Calcium Regulation of Transcription in Plants
Professor Hillel Fromm (PhD)

Lecture topics

- Overview:
  - Origin and status of Ca\(^{2+}\) in plant cells
  - Ca\(^{2+}\) signals in the cytosol and in the nucleus
- How calcium signals regulate gene expression
- Roles of calcium-regulated transcription in plant defenses (biotic & abiotic stresses)
- Calcium as a modulator of hormonal function and plant growth:
  - key role in plant phenotypic plasticity

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Early cellular evolution
- On rate binding: $10^{-9}$
- Strong binding
- High availability
- Limit of solubility: $10^{-5}$ M

1-10 mM Ca$^{2+}$

Sensors

Effectors

Ca$^{2+}$

200 nM

1-10 mM Ca$^{2+}$

Ca$^{2+}$ pump

Ca$^{2+}$ channel

Apoplast Ca$^{2+}$ 1-10 mM

Vacuole 1-10 mM Ca$^{2+}$

Cytosolic Ca$^{2+}$ ~ 100-200 nM

ATP ADP

1 mM

Ca$^{2+}$ pump

Ca$^{2+}$ channel

Nucleus ~ 50 nM

1 μM

Ca$^{2+}$ signals in the cell nucleus

Ca$^{2+}$ pump

Ca$^{2+}$ channel

Cyotosol

Nucleosol

Pauly et al. (2000)

Mcllvanan et al. (2003)

Jaiswal (2001); Williams (2006)

Kudla et al. (2010); Dodd et al. (2010);

Reddy et al. (2011); McAinsh & Pittman (2009)

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Cytoplasm Nucleoplasm
Ca²⁺-regulated gene expression

TFs cis-elements Transcriptome

Sensor

Genes

Pauly et al. (2000)
Bootman et al. (2009)

Ca²⁺/Calmodulin

Structures are derived from X-ray diffraction analysis of crystal structures;
Figures were prepared with the MOLMOL program; Bouché et al. (2005).

Plant calmodulins (CaMs) and calmodulin-like (CMLs) proteins

CaM

Anastrepsis TCH3

EF-hand Ca²⁺-binding site

Wheat CaM-1

Rice CaM11/Petunia CaM3

Arabidopsis TCH1

Snedden & Fromm, 1998

**Rodriguez-Concepcion et al. 1999

The Anastrepsis genome contains ~ 50 genes encoding CaMs and CalM-like (CML) proteins (McCormack et al. 2005), and ~ 250 different putative EF-hand calcium-binding proteins (Ray et al., 2002).
Plant proteins associated with calmodulin

- **Response to pathogens**
  - MLO protein (barley PM receptor)
- **DNA binding**
  - CAMTA
  - Myb2
  - WRKY10
  - GT1
- **Metabolism**
  - Glutamate decarboxylase (GAD)
  - NAD kinase
  - Catalase
  - Phospholipids
- **DNA binding domain purification**
  - IQ
  - DNABD
  - CMBD

CAMTA binding sites

- CAMTA binds to DNA through its DNA-binding domain.
- CAMTA proteins include CAMTA1-147 His-Tag DNA binding domain purification.
- CAMTA binding sites include G-box ABRE CA CGTG(T/G/C)
- Weak binding includes (CT) (CA/CCTG T/G/C)
- Strong binding includes ABRE-CG motif (A/G) CGCG(T/G/C)
- Probe includes CGCG and CATG.
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**CM2: a CAMTA binding site in DREB1c (CBF2) promoters required for freezing tolerance**

- DREB1 - drought responsive element binding factor 1 that binds to DRE (dehydration-responsive regulatory element)
- ABRE – ABA-response element (ACGT)

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**How Ca²⁺ regulates transcription: principal mechanisms**

Ca²⁺ may function to activate or repress transcription depending on the TF, cis-elements, and physiological context.

Examples: Arabidopsis CaM7 and NIG1, human DREAM protein

- Z-box: ATACGTG
- G-box: CACGTG
- E-box: CANNTG

Ca²⁺-regulated TFs

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**Arabidopsis CaM7 functions as a Ca²⁺-regulated TF**

Light-regulated genes

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**Ca^2+/-CaM-regulated TFs**

Examples of DNA binding sites of CaM-binding TFs in plants:

<table>
<thead>
<tr>
<th>TF</th>
<th>Binding Motif</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBP90</td>
<td>(C/A)CGCG</td>
</tr>
<tr>
<td>WRKY1</td>
<td>(C/T)TCG</td>
</tr>
<tr>
<td>MYC52</td>
<td>(C/T)ACG</td>
</tr>
<tr>
<td>NAC1</td>
<td>TTGCTT</td>
</tr>
<tr>
<td>ZBP1</td>
<td>(T/G)ACGT</td>
</tr>
<tr>
<td>MADS box</td>
<td>CGAG/GG</td>
</tr>
</tbody>
</table>

*Most of these TFs are members of gene families; not all members of these families bind CaM, and their DNA-binding specificities may also differ from the above indicated sequences.*

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CAMTA3 is a negative regulator of defense responses; Ca^2+/-CaM binding to CAMTA3 relieves the repression of expression of defense genes.

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Transcriptome analysis of *camta3* mutants

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*camta3* T-DNA insertion mutants have necrotic lesions on leaves

![Wild type, camta3-1, camta3-2 plants](image)

**Galon et al. (2008)**

*camta3* mutants are hyper-resistant to fungal and bacteria pathogens

**Botrytis cinerea**

Pathogenic fungus

**Pseudomonas syringae**

Pathogenic bacteria

**Galon et al. (2008)**

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**Complex Ca^{2+}-regulated transcription networks in plant defenses**

The Ca^{2+}-dependent calmodulin-binding CBP60g transcription factors is a positive regulator of salicylic acid mediated defense responses downstream of EDS1 (Wang et al. 2009; 2011)

![CBP60g diagram](image)

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**Ca^{2+}-regulated TFs in abiotic stress responses**

<table>
<thead>
<tr>
<th>Salt tolerance</th>
<th>Drought tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myb2</td>
<td>GTL1</td>
</tr>
<tr>
<td>(C/T)AAC(C/T)(G/A)</td>
<td></td>
</tr>
<tr>
<td>NIG</td>
<td></td>
</tr>
<tr>
<td>CANNYTG (E-box)</td>
<td></td>
</tr>
<tr>
<td>Transcription activators</td>
<td>Stomata number</td>
</tr>
<tr>
<td></td>
<td>Drought tolerance</td>
</tr>
</tbody>
</table>

Reddy et al. (2011)  
Yu et al. (2010)

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**Ca^{2+}-regulated protein kinases and phosphatases modifying TFs**

- CDPKs
- CCMKs
- CaMBK
- CIPKs
- CBLs
- TF

| CBL = Calcineurin B-like
| CIPK = CBL-interacting protein kinase

Reddy et al. (2010)  
Kudla et al. (2010)  
De Falco et al. (2010)

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**Ca^{2+}-dependent thermotolerance**

Heat

Ca^{2+}-dependent phosphorylation of heat shock TF

Ca^{2+}-dependent de-phosphorylation of heat shock TF

Enhanced CAMTA1 expression

Galon et al. (2010a)

Reddy et al. (2011)

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Cis-elements mediating Ca\(^{2+}\)-regulated transcription

Bioinformatic analysis of promoters of Ca\(^{2+}\)-up-regulated genes

- Align ACE motifs
- Finds over-represented exact short sequences within the set of promoters, compared to whole genome regulatory domains
- RSA-tools: oligo-analysis motifs
- Finds short non-exact repetitive motifs within the set of promoters

\[ \text{ABRE} = \text{ABA-responsive element} \]
\[ \text{ABRE/T} = \text{Weak CAMTA binding sites} \]
\[ \text{ABRE/CE} = \text{Strong CAMTA binding sites} \]

Experimental assessment of bioinformatic predictions

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**Cis-elements, transcription factors, and target genes in Ca²⁺ signaling pathways**

<table>
<thead>
<tr>
<th>Motif</th>
<th>cis-element</th>
<th>TF</th>
<th>Target genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA²⁺ response element</td>
<td>[ATTG]</td>
<td>Ca²⁺-regulated TFs</td>
<td>RD29A, RD29B, RAB18, KAT1, KAT2, CHS, RBCS, ABF1-4 (bZIP); Ca²⁺-regulated kinas e (CDPK)</td>
</tr>
<tr>
<td>CAMTA-binding element</td>
<td>[ATTTG]</td>
<td>CAMTA, APX</td>
<td>Light-stress response genes, growth regulation, CAMTA-binding genes</td>
</tr>
<tr>
<td>Other</td>
<td>[TCTG]</td>
<td>Ca²⁺-regulated TFs</td>
<td>RD29A, RD29B, RAB18, KAT1, KAT2, CHS, RBCS, ABF1-4 (bZIP); Ca²⁺-regulated kinas e (CDPK)</td>
</tr>
</tbody>
</table>

**Continued...cis-elements, TFs, and target genes in Ca²⁺ signaling pathways**

<table>
<thead>
<tr>
<th>Motif</th>
<th>cis-element</th>
<th>TF</th>
<th>Target genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BARE1T retention site</td>
<td>[TATTIC]</td>
<td>CAMTA, APX</td>
<td>Light-stress response genes, growth regulation, CAMTA-binding genes</td>
</tr>
<tr>
<td>BARE1T retention site</td>
<td>[TATTIC]</td>
<td>CAMTA, APX</td>
<td>Light-stress response genes, growth regulation, CAMTA-binding genes</td>
</tr>
<tr>
<td>Site II</td>
<td>[ATGGGCCCT]</td>
<td>TCPs</td>
<td>Possibly TCPs, Ca²⁺-dependent mechanisms, Ca²⁺-regulated TFs</td>
</tr>
</tbody>
</table>

**Ca²⁺-regulated transcription factors and plant growth**

CAMTA1 expression profile is reminiscent of auxin-responsive genes.
Reverse-genetics approaches to elucidate the function and mode of operation of calcium-regulated TFs

![Diagram of T-DNA insertion mutants]

CAMTA1-SRDX: dominant negative transcriptional repressor

![Diagram of CAMTA1-SRDX construct]

Hyper-responsiveness to auxin of dark-grown hypocotyl elongation in *camta1* mutants and CAMTA1-repressor line

![Diagram of auxin response experiment]

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CAMTA1 gene expression responds to abiotic stresses

150mM NaCl

Galon et al. (2010a)

Complex interactions among CAMTA genes

Hormone-dependent growth responses

CAMTA1 CAMTA2 CAMTA3 CAMTA4 CAMTA5 CAMTA6

Abiotic stimuli Biotic stimuli

Senescence

Galon et al. (2010b)

Developmental plasticity model

Environmental cues (biotic and abiotic)

Ca²⁺ signaling

Ca²⁺-regulated TFs

"Biotic & abiotic stress responses"

Hormones

Developmental cues Intrinsic pathways

From cell proliferation to senescence

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