lution has selected for multiple parallel pathways to ensure that a replicated chromosome does not erroneously reinitiate replication until after chromosome segregation is completed in mitosis. Cdks are believed to prevent replication initiation by phosphorylating CDC6 to target it either for proteolysis (in yeasts) or for nuclear export (in higher eukaryotes) (*6*, *21*–*23*). In addition, phosphorylation of MCM proteins by cdk is concordant with their dissociation from chromatin (*3*). However, in response to checkpoint activation, e.g., after DNA damage, cdk activity is repressed to block cell cycle progression until DNA repair is completed (*24*, *25*). The decrease in cdk activity could allow inappropriate pre-RC assembly on already fired origins, particularly because higher eukaryotes do not degrade CDC6 at the G_1-S transition. Therefore geminin, a second negative factor for DNA replication in S and G_2, may have evolved in higher eukaryotes to prevent re-replication in the event that cdk activity is lowered during a checkpoint response. It is also interesting that geminin targets a different initiator protein, Cdt1, in comparison to the CDC6 or MCM targeted by cdks. This diversity of targets prevents escape from re-replication control through mutation in the gene of a single target protein.

Note added in proof: While this manuscript was under review, we learned that Tada *et al.* have attained similar results (*27*).

References and Notes

1. P. N. Rao, R. T. Johnson, *Nature* **225**, 159 (1970).
2. B. Stillman, *Science* **274**, 1659 (1996).
3. A. Dutta, S. P. Bell, *Annu. Rev. Cell Dev. Biol.* **13**, 293 (1997).
4. J. Correa-Bordes, P. Nurse, *Cell* **83**, 1001 (1995).
5. J. Hayles, D. Fisher, A. Woollard, P. Nurse, *Cell* **78**, 813 (1994).
6. S. Piatti, T. Bohm, J. H. Cocker, J. F. Diffley, K. Nasmyth, *Genes Dev.* **10**, 1516 (1996).
7. T. J. McGarry, M. W. Kirschner, *Cell* **93**, 1043 (1998).
8. Full-length human geminin (amino acids 1 to 209) was polymerase chain reaction (PCR) amplified from IMAGE clone #727990 using primers containing a Nde I site in the 5′ primer and a Bam HI site in the 3′ primer. The PCR product was digested with Nde I and Bam HI and was ligated to pET14b (Novagen, Madison, WI) that had been cut with the same enzymes. This plasmid was transformed into BL21(DE3), and protein expression was induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. The cells were lysed by sonication and His6-tagged geminin purified over a nickel–nitrilotriacetic acid (nickel-NTA agarose) column (Qiagen, Valencia, CA) according to the manufacturer's instructions. Fractions containing human geminin were pooled and dialyzed against a buffer composed of 50 mM tris, pH 8.0, 50 mM NaCl, and 10% glycerol. Rabbit antibodies against this protein were generated (Cocalico Biologicals, Reamstown, PA) and reacted to the recombinant His6- geminin.
9. For metabolic labeling with ^{35}S-methionine, 293T cells were incubated for 2 hours in media lacking methionine. 293T cells were then incubated for two additional hours in media containing Express ^{35}S Protein Labeling Mix (200 μCi) (NEN Biochemicals, Boston, MA). Extracts were prepared by lysing the cells in a NP-40 lysis buffer containing 50 mM tris, pH 8.0, 0.1% Nonidet P-40, 150 mM NaCl, 50 mM NaF, 1 mM Na_3VO_4, 5 mM EDTA, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, pepstatin A (2 μg/ml), leupeptin (2 μg/ml), and aprotinin (5 μg/ml). Immunoprecipitations were done as described in (*26*), were washed five times in NP-40 lysis buffer, and were analyzed using SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography.
10. D. Maiorano, J. Moreau, M. Mechali, *Nature* **404**, 622 (2000).
11. H. Nishitani, Z. Lygerou, T. Nishimoto, P. Nurse, *Nature* **404**, 625 (2000).
12. A. J. Whittaker, I. Royzman, T. L. Orr-Weaver, *Genes Dev.* **14**, 1765 (2000).
13. Whittaker *et al.* (*12*) identified the cDNA clone AF070552 as the human Cdt1 homolog. Due to a frameshift error and incompleteness of the cDNA at the 5′ end, the published Cdt1 clone lacks amino acids 1 to 145 of the full-length human Cdt1. Amino acids 1 to 140 of human Cdt1 were discovered by using XCdt1 to identify a murine EST (BE303631) that was homologous to the NH_2 terminus of XCdt1 but missing from the published human Cdt1 sequence. Further database searches using BE303631 as the query sequence identified a human EST, BE018212, which was found after sequencing to contain the entire human Cdt1 sequence.
14. The plasmid expressing a fragment of Cdt1 (residues 238-546) was constructed by ligating the Msc I–Not I fragment of Image clone #24767 (AF070552) between the Not I and Bam HI sites of pET28a (Novagen). The protein was then expressed in the bacterial strain BL21(DE3), solubilized in 8M urea, and purified over a nickel-NTA agarose column as described by the manufacturer (Qiagen). Rabbits were immunized with this antigen (Cocalico Biologicals), and the antibody was shown to react specifically to His6-Cdt1 (238-546).
15. The plasmid expressing full-length GST-tagged geminin was constructed by PCR amplifying full-length geminin containing a 5′ Bam HI site and a 3′ Xho I site (restriction sites were contained in the primer) and the cloned into pGEX 5X-3 between the Bam HI and Xho I sites. The fusion protein was then expressed in bacteria and purified according to the manufacturer's instructions (Pharmacia Biotech, Piscataway, NJ). In vitro transcription and translation reactions were performed using the Promega TNT System (Madison, WI). Pull-down assays on glutathione agarose beads were done as described previously (*26*).
16. J. Walter, L. Sun, J. Newport, *Mol. Cell* **1**, 519 (1998).
17. Full-length human Cdt1 was PCR amplified from clone BE018212 using PCR primers containing a 5′ Eco RI site and a 3′ Not I site. This PCR product was cloned into pGEX 5X-1 between Eco RI and Not I and expressed in bacteria. The GST-fusion protein was purified according to the manufacturer's direction (Pharmacia).
18. J. F. Diffley, J. H. Cocker, S. J. Dowell, A. Rowley, *Cell* **78**, 303 (1994).
19. D. A. Natale, C. J. Li, W. H. Sun, M. L. DePamphilis, *EMBO J.* **19**, 2728 (2000).
20. HeLa cells were synchronized in mitosis using nocodazole (40 ng/ml) for 24 hours and then were released into drug-free media. Whole cell extracts were prepared every 3 hours until 24 hours after release from the mitotic block. The extracts were then immunoblotted with antibodies to geminin (1:2000), Cdt1 (1:2000), cyclin A (1:2000), or ORC2 (1:2000).
21. W. Jiang, N. J. Wells, T. Hunter, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 6193 (1999).
22. B. O. Petersen, J. Lukas, C. S. Sorensen, J. Bartek, K. Helin, *EMBO J.* **18**, 396 (1999).
23. P. Saha *et al.*, *Mol. Cell. Biol.* **18**, 2758 (1998).
24. V. Costanzo *et al.*, *Mol. Cell.* **6**, 649 (2000).
25. Y. Zeng *et al.*, *Nature* **395**, 507 (1998).
26. Y. L. Lin, C. Chen, K. F. Keshav, E. Winchester, A. Dutta, *J. Biol. Chem.* **271**, 17190 (1996).
27. S. Tada, A. Li, D. Maiorano, M. Mechali, J. Blow, *Nature Cell Biol.*, in press.

28. This work was supported by grant CA60499 to A.D. J.A.W. was supported by an NDSEG predoctoral fellowship. S.K.D. was supported by a U.S. Army postdoctoral fellowship DAMD17-00-1-0166. J.C.W. was supported by a Giovanni Armenise Grant and a Burroughs Wellcome Career Award. We thank M. Mechali for the antibodies to XCdt1 and M. Edwards for the *Xenopus* extracts.

23 October 2000; accepted 20 November 2000

Distinct Roles for TBP and TBP-Like Factor in Early Embryonic Gene Transcription in *Xenopus*

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The TATA-binding protein (TBP) is believed to function as a key component of the general transcription machinery. We tested the role of TBP during the onset of embryonic transcription by antisense oligonucleotide–mediated turnover of maternal TBP messenger RNA. Embryos without detectable TBP initiated gastrulation but died before completing gastrulation. The expression of many genes transcribed by RNA polymerase II and III was reduced; however, some genes were transcribed with an efficiency identical to that of TBP-containing embryos. Using a similar antisense strategy, we found that the TBP-like factor TLF/TRF2 is essential for development past the mid-blastula stage. Because TBP and a TLF factor play complementary roles in embryonic development, our results indicate that although similar mechanistic roles exist in common, TBP and TLF function differentially to control transcription of specific genes.

The TATA-binding protein (TBP) is often considered an essential component of the general transcription machinery, being involved in transcription by all three eukaryotic RNA polymerases. TBP is essential in yeast, binds to a variety of TATA boxes, and is recruited to TATA-less promoters via protein-protein interactions [reviewed in (*1*)]. Recently, however, TBP-related factors have been identified in metazoans. *Drosophila* has two such proteins (TRF1 and TRF2). TRF1 plays a role in the transcription of *tudor* and

transfer RNA (tRNA) genes (*2*, *3*). TRF1 has thus far not been identified in human, mouse, *Xenopus*, or *Caenorhabditis elegans*. TRF2, also known as TBP-like factor (TLF), is found in many metazoans, including *Xenopus* (*4*). The protein interacts with TFIIA and TFIIB and does not bind to canonical TATA boxes but may positively contribute to promoters similar to the SV40 promoter (*5*–*7*).

Here we examine whether either TBP or TLF/TRF2 is required for the onset of embryonic transcription or embryogenesis of *Xenopus laevis*. In *Xenopus* embryos, transcription begins after the egg has divided into 4000 cells, at the mid-blastula stage (*8*, *9*). Before the mid-blastula transition (MBT), embryos contain very little TBP (*10*, *11*). However, TBP is translated from maternally stored RNA just before the MBT to better match the levels of other basal transcription factors (*11*). TBP is rate-limiting for transcription before the MBT in both extracts and embryos (*11*–*13*), and precocious translation of synthetic TBP RNA in the embryo facilitates transcription before the MBT (*11*). The rapid rise in TBP protein levels correlates well with the onset of embryonic transcription.

TBP protein accumulation was prevented by an antisense oligonucleotide–based approach. We injected embryos with chemically modified oligonucleotides that are more stable in vivo than unmodified oligonucleotides, in order to reduce the amount of oligonucleotide required for effective mRNA turnover. These oligonucleotides contained a backbone modified with a cationic diethylethylenediamine (DEED) group (*14*), except for six or seven nucleotides in the middle that have normal phosphodiester bonds in their backbone. The 5′ and 3′ modified bases protect against exonuclease activity, whereas six phosphodiester bonds in the middle fulfill ribonuclease H (RNase H) substrate requirements to cleave the targeted mRNA (*15*). One of these oligonucleotides, TBP-AS3 (Fig. 1A), efficiently degraded maternal TBP mRNA in the embryo (Figs. 1B and 3B), effectively preventing the accumulation of TBP protein at the mid-blastula transition (Fig. 1C). Although these embryos lacked detectable levels of TBP, they were able to initiate gastrulation (Fig. 2C). However, development arrested before the completion of gastrulation (Fig. 2D). We have also tested a more fully DEED-modified TBP-AS3 oligonucleotide. By leaving only three instead of six phosphodiester bonds in the middle of the oligonucleotide, hybrids formed with mRNA are not degraded by RNase H (*15*). Embryos injected with this oligonucleotide developed normally (*16*). In addition, we constructed a mutant TBP RNA that encodes the wild-type TBP protein but is resistant to TBP-AS3–mediated degradation (Fig. 1B). This mutant RNA was translated in the embryo (Fig. 1C) and rescued the effects of the TBP-AS3 depletion of endogenous TBP mRNA. Rescued embryos not only completed gastrulation but developed into larvae (Fig. 2, E and F). Because transcription is required for the onset of gastrulation (*8*, *17*), it is remarkable that embryos without detectable levels of TBP initiated gastrulation. It is possible that levels of TBP that are undetectable by Western blotting contribute to the initiation of gastrulation in TBP-AS3 embryos. However, the significant reduction of transcription from a number of genes well before the developmental

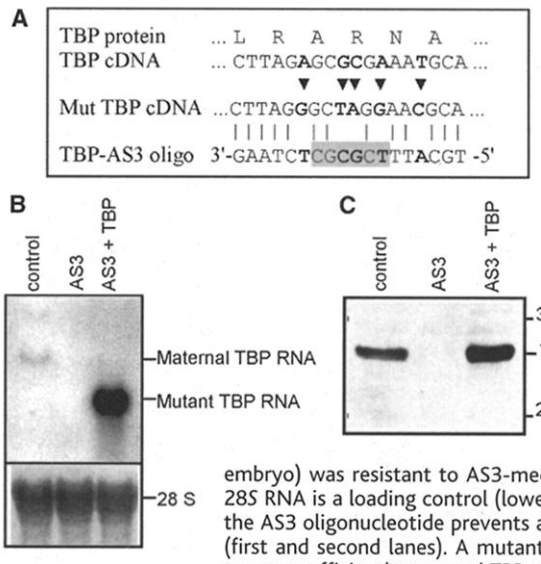

Fig. 1. The TBP-AS3 antisense oligonucleotide abolishes TBP protein accumulation during early embryogenesis. (**A**) Region of the TBP RNA targeted with the AS3 oligonucleotide. Point mutations were made (residues shown in bold) to render a synthetic RNA resistant to AS3-dependent turnover. The six nucleotides in the middle of the AS3 oligonucleotide feature normal phosphodiester bonds (shaded box). (**B**) Northern blot showing that the maternal TBP message (first lane) was effectively degraded in pre-gastrula embryos by TBP-AS3 oligonucleotide (second lane). A synthetic mutant TBP mRNA (0.5 ng per embryo) was resistant to AS3-mediated turnover (third lane). Staining of 28*S* RNA is a loading control (lower panel). (**C**) Western blot showing that the AS3 oligonucleotide prevents accumulation of TBP protein at the MBT (first and second lanes). A mutant TBP mRNA resistant to AS3-mediated turnover efficiently restored TBP protein levels to the embryo (third lane). Details on the methods are provided in (*26*).

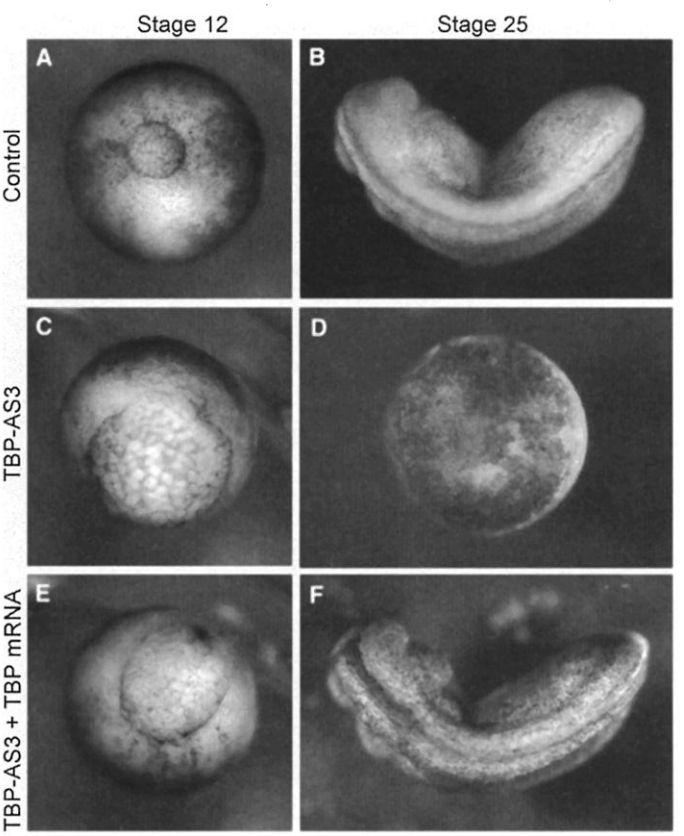

Fig. 2. TBP accumulation is required for embryonic development but is not essential for the onset of gastrulation. Embryos injected at the one-cell stage with water (**A** and **B**), 2 ng of partially modified TBP-AS3 oligonucleotide (**C** and **D**), or 2 ng of TBP-AS3 plus 0.25 ng of mutant TBP RNA (**E** and **F**) are shown at stages 12 (advanced gastrula, left panels) and 25 (larva, right panels). More than 95% of TBP-AS3–injected embryos showed a phenotype similar to the phenotype shown in (C) and (D).

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arrest suggests that TBP was functionally deficient in these embryos.

To determine to what extent transcription was affected in TBP-AS3–injected embryos, α^{32}P-UTP was injected to visualize the most abundant transcripts at the MBT. TBP-AS3 reduced most transcripts (Fig. 3A). Specifically, tRNA synthesis, which accounts for more than 85% of all transcription in the embryo after the MBT, decreased about 10-fold in TBP-deficient embryos. As for the other identifiable RNA polymerase III–dependent transcripts, 7*S* but not 5*S* transcription was down-regulated in AS3-injected embryos. U1 and U2, two abundant RNA polymerase II–dependent transcripts involved in splicing, were also affected in these embryos (Fig. 3A). Northern blot analysis was performed to investigate the requirement for TBP protein accumulation for the embryonic onset of transcription of protein-encoding genes (Fig. 3B). The genes selected for analysis are transcribed primarily after the MBT. *Xbra* is a frog *brachyury* transcription factor homolog involved in mesoderm formation (*18*), whereas *GS17* is a gene of unknown function that is activated at the MBT (*19*). Neither gene was significantly affected in TBP-AS3–injected embryos (Fig. 3B). In contrast, transcription of the genes encoding translation elongation factor EF1α (*20*), *Xenopus* keratins XK81A1 and XK70A (*21*), and MyoD (*22*) decreased significantly in embryos lacking detectable amounts of TBP (Fig. 3B). These effects were rescued by injected TBP message (Fig. 3C).

The observation that not all embryonic transcription is TBP-dependent raised the possibility of an important developmental role of TLF (TRF2). The *Xenopus* homolog of TLF was isolated by reverse transcription–polymerase chain reaction (RT-PCR). We determined the expression of TLF during embryogenesis by quantitative RT-PCR. TLF

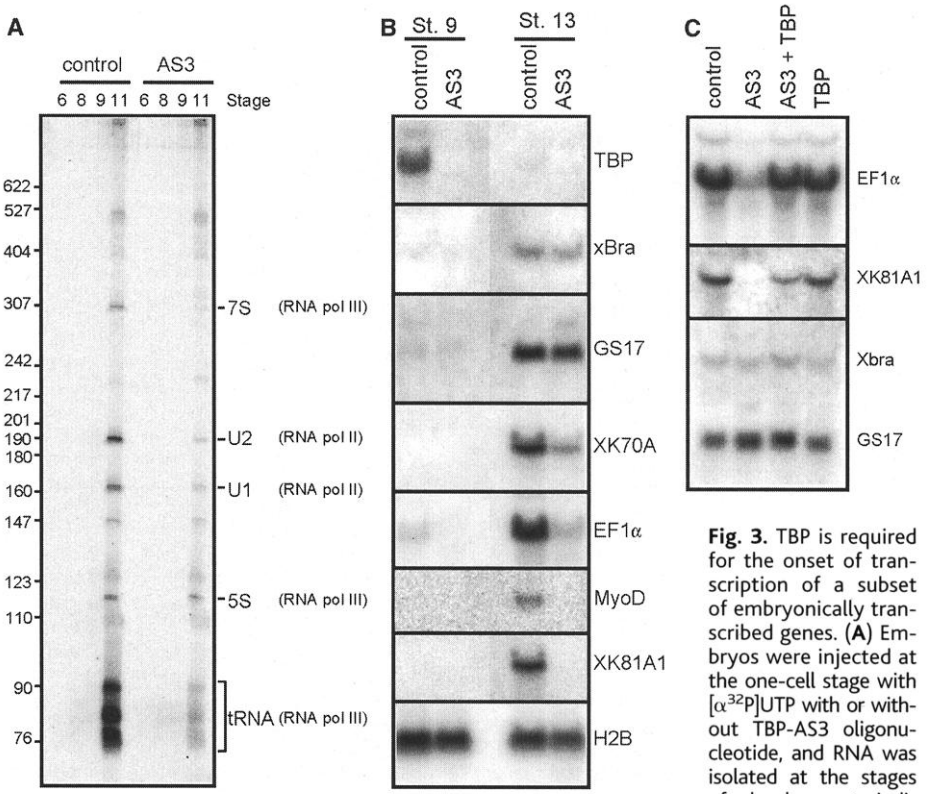

Fig. 3. TBP is required for the onset of transcription of a subset of embryonically transcribed genes. (**A**) Embryos were injected at the one-cell stage with [α^{32}P]UTP with or without TBP-AS3 oligonucleotide, and RNA was isolated at the stages of development indicated. Developmental stages are as follows: Stages 6 to 9, blastula; stage 8.5, mid-blastula; stages 10.5 to 13, gastrula. Transcripts were identified by size as previously described (*8*, *9*). (**B**) Northern blots were labeled, stripped, and reprobed multiple times with DNA fragments of the genes shown on the right. Three embryo equivalents of RNA from uninjected (first and third lanes) or TBP-AS3 oligonucleotide–injected (second and fourth lanes) embryos were loaded per lane. (**C**) TBP mRNA rescues the effects of the AS3 oligonucleotide on gene expression.

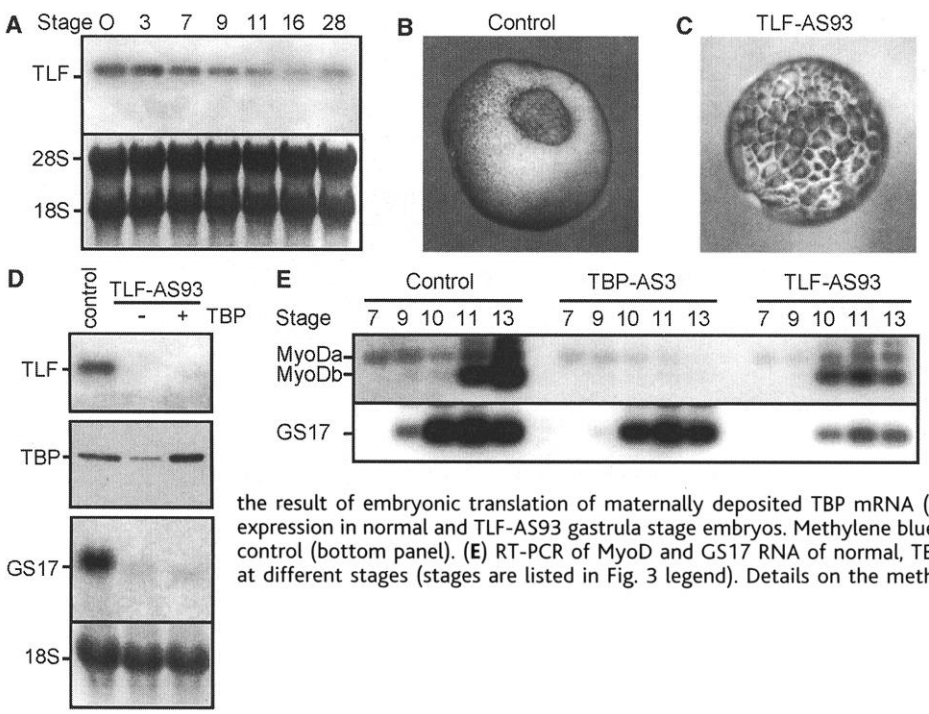

Fig. 4. Expression and function of TLF in *Xenopus* embryogenesis. (**A**) TLF mRNA abundance in oocytes (O), and cleavage (stage 3), early and late blastula (stages 7 and 9), gastrula (stage 11), neurula (stage 16), and tailbud stage (stage 28) embryos, as detected by quantitative RT-PCR (*26*). Staining of 28*S* and 18*S* RNA was performed as a control for RNA amount (lower panel). (**B** and **C**) Phenotypes of normal and TLF-AS93–injected embryos at stage 12 (gastrula). The arrest was observed in close to 100% of all TLF-AS93–injected embryos. (**D**) Top panel: TLF mRNA is efficiently degraded in a TLF-AS93–dependent fashion in vivo, as assessed by quantitative RT-PCR. Second panel: TBP expression in control embryos (first lane), TLF-AS93 oligonucleotide–injected embryos (second lane), and embryos injected with TLF-AS93 and TBP RNA (third lane). Any TBP detected in the first and second lanes is the result of embryonic translation of maternally deposited TBP mRNA (*11*). Third panel: Northern blot analysis of *GS17* expression in normal and TLF-AS93 gastrula stage embryos. Methylene blue staining of 18*S* RNA was performed as a loading control (bottom panel). (**E**) RT-PCR of MyoD and GS17 RNA of normal, TBP-AS3–injected, and TLF-AS93–injected embryos at different stages (stages are listed in Fig. 3 legend). Details on the methods are provided in (*26*).

mRNA was detected in oocytes and embryos, being relatively abundant during cleavage and blastula stages (Fig. 4A). TLF is widely expressed in the embryo, as was shown in a large-scale in situ hybridization screen using randomly picked cDNAs, one of which was TLF (clone 3.39) (*23*). We targeted the endogenous TLF mRNA for degradation with a DEED-modified oligonucleotide, TLF-AS93, similar to the way in which we targeted TBP mRNA with TBP-AS3. TLF-AS93 mediated efficient degradation of TLF mRNA in vivo (Fig. 4D). TLF-AS93–injected embryos developed normally until the onset of embryonic transcription between stage 8 and 9, the stage at which they arrested (Fig. 4, B and C). TLF-AS57, a different oligonucleotide capable of degrading TLF mRNA in vivo, caused a similar developmental arrest, whereas control oligonucleotides did not (*16*). Recently, a similar early embryonic arrest was observed in *tlf-1* (*RNAi*) embryos of *C. elegans* (*24*, *25*). We examined the expression of embryonically transcribed genes in TLF-AS93–injected embryos, and we could not detect embryonic transcription of *Xbra* or *EF1*α, and *GS17* RNA was also severely reduced [Fig. 4, D and E (*16*)]. Maternal TBP mRNA, which in normal embryos is translated at the MBT (*11*), was translated in TLF-AS93 embryos (Fig. 4D), albeit with slightly lower efficiency. We ruled out a possible contribution of lower TBP levels to the TLF-AS93 phenotype by overexpressing TBP with a synthetic TBP mRNA. Both the TLF-AS93–mediated developmental arrest and the transcriptional impairment of GS17 were independent of TBP levels (Fig. 4D). The expression of MyoD and GS17 was examined in more detail. In the absence of detectable amounts of TBP, MyoD was not transcribed de novo, although low levels of maternally deposited MyoD RNA were observed (Fig. 4E). In TLF-AS93–injected embryos, on the other hand, MyoD was expressed after their developmental arrest, most notably at the time normal embryos start gastrulation [stage 10 to 11 (Fig. 4E)]. GS17 expression by comparison was significantly affected in TLF-AS93 embryos (Fig. 4, D and E) but much less so in TBP-AS3 embryos (Figs. 3B and 4E).

These data establish that TBP is essential for transcription of some but not all class II and class III genes during early embryogenesis. TBP is dispensable for transcription of a subset of genes and for the onset of gastrulation, whereas it is required for sustaining embryonic development. TLF, on the other hand, is essential for development beyond the mid-blastula stage. Our analysis of TBP and TLF function suggests that a remarkable functional dichotomy exists regarding general transcription factor requirements. Important developmental genes such as *Xbra* are transcribed in the absence of TBP, whereas a gene such as *MyoD* is strictly TBP-dependent. TBP and TLF fulfill distinct requirements for transcription and embryogenesis.

References and Notes

1. N. Hernandez, *Genes Dev.* **7**, 1291 (1993).
2. S. Takada, J. T. Lis, S. Zhou, R. Tjian, *Cell* **101**, 459 (2000).
3. M. C. Holmes, R. Tjian, *Science* **288**, 867 (2000).
4. J. C. Dantonel, J. M. Wurtz, O. Poch, D. Moras, L. Tora, *Trends Biochem. Sci.* **24**, 335 (1999).
5. M. Teichmann *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 13720 (1999).
6. P. A. Moore *et al.*, *Mol. Cell. Biol.* **19**, 7610 (1999).
7. M. D. Rabenstein, S. Zhou, J. T. Lis, R. Tjian, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 4791 (1999).
8. J. Newport, M. Kirschner, *Cell* **30**, 675 (1982).
9. ———, *Cell* **30**, 687 (1982).
10. P. Bell, U. Scheer, *Exp. Cell Res.* **248**, 122 (1999).
11. G. J. C. Veenstra, O. H. J. Destrée, A. P. Wolffe, *Mol. Cell. Biol.* **19**, 7972 (1999).
12. M.-N. Prioleau, J. Huet, A. Sentenac, M. Méchali, *Cell* **77**, 439 (1994).
13. G. Almouzni, A. P. Wolffe, *EMBO J.* **14**, 1752 (1995).
14. J. M. Dagle, J. L. Littig, L. B. Sutherland, D. L. Weeks, *Nucleic Acids Res.* **28**, 2153 (2000).
15. J. M. Dagle, D. L. Weeks, *Methods Enzymol.* **313**, 420 (2000).
16. G. J. C. Veenstra, D. L. Weeks, A. P. Wolffe, data not shown.
17. J. C. Sible, J. A. Anderson, A. L. Lewellyn, J. L. Maller, *Dev. Biol.* **189**, 335 (1997).
18. J. Smith, *Trends Genet.* **15**, 154 (1999).
19. P. A. Krieg, D. A. Melton, *EMBO J.* **4**, 3463 (1985).
20. P. A. Krieg, S. M. Varnum, W. M. Wormington, D. A. Melton, *Dev. Biol.* **133**, 93 (1989).
21. M. Jamrich, T. D. Sargent, I. B. Dawid, *Genes Dev.* **1**, 124 (1987).
22. R. A. W. Rupp, H. Weintraub, *Cell* **65**, 927 (1991).
23. V. Gawantka *et al.*, *Mech. Dev.* **77**, 95 (1998).
24. J. C. Dantonel, S. Quintin, L. Lakatos, M. Labouesse, L. Tora, *Mol. Cell.* **6**, 715 (2000).
25. L. Kaltenbach, M. A. Horner, J. H. Rothman, S. E. Mango, *Mol. Cell.* **6**, 705 (2000).
26. Supplementary Web information on materials and methods is available on *Science* Online at www.sciencemag.org/cgi/content/full/290/5500/2312/DC1.

27. We thank T. D. Sargent for constructs, C. Greaves for synthesis of DEED-modified oligonucleotides, E. Schlag for sequencing, and I. B. Dawid and J. M. Dagle for helpful discussions. D.L.W. was supported by grants from NIH.

20 September 2000; accepted 21 November 2000

Cholinergic Enhancement and Increased Selectivity of Perceptual Processing During Working Memory

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Using functional magnetic resonance imaging, we investigated the mechanism by which cholinergic enhancement improves working memory. We studied the effect of the cholinesterase inhibitor physostigmine on subcomponents of this complex function. Cholinergic enhancement increased the selectivity of neural responses in extrastriate cortices during visual working memory, particularly during encoding. It also increased the participation of ventral extrastriate cortex during memory maintenance and decreased the participation of anterior prefrontal cortex. These results indicate that cholinergic enhancement improves memory performance by augmenting the selectivity of perceptual processing during encoding, thereby simplifying processing demands during memory maintenance and reducing the need for prefrontal participation.

Working memory (WM) (*1*) is mediated by a widely distributed neural system in the human brain. Modulation of cholinergic neurotransmission alters memory function, including WM. Performance on WM tasks is improved by pharmacologic agents that enhance cholinergic function and is impaired by agents that block cholinergic function (*2–4*). The mechanism by which cholinergic modulation alters WM, however, is unclear.

Different regions in the distributed neural system for WM support dissociable cognitive subcomponents of this complex function. With functional magnetic resonance imaging (fMRI) the neural activity associated with WM can be decomposed into task subcomponents that are separated in time and space, namely the responses during perceptual encoding, activity during memory delays, and the responses during recognition testing (*5, 6*).

Prefrontal cortex plays a central role in maintaining and manipulating the contents of WM (*7, 8*), but previous work suggests this region is not the site where cholinergic enhancement improves processing efficiency. Cholinergically mediated improvement in WM performance is correlated with reduced activity in right prefrontal cortex (*9, 10*). Reduced activity in this region likely reflects reduced WM load or task difficulty (*11, 12*)

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