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Transcriptional read-through of the long non-coding RNA *SVALKA* governs plant cold acclimation

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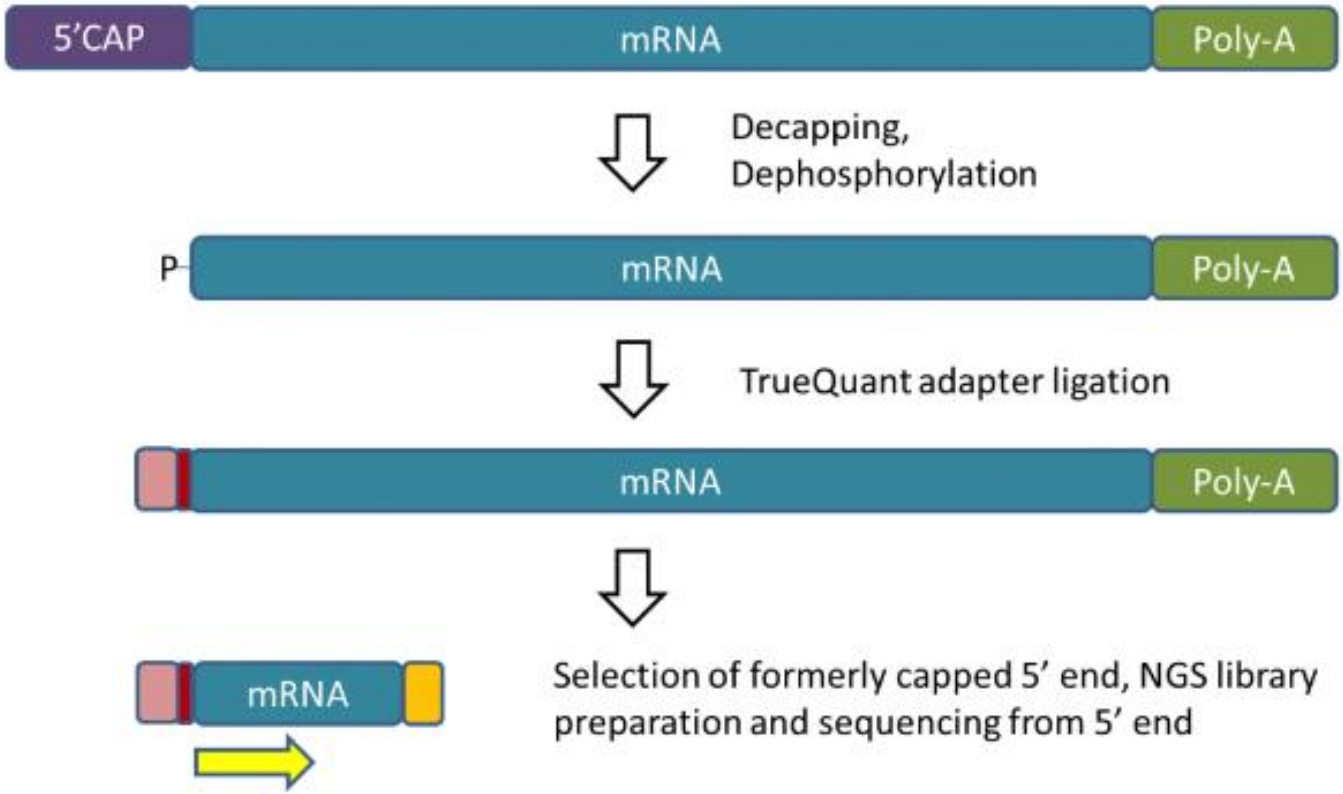
The Background

- ✓ **The biological significance of most lncRNAs is largely unclear**
- ✓ **Expression of lncRNAs is highly specific to environmental conditions, tissue or cell types**

- ✓ **The *cold-mediated* transition from vegetative-to-reproductive stage is strictly controlled at epigenetic level with the involvement of several lncRNAs:**
  - **COOLAIR**
  - **COLDAIR, associated with PRC2**
  - **COLDWRAP, PRC2-associated, derived from the repressed promoter of *FLC***
- ✓ ***COOLAIR* is induced by CBFs, the main players of cold stress response**

The Aim is……..

- ✓ **To identify transcription initiation events that respond to cold temperature in *Arabidopsis*, we performed transcription start site (TSS)-sequencing(TSS-seq)**



TSS-seq

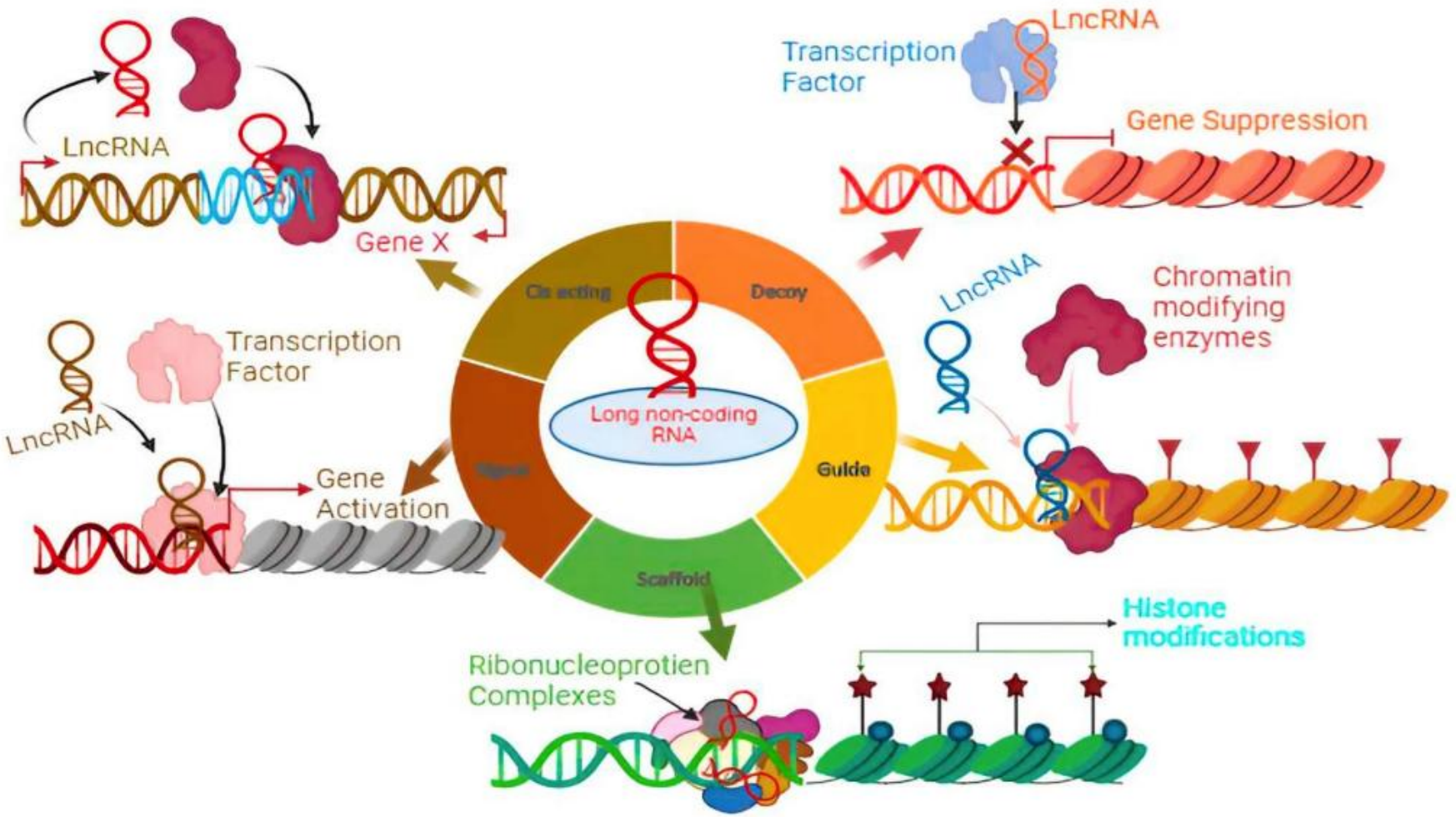
5'mRNA sequencing aims at sequencing the 5′ ends of formally 7-methylguanylate capped mRNAs.

This method employs a series of enzymatic reactions, named 'oligo-capping,' to label the cap structure.

Long non-coding (lnc) RNAs

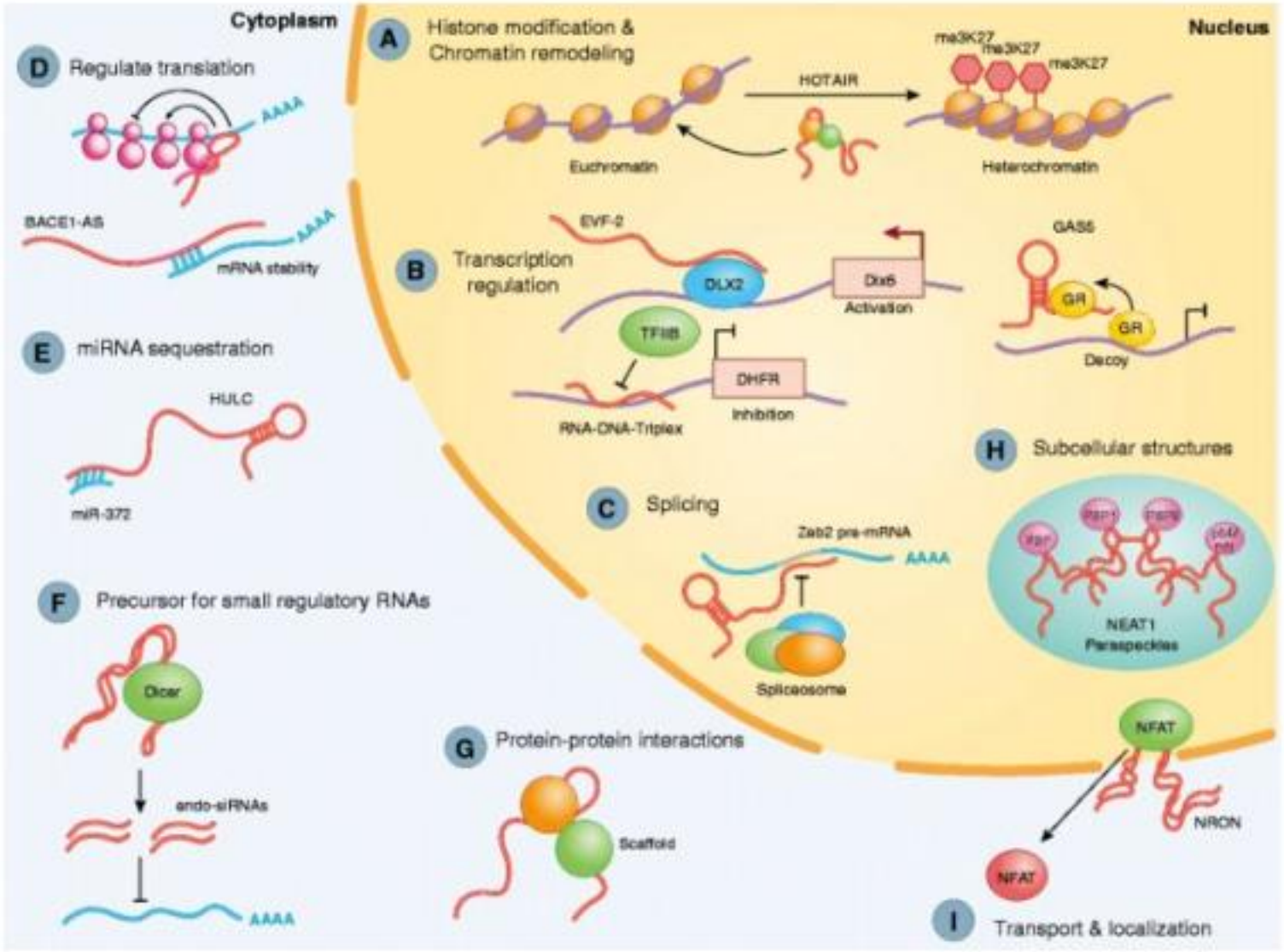
- ✓ **non-coding RNAs longer than 200 nt**
- ✓ **primarily interact with mRNA, DNA, protein, and miRNA**
- ✓ **regulate gene expression at epigenetic, transcriptional, post-transcriptional, translational, post-translational levels**
- ✓ **play important roles in biological processes such as chromatin remodeling, transcriptional activation, transcriptional interference, RNA processing, and mRNA translation**
- ✓ **have important functions in plant growth and development, biotic and abiotic stress responses, control of cell differentiation**
- ✓ **related to the occurrence of many diseases in humans and animals**

Conserved Functions of lncRNAs……

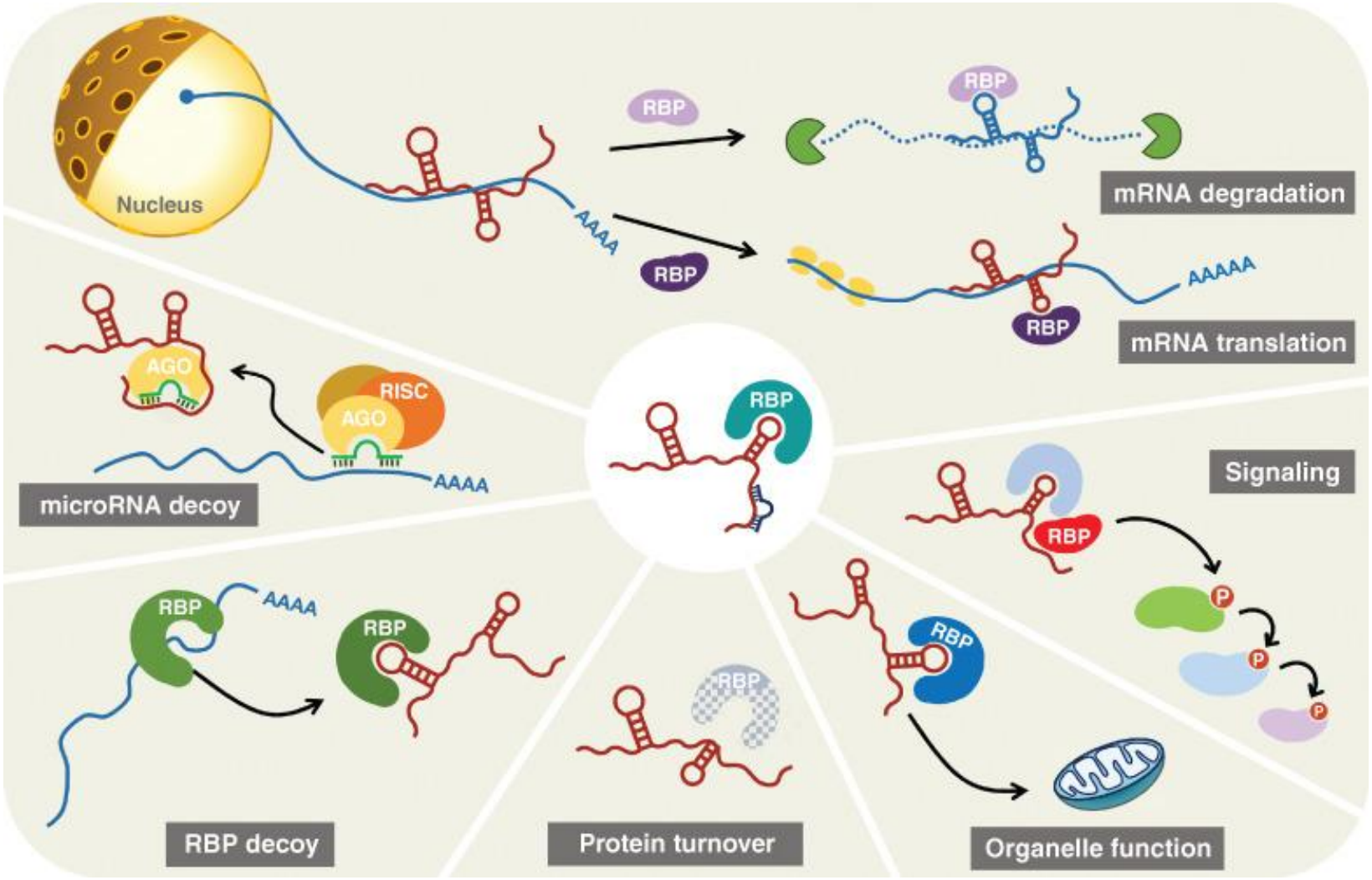


LncRNAs acts as structural decoys and interact with TF, miRNA and attenuate expression of genes. By guiding chromatin remodellers interaction with ribonucleoprotein complexes lncRNA indirectly modifies the histone code of epigenome and regulate the gene expression. Scaffolds facilitate the temporary assembly of protein complexes at genomic sites which can induce histone alterations and DNA methylation

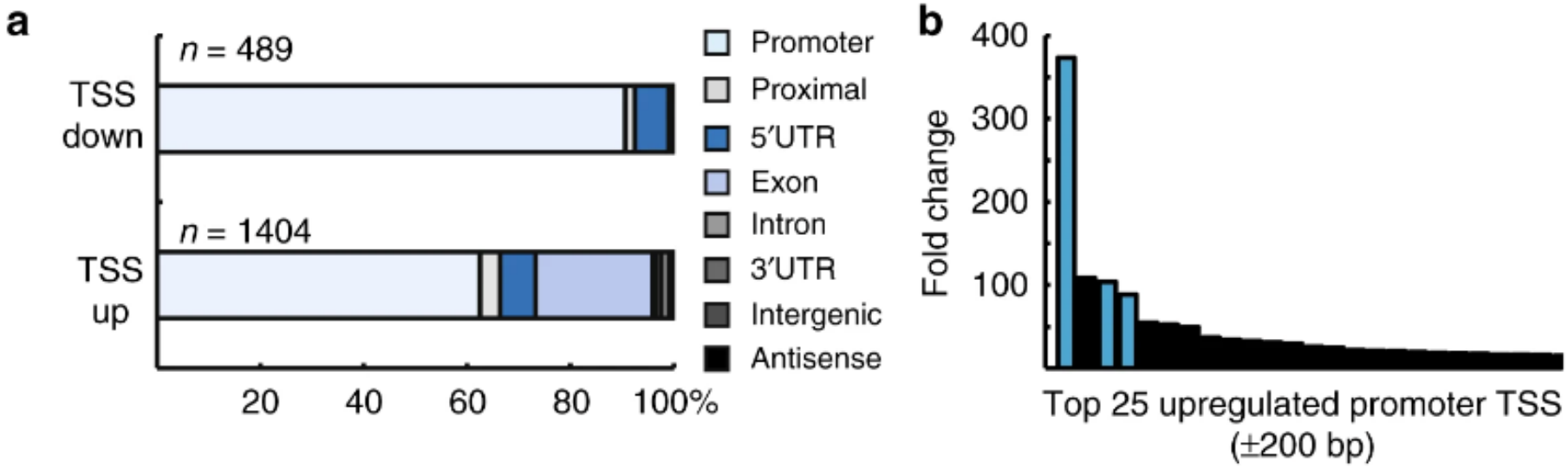
Where and what do lnc RNAs do??????



Cytoplasmic lnc RNAs



Identification of the lncRNA SVALKA

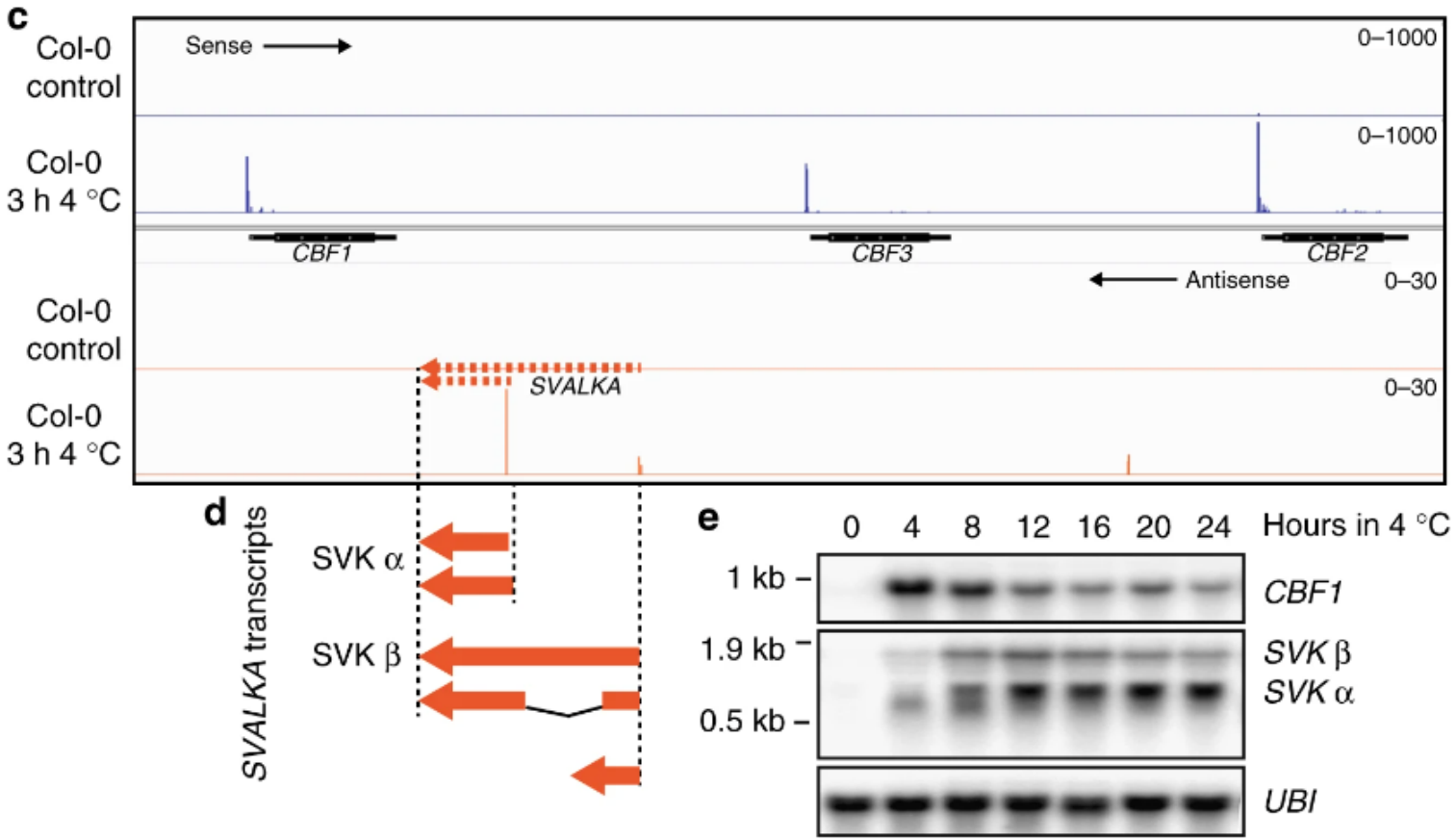


Experimental Set-up

two biological replicates at 22 °C and *two* biological replicates at 4°C 3 h

489 down-regulated, 1404 up-regulated

TSS classified according to their position relative to gene bodies

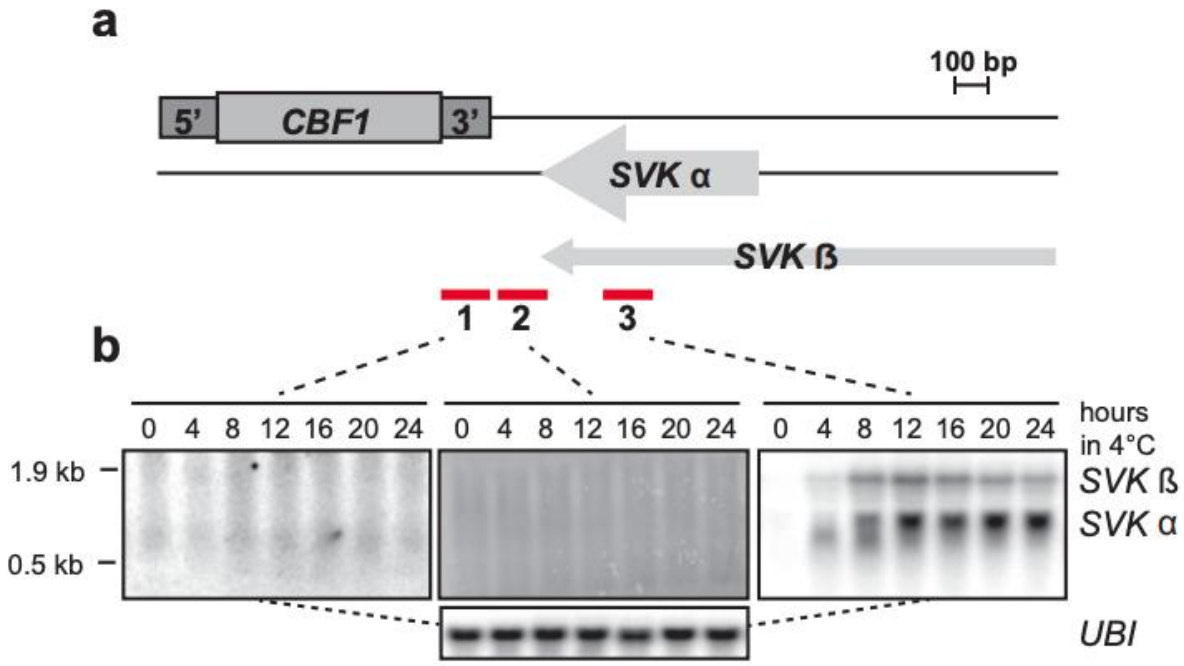


***CBF* genes were upregulated 100–400 fold making the CBF genomic region by far the most cold-responsive region in the genome**

Identified also a cold-responsive lncRNA,

transcribed on the as strand between *CBF3* and *CBF1*, named SVALKA

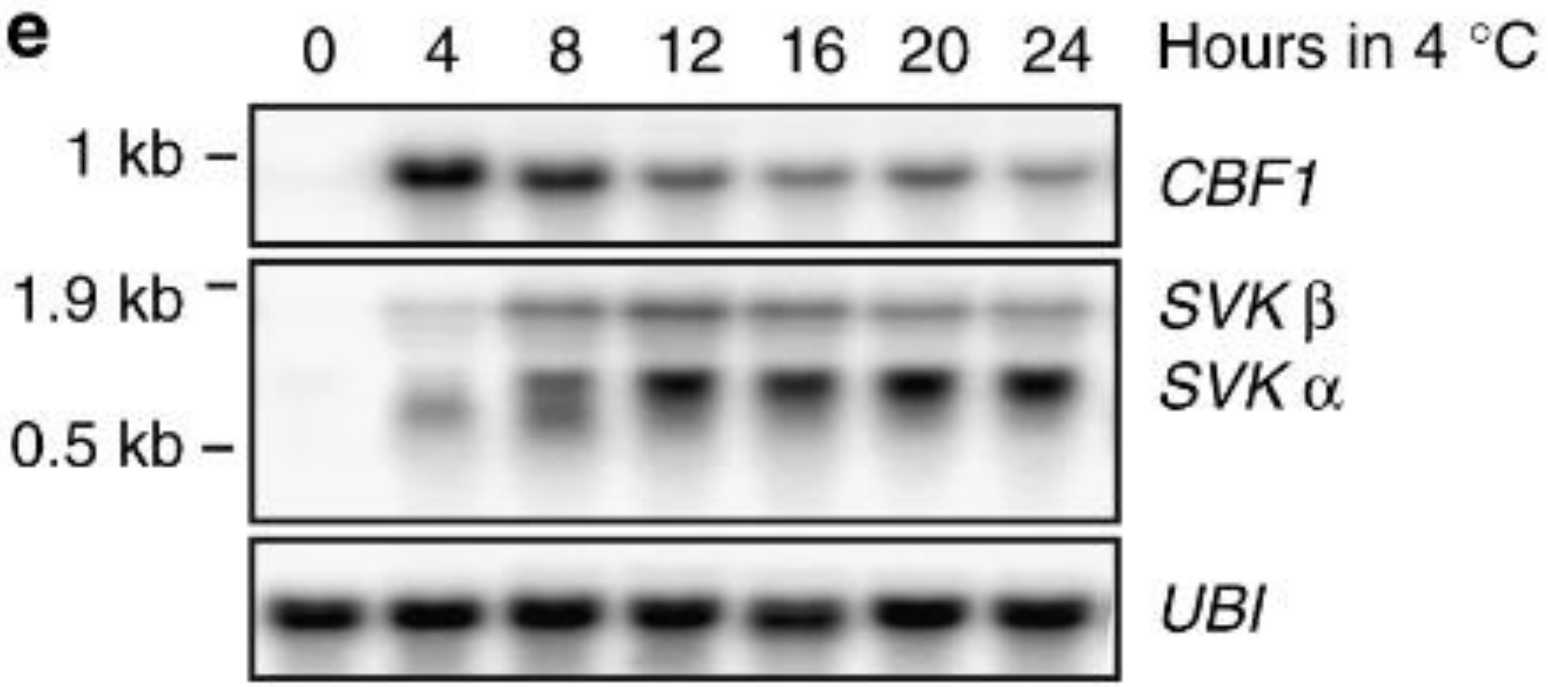
Mapping of the *SVALKA* variants



Supplementary Figure 1. Stable *SVK* transcription does not reach the 3'UTR of *CBF1*.
a) Graphical representation of the *CBF1-SVK* genomic region. The probes used in b) are shown with red lines.
b) Representative Northern blots of a cold exposure time series in WT. Blots were repeated with three biological replicates with similar results. Presented are results from the same membrane hybridized with the different probes shown in a). *SVK* transcripts could only be found with probe 3. For probe 1 and 2, membranes were exposed for twice the time as probe 3. No signal (i.e. stable transcripts) was detected further downstream of the identified polyA signal of *SVK*. *UBI* was used as loading control. Uncropped blots can be found in the Source Data file.

Expression profile of *CBF1* and *SVALKA*

Under cold induction

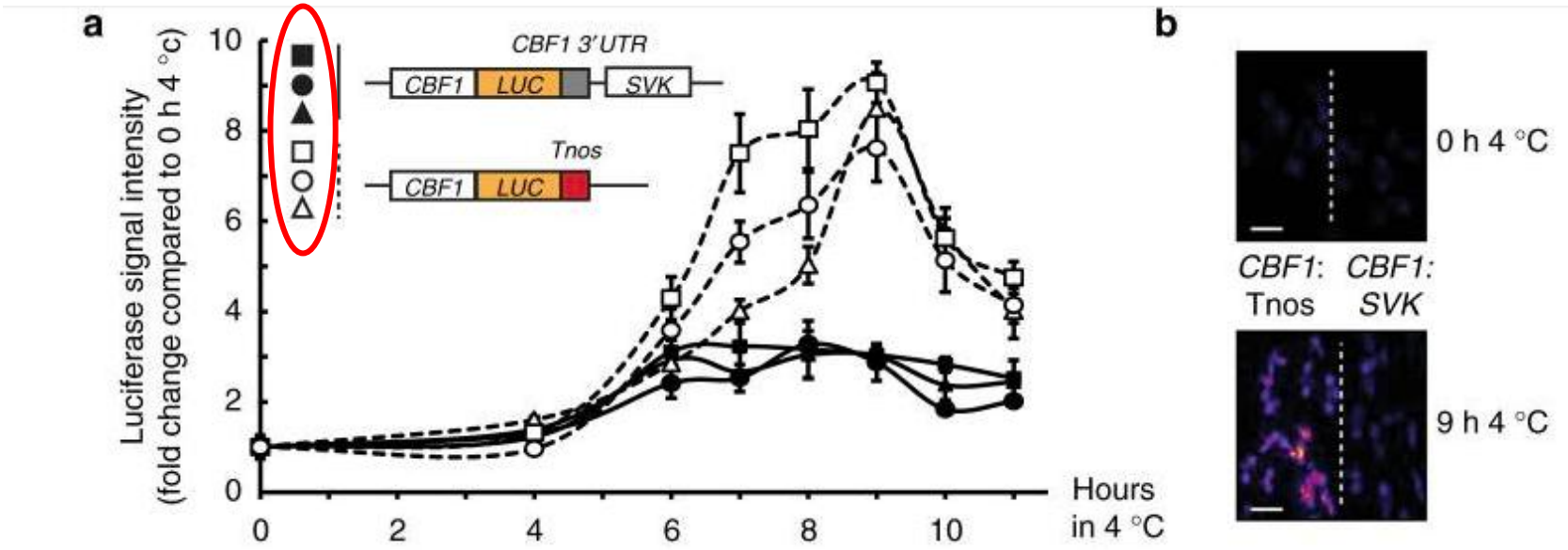


***UBI* is used as a loading control**

Experiments done with 3 biological replicates *showing similar results*

***Uncropped blots* can be found in the Source Data file**

Is *SVK* involved in *CBF1* repression?

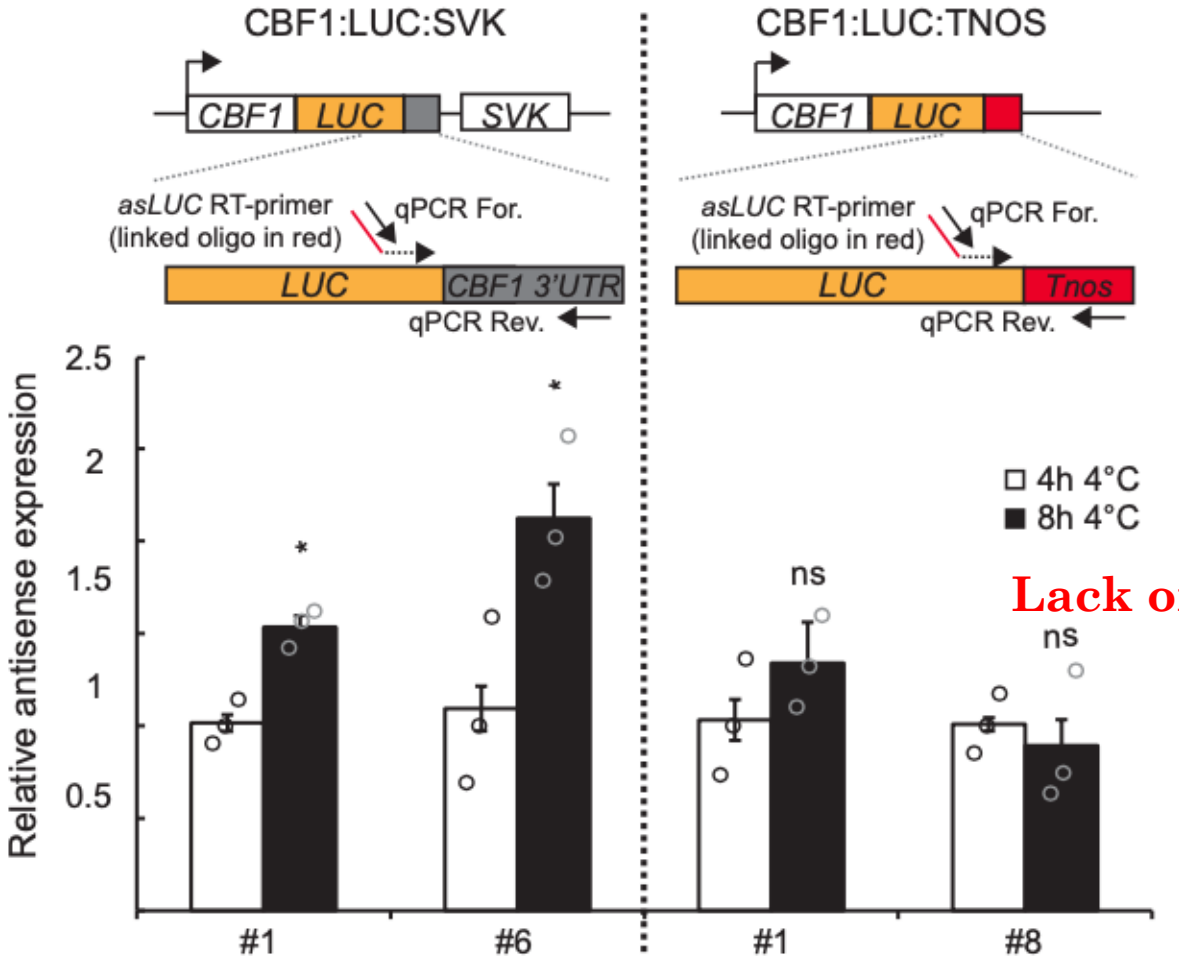


2 LUCIFERASE reporter lines of *CBF1* with different termination sequences

3 independent lines

SVK represses LUC activity

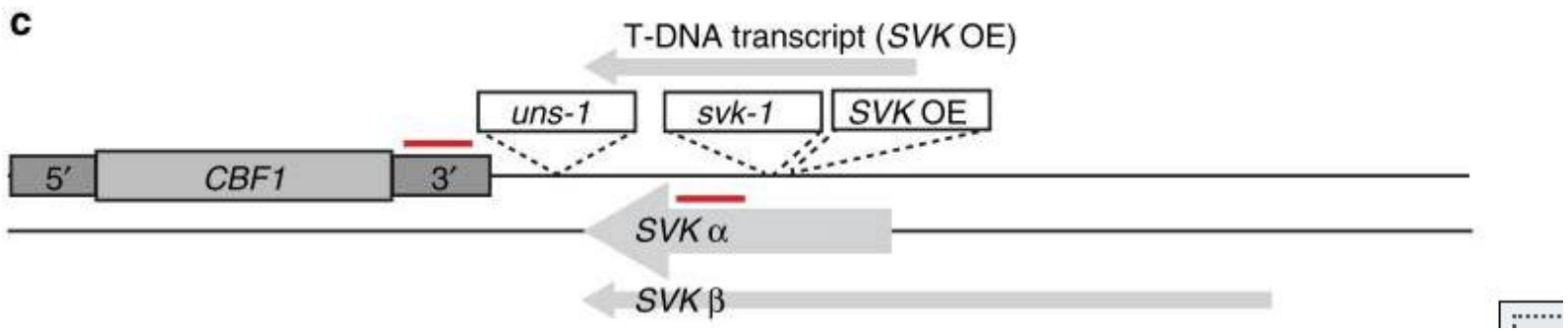
Cold-induced antisense transcription involved in regulating endogenous *CBF1* expression



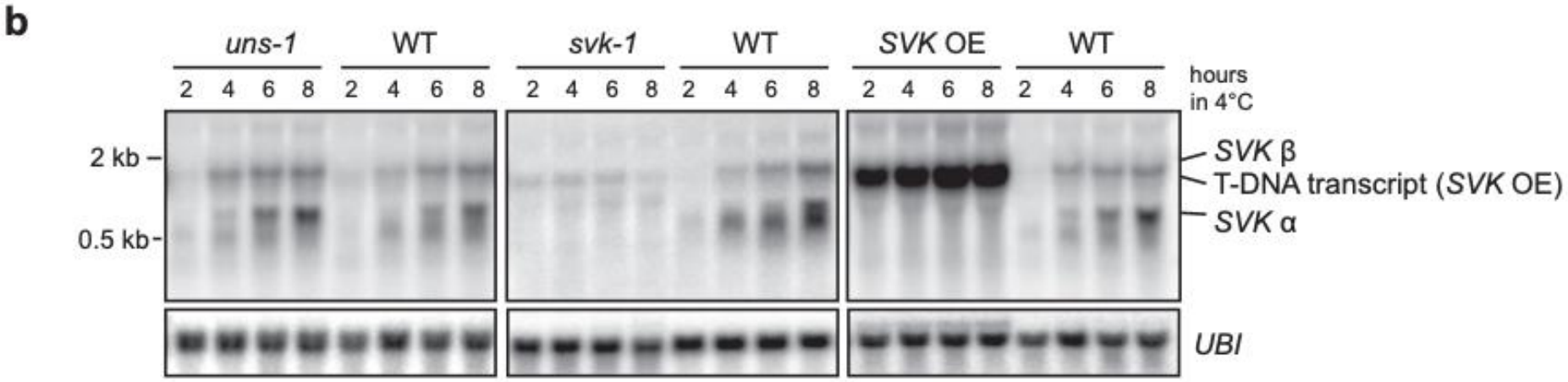
Lack of proper control: 0h 4°C

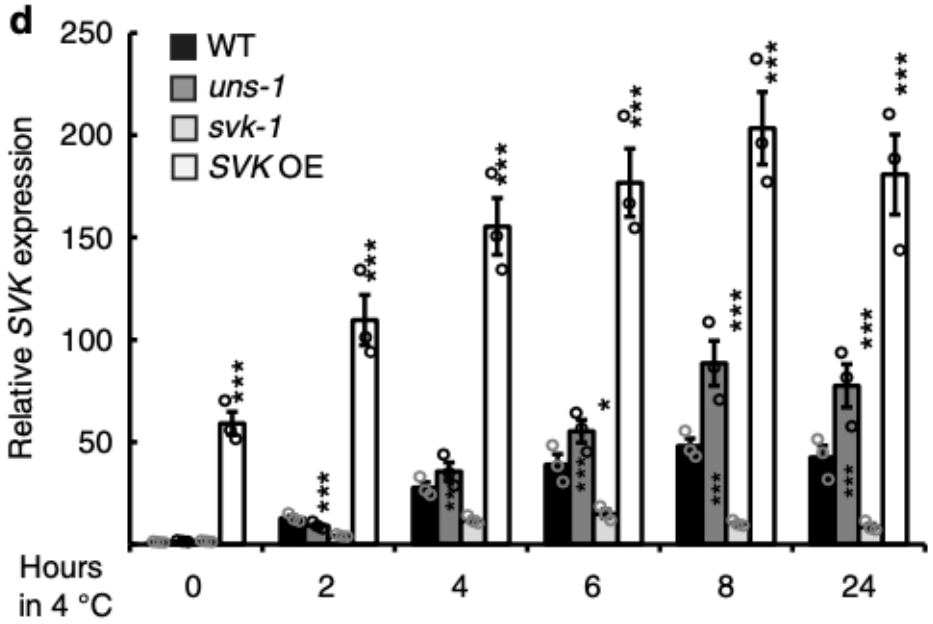
RT-qPCR of antisense transcripts in response to cold exposure in two independent lines from each LUC construct

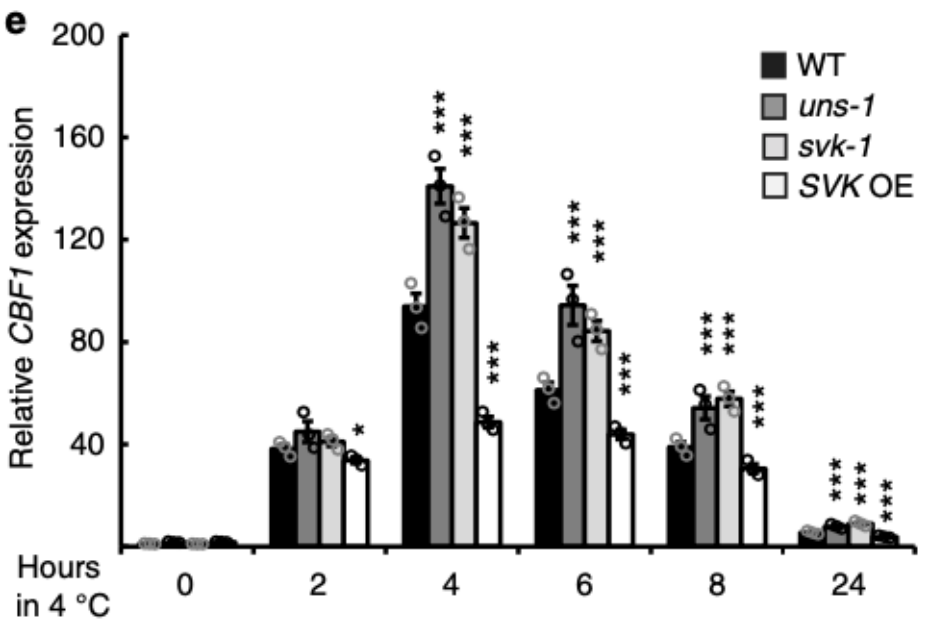
Cold-induced antisense transcription involved in regulating endogenous *CBF1* expression



- ✓ a line that disrupted *SVK* (*svk-1*)
- ✓ a line increasing the distance of SVK transcription from CBF1 (*uncoupling svalka-1*, *uns-1*)
- ✓ a line overexpressing *SVK* (*SVK OE*) 35S promoter close to the LB of the T-DNA drives expression of the transcripts seen in the SVK OE mutant

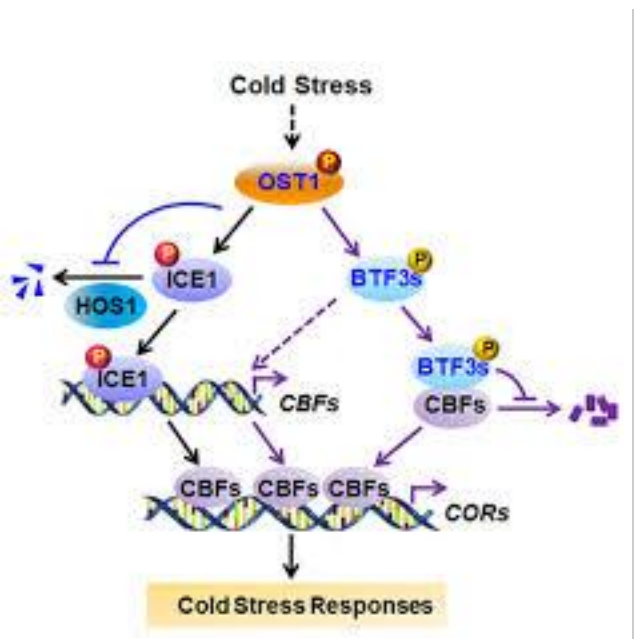


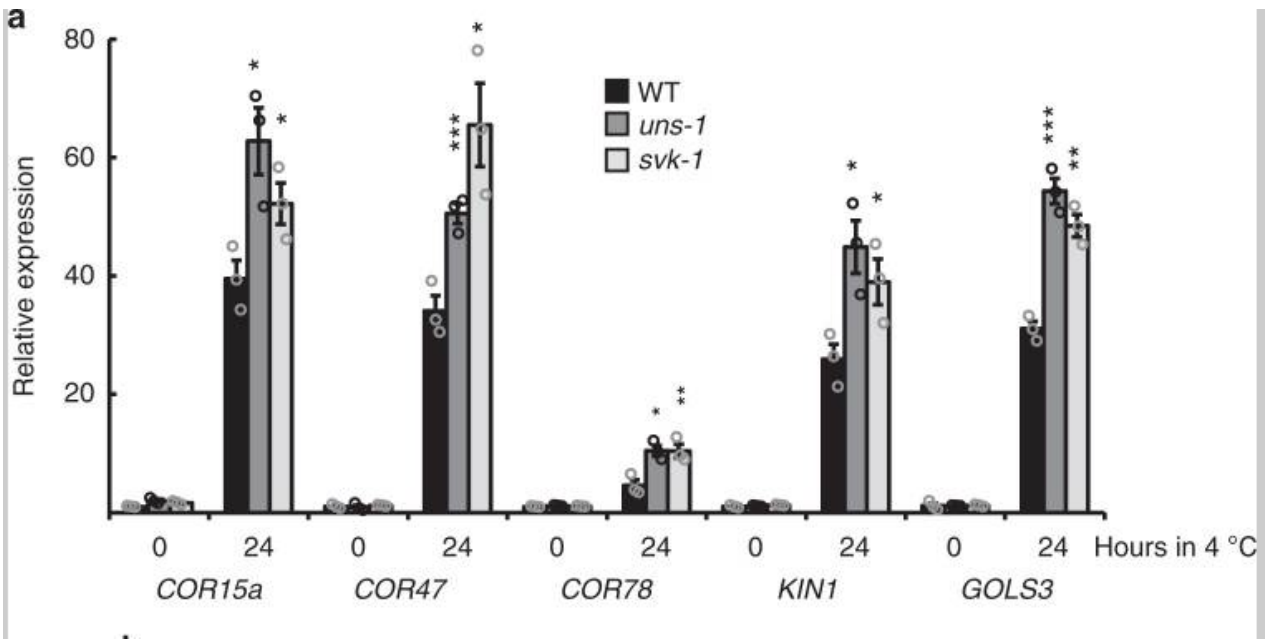




- ✓ **In *svk-1* *reduced* expression of *SVK***
- ✓ **In *uns-1* slightly elevated levels of *SVK* compared to WT**
- ✓ ***CBF1* mis-regulation in all three mutants**
- ✓ In ***uns-1***, *SVK* is expressed 4–5 kb away from *CBF1* (110 bp in WT) but *CBF1* expression is still increased respect to WT:

trans-acting function of SVK



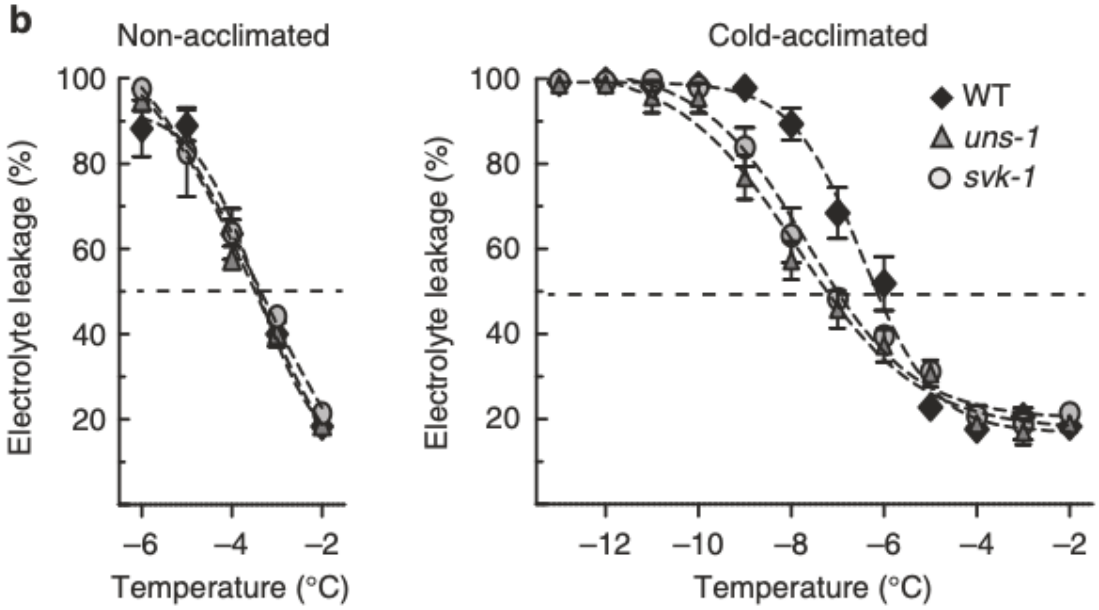


Increased *CBF1* in *uns-1* and *svk-1* mutants lead to greater induction of CBF-activated *COR* genes

Resulting in

increased freezing tolerance

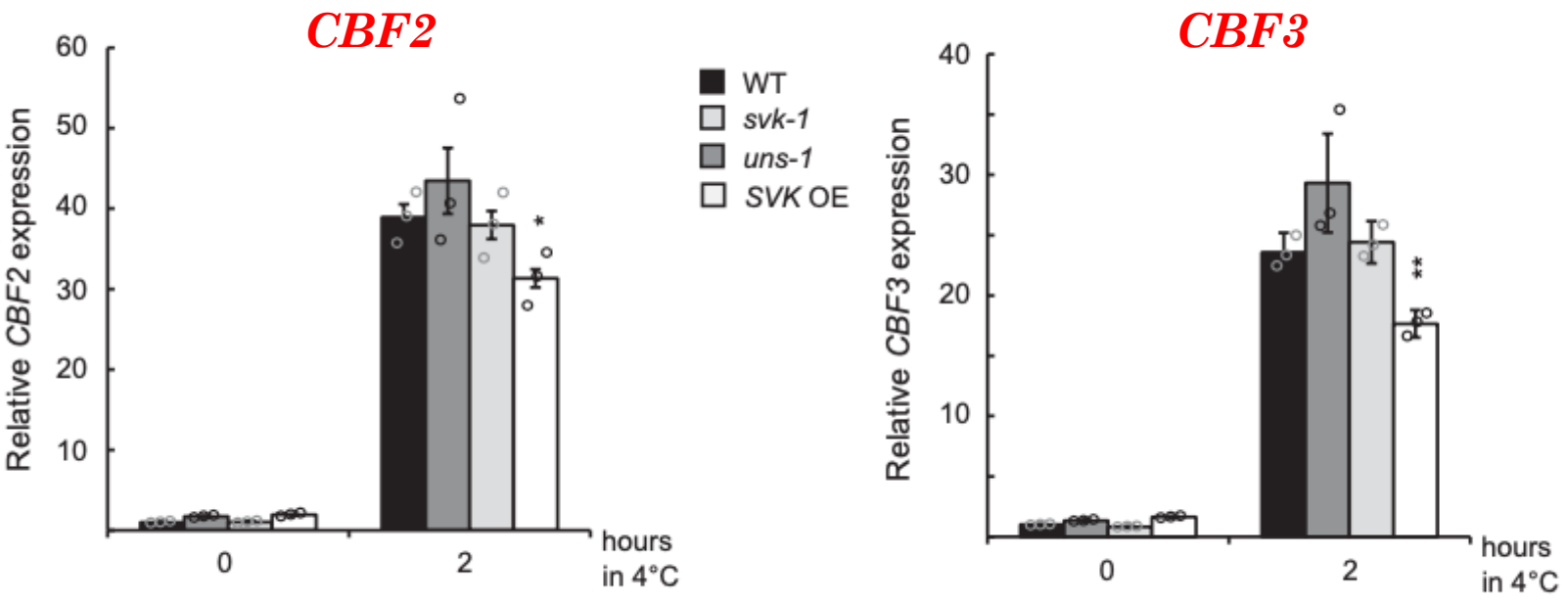
Electrolyte leakage is a measure of plasma membrane disruption



SVK represses cold-induced *CBF1* expression and has a biologically relevant effect on cold acclimation and freezing tolerance

nuclear exoribonucleases XRN3

Expression of the *CBF2* and *3* homologous genes are not affected by the *svk-1* and *uns-1* mutations



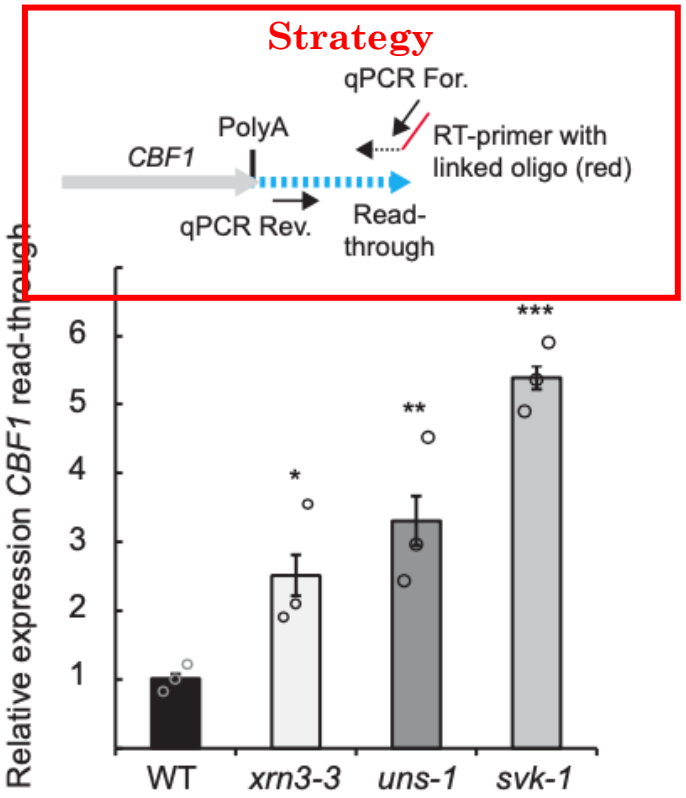
Question:

Why the *uns-1* mutation results in the same molecular effect of the *svk-1* one?

Hypothesis:

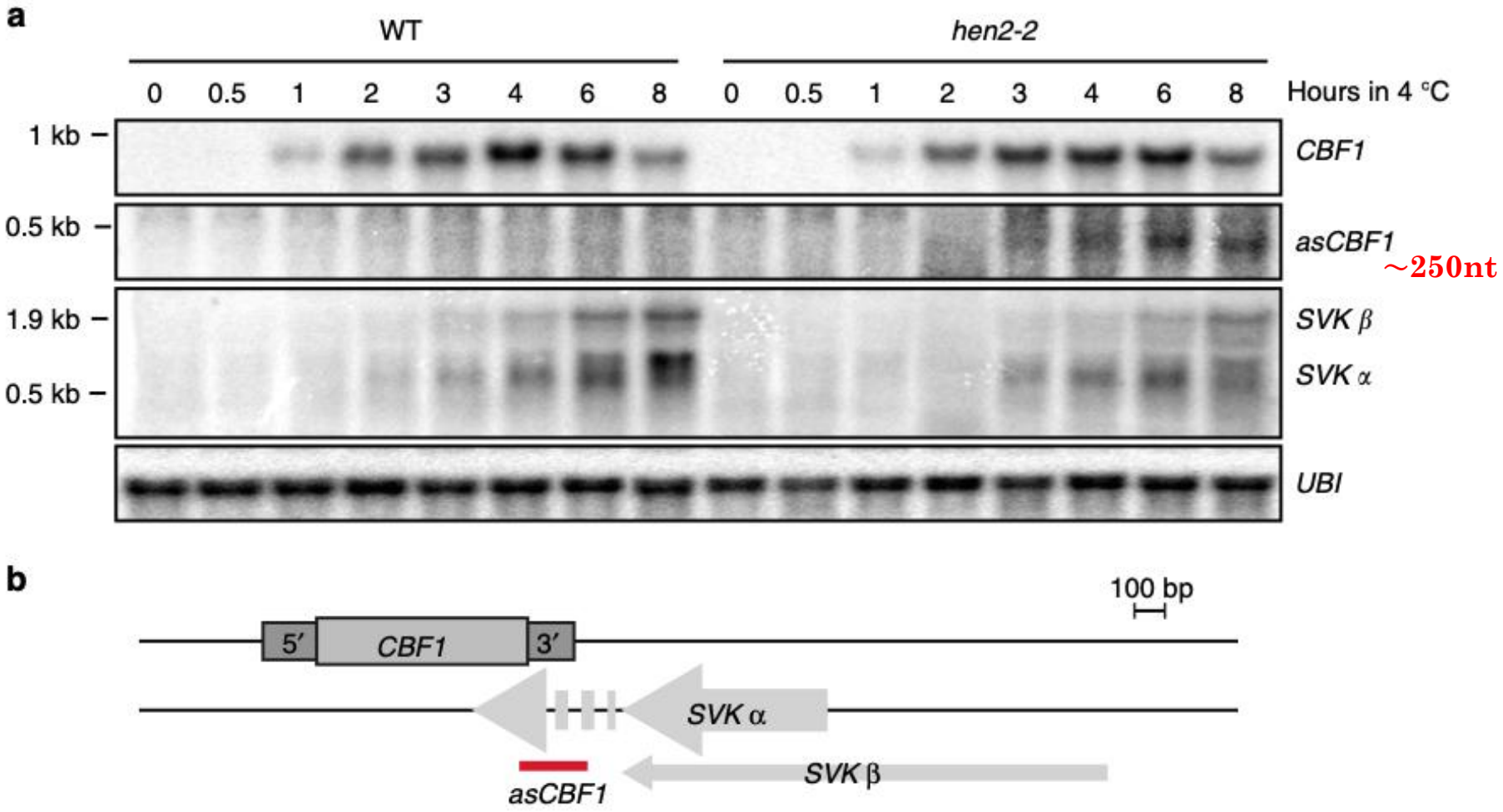
Read-through transcription of *CBF1* is reduced by the T-DNA insertions in *svk-1* and *uns-1*, thus increasing the stability of *CBF1* mRNA

- ✓ **Increased *CBF1* read-through transcription in the exoribonucleases mutant (*xrn3*) (XRN3 mediates transcriptional termination)**
- ✓ **NOT decreased read-through transcription in *svk-* and *uns-1***



New Hypothesis:

SVK promotes transcription of a cryptic as transcript into the *CBF1* gene body, which would be disrupted in *uns-1* and *svk-1*



HEN2 is part of the nucleoplasmic 3′ to 5′ exosome responsible for degrading many types of non-coding RNAPII transcripts

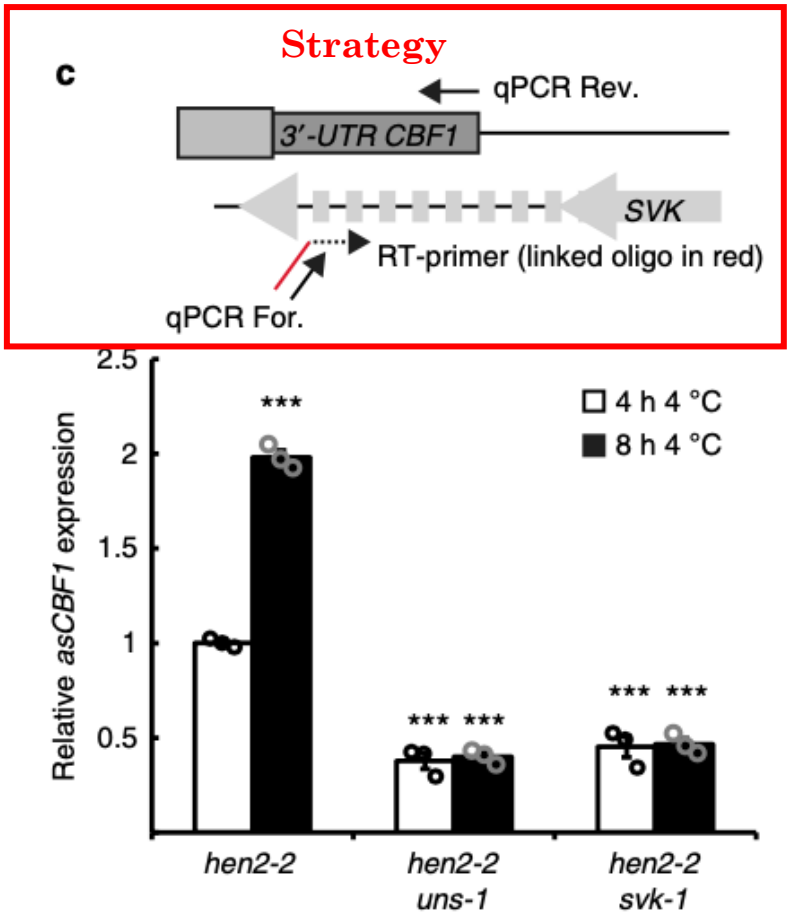
Lack of HEN2 results in accumulation of as transcripts

Question:

Does asCBF1 depend on SVALKA transcription?

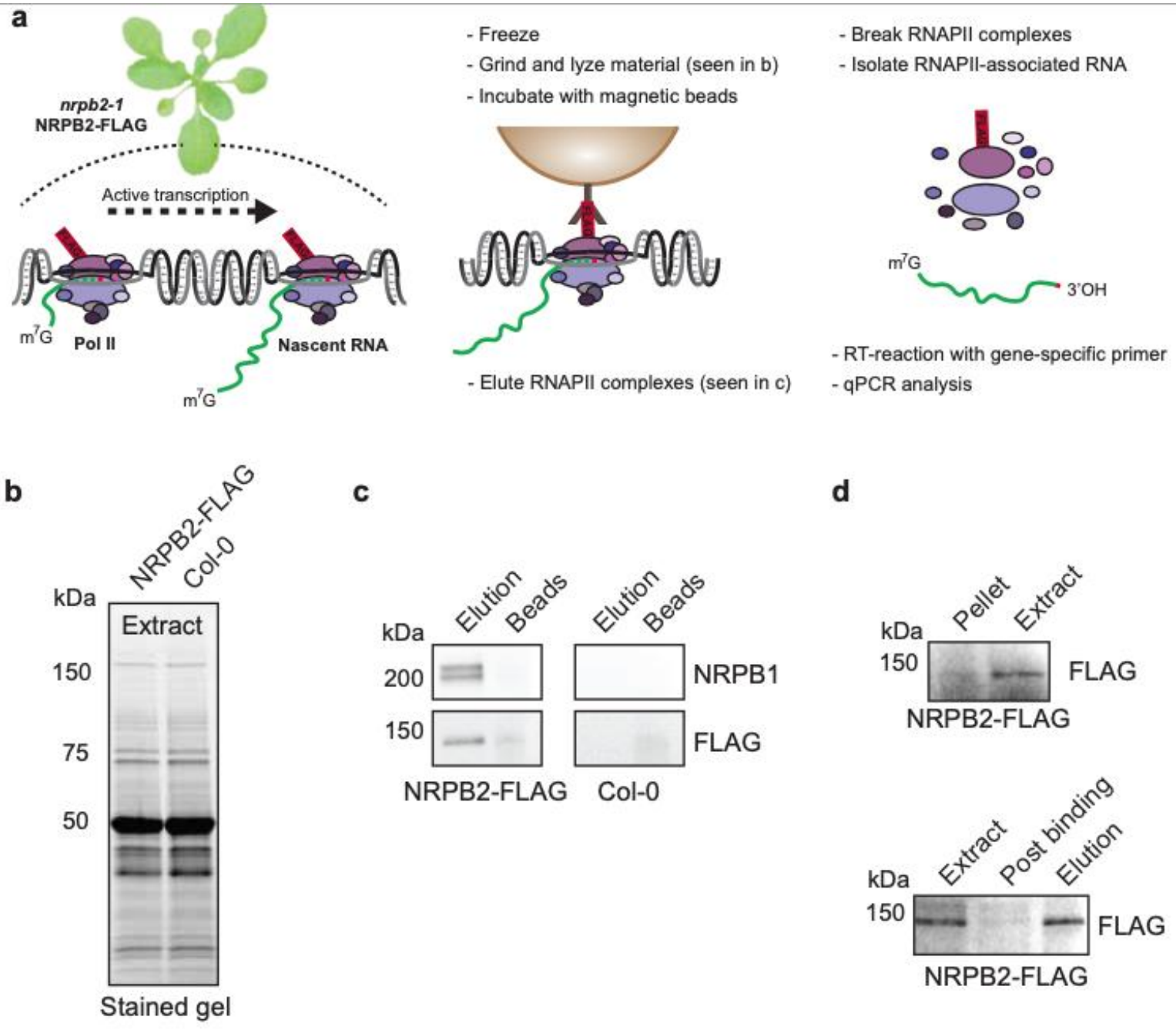
Experimental «answer»:

***hen2-2uns-1* and *hen2-2svk-1* double mutant analysis**



RNAPII-IP (RNA attached to RNAPII) vs total RNA (see next)

Flow-chart of purification of nascent RNA

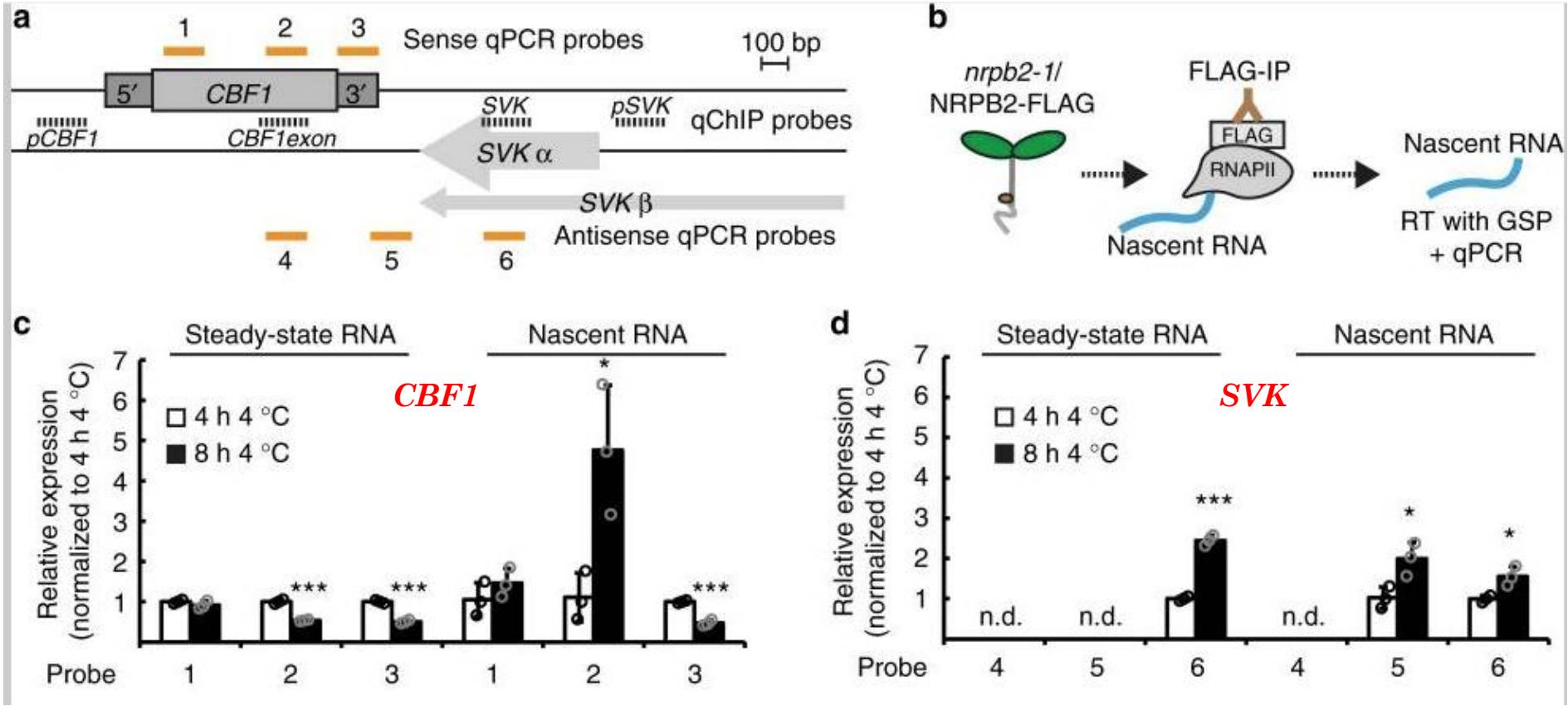


Question:

Which is the mechanism of SVK-mediated effect on *CBF1* expression?

Hypothesis:

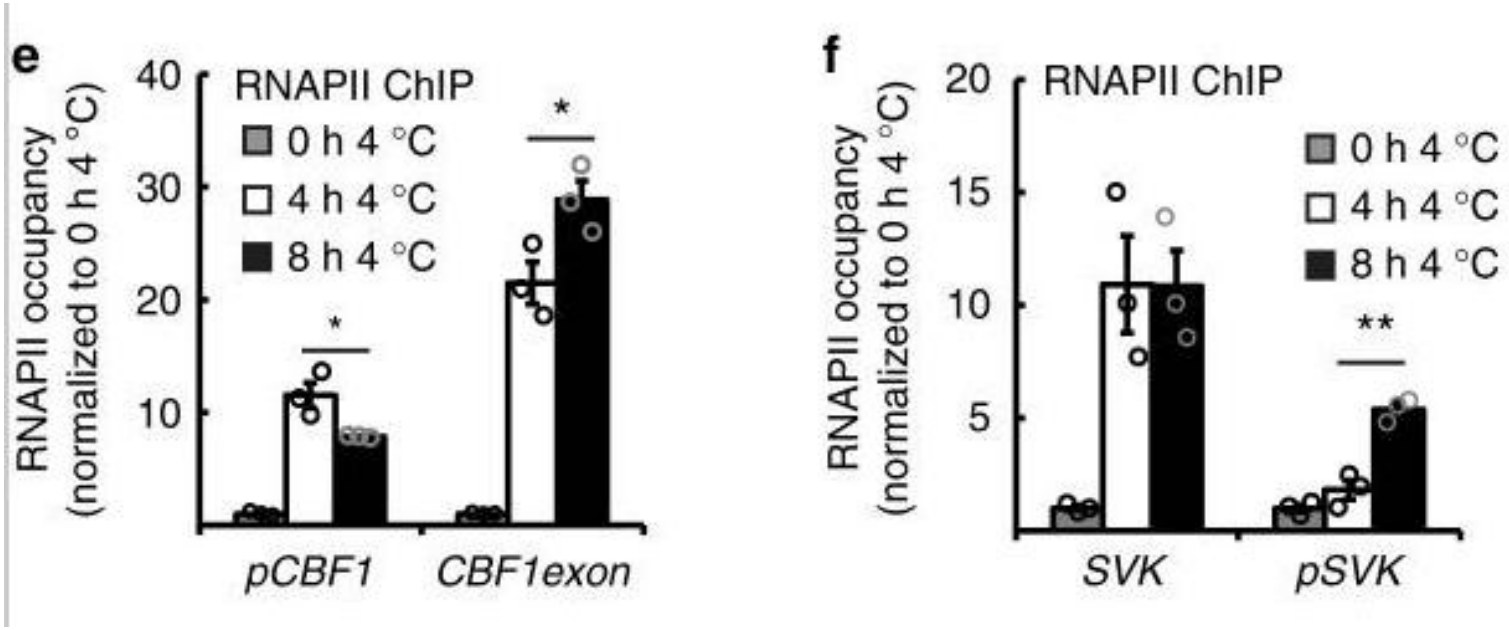
A RNAPII collision model, predicting a discrepancy of transcription between the *CBF1* 5′- and 3′-end due to stalled RNAPII complexes in the *CBF1* 3′-end



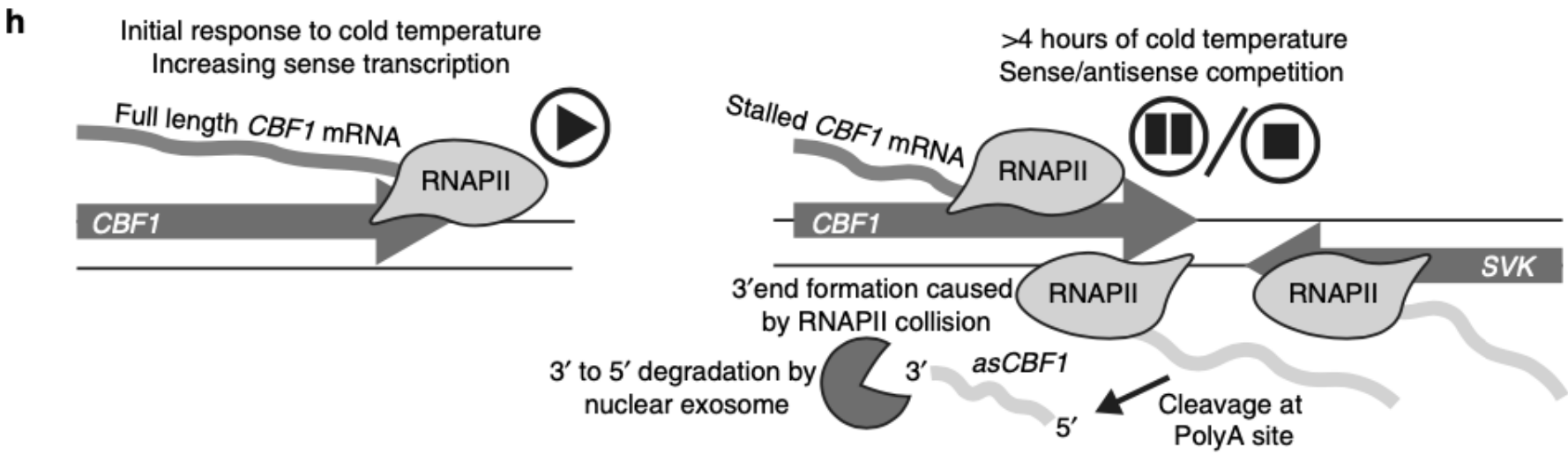
RNAPII complexes are terminated before transcription reaches further into the CBF1 exon, consistent with *asCBF1* size

To corroborate these results….

Total RNAPII quantitative Chromatin Immuno-Precipitation (qChIP)



- ✓ **Higher RNAPII occupancy over the CBF1 exon at 8 h 4°**
- ✓ **Higher RNAPII occupancy over the SVK exon at both 4 and 8 h 4° compared to ctrl**
- ✓ **RNAPII complexes stalled in *CBF1* 3′ as fl *CBF1* levels decrease at 8 h 4°**



Mechanistic model of how SVK transcription represses s*CBF1* transcription. During early cold exposure, *SVK* is not expressed and *sCBF1* can be transcribed (left). *CBF1* expression peaks at 4 h cold. Simultaneously, *SVK* expression is increased (right). SVK Read-through transcription results in transcription antisense to *CBF1* 3′-end of and increase of RNAPII occupancy on both strands. This creates RNAPII collision and stalling of *sCBF1* transcription.

Outcome: decrease of full-length *CBF1* mRNA to prevent an over-respone to cold


The End