Escherichia coli mazEF Toxin-Antitoxin System as a Tool to Target Cell Ablation in Plants

Fabien Baldacci-Cresp^a Anaxi Houbaert^a Amana Metuor Dabire^a
Adeline Mol^a Daniel Monteyne^{b, c} Mondher El Jaziri^a Laurence Van Melderén^d
Marie Baucher^a

^aLaboratoire de Biotechnologie Végétale, ^bLaboratoire de Parasitologie Moléculaire, IBMM, ^cCenter for Microscopy and Molecular Imaging – CMMI and ^dLaboratoire de Génétique et Physiologie Bactérienne, Faculté des Sciences, Université libre de Bruxelles (ULB), Gosselies, Belgium

Key Words

Toxin-antitoxin system · MazF endoribonuclease · *Escherichia coli* · *Nicotiana tabacum* · Tapetum · Targeted cell ablation

Abstract

Background/Aims: The *Escherichia coli* MazF is an endoribonuclease that cleaves mRNA at ACA sequences, thereby triggering inhibition of protein synthesis. The aim of this study is to evaluate the efficiency of the *mazEF* toxin-antitoxin system in plants to develop biotechnological tools for targeted cell ablation. **Methods:** A double transformation strategy, combining expression of the *mazE* antitoxin gene under the control of the *CaMV 35S* promoter, reported to drive expression in all plant cells except within the tapetum, together with the expression of the *mazF* gene under the control of the *TA29* tapetum-specific promoter in transgenic tobacco, was applied. **Results:** No transgenic *TA29-mazF* line could be regenerated, suggesting that the *TA29* promoter is not strictly tapetum specific and that MazF is toxic for plant cells. The regenerated *35S-mazE/TA29-mazF* double-transformed lines gave a unique phenotype where the tapetal cell layer

was necrosed resulting in the absence of pollen. **Conclusion:** These results show that the *E. coli mazEF* system can be used to induce death of specific plant cell types and can provide a new tool to plant cell ablation.

© 2016 S. Karger AG, Basel

Introduction

Toxin-antitoxin (TA) systems are small genetic modules widespread in bacterial and archaea chromosomes and mobile genetic elements [Van Melderén, 2010]. They are divided into 5 groups (types I–V) depending on the nature and the mechanism of action of the antitoxin [Goeders and Van Melderén, 2014]. Several potential roles have been attributed to TA systems such as addictive genomic modules, stabilization of genomic parasites, selfish alleles, gene regulation, growth control, persister cell formation, programmed cell arrest, programmed cell death and antiphage measures [Hayes and Van Melderén, 2011; Wang and Wood, 2011].

L.V.M. and M.B. contributed equally to this paper.

The *mazEF* TA system, located on the chromosome of *Escherichia coli*, is one of the best-characterized bacterial type II TA systems [Goeders and Van Melder, 2014]. The *mazE* and *mazF* genes are organized in an operon and code for a 9-kDa antitoxin and a 12-kDa toxin protein, respectively. The MazF toxin is stable while the MazE antitoxin is labile and degraded by ATP-dependent proteases [Aizenman et al., 1996; Christensen et al., 2003]. MazF has been demonstrated to be an endoribonuclease (or mRNA interferase) that functions independently of ribosomes and codon context and cuts single-stranded mRNA at the specific ACA sequence triggering inhibition of protein synthesis [Zhang et al., 2005]. The X-ray structure of the MazF-MazE complex has been determined, and this complex forms a heterohexamer composed of alternating toxin and antitoxin homodimers (MazF₂-MazE₂-MazF₂) [Kamada et al., 2003].

MazF has been shown to be a powerful biotechnological tool for several applications. By engineering genes to be transcribed in mRNAs devoid of any ACA sequences and concomitantly expressing *mazF*, Suzuki et al. [2005] could selectively produce a single recombinant protein in *E. coli*. MazF has also been used as counterselectable marker for unmarked genetic modification of the yeast *Pichia pastoris* where its expression halted cell growth [Yang et al., 2009]. It has also been proven to be a powerful tool to suppress HIV-1 replication following viral infection into CD4+ T-lymphoid CEM-SS cells [Chono et al., 2011] as well as antiviral therapies for the hepatitis C virus [Shapira et al., 2012]. Expression of *mazF* in mammalian cells has been shown to lead to apoptotic cell death [Shimazu et al., 2007] and to induce regression of solid tumours, when expressed in mice [Shimazu et al., 2014].

The aim of this study is to evaluate the ability of the *mazEF* system to induce death in plant cells to develop biotechnological tools for targeted cell ablation. A well-characterized model is the use of the *TA29* promoter in *Nicotiana tabacum* (tobacco) to target gene expression within the tapetum, a nutritive tissue within the sporangium of anthers essential for the development of pollen grains [Koltunow et al., 1990]. Previously, it was shown that several cytotoxic genes such as the *Bacillus amyloliquefaciens* barnase, the diphtheria toxin A-chain or the RNase T1, when targeted to the tapetum under the control of the *TA29* promoter, cause male sterility in tobacco plants [Koltunow et al., 1990; Mariani et al., 1990]. In this study, expression of *mazF* under the control of the *TA29* promoter was used to demonstrate the ability of MazF to induce plant cell death.

Fig. 1. RT-qPCR expression analysis of *TA29*, *mazE* and *mazF* in aerial parts of 1-month-old in vitro-grown WT and 35S-*mazE* transgenic lines. The line 35S-*mazE*4 was used for double transformation. C_t = Threshold cycle; white bars = *TA29*; grey bars = *mazE*; # = *mazE* not detected; \$ = *mazF* not detected.

Results and Discussion

No Transgenic Plant Expressing *mazF* under the Control of the *TA29* Could Be Generated

A 600-bp *TA29* promoter fragment (−616 to −17 relative to the start codon) was amplified from tobacco genomic DNA, and transgenic *TA29-GUS* T2 lines were regenerated. *GUS* expression mediated by the *TA29* promoter was assessed at different stages of development of the transgenic tobacco plants. *GUS* expression was detected neither in in vitro plants nor in vegetative tissues of phytotron-grown plants. In accordance with the literature [Koltunow et al., 1990], *GUS* expression was found in the tapetum tissue of anthers of transgenic *TA29-GUS* plants (online suppl. fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000446112). No transgenic lines expressing the *TA29-mazF* construct could be regenerated, indicating that MazF is highly toxic in plant cells and that leaky expression during plant regeneration or development is sufficient to kill plant cells. To further document this hypothesis, endogenous *TA29* gene expression was evaluated by real-time quantitative reverse transcription PCR (RT-qPCR) on the aerial part of 1-month-old in vitro grown wild-type (WT) plants (fig. 1). Surprisingly, detectable *TA29* expression was dis-

covered in young tobacco plants, although it was previously found to be restricted to the tapetum tissue as measured by Northern blot or by promoter-*GUS* analysis [Koltunow et al., 1990; Mariani et al., 1990]. However, there is no previous report on *TA29* expression using PCR-based derived techniques. This discrepancy may be explained by the higher sensitivity of PCR compared to the two other methods.

Accordingly, a first hypothesis to explain our results compared to those of Mariani et al. [1990] using the barnase toxin-encoding gene driven by the *TA29* promoter would be that plant cells are more sensitive to MazF than to barnase. A second hypothesis is related to the size of the *TA29* promoter used to drive expression within the tapetum. For instance, Mariani et al. [1990] obtained male sterility in tobacco plants by fusing a 1,500-bp *TA29* promoter to either *Aspergillus oryzae* RNase-T1 or barnase-encoding genes. Besides, Kriete et al. [1996] showed that male sterility could be induced in tobacco plants by application of the non-phytotoxic compound N-acetyl-L-phosphinothricin to transgenic plants containing the *E. coli argE* gene under the control of an 850-bp fragment of the *TA29* promoter. *argE* encodes an N-acetyl-L-ornithine deacetylase which removes the acetyl group from N-acetyl-L-phosphinothricin giving the cytotoxic compound L-phosphinothricin. Yang et al. [1997] could express specifically the *iaaL* gene, encoding indoleacetic acid-lysine synthetase, in tobacco tapetum, by using a 291-bp *TA29* promoter. In the present study, a 600-bp *TA29* promoter was used. Nevertheless, the length of the *TA29* promoter should not affect the results as it was shown, by promoter deletion analysis, that *GUS* mRNA levels were similar in transformants containing a *TA29-GUS* gene construct with 5,270 to 279 nucleotides of the *TA29* 5'-region [Koltunow et al., 1990]. Since there were no reports on loss of tapetum specificity when using different lengths of *TA29* promoters, the second hypothesis is not supported.

MazF Is Toxic to Plant Cells

In order to further evidence the toxicity of MazF in plant cells, a double transformation strategy was used. As reported in the literature, the *CaMV* 35S promoter drives expression in almost all plant cell types except in the tapetal and sporogenous tissues of anthers [Plegt and Bino, 1989]. Therefore, to combine a protective expression of *mazE* in all plant tissues except tapetum with a toxic expression of *mazF* in the tapetum, transformation of the *TA29-mazF* construct was performed in a *35S-mazE* background. Five independent homozygous

T2 transgenic lines were obtained (see online suppl. table 1). The expression of *mazE* in 3 of them, designated *35S-mazE*1, 4 and 5, was confirmed by RT-qPCR analysis performed on the aerial part of 1-month-old in vitro grown plants. No amplicon was detected in the WT (fig. 1). No phenotypical differences were observed for the *T0*, *T1* or *T2* *35S-mazE* lines, when compared to the WT. These lines did produce pollen and were fertile similarly to the WT.

The line *35S-mazE*4 was then transformed with the *TA29-mazF* construct. Twenty double-transformed (homozygous for *35S-mazE* construct and hemizygous for *TA29-mazF* construct) transgenic lines were regenerated and transferred in soil. After 3 months of growth, both WT and double-transformed lines had flowers. As shown in figure 2, double-transformed lines were characterized by the absence of pollen after dehiscence of the anthers (fig. 2a) compared to WT (fig. 2b) that produced abundant pollen grains. In total 5 double-transformed lines presented this phenotype, and 3 of them are shown in the online supplementary figure 2. Presence and expression of transgenes was demonstrated by PCR amplification of the *mazE* and *mazF* genes (online suppl. fig. 3) as well as by RT-qPCR analysis performed on leaves of 3-month-old soil-grown plants (fig. 3). An endogenous *TA29* expression was observed at various levels in both WT and transgenic lines. *mazE* was expressed in the 3 analysed *35S-mazE/TA29-mazF* transgenic lines but not in WT plants. In addition, *mazF* was found to be expressed in the 3 analysed double-transformed transgenic lines but not in the *35S-mazE* (fig. 1) or in the WT (fig. 1, 3) lines. In order to verify that the absence of pollen in the double-transformed lines was caused by a defect in the tapetum, cross-sections were made in young developing anther of a *35S-mazE/TA29-mazF* line. As shown in the online supplementary figure 4, the tapetum was necrosed in this transgenic line. Observation of transverse sections of anthers harvested prior to dehiscence and stained with toluidine blue showed that transgenic anthers (fig. 2c) are empty whereas those of the WT are filled with pollen grains (fig. 2d). Scanning electron microscopy examination (fig. 2e–h) confirmed this observation. All flowers from the 5 analysed double-transformed lines were male sterile, and no capsules resulting from autofertilization were formed throughout the plant life cycle (about 10 months) and, therefore, no progeny was produced. Capsules and seeds were however obtained after manual fertilization of double-transformed *35S-mazE/TA29-mazF* plants with WT pollen, showing that these plants were female fertile.

Fig. 2. Anther phenotype of 3-month-old WT and double-transformed *TA29-mazF/35S-mazE* transgenic tobacco plants. **a, c, e, g** Transgenic-line anthers. **b, d, f, h** WT anthers. **a, b** Anthers at dehiscence stage. **c, d** Cross-sections through anther prior to dehiscence stage. **e-h** Scanning electron micrographs of cross-sectioned anthers. c = Connective tissue; ep = epidermis; end = endothecium; p = pollen; s = stomium; vb = vascular bundle. **a-f** Bars = 500 μ m. **g, h** Bars = 100 μ m.

Fig. 3. RT-qPCR expression analysis of *TA29*, *mazE* and *mazF* in leaves of 3-month-old WT and double-transformed *TA29-mazF/35S-mazE* grown in the phytotron. C_t = Threshold cycle; white bars = *TA29*; grey bars = *mazE*; dark grey bars = *mazF*; # = *mazE* not detected; § = *mazF* not detected.

In conclusion, a specific degradation of the tapetum was obtained when *mazF* was expressed under the control of the *TA29* promoter together with *mazE* under the control of the *CaMV 35S* promoter. This strategy allowed circumventing the *TA29* promoter leaky activity in other cell types than tapetum. A similar double transformation strategy using two different promoters with distinct but overlapping specificities driving the expression of either barnase or barstar, respectively, has been previously reported to ablate specific anther cell types and to overcome regeneration problems [Beals and Goldberg, 1997; Goldberg et al., 1995]. In this study, the *TA56* promoter, driving expression successively in the circular-cell cluster, in the stomium and in the connective tissue of anthers but also within regenerating callus, was fused to barnase. The soybean lectin promoter, driving expression in regenerating callus, in vegetative and floral organs and at low level in anthers of transgenic tobacco as well as in most anther cell types at different stages of development, was fused to barstar. When introduced together into transgenic tobacco, a selective ablation of the stomium and circular-cell cluster region resulted in anthers that failed to dehisce.

Nowadays, there is an increasing interest in the development of biotechnological tools able to induce cell death for counterselection of genetic modifications, phytopa-

thology or gene containment purposes. In this context, prokaryote TA systems provide promising toxin candidates as demonstrated for *mazEF* (this study) and also as recently evidenced by the use of the inducible expression of the YoeB toxin [Bakar et al., 2015], from the *yefM-yoeB* type II TA system from *Streptococcus pneumoniae*, that was found to be lethal for *Arabidopsis thaliana*.

Experimental Procedures

Plant Material and Growth Conditions

Non-transgenic and transgenic tobacco plants (*N. tabacum* cv. Havana) were grown aseptically, and sown seeds or acclimatized plants were cultivated as described in Baucher et al. [2011].

Constructing the Yeast-Compatible pMDC162y Vector

The approach used to clone the toxin-encoding gene under the tapetum-specific *TA29* promoter into the Gateway-compatible T-DNA destination vector pMDC162 containing *gusA* [Curtis and Grossniklaus, 2003] is the cloning through in vivo recombination in yeast. Firstly, the yeast 2- μ m replication origin as well as the *URA3* gene (to complement the *ura3* mutation in yeast) were introduced into the pMDC162 plasmid. These parts were amplified by PCR using the primer pair pMDCsacII (see online suppl. table 2) using the *Saccharomyces/E. coli* cloning vector pFL44S as template [Bonneaud et al., 1991]. The PCR product, bordered by 40 bp homologous to 40 bp on the pMDC162 vector (close to the *SacII* restriction site), was purified using the Genelute gel extraction (Sigma-Aldrich; www.sigmaaldrich.com). The pMDC162 vector was restricted by *SacII*, and both the linearized vector and the insert were used for in vivo recombination by cotransformation of yeast according to Schiestl and Gietz [1989]. DNA was extracted from colonies growing on selective medium, and a PCR with the pMDC162-specific primers pMDChyg forward and reverse (online suppl. table 2) was performed to verify the presence of the plasmid in the yeast transformants. After dialysis against water on mixed cellulose ester membrane (pore size 0.025 μ m, MF-Millipore), DNA was electroporated into One Shot[®] ccdB Survival[™] 2 T1R Competent Cells (Invitrogen; <http://www.lifetechnologies.com>), and the presence of the pMDC162y plasmid in *E. coli* transformants was verified by PCR.

Cloning of *TA29* Promoter in pMDC162y

Genomic DNA was extracted from young leaves of 3-month-old tobacco plants using the Genelute plant genomic DNA miniprep kit (Sigma-Aldrich). The *TA29* promoter (GenBank: X52283) was amplified by PCR using the pTA29 primer pair (online suppl. table 2). This fragment was flanked by *attB1* and *attB2* recombination sites by 2 successive PCRs, the first using the *AttBpTA29* primer pair (online suppl. table 2) and the second with the primers amplifying the entire *attB1* and *attB2* sequences according to the supplier's instructions (Invitrogen). Gateway cloning by recombination into the vector pDONR221 (Invitrogen) followed by a second recombination with pMDC162y was made in reactions mediated by the Gateway[™] BP and LR Clonase[™] Enzyme Mix, respectively (Invitrogen). All constructs were verified with sequencing.

Cloning *mazF* in pMDC162y

mazF (EcoGene: EG11249) was amplified by PCR from the K-12 *E. coli* strain MC1061 [Casadaban and Cohen, 1980] using the MazF primer pair allowing to flank the PCR product by 40 nucleotides homologous to the 40 bp bordering the *gusA* gene on pMDC162yTA29. The plasmid pMDC162yTA29 was restricted by *Mlu*I, cutting 3 times in the *gusA* gene, removing a fragment of 18 and 698 bp, thus linearizing the vectors. The PCR product of *mazF* and the restricted vector were consecutively transformed in *Saccharomyces cerevisiae*. DNA extracted from yeast transformants was electroporated into *E. coli* strain DH5α (Invitrogen).

Cloning *mazE* in pK7WG2

mazE (EcoGene: EG10571) was amplified by PCR from the K-12 *E. coli* strain MC1061 and flanked with the *attB* sequences necessary for the Gateway™ BP recombination reaction in two successive PCR reactions, the first using the AttBMazE primer pair (online suppl. table 2) and the second as described above. The PCR fragment was cloned into the vector pDONR221 which was recombined with the destination vector pK7WG2 [Karimi et al., 2002] and sequenced.

Transformation of *N. tabacum*

After mobilization of the plasmid by the *Agrobacterium* strain C58C1^{Rif}, containing the helper plasmid pMP90, *N. tabacum* was transformed according to Mukoko Bopopi et al. [2010]. In a first transformation step, a minimum of 20 transgenic tobacco lines harbouring 35S-*mazE* were generated and selected based on kanamycin (200 mg/l) resistance conferred by the *nptII* gene in pK7WG2. Transgenic 35S-*mazE* lines whose T1 progeny phenotypes displayed likely single-copy T-DNA inserts, based on 3:1 segregation on selective medium (MS supplemented with 200 mg/l kanamycin), were selected. The progeny analysis is shown in the online supplementary table 1. Eight T1 seedlings of each 1-insert line were grown in soil, and their seeds were sown on selective medium to identify homozygous lines (T2). One of these lines, 35S-*mazE*4, was used for a second transformation with the TA29-*mazF* construct using hygromycin (50 mg/l) as selectable marker. T2 transgenic TA29-*GUS* lines with likely 1 T-DNA insert were obtained after segregation analysis of T1 transgenic lines on hygromycin (50 mg/l) (online suppl. table 1).

Histochemical GUS Staining

Histochemical GUS staining was performed as described by Hemerly et al. [1993] on anthers from soil-grown plants sectioned in 100- to 120-μm slices using an HM 650V vibratome. Samples were observed under an SZW-ILLK200 light binocular (Olympus;

<http://www.olympus.be>), and images were acquired with a Color-view II camera and CELLSSENS imaging software (Olympus).

Morphological Analysis with Light and Scanning Electron Microscopy

Preparation of samples (fixing, dehydrating, embedding, sectioning) for light microscopy and staining with toluidine blue was made as described by Baldacci-Cresp et al. [2012] with the use of an RM2155 rotary microtome (Leica Microsystems; <http://www.leica-microsystems.com>). Scanning electron microscopy of cross-sectioned anthers was made as described in Baldacci-Cresp et al. [2015] with the same equipment.

RT-qPCR Analysis

Total RNA from either 1-month-old aerial parts of 35S-*mazE* lines cultivated in vitro or young leaves of 3-month-old 35S-*mazE*/TA29-*mazF* double-transformed transgenic plants grown in soil and respective WT was extracted using the Plant RNA Isolation minikit (Agilent; <http://www.agilent.com>). One microgram of total RNA was reverse transcribed using the Reverse Transcription System (Promega; www.promega.com). RT-qPCR reactions were performed using the Ligh Cycler 480 Master Mix SYBER green (Roche; <https://lifescience.roche.com>) on a Ligh Cycler 480 thermocycler with specific primer couple *mazE*-qPCR, *mazF*-qPCR, TA29-qPCR and NtEF1α-qPCR to amplify *mazE*, *mazF*, TA29 and *elongation factor-1α* (*ef1α*, GenBank: D63396), respectively (online suppl. table 2).

Acknowledgements

The authors thank Safya Balil for anther sectioning. F.B.-C. is a Postdoctoral Researcher of the Fonds de la Recherche Scientifique (FNRS-FRS) in the frame of the research project T.0004.14. M.B. is Senior Research Associate of the FRS-FNRS. M.B. and F.B.-C. were supported by the David and Alice Van Buuren Foundation. Research in the LVM laboratory is funded by the FRS-FNRS FRSM 3.4621.1, the Interuniversity Attraction Poles Programme initiated by the Belgian Science Policy Office (MICRODEV), the Jean Brachet Fund and the David and Alice Van Buuren Foundation.

Disclosure Statement

All authors declare no conflict of interest.

References

- Aizenman E, Engelberg-Kulka H, Glaser G: An *Escherichia coli* chromosomal 'addiction module' regulated by 3',5'-bispyrophosphate: a model for programmed bacterial cell death. *Proc Natl Acad Sci USA* 1996;93:6059–6063.
- Bakar FA, Yeo CC, Harikrishna JA: Expression of the *Streptococcus pneumoniae yoeB* chromosomal toxin gene causes cell death in the model plant *Arabidopsis thaliana*. *BMC Biotechnol* 2015;15:26.
- Baldacci-Cresp F, Chang C, Maucourt M, Deborde C, Hopkins J, Lecomte P, Bernillon S, Brouquisse R, Moing A, Abad P, Hérouart D, Puppo A, Favory B, Frendo P: (Homo)glutathione deficiency impairs root-knot nematode development in *Medicago truncatula*. *PLoS Pathog* 2012;8:e1002471.
- Baldacci-Cresp F, Moussawi J, Leplé J-C, Van Acker R, Kohler A, Candiracci J, Twyffels L, Spokevicius AV, Bossinger G, Laurans F, Brunel N, Vermeersch M, Boerjan W, El Jaziri M, Baucher M: PtaRHE1, a *Populus tremula* × *Populus alba* RING-H2 protein of the ATL family, has a regulatory role in secondary phloem fibre development. *Plant J* 2015;82: 978–990.

- Baucher M, Oukouomi Lowe Y, Vandeputte OM, Mukoko Bopopi J, Moussawi J, Vermeersch M, Mol A, El Jaziri M, Homblé F, Pérez-Morga D: Ntann12 annexin expression is induced by auxin in tobacco roots. *J Exp Bot* 2011;62:4055–4065.
- Beals TP, Goldberg RB: A novel cell ablation strategy blocks tobacco anther dehiscence. *Plant Cell* 1997;9:1527–1545.
- Bonneaud N, Ozier-Kalogeropoulos O, Li G, Labouesse M, Minvielle-Sebastia L, Lacroute F: A family of low and high copy replicative, integrative and single-stranded *S. cerevisiae*/ *E. coli* shuttle vectors. *Yeast* 1991;7:609–615.
- Casadaban MJ, Cohen NC: Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. *J Mol Biol* 1980;138:179–207.
- Chono H, Matsumoto K, Tsuda H, Saito N, Lee K, Kim S, Shibata H, Ageyama N, Terao K, Yasutomi Y, Mineno J, Kim S, Inouye M, Kato I: Acquisition of HIV-1 resistance in T lymphocytes using an ACA-specific *E. coli* mRNA interferase. *Hum Gene Ther* 2011;22:35–43.
- Christensen SK, Pedersen K, Hansen FG, Gerdes K: Toxin-antitoxin loci as stress-response elements: ChpAK/MazF and ChpBK cleave translated RNAs and are counteracted by tmRNA. *J Mol Biol* 2003;332:809–819.
- Curtis MD, Grossniklaus U: A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant Physiol* 2003;133:462–469.
- Goeders N, Van Melderden L: Toxin-antitoxin systems as multilevel interaction systems. *Toxins* 2014;6:304–324.
- Goldberg RB, Sanders PM, Beals TP: A novel cell-ablation strategy for studying plant development. *Philos Trans R Soc Lond B Biol Sci* 1995;350:5–17.
- Hayes F, Van Melderden L: Toxins-antitoxins: diversity, evolution and function. *Crit Rev Biochem Mol Biol* 2011;46:386–408.
- Hemerly AS, Ferreira P, de Almeida Engler J, Van Montagu M, Engler G, Inzé D: *cdc2a* expression in Arabidopsis is linked with competence for cell division. *Plant Cell* 1993;5:1711–1723.
- Kamada K, Hanaoka F, Burley SK: Crystal structure of the MazE/MazF complex: molecular bases of antidote-toxin recognition. *Mol Cell* 2003;11:875–884.
- Karimi M, Inzé D, Depicker A: GATEWAY™ vectors for *Agrobacterium*-mediated plant transformation. *Trends Plant Sci* 2002;7:193–195.
- Koltunow AM, Truettner J, Cox KH, Wallroth M, Goldberg R: Different temporal and spatial gene expression patterns occur during anther development. *Plant Cell* 1990;2:1201–1224.
- Kriete G, Niehaus K, Perlick AM, Pühler A, Broer I: Male sterility in transgenic tobacco plants induced by tapetum-specific deacetylation of the externally applied non-toxic compound N-acetyl-L-phosphinothricin. *Plant J* 1996;9:809–818.
- Mariani C, De Beuckeleer M, Truettner J, Lee-mans J, Goldberg RB: Induction of male sterility in plants by a chimeric ribonuclease gene. *Nature* 1990;347:737–741.
- Mukoko Bopopi J, Vandeputte OM, Himanen K, Mol A, Vaessen Q, El Jaziri M, Baucher M: Ectopic expression of *PtaRHE1*, encoding a poplar RING-H2 protein with E3 ligase activity, alters plant development and induces defence-related responses. *J Exp Bot* 2010;61:297–310.
- Plegt L, Bino RJ: β -Glucuronidase activity during development of the male gametophyte from transgenic and non-transgenic plants. *Mol Gen Genet* 1989;216:321–327.
- Schiestl RH, Gietz RD: High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. *Curr Genet* 1989;16:339–346.
- Shapira A, Shapira S, Gal-Tanamy M, Zemel R, Tur-Kaspa R, Benhar I: Removal of hepatitis C virus-infected cells by a zymogenized bacterial toxin. *PLoS One* 2012;7:e32320.
- Shimazu T, Degenhardt K, Nur-E-Kamal A, Zhang J, Yoshida T, Zhang Y, Mathew R, White E, Inouye M: NBK/BIK antagonizes MCL-1 and BCL-X_L and activates BAK-mediated apoptosis in response to protein synthesis inhibition. *Genes Dev* 2007;21:929–941.
- Shimazu T, Mirochnitchenko O, Phadtare S, Inouye M: Regression of solid tumors by induction of MazF, a bacterial mRNA endoribonuclease. *J Mol Microbiol Biotechnol* 2014;24:228–233.
- Suzuki M, Zhang J, Liu M, Woychik NA, Inouye M: Single protein production in living cells facilitated by an mRNA Interferase. *Mol Cell* 2005;18:253–261.
- Van Melderden L: Toxin-antitoxin systems: why so many, what for? *Curr Opin Microbiol* 2010;13:781–785.
- Wang X, Wood TK: Toxin-antitoxin systems influence biofilm and persister cell formation and the general stress response. *Appl Environ Microbiol* 2011;77:5577–5583.
- Yang H, Wei Z, Xu Z: Effects of specific expression of *iaaL* gene in tobacco tapetum on pollen embryogenesis. *Sci China Life Sci* 1997;40:384–391.
- Yang J, Jiang W, Yang S: mazF as a counter-selectable marker for unmarked genetic modification of *Pichia pastoris*. *FEMS Yeast Res* 2009;9:600–609.
- Zhang Y, Zhang J, Hara H, Kato I, Inouye M: Insights into the mRNA cleavage mechanism by MazF, an mRNA interferase. *J Biol Chem* 2005;280:3143–3150.