Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 8393–8397, August 1994
Evolution

Structure of homeobox–leucine zipper genes suggests a model for the evolution of gene families

(transcription factor/exon capture/gene duplication/*Arabidopsis thaliana*)

MARK SCHENA* AND RONALD W. DAVIS

Department of Biochemistry, Beckman Center, Stanford University Medical Center, Stanford, CA 94305-5307

Contributed by Ronald W. Davis, June 2, 1994

ABSTRACT **Homeobox genes are present in both plants and animals. Homeobox–leucine zipper genes, however, have been identified thus far only in the small mustard plant *Arabidopsis thaliana*. This observation suggests that homeobox–leucine zipper genes evolved after the divergence of plants and animals, perhaps to mediate specific regulatory events. To better understand this gene family, we isolated several sequences containing the homeobox–leucine zipper motif and carried out a comparative analysis of nine homeobox–leucine zipper genes (*HAT1*, *HAT2*, *HAT3*, *HAT4*, *HAT5*, *HAT7*, *HAT9*, *HAT14*, and *HAT22*). Gene structures, sequence comparisons, and chromosomal locations suggest a simple model for the evolution of these genes. The model postulates that a primordial homeobox gene acquired a leucine zipper by exon capture. The nascent homeobox–leucine zipper gene then appears to have undergone a series of gene duplication and chromosomal translocation events, leading to the formation of the *HAT* gene family. This work has general implications for the evolution of regulatory genes.**

Gene families in higher organisms tend to be more complex than those found in simple eukaryotes (1), suggesting a correlation between gene family size and regulatory complexity. Studies on actin, for example, reveal the presence of multiple actin genes in all multicellular organisms, whereas only a single actin gene is found in yeast (2). Sequence comparisons of related genes suggest molecular models for the evolution of gene families. Members of the steroid receptor superfamily, for example, are thought to have arisen from an ancestral gene through a series of gene duplication and chromosomal translocation events (3). Gene conversion (4), exon shuffling (5), and unequal crossing-over (6) have also been implicated in the evolution of genes in animal systems.

Higher plants afford the opportunity to examine gene family evolution in organisms whose biology differs significantly from members of the animal kingdom. Of particular interest are genes that are unique to higher plants. One class of genes that has been described only in plants is the homeobox–leucine zipper gene family from *Arabidopsis thaliana* (7–9). Homeobox–leucine zipper genes encode homeodomain proteins with a tightly linked leucine zipper motif. These genes constitute a subset of the *Arabidopsis* homeobox gene superfamily estimated to contain as many as 50 members (9). Nearly 350 homeobox genes have been characterized from animal systems (10), and none of these contains a leucine zipper; thus, it seems likely that the homeobox–leucine zipper motif arose after the divergence of plants and animals.

The goal of this work focuses on understanding how homeobox–leucine zipper genes evolved in higher plants. The interest in this gene family is underscored by previous experiments that suggest an important developmental regulatory role for these genes (11). Ectopic expression of the homeobox–leucine zipper gene *HAT4* causes a series of developmental changes in plants including hypocotyl elongation, early flowering, altered leaf morphology, and dark green pigmentation. To understand this gene family in greater detail, we isolated and characterized additional homeobox–leucine zipper genes. Reported here is a comparative study of a homeobox–leucine zipper gene family.†

MATERIALS AND METHODS

Screening the cDNA Library. A λ-YES cDNA library (9) prepared from adult *A. thaliana* plants (Col-O) was plated at a density of 7500 plaques per 15-cm dish. A total of 225,000 plaques were transferred to Colony/Plaque Screen filters (DuPont) and screened exactly as described (9), except that the probe used to screen the library was a 512-fold degenerate 23-mer (5′-GTNGAA/GGTNTGGTTT/CCAA/GAAT/CA/CG) designated PAN39. Of the 70 clones that scored positive initially, 67 scored positive upon rescreening with PAN39. The 67 λ clones were converted to plasmids and blotted onto a Nytran filter (DuPont), and the filter was subjected to a series of successive hybridizations using each of the newly isolated sequences as probes. This analysis revealed that the 67 positives represented 10 unique sequences, 7 of which were shown to contain a homeobox–leucine zipper motif. None of the clones exhibited cross hybridization under the wash conditions employed (0.1× SSPE/1% SDS at 65°C for 30 min).

Isolating Genomic Sequences. The *HAT* genes were amplified from *A. thaliana* (Col-O) genomic DNA using PCR under standard conditions (12). The primer pairs used for the amplification were as follows: *HAT1* was amplified using PAN47 (5′-GCGGCGTCTAGATATTCTCATTTTCTACCAAG) and PAN49 (5′-GGGCGGGAGCTCACAATGACCCACACACAACCC), *HAT3* was amplified using PAN59 (5′-GGGCCGTCTAGAAATTCTTCTTTCTTCTTTCTG) and PAN60 (5′-GGCGGGGAGCTCCACCAAACCTATTATCTTTAG), *HAT9* was amplified using PAN57 (5′-GGGCGCTCTAGAAGGCATATTTTTTTCATTTCG) and PAN58 (5′-GCCCGGGAGCTCCGAATTAACTATTCAACAAGC), and *HAT22* was amplified using PAN55 (5′-GCCGGGTCTAGATACGAAGAGAAAATTCAAATGG) and PAN56 (5′-GGGGCCGAGCTCATTTATCTTCTAACTAATTCC).

DNA Sequencing. The cDNA clones were liberated from the vector with *Eco*RI, subcloned into plasmid pBS+ (Stratagene), and sequenced on both strands using an automated

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: UPGMA, unweighted pair group method with arithmetic mean; cM, centimorgan(s).

*To whom reprint requests should be addressed.

†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U09332–U09342).

sequencer (Applied Biosystems). The genomic clones were cleaved with *Xba* I and *Sac* I (sites introduced by the 5′ and 3′ primers, respectively), and inserted into pBS+. Nucleotide sequences were determined as above, except that genomic samples contained at least 100 clones to avoid sequencing errors resulting from the PCR. Nested deletions spanning each of the sequences were made using restriction enzymes followed by religation.

Sequence Alignments. Basic sequence manipulations were carried out using DNA STRIDER 2.1 (Apple). Sequence comparisons and evolutionary analyses were performed using GENEWORKS software (IntelliGenetics).

Gene Mapping. Chromosome assignments for *HAT1*, *HAT2*, and *HAT22* were made using restriction fragment length polymorphisms (13).

RESULTS

Isolation of a cDNA Family. A 512-fold degenerate oligonucleotide probe (PAN39) was used to screen an *Arabidopsis* cDNA library. The degenerate 23-mer was designed to detect sequences encoding residues VEVWFQNR of the HAT4 homeodomain (9). Under conditions of high stringency, the degenerate PAN39 oligonucleotide hybridized to 67 plaques out of a total of 225,000 screened (≈0.03% of the clones in the cDNA library). These 67 λ clones were converted to plasmid DNA and the cDNA inserts were analyzed by hybridization and DNA sequencing. Of the 67 clones isolated, 51 contained a homeobox–leucine zipper motif; the remaining 16 clones exhibited extensive homology to the PAN39 probe on the noncoding strand but did not contain a homeobox or a leucine zipper (unpublished data). Of the 51 clones that contained a homeobox–leucine zipper motif, 34 corresponded to cDNAs reported previously (9); in particular, 31 hybridized to *HAT4* and 3 hybridized to *HAT22*. The 17 remaining clones represented five additional genes designated *HAT1*, *HAT2*, *HAT3*, *HAT9*, and *HAT14* (Fig. 1). The extent to which these five sequences corresponded to cDNAs isolated previously (9) was not evaluated. The *HAT5* and *HAT7* cDNAs, which contain several mismatches to PAN39, were not recovered in the screen (data not shown).

A.

```
conserved  ----R----------L---F-----L------------L----V-IWF-N-R-K-K---
invariant  ---KK-RL---Q---LE--F-----L-P--K--LA--L-L--RQV--WFQNRR-R-K-KQ
HAT1       TCRKKLRLSKDQSAVLEDTFKEHNTLNPKQKLALAKKLGLTARQVEVWFQNRRARTKLKQ
HAT2       TSRKKLRLSKDQSAFLEETFKEHNTLNPKQKLALAKKLNLTARQVEVWFQNRRARTKLKQ
HAT3       SSRKKLRLSKEQALVLEETFKEHSTLNPKQKMALAKQLNLRTRQVEVWFQNRRQRTKLKQ
HAT4       NSRKKLRLSKDQSAILEETFKDHSTLNPKQKQALAKQLGLRARQVEVWFQNRRARTKLKQ
HAT5       LPEKKRRLTTEQVHLLEKSFETENKLEPERKTQLAKKLGLQPRQVAVWFQNRRARWKTKQ
HAT7       LGEKKKRLNLEQVRALEKSFELGNKLEPERKMQLAKALGLQPRQIAIWFQNRRARWKTKQ
HAT9       SARKKLRLTKQQSALLEESFKDHSTLNPKQKQVLARQLNLRPRQVEVWFQNRRARTKLKQ
HAT14      STRKKLRLSKDQSAFLEDSFKEHSTLNPKQKIALAKQLNLRPRQVEVWFQNRRARTKLKQ
HAT22      SARKKLRLTKQQSALLEDNFKLHSTLNPKQKQALARQLNLRPRQVEVWFQNRRARTKLKQ

Athb-1     LPEKKRRLTTEQVHLLEKSFETENKLEPERKTQLAKKLGLQPRQVAVWFQNRRARWKTKQ
Athb-2     NSRKKLRLSKDQSAILEETFKDHSTLNPKQKQALAKQLGLRARQVEVWFQNRRARTKLKQ
Athb-3     LGEKKKRLNLEQVRALEKSFELGNKLEPERKMQLAKALGLQPRQIAIWFQNRRARWKTKQ
Athb-4     GSRKKLRLSKDQALVLEETFKEHSTLNPKQKLALAKQLNLRARQVEVWFQNRRARTKLKQ
           ........10........20........30........40........50........60
```

B.

```
invariant  -E-D---L------L-------------L-------
HAT1       TEVDCEYLKRCVEKLTEENRRLEKEAAELRALKLSP
HAT2       TEVDCEYLKRCVEKLTEENRRLQKEAMELRTLKLSP
HAT3       TEVDCEYLKRCCENLTDENRRLQKEVSELRALKLSP
HAT4       TEVDCEFLRRCCENLTEENRRLQKEVTELRVLKLSP
HAT5       LERDYDLLKSTYDQLLSNYDSIVMDNDKLRSEVTSL
HAT7       LERDYDSLKKQFDVLKSDNDSLLAHNKKLHAELVAL
HAT9       TEVDCEFLKKCCETLADENIRLQKEIQELKTLKLTQ
HAT14      TEVDCEYLKRCCESLTEENRRLQKEVKELRTLKTST
HAT22      TEVDCEFLKKCCETLTDENRRLQKELQDLKALKLSQ

Athb-1     LERDYDLLKSTYDQLLSNYDSIVMDNDKLRSEVTSL
Athb-2     TEVDCEFLRRCCENLTEENRRLQKEVTELRALKLSP
Athb-3     LERDYDSLKKQFDVLKSDNDSLLAHNKKLHAEVYNI
Athb-4     TEVDCEYLKRCCDNLTEENRRLQKEVSELRALKLSP
           ........10........20........30......
```

FIG. 1. Homeodomain and leucine zipper sequences. (*A*) The presumptive homeodomains of the HAT and Athb proteins. Residues that are invariant in the homeodomains (second line) are given. The 13 most highly conserved residues among 346 homeodomain sequences are also shown (top line). Numbers (bottom line) demarcate the 60 residues of the homeodomain. (*B*) The presumptive leucine zippers of the HAT and Athb proteins. The invariant residues (top line) and heptadic leucines (boldface type) are shown. Note that HAT5 and Athb-1 contain an isoleucine at the presumptive third leucine. Numbers (bottom line) demarcate the 36 residues encompassing the leucine zipper.

Sequence Alignments of the Proteins. The *HAT* genes encode proteins that contain invariant residues at 28 of 60 positions (≈47%) within the homeodomain (Fig. 1*A*). Nearly all of the invariant residues are also highly conserved in homeodomains from other organisms (10). The presumptive leucine zippers exhibited less sequence identity than the homeodomains; only 5 of the 36 residues (≈14%) within the leucine zippers are conserved in all of the HAT proteins (Fig. 1*B*). Three of the five invariant residues within the leucine zipper correspond to the heptadic leucines.

To assess the evolutionary relatedness of the HAT and other homeodomain–leucine zipper proteins, an unweighted pair group method with arithmetic mean (UPGMA) analysis (14, 15) was performed. This analysis indicates that the currently identified HAT proteins make up two evolutionarily distinct subfamilies (Fig. 2). The UPGMA dendrogram contains a major branch that separates subfamily 1 (HAT1, HAT2, HAT3, HAT4, HAT9, HAT14, and HAT22) and subfamily 2 (HAT5 and HAT7). Direct sequence comparisons of the homeodomain–leucine zippers corroborate the UPGMA data (Fig. 1). In all cases, members of subfamily 1 exhibit greater sequence identity to each other (69–93%) than to members of subfamily 2 (31–44%). It is also noteworthy that the UPGMA diagram suggests a pairwise grouping of HAT1 and HAT2 and of HAT9 and HAT22 (Fig. 2). An evolutionary tree using the neighbor-joining method (16) yielded results similar to the UPGMA analysis (data not shown).

Comparisons of intact proteins reveals that the homeodomain–leucine zipper region bears the highest degree of amino acid identity between any two proteins. Alignment of HAT9 and HAT22, for example, reveals 90% identity in the homeodomain–leucine zipper region and 71% identity overall (data not shown). Except for HAT9 and HAT22, little homology is seen outside of the homeodomain–leucine zipper region for any two proteins (data not shown).

Sequence Determination of Intact cDNAs and Genes. The largest cDNA from each gene was subcloned and sequenced. Sequence analysis revealed that a cDNA encompassing the entire coding region had been identified for *HAT1*, *HAT3*, *HAT9*, and *HAT22*. The cDNAs ranged in size from 1.0 to 1.3 kb and encoded proteins of 274–315 residues. The homeodomain–leucine zipper motif in each characterized sequence, as in HAT4, mapped to the central portion of the coding region.

The polymerase chain reaction was used to isolate genomic sequences for *HAT1*, *HAT3*, *HAT9*, and *HAT22*. Primers derived from the cDNAs yielded amplification products with *Arabidopsis* genomic DNA ranging in size from 1.1 to 1.6 kb. Nucleotide sequence determination of genomic clones revealed the presence of three introns in *HAT1* and *HAT3*, and two introns in *HAT9* and *HAT22* (Fig. 3). The splice sites for the intervening sequences in each gene conformed closely to the AG/GT consensus. The positions of the introns in the homeobox–leucine zipper regions of *HAT1*, *HAT3*, *HAT9*, and *HAT22* were identical to those found in the *HAT4* gene (11); thus, all five genes share a similar intron/exon organization (Fig. 3).

Chromosomal Locations of the Genes. To determine the map positions of the *HAT* genes, each of the cDNAs (Fig. 1) was used to detect restriction fragment length polymorphisms in DNA prepared from crosses of various *Arabidopsis*

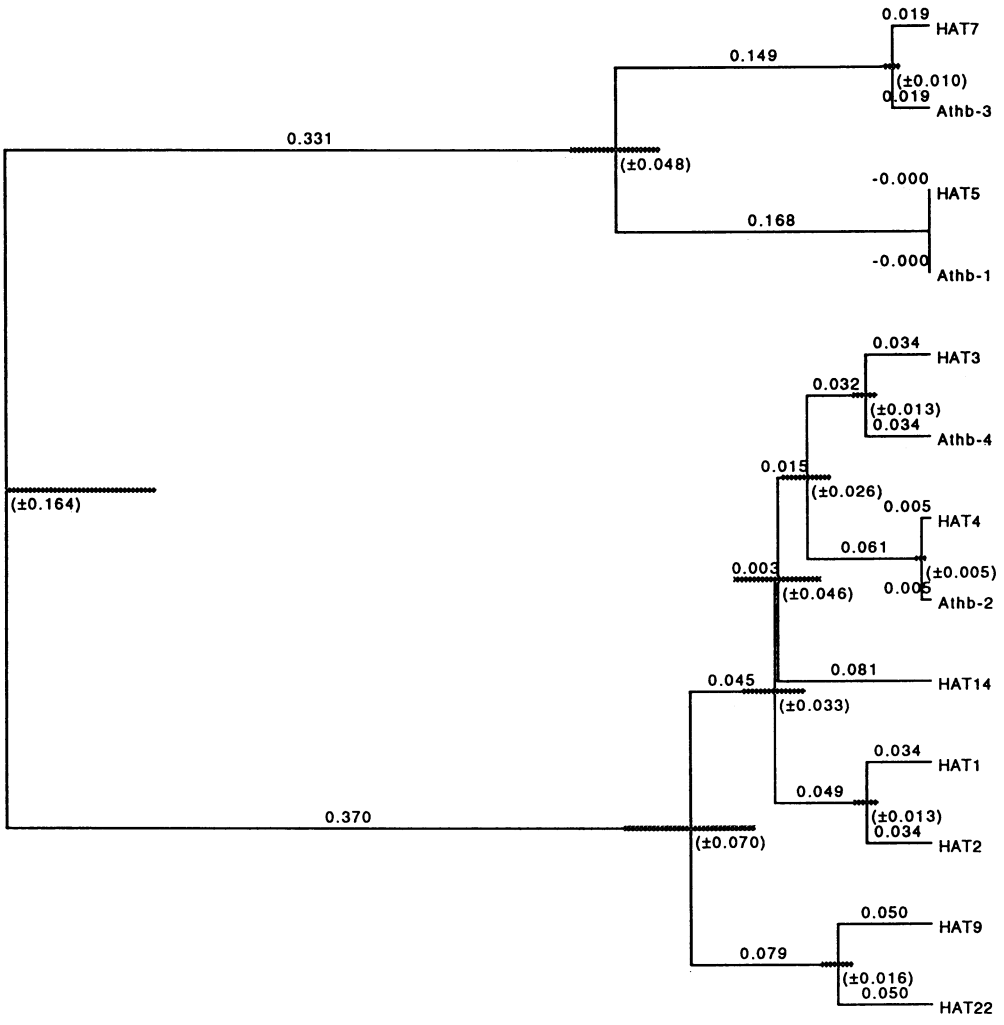

FIG. 2. Evolutionary relationships of homeodomain–leucine zipper proteins. Shown is a UPGMA dendrogram depicting the calculated evolutionary relationships of the HAT and Athb protein sequences. A 96-amino acid segment encompassing the homeodomain–leucine zipper motif (Fig. 1) from each protein was aligned. The estimated genetic distance between the sequences is proportional to the length of the horizontal lines connecting the sequences. The error bars (hatched lines) at branch points in the dendrogram denote the standard error of each branch position.

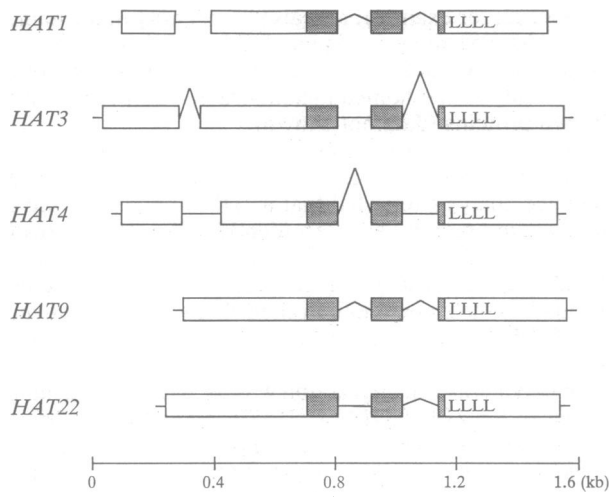

FIG. 3. Schematic representation of a *HAT* gene subfamily. Shown are diagrammatic representations of the *HAT* genes for which the complete nucleotide sequence is known. The exons (boxes), introns (lines), homeodomain (shaded), and heptadic leucines of the leucine zipper are depicted. The approximate size of the genes in kilobases is shown.

ecotypes (13). Restriction fragment length polymorphisms were detected with *HAT1*, *HAT2*, and *HAT22* (data not shown). The *HAT22* gene maps to end of chromosome 4 at 75.1 centimorgans (cM) (Fig. 4), which is ≈1.2 cM distal to compacta-3 (17). The *HAT2* gene maps to chromosome 5 at 83.5 cM, ≈0.6 cM distal to leafy (18).

DISCUSSION

This work was undertaken to investigate the evolution of homeobox–leucine zipper genes in *A. thaliana*. Homeobox–leucine zipper genes are of evolutionary intrigue in that this multigene family appears to be unique to higher plants. Ectopic expression causes a diverse array of developmental changes in plants, suggesting that these genes probably play an important regulatory function. A simple model described here may explain the evolution of a homeobox–leucine zipper gene subfamily.

Several lines of evidence implicate homeobox–leucine zipper genes in developmental control (11). Perhaps the most compelling of these is the observation that overexpression of *HAT1* or *HAT4* causes a series of developmental changes that include early flowering, dark green pigmentation, hypocotyl elongation, and altered leaf morphology. Plants overexpressing other transcription factors do not show any of the phenotypes that are observed upon *HAT1* or *HAT4* overexpression (19–21); thus, it is likely that the phenotypes observed with *HAT1* and *HAT4* overexpression provide clues to the biological role(s) of the genes. It will be informative to determine the chromosomal locations of all of the *HAT* genes. These map positions may reveal linkage to previously characterized mutations.

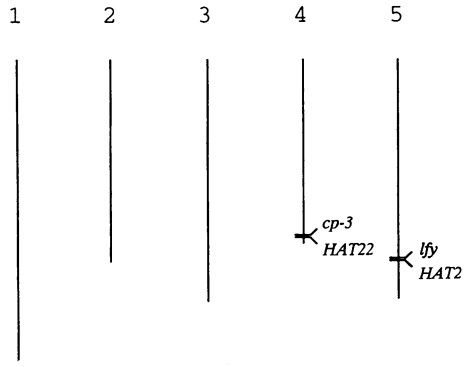

FIG. 4. Map positions of two *HAT* genes. Shown is a diagrammatic representation of the five *Arabidopsis* chromosomes. The chromosomal locations of *HAT22* and *HAT2* are shown relative the compacta-3 (*cp-3*) and leafy (*lfy*) mutations, respectively.

The proteins encoded by the *HAT* gene family share amino acid identity at 28 of 60 positions within the homeodomain. It is noteworthy that 10 of these positions correspond to the 13 most highly conserved residues in homeodomains from animals systems (10). This observation, coupled with some genetic evidence (11), suggests that the HAT proteins probably contain a prototypic homeodomain. These results extend the observation that transcription factor function has been highly conserved in eukaryotic organisms (22–25).

The sequence conservation of plant and animal homeoboxes suggests that all of these genes probably arose from a common ancestral homeobox gene prior to the separation of the plant and animal kingdoms. It is interesting, however, that the positions of the introns in the *HAT* homeoboxes (*HAT1*, *HAT3*, *HAT4*, *HAT9*, and *HAT22*) do not correspond to the sites of intron insertion of any of the animal homeoboxes (10). It may be that the primordial homeobox gene has yet to be identified or that the *HAT* genes underwent rearrangement during their evolution. It seems less likely that homeobox genes in plants and animals arose by convergent evolution.

It is surprising that many of the homeobox genes from *A. thaliana* contain a leucine zipper motif (7–9, 26, 27). This is markedly different from the case in animal systems in which none of the nearly 350 homeobox genes examined contains a leucine zipper (10). Leucine zippers would potentially allow homeodomain–leucine zipper proteins to interact with each other and with other leucine zipper proteins (28). Several of the HAT proteins (e.g., HAT9 and HAT22) show extensive sequence identity in the leucine zipper region. This observation and the fact that many of the *HAT* genes have overlapping expression patterns (unpublished data) suggest that homo- and heterodimer interactions may be important in the function of these proteins.

The similar structure of a homeobox–leucine zipper gene subset (*HAT1*, *HAT3*, *HAT4*, *HAT9*, and *HAT22*) suggests that these genes share a common evolutionary history. The fact that the leucine zipper motif lies on a separate exon raises the possibility that this domain was acquired by a primordial homeobox gene through exon capture (29). A subsequent series of gene duplication and chromosomal translocation events is a plausible model to explain the genesis of this gene family (Fig. 5). This model is attractive in that all of its main features can be subjected to molecular analysis.

It will be instructive to determine whether any of the homeobox–leucine zipper genes are clustered like the animal homeobox genes (30) or genetically dispersed as is seen for the maize knotted genes (31). Given that at least two *HAT* genes map to different chromosomes suggests that translocations and gene duplications played a role in the evolution of this gene family (Fig. 5). It is interesting that *HAT9* and *HAT22* lack an amino-terminal exon present in *HAT1*, *HAT3*, and *HAT4*. One explanation is that the genes with two introns (*HAT9* and *HAT22*) arose by a relatively recent intron deletion event from one of the genes that contains three introns (*HAT1*, *HAT3*, or *HAT4*). Consistent with this proposal, *HAT9* and *HAT22* share the greatest degree of sequence identity of any of the *HAT* genes examined thus far.

Previous experiments suggest that *Arabidopsis* may contain as many as 50 homeobox genes (9). It will be interesting to examine the sequences, gene structures, and chromosomal locations of additional homeobox–leucine zipper sequences.

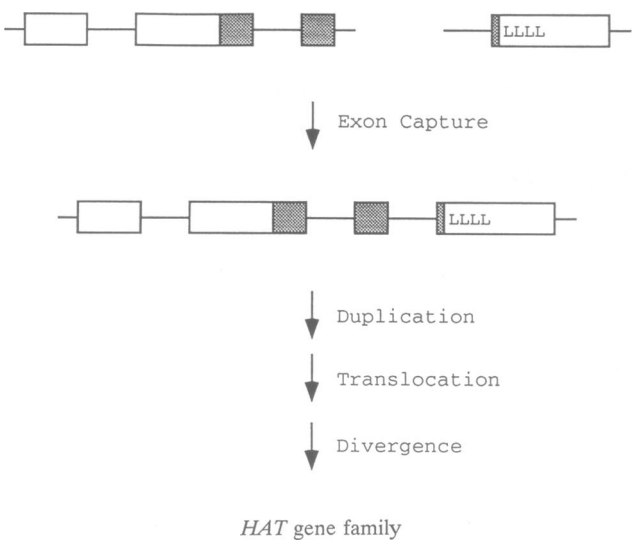

FIG. 5. One model for the evolution of the *HAT* gene family. Shown is one possible scenario depicting the evolution of a homeobox–leucine zipper gene subfamily. A primordial gene containing a homeobox (shaded), three exons (boxes), and three introns (lines) recombines with a leucine zipper gene resulting in the capture of a leucine zipper-containing exon (LLLL). The resulting homeobox–leucine zipper gene is then duplicated, translocated, and functionally altered. A repeated series of such events would give rise to the *HAT* gene family.

A detailed view of the evolution of this gene family would be greatly aided by the sequence determination of the *Arabidopsis* genome.

We thank all members of the Davis laboratory for critical comments on the manuscript and other members of the Department of Biochemistry, Stanford University, for helpful insights. We also thank Tod Klingler and Holly Goodson for computer assistance, the PAN facility at Stanford University for oligonucleotide synthesis and DNA sequencing, and the Meyerowitz laboratory for gene mapping. This work was supported by grants from the National Science Foundation (DMR-91060-11) and the National Institutes of Health (8 R37 HG00198-17) to R.W.D. M.S. held a National Science Foundation Postdoctoral Fellowship in Plant Biology during the completion of part of this work.

1. Ohta, T. (1990) *Theor. Pop. Biol.* **37**, 213–219.
2. Hightower, R. C. & Meagher, R. B. (1986) *Genetics* **114**, 315–332.
3. Laudet, V., Hänni, C., Coll, J., Catzeflis, F. & Stehelin, D. (1992) *EMBO J.* **11**, 1003–1013.
4. Wines, D. R., Brady, J. M., Southard, E. M. & MacDonald, R. J. (1991) *J. Mol. Evol.* **32**, 476–492.
5. Lawler, J., Duquette, M., Urry, L., McHenry, K. & Smith, T. F. (1993) *J. Mol. Evol.* **36**, 509–516.
6. Groot, P. C., Mager, W. H., Henriquez, N. V., Pronk, J. C., Arwert, F., Planta, R. J., Eriksson, A. W. & Frants, R. R. (1990) *Genomics* **8**, 97–105.
7. Ruberti, I., Sessa, G., Lucchetti, S. & Morelli, G. (1991) *EMBO J.* **10**, 1787–1791.
8. Mattsson, J., Söderman, E., Svenson, M., Borkird, C. & Engström, P. (1992) *Plant Mol. Biol.* **18**, 1019–1022.
9. Schena, M. & Davis, R. W. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3894–3898.
10. Bürglin, T. (1994) *A Guidebook to Homeobox Genes* (Oxford Univ. Press, Oxford), pp. 25–71.
11. Schena, M., Lloyd, A. M. & Davis, R. W. (1993) *Genes Dev.* **7**, 367–379.
12. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
13. Chang, C., Bowman, J. L., DeJohn, A. W., Lander, E. S. & Meyerowitz, E. M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6856–6860.
14. Sokal, R. R. & Michener, C. D. (1958) *Univ. Kansas Sci. Bull.* **38**, 1409–1438.
15. Nei, M. (1987) *Molecular Evolutionary Genetics* (Columbia Univ. Press, New York).
16. Saitou, N. & Nei, M. (1987) *Mol. Biol. Evol.* **4**, 406–425.
17. Koornneef, M., Hanhart, C. J. & van der Veen, J. H. (1991) *Mol. Gen. Genet.* **229**, 57–66.
18. Weigel, D., Alvarez, J., Smyth, D. R., Yanofsky, M. F. & Meyerowitz, E. M. (1992) *Cell* **69**, 843–859.
19. Matsuoka, M., Ichikawa, H., Saito, A., Tada, Y., Fujimura, T. & Kano-Murakami, Y. (1993) *Plant Cell* **5**, 1039–1048.
20. Sinha, N. R., Williams, R. E. & Hake, S. (1993) *Genes Dev.* **7**, 787–795.
21. Mizukami, Y. & Ma, H. (1992) *Cell* **71**, 119–131.
22. Schena, M. & Yamamoto, K. R. (1988) *Science* **241**, 965–967.
23. Ptashne, M. & Gann, A. A. F. (1990) *Nature (London)* **346**, 329–331.
24. Schena, M., Lloyd, A. M. & Davis, R. W. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10421–10425.
25. Struhl, K. (1994) *Science* **263**, 1103–1104.
26. Carabelli, M., Sessa, G., Baima, S., Morelli, G. & Ruberti, I. (1993) *Plant J.* **4**, 469–479.
27. Schindler, U., Beckmann, H. & Cashmore, A. R. (1993) *Plant J.* **4**, 137–150.
28. Landschulz, W. H., Johnson, P. F. & McKnight, S. L. (1988) *Science* **240**, 1759–1764.
29. Dorit, R. L., Schoenbach, L. & Gilbert, W. (1990) *Science* **250**, 1377–1382.
30. McGinnis, W. & Krumlauf, R. (1992) *Cell* **68**, 283–302.
31. Vollbrecht, E., Veit, B., Sinha, N. & Hake, S. (1991) *Nature (London)* **350**, 241–243.