once animals with skeletons appeared on the evolutionary stage. This is consistent with the later rapid exploitation of more narrowly defined morphospaces, documented for crinoids and other taxa, in the Ordovician (*22*).

2) Predominance of serial segmentation and design elements broadly comparable with those of arthropods indicates strong, early selection for rapid duplication and subsequent specialization of structural subunits (*7, 23*).

3) The disparate types of skeletal elements that occur together in some organisms (*14*) suggest a low level of morphological integration that would later be streamlined by more precise gene regulation (*24*). However, even the most seemingly bizarre taxa, such as *Anomalocaris* (*25, 26*), do not have more distinct types of skeletal elements than contemporary arthropods assigned to the Crustacea. Some Burgess Shale crustaceans already had skeletons as structurally specialized as those of their close living relatives (*27*).

4) Skeletons of terminal Proterozoic and earliest Cambrian animals (*28*) consisted of scales and spicules, weakly mineralized shells, and structures built largely with radiating aggregates of crystals. The rapid, late Early Cambrian exploitation of opportunities presented by the Skeleton Space was facilitated by the parallel evolution of complex, biologically tailored multilayer composites constructed from a variety of organic and inorganic materials. Recurring features of these structural materials suggest that their development is controlled by a common regulatory network of genes that was already established in ancestral stem-group bilaterians (*29*).

5) Internal skeletons and growth by remodeling are uncommon among the Burgess Shale animals. These options have since been extensively exploited by vertebrates, after two duplications of the *Hox* gene cluster (*30*). No causal relation between regulatory functions of *Hox* genes and the emergence of internal skeletons has yet been established. However, the duplication of high-level regulatory genes would have made it possible to bypass constraints set by established lower-level linkages, opening the way for the establishment of a novel *Bauplan* with more independent controls over the development of local structural units. Basal agnathan chordates are now known from the Lower Cambrian of Chenjiang (*31*), so the fossil record is consistent with two phases of vertebrate *Hox* duplication, one preceding the early Cambrian radiation and the other that could come much later, in the Ordovician, if it was associated with the development of bony endoskeletons.

After the divergence of protostomes and deuterostomes, a major clade within each group went in for active locomotion, evolving strong anterior-posterior differentiation and jointed-lever skeletons. In arthropods, these emerged as exoskeletons; in vertebrates, they are predominantly internal. These distinctive types of skeletal development reflect prescriptive patterns of embryogenesis that appear to have evolved long before hard skeletons emerged (*29*). Convergent patterns of evolutionary diversification in the two metazoan clades with the most varied modes of life reflect common geometric constraints of growth process and mechanical function on skeletons with radically different origins.

Viable design options are fixed point attractors that actual skeletons must approach, leading to the evolutionary convergence emphasized by Conway Morris (*14*). Real animals evolve as strange attractors, far-from-equilibrium systems with combinations of properties that are unpredictable in detail. Rapid exploitation of the Skeleton Space by early Cambrian animals confirms that evolution follows rational and consequently predictable patterns, as Niklas (*32*) and McGhee (*8*) have shown for land plants and a variety of animals in the context of analogous theoretical morphospaces.

References and Notes

1. J. W. Valentine, D. Jablonski, D. H. Erwin, *Development* **126**, 851 (1999).
2. A. M. A. Aguinaldo *et al.*, *Nature* **387**, 489 (1997).
3. K. M. Halanych *et al.*, *Science* **267**, 1641 (1995).
4. J. W. Valentine, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 8001 (1997).
5. R. Rosa *et al.*, *Nature* **399**, 772 (1999).
6. R. D. K. Thomas and W. E. Reif, in *Constructional Morphology and Evolution*, N. Schmidt-Kittler and K. Vogel, Eds. (Springer-Verlag, Berlin, 1991), pp. 283–294.
7. ———, *Evolution* **47**, 341 (1993).
8. G. R. McGhee, *Theoretical Morphology: The Concept and Its Applications* (Columbia Univ. Press, New York, 1999).
9. S. A. Bowring and D. H. Erwin, *GSA Today* **8** (no. 9), 1 (1998).
10. J. Chen, Y. Cheng, H. V. Iten, Eds., *The Cambrian Explosion and the Fossil Record* [*Bull. Nat. Mus. Nat. Sci. Taiwan* **10** (1997)].
11. H. B. Whittington, *The Burgess Shale* (Yale Univ. Press, New Haven, CT, 1985).
12. S. J. Gould, *Wonderful Life* (Norton, New York, 1989).
13. D. E. G. Briggs, D. H. Erwin, F. J. Collier, *The Fossils of the Burgess Shale* (Smithsonian Institution Press, Washington, DC, 1994).
14. S. Conway Morris, *The Crucible of Creation: The Burgess Shale and the Rise of Animals* (Oxford Univ. Press, New York, 1998).
15. A. H. Knoll and S. B. Carroll, *Science* **284**, 2129 (1999).
16. Database available at www.fandm.edu/Departments/Geosciences/faculty/r_thomas.html
17. A. B. Smith, *Evol. Dev.* **1**, 138 (1999).
18. W. D. Stewart, O. A. Dixon, B. R. Rust, *Geology* **21**, 687 (1993).
19. P. D. Kruse, Yu. Zhuravlev, N. P. James, *Palaios* **10**, 291 (1995).
20. P. W. Signor and G. J. Vermeij, *Paleobiology* **20**, 297 (1994).
21. N. J. Butterfield, *Paleobiology* **23**, 247 (1994).
22. M. Foote, *Annu. Rev. Ecol. Syst.* **28**, 129 (1997).
23. D. K. Jacobs, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4406 (1990).
24. G. E. Budd, *Bioessays* **21**, 326 (1999).
25. H. B. Whittington and D. E. G. Briggs, *Philos. Trans. R. Soc. London Ser. B* **309**, 569 (1985).
26. D. Collins, *J. Paleontol.* **70**, 280 (1996).
27. M. A. Wills, D. E. G. Briggs, R. A. Fortey, *Paleobiology* **20**, 93 (1994).
28. S. Bengtson, in *Early Life on Earth*, S. Bengtson, Ed. (Columbia Univ. Press, New York, 1994), pp. 412–425.
29. J. W. Valentine, D. H. Erwin, D. Jablonski, *Dev. Biol.* **173**, 373 (1996).
30. P. W. H. Holland, J. Garcia-Fernàndez, N. A. Williams, A. Sidow, *Development* (suppl.), 124 (1994).
31. D.-G. Shu *et al.*, *Nature* **402**, 42 (1999).
32. K. J. Niklas, *The Evolutionary Biology of Plants* (Univ. of Chicago Press, Chicago, 1997).

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Distinct Classes of Yeast Promoters Revealed by Differential TAF Recruitment

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The transcription factor TFIID contains the TATA box binding protein (TBP) and multiple TBP-associated factors (TAFs). Here, the association of TFIID components with promoters that either are dependent on multiple TAFs (TAF_{dep}) or have no apparent TAF requirement (TAF_{ind}) is analyzed in yeast. At TAF_{dep} promoters, TAFs are present at levels comparable to that of TBP, whereas at TAF_{ind} promoters, TAFs are present at levels that approximate background. After inactivation of several general transcription factors, including TBP, TAFs are still recruited by activators to TAF_{dep} promoters. The results reveal two classes of promoters: at TAF_{ind} promoters, TBP is recruited in the apparent absence of TAFs, whereas at TAF_{dep} promoters, TAFs are co-recruited with TBP in a manner consistent with direct activator-TAF interactions.

TFIID is a general Pol II transcription factor (GTF) that initiates transcription complex assembly by binding to the TATA box through its TBP subunit. TFIID has also been implicated in the mechanism of action of certain promoter-specific activator proteins (activators) (*1*). Whereas TBP is a general factor, TAFs are highly promoter selective, which raises the

question of whether TAFs are co-recruited with TBP to all promoters.

To address this issue we have used TAF temperature-sensitive (ts) mutants (*2–7*) to identify yeast genes that depend on multiple TAFs or do not require TAFs in general. Results for four representative yeast TAFs—yTAF$_{II}$145 (*2*), yTAF$_{II}$60, yTAF$_{II}$61/68 (*7*), and yTAF$_{II}$17 (*4*)—are shown in Fig. 1. After ts inactivation of these TAFs, transcription of the *RPS5*, *RPS30*, *SSB1*, *ACT1*, and *RPL25* genes rapidly ceased, whereas transcription of the *ADH1*, *SED1*, *PGK1*, *GAL1* (+Gal), and *CUP1* (+Cu^{2+}) genes was unaffected (Fig. 1) (*8*). We refer to these as TAF-dependent (TAF$_{dep}$) and TAF-independent (TAF$_{ind}$) promoters.

To determine whether TAFs are differentially recruited to the two classes of promoters, we performed formaldehyde DNA-crosslinking and immunoprecipitation experiments (*9*). Either α-TAF polyclonal antibodies (Fig. 2A) or an α-influenza hemagglutinin (HA) mouse monoclonal antibody and yeast strains expressing an NH_2-terminal triple HA-tagged TAF (*10*) (Fig. 2B) were used. As expected, TBP bound to all promoters at levels correlating with transcriptional activity (*11, 12*). However, TAF binding differed according to promoter class. For TAF$_{dep}$ promoters, each TAF bound at levels comparable to that of TBP (Fig. 2A, compare lane 2 with lanes 3 to 6). In contrast, at TAF$_{ind}$ promoters, each TAF bound at levels only slightly above background and much lower than that of TBP (Fig. 2A, compare lane 8 with lanes 9 to 12) (*13*). Other GTFs, such as Pol II, bound to both classes of promoters at comparable levels (Fig. 2C). These results indicate that TAFs are differentially recruited to the two classes of promoters in a manner that correlates with their transcriptional requirement. This general conclusion has been independently reached in a related study (*14*).

We next investigated the role of TBP and other GTFs in TAF recruitment. Inactivation of TBP by a ts mutant (*15*) caused a substantial decrease in TBP binding to the *ADH1*, *ACT1*, and *RPS5* promoters (Fig. 3A, compare lanes 2 and 8); a decrease in Pol II binding (Fig. 3B, compare lanes 3 and 4); and a decrease in transcription. Remarkably, however, inactivation of TBP did not abolish binding of TAFs to the TAF$_{dep}$ promoters of the *RPS5* and *ACT1* genes (Fig. 3A, compare lanes 3 to 6 with lanes 9 to 12) (*16*).

The unexpected finding that recruitment of TAFs to TAF$_{dep}$ promoters does not require TBP raised the possibility that TAFs might be constitutively bound to the core promoter, an element that in some cases confers TAF dependence (*17*). To address this possibility, we asked whether promoter-specific transcriptional activators were required for association of TAFs with the *RPS5* promoter. Association of TAFs and TBP was lost after removal of the activator binding sites from the *RPS5* promoter (Fig. 3C), which indicates that activators are required for TAF recruitment.

Finally, we analyzed the role of representative GTFs in recruitment of TBP and TAFs. Inactivation of TFIIB (*11*) or suppressor of RNA polymerase B-4 (Srb4) (*18*) dramatically reduced binding of TBP to the TAF$_{ind}$ *ADH1* promoter, as previously reported (*11*),

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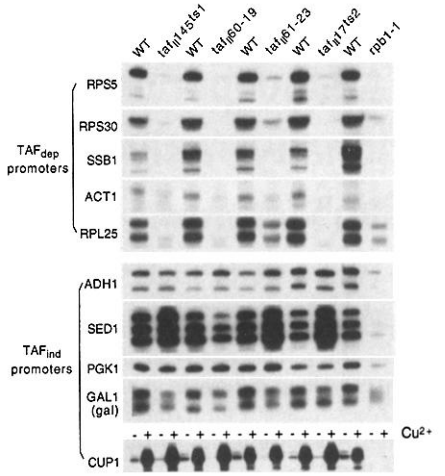

Fig. 1. Two classes of promoters defined by their transcriptional requirement for TAFs. Yeast strains harboring ts mutations in one of the TAFs (*2, 4, 7*) or the Pol II large subunit (*22*) and their corresponding isogenic wild-type strains were grown at 23°C in 1% yeast extract containing 2% peptone plus glucose or galactose as indicated. After shift to the nonpermissive temperature (37°C) for 1 hour, cells were harvested, and total cellular RNA was prepared. Transcripts were quantitated by primer extension using primers near the transcription start site and the products were separated by denaturing polyacrylamide gel electrophoresis. The *CUP1* gene was induced for 15 min by addition of $CuSO_4$ (final concentration, 1 mM) 45 min after the temperature shift.

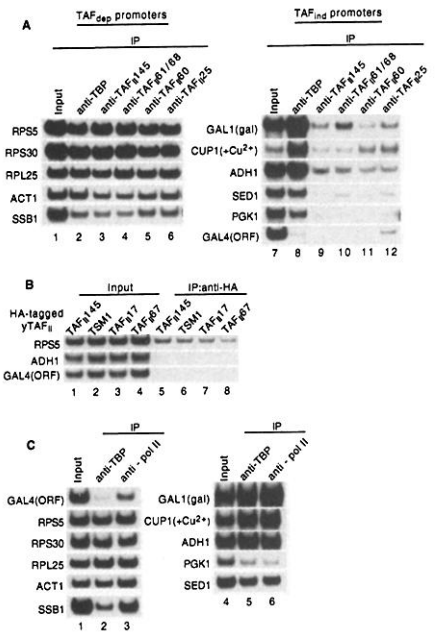

Fig. 2. Differential association of TAFs with TAF$_{dep}$ and TAF$_{ind}$ promoters. Formaldehyde DNA-crosslinking and immunoprecipitation (IP) analysis (*9*) was carried out in the wild-type yeast strain W303a (**A** and **C**) or in strains expressing a TAF with an NH_2-terminal triple influenza HA epitope tag (*10*). (**B**) Immunoprecipitation was done with polyclonal antibodies against TBP or a TAF, the mouse monoclonal antibody 16B12 (Covance, Princeton, New Jersey) against HA, or the mouse monoclonal antibody 8WG16 (Covance) against the COOH-terminal domain of Pol II large subunit. Primer pairs located in the core promoter region of each gene were used in the PCR analysis of the immunoprecipitated DNA samples. A PCR fragment corresponding to the internal transcribed region of the *GAL4* gene was used as a control for background binding. IP = immunoprecipitation; ORF = open reading frame.

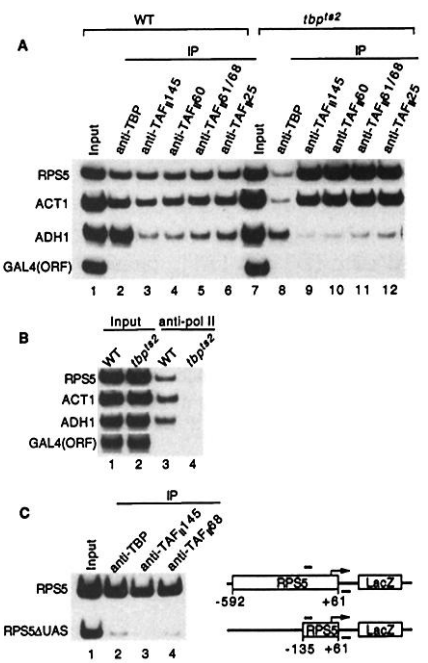

Fig. 3. Requirement of TBP and activators for recruitment of TAFs to TAF$_{dep}$ promoters. (**A**) Requirement of TBP for recruitment of TAFs. The TBP ts mutant (*15*) and isogenic wild-type yeast strains were grown at 23°C and shifted to 37°C for 1 hour before formaldehyde crosslinking. (**B**) Requirement of TBP for recruitment of Pol II. (**C**) Requirement of activators for recruitment of TBP and TAFs. Promoter derivatives containing the intact RPS5 promoter (−592 to +59) or the core promoter sequence (−135 to +59) fused to a *LacZ* sequence were integrated at the *URA3* locus. The resulting yeast strains were used in formaldehyde crosslinking and immunoprecipitation analysis. The primers used for the PCRs are indicated.

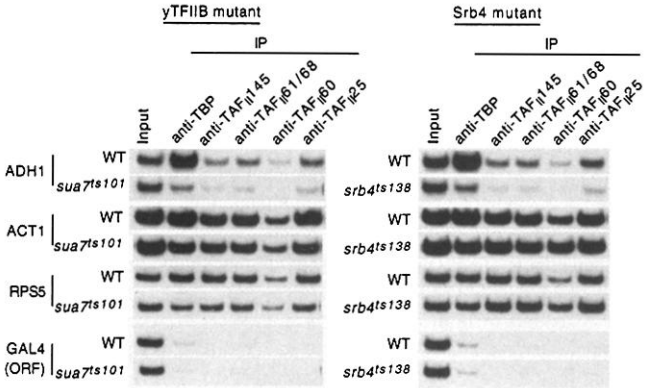

Fig. 4. Requirement of TFIIB and Srb4 for recruitment of TBP and TAFs to TAF_{dep} promoters. TFIIB (*11*) and Srb4 (*18*) ts mutant strains were grown at 23°C and shifted to 37°C for 1 hour; association of TBP and TAFs was analyzed as in Fig. 3.

but had little effect on binding of TBP to the TAF_{dep} promoters of the *ACT1* and *RPS5* genes (Fig. 4).

In summary, we have identified two distinct classes of yeast promoters based on their requirement for TAFs. At TAF_{dep} promoters, TAFs are recruited and are required for delivery of TBP (*19*). Recruitment of TAFs to TAF_{dep} promoters is activator dependent but appears to be relatively independent of other GTFs—surprisingly, even TBP. These data are consistent with the possibility that, at TAF_{dep} promoters, TAFs are directly targeted by activators, which results in recruitment of TAFs and TBP. The notion that TAFs may be direct targets of some activators is consistent with a variety of biochemical studies (*1*).

For TAF_{ind} promoters, TAFs are not required for transcriptional activity or for TBP recruitment (*19*). The same assay that revealed the approximate stoichiometric association of TAFs and TBP with TAF_{dep} promoters showed that the level of TAF association with these promoters is close to background. These results strongly suggest that TBP is recruited to TAF_{ind} promoters in the absence of TAFs, perhaps alone or in a complex with other proteins (*20*). The absence of TAFs on TAF_{ind} promoters in wild-type cells confirms their dispensability for transcription of certain genes, a conclusion independently derived from yeast TAF inactivation studies (*2–6, 21*).

Although recruitment of TBP to TAF_{ind} promoters does not require TAFs, there is a strong dependence on GTFs, such as TFIIB and Srb4. These results are consistent with the possibility that, at TAF_{ind} promoters, the activator targets one of these GTFs, either directly or through another component, which through cooperative interactions ultimately promotes TBP binding and transcription.

References and Notes

1. C. P. Verrijzer and R. Tjian, *Trends Biochem. Sci.* **21**, 338 (1996); S. K. Burley and R. G. Roeder, *Annu. Rev. Biochem.* **65**, 769 (1996); M. R. Green, *Trends Biochem. Sci.* **25**, 59 (2000).
2. S. S. Walker, J. C. Reese, L. M. Apone, M. R. Green, *Nature* **383**, 185 (1996).
3. L. M. Apone, C. M. Virbasius, J. C. Reese, M. R. Green, *Genes Dev.* **10**, 2368 (1996).
4. L. M. Apone *et al.*, *Mol. Cell* **2**, 653 (1998).
5. F. C. Holstege *et al.*, *Cell* **95**, 717 (1998).
6. T. I. Lee *et al.*, *Nature*, in press.
7. B. Michel, P. Komarnitsky, S. Buratowski, *Mol. Cell* **2**, 663 (1998).
8. The ts mutants of $yTAF_{II}60$ and $yTAF_{II}61/68$ were those used in (*7*). Although these TAFs were reported to be generally required for transcription, our results (Fig. 1) and results of genome-wide expression analysis (*6*) indicate that they are highly selective. Transcription of the *ADH1*, *SED1*, *PGK1*, *GAL1* (+Gal), and *CUP1* ($+Cu^{2+}$) genes also was not affected after inactivation of any of the other known yeast TAFs (data not shown).
9. In brief, the crosslinking procedure is as follows. Live yeast cells were treated with 1% formaldehyde to induce crosslinks and disrupted with glass beads; the resultant extract was sonicated to generate DNA fragments with an average size of about 500 base pairs. Crosslinked complexes were immunoprecipitated with the specified antibodies, and proteinase K was used to release immunoprecipitated DNA from protein A–agarose beads. DNA samples were incubated at 65°C for 6 hours to reverse the crosslinks, purified, and used for quantitative polymerase chain reaction (PCR) analysis. Under these conditions, the crosslinking assay provides a quantitative measure of factor association with the promoter (*11*).
10. Z. Moqtaderi, J. D. Yale, K. Struhl, S. Buratowski, *Proc. Natl. Acad. Sci. U.S.A.* **93**,14654 (1996).
11. X. Y. Li, A. Virbasius, X. Zhu, M. R. Green, *Nature* **399**, 605 (1999).
12. L. Kuras and K. Struhl, *Nature* **399**, 609 (1999).
13. Quantitation of the data in Fig. 2A (using the NIH image 1.62 program after scanning the autoradiogram) indicates that $TAFs_{average}$/TBP ratios were as follows: TAF_{dep} promoters—*RPS5*, 0.94; *RPS30*, 0.91; *RPL25*, 0.89; *ACT1*, 0.80; *SSB1*, 0.86; TAF_{ind} promoters—*GAL1*, 0.11; *CUP1*, 0.16; *ADH1*, 0.14; *SED1*, 0.16; *PGK1*, 0.09.
14. L. Kuras, P. Kosa, M. Mencia, K. Struhl, *Science* **288**, 1244 (2000).
15. B. P. Cormack and K. Struhl, *Cell* **69**, 685 (1992).
16. Similar results were observed with the *RPS30* and *SSB1* genes (data not shown).
17. W. C. Shen and M. R. Green, *Cell* **90**, 615 (1997).
18. C. M. Thompson and R. A. Young, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 4587 (1995).
19. Inactivation of TAFs substantially decreased binding of TBP to TAF_{dep} but not to TAF_{ind} promoters (data not shown).
20. An alternative possibility, that TAFs are bound but not crosslinked to TAF_{ind} promoters, is unlikely for several reasons. First, the crosslinking procedure has been successfully used to detect the association of a wide variety of factors, some of which bind DNA and others do not, with the promoter. Second, and more importantly, the crosslinking data are consistent with the dispensability of TAFs for TAF_{ind} promoters.
21. Z. Moqtaderi, Y. Bai, D. Poon, P. A. Weil, K. Struhl, *Nature* **383**, 188 (1996).
22. M. Nonet, C. Scafe, J. Sexton, R. A. Young, *Mol. Cell. Biol.* **7**, 1602 (1987).

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TAF-Containing and TAF-Independent Forms of Transcriptionally Active TBP in Vivo

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Transcriptional activity in yeast strongly correlates with promoter occupancy by general factors such as TATA binding protein (TBP), TFIIA, and TFIIB, but not with occupancy by TBP-associated factors (TAFs). Thus, TBP exists in at least two transcriptionally active forms in vivo. The TAF-containing form corresponds to the TFIID complex, whereas the form lacking TAFs corresponds to TBP itself or to some other TBP complex. Heat shock treatment altered the relative utilization of these TBP forms, with TFIID being favored. Promoter-specific variations in the association of these distinct forms of TBP may explain why only some yeast genes require TFIID for transcriptional activity in vivo.

Eukaryotic RNA polymerase II (Pol II) requires auxiliary factors to recognize promoters. The primary promoter recognition factor is TFIID, a complex that consists of TBP and about 10 TAFs (*1*). TBP binds TATA elements, which are found in most promoters, and it interacts with general transcription factors TFIIA and TFIIB (*2*). In yeast, TBP is generally required for Pol II transcription (*3*), and the level of TBP occupancy of promoters is correlated with transcriptional activity (*4*, *5*). In the context of TFIID, certain TAFs directly contact initiator or downstream promoter elements (*6*). For this reason, TAFs are

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