–256.

Bienz,M. (1986) A CCAAT box confers cell-type-specific regulation on the *Xenopus hsp70* gene in oocytes. *Cell*, **46**, 1037–1042.

Burch,J.B.E. and Weintraub,H. (1983) Temporal order of chromatin structural changes associated with activation of the major chicken vitellogenin gene. *Cell*, **33**, 65–76.

Burton,N., Cavallini,B., Kanno,M., Moncollier,V. and Egly,J.M. (1991) Purification and properties of the yeast general transcription factor TFIID. *Prot. Expression Purification*, **2**, 432–441.

Chasmann,D.I., Leatherwood,M., Carey,M., Ptashne,M. and Kornberg,R. (1989) Activation of yeast polymerase II transcription by herpes virus VP16 and Gal4 derivatives in vitro. *Mol. Cell. Biol.*, **9**, 4746–4749.

Davidson,E.H. (1986) *Gene Activity in Early Development*. Academic Press, NY.

Dyson,P.J. and Rabbits,T.H. (1985) Chromatin structure around the c-*myc* gene in Burkitt lymphomas with upstream and downstream location points. *Proc. Natl Acad. Sci. USA*, **82**, 1984–1988.

Etkin,L.D. and Maxson,R.E. (1980) The synthesis of authentic sea urchin transcriptional and translational products by sea urchin histone genes injected into *Xenopus laevis* oocytes. *Dev. Biol.*, **75**, 13–25.

Felsenfeld,G. (1992) Chromatin as an essential part of the transcriptional mechanism. *Nature*, **355**, 219–224.

Gimlich,R.L. and Gerhart,J.C. (1984) Early cellular interactions promote embryonic axis formation in *Xenopus laevis*. *Dev. Biol.*, **104**, 117–130.

Green,J., Brady,J. and Khoury,G. (1987) 72-bp element contains a critical control region for SV40 late expression in *Xenopus laevis* oocytes. *Virology*, **159**, 339–349.

Grosschedl,R. and Birnstiel,M.L. (1980) Identification of regulatory sequences in the prelude sequences of an H2A histone gene by the study of specific deletion mutants *in vivo*. *Proc. Natl Acad. Sci. USA*, **77**, 1432–1436.

Gurdon,J.B. and Melton,D.A. (1981) Gene transfer in amphibian eggs and oocytes. *Annu. Rev. Genet.*, **15**, 189–218.

Gurdon,J.B., Mohun,T.J., Fairman,S. and Brennan,S. (1985) All components required for the eventual activation of muscle-specific actin genes are localized in the subequatorial region of an uncleaved amphibian egg. *Proc. Natl Acad. Sci. USA*, **82**, 139–143.

King,M.W., Roberts,J.M. and Eisenman,R.N. (1986) Expression of the c-*myc* proto-oncogene during development of *Xenopus laevis*. *Mol. Cell. Biol.*, **6**, 4499–4508.

Laskey,R.A., Mills,A.D. and Morris,N.R. (1977) Assembly of SV40 chromatin in a cell free system from *Xenopus* eggs. *Cell*, **10**, 237–243.

Lin,Y.S., Carey,M.F., Ptashne,M. and Green,M.R. (1988) GAL-4 derivatives function alone and synergistically with mammalian activators in-vitro. *Cell*, **54**, 659–664.

Lowenhaupt,K., Cartwright,I.L., Keene,M.A., Zimmerman,J.L.C. and Elgin,S.C.R. (1983) Chromatin structure in pre- and post-blastula embryos of *Drosophila*. *Dev. Biol.*, **99**, 194–201.

Mango,S.E., Schuler,G.D., Steele,M.E.R. and Cole,M.D. (1989) Germ line c-*myc* is not down-regulated by loss or exclusion of activating factors in c-*myc*-induced macrophage tumors. *Mol. Cell. Biol.*, **9**, 3482–3490.

Mattaj,I.W., Lienhard,S., Jiricny,J. and De Robertis,E.M. (1985) An enhancer-like sequence within the *Xenopus* U2 gene promoter facilitates the formation of stable transcription complexes. *Nature*, **316**, 163–167.

McKnight,S.L. and Gavis,E.R. (1980) Expression of the Herpes thymidine kinase gene in *Xenopus laevis* oocytes: an assay for the study of deletion mutants constructed in vitro. *Nucleic Acids Res.*, **8**, 5931–5948.

Modak,S.P., Principaud,E. and Spohr,G. (1993) Regulation of *Xenopus* c-*myc* promoter activity in oocytes and embryos. *Oncogene*, **8**, 645–654.

Mohun,T.J., Garrett,N. and Gurdon,J.B. (1986) Upstream sequences required for tissue specific activation of the cardiac actin gene in *Xenopus laevis* embryos. *EMBO J.*, **5**, 3185–3193.

Newport,J. and Kirschner,M. (1982) A major developmental transition in early *Xenopus* embryos: II. Control of the onset of transcription. *Cell*, **30**, 687–696.

Nishikura,K. (1986) Sequences involved in accurate and efficient transcription of human c-*myc* genes microinjected into frog oocytes. *Mol. Cell. Biol.*, **6**, 4093–4098.

Ovsenek,N. and Heikkila,J.J. (1990) DNA sequence specific binding activity of the heat shock transcription factor is heat-inducible before the midblastula transition of early *Xenopus* development. *Development*, **110**, 427–433.

Ovsenek,N., Karn,H.A. and Heikkila,J.J. (1991) Analysis of CCAAT box transcription factor binding activity during early *Xenopus laevis* embryogenesis. *Dev. Biol.*, **145**, 323–327.

Ovsenek,N., Zorn,A.M. and Krieg,P.A. (1992) A maternal factor, OZ-1, activates embryonic transcription of the *Xenopus laevis* GS17 gene. *Development*, **115**, 829–846.

Paranjape,S., Kamakaka,R.T. and Kadonaga,J.T. (1994) Role of chromatin structure in the regulation of transcription by RNA polymerase II. *Annu. Rev. Biochem.*, **63**, 265–267.

Perlmann,T. and Wrange,O. (1991) Inhibition of chromatin assembly in *Xenopus* oocytes correlates with derepression of the mouse mammary tumor virus promoter. *Mol. Cell. Biol.*, **11**, 5259–5265.

Prioleau,M.-N., Huet,J., Sentenac,A. and Méchali,M. (1994) Competition between chromatin and transcription complex assembly regulates gene expression during early development. *Cell*, **77**, 439–449.

Rougvie,A.E. and Lis,J.T. (1988) The RNA polymerase II molecule at the 5′ end of the uninduced hsp70 gene of *D.melanogaster* is transcriptionally engaged. *Cell*, **54**, 795–804.

Rozek,D. and Pfeifer,G.P. (1993) *In vivo* protein–DNA interactions at the c-*jun* promoter: preformed complexes mediate the UV response. *Mol. Cell. Biol.*, **13**, 5490–5499.

Sealy,L., Cotten,M. and Chalkley,R. (1986) *Xenopus* nucleoplasmin: egg vs. oocyte. *Biochemistry*, **25**, 3064–3072.

Steinbesser,H., Hofmann,A., Stutz,F. and Trendelenburg,M.F. (1988) Different regulatory elements are required for cell-type and stage specific expression of the *Xenopus laevis* skeletal muscle actin gene upon injection in *X.laevis* oocytes and embryos. *Nucleic Acids Res.*, **16**, 3185–3193.

Taylor,I.C.A., Workman,J.L. and Kingston,R.E. (1991) Facilitated binding of GAL4 and heat shock factor to nucleosomal templates: differential function of DNA-binding domains. *Genes Dev.*, **5**, 1285–1298.

Taylor,M.V., Gusse,M., Evan,G.J., Dathan,N. and Méchali,M. (1986) *Xenopus myc* proto-oncogene during development: expression as a stable maternal mRNA uncoupled from cell division. *EMBO J.*, **5**, 3563–3570.

Thomas,G.H. and Elgin,S.C.R. (1988) Protein/DNA architecture of the DNase I hypersensitive region of the *Drosophila* hsp26 promoter. *EMBO J.*, **7**, 2191–2201.

Tsukiyama,T., Becker,P.B. and Wu,C. (1994) ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. *Nature*, **367**, 525–532.

Vashee,S., Xu,H., Johnston,S.A. and Kodadek,T. (1993) How do 'Zn2 cys6' proteins distinguish between similar upstream activation sites? Comparison of the DNA-binding specificity of the GAL4 protein *in vitro* and *in vivo*. *J. Biol. Chem.*, **268**, 24699–24706.

Vriz,S., Taylor,M. and Méchali,M. (1989) Differential expression of two *Xenopus* c-*myc* proto-oncogenes during development. *EMBO J.*, **8**, 4091–4097.

Wallrath,L.L., Lu,Q., Granok,H. and Elgin,S.R.C. (1994) Architectural variations of inducible eukaryotic promoters: preset and remodeling chromatin structures. *BioEssays*, **16**, 165–170.

Wolffe,A.P. (1994) Nucleosome positioning and modification: chromatin structures that potentiate transcription. *Trends Biochem. Sci.*, **19**, 240–244.

Woodland,H.R. and Adamson,E.P. (1977) The synthesis and storage of histone during the oogenesis of *Xenopus laevis*. *Dev. Biol.*, **57**, 118–135.

Workman,J.L. and Buchman,A.R. (1993) Multiple functions of nucleosomes and regulatory factors in transcription. *Trends Biochem. Sci.*, **18**, 90–95.

Workman,J.L. and Kingston,R.E. (1992) Nucleosome core displacement *in vitro* via a metastable transcription factor–nucleosome complex. *Science*, **258**, 1780–1784.

Workman,J.L. and Roeder,G.R. (1987) Binding of transcription factor TFIID to the major late promoter during *in vivo* nucleosome assembly potentiates subsequent initiation by RNA polymerase II. *Cell*, **51**, 613–622.

Workman,J.L., Taylor,I.A. and Kingston,R.E. (1991) Activation domains of stably bound GAL4 derivatives alleviate repression of promoters by nucleosomes. *Cell*, **64**, 533–544.

Wyllie,A.M., Laskey,R.A., Finch,J.T. and Gurdon,J.B. (1978) Selective DNA conservation and chromatin assembly after injection of SV40 DNA into *Xenopus* oocytes. *Dev. Biol.*, **64**, 178–188.

Xu,L., Rungger,D., Georgiev,O., Seipel,K. and Schaffner,W. (1994) Different potential of cellular and viral activators of transcription revealed in oocytes and early embryos of *Xenopus laevis*. *Biol. Chem. Hoppe Seyler*, **375**, 105–112.

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