The thyroid transcription factor 1 (TTF-1) is a home-domain-containing protein implicated in the activation of thyroid-specific gene expression. Here we report that TTF-1 is capable of activating transcription from thyroglobulin and, to a lesser extent, thyroperoxidase gene promoters in nonthyroid cells. Full transcriptional activation of the thyroperoxidase promoter by TTF-1 requires the presence of at least two TTF-1 binding sites. TTF-1 activates transcription via two functionally redundant transcriptional activation domains that as suggested by competition experiments, could use a common intermediary factor.

Regulation of transcription is mediated by protein factors that interact with specific DNA sequences located, in promoter and enhancer elements, at variable distances from the site of assembly of the basal transcription complex (1, 2). The recognition of specific DNA sequences and the ability to stimulate transcription impinge on structurally distinct domains of transcription factors (3). The DNA binding function is determined by one of several structural motifs such as homeodomains, POU domains, zinc fingers, basic-leucine zippers, and basic helix-loop-helix domains (4). Detailed functional and structural studies have demonstrated a specific interaction between these motifs and the cognate DNA sequences (5). In contrast, protein domains with transcriptional activating properties are not as well defined. Several apparently unrelated motifs capable of transcriptional activation have been identified. Some of them depend on the presence of sequences rich in a particular amino acid (acidic residues, glutamine-rich, proline-rich), but many others cannot be grouped in any particular class (6–8). The structures of these motifs are not known; it has been suggested that the integrity of either amphipathic α-helices (VP16 mutants) (9, 10) or β-sheets (11, 12) could be essential for transcriptional activity.

The activity of eukaryotic transcription factors can be regulated by various mechanisms including phosphorylation (13–15), control of the redox state (16, 17), and interaction with other factors (18). Furthermore, several lines of evidence suggest that the activity of some transcription factors and the selection of promoters to be activated by the same factor may depend on a specific cellular environment. Transcriptional activators contact the basal transcriptional machinery through intermediates called co-activators that could be subjected to regulation themselves (19–21). Some of these co-activators can be relevant in determining the specific action of a transcription factor (22–24).

The thyroid transcription factor 1 (TTF-1) could provide a useful model to study the regulatory interactions controlling the functional specificity of a transcription factor. TTF-1 was originally identified as a protein binding to sequences present in multiple copies in the promoters of the thyroglobulin (Tg) (25) and thyroperoxidase (TPO) (26) genes, both of which are exclusively expressed in the thyroid follicular cells (27). However, the presence of TTF-1 in lung (28), in restricted regions of the fetal brain, and in thyroid cell precursors (29), where the Tg and TPO genes are silent, indicates that the function of TTF-1 is not restricted to adult thyroid tissue. In support of this notion, TTF-1 has recently been implicated in the transcriptional activation of genes exclusively transcribed in lung epithelium, such as those encoding surfactant proteins A and B (30, 31). The multiplicity of roles that TTF-1 plays in vivo suggests that its activity is subjected to regulation. In this respect we have already shown that TTF-1 is a phosphoprotein (32) and that its DNA binding activity is under redox control (33).

In this paper we show that TTF-1 transactivates the Tg and, albeit to a lesser extent, the TPO promoter in nonthyroid cells. We demonstrate that two independent domains, located on either side of the TTF-1 homeodomain, contribute to the transcriptional activity obtained with the entire protein. Competition experiments suggest that the two transcriptional activating domains of TTF-1 could converge on a common pathway.

**MATERIALS AND METHODS**

Plasmids—The plasmid CMV-TTF1 was generated by inserting the entire TTF-1 cDNA (28) in the expression vector Rc-CMV (Invitrogen). The TTF-1 coding sequence extended for one ATG codon upstream of the sequence published in Ref. 28, and was deduced by Lonigro et al. (2). TTF-1 deletion mutants were constructed by polymerase chain reaction using primers encoding the amino acids at the ends of each fragment (see below); all deletions were done replacing the full length cDNA between the HindIII and XbaI sites of CMV-TTF1. The primer always included either HindIII or XbaI restriction sites to facilitate cloning. For all deletions of TTF-1 amino terminus the sequence 5′-CCAC-
CAATG-3' was added to provide a ribosome entry site (34) and an ATG codon for translation initiation. Plasmid M1 was derived from RC-CMV by deletion of a BamHI fragment containing the neomycin resistance and was used as control.

GAL4-TTF-1 chimeras were constructed in the plasmid CMV-SG424, which contains the DNA-binding domain of GAL4 (residues 1-147) under the control of the human cytomegalovirus enhancer promoter (33). CMV-SG424 is derived from pSG424 (36) with the CMV promoter replacing the SV40 early promoter. DNA fragments, generated by polymerase chain reaction as described above and encoding different segments of TFF-1, were inserted between the EcoRI and XbaI restriction sites of CMV-SG424. The reporter plasmid for the GAL4 fusions, pCMV-GAL4, has been described (20). To generate C5 E1b, the GAL4 binding sites of G5E1b were replaced with a pentamer of the TTF-1 binding sequence (5’-CCCAGTCAAGTGTTCTT-3’) that was inserted between the PstI and XbaI restriction sites. E1b contains only the E1b TATA box in front of the CAT coding sequence.

The plasmids TPO-Luc, TPO-Em (26), pTACAT3, pTACAT11.5, pTACAT13 (37), A-core, B-core, C-core, and CBC (25) have been described. The plasmids CMV-Luc and RSV-CAT used as internal control in transfection assays were kindly provided by U. Deuschle and G. Morrone, respectively.

Cell Culture and Transfection—HeLa cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. For transient expression assays, cells were plated at 3 x 10^5 cells/60-mm tissue culture dish 4–6 h prior to transfection. Transfections were carried out by the calcium phosphate co-precipitation technique (38). After exposure to calcium phosphate-DNA precipitate for 12–14 h, the cells were washed with phosphate-buffered saline and grown for 48 h. CAT (39) and luciferase activities (40) on cell extracts were determined as described.

FRTL-5 cells were grown as described by Ambesi-Impiombato and Coon (41). For the transient expression assay, cells were plated at 5 x 10^5 cells/60-mm tissue culture dish 48 h prior to transfection. 3 h prior to transfection, the medium was changed to Dulbecco’s modified Eagle’s medium containing 5% calf serum and growth factors. Transfections were carried out by calcium phosphate co-precipitation as described (26).

Bandshift Assay—Cellular extracts were prepared as described (25). The binding reaction was carried out in a buffer containing 40 mM Hepes, pH 7.9, 200 mM KCl, 0.5 mM dithiothreitol, and 0.3 mg/ml poly(dI-dC). After 30 min of incubation at room temperature, free DNA and DNA-protein complexes were resolved on a 6% polyacrylamide gel run in 0.5 x TBE (2 mM EDTA, 90 mM boric acid, 90 mM Tris-HCl, pH 8.0) for 2–3 h at 4°C. The gel was dried and then exposed to x-ray film at –80°C. Oligonucleotide C, used to measure TTF-1 binding activity, has been described (28). GAL4 DNA binding activity was measured using a double-stranded nucleotide with the sequence 5’-GATCCGGAGGGACATCCGGCTAG-3’ (top strand).

RNase Protection Assay—Total RNA from cultured cells was prepared by the acid guanidinium thiocyanate phenol procedure (42). The probe for RNase protection was synthesized by T7 polymerase transcription of SacI-linearized pBSLuc (26) and used to control for transfection efficiency. Hybridization and RNase treatment were as described (43).

Western Blot—Extracts of HeLa transfected cells, prepared as described previously (44), were boiled in Laemmli sample buffer and resolved by 10% SDS-polyacrylamide gel electrophoresis (43). After electro blotting onto nitrocellulose membrane, TTF-1 was localized with a specific antibody (29) using an ECL kit (Amersham Corp.).

RESULTS

TTF-1 Activates Transcription of Tg and TPO Promoters—Plasmids containing either Tg (pTACAT3 (37) or TPO (TPO-Luc) (26) promoter, fused to CAT or luciferase (Luc) coding sequences (Fig. 1A), respectively, were transfected in HeLa cells, which do not contain any detectable TTF-1. As expected, given the demonstrated thyroid-specific expression of both promoters (45), very little transcriptional activity could be detected from either of them. A dose-dependent activation of both promoters was observed (Fig. 1B) upon co-transfection with increasing amounts of a TTF-1 expression vector containing the TTF-1 cistron under the control of the human cytomegalovirus promoter (CMV-TTF-1). In the case of the Tg promoter, maximal activation was obtained with 0.25 μg of expression vector, whereas the TPO promoter was only activated when 4 μg of expression vector were used. Mutants of Tg (C-core) and TPO (TPO-Em) promoters, where the TATA proximal TTF-1 binding sites are mutated and do not bind TTF-1 any longer, are poorly activated, indicating that the transcriptional activation obtained with wild-type promoters depends on TTF-1 bind-
Transcriptional activation by TTF-1 appears to be restricted to exogenous, co-transfected promoters, because neither Tg nor TPO mRNA could ever be detected in TTF-1-producing HeLa cells (data not shown). Further studies were only carried out on the more responsive Tg promoter. To test whether Tg transcription in HeLa cells uses the same basal signals as in thyroid, we carried out the RNase mapping experiment shown in Fig. 1C, which demonstrates that the transcripts produced in HeLa cells upon TTF-1 stimulation initiate at the same site used by the Tg promoter in differentiated thyroid cells.

We next asked whether the template requirements for TTF-1 activation of the Tg promoter were similar between HeLa and differentiated thyroid cells. To this end, several promoter mutants were assayed in a co-transfection assay. The minimal Tg promoter contains three TTF-1 binding sites (Fig. 2, A, B, and C), two of which (A and C) are required for transcription to occur in thyroid cells. Site A also binds the ubiquitous factor ubiquitous factor A (25). However, it could be shown that, in thyroid cells, this binding is dispensable because a mutated promoter (CBC in Fig. 2) that abolishes ubiquitous factor A but maintains TTF-1 binding at A site is even more active than the wild-type promoter (25). As shown in Fig. 2, mutants A-core and C-core have a highly reduced transcriptional activity in TTF-1-transfected HeLa cells, whereas the B-core mutation does not significantly interfere with the transactivation by TTF-1. Conversely, mutant CBC shows a transcriptional activity about 50% higher than the wild-type Tg promoter. These results go in the same direction as those obtained in the FRTL-5 cell line (Fig. 2 and Ref. 37), suggesting that the template requirements for transcriptional activation by TTF-1 are the same between thyroid and HeLa cells.

In addition to TTF-1 binding sites, at least two other DNA elements are important for Tg promoter activity in thyroid cells: site K, which is bound by the thyroid transcription factor 2 (25), and the site defined by mutation 13 (37). Plasmid pTACAT11.5 contains a mutated Tg promoter where TTF-2 binding to site K has been abolished (37), and in agreement with previous results, the transcriptional activity of this mutant in FRTL-5 cells is 50% of the wild-type promoter. The mutation 13, which does not interfere with TTF-1 binding, results in an even larger decrease of Tg promoter transcription (Fig. 3A and Ref. 37). However, mutations 11.5 and 13 have no effect on Tg promoter transcription when tested in TTF-1-transfected HeLa cells (Fig. 3A). These data indicate that both TTF-2 and the protein recognizing the site defined by mutation 13 are thyroid-specific and that they contribute to the full activity of the Tg promoter in thyroid cells. Furthermore, these data suggest that the TTF-1-induced Tg promoter activity, which we are measuring in the co-transfection assay, is much lower than that expressed by the same promoter in thyroid cells. Nonetheless, it can be concluded that TTF-1 can activate transcription in the absence of other thyroid-specific proteins. To provide further support for this notion, we constructed C5 E1b (Fig. 3B), a promoter containing five TTF-1 binding sites, arranged head to tail upstream of the E1b TATA box. C5 E1b is at least as
effcient as the Tg promoter in FRTL-5 cells, and in HeLa cells it is efficiently transcribed only upon co-transfection of a TTF-1 expression vector.

Several Parts of TTF-1 Contribute to Its Transactivating Function—To map TTF-1 sequences involved in transcriptional activation, expression vectors encoding deletion mutants of TTF-1 (Fig. 4A) were constructed and transfected in HeLa cells. Comparable protein levels (Fig. 4B) corresponding to approximately equal DNA binding activity (Fig. 4C) were obtained for each of the mutants tested, indicating that differences in transcriptional activity among TTF-1 deletion mutants are not due to either reduced protein concentration or impaired DNA binding.

The ability of various TTF-1 deletions to activate transcription from the Tg promoter is shown in Fig. 5. The decreased transcriptional activity of TTF-1 deletions Δ1, Δ33, Δ2, and Δ3 compared with the wild-type protein indicate the presence of a transcriptional activating domain (domain N) NH2-terminal to the homeodomain. A critical part of this domain is contained in the amino acids missing in the Δ1 mutant, because more extensive deletions, such as those of mutants Δ2, Δ33, and Δ3, do not decrease the transcriptional activity any further. Furthermore, the considerable residual activity of these deletion mutants indicates that other parts of TTF-1 are capable of transcriptional activation.

The decreased transcriptional activity of mutant Δ6 indicates that an activating domain (domain C) resides in this region of TTF-1. However, extending the deletion of mutant Δ6, such as in mutant Δ14, a transcriptional activity comparable with that of the wild-type protein is obtained. These results are consistent with the presence of an inhibitory region located between amino acids 221 and 295 (domain I). The behavior of deletion mutants Δ26 and Δ35, where the putative inhibitory region was removed, supports this notion because both mutants show a higher activity of the corresponding proteins that contain this domain (wild-type TTF-1 and mutant Δ33, respectively). Interestingly, this inhibitory region is glutamine-rich, even though glutamine-rich regions are very often found within transcriptional activating domains (46). Mutant Δ36, where both the amino- and the carboxyl-terminal transactivating domains were disrupted, shows the least ability to activate transcription, thus confirming the redundancy of the two activating domains.

TTF-1 deletion mutants were also tested using the artificial

Fig. 3. Additional thyroid-specific elements different from TTF-1 are required for full transcriptional activity of the Tg promoter. A, the mutated Tg promoters (3 µg) were transiently transfected in FRTL-5 and HeLa cells (in the latter case 2 µg of CMV-TTF1 were co-transfected). In each cell line the activity of wild-type promoter pTACAT3 was taken as 100%, and the relative expression of the mutants was assessed. CMV-Luc was co-transfected to normalize for transfection efficiency. The values represent the averages of at least three independent determinations. The error bars show the standard deviation of the mean. B, C5 E1b or E1b promoters (3 µg) were transiently transfected in FRTL-5 and HeLa (in the latter case 2 µg of CMV-TTF1 were co-transfected). Data are presented as described for A.
Transcriptional Activation by TTF-1

The Activation Domains of TTF-1 Are Also Active If Fused to Heterologous DNA-binding Domain—To obtain an independent assessment of the functional domain in TTF-1, several constructs encoding chimeric proteins containing different segments of TTF-1 fused to the GAL4 DNA-binding domain were prepared. TTF-1/GAL4 fusions were tested on the G5E1b reporter construct (20) containing five GAL4 binding sites upstream to the E1b TATA box (Fig. 6). The GAL4 DNA-binding domain alone was unable to activate the expression of the reporter gene in this assay, and its activity was considered as a reference value for the chimeric constructs. Consistent with the observations reported in the previous paragraph, the region of TTF-1 NH2-terminal to the homeodomain contains an autonomous transactivating domain (see constructs from ΔG7 to ΔG21). The limits of this domain can be located, at the NH2-terminal side, between amino acids 51 and 102 (compare ΔG18 and ΔG19) and, at the COOH terminus, between amino acids 90 and 123 (compare ΔG1 and ΔG2). In agreement with the results obtained with the deletion mutants on the C5 E1b promoter, removal of the region spanning between amino acids 1 and 51 determines an increase in transcriptional activity (compare ΔG7 with ΔG18 and ΔG2 with ΔG21). Thus, in two different contexts, region 1–51 displays an inhibitory effect on TTF-1 transcriptional activation. However, in FTTL-5 cells this domain seems to have an activating function, because ΔG21 is much less active than ΔG7. These results suggest that this region of TTF-1 is capable of functioning in both a cell type- and promoter-dependent manner.

Although the entire COOH-terminal region of TTF-1 (ΔG11) is unable to activate transcription when fused to GAL4, deletion of the glutamine-rich region (amino acids 221–299) demonstrates the transcriptional activity of the C domain (ΔG13). Moreover, the glutamine-rich region does not have any transcriptional activity even when fused to the GAL4 DNA-binding domain (ΔG12). The COOH-terminal border of the transcriptional activating domain starting from amino acid 295 is very close to the end of the molecule because a deletion of the last 20 amino acids results in complete loss in transcriptional activity (ΔG15).

Functional Interplay between the Different Domains of TTF-1—The functional redundancy of the NH2- and COOH-terminal domains suggests that they might interact with a common intermediary factor or co-activator. To test this hypothesis, we verified whether the amino-terminal domain could compete with the carboxyl-terminal one and vice versa. This inhibition or “squelching” effect (47) would result from the NH2- and COOH-terminal domain competing for and thereby titrating away a common co-activator. Results of these squelching experiments are shown in Fig. 7. The deletion mutant Δ14 (containing the NH2-terminal domain, up to and including the homeodomain) is able to inhibit the transcriptional activation induced by either the NH2-terminal domain (ΔG21) or the COOH-terminal domain (ΔG13) of TTF-1 fused to the DNA-binding domain of GAL4 on the GSE1b reporter. No reduction was observed in transcription from the CMV-Luc reporter used to normalize for transcription efficiency, demonstrating that the construct Δ14 did not inhibit transcription in a nonspecific manner. The ability of Δ14 to inhibit TTF-1-dependent transcription strongly argues for the existence of a co-factor that is not a component of the basal transcriptional machinery but it is essential for TTF-1 function. Thus, both the NH2- and COOH-terminal domains could activate the basal transcriptional machinery through an essential intermediary factor that is present in the cells at a relatively low concentration, and it can thereby be titrated away by the NH2-terminal domain over-expressed in a form unable to bind the target promoter. Surprisingly, Δ3, which contains the COOH-terminal region of TTF-1 up to and including the homeodomain, is unable to inhibit either ΔG21 or ΔG13 transcriptional activity. Even though the level of expression of Δ3 in the cells is comparable with that of Δ14 as measured by DNA binding assays (data not shown), it is still conceivable that the interaction of Δ3 with the intermediary factor is subordinate to a limiting step (e.g. inter-
action with another protein, phosphorylation, etc.) that does not allow the attainment of sufficient concentration of the D3 species "competent" for squelching.

**DISCUSSION**

The present study demonstrates that TTF-1 is capable of activating transcription from thyroid-specific promoters in nonthyroid cells, thus providing strong support to the notion that this factor plays an important role in the differentiation of thyroid follicular cells. The HeLa cell co-transfection assay used in this paper reflects some very important functional properties of TTF-1. In thyroid cells TTF-1 requires at least two properly spaced binding sites in order to activate transcription from the Tg promoter (45). Such a requirement is maintained in HeLa cells, as demonstrated by the sensitivity of transcriptional activation to Tg promoter mutations. Furthermore, as in FRTL-5 cells, TTF-1 is capable of activating transcription in HeLa (and several other) cells from C5 E1b, an artificial promoter containing a viral TATA box (20) and a multimerized TTF-1 binding site, thus revealing the intrinsic capacity of TTF-1 to activate transcription in the absence of other cell type-specific components. Nonetheless, in defining the regions of TTF-1 necessary for transcriptional activation, we have discovered that this protein is capable of functioning in a promoter and cell type-specific manner. The ability of TTF-1 to distinguish between different promoters and cell types is revealed by the deletion that removes 50 amino acids at the amino terminus (Δ1). When the activity of Δ1 is compared with that of wild-type TTF-1 in HeLa cells, a 2-fold reduction in the ability to activate the Tg promoter but a 3-fold improvement in transcription from C5E1b are observed, suggesting that TTF-1 is sensitive to promoter structure and adjusts the activity of its amino-terminal region accordingly. The ability of a transcription factor to function as activator or repressor, depending on the promoter context, has been previously reported for c-ErBα (48) and Rap1 (49). The sensitivity of TTF-1 to promoter structure is also suggested by the much higher transactivation observed with the Tg than with the TPO promoter. Even though the number and spacing of binding sites is quite similar in the regulatory regions of both genes, the orientation of the binding sites and their affinity toward TTF-1 are not identical between the two promoters (45), suggesting that these features could be important modulators of TTF-1 activity. Interestingly, this also appears to be the case for the thyroid-specific transcriptional factor Pax-8, which binds a single site in both Tg and TPO promoters. In both cases the Pax-8 binding site overlaps with the TTF-1 binding site proximal to the TATA box. However, Pax-8 activates the TPO promoter more efficiently of the Tg promoter (44).

In order to define the transcriptional activation domains of TTF-1, we have analyzed the activity of chimeric proteins con-
some general characteristics of these domains have been elu-
cidated (46). However, an inhibitory glutamine-rich region has
ever been described. It would be of interest to test whether the
inhibitory domain of TTF-1 interacts with the same specific
subset of TBP-associated factors that has been shown to be
required for transcriptional activation by the glutamine-rich
Sp1 (47).

The functional redundancy existing between the NH₂- and
COOH-terminal activating domains of TTF-1 suggests that
they could converge on the same functional pathway or inter-
mediary factor. Transcriptional co-factors are, of course, rele-
vant for all transcription factors, but they could play an essen-
tial role in the cell type- and promoter-specific transcriptional
activation. The best example is the B-cell-specific activation of
immunoglobulin promoters by the ubiquitous factor Oct-1,
which is mediated by B-cell-specific transcriptional co-factors
(22–24). TTF-1 presents a similar different problem, because
even though it shows a very restricted tissue distribution, it
nonetheless has different functions in thyroid and lung, two of
the three cell types where it is expressed. Unfortunately, no
information is available on putative targets of TTF-1 in devel-
oping diencephalic neurons, the third cell type known to con-
tain TTF-1 proteins. TTF-1 is able to activate transcription of
Tg and TPO genes in thyroid but not in lung tissue and,
conversely, it is able to activate transcription of the SPB gene
in lung but not in thyroid tissue. HeLa cells seem to be a
neutral environment, because TTF-1 is able to activate both
thyroid (this study) and lung targets (30, 31) in this cellular
context. It is conceivable that this loss of specificity could be
due to the transient transfection assay, as it has been sug-
gested that at high levels of protein some cell type-specific
requirements could be lost (52). Experiments are in progress
to test whether some of the specificity could be obtained in stable
transformants expressing lower levels of TTF-1. Artificial pro-
moters containing only TTF-1 binding sites are being con-
structed, and some of them do show a better transcription in
lung or thyroid, suggesting that promoter architecture is an
important component in cell type-specific activation by TTF-1. 3

Nonetheless, the squelching data presented in this paper sug-
gest the presence of TTF-1-specific co-activators. Thyroid- and
lung-specific TTF-1 co-activators could be responsible for acti-
vation of different promoters by TTF-1 in the two tissues. The
isolation of such co-activators from both tissues is in progress
and may reveal interesting mechanisms behind in the differentia-
tion of endodermal derivatives.

REFERENCES
1. Mitchell, P. J., and Tjian, R. (1989) Science 245, 371–378
2. Tjian, R., and Maniatis, T. (1994) Cell 77, 5–8
3. Brent, R., and Ptashne, M. (1983) Cell 34, 729–736
4. Harrison, S. C. (1992) Nature 353, 715–719
5. Pabo, C. O., and Sauer, R. T. (1992) Annu. Rev. Biochem. 61, 1053–1095
6. Courey, A. J., and Tjian, R. (1988) Cell 55, 887–898
7. Ptashne, M. (1988) Nature 335, 683–689
8. Triezenberg, S. J. (1995) Curr. Opin. Cell Biol. 5, 190–196
9. Quong, M. W., Massari, M. E., Zwart, R., and Murre, C. (1993) Mol. Cell. Biol. 13, 792–800
10. Cres, W. D., and Triezenberg, J. S. (1991) Science 251, 87–90
11. Van Hoy, M., Leuther, K. K., Kodadé, T., and Joehnsten, S. A. (1993) Cell 72, 587–594
12. Leuther, K. K., Salmeron, J. M., and Joehnsten, S. A. (1993) Cell 72, 575–585
13. Hunter, T., and Karin, M. (1992) Cell 70, 375–387
14. Gonzalez, G. A., and Montminy, M. R. (1989) Cell 59, 675–680
15. Trautwein, C., Caelles, C., van der Geer, P., Hunter, T., Karin, M., and Chojkier, M. (1993) Nature 364, 544–547
16. Abate, C., Patel, L., Ruascher, F. J. I., and Curran, T. (1990) Science 249, 1157–1161
17. Meyer, M., Schreck, R., and Baeuerle, P. (1993) EMBO J. 12, 2005–2015
18. Chan, S.-K., Jaffe, L., Capovilla, M., Botas, J., and Mann, R. S. (1994) Cell 78, 615–619
19. Pugh, B. F., and Tjian, R. (1990) Cell 61, 1187–1197
20. Martin, K. J., Lille, J. W., and Green, M. R. (1990) Nature 346, 147–152
21. Berger, S. L., Cress, W. D., Cress, A., Triezenberg, S. J., and Guarente, L.
3. G. Cobellis, M. De Féliex, and R. Di Lauro, unpublished results.
