YTH结构域蛋白ECT2-3-4通过招募polyA结合蛋白来稳定包含m6A的RNA

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肖超玲
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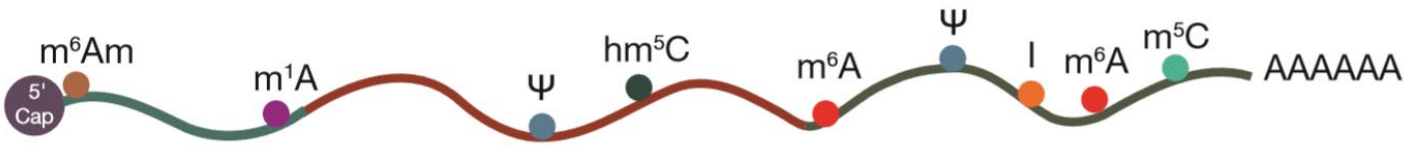
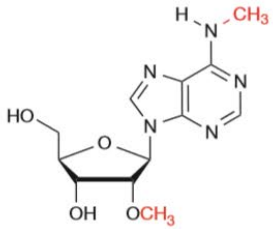
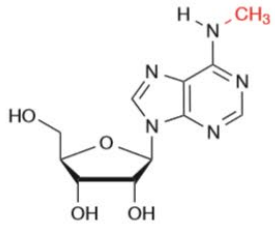
背景：mRNA的化学修饰



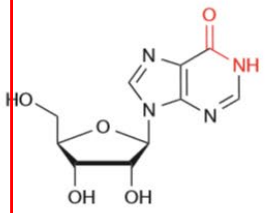
the most abundant mRNA modification



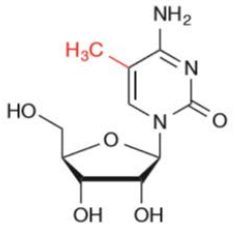
N^6,2'-O-dimethyladenosine (m^6Am)



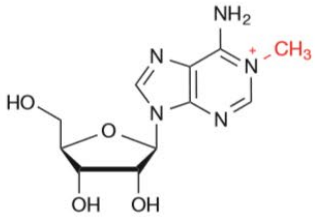
N^6-methyladenosine (m^6A)



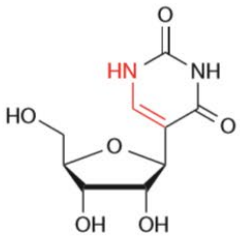
Inosine (I)



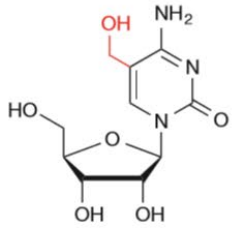
5-methylcytidine (m^5C)



N^1-methyladenosine (m^1A)



Pseudouridine (Ψ)



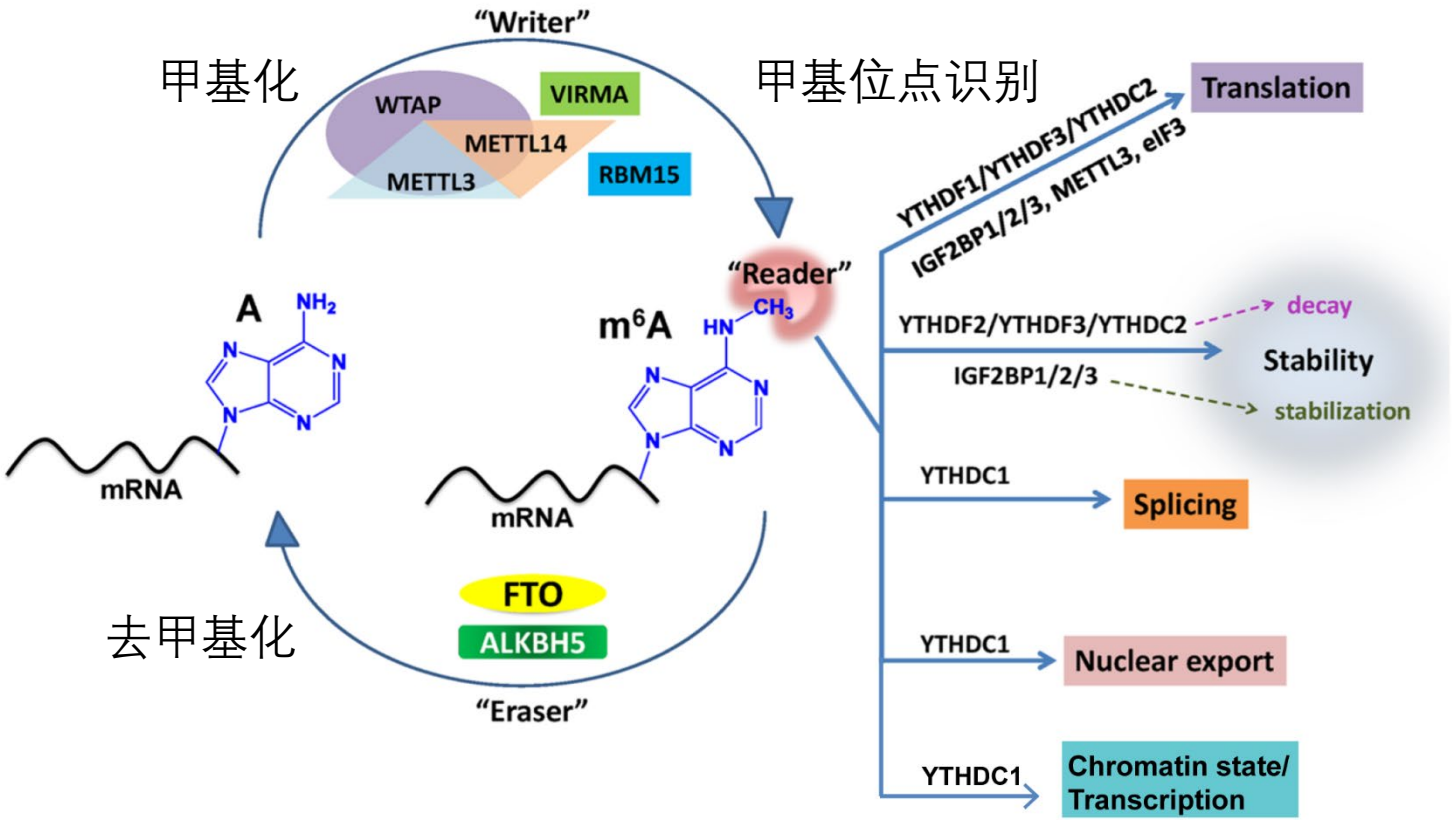
5-hydroxylmethylcytidine (hm^5C)

迄今为止，已经有100多种RNA修饰被鉴定出来，在RNA的代谢中发挥了重要的作用，其中6-甲基腺嘌呤的修饰最为普遍。

LI X, *et al.* Nat. Methods 2014

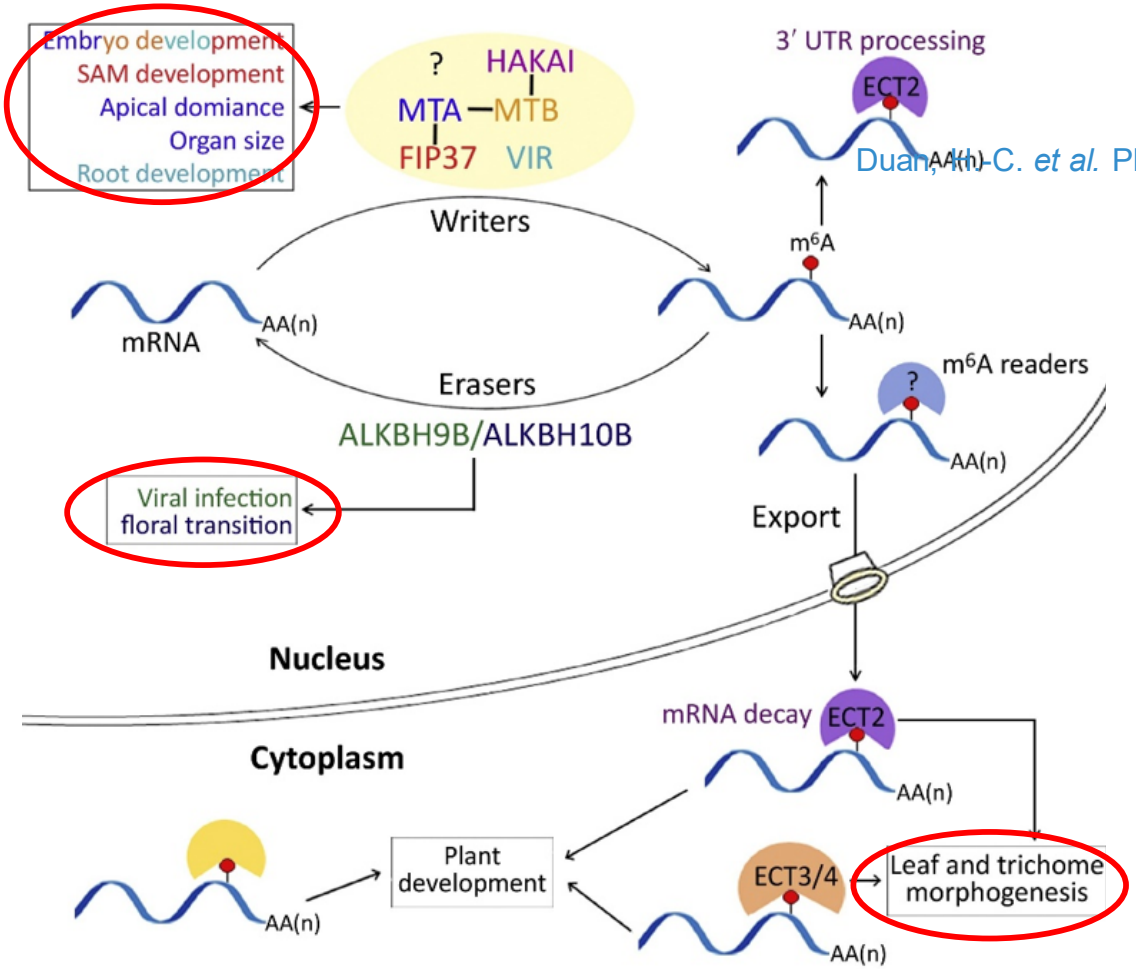
哺乳动物细胞中m6A的功能

N^6-methyladenosine (m^6A)



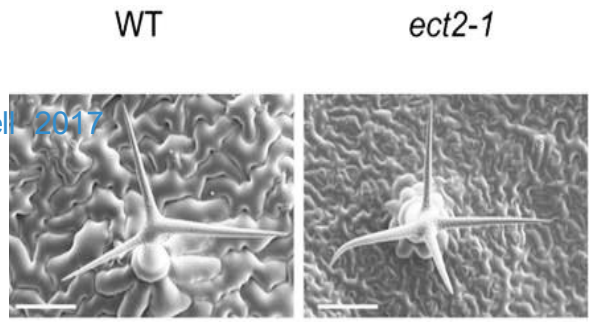
Wang, X. *et al.* Cell 2015
Xiao, W. *et al.* Mol. Cell 2016
Liu, J.*et al.* Science. 2020

拟南芥中m6A的功能

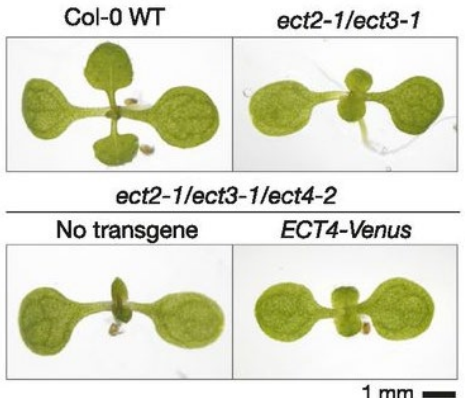


Shen, L.*et al.* Trends Plant. Sci 2019

Duan, H.-C. *et al.* Plant Cell 2017



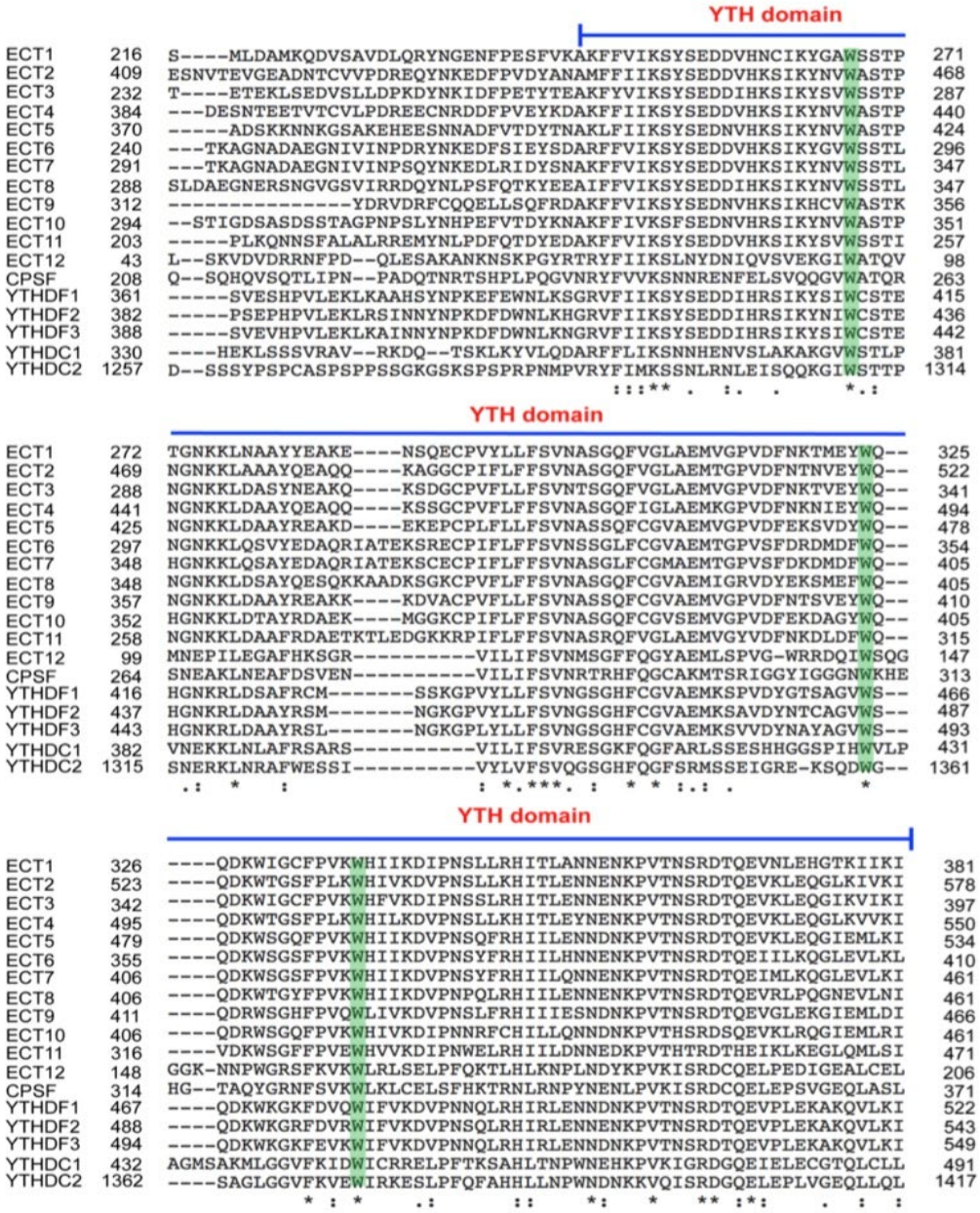
Wei, L.-H. *et al.* Plant Cell 2018



Arribas-Hernández, L. *et al.* Plant Cell 2018

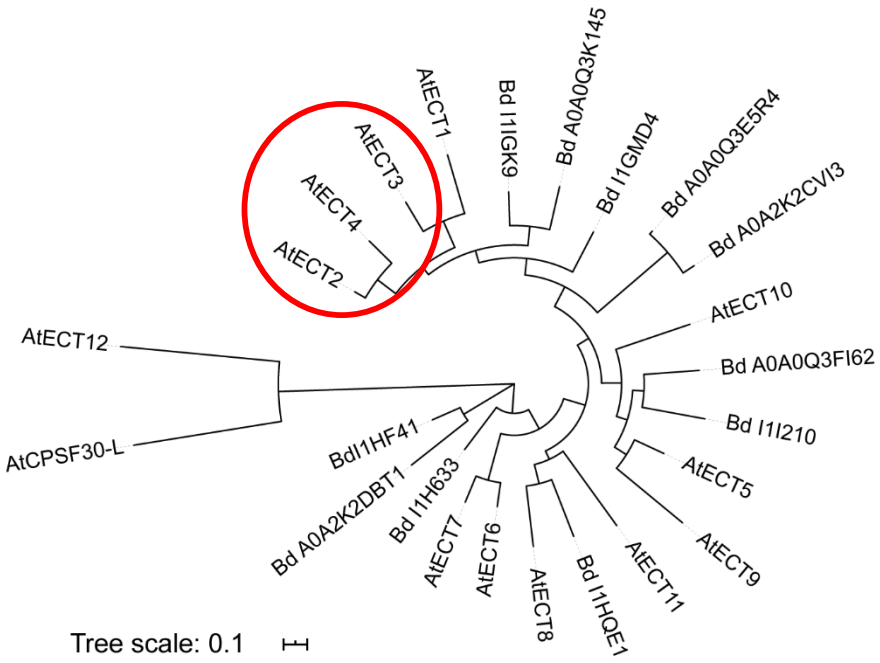
Sequence alignment of YTH domain family proteins in Arabidopsis and human.

三个保守的色氨酸位点与蛋白功能紧密相关。突变会导致蛋白功能的丧失



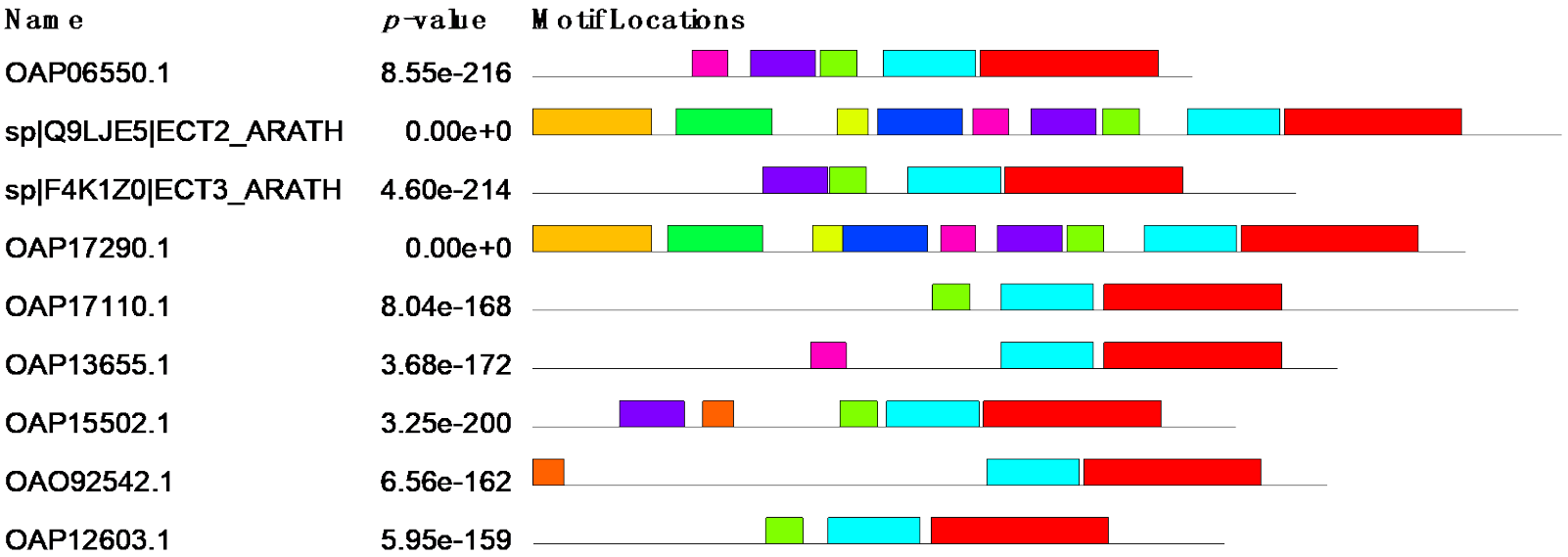
Wei LH, et al.. *Plant Cell*. 2018;30(5):968-985.

Phylogenetic tree of ECT2 between *Arabidopsis* and *Brachypodium distachyon*



PNT71737	BRADI_2g34785v3	A0A2K2DBT1_BRADI
PNT66034	BRADI_3g06027v3	A0A2K2CVI3_BRADI
KQJ81540	BRADI_5g01317v3	A0A0Q3E5R4_BRADI
KQK09193	BRADI_2g46590v3	I1HQE1
KQK23555	BRADI_1g74560v3	A0A0Q3K145_BRADI
KQJ85870	BRADI_4g02150v3	I1IGK9_BRADI
KQK12786	BRADI_1g05970v3	I1GMD4_BRADI
KQJ99327	BRADI_3g42640v3	A0A0Q3FI62_BRADI
KQJ95618	BRADI_3g18190v3	I1I210_BRADI
KQK21941	BRADI_1g64100v3	I1H633_BRADI
KQK04213	BRADI_2g12380v3	I1HF41_BRADI

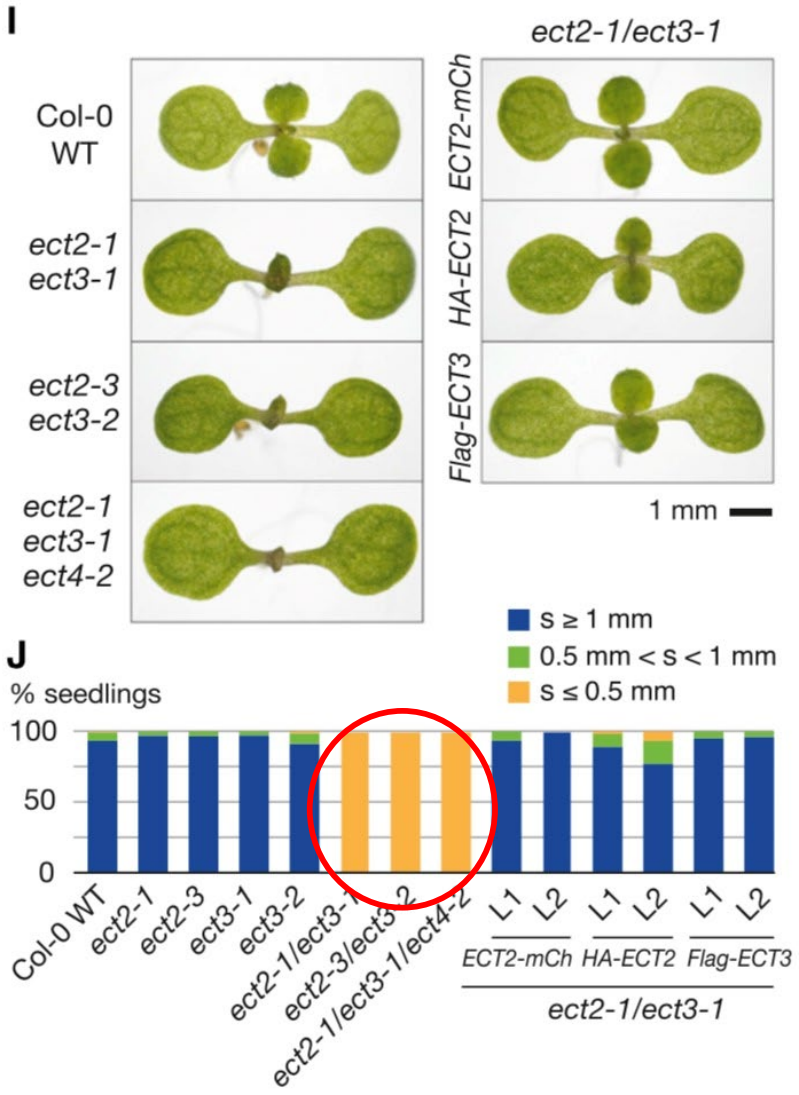
Similar motifs discovered of Arabidopsis thaliana ECT proteins



Motif	Symbol	Motif Consensus
1.		CPVFLFFSVNASGQFVGLAEMVGPVDFNKTVEYWQQDKWSGSFPVKWHIVKDVPNSLLRHIILENNENKPVTNSRDTQEVKLEQGJEVLKIFKEYESKT SILDDFSFYEERQKVI
2.		PDPEQYNKEDFPTDYKDAKFFVIKSYSEDDVHKSIKYNVWSSTPNGNKKLDAAYQEAQKK
3.		YGNENIDGLNELNRGPRAKGLKNQ
4.		YGQYGSPIRSGYGYGSYGYDSRTNGRGWYAVDNKYRSRGRGH
5.		MATVAPPADQAADLLKKLSLDSKAKALEIPEPNKKTGVYQYGGMDVNGQVPSFDRSLSPMLPSDAADPSVCYVPNPY
6.		DYPGYTNPEGVDMNSGIYGENGSLVYPQGYGYAAFPYSPATSPAPQLGGEGQLYGAQQYQYP
7.		LYGNGAPGGGLAAGYQDPRYAYEGFYAPVPWHDGSKFSDVQRPVSGSGVASSYSK
8.		RNQNYSSNSHYTNVHQPKSVTGY
9.		MFPPLAGERPCMRDNGVQZL
10.		AGIPKGMNGSAPVKPLNQAA

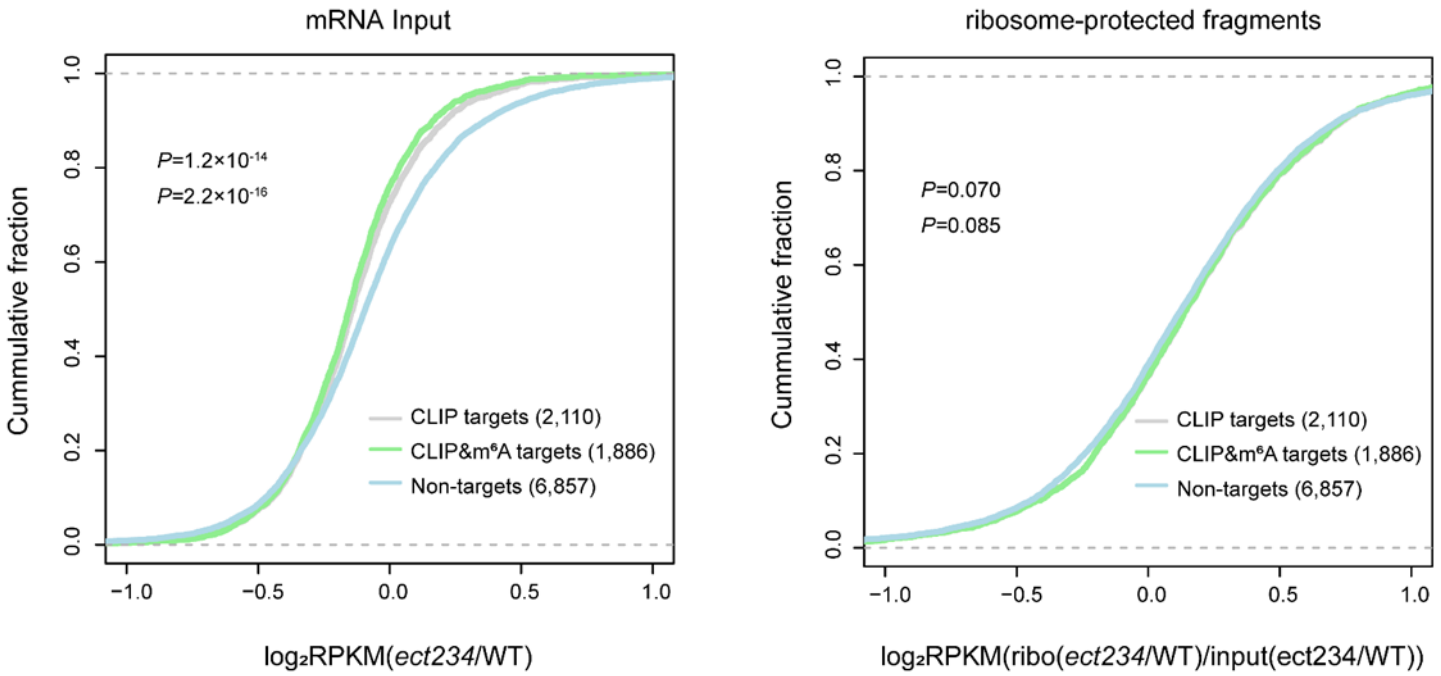
YTH proteins in *Arabidopsis*

- Eight-day-old seedlings
- Quantification of the length of the first true leaves at 8 d after germination in the indicated genotypes.
- ECT2/3/4 works together to mediate the timing of leaf formation.



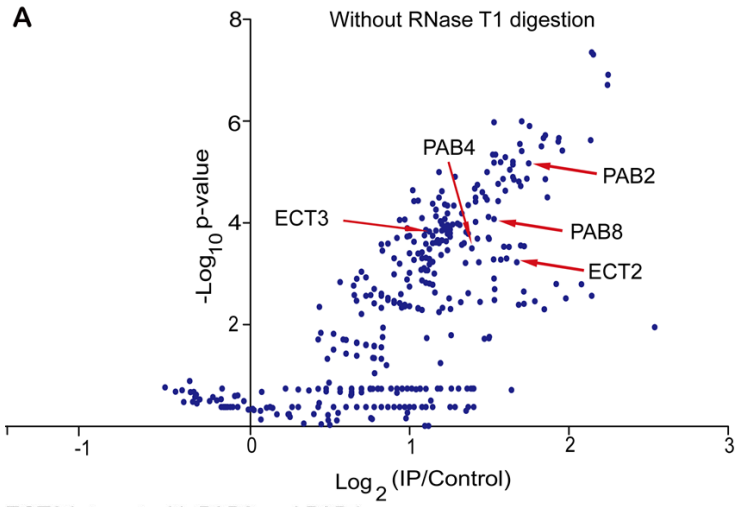
Arriba's-Hernández, L. *et al.* Plant Cell 2018

ECT2-3-4 regulate transcriptome-wide mRNA levels?

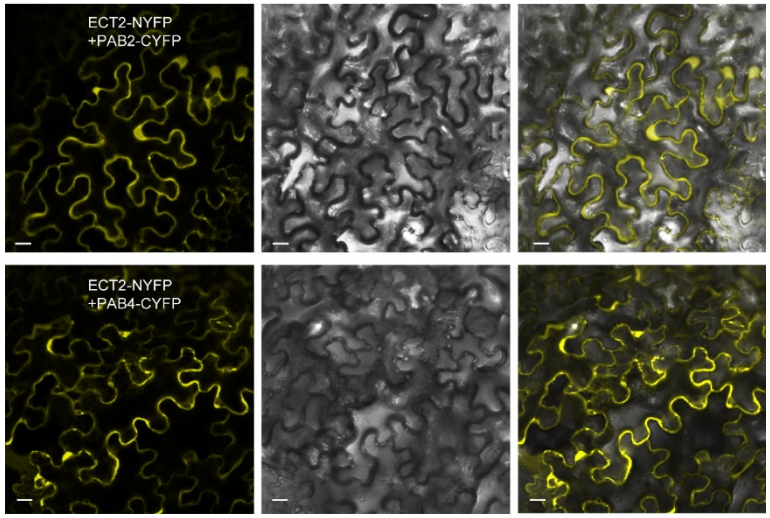


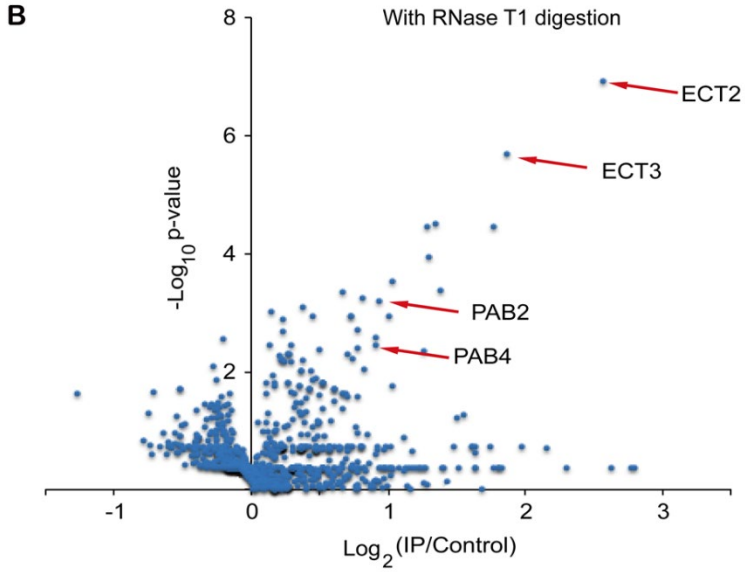
ECT2/3/4 stabilizes its cognate mRNAs, but has no effect on translation efficiency.

ECT2 directly intact with PAB2.

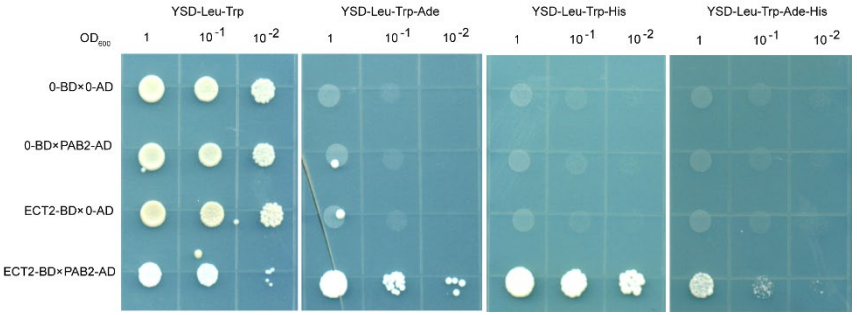


ECT2 interact with PAB2 and PAB4

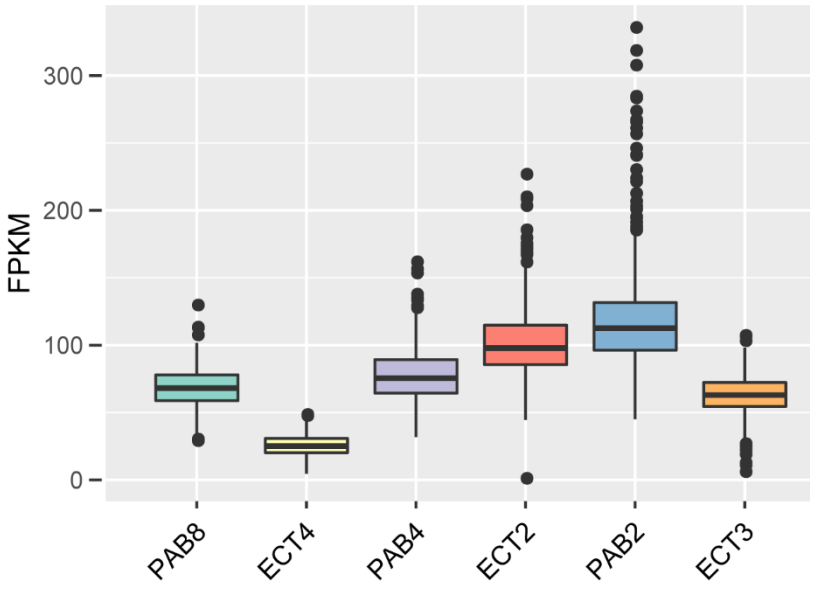


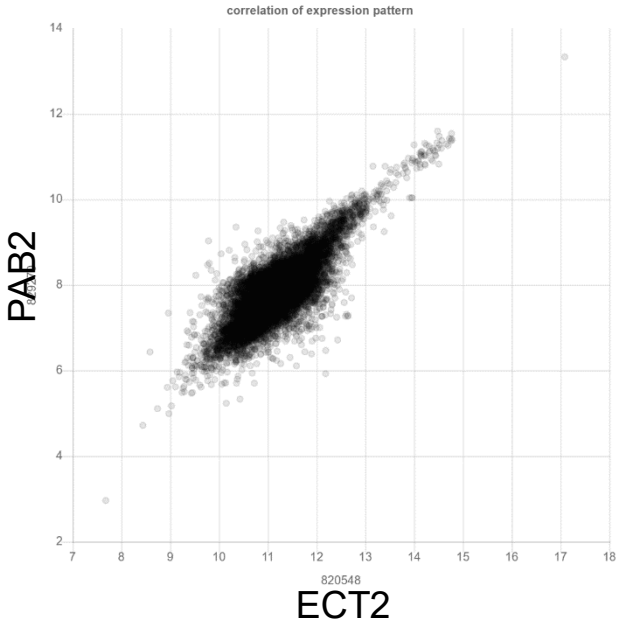


ECT2 interact with PAB2



PAB2 was highly co-expressed with ECT2





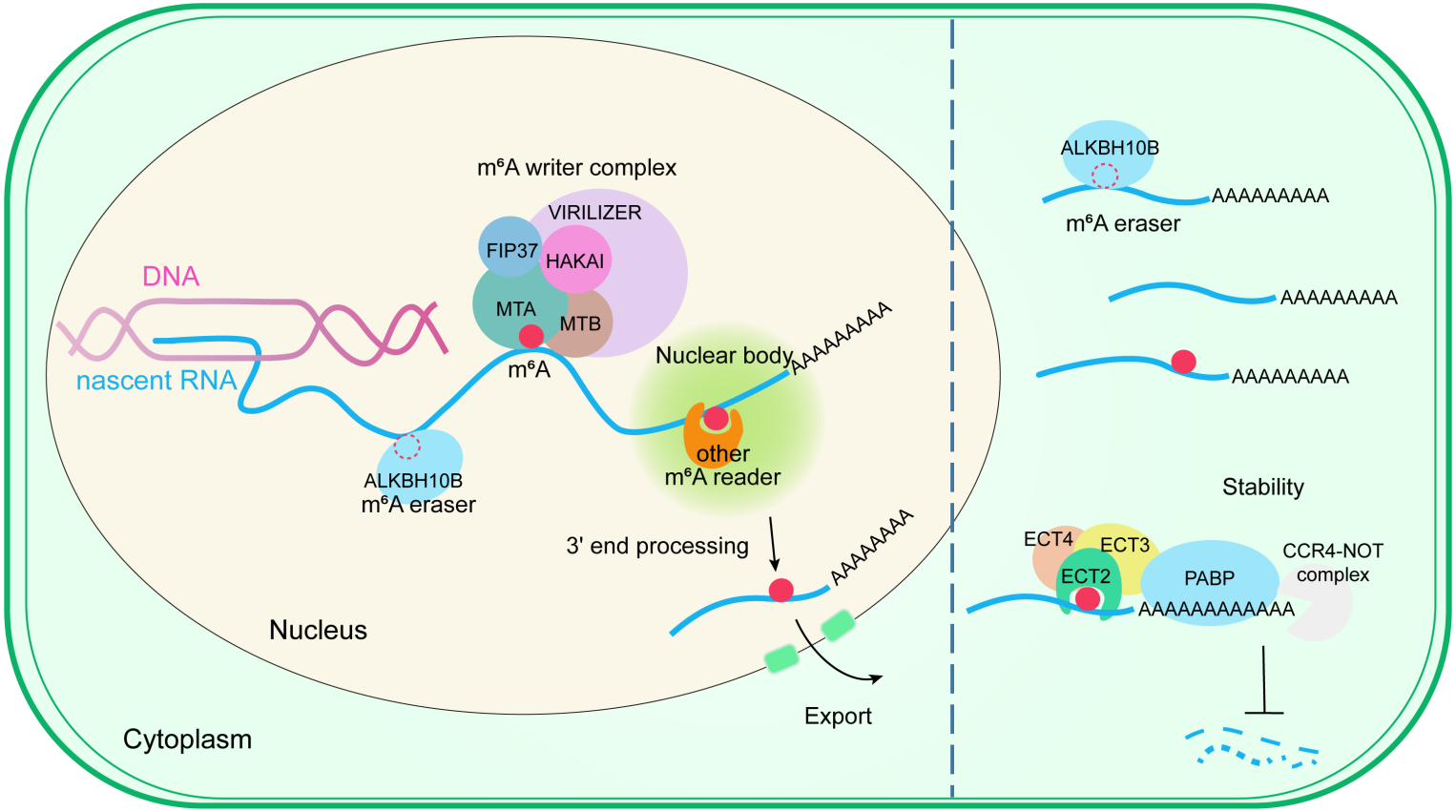
Eight PABP genes have been identified in *Arabidopsis thaliana*, which are divided into four classes based on gene expression and similarity. Class II genes (**PAB2, PAB4** and **PAB8**) are highly and broadly expressed and probably encode the bulk of PABP required for cellular functions.

According to the **ATTED-II** Plant Coexpression Database, PAB2 was co-expressed with ECT2. However, either ECT3 or ECT4 was co-expressed with both PAB4 and PAB8, but not PAB2.

猜想：ECT2-3-4 稳定其相互作用的RNA。

Co-transcriptional Regulation

Post-transcriptional Regulation



Experimental Methods

- Arabidopsis thaliana: WT & ECT2/3/4 敲除
- ActD treatment: 抑制转录
- RNA-seq for mRNA lifetime: 对抑制转录后0h/2h/4h/6h时mRNA存量进行检测
- Data analysis of seq-data : 计算所有基因表达量RPKM（即mRNA的量）

Stability assay

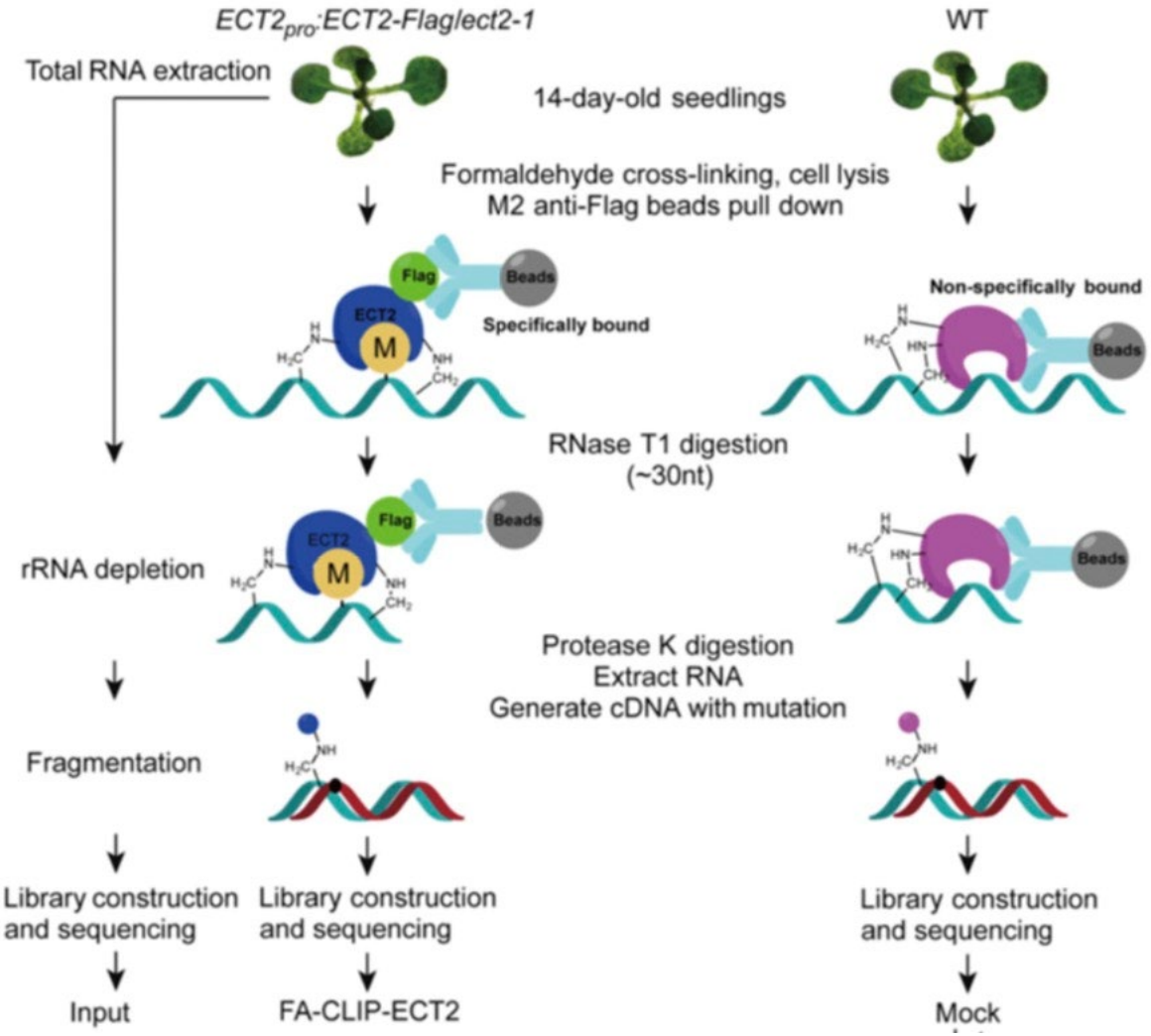
ActD treatment:

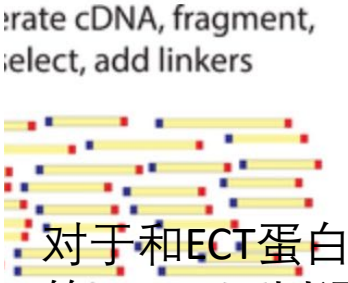
An mRNA stability measurement assay was performed as previously described with minor modification. Briefly, 7-d-old wild-type and *ect2-3-4 Arabidopsis* seedlings grown on 1/2 MS medium were transferred to 10-cm Petri dishes containing 10 mL 1/2 MS liquid medium. After 30 min incubation, 0.2 mM actinomycin D was added to the buffer. For the global mRNA decay study, triplicate experiments were performed where, seedlings (10 seedlings each sample) were collected prior to ActD treatment (0 h) and then 2h, 4h and 6h after treatment. Seedlings were collected and immediately frozen in liquid nitrogen. The tissues were stored at −80°C or subjected to total RNA extraction.

mRNA enrichment:

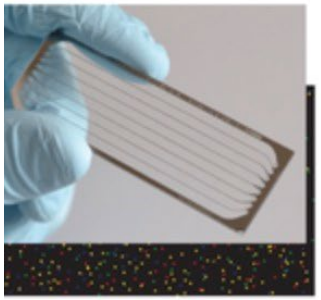
A's number	Numbers ERCC spike-ins
>=20	***92***
>=21	***91***
>=22	***88***
>=23	***80***
>=24	***73***

RNAseq steps





对于和ECT蛋白有结合的RNA，可以通过FA-CLIP的方式提取



quantitative analysis of gene expression: RPKM

Reads Per Kilobase per Million mapped reads，代表每百万reads中来自于某基因每千碱基长度的reads数

$$\mathrm{RPKM}_g = \frac{\frac{r_g 10^3}{\mathrm{fl}_g}}{\frac{R}{10^6}} = \frac{r_g \times 10^9}{\mathrm{fl}_g \times R}$$

目标基因的数值

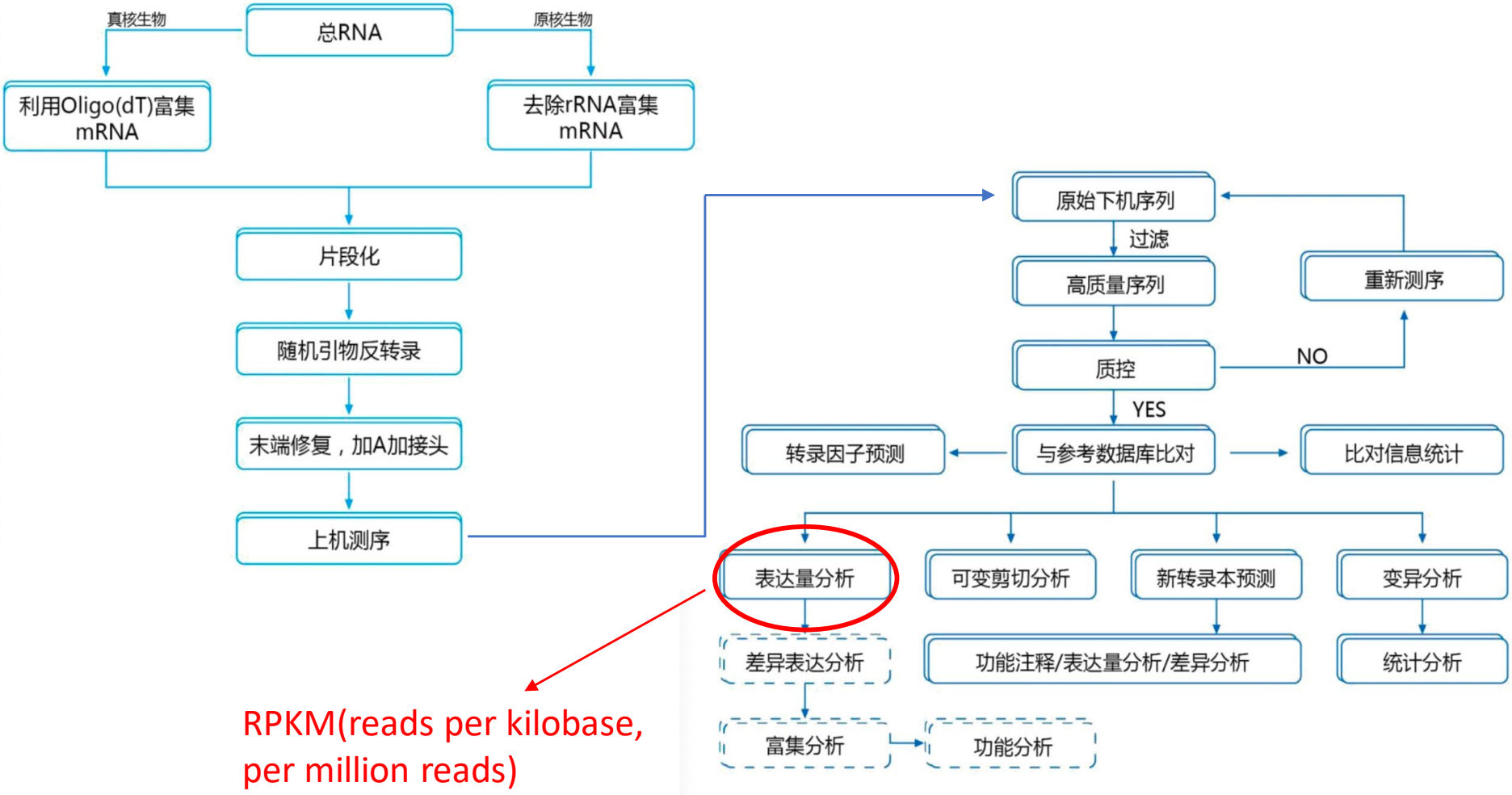
r_g:每个基因的reads数;
fl_g:每个基因的长度

参照的数值

R:样本测序得到的总reads数;

- 意义：在随机抽样的情况下，序列较长的基因被抽到的机率本来就会比序列短的基因较高，如此一来，序列长的基因永远会被认为表达量较高
- 对基因长度(基因间的比较)和总数据量(样本间的比较) 做校正;

实验&分析的步骤


真核生物
总RNA
原核生物
利用Oligo(dT)富集mRNA
去除rRNA富集mRNA
片段化
随机引物反转录
末端修复，加A加接头
上机测序
原始下机序列
过滤
高质量序列
重新测序
质控
NO
YES
转录因子预测
与参考数据库比对
比对信息统计
表达量分析
可变剪切分析
新转录本预测
变异分析
差异表达分析
功能注释/表达量分析/差异分析
统计分析
富集分析
功能分析
RPKM(reads per kilobase, per million reads)

Results of data analysis： RPKM to mRNA lifetime

The degradation rate of RNA k was estimated by

$$\log_2\left(\frac{A_t}{A_0}\right) = -kt$$

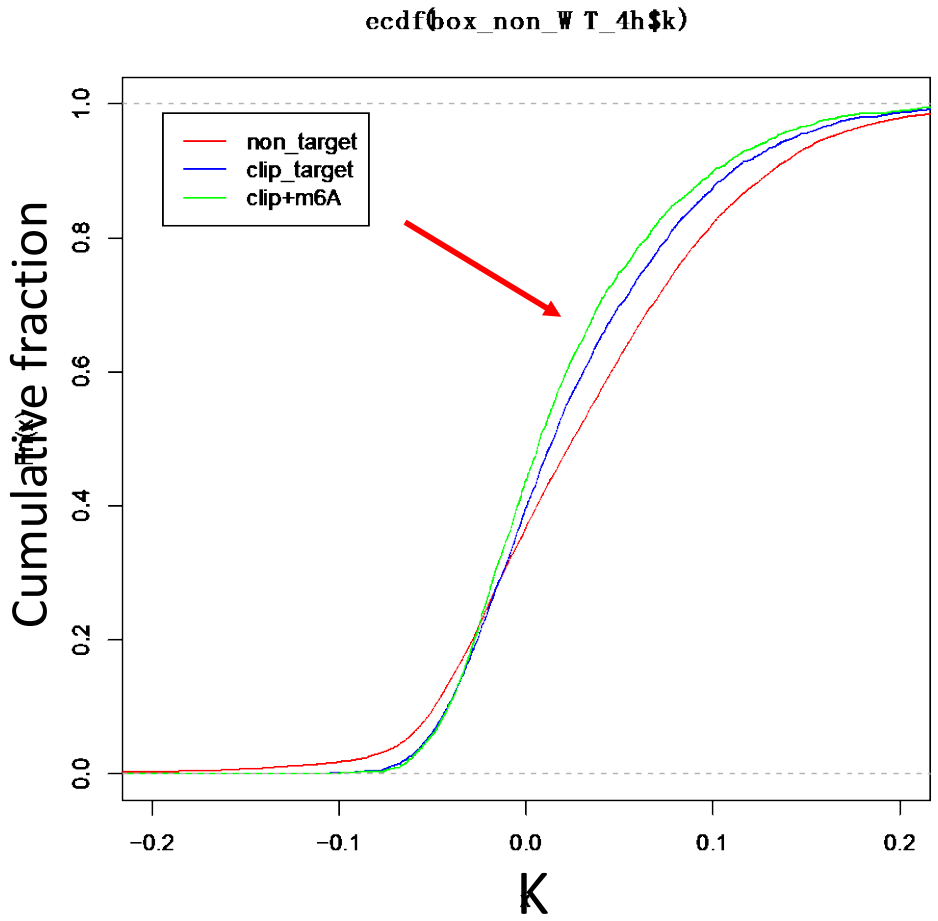
where t is transcription inhibition time (h), A_t and A_0 represent mRNA quantity (attomole) at time t and time 0. Two k values were calculated: time 3 h versus time 0 h, and time 6 h versus time 0 h. The final lifetime was calculated by using the average of $k_{3\,\mathrm{h}}$ and $k_{6\,\mathrm{h}}$.

$$t_{\frac{1}{2}} = \frac{2}{k_{3\mathrm{h}} + k_{6\mathrm{h}}}$$

通过比对WT和ECT2/3/4 mutation中所有基因的degradation rate K或者mRNA半衰期来判断ECT2/3/4是否可以稳定细胞mRNA。

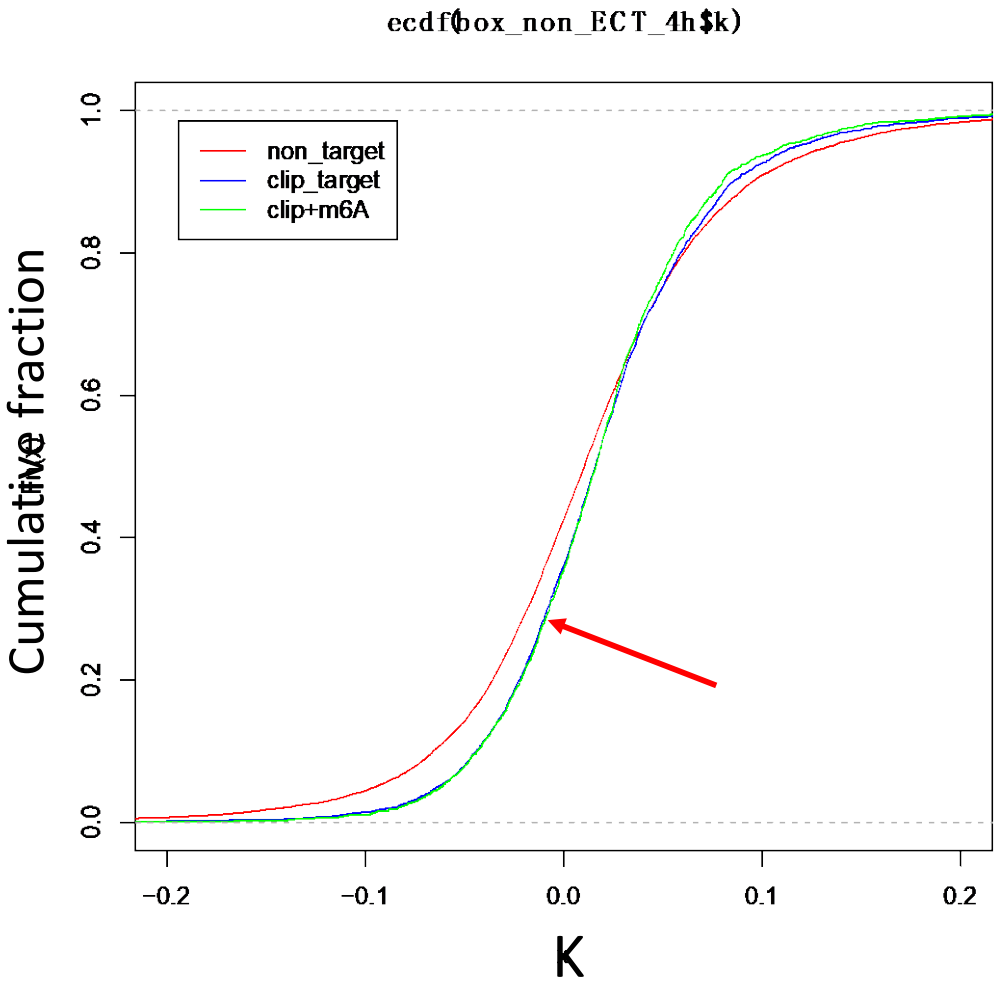
Wang X. Nature. 2014 Jan 2;505(7481):117-20.

Results of data analysis： WT vs mutation



在野生型中，和ECT2蛋白有作用的mRNA降解速率较慢，说明其较稳定

Results of data analysis： WT vs mutation



在突变体中，和ECT2蛋白有作用的mRNA降解速率加快，说明其失去了ECT蛋白的保护作用。

小结：ECT2/3/4蛋白

- 从目前的结果来看，对野生型和突变体中RNA的分解速率的分析可以有效的帮助我们研究一些与RNA稳定性相关的蛋白功能。

- 在拟南芥中，招募m6A识别蛋白到甲基化修饰的转录物上是一种基因表达调控的方式，以ECT2/3/4蛋白为例，可以有效的提高RNA的稳定性。

研究计划

- 现已知不同的基因上m6A修饰的数量也有差别，有的基因上会有多个甲基化修饰。与ECT2蛋白有相互作用的RNA位点数量也存在不同。本课题计划接下来根据m6A数和ECT2 clip目标位点结合数将基因进行细分，对RNA稳定性的变化进行探究。