The methods of miR169 regulates its target gene *NFYA5* in *Arabidopsis*

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Why we are interested in miRNA

成熟的microRNA（miRNA）是一种大小约21—23个碱基的单链小分子RNA，广泛存在于动物、植物和人类等真核生物细胞中。miRNA由RNA聚合酶Ⅱ转录生成（Lee et al.,2004）。许多植物miRNA具有高度的保守性、时序性、组织特异性及逆境特异性。

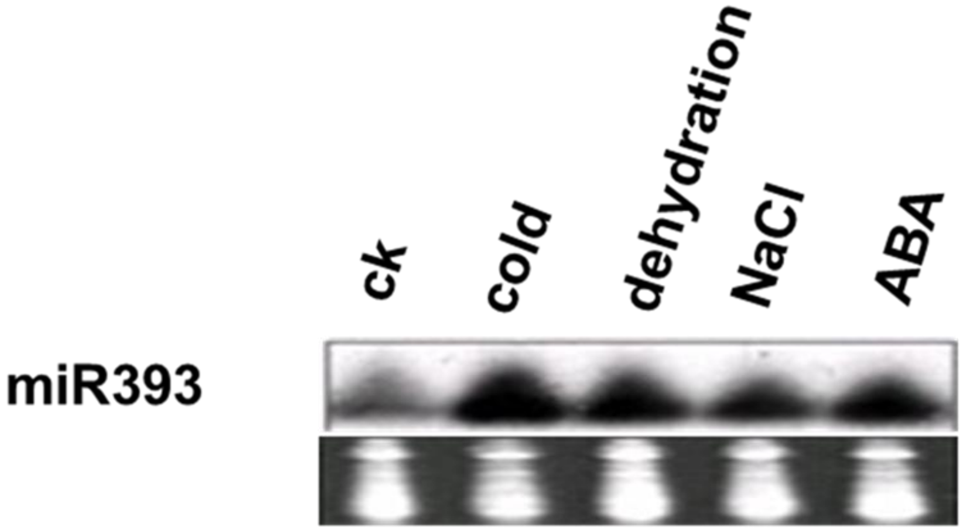
The functions of miRNA

近年来，越来越多的研究结果表明miRNA也参与植物感受环境胁迫的信号转导过程。miRNA不仅参与植物感应冷、盐、快速失水等非生物胁迫（Sunkar et al.,2004），还参与感应真菌等生物胁迫（Navarro et al.,2006），近年来更多研究的注意力多集中在植物miRNA与营养胁迫上（Chiou et al.,2006；Lin et al., 2008；Li et al., 2008）。植物在生长过程中，难免会遭受到各种各样的胁迫。在面对胁迫时，植物细胞或者恢复细胞变化前的细胞内平衡，或者适应已经发生改变的新环境，miRNA在这些过程中起着重要作用（Anthony et al.,2010）。

miR166

Juarez et al., 2004

miRNA一个显著特征是参与植物整个生长发育进程（Chen,2005b）。在植物中主要调控花器官的建成、胚的发育、植物由营养生长向生殖生长的转化、根瘤的形成等过程（Carrington et al.,2003;Jones-Rhoades et al.,2006）。

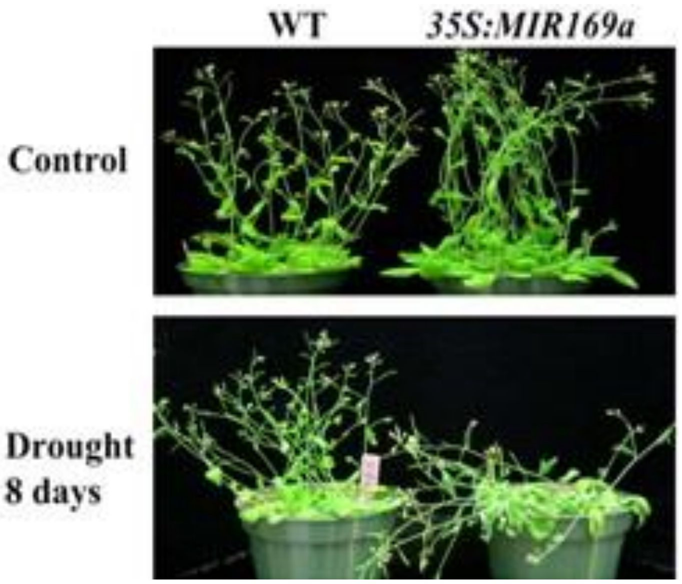


Sunkar R et al., 2004

大量研究表明，植物中不同的miRNA对多种逆境胁迫发生响应，并证实某些miRNA在植物感受逆境胁迫并产生适应性的过程中发挥重要作用（Jian et al.,2010;Liu et al.,2008a）。

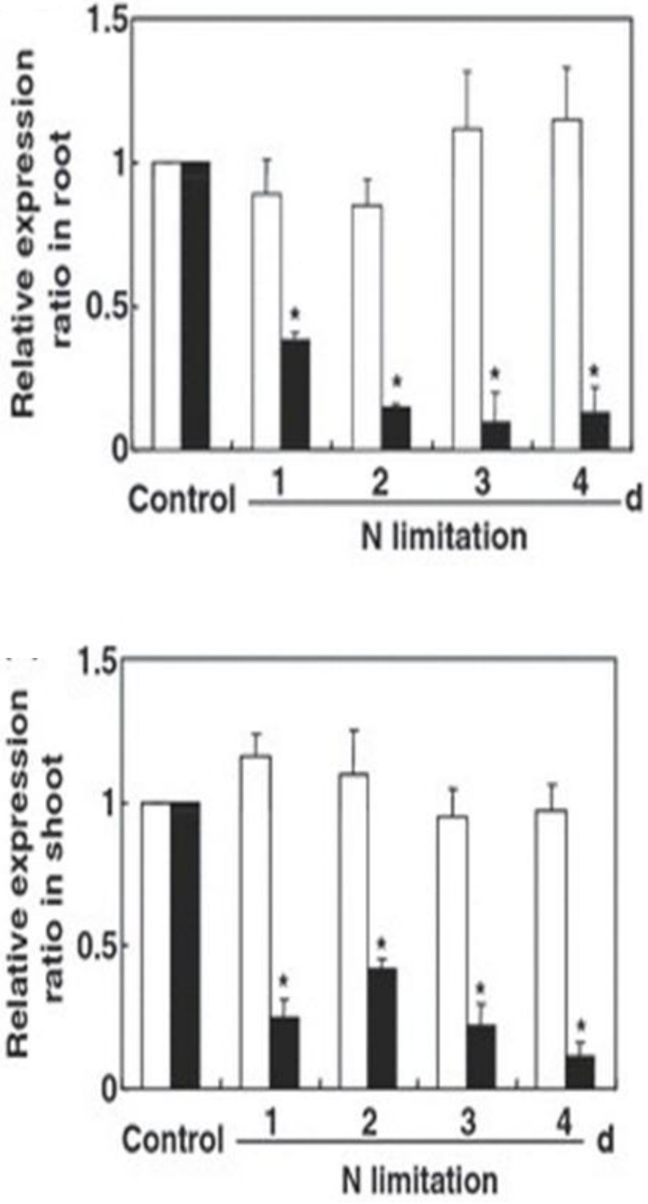
miR169 fundamental information

miR169是在植物中首批发现的小RNA，在进化上具有高度的保守性，是拟南芥中最大的miRNA家族，由14个成员组成。miR169的靶基因为核因子YA（nuclear factor YA）的家族成员。核因子Y存在于所有真核生物，进化上非常保守，为异源三聚体转录因子，由NF-YA（CBF-B/HAP2）、NF-YB（CBF-A/HAP3）和 NF-YC（CBF-C/HAP5）三个亚基组成（Romier *et al.*,2003）。



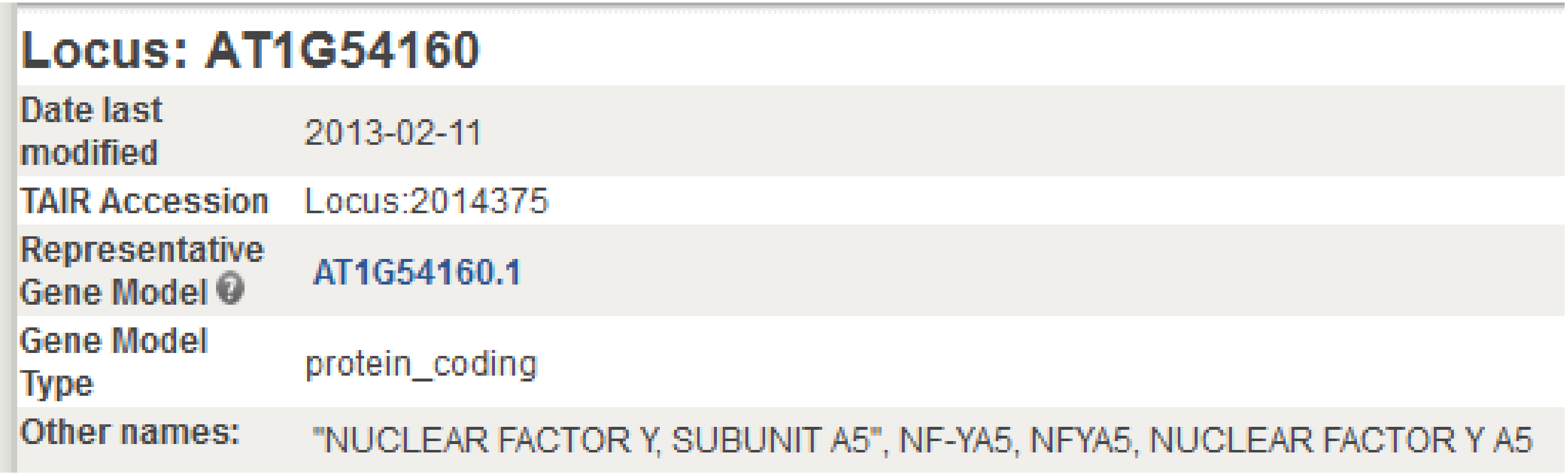
Li et al., 2008

Wen-Xue Li et al.(2008)报道：*AtNFYA5*在拟南芥抗水分胁迫过程中起到重要作用，其表达受到转录和转录后水平的双重调控，转录后水平的调控主要是通过*miR169*来实现，其中以*miR169*a起主要调控作用。



Zhao et al., 2011

NFYA5 fundamental information

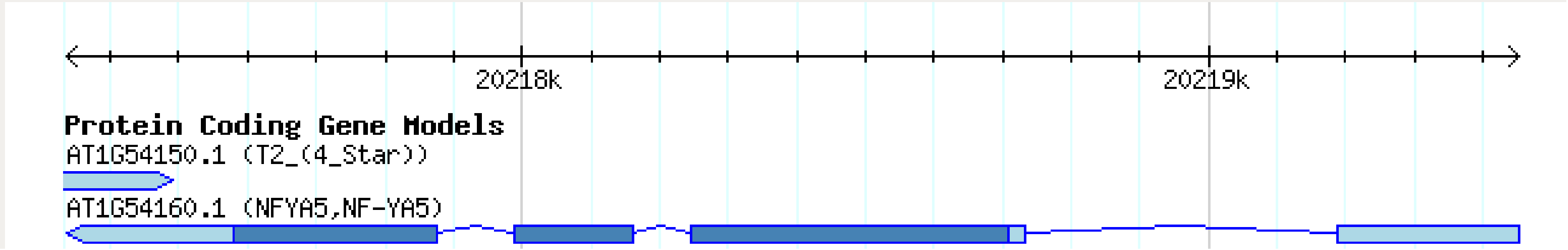


Locus: AT1G54160

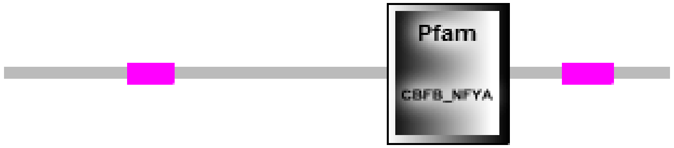
Date last modified	2013-02-11
TAIR Accession	Locus:2014375
Representative Gene Model	AT1G54160.1
Gene Model Type	protein_coding
Other names:	"NUCLEAR FACTOR Y, SUBUNIT A5", NF-YA5, NFYA5, NUCLEAR FACTOR Y A5

Function: Encodes a member of the CCAAT-binding transcription factor (CBF-B/NF-YA) family. Expression is upregulated in response to ABA and drought. This regulation appears to be mediated by MIR169A which is downregulated in response to drought. NFYA5 is a target of MIR169A. Loss of function mutations are hypersensitive to drought.

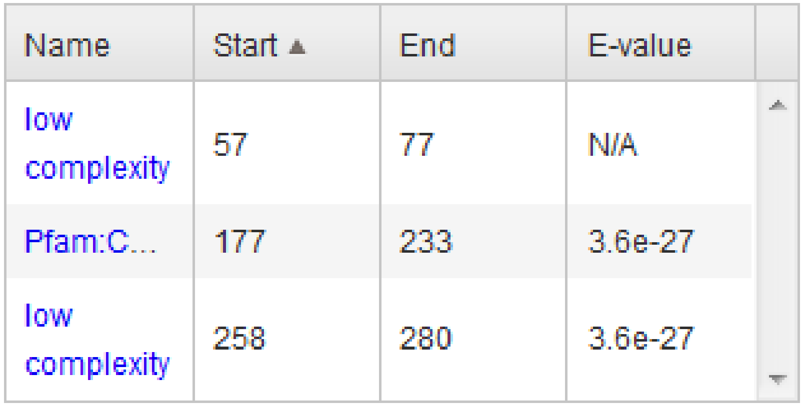
NFYA5 gene structure



Domation prediction of NFYA5 protein

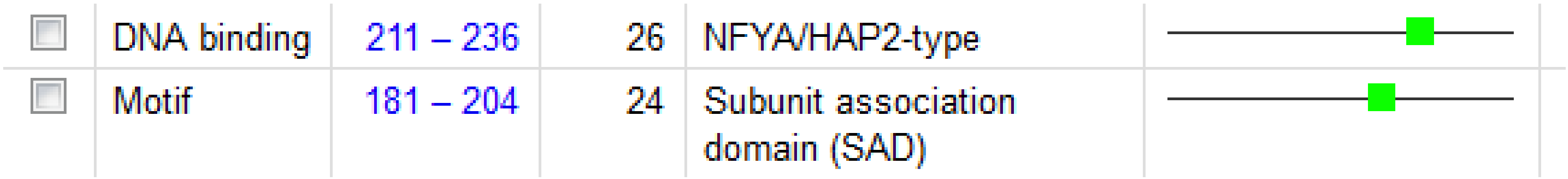


Confidently predicted domains, repeats, motifs and features:



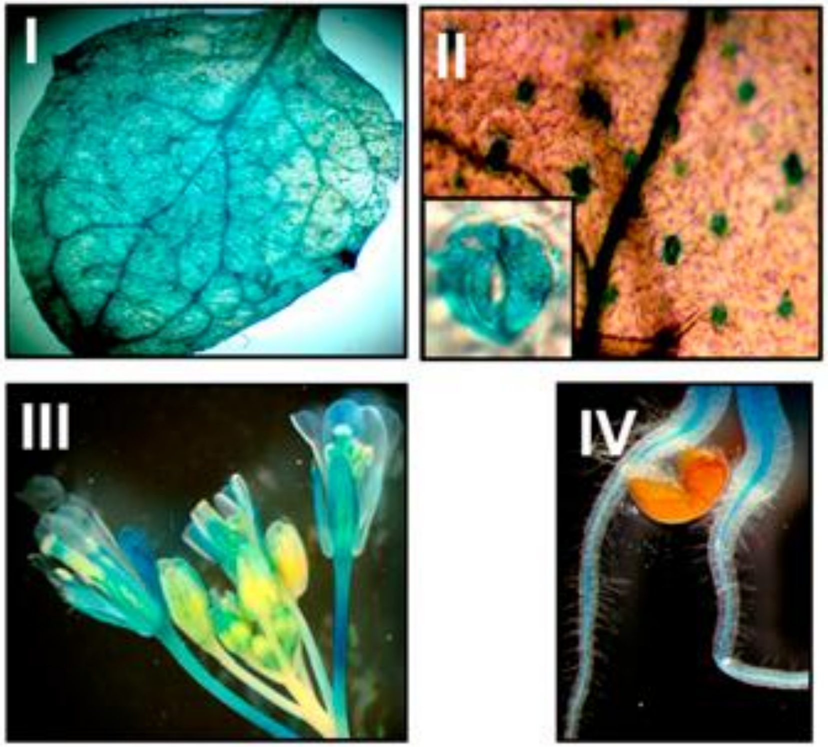
Name	Start ▲	End	E-value
low complexity	57	77	N/A
Pfam:C...	177	233	3.6e-27
low complexity	258	280	3.6e-27

Regions



☐	DNA binding	211 – 236	26	NFYA/HAP2-type
☐	Motif	181 – 204	24	Subunit association domain (SAD)

Tissue expression pattern of NFYA5

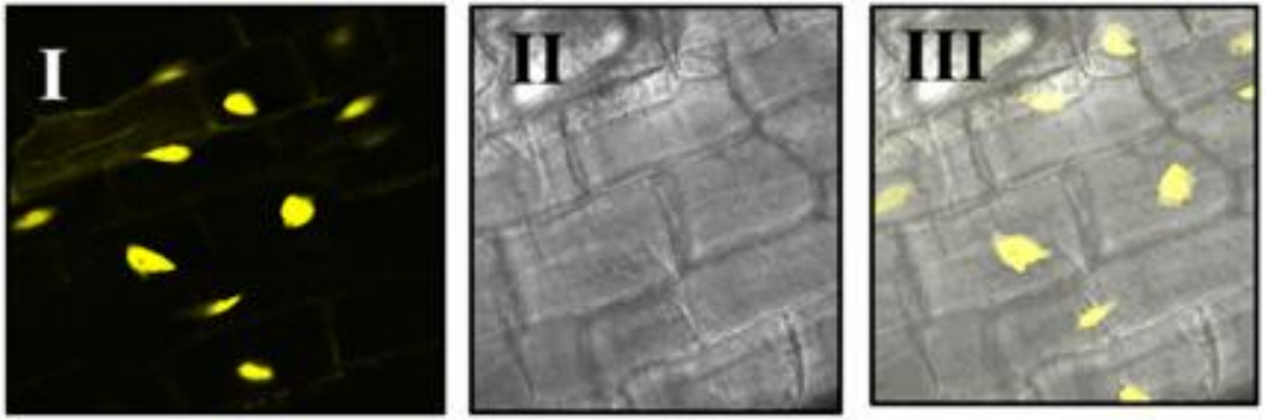


NFYA5p:GUS expression pattern in various tissues.

The staining was prominent in the vascular tissues (I) and guard cells (II) of leaves. The staining was also visible in floral tissues of the inflorescence (III) and root vascular system (IV).

(Li et al.2008)

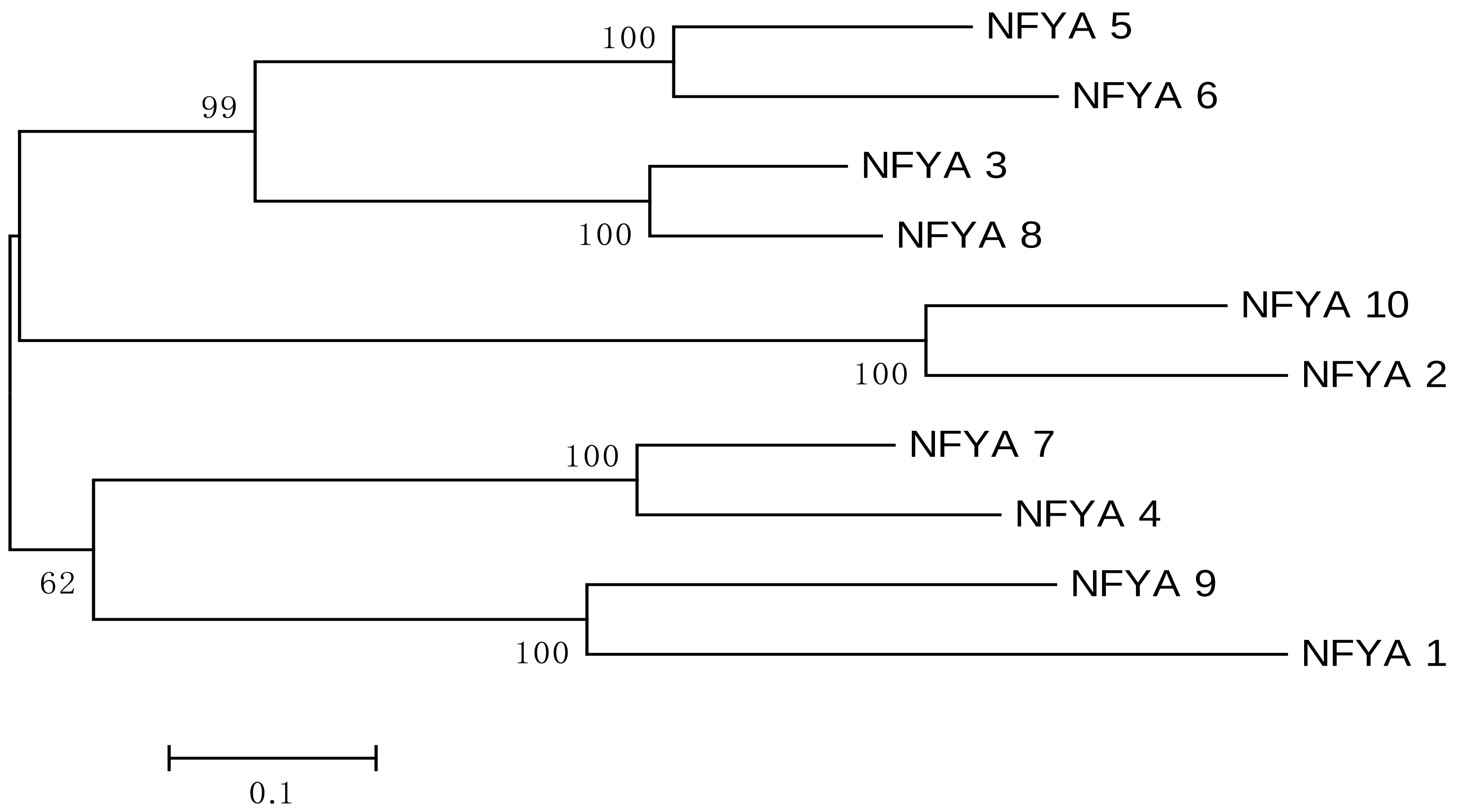
Subcellular localization of *NFYA5*



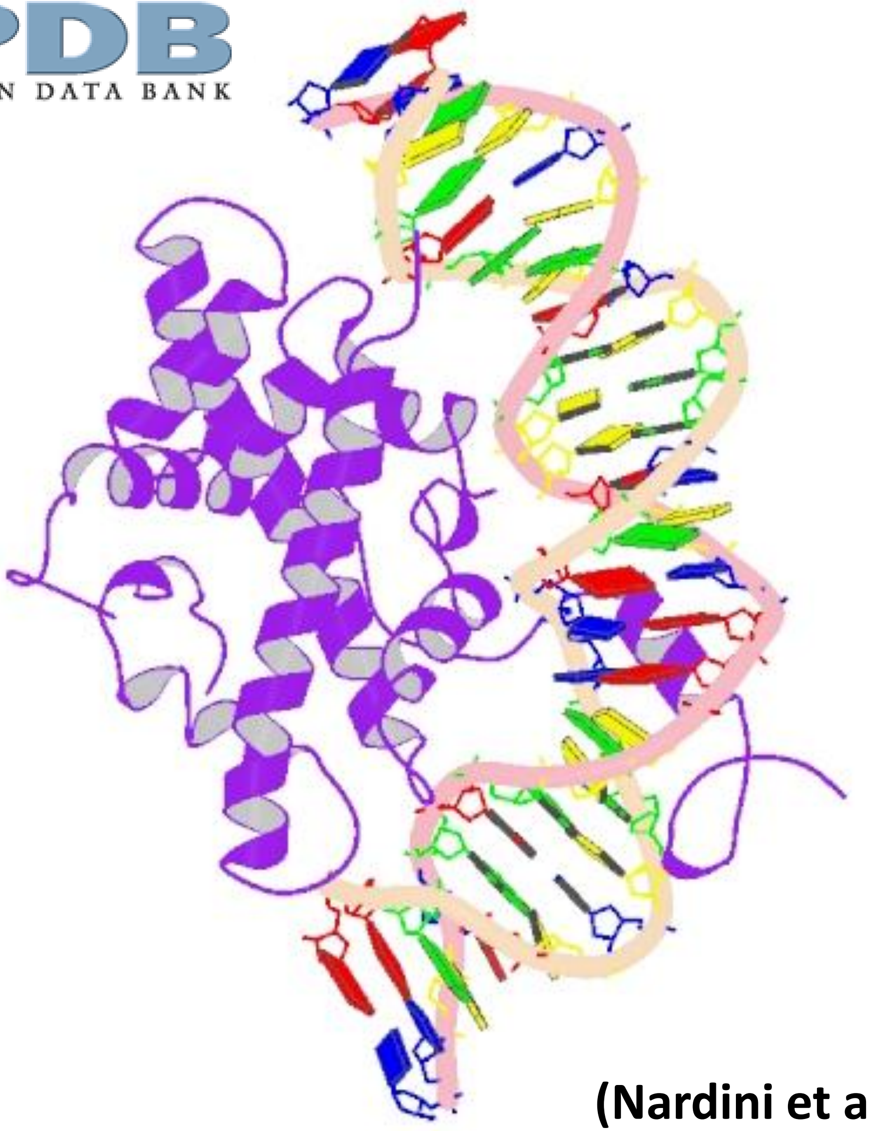
(Li et al.2008)

The *NFYA5-YFP* fusion construct was expressed in transgenic *Arabidopsis* under the control of the cauliflower mosaic virus 35S promoter, and the plant roots were observed under a confocal microscope. The photographs were taken in the dark field for yellow fluorescence (I), in the bright field for the morphology of the cells (II), and in combination (III).

Phyloanalysis of NFYA family members in Arabidopsis thaliana



Interaction between NFY and DNA



The sequence-specific transcription factor NF-Y binds the CCAAT box, one of the sequence elements most frequently found in eukaryotic promoters. NF-Y is composed of the NF-YA and NF-YB/NF-YC subunits.NF-YA both binds to NF-YB/NF-YC and inserts an α helix deeply into the DNA minor groove, providing sequence-specific contacts to the CCAAT box.

(Nardini et al.2013)

Plant miRNA control methods

植物miRNA在多种胁迫中是通过翻译抑制、降解靶基因mRNA这两种调控方式参与植物抵抗逆境胁迫的过程。因此，通过不同的逆境或者相同逆境不同胁迫程度来处理植物，通过构建各种与miRNA相关的超表达突变株，并进行一系列指标的检测、表型鉴定等，将会使miRNA的作用机制、植物对环境胁迫的适应性机制更加清楚。

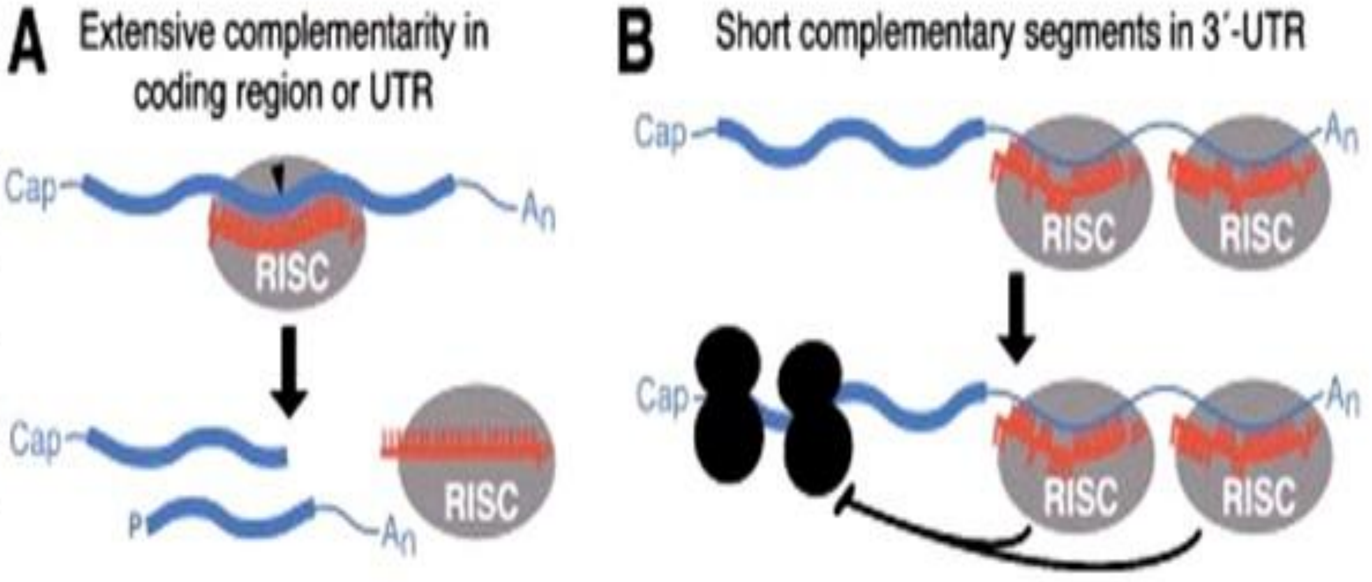


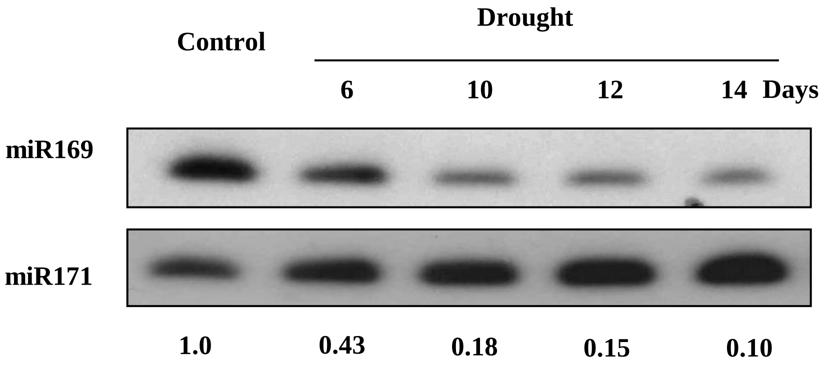
图1.2　microRNA的作用方式　（Bartel, 2004）

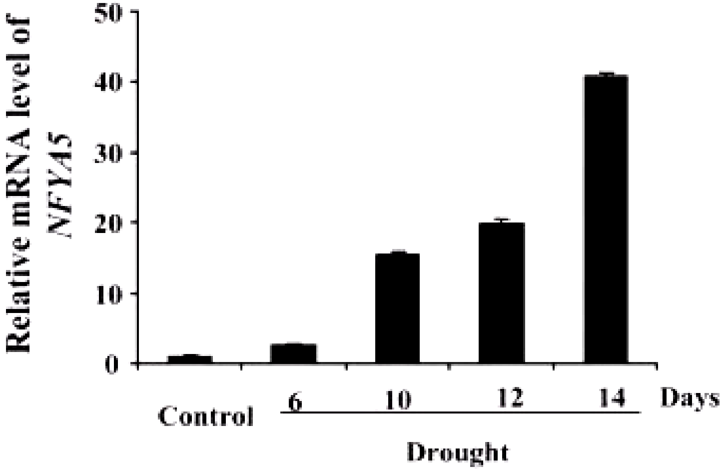
Figure1.2 The actions of microRNA.（Bartel, 2004）

注：（A）mRNA被miRNA特异性切割过程，黑色箭头代表切割位点。（B）miRNA引导的翻译抑制过程.

Note:（A）The process of messenger RNA cleavage specified by a miRNA. Black arrowhead indicates site of cleavage.（B）The process of translational repression specified by miRNAs..

Regulation pattern between miR169 and its target gene





Li et al., 2008

Future work

- 对拟南芥进行不同程度的快速失水处理，检测miR169及其靶基因的而变化，确定miR169在快速失水条件下对靶基因*NFYA5*的调控方式。
- 构建各种超表达突变株进行各项指标的检测以及表型鉴定，进一步证实在快速失水条件下，miR169对靶基因*NFYA5*的调控方式。

THANK YOU !