ES/embryo body system. Embryo cell suspensions were plated in 1% methylcellulose containing 10% FCS (Summit), VEGF (5 ng ml$^{-1}$), insulin-like growth factor-1 (IGF-1; 50 ng ml$^{-1}$), leukemia inhibitory factor (LIF; 1 ng ml$^{-1}$), interleukin-6 (IL-6; 6.5 ng ml$^{-1}$) and 25% D4T endothelial cell conditioned medium$^2$. Colonies were grown in either 10 mm dishes or 24-well or 96-well plates in low oxygen incubators (5% oxygen). Haematopoietic colonies were further expanded using previously published expansion conditions$^1$. Haematopoietic progenitors were assayed in 1% methylcellulose containing 10% protein-free hydromixia (Gibco/BRL), 15% plasma-derived serum (Antech), c-kit ligand (K1; 1% conditioned medium), IL-3 (1% conditioned medium), granulocyte-macrophage colony-stimulating factor (GM-CSF; 3 ng ml$^{-1}$), IL-11 (5 ng ml$^{-1}$), erythropoietin (2 U ml$^{-1}$), IL-6 (5 ng ml$^{-1}$) and thrombopoietin (1% conditioned medium). Primitive erythroid colonies were counted from day 4 whereas definitive macrophage, erythro-macrophage and mast colonies were counted after 7–10 days of culture.

**Gene expression analysis**

Expression analyses of haematopoietblast colonies, the non-adherent and adherent cell populations from expanded haematopoietblast colonies, and sorted cell populations were performed using a modified global cDNA amplification protocol$^{12,13}$. Total RNA from pooled fragments of dissected neural plates stage embryos was harvested using the Absolutely RNA Microprep kit (Stratagene) and reverse transcribed with Omniscript RT (Qiagen) to generate cDNA. Primers used for PCR amplification of brachyury, Flk-1 and β-actin were as previously reported$^{10}$. The following additional primers were also used: Gata2 (forward) 5′-CATGCGGCTTTGTCGAGAAGA-3′, Gata2 (reverse) 5′-ACCTGATTGACCTGTAAGAGGG-3′, Cer1 (forward) 5′-CCAGGTTGGAGAATCTGGAAGA-3′, Cer1 (reverse) 5′-GCTTCACCACTGCAGCAGAATTG-3′, CTCGTT-3′, Cer1 (forward) 5′-GCTTCACCACTGCAGAATTG-3′, Moep (reverse) 5′-CAAGGACTCTGTTGAGATCATG-3′. Neomycin and hygromycin gene detection PCR reactions for the neomycin and hygromycin genes were performed using the Advantage 2 PCR system (BD Biosciences Clontech) on genomic DNA isolated from the non-adherent and adherent cell populations of the liquid-expanded haematopoietblast colonies. The primers used were as previously published$^{12}$. Fluorescence-activated cell sorting Embryos at a concentration of 1 × 10$^4$ ml$^{-1}$ were incubated with a biotinylated anti-Flik-1 antibody followed by incubation with streptavidin–PE-Cy5 (BD Pharmingen) and sorted on a MoFlo high-speed cell sorter (Cytometry). For the limiting dilution analysis (Fig. 3f), GFP Flk-1$^+$ and GFP Flk-1$^-$ cells were sorted directly into haematopoietblast conditions in 96-well plates. Out of three experiments, one was performed using duplicate wells containing 3,500, 5,700 or 10,000 cells each, and two experiments were performed using only one well for each condition. Immunofluorescence for CD31 and SMA expression Haematopoietblast colonies were cultured on fibronectin-coated glass coverslips in IMDM medium containing VEGF (5 ng ml$^{-1}$) and basic fibroblast growth factor (bFGF; 10 ng ml$^{-1}$) for 5–8 days. The coverslips were fixed with 4% paraformaldehyde for 15 min at room temperature, incubated with biotinylated anti-mouse CD31 (BD Pharmingen) and anti-mouse SMA (NeoMarkers) in 1× PBS buffer for 1 h, washed five times (10 min washes) and incubated with streptavidin–Cy3 (Sigma) and anti-mouse–FITC (Biosource International) for 1 h. After five washes the coverslips were inverted onto a drop of 4,6-diamidino-2-phenylindole (DAPI, Vector Laboratories, Inc.) on slides and viewed under an inverted fluorescence microscope.

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initiation of all aerial lateral meristems in maize. *barren stalk* represents one of the earliest genes involved in the patterning of maize inflorescences, and, together with the *teosinte branched* gene, it regulates vegetative lateral meristem development. The architecture of maize has been a major target of selection for early agriculturalists and modern farmers, because it influences harvesting, breeding strategies and mechanization. By sampling nucleotide diversity in the *barren stalk* region, we show that two haplotypes entered the maize gene pool from its wild progenitor, *teosinte*, and that only one was incorporated throughout modern inbreds, suggesting that *barren stalk* was selected for agronomic purposes.

*barren stalk* (*ba1*) is a spontaneous recessive mutation identified in 1928 (*ba1-ref*) from material collected by R. A. Emerson in South America. Homozygous *ba1* mutant plants are unable to produce vegetative branches (tillers), female inflorescences (ears) and a normal apical male inflorescence, the tassel. The tassel of *ba1* mutants is unbranched, shortened and predominantly sterile owing to the often complete lack of spikelets, the short branches bearing florets that represent the basic unit of grass inflorescences (Fig. 1a–d, l). Double mutant analysis with *teosinte branched* (*tb1*), an enhanced tillering mutant, and *in situ* hybridization on immature tassels with *knotted1*, a marker for meristematic cells, showed that lateral meristems in the aerial portion of *ba1* mutant plants fail to initiate during both vegetative and reproductive development, whereas the shoot apical meristem and inflorescence meristem, which define the main axis of aerial growth, develop normally.

We isolated the *ba1* gene through a candidate gene approach, using the recently isolated LAX gene of rice to probe an immature tassel complementary DNA library and a bacterial artificial chromosome (BAC) library. The *lax* mutant fails to initiate lateral meristems during the reproductive phase, resulting in an almost completely unbranched inflorescence with few or no spikelets. Vegetative lateral meristems, however, are unaffected. Even though *ba1*, unlike *lax*, affects both vegetative and reproductive development, the map positions of *ba1* and *lax* are in syntenic regions of chromosomes 3L and 1L, respectively, suggesting that both mutants...
could carry defects in orthologous genes. Library screens led us to identify a single gene. We subsequently cloned its corresponding genomic region in the bal-ref mutant and identified a 6.5-kilobase (kb) insertion in the proximal regulatory region at −306 base pairs (bp) from the predicted start codon (Fig. 2a). This insertion is a Helitron; that is, a recently discovered class of transposable elements. As the bal-ref allele is the only known allele in mutant collections, we searched for mutations in the candidate gene by screening a Mutator-tagged population (Trait Utility System Corn, Pioneer Hi-Bred International) and by sequencing the gene from a bal-like mutant found to be segregating in an ethylmethanesulphonate-induced M2 family. The identification of three insertions and a point mutation in the same gene, each showing a bal-like tassel phenotype (Fig. 1a, c) and suppression of ear and tiller formation, confirmed that we had isolated the bal gene. These alleles were named bal-mum1, bal-mum2, bal-mum3 and bal-IL, respectively (Fig. 2a).

The bal gene is intron-less, as is LAX, and the cDNA sequence presents an open reading frame (ORF) of 660 bp. The comparison between BA1 and LAX putative amino acid sequences reveals an overall identity of 62% (Fig. 2b). The bal gene is predicted to encode a small protein of 219 amino acids with a basic helix–loop–helix (bHLH) domain, which is the defining feature of the bHLH family of transcription factors. This domain is 100% conserved when compared to LAX (Fig. 2b,c). The bHLH domain consists of an amino-terminal basic region involved in DNA binding, and a carboxy-terminal HLH region, which leads to the formation of homo- or heterodimers. bHLH transcription factors are important components of eukaryotic transcription networks in many biological pathways. Although this family of transcription factors represents the second largest in the Arabidopsis genome, only a few members involved in developmental processes have been characterized in detail. The predicted BA1 bHLH domain lacks the glutamic acid at position 9 in the basic region that was demonstrated in non-plant bHLH proteins to be necessary for DNA binding to the canonical E-box site (Fig. 2c). Although no significant similarities are observed outside of the bHLH domain, BA1 as well as the recently characterized INDEHISCENT (IND) protein in Arabidopsis belong to a subgroup of proteins that share an atypical bHLH domain that is suggested to bind DNA through recognition of a different motif or together with a partner protein (Fig. 2c).

The earliest events in tassel development include the formation on the flanks of the main inflorescence meristem of a few branch meristems that develop into the long branches of mature tassels, and of several rows of spikelet-pair meristems along the sides of the branches and the central spike. Spikelet-pair meristems give rise to two spikelet meristems, each of which initiates two floral meristems: the upper and lower floral meristems (Fig. 1fg). This specific sequence of branching events by intermediate lateral meristems eventually leads to the formation of two paired spikelets (pedicellate and sessile spikelets), each consisting of a pair of glumes (modified leaves) enclosing a short branch terminating in an upper and a lower floret (Fig. 1i) composed of lemma, palea, lodicules and stamens.

Homozygous bal-mum1 and bal-mum3 plants manifest a weaker phenotype than the bal-ref mutant, typically having a central spike of normal length with spikelets forming along the upper portion, but still lacking basal branches (Fig. 1e). bal-mum2 and bal-IL tassels are, however, as severely affected in branching and spikelet formation as seen in the bal-ref tassels. The spikelets that form in the upper portion of bal-mum1 and bal-mum3 tassels manifest a wide range of defects. Scanning electron microscopy (SEM) on immature bal-mum1 and bal-mum3 tassels reveals that the development of all lateral meristems is affected. No branch meristems are formed, and several spikelet-pair meristems remain undeveloped, originate single spikelets or divide abnormally (Fig. 1h–k). Some spikelet meristems appear smaller and produce tubular structures in place of glumes (Fig. 1i–k). Once mature, mutant spikelets are either empty or bear a varying number of floral organs in just one of the two florets or in both florets (Fig. 1m, n). These defects indicate that a functional bal gene is required early in inflorescence development for branch meristem and spikelet-pair meristem initiation, and later for the correct establishment of all subsequent lateral meristems, either having a role in their maintenance or possibly regulating organ formation during spikelet and floret development. As meristem size correlates with the number of organs formed, we favour the view that bal is necessary for establishing the appropriate population of meristematic cells, which is in turn required for the correct partitioning of these cells during all branching events, as already suggested for barren inflorescence2, a mutant similarly impaired in lateral meristem formation.

Expression analysis via in situ hybridizations during vegetative and reproductive development supports these deduced functions of BA1 (Fig. 3). Sections of young seedlings show a defined signal on the adaxial (facing towards the stem) side of initiating axillary meristems, whereas no expression is observed in the shoot apical meristem (Fig. 3a). After the transition to the reproductive phase, bal is first detected in a narrow arc of cells immediately above the region where branch meristems and spikelet-pair meristems will initiate (Fig. 3b, c). As these branch meristems and spikelet-pair meristems become visible, the expression persists in a position adjacent to the initiating meristems (Fig. 3b, c) and is later localized in the dividing spikelet-pair meristems, in the region between the two developing spikelet meristems (Fig. 3d). During floral meristem formation, we detected the transcript in a region of cells between the upper floral meristem and lower floral meristem, and in a more diffuse pattern in the upper floral meristem (Fig. 3e). As spikelets develop, bal expression remains localized to the sides and to the base of both spikelets (Fig. 3f). In sections through ear spikelets,
when the reproductive organs are initiating, ba1 expression is observed on the adaxial side of the lower floret but not in developing floral organ primordia (Fig. 3g). A comparatively faint signal is present in developing anthers of young tassel spikelets (Fig. 3h). No expression is seen in sections of ba1-ref tassels, but a shortened transcript is weakly detectable through northern hybridizations (data not shown), suggesting that the smaller transcript is unstable.

In every branching event, from the formation of branch meristems to the formation of floral meristems, ba1 retains a clear and specific expression pattern, localized in the regions where this branching occurs. These observations, together with the mutant phenotypes, suggest that a conserved mechanism in maize regulates branching occurs. These observations, together with the mutant specific expression pattern, localized in the regions where this signalling. Polarized gradients of auxin were recently proposed as a mechanism that might be involved in transmitting an endogenous signal to axillary buds along the main stem1. Accordingly, expression of tbi is first detected during the formation of vegetative axillary meristems11. barren stalk1 maps to the genomic region encompassing QTL 3L, and the ba1 mutation is epistatic to tbi, suppressing tiller formation in double mutant plants, suggesting that ba1 acts upstream of tbi in the pathway of vegetative lateral meristem formation6. In order to clarify the relationship between these two genes, we quantitatively analysed tbi expression in ba1 mutant shoots and developing tassels, and ba1 expression in tbi mutant seedlings (Fig. 4a). The reduction in tbi transcript levels in both ba1-ref seedling and tassel samples confirms that ba1 activity is required for normal levels of tbi expression. Furthermore, the normal level of expression of ba1 in tbi mutant seedlings, together with the ba1 expression pattern adjacent to initiating meristems, suggest that ba1 is required for axillary meristem formation but not for axillary shoot elongation.

We then investigated whether selection acted on ba1 during maize domestication or improvement. One expectation for a selected gene is a change in the pattern of nucleotide diversity, as the favourable allele(s) is brought to higher frequency by human selection25,22.24. We analysed sequence diversity at ba1 in 14 diverse maize inbreds, 16 diverse maize landraces, and 14–17 teosinte samples (Zea mays parviglumis and Z. mays mexicana) for three distinct regions of ba1 (Fig. 4b, c). Two standard selection tests (Tajima's P0 and the Hudson–Kreitman–Aguade26) were consistent with neutral evolution at ba1 in maize landraces, but the HKA test showed a highly significant deviation from neutrality for inbreds (P < 0.0002). Moreover, for ba1, maize inbreds possess only 3% of the diversity found in teosinte (Fig. 4b), a value that is much smaller that the 30% figure for starch pathway genes27, which were targets of human selection, and nearly as small as that for the domestication gene tbi (ref. 22). The low diversity among inbreds is a function of the large single haplotype, as shown by a survey of 86 diverse maize inbreds (Supplementary Data). Thus, although the standard tests provide no evidence that ba1 was under selection during domestication, they suggest that it was under selection during maize improvement, reducing modern maize inbreds to a single basic haplotype (haplotype I, see below).

Although the standard tests of selection during domestication for ba1 were not significant, other aspects of the sequence data are atypical of genes that passed through the domestication process in a neutral fashion. First, maize landraces possess only two basic haplotypes (I and II; Fig. 4c) in contrast to the multiple haplotypes typically observed at neutral loci in maize25. Haplotype I is found in maize from throughout the Americas, but haplotype II is found only in the central Mexican highlands. Second, these two maize haplotypes occur with a patterned distribution in teosinte. The populations of the parviglumis subspecies from the region of maize domestication (southwestern Mexican lowlands20) possess haplotype I plus multiple other haplotypes but not haplotype II. In contrast, both haplotypes I and II occur in the mexicana subspecies from the central Mexican highlands. Third, in maize there is no evidence of recombination between haplotypes I and II, and accordingly linkage disequilibrium (LD) at ba1 in maize is
very high \( r^2 = 0.96 - 1.0 \) whereas in teosinte it is modest \( r^2 = 0.06 - 0.31 \).

Although uncertainty remains about the role of \( ba1 \) during the domestication process, these observations can be explained as follows. Initially, \( ba1 \) was a target of selection during maize domestication from the \( parviglumis \) subspecies in the Mexican lowlands and as a consequence only haplotype I entered the maize gene pool. Later after maize spread to the highlands, maize acquired haplotype II from the \( mexicana \) subspecies via introgression. Thus, haplotype II is found in sympatric populations of maize and the \( mexicana \) subspecies at high elevations in central Mexico. The high LD observed for \( ba1 \) in maize is a consequence of the admixture with the \( mexicana \) subspecies. Finally, because of the admixture with the \( mexicana \) subspecies, the standard tests of selection are nonsignificant for maize landraces, despite the fact that \( ba1 \) was under selection during the domestication process. If this model is correct, then future analyses should show that both haplotypes I and II are associated with a more maize-like phenotype as compared with the multiple other haplotypes found only in teosinte.

The evidence suggests that \( ba1 \) was under selection during maize improvement and encourages further investigation to determine whether \( ba1 \) corresponds to the QTL 3L implicated in maize domestication\(^{19,23} \). Mutations in \( ba1 \) produce a plant manifesting strong apical dominance, whereas mutations in \( tb1 \) cause the opposite effect, namely a severe loss of apical dominance. A balance between the activities of these two genes may have been an important contributor to the domestication and improvement of maize.

**Methods**

**Gene cloning**

A full-length \( LAX \) cDNA clone was used to screen at medium stringency (\( \approx 55^\circ C \)) a cDNA library from RNA of immature tassels (1–3 mm), cloned in HybZAP-2.1 vector (Stratagene). Hybridizing clones were recovered according to manufacturer’s instructions (Stratagene). The same probe was used to isolate a 4.5-kb fragment of the genomic region harbouring the \( ba1 \) gene by screening a BAC library (NSF B73, Clemson University Genomic Institute). The mutant \( ba1-ref \) locus was characterized as follows. The coding sequence was amplified by polymerase chain reaction (PCR). Southern analysis on...
The BCL6 proto-oncogene suppresses p53 expression in germinal-centre B cells

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The human proto-oncogene BCL6 encodes a BTB/POZ-zinc-finger transcriptional repressor that is necessary for germinal-centre formation and is implicated in the pathogenesis of B-cell lymphoma1,2. The precise function of BCL6 in germinal-centre development and lymphomagenesis is unclear because very few direct BCL6 target genes have been identified3–5. Here we report that BCL6 suppresses the expression of the p53 (also known as TP53) tumour suppressor gene and modulates DNA damage-induced apoptotic responses in germinal-centre B cells. BCL6 represses p53 transcription by binding two specific DNA sites within the p53 promoter region and, accordingly, p53 expression is absent in germinal-centre B cells where BCL6 is highly expressed. Suppression of BCL6 expression via specific short interfering RNA leads to increased levels of p53 messenger RNA and protein both under basal conditions and in response to DNA damage. Most notably, constitutive expression of BCL6 protects B cell lines from apoptosis induced by DNA damage. These results suggest that an important function of BCL6 is to allow germinal-centre B cells to tolerate the physiological DNA breaks required for immunoglobulin class switch recombination and somatic hypermutation without inducing a p53-dependent apoptotic response. These findings also imply that deregulated BCL6 expression contributes to lymphomagenesis in part by functional inactivation of p53.


Supplementary Information accompanies the paper on www.nature.com/nature.

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