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Phylogenetic classification and molecular evolution of *knotted1* homeobox genes

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Abstract Homeobox genes encode a family of DNA-binding regulatory proteins which are crucial for development. The first plant homeobox gene identified was *knotted1* which plays a major role in leaf development. The *knotted1* gene has a homeobox which encodes a homeodomain (HD) and HD proteins have been shown to function as transcription factors. A phylogenetic classification of the KNOTTED1 HD is presented. Here, we report six *kn1* HDs from the cereals oat, barley, wheat, rye and rice. The KN1 class-I and -II genes can be divided into two distinct clades. Further, we hypothesize that KN1 and BELL1/MEIS HDs, (the closest non-KN1 class HDs) evolved from a common ancestor after divergence from the common precursor of all the homeobox genes. Our analysis clearly shows the presence of an ancestral KN1 HD from which all the known plant *kn1* class of genes evolved.

Key words Homeobox gene · KNOTTED1 · Evolution

Introduction

Homeobox genes, also referred as master control genes, are found throughout the plant and animal kingdoms and play a pivotal role in the development of an organism (Gehring et al. 1994 a). They are derived from a helix-turn helix motif and act as transcriptional factors wherein the DNA-binding and functional specificity reside in the third helix (recognition helix) of the homeodomain (HD) of these genes (Gehring et al. 1994 b). Regulation of gene expression by HD proteins may represent a general mechanism that is widely conserved in evolution. HD proteins play major roles in evolutionarily diverse organisms ranging from Antennapedia (Antp) in the patterning of the anterior posterior body axis in *Drosophila* to BELL1 in ovule primordium formation and GLABRA2 in trichome development in *Arabidopsis* (Manak and Scott 1994; Rerie et al. 1994; Reiser et al. 1995). Flies with legs replacing antennae or *Arabidopsis* plants lacking meristems are only two examples of the kind of abnormalities that arise from mutations in homeobox genes.

In plants, the entire range of diverse roles which the HD proteins play is not fully understood. The maize *knotted1* (*kn1*) gene, isolated by transposon tagging, has been the founding member of a large group of similar genes in plants (Hake et al. 1989; Vollbrecht et al. 1991; Matsuoka et al. 1993; Kerstetter et al. 1994; Lincoln et al. 1994). These genes have been shown to be involved in meristem maintenance or initiation in maize, rice and barley among the monocots. The *kn1* group of HDs is divided into class 1 which is 73–89% identical to *kn1* and class 2 which is 55–58% identical to *kn1*. ROUGH SHEATH1 (RS1), a class-1 gene of maize has 89% identity to *kn1* while the KNOX6 and KNOX7 class-2 genes of maize have 55% identity to *kn1*. The HD contains three helices, 1, 2 and 3, and an intron is present in helix 2. Between helices 2 and 3, there is an invariant amino-acid stretch, PYP. Helix 1 harbors most of the taxa-specific replacements.

The establishment of developmentally important functions and their regulation can lead to the

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appearance of novel structures by the alteration of homeobox genes. In barley, for example, the evolution of seed morphology from the awned to the hooded phenotype is associated with a 305-bp tandem duplication in intron4 in Hvknox3, a *kn1* homologue (Muller et al. 1995). Moreover, the floral homeotic mutations, *ovulata* in *Antirrhinum* and *agamous* in *Arabidopsis*, are both caused by insertion of a DNA sequence within the introns (Coen and Meyerowitz 1991). Thus, it is not only the homeobox region but also the intron within it that plays an important role in the developmental changes associated with evolution. The sequence similarity of genes involved in development can reveal the relatedness of organisms (Kappen et al. 1993; Munster et al. 1997) and provide valuable information on evolution associated with morphology.

Since the kn1 homeobox genes have been isolated from both monocots and dicots, evolutionary analysis of the sequence arrangement of the kn1 homeobox can provide a powerful means to address a variety of questions regarding gene function, as shown in the case of MADS-box genes (Meyerowitz 1994; Purugganan et al. 1995). Previously, we have shown the evolutionary and polymorphic organization of the *kn1* homeobox in plants (Deshpande et al. 1998). In the present study, we report the *kn1* homeobox sequence from rice, barley, wheat, rye and oat and have carried out phylogenetic reconstructions with all available *kn1* genes to analyze the events associated with their evolution and to classify them in a proper phylogenetic context.

Materials and methods

Plant material

Seeds of oat, barley, wheat and rye and their respective wild relatives were obtained from USDA-ARS, National Small Grains Collection, Aberdeen, USA, while those of barley were obtained from the Swedish Agricultural University. Seeds of the remaining came from various agricultural research stations in India.

PCR amplification, cloning and sequencing

Total genomic DNA was extracted from the leaves of different cereals by the CTAB method as described previously (Ramakrishna et al. 1994). Primers from the basic region (5′ AAAGGGAAGCTCCCCAAGGA 3′) and the helix-3 region (5′ GGCTTCCAGTGCC GCTTCCG 3′) were synthesized and used for PCR amplification in a vol of 100 µl containing 500 ng of DNA, each primer at 1.5 µM, each dNTP at 200 µM, 2.4 U of *Taq* DNA polymerase, 50 mM KCl, 10 mM Tris·HCl (pH 8) and 1.5 mM $MgCl_2$. DNA amplifications were performed in a Perkin-Elmer Cetus thermal cycler with the following profile: 94°C for 4 min for one cycle, 94°C for 1 min, 55°C for 1 min and 72°C for 2 min for 30 cycles, and 72°C for 5 min for one cycle. PCR products from different species were separated on agarose gels, eluted and cloned into either pGEM T-vector (Promega) or pMOS T-vector (Amersham). Sequencing was performed using the Sequenase version 2.0 DNA sequencing kit (US Biochemical Corporation).

Table 1 List of KN1 and related sequences used in the present study

Name	Accession no.	Source
KN1	123183	*Zea mays* (maize)
OOF1	AF011554	*Oryza officinalis* (rice)
KNHM1	AF011552	*Hordeum marinum* (seaside barley)
KNHM2	AF016645	*Hordeum marinum* (seaside barley)
KNSCA1	AF016646	*Secale cereale* (rye)
KNAL1	AF003526	*Aegilops longissima* (wheat)
KNAV1	AF003527	*Avena vaviloviana* (oat)
RS1	1008879	*Zea mays* (maize)
OSH1	1171924	*Oryza sativa* (rice)
SBH1	1170312	*Gycine max* (soybean)
KNOX3	2130040	*Hordeum vulgare* (barley)
TKN1	1256575	*Lycopersicon esculentum* (tomato)
LeT6	2529701	*Lycopersicon esculentum* (tomato)
LeT12	2529703	*Lycopersicon esculentum* (tomato)
KNAP1	1946220	*Malus domestica* (apple)
KNAP2	1946218	*Malus domestica* (apple)
STM	1586022	*Arabidopsis thaliana* (thale cress)
ATK1	1361991	*Arabidopsis thaliana* (thale cress)
KNAT1	1170676	*Arabidopsis thaliana* (thale cress)
KNAT2	1170677	*Arabidopsis thaliana* (thale cress)
KNAT3	1045042	*Arabidopsis thaliana* (thale cress)
KNAT4	1045044	*Arabidopsis thaliana* (thale cress)
KNAT5	1045046	*Arabidopsis thaliana* (thale cress)
POTH1	1814234	*Solanum tuberosum* (potato)
PKNOX1	2495289	*Homo sapiens* (human)
KNOX4	913142	*Zea mays* (maize)
KNOX10	913143	*Zea mays* (maize)
BnHD1	1170191	*Brassica napus* (rape seed)
KNOX6	913144	*Zea mays* (maize)
KNOX7	913145	*Zea mays* (maize)
OSH42	1805619	*Oryza sativa* (rice)
OSH44	1805617	*Oryza sativa* (rice)
OSH45	1805618	*Oryza sativa* (rice)
Homothorax	2564942	*Drosophila melanogaster* (fruit fly)
BEL1	2129613	*Arabidopsis thaliana* (thale cress)
MEIS1	2495285	*Mus musculus* (house mouse)

Phylogenetic analysis

The sequences reported in the present study are deposited in the Genebank database. Access to databases was facilitated by using ENTREZ (at http://www.ncbi.nlm.nih.gov/Entrez). The nucleotide sequences were translated into amino-acid sequences using the computer software package, SEQUAID II. Other sequences used in the phylogenetic analysis were retrieved from the databank and only the HD was retained. The name, accession number and source are shown in Table 1. The nucleotide and amino-acid sequences were aligned using the multiple sequence alignment CLUSTAL W package (Thompson et al. 1994). The aligned sequences were used to construct phylogenetic trees based on p-distance using neighbour joining (NJ) and the unweighted pair group method of analysis (UPGMA) with the Molecular Evolutionary Genetic Analysis (MEGA) package (Kumar et al. 1994). Bootstrapping was performed to quantify the relative support for branches of the inferred phylogenetic tree.

Results

Identification of new homeobox genes from cereals

In an attempt to determine the phylogeny and sequence organisation of the knotted1 homeobox in diverse

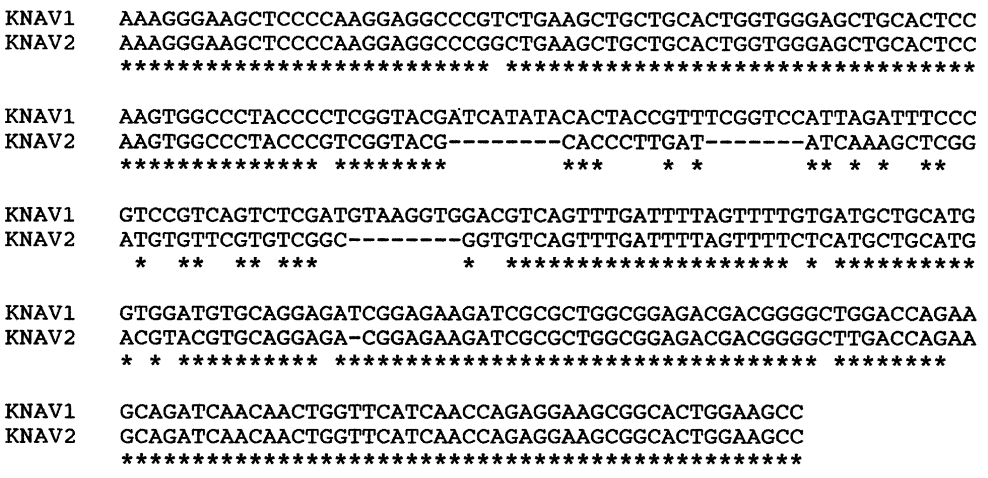

Fig. 1 Alignment of the two oat kn1 homeobox sequences, KNAV1 and KNAV2, along with the introns. Nucleotides that are identical are shown by *stars*

cereals, primers based on published maize and rice sequences spanning the basic end and the helix-3 region were used to specifically amplify the knotted1 homeobox from diverse cereals which were then cloned and sequenced. We identified two kn1 homologues from oat (KNAV1 and 2) which differed by three nucleotide changes within their homeoboxes while they were conserved at the amino-acid level. The introns in these two sequences were divergent both in length and composition. As shown in Fig. 1, KNAV1 had an intron of 105 bp whereas KNAV2 had an intron of 91 bp which clearly indicates that KNAV1 and KNAV2 are two distinct homeobox genes. In the case of barley, two kn1 homologues (KNHM1 and 2) showed two nucleotide differences in the homeobox which resulted in a single amino-acid change in the recognition helix. The introns in these two sequences also differed in length. The rice sequence (OOF1) differed at three nucleotide positions with reference to the homeobox reported earlier in rice, OSH1 (Matsuoka et al. 1993), but the amino-acid sequence was conserved. Wheat (KNAL1) and rye (KNSCA1) sequences differed from the other kn1 homologues we isolated, suggesting that they were kn1-like and were diverse compared to other kn1 homologues. A comparison among different cereals showed a minimum of six substitutions between oat and barley (KNAV1 and KNHM2), whereas a maximum of 47 substitutions were observed between wheat and barley (KNAL1 and KNHM2). The intron in the homeobox was remarkably different in both composition and length in the cereals and may represent an important evolutionary step. Previously, we have shown the presence of a large intron (about 1 kb) in rice and a wild wheat species, while it was relatively smaller (about 80–300 bp) in the other cereals studied (Deshpande et al. 1998). The introns are either divergent or non-homologous which makes them un-informative for the construction of a phylogenetic tree; therefore, only the HD was used in our analysis.

Phylogeny of the KN1 HD

In order to shed light on the evolution of the kn1 class of homeobox genes, we performed an extensive survey by analysing all HDs related to the kn1 HD retrieved from the databank (Table 1). Phylogenetic analysis of the KN1 HD was done using neighbour joining (NJ) and the unweighted pair group method of analysis (UPGMA), methods based on the p-distance, to establish the relationships between different KN1 and KN1-like HDs. Bootstrapping was performed 1000 times to obtain information on the statistical reliability of individual nodes in the tree. Figures 2 and 3 show the phylogenetic trees made using NJ and UPGMA analysis respectively where class-I genes are divided into two distinct clades, IA and IB. The topology of both the NJ and UPGMA trees are similar. Clade IA is divided into several distinct subclades which appear on both trees. Kn1 of maize, Hvknox3 of barley, OSH1 and OOF1 of rice are grouped together while the dicot kn1 homologues, SBH1 from soybean (Ma et al. 1994), STM from *Arabidopsis* (Long et al. 1996) and LeT6 from tomato, are grouped together. The ROUGH SHEATH1 HD of maize has more homology to the class-I genes, such as the maize KNOX4 and KNOX8, barley KNHM1 and KNHM2 and oat KNAV1 HDs, identified in the present study, since they are present in the same subclade. Apple KNAP1 and KNAP2 (Watillon et al. 1997), *Arabidopsis* KNAT1 and tomato TKN1 are classified together in the same subclade. Although *Arabidopsis* KNAT1 and KNAT2 have been classified as class-I genes, they belong to distinct subclades with KNAT2 forming a seperate subclade with ATK1 of *Arabidopsis*. The wheat and rye KN1 HDs reported in the present study form the second clade, designated as IB, which includes LIGULELESS3 (LG3), KNOX5 and KNOX11.

Class-II genes have two distinct clades strongly supported by bootstrap analysis. Clade I (designated as

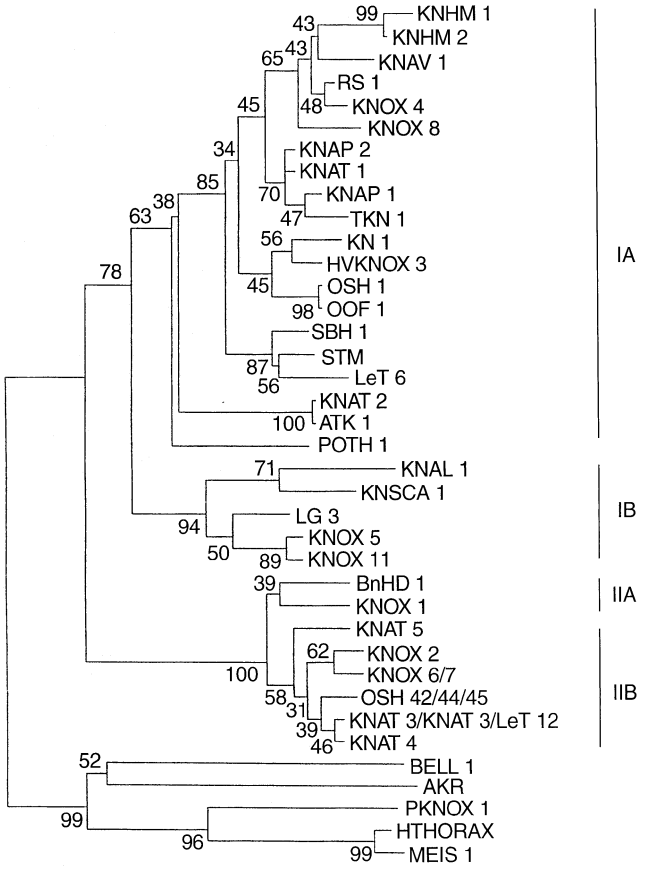

Fig. 2 Neighbour-joining phylogenetic tree showing the relationships of the KN1 and related HDs. Subclasses proposed in the present study are shown at the right margin. Bootstrap values based on 1000 replications are indicated at the nodes. In some cases the kn1 HDs from different plants were identical and are grouped as a single sequence in the tree and separated by /

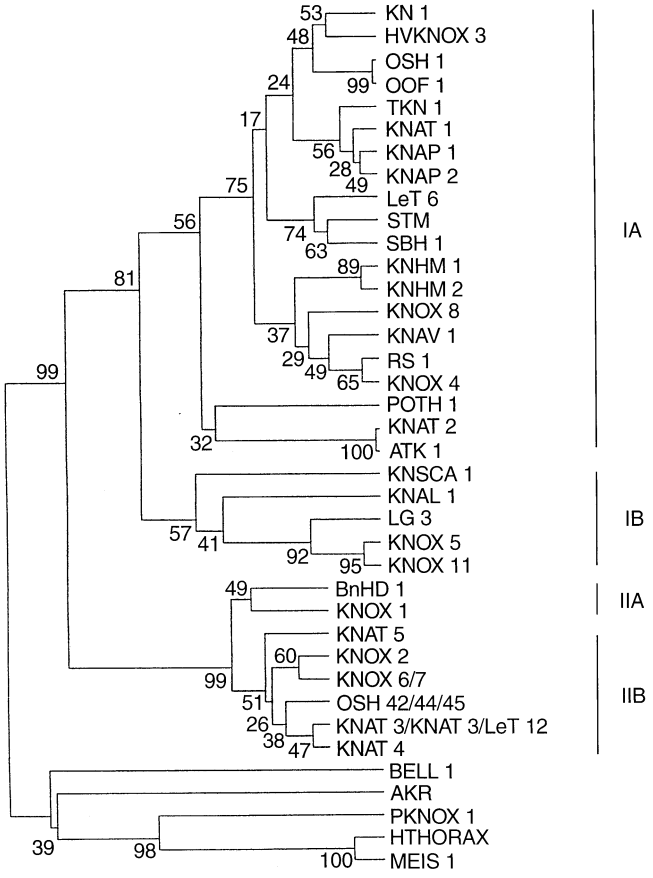

Fig. 3 The phylogenetic tree constructed using the UPGMA algorithm showing the relationships of KN1 and related HDs. Subclasses proposed in the present study are shown at the right margin. Bootstrap values based on 1000 replications are indicated at the nodes. In some cases the kn1 HDs from different plants were identical and are grouped as a single sequence in the tree and separated by /

IIA) includes KNOX1 of maize and BnHD1 of *Brassica* (Biovin et al. 1994). Clade II (designated as IIB) shows the previously identified kn1 homologues from rice (OSH 42, OSH 44, OSH 45) to belong to class-II genes. Similarly, *Arabidopsis* KNAT3, KNAT4 and KNAT5 belong to class-II genes. KNAT3 of *Arabidopsis*, LeT12 of tomato (Janssen et al. 1998) and KNAP3 of apple, all of which belong to class II, have identical HDs. Since *Arabidopsis* KNAT3 is closer to these genes than to KNAT4 and KNAT5, they can all be considered as a different subset of kn1-like genes belonging to class II, and have diverged prior to the divergence of tomato and apple from *Arabidopsis*. The non-kn1 homologues which are nearest to the kn1 class of genes in the phylogenetic tree are *Arabidopsis* BELL1, HOMOTHORAX of *Drosophila*, MEIS1 of mouse, PKNOX1 of human and the avian knotted1-related AKR (Ryan et al. 1995).

The KN1 HD can be used to study phylogenetic relationships among monocot and dicots in a limited manner when comparing exact orthologs. From Fig. 2 and 3 we can infer that maize (KN1) is closer to barley (HvKNOX3) than to rice (OSH1 and OOF1). Similarly, in the lineage leading to RS1, it can be inferred that oat (KNAV1 and KNAV2) and barley (KNHM1 and KNHM2) are closer to each other than to maize (RS1). The position of the Bambusoid (rice) subfamily of the Poaceae in relation to the Panicoid (maize) and Pooid (barley) types is not clear. Our data is in agreement with the phylogeny proposed using rDNA and rbcL gene sequences (Barker and Linder 1995; Hamby and Zimmer 1998). Similar conclusions can be made for dicot kn1 homologs, where *Arabidopsis* (STM) is closer to tomato (LeT6) than to soybean (SBH1). Such inferences cannot, however, be made for class-II genes due to the presence of many subclasses of kn1-like genes, as illustrated by several maize and *Arabidopsis* genes in clade IIB.

Discussion

Different homeobox gene subfamilies in evolutionarily distinct organisms have been postulated to arise from

a common ancestor and to represent a distinct monophyletic gene clade (Kenyon 1994). The common precursor of *Drosophila*, vertebrates and plants might have possessed a homeobox gene which was duplicated and then diverged in the process of evolution to give rise to the different classes of homeobox genes. Phylogenetic analysis of known homeobox genes that occur in such diverse species as yeast and man indicates that a vast majority of subclass members appear as distinct groups in phylogenetic trees. After the establishment of different subclasses, additional gene duplications led to a further increase in the number of homeobox genes. Independent gene duplications have resulted in young paralogs, as indicated by the presence of more than one gene in the same species.

The *knotted1* homeobox gene-lineages might have originated prior to the dicot – monocot divergence (200 mya) since the divergent subclasses of kn-1-like genes are present in both monocots and dicots. The common precursor of monocots and dicots probably had at least one gene corresponding to each of the seperate clades seen in the tree. After the ancestors of angiosperms diverged, duplication of the kn1 genes might have occurred several times to give rise to the different classes of kn1 genes which evolved diverse functions. Once established a distinct homeobox gene family is strongly conserved in its sequence. Some of the homeobox genes from rice (OSH42, OSH44, OSH45), which were previously classified as kn1 homologs, are significantly different from kn1 and belong to class-II genes as shown in the phylogenetic trees (see Figs. 2 and 3). Rice and maize diverged 60 mya. Different maize and rice knotted1 paralogs might have arisen prior to this diversification from their last common ancestor and were then recruited to perform novel developmental functions. Similarly, the apple KNAP1, KNAP2 and KNAP3 are paralogs since KNAP1 is closer to tomato TKN1 than to KNAP2 in IB and KNAP3 in IIB. The speed of evolution of the knotted1 homeobox gene-family members varies in different genes. For example, class-II genes of tomato (LeT12), apple (KNAP3) and *Arabidopsis* (KNAT3) have an identical HD which might have evolved in the ancestor of tomato, apple and *Arabidopsis* whereas some changes in the lineage leading to *Arabidopsis*, have given rise to KNAT4 and KNAT5. The shared evolutionary history of this gene family reflects distinct functional roles. Since the sequence of the KN1 HD directly determines functional aspects like DNA binding, the establishment of different subclasses was important in the establishment of novel structures and functions in plants.

MEIS1 (murine proto-oncogene) is a homeobox gene involved in myeloid leukemia in mice (Moskow et al. 1995) whereas HOMOTHORAX is an extradenticle-related HD protein which is required for nuclear translocation of the extradenticle in *Drosophila* (Rieckhof et al. 1997). Meis1 HD has been shown to be 65% similar to KN1 HD. Recently, a *kn1*-like gene (PKNOX1) was identified from humans whose HD was closer to KN1 (Chen et al. 1997 a) and mouse MEIS1. The present study shows PKNOX1 to be closer to MEIS1 than to KN1, which is well supported by the common DNA-binding sites of PKNOX1 and MEIS1 with PBX1 (Knoepfler et al. 1997). The avian Kn-related protein (AKR) was reported to be most closely related to KN1 than to any other HD when BELL1 was not included in the analysis. Our analysis shows AKR to be a BELL1 homolog and human PKNOX1 to be a MEIS/HOMOTHORAX homolog in a proper phylogenetic context. The common ancestor of homeobox genes seems to have diverged to give rise to the *knotted1* class of genes and other homeobox genes in separate evolutionary events.

KN1 HD subclasses appear as distinct subclades of phylogenetic trees, which suggests that the evolution of these subclasses might play a pivotal role in the evolution of specific functions. In the present phylogenetic analysis, we have identified several cereal kn1-class genes. For example, kn1-like genes of barley (KNHM1 and KNHM2) and oat (KNAV1) appear to be orthologs of RS1, that of rice (OOF1) to be an ortholog of kn1, while those of rye (KNSCA1) and wheat (KNAL1) are orthologs of lg3. Here, we have shown that rye and wheat contain kn1-like HDs closer to KNOX5, KNOX11 (which show tight linkage to LIGULELESS4) and LG3, and suggest evolutionary and possibly functional relationships. Understanding the phylogenetic relationships of the diverse kn1 class of genes can suggest the function of the relevant proteins in gene regulation and development. In tomato, for example, the two class-I genes reported have diverse functions and are phylogenetically distinct. LeT6 (Chen et al. 1997 b) is a kn1 homolog which has been suggested to be associated with the mouse-ears (Me) mutation in tomato whereas TKN1, involved in compound leaf formation (Hareven et al. 1996), is a kn1-like gene which is closer to KNAP1 of apple. It can be speculated that the diverse and distinct clades of KN1 subclasses represent specific characteristics of crop plants. Such a correlation was observed in the MADS-box genes of ferns in which ovules are absent (Munster et al. 1997). Lineage-specific increases in the number of genes during evolution can be observed with special reference to the class-I genes. A single plant species like maize, rice, tomato and apple show at least two members of the kn1 class of genes with continued diversification.

The overall broad function of the *knotted1* homeobox genes is the determination of cell fate, the overexpression of which causes a change from a determinate to an indeterminate cell type. Hence, it is possible to assume that the coding region of this homeobox gene could have been copied during an event of gene duplication which later came under the control of a totally different promoter. Further lines of evidence show that rice OSH45 has two different promoters and three

alternating splice sites, similar to *Drosophila* genes (Tamaoki et al. 1995), which account for the differential functioning of these homeobox genes. Gene fusion can explain how the expression patterns of a protein involved in morphogenesis can be changed in a single step leading to a dramatic alteration in phenotype. Exon shuffling by gene fusions, as observed in human cancers, was shown for LeT6 of tomato, accounting for the morphological variations as seen in the leaves of relatives of the cultivated tomato (Chen et al. 1997 b). The molecular mechanisms that generate morphological variability during the process of evolution, by the creation of new genes through gene duplication and the diversification and establishment of new regulatory interactions between existing genes, have been proposed for MADS-box genes to account for the generation of novel reproductive structures (TheiBen and Saedler 1995). That is, new gene subclasses are created by the duplication of an ancestral gene followed by mutations to adapt for new functions. The phylogenetic classification of the *kn1* class of genes seems to correlate well over a broad range with their expression and probable function.

The number of homeobox genes identified in plants is relatively small and many more with diverse functions may exist. It is likely that orthologs of *kn1*-like genes, corresponding to many of the known genes in some monocots and dicots, have yet to be identified in others. Alternatively, the large number of genes as observed in maize can be a result of gene duplication and diversification within a species. A study of the entire range of homeobox genes in plants provides an opportunity to account for the diverse morphological patterns seen in plants. For instance, patterns of development in winged insects can be traced to differences in regulation and in target genes of the homeobox genes like Ubx (Carroll 1994). Homeobox genes are, therefore, interesting not only because they control key biological pathways but also because they provide valuable information on evolution and the direction in which evolution may proceed in future.

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References

Barker N, Linder P (1995) Polyphyly of Arundinoideae (Poaceae): evidence from rbcL sequence data. Systematic Bot 20 : 423–435

Biovin R, Hamel F, Beauseigle D, Bellemare G (1994) Stage-specific transcription of the homeobox gene *Bnhd1* in young tissues and flowers of *Brassica napus*. Biochim Biophy Acta 1219 : 201–204

Carroll S (1994) Developmental regulatory mechanisms in the evolution of insect diversity. Development 1994 (Suppl) : 217–223

Chen H, Rossier C, Nakamura Y, Lynn A, Chakravarti A, Antonarakis S (1997 a) Cloning of a novel homeobox-containing gene, *PKNOX1*, and mapping to human chromosome 21q22.3. Genomics 41 : 193–200

Chen J, Janssen B, Williams A, Sinha N (1997 b) A gene fusion at a homeobox locus: alterations in leaf shape and implications for morphological evolution. Plant Cell 9 : 1289–1304

Coen ES, Meyerowitz EM (1991) The war of the whorls: genetic interactions controlling flower development. Nature 353 : 31–37

Deshpande A, Ramakrishna W, Mulay GP, Gupta VS, Ranjekar PK (1998) Evolutionary and polymorphic organisation of the *knotted1* homeobox in cereals. Theor Appl Genet 97 : 135–140

Gehring WJ, Affolter M, Burglin TR (1994 a) Homeodomain proteins. Annu Rev Biochem 63 : 487–526

Gehring W, Qian Y, Billeter M, Tokunaga K, Schier A, Diana P, Affolter M, Otting G, Wuthrich K (1994 b) Homeodomain – DNA recognition. Cell 78 : 211–223

Hake S, Vollbrecht E, Freeling M (1989) cloning Knotted, the dominant morphological mutant in maize using Ds2 as a transposon tag. EMBO J 8 : 15–22

Hamby RK, Zimmer EA (1998) Ribosomal RNA sequences for inferring phylogeny within the grass family (Poaceae). Plant Syst Evol 160 : 29–37

Haraven D, Gutfinger T, Parnis A, Eshed Y, Lifschitz E (1996) The making of a compound leaf : genetic manipulation of leaf architecture in tomato. Cell 4 : 735–743

Janssen BJ, Williams A, Chen JJ, Mathern J, Hake S, Sinha N (1998) Isolation and characterzation of two knotted-like genes from tomato. Plant Mol Biol 36 : 417–425

Kappen C, Schughart K, Ruddle F (1993) Early evolutionary origin of major homeodomain sequence classes. Genomics 18 : 54–70

Kenyon C (1994) If birds can fly, why can't we? Homeotic genes and evolution. Cell 78 : 175–180

Kerstetter R, Vollbrecht E, Lowe B, Veit B, Yamaguchi J, Hake S (1994) Sequence analysis and expression patterns divide the maize Knotted-1-like homeobox genes into two classes. Plant Cell 6 : 1877–1887

Knoepfler P, Calvo K, Chen H, Antonarakis S, Kamps M (1997) Meis1 and pKnox1 bind DNA cooperatively with Pbx1 utilizing an interaction surface disrupted in oncoprotein E2a–Pbx1. Proc Natl Acad Sci USA 94 : 14553–14558

Kumar S, Tamura K, Nei M (1994) MEGA: Molecular evolutionary genetics analysis software for microcomputers. Comput Appl Biosci 10 : 189–195

Lincoln C, Long J, Yamaguche J, Serikawa K, Hake S (1994) A knotted-1 homeobox gene in *Arabidopsis* is expressed in the vegetative meristem and dramatically alters leaf morphology when over expressed in transgenic plants. Plant Cell 6 : 1859–1876

Long J, Moan E, Medford J, Barton K (1996) A member of the KNOTTED class of homeodomain proteins encoded by the STM gene in *Arabidopsis*. Nature 79 : 66–69

Ma H, McMullen MD, Finer JJ (1994) Identification of a homeobox-containing gene with enhanced expression during soybean (*Glycine max* L.) somatic embryo development. Plant Mol Biol 24 : 465–473

Manak JR, Scott MP (1994) A class act: conservation of the homeodomain protein functions. Development Suppl S : 61–77

Matsuoka M, Ichikawa H, Saito A, Tade Y, Fujimura T, Kano-Murakami (1993) Expression of a rice homeobox gene causes altered morphology of transgenic plants. Plant Cell 5 : 1039–1048

Meyerowitz EM (1994) Floral development and evolution: new answers and new questions. Proc Natl Acad Sci USA 91 : 5735–5737

Moskow J, Bullrich F, Huebner K, Daar I, Buchberg A (1995) Meis1, a PBX-related homeobox gene involved in myeloid leukemia in BXH-2 Mice. Mol Cell Biol 15 : 5435–5443

Muller KJ, Romano N, Gerstner O, Garcia-Maroto F, Pozzi C, Salamini F, Rohde W (1995) The barley Hooded mutation caused by a duplication in a homeobox gene intron. Nature 374: 727–730

Munster T, Pahnke J, Rosa A, Kim J, Martin W, Saedler H, Theissen G (1997) Floral homeotic genes were recruited from homologous MADS box genes preexisting in the common ancestor of ferns and seed plants. Proc Natl Acad Sci USA 4: 2415–2420

Purugganan M, Rounsley S, Schmidt R, Yanofsky M (1995) Molecular evolution of flower development: Diversification of the plant MADS-box regulatory gene family. Genetics 140: 345–356

Ramakrishna W, Lagu MD, Gupta VS, Ranjekar PK (1994) DNA fingerprinting in rice using oligonucleotide probes specific for repetitive DNA sequences. Theor Appl Genet 90: 1000–1006

Reiser L, Modrusan Z, Margossian L, Samach A, Ohad N, Haughn GW, Fischer RL (1995) The *BELL1* gene encodes a homeodomain protein involved in pattern formation in the *Arabidopsis* ovule primordium. Cell 83: 735–742

Rerie WG, Feldmann KA, Marks MD (1994) The *GLABRA2* gene encodes a homeodomain required for normal trichome development in *Arabidopsis*. Genes Dev 8: 1388–1399

Rieckhof G, Casares F, Ryoo H, Abu-Shaar M, Mann R (1997) Nuclear translocation of extradenticle requires homothorax, which encodes an extradenticle-related homeodomain protein. Cell 91: 171–183

Ryan A, Tejada M, May D, Dubaova M, Deeley R (1995) Isolation and characterisation of the chicken homeodomain protein AKR. Nucleic Acids Res 23: 3253–3259

Tamaoki M, Tsugawa H, Minami E, Kayano T, Yamamoto N, Kano-Murakami Y, Matsuoka M (1995) Alternative RNA products from a rice homeobox gene. Plant J 7: 927–938

TheiBen G, Saedler H (1995) MADS-box genes in plant ontogeny and phylogeny: Haeckel's 'biogenetic law' revisited. Curr Opin Genet Dev 5: 628–639

Thompson JD, Higgins GD, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673–4680

Vollbrecht E, Veit B, Sinha N, Hake S (1991) The developmental gene *knotted-1* is a member of a maize homeobox gene family. Nature 350: 241–243

Watillon B, Kettmann R, Boxus P, Burny A (1997) *Knotted1*-like homeobox genes are expressed during apple tree (*Malus domestica* [L.] Borkh) growth and development. Plant Mol Biol 33: 757–763