ES/embryoid body system. Embryo cell suspensions were plated in 1% methycellulose containing 10% FCS (Summit), VEGF (5 ng ml^{-1}), insulin-like growth factor-1 (IGF-1; 50 ng ml^{-1}), leukaemia inhibitory factor (LIF; 1 ng ml^{-1}), interleukin-6 (IL-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). Haemangioblast colonies were further expanded using previously published expansion conditions[1]. Haematopoietic progenitors were assayed in 1% methycellulose containing 10% protein-free hybridoma medium (Gibco/BRL), 15% plasma-derived serum (Antech), c-kit ligand (KL; 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 4–5 whereas definitive macrophage, erythroid–macrophage and mast colonies were counted after 7–10 days of culture.

Gene expression analysis

Expression analyses of haemangioblast/blast colonies, the non-adherent and adherent cell populations from expanded haemangioblast colonies, and sorted cell populations were performed using a modified global cDNA amplification protocol[2,28].

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[17]. The following additional primers were also used: *Gata1* (forward) 5′-CATTGGCCCCTTGTGAGGCCAGAGA-3′, *Gata1* (reverse) 5′-ACCTGATGGAGCTTGAAATAGAGGC-3′; *Cer1* (forward) 5′-CCAG GCTTGGAAGATTCTGGAAGA-3′, *Cer1* (reverse) 5′-GTCTTCACCATGCACTGACA CTCT-3′; *Mesp1* (forward) 5′-GTTCCTGTACGCAGAAACAGCATC-3′, *Mesp1* (reverse) 5′-CAAGGAGGGTTGGAATGGTACAGT-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 haemangioblast colonies. The primers used were as previously published[2].

Fluorescence-activated cell sorting

Embryo cells at a concentration of 1×10^6 ml^{-1} were incubated with a biotinylated anti-Flk-1 antibody followed by incubation with streptavidin-PE-Cy5 (BD Pharmingen) and sorted on a MoFlo high-speed cell sorter (Cytomation). For the limiting dilution analysis (Fig. 3f), GFP$^-$Flk-1$^+$, GFP$^+$Flk-1$^+$ and GFP$^+$Flk-1$^-$ cells were sorted directly into haemangioblast conditions in 96-well plates. Out of three experiments, one was performed using duplicate wells containing 3,300, 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

Haemangioblast 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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The role of *barren stalk1* in the architecture of maize

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The architecture of higher plants is established through the activity of lateral meristems—small groups of stem cells formed during vegetative and reproductive development. Lateral meristems generate branches and inflorescence structures, which define the overall form of a plant[1–3], and are largely responsible for the evolution of different plant architectures[3]. Here, we report the isolation of the *barren stalk1* gene, which encodes a non-canonical basic helix–loop–helix protein required for the

initiation of all aerial lateral meristems in maize. *barren stalk1* represents one of the earliest genes involved in the patterning of maize inflorescences, and, together with the *teosinte branched1* gene[4], 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 stalk1* 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 stalk1* was selected for agronomic purposes.

barren stalk1 (*ba1*) is a spontaneous recessive mutation identified in 1928 (*ba1-ref*) from material collected by R. A. Emerson in South America[5]. Homozygous *ba1* mutant plants are unable to produce vegetative branches (tillers), female inflorescences (ears) and a normal apical male inflorescence, the tassel[6]. 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 branched1* (*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[6].

We isolated the *ba1* gene through a candidate gene approach, using the recently isolated *LAX* gene of rice[2] 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[2]. 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

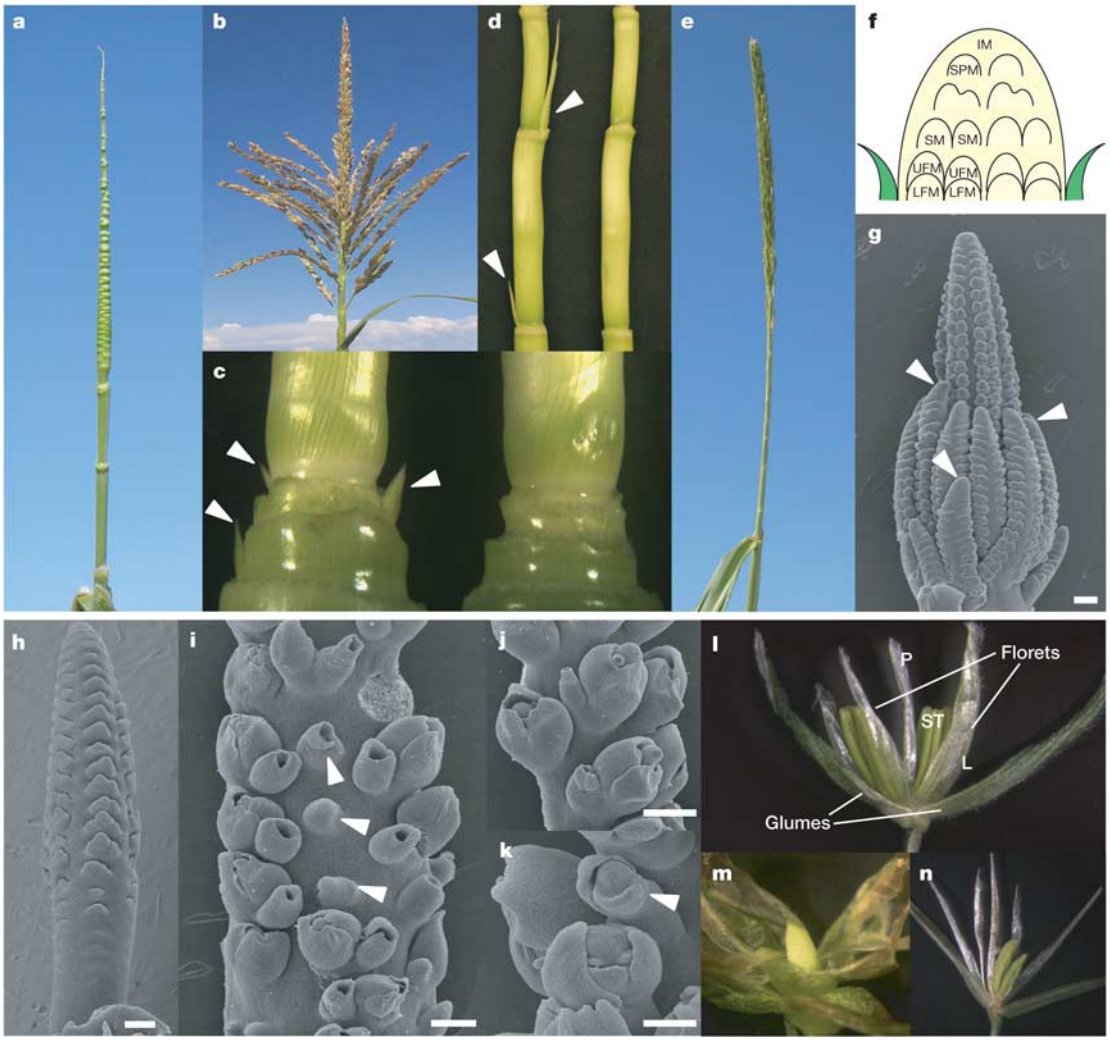

Figure 1 Effects of *ba1* mutations on maize development. **a**, *ba1-ref* tassel. **b**, Wild-type tassel. **c**, **d**, Axillary buds during vegetative (**c**) and reproductive (**d**) development in wild-type (left) and *ba1-ref* mutant plants (right). Upon removal of leaves, axillary buds (arrowheads) are only visible in wild-type plants. **e**, *ba1-mum1* mutant tassel. Spikelets are borne on the central rachis. **f**, Schematic representation of the acropetal development of maize inflorescences and of the lateral meristems involved. IM, inflorescence meristem; SPM, spikelet-pair meristem; SM, spikelet meristem; UFM/LFM, upper/lower floral meristem. **g**–**k**, SEM of immature tassels. **g**, Wild type showing branch meristems (arrowheads). **h**, *ba1-mum1*. **i**, *ba1-mum1* (later stage); several meristems are arrested in development (arrowheads). **j**, *ba1-mum1* spikelet pairs. **k**, *ba1-mum3* spikelets; white arrowhead points to an undeveloped spikelet meristem. **l**, Wild-type tassel spikelet. Each floret is composed of lemma (L), palea (P) and three stamens (ST). **m**, *ba1-mum1* mature spikelet. A sterile structure is produced in place of reproductive organs. **n**, *ba1-mum3* spikelet. The upper floret is missing; the lower floret has a reduced number of stamens. Scale bars, 200 μm.

could carry defects in orthologous genes. Library screens led us to identify a single gene. We subsequently cloned its corresponding genomic region in the *ba1-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[7]. As the *ba1-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 *ba1*-like mutant found to be segregating in an ethylmethane-sulphonate-induced M2 family. The identification of three insertions and a point mutation in the same gene, each showing a *ba1*-like tassel phenotype (Fig. 1a, e) and suppression of ear and tiller formation, confirmed that we had isolated the *ba1* gene. These alleles were named *ba1-mum1*, *ba1-mum2*, *ba1-mum3* and *ba1-IL*, respectively (Fig. 2a).

The *ba1* gene is intron-less, as is *LAX*[2], 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 *ba1* 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[8,9]. bHLH transcription factors are important components of eukaryotic transcription networks in many biological pathways[8,9]. 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[9,10]. 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)[8–10]. Although no significant similarities are observed outside of the bHLH domain, BA1 as well as the recently characterized INDEHISCENT (IND) protein in *Arabidopsis*[10] 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[8–10] (Fig. 2c).

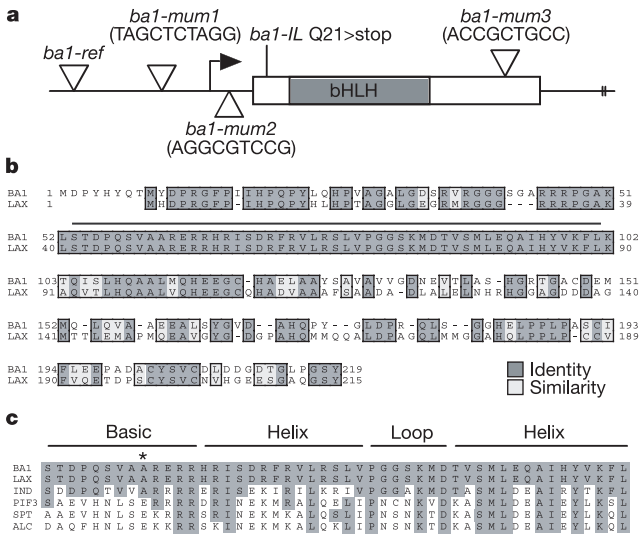

Figure 2 *barren stalk1* encodes a non-canonical bHLH protein. **a**, *ba1* gene schematic showing the location of mutations in characterized alleles. The rectangle represents the coding region with the bHLH domain shown in grey. The arrow and vertical bars represent the transcription start and end, respectively. In parenthesis are the sequences of the target site duplication resulting from *Mutator* insertions. **b**, Comparison of BA1 and LAX predicted amino acid sequences. The bHLH domain is indicated by an overhead horizontal line. **c**, Sequence alignment of BA1 bHLH domain with several related proteins characterized in plants: LAX, IND, PHYTOCHROME INTERACTING FACTOR3 (PIF3), SPATULA (SPT) and ALCATRAZ (ALC)[2,9,10]. The asterisk marks the alanine residue at position 9 of BA1, LAX and IND basic domain.

Homozygous *ba1-mum1* and *ba1-mum3* plants manifest a weaker phenotype than the *ba1-ref* mutant, typically having a central spike of normal length with spikelets forming along the upper portion, but still lacking basal branches (Fig. 1e). *ba1-mum2* and *ba1-IL* tassels are, however, as severely affected in branching and spikelet formation as seen in the *ba1-ref* tassels. The spikelets that form in the upper portion of *ba1-mum1* and *ba1-mum3* tassels manifest a wide range of defects. Scanning electron microscopy (SEM) on immature *ba1-mum1* and *ba1-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 matured, 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 *ba1* 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[14], we favour the view that *ba1* 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[15].

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, *ba1* 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, *ba1* 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 the formation of lateral meristems during both vegetative and reproductive development. The reiterative adaxialized expression domain observed during the initiation of all lateral meristems indicates that these *ba1*-expressing cells have a specific identity and might be involved in transmitting an endogenous signal to regulate downstream genes controlling lateral meristem initiation and outgrowth. Several mutants affected in lateral meristem formation have been related to failures in polar auxin transport or signalling. Polarized gradients of auxin were recently proposed as a common module for several developmental processes[16,17], raising the possibility that *ba1* could be involved in an auxin signalling pathway.

The morphology of modern maize is derived from that of its wild progenitor teosinte through a domestication process that is still poorly understood[4,18–20]. A quantitative trait locus (QTL) analysis of maize and teosinte identified five major chromosomal regions that explain most of their morphological differences[4,19], essentially accounting for lateral branch development and for the positioning and morphology of female inflorescences[4,19,21]. So far, only the *teosinte branched1* gene, a TCP transcription factor, has been clearly implicated in the domestication of maize architecture by its identity with the QTL on the long arm of chromosome 1 (refs 4, 19–22). Genetic evidence supports the existence of an epistatic interaction between another major QTL on the long arm of chromosome 3 (QTL 3L) and *tb1* (refs 19, 23). QTL 3L was proposed as an upstream regulator of *tb1* (ref. 23). TB1 is a repressor of lateral meristem outgrowth and the *teosinte branched1* mutant is characterized by a severe loss of apical dominance, which results in an unrestrained outgrowth of axillary buds along the main stem[4]. Accordingly, expression of *tb1* is first detected during the formation of vegetative axillary meristems[21]. *barren stalk1* maps to the genomic region encompassing QTL 3L, and the *ba1* mutation is epistatic to *tb1*, suppressing tiller formation in double mutant plants, suggesting that *ba1* acts upstream of *tb1* in the pathway of vegetative lateral meristem formation[6]. In order to clarify the relationship between these two genes, we quantitatively analysed *tb1* expression in *ba1* mutant shoots and developing tassels, and *ba1* expression in *tb1* mutant seedlings (Fig. 4a). The reduction in *tb1* transcript levels in both *ba1-ref* seedling and tassel samples confirms that *ba1* activity is required for normal levels of *tb1* expression. Furthermore, the normal level of expression of *ba1* in *tb1* 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 selection[20,22,24]. 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 D[25] and the Hudson–Kreitman–Aguadé (HKA) test[26]) 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 genes[27], which were targets of human selection, and nearly as small as that for the domestication gene *tb1* (ref. 22). The low diversity among inbreds is a function of their possessing a 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 maize[24]. 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 lowlands[20]) 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

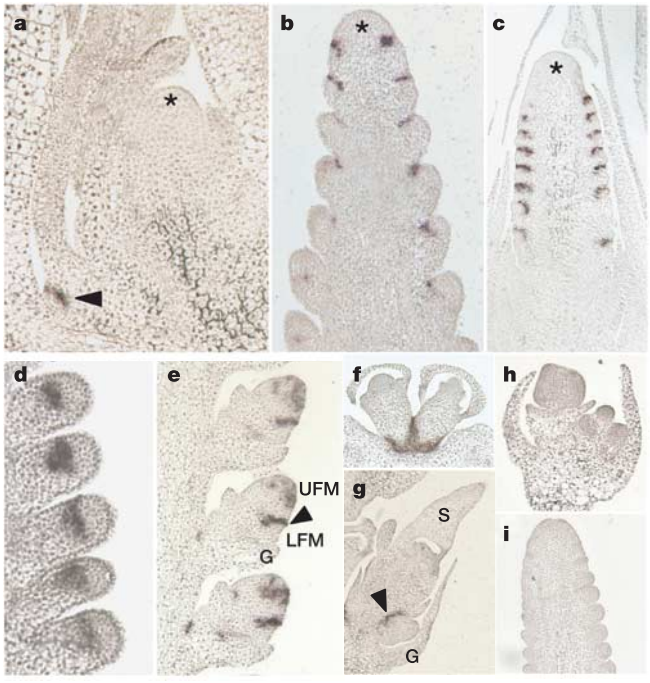

Figure 3 *barren stalk1* expression by *in situ* hybridization. **a**, Longitudinal section of a 5-day-old seedling showing *ba1* expression on the adaxial side of the initiating vegetative axillary meristem (arrowhead). Asterisk, shoot apical meristem. **b**, **c**, Longitudinal sections of immature tassel (**b**) and ear (**c**). Asterisk marks the inflorescence meristem. **d**, Longitudinal oblique section of a series of dividing spikelet-pair meristems. **e**, Longitudinal section of spikelets in which upper floral meristems and lower floral meristems are forming. Signal is detected in the upper floral meristem and in the region demarcating the upper and lower floral meristems (arrowhead). G, glume primordia. **f**, Cross-section of an ear spikelet pair. **g**, Longitudinal section of ear spikelets. Strong expression is observed adaxial to the lower floret (arrowhead). S, developing silk. **h**, Tassel spikelet. **i**, Ear, sense control.

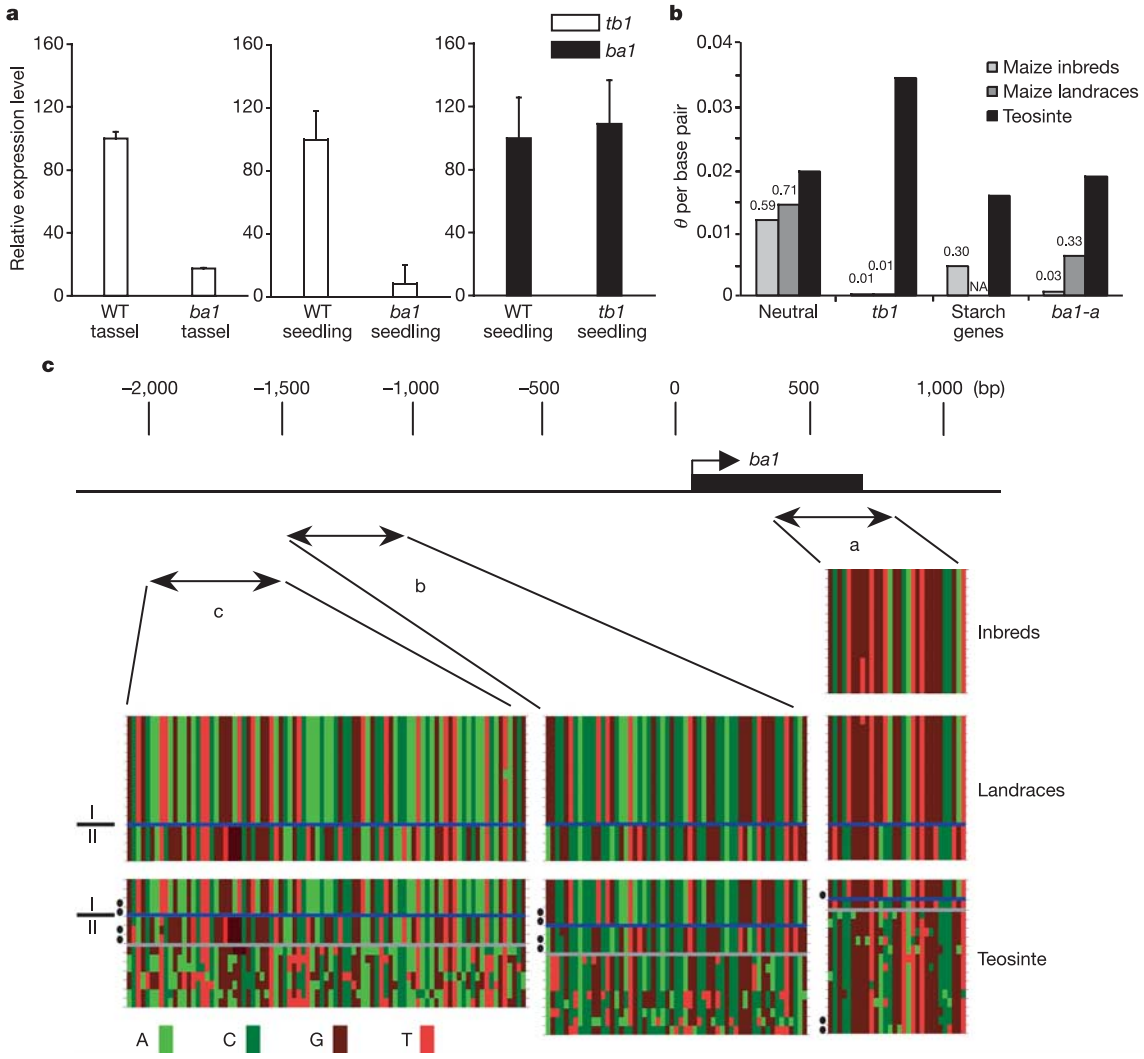

Figure 4 *barren stalk1* as a candidate for QTL 3L. **a**, Expression analysis of *tb1* and *ba1* in *ba1* and *tb1* mutants, respectively, by RT–quantitative PCR. Values for the *y* axis are arbitrary units of expression level relative to actin. Error bars indicate standard deviation of at least four replicates. WT, wild type. **b**, Comparison of silent nucleotide polymorphism (θ) between maize and teosinte in the coding region of the *ba1* locus (*ba1-a*). The numbers above the bars indicate the fold reduction in diversity between maize and teosinte. Values for neutral genes (*csu1171*, *asg11*, *csu1132*, *bz2*, *glb1*, *adh1* and *csu381*)[24], maize starch pathway genes (*ae1*, *bt2* and *su1*)[27] and the domestication gene *tb1* are indicated. **c**, Single nucleotide polymorphism survey between maize and teosinte. Three different regions of the *ba1* locus (a, b, c) were analysed. Each array consists of sequenced samples (rows) and polymorphic sites (columns). Haplotypes I and II are separated by the blue lines; other teosinte haplotypes appear below the grey lines. Dots mark the *mexicana* subspecies sequences.

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 (~57 °C) a cDNA library from RNA of immature tassels (1–3 mm), cloned in HybriZAP-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

genomic DNA (*Bam*HI digest) revealed an insertion in the proximal promoter region, whose sequence and location were subsequently determined. A proximal 1.5-kb *Bam*HI fragment was isolated through iPCR. The distal *Bam*HI fragment was cloned using the ZAP Express predigested vector kit (Stratagene), following the manufacturer's instructions. The whole insertion was eventually obtained using the Elongase amplification system (Invitrogen).

New *ba1* mutant alleles were identified at Pioneer Hi-Bred International, as previously described[28], via PCR (Supplementary Methods). New material is available for non-commercial research purposes upon acceptance and signing of an appropriate material transfer agreement. The *ba1-IL* (03IL-A619TR-996) point mutation was identified by sequencing the *ba1* gene from mutant plants segregating in an ethylmethane-sulphonate-generated M2 family (http://www.maizegdb.org/mip). We used ClustalW (MacVector 6.5 [K]) for sequence comparisons.

Expression analysis and SEM

Tissue samples were fixed for both *in situ* sectioning and SEM as previously described[13]. SEM specimens were viewed on a Quanta 600 microscope. *In situ* hybridizations were performed as previously reported[29]. Antisense probes for *ba1* were synthesized with T7 polymerase using DIG RNA labelling mixture (Roche). Both *Hin*dIII and *Pst*I 3′ fragments of the *ba1* cDNA (~500 and 300 bp, respectively) were used for *in situ* hybridizations. *ba1* sense probe template was amplified from the same *Hin*dIII fragment using a chimaeric T7 promoter primer.

Total RNA from pooled seedlings (20 days old) and immature tassels (1.5–2.5 cm) was extracted using standard procedures. RNA samples were further purified (Qiagen RNeasy mini kit) and 2–5 μg were treated with DNase and subsequently reverse-transcribed using the Superscript first-strand synthesis system for RT–PCR, following the manufacturer's instructions (Invitrogen). RT–PCR reactions were run in a LightCycler (Roche) using Hot Start SYBR green reaction mix (Roche) and analysed using the LightCycler relative quantification software (version 1.0, Roche). Samples for expression level comparison are in the same genetic background. Primer sequences are available on request.

Nucleotide diversity survey and statistical analysis

Nucleotide diversity in the *ba1* locus was determined for three fragments (regions a, b and c) from 16 maize landraces previously described[24], 14 inbreds and 14–17 teosinte individuals (Supplementary Methods and Supplementary Table 1). Nucleotide polymorphism (θ), linkage disequilibrium (r^2) and Tajima's D statistic were calculated using DnaSP version 4.0 (ref. 30). The HKA test was performed using *Tripsacum* as the preferred outgroup for the divergence estimate, and *Zea diploperennis* in cases where the *Tripsacum* sequence could not be obtained. PCR products for *Z. diploperennis* and *Tripsacum bravum*, potentially heterozygous for *ba1*, were cloned using the pCR 2.1-TOPO kit (Invitrogen) and at least four clones were sequenced. A joint HKA test across the six neutral loci was obtained by summing the individual χ^2 values.

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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 lymphoma[1–3]. The precise function of BCL6 in germinal-centre development and lymphomagenesis is unclear because very few direct BCL6 target genes have been identified[4–7]. 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.