Basal Signaling Regulates Plant Growth and Development[1]

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The term signal transduction refers to the classical paradigm where an external stimulus is sensed and initiates an increase in second messengers. Each second messenger transmits and amplifies the signal by activating a subset of downstream pathways. This complex network of interwoven downstream events ultimately converges to produce measurable responses. While the paradigms for signal transduction are well known, little consideration has been given to the second messengers in the nonstimulated cell, the basal signal, and yet, basal signals also impact plant growth and development.

Basal signals are low levels of oscillating second messengers that are being sensed by the cell. These are difficult to measure and are often considered to be background noise. Not surprisingly, there is a dearth of knowledge about how plants respond to this constant flux of signaling metabolites or about how basal signals define homeostasis or contribute to species diversity. Both genetics and environment will define the basal signal of a cell. To truly understand how plants regulate growth and development, future research must focus on understanding how basal signals regulate fundamental metabolism.

Our hypothesis is that in a nonstimulated cell, a low level of basal signal will constitutively repress some downstream events and stimulate others. If this is true, then lowering the basal level of a second messenger should derepress or enhance those downstream events specifically targeted by this second messenger, while events that depend on the basal level of second messenger will not be activated (Fig. 1).

Monitoring rapid, transient changes in second messengers within stimulated cells is challenge enough. The problem is amplified when trying to measure rapidly oscillating basal signals. There are methods for monitoring external oscillations of ions and metabolites using vibrating probes (Jaffe and Nuccitelli, 1974; Shabala et al., 1997) and recently developed, self-referencing biosensors (Porterfield, 2007; McLamore et al., 2010), respectively. Similar technical advances are needed for studying fluxes within cells. In vivo fluorescent reporters can indicate changes in stimulus-induced oscillations within single cells (Monshausen et al., 2008, 2009); however, the fluorophores, by necessity, are selected to report signals above the background noise so that the basal signals of a nonstimulated cell are often below the limits of detection. Furthermore, the basal levels of second messengers are usually at or below the limits of detection for metabolomic analyses, and metabolic fluxes are difficult to assess with metabolomics (Fernie et al., 2005).

Alternative approaches are needed to assess the impact of basal signals. In principle, if one lowered a basal signal, downstream events regulated by the second messenger in the nonstimulated cell would be revealed. Because basal signals are inherently a part of normal metabolism, it is difficult to lower basal signals; however, there are several examples where expression of genes from heterologous systems has led to important insights. One of the first studies demonstrating the effect of lowering a basal signal came from the expression of the bacterial gene *nahG*, encoding salicylate hydroxylase, which metabolizes salicylic acid (SA; Gaffney et al., 1993; Delaney et al., 1994). Expression of *nahG* constitutively lowered the endogenous levels of SA and prevented a stimulus-induced increase, essentially dampening the signal. *NahG*-expressing plants have become the accepted test for determining whether SA is essential for a given response. Importantly, under nonstressed conditions, the *nahG*-expressing plants had significantly greater seed production (Cipollini, 2002). While it is not surprising that under stress conditions, diverting resources to defense-related pathways would decrease biomass production (Baldwin, 1998; De Block et al., 2005; Vanderauwera et al., 2007; Wasternack, 2007), the work also indicates that under nonstressed conditions
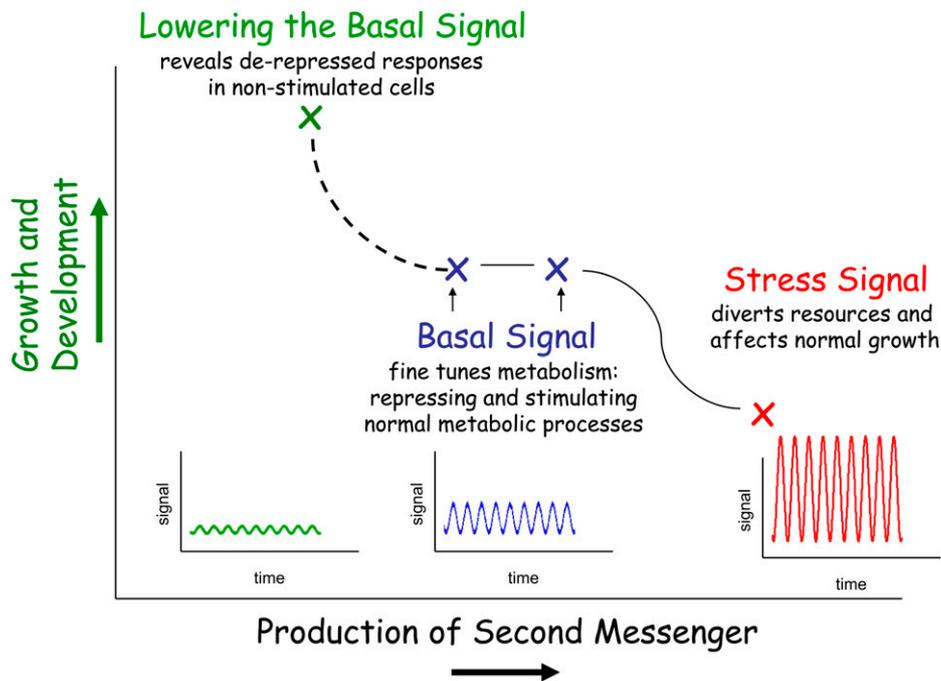
Figure 1. Model illustrating how lowering basal signaling could impact growth and development. When the cell senses changes in extracellular cues, stimulus-induced signaling diverts resources from primary metabolism and may adversely affect normal plant growth (Stress Signal, red). In nonstimulated cells there is a basal flux of metabolism that generates low, oscillating signals (Basal Signal, blue). These basal signals are determined by the genetics and environment of a given organism and fine tune metabolism by repressing or enhancing metabolic events within the cell. If the basal signals are lowered (Lowering the Basal Signal, green), downstream events that were repressed should now be derepressed, and those that were stimulated by the basal signal should decrease. In this manner, lowering the basal signal could enhance growth and development. Whether the plants with lower basal signaling would be more or less tolerant of stress would depend on whether the second messenger was necessary for stress tolerance and whether compensatory tolerance pathways were induced.

lowering the basal signal (in this instance SA) increased biomass.

Constitutive lowering of second messengers can be used to gain insights into events downstream of basal signals as well as stimulus-induced signaling events. There are several reports where second messengers such as calcium have been altered by sequestering or chemically removing them from cells (Hirschi, 1999, 2004; Persson et al., 2001; Wu et al., 2002; Wyatt et al., 2002; Mei et al., 2007); however, for the purpose of this discussion and to illustrate the effects of lowering basal signaling, we will focus on two examples where, like *nahG*, heterologous gene expression was used to effectively dampen basal signaling by increasing the metabolism of a specific second messenger. While some of the plant phenotypes might have been predicted based on signal transduction paradigms, others were unexpected, and it was these results that prompted us to look closer at the effects of basal signaling.

In the first example, Arabidopsis (*Arabidopsis thaliana*) plants expressing the human type I inositol polyphosphate 5-phosphatase (InsP 5-ptase) had the second messenger, inositol (1,4,5) trisphosphate (InsP$_3$) reduced to about 2% to 5% of wild-type plants. Even when the plants were stimulated, the InsP$_3$ never rose above the basal levels of the wild-type plants

(Perera et al., 2006). Lowering the InsP$_3$-mediated signals did not visibly affect growth under normal conditions, suggesting that in Arabidopsis, InsP$_3$ was not a limiting factor for normal growth. Based on the normal growth phenotype, it would be easy to dismiss InsP$_3$-mediated signaling as unimportant for plants, but this would be short sighted. For example, predicted InsP$_3$-mediated responses such as gravitropism were delayed in the InsP 5-ptase transgenic plants (Perera et al., 2006). The delayed response to gravity was evident in both shoots and roots, indicating that InsP$_3$ was a universal signal that contributed to gravitropic bending. Further studies also revealed that about 30% of the total calcium signal induced by osmotic stress and cold shock was either directly or indirectly mediated by InsP$_3$ (Perera et al., 2008). These results are predicted by the classical InsP$_3$ signaling paradigms. However, the paradigms also predict that constitutively decreasing the levels of InsP$_3$ and calcium would decrease stomatal closure and decrease drought tolerance.

Paradoxically, the human InsP 5-ptase plants were more drought tolerant (Perera et al., 2008). Another puzzling observation was the delayed production of abscisic acid when the InsP 5-ptase plants were not watered. What was discovered from these studies was

that under normal growth conditions, unpredicted, compensatory pathways increased when $InsP_3$ was constitutively lowered. The induced pathways appeared to enhance drought tolerance in spite of the lower abscisic acid and decrease in $InsP_3$-mediated calcium signal. One contributing factor to drought tolerance was that a subset of DREB2A-regulated transcripts was up-regulated in the human InsP 5-ptase plants. A total of seven transcripts associated with enhanced drought tolerance were constitutively increased. These transcripts had not been previously associated with $InsP_3$-mediated signaling. One interpretation of these results is that lowering the basal $InsP_3$ level led to increased expression of selective drought-stress related genes. This would suggest that the basal levels of $InsP_3$ and $InsP_3$-mediated events are normally involved in repressing the expression of these genes. The insights from these studies revealed potential targets for improving drought tolerance without affecting normal growth.

In addition to inducing compensatory pathways in Arabidopsis at the transcript level, in rapidly growing tobacco (*Nicotiana tabacum*) suspension culture cells, the increased metabolism of $InsP_3$ by the human InsP 5-ptase increased the flux through the phosphoinositide pathway and lowered the total $PtdInsP_2$ (Perera et al., 2002). When the outward rectifying K^+ channel (NtORK) activity was monitored using protoplasts in the whole-cell patch-clamp configuration, the InsP 5-ptase-expressing tobacco cells had an elevated NtORK channel activity (Ma et al., 2009). The change in channel activity is consistent with increased drought tolerance (Perera et al., 2008). Moreover, the data demonstrate that increasing the metabolism of $InsP_3$ went well beyond lowering cytosolic $InsP_3$ levels and had a specific effect on membrane proteins.

While Arabidopsis and tobacco are good model systems, studies using tomatoes (*Solanum lycopersicum* 'Micro-Tom') provided evidence that altering basal signaling in planta affects carbon partitioning. Khodakovskaya et al. (2010) showed that lowering basal $InsP_3$ in tomatoes also resulted in a drought-tolerant phenotype. However, the underlying mechanisms controlling the response appeared to be quite different. Transgenic tomato plants expressing the human InsP 5-ptase gene did not show the same changes in transcript profiles observed in Arabidopsis. There was no evidence for induction of the DREB2A-regulated pathway. Rather, the tomatoes had increased Glc and Fru (potential osmolytes) and when grown in nonlimiting phosphate (0.25 mM) had increased leaf and root dry weight (2- to 4-fold and 4- to 7-fold, respectively). All of these changes would contribute to the enhanced drought tolerance.

The tomato InsP 5-ptase transgenics, like the *nahG* transgenics, revealed that lowering the basal $InsP_3$ signal can affect primary metabolism and biomass production. The increase in stress tolerance and biomass production in the InsP 5-ptase tomato plants could have resulted from delayed $InsP_3$-mediated signaling, an increase in responses normally repressed by basal $InsP_3$, induction of other compensatory pathways, or all of the above. Clearly, more research is needed to understand the underlying mechanisms involved; however, there is no doubt that in both tomato and Arabidopsis drought tolerance was increased without sacrificing normal growth under optimal conditions. These studies provide fundamental insights into which pathways were directly or indirectly affected by increasing $InsP_3$ catabolism and lowering basal $InsP_3$.

Another example of lowering basal signaling and increasing stress tolerance is the expression of superoxide reductase (SOR) from a hyperthermophilic archaeon, *Pyrococcus furiosus* in plants. All aerobic organisms have superoxide detoxification mechanisms, usually superoxide dismutases. However, *P. furiosus*, an anaerobe that normally grows in deep thermal ocean vents, can be ejected in vent fluids into cold, aerated seawater, and when this happens, it uses SOR to reduce superoxide. SOR is more effective and efficient in removing superoxide than superoxide dismutase (Jenney et al., 1999; Emerson et al., 2003). Furthermore, SOR reduces superoxide to hydrogen peroxide without producing oxygen, and in the presence of ferrocyanide cofactors can reduce superoxide to water (Molina-Heredia et al., 2006). Arabidopsis plants expressing a fusion protein consisting of GFP and SOR (GFP-SOR) were more tolerant of heat stress than wild type or *GFP*-expressing plants (Im et al., 2009). Interestingly, both transcript and protein profiles of the GFP-SOR plants indicated a delayed response to heat stress. Stress-induced proteins such as heat shock protein 70 and the endoplasmic reticulum binding protein and chaperone (BiP) were lower in the SOR plants compared to wild-type plants under normal conditions, and induction of stress-protein production in SOR plants was delayed under heat stress conditions. In addition, the induction of transcripts encoding reactive oxygen species (ROS)-mediated transcription factors as well as ROS-scavenging enzymes such as ascorbic acid peroxidase was delayed in the SOR transgenic plants compared to wild-type controls. Because superoxide is both a potential second messenger and a toxic stress-induced compound, lowering superoxide may have simply decreased toxicity; however, ROS-sensitive transcript profiles indicate a delayed response to heat in a heat stress time course study. In spite of this delay, the GFP-SOR plants were more heat tolerant.

What caused the heat tolerance? The plants responded as though the rapid reduction of O_2^- decreased their ability to sense the heat stress and yet, they were more heat tolerant. It is possible that, as with the InsP 5-ptase plants, which seemed to be slower to sense drought stress, other compensatory pathways were induced in the GFP-SOR plants and that these pathways enhanced heat tolerance. More extensive molecular and biochemical studies are necessary to understand the full

impact of expressing GFP-SOR in planta and lowering
O_2^-. Importantly, because of the transient nature of
second messengers in general and of the difficulties of
studying reactive molecules such as superoxide, the
GFP-SOR plants provide a model system to identify
superoxide-mediated responses. Through this type of
study, downstream events normally induced by ROS
during stress signaling, as well as those repressed by
basal ROS, will become evident. These fundamental
insights will advance our basic understanding of signal
transduction while expanding potential approaches for
increasing stress tolerance without negatively impact-
ing biomass production.

Acclimating plants by incrementally increasing an
environmental stress usually increases their tolerance
to a subsequent stress. Centuries of empirical data
support the theory of plant acclimation. Based on
this, our initial prediction was that plants with com-
promised signaling would be less tolerant of stress.
However, we found that in many instances, this was
not true. One reason may be the evolutionary pres-
sure on sessile organisms to process and interpret
multiple second messenger signals prior to mounting
a response. In this case, if one signal is missing, others
will compensate or perhaps be enhanced so that
the plants will have a greater propensity for stress
tolerance.

It is intuitive to consider that plants, like humans,
can survive better in a stressful environment, when
they are strong and in good health. If lowering basal
signaling derepressed a subset of downstream events
that enhanced growth under nonstress conditions and
if the loss of the second messenger did not directly
compromise the response when the stress is imposed,
then plants with lower basal signaling would be more
stress tolerant (Fig. 1). There will be instances when a
particular second messenger is essential and limiting
for a specific stress response and when compensatory
pathways are not sufficient to ameliorate the response.
One example is SA in disease resistance. The *nahG*
plants have less SA and are less tolerant of pathogens.
Obviously, the outcome of lowering second messen-
gers will depend upon the second messenger that is
reduced, the compensatory pathways induced, and
the stimulus given.

Lowering basal signaling can reveal subtle, meta-
bolic, and transcriptional changes that would other-
wise be masked by normal metabolism. Furthermore,
when studying signal transduction pathways where
second messenger production is both rapid and tran-
sient, dampening or removing a signal to reveal
downstream events can be a useful tool for gaining
fundamental insights into the dynamic processes of
the plant signalsome.

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