# Calcium Signaling: Deciphering the Calcium–NFAT Pathway

**Rapid cellular calcium oscillations activate gene expression hours later. How this temporal response amplification is achieved has until now been largely a mystery. An elegant combination of experimental strategies and a model that encompasses non-linear inputs and outputs now sheds new light on this long-standing problem.**

**James W. Putney**

At some point in their growth, differentiation or function, every cell in the body is affected by calcium signaling, a process whereby external signals interact with cells to cause their cytoplasmic $Ca^{2+}$ to rise. This rise in cytoplasmic $Ca^{2+}$ triggers cellular responses over time courses that range from subsecond (neurotransmitter release for example) to hours or days (gene regulation and differentiation). In this issue of *Current Biology*, Kar *et al.* [1] describe a new and intriguing level of complexity in the process by which $Ca^{2+}$ signals regulate gene expression.

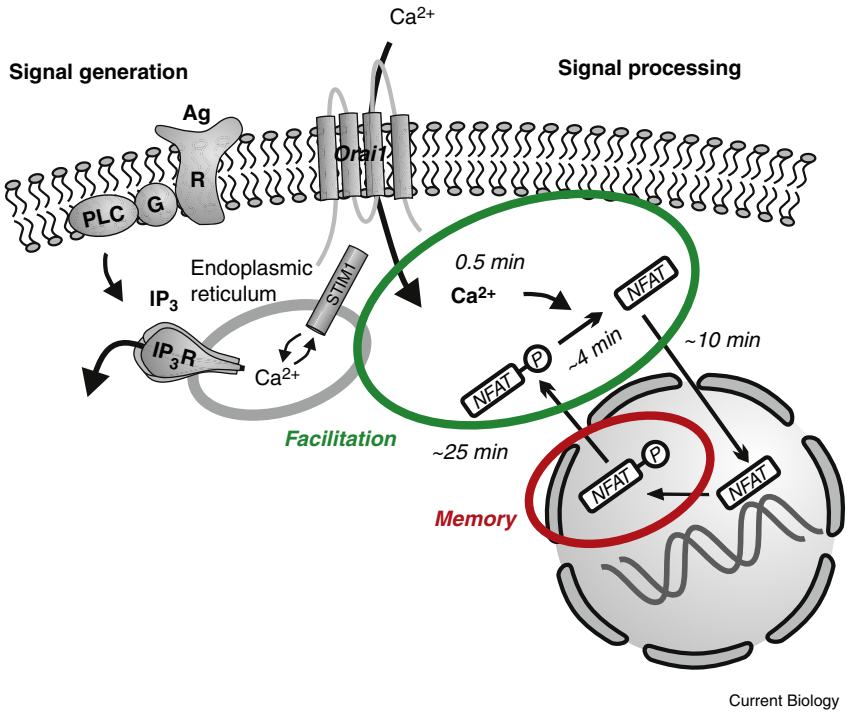


Figure 1. Pathway of calcium–NFAT signaling.

Calcium signaling mechanisms typically involve two distinct sets of reactions: one responsible for signal generation (producing the $Ca^{2+}$ signal) and one responsible for processing the signal into an appropriate downstream event (for example, gene transcription). In the study by Kar *et al.* [1], the pathway is initiated by a receptor (R)/G-protein (G)/phospholipase C (PLC) mechanism, resulting in the production of $IP_3$ and discharge of $Ca^{2+}$ stored in the endoplasmic reticulum via the $IP_3$ receptor ($IP_3R$). This leads to the activation of the $Ca^{2+}$ sensor STIM1, which in turn activates plasma membrane store-operated channels, comprising Orai1 subunits. $Ca^{2+}$ entering through the store-operated channels catalyzes the dephosphorylation of NFAT. Dephosphorylated NFAT translocates to the nucleus where it acts as a transcription factor for a specific set of genes. The process is reversible, as NFAT becomes re-phosphorylated and exits the nucleus. The findings of Kar *et al.* [1] define two critical non-linear components of the signal processing cascade. First, the $Ca^{2+}$ oscillatory period determines the rate of net cytoplasmic NFAT dephosphorylation and subsequent accumulation in the nucleus, a process of facilitation. Second, the exit rate of NFAT from the nucleus is slow, permitting a second level of integration, or 'memory', allotting sufficient time to activate gene transcription long after the $Ca^{2+}$ signal has ended.

The role of $Ca^{2+}$ signaling in muscle contraction has been appreciated for over a century [2], and its role in other relatively rapid responses for over fifty years [3]. The ability of $Ca^{2+}$ signals to activate gene expression has a more recent history (e.g. [4]), probably owing to difficulties in linking rapid cytoplasmic $Ca^{2+}$ elevations to substantially delayed biochemical events. However, it is now generally appreciated that $Ca^{2+}$ regulation of gene expression is a widely encountered signaling mechanism [5]. When $Ca^{2+}$ rises in the cytoplasm, it binds to its most common cognate receptor, calmodulin, which in turn can activate one of three major pathways. Two of these pathways culminate in activation of the transcriptional regulator CREB: one involves a calmodulin-dependent kinase cascade, and the other activates Ras followed by recruitment of the ERK pathway. In the third pathway, $Ca^{2+}$–calmodulin activates the widely expressed protein phosphatase calcineurin, leading to dephosphorylation of the NFAT transcriptional regulators, which permits their translocation into the nucleus to activate a distinct set of genes.

NFATs are a family of $Ca^{2+}$-dependent transcription factors that play a central role in the morphogenesis, development and physiological activities of numerous distinct cell types and organ systems. Four members of the NFAT family (NFAT1–4) are stimulated by a rise in cytoplasmic $Ca^{2+}$. In immune cells, activated NFAT regulates numerous inducible genes encoding cytokines and cell-surface receptors that are essential for T-cell development and effective immune responses. Calcineurin, a key element in the NFAT activation mechanism, is a major target for immunosuppressants like cyclosporin A.

Attention in recent years has turned to the nature (i.e. kinetics and localization) of the $Ca^{2+}$ signals that regulate gene expression. In the vast majority of instances, $Ca^{2+}$ signals involve a combination of $Ca^{2+}$ release from internal stores and $Ca^{2+}$ influx across the plasma membrane. And typically these two modes of signaling interact and regulate one another. In excitable cells, a common theme is activation of $Ca^{2+}$ release by $Ca^{2+}$ entering through voltage-activated $Ca^{2+}$ channels, a process known as calcium-induced calcium release [6]. In non-excitable cells, the most common scenario is an initial release of $Ca^{2+}$ stored in the endoplasmic reticulum, induced by inositol 1,4,5-trisphosphate ($IP_3$), which leads to the activation of plasma membrane store-operated $Ca^{2+}$ channels [7]. Under conditions of maximal activation, the combination of these two modes of $Ca^{2+}$ mobilization results in either a sustained elevation of cytoplasmic $Ca^{2+}$ or an elevation that declines slowly over time. However, it is unlikely that such monotonic signals occur often in cells under conditions of more physiological levels of activation. Rather, $Ca^{2+}$ signals more typically take on the appearance of transient, episodic elevations termed $Ca^{2+}$ spikes or $Ca^{2+}$ oscillations [8]. Oscillatory behavior generally reflects a regenerative, all-or-none component. In excitable cells this is usually a voltage-dependent $Ca^{2+}$ channel, or the intracellular calcium-induced calcium release ryanodine receptor. In non-excitable cells, there are two models for the mechanism of $Ca^{2+}$ oscillations. One involves regenerative and episodic activation of phospholipase C by $Ca^{2+}$, leading to bursts of $IP_3$ production. In an alternative model, $Ca^{2+}$ augmentation of $IP_3$-induced $Ca^{2+}$ discharge generates spikes of intracellular $Ca^{2+}$ release.

Regardless of the mechanism of $Ca^{2+}$ oscillations, there is a general consensus that they provide a digital mode of $Ca^{2+}$ signaling, resulting in a high signal-to-noise ratio. Previous studies have demonstrated that

artificially imposed $Ca^{2+}$ oscillations more efficiently activate downstream gene expression than sustained signals that provide a similar average $Ca^{2+}$ rise [9]. Digital all-or-none signals reduce ambiguity in signaling and provide checkpoints to prevent spurious low-level outputs. The simplest way in which this can be accomplished is by setting a significant threshold for detection at one or more steps in the pathway. Thus, in the case of $Ca^{2+}$ signaling, minor fluctuations in cytoplasmic $Ca^{2+}$ have no effect, but the regenerative nature of $Ca^{2+}$ oscillations assures some degree of signal recognition, even at low frequencies of oscillations.

In non-excitable cells, the burst of cytoplasmic $Ca^{2+}$ can be attributed almost exclusively to the release of store $Ca^{2+}$ by $IP_3$. Yet, in the absence of external $Ca^{2+}$, and thus without store-operated $Ca^{2+}$ entry, the oscillations run down and signaling fails. And, despite the fact that the visible cytoplasmic $Ca^{2+}$ signal results from intracellular release, there is strong evidence that, for the regulation of certain genes, it is the $Ca^{2+}$ entering through the store-operated channels that triggers the critical downstream pathways [10]. This requirement for $Ca^{2+}$ entering through the store-operated channels was specifically demonstrated for the NFAT pathway [11,12]. This must mean that spatially restricted signaling domains exist near the mouth of the store-operated channels, resulting from both a localization of the $Ca^{2+}$ sensor and high $Ca^{2+}$ levels close to the channels. In the new work by Kar *et al.* [1] an additional level of complexity and specifity in this signaling mechanism is revealed. Gene expression in the NFAT pathway was followed using an NFAT promoter–GFP reporter assay. While the total amount of GFP formed was a graded function of agonist concentration, gene expression occurred in an all-or-none manner at the single-cell level.

As alluded to above, a long-standing problem has been the mechanism of linking rapid and relatively transient $Ca^{2+}$ signals on a timescale of seconds/minutes to gene regulation occurring several hours later. Kar *et al.* [1] present novel kinetic data which they use to construct a feasible model demonstrating a series of integrating steps in the $Ca^{2+}$–NFAT–gene expression pathway (Figure 1). This pathway depends upon serial steps with short-term and long-term memories. That such memory occurs in the early steps of the pathway was elegantly demonstrated by experiments utilizing a paired-pulse strategy; paired $Ca^{2+}$ signals produced markedly supra-additive responses. Kar *et al.* [1] reason that such behavior can be readily reconciled with the relatively rapid kinetics of NFAT dephosphorylation and relatively slow export of NFAT from the nucleus.

Interestingly, all-or-none activation of gene expression was observed with a physiological agonist (leukotriene $C_4$), or with the $Ca^{2+}$ pump inhibitor thapsigargin, under conditions of graded $Ca^{2+}$ influx. Thus, the physiological pathway leading to gene expression may have multiple all-or-none checkpoints. There is evidence that the all-or-none $Ca^{2+}$ discharge in oscillating cells is necessary to reach a threshold for activation of store-operated channels [13]. In the current study from Kar *et al.* [1], a threshold of $Ca^{2+}$ elevation in the vicinity of the store-operated channels seems necessary to initiate the pathway, probably by activation of spatially sequestered calmodulin. This then is linked to an all-or-none activation of NFAT-regulated gene expression, apparently linked to a threshold of NFAT dephosphorylation and translocation. This complexity can provide multiple points for regulation, as well as a high safety factor for assuring appropriate timing of gene regulation pathways.

## References

1. Kar, P., Nelson, C., and Parekh, A.B. (2012). CRAC channels drive digital activation and provide analog control and synergy to $Ca^{2+}$-dependent gene regulation. Curr. Biol. *22*, 242–247.
2. Ringer, S. (1883). A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. J. Physiol. *4*, 29–42.
3. Douglas, W.W., and Rubin, R.P. (1961). The role of calcium in the secretory response of the adrenal medulla to acetylcholine. J. Physiol. *159*, 40–57.
4. Curran, T., Bravo, R., and Muller, R. (1985). Transient induction of c-fos and c-myc in an immediate consequence of growth factor stimulation. Cancer Surv. *4*, 655–681.
5. Bading, H., Hardingham, G.E., Johnson, C.M., and Chawla, S. (1997). Gene regulation by nuclear and cytoplasmic calcium signals. Biochem. Biophys. Res. Comm. *236*, 541–543.
6. Fill, M., and Copello, J.A. (2002). Ryanodine receptor calcium release channels. Physiol. Rev. *82*, 893–922.
7. Parekh, A.B., and Putney, J.W. (2005). Store-operated calcium channels. Physiol. Rev. *85*, 757–810.
8. Dupont, G., Combettes, L., Bird, G.S., and Putney, J.W. (2011). Calcium oscillations. Cold Spring Harb. Perspect. Biol. *3*, doi: 10.1101/cshperspect.a004226.
9. Dolmetsch, R.E., Xu, K.L., and Lewis, R.S. (1998). Calcium oscillations increase the efficiency and specificity of gene expression. Nature *392*, 933–936.
10. Di Capite, J., Ng, S.W., and Parekh, A.B. (2009). Decoding of cytoplasmic Ca(2+) oscillations through the spatial signature drives gene expression. Curr. Biol. *19*, 853–858.
11. Kar, P., Nelson, C., and Parekh, A.B. (2011). Selective activation of the transcription factor NFAT1 by calcium microdomains near Ca2+ release-activated Ca2+ (CRAC) channels. J. Biol. Chem. *286*, 14795–14803.
12. Feske, S., Gwack, Y., Prakriya, M., Srikanth, S., Puppel, S.H., Tanasa, B., Hogan, P.G., Lewis, R.S., Daly, M., and Rao, A. (2006). A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature *441*, 179–185.
13. Bird, G.S., Hwang, S.Y., Smyth, J.T., Fukushima, M., Boyles, R.R., and Putney, J.W., Jr. (2009). STIM1 is a calcium sensor specialized for digital signaling. Curr. Biol. *19*, 1724–1729.

National Institute of Environmental Health Sciences – NIH, Department of Health and Human Services, PO Box 12233, Research Triangle Park, NC 27709, USA.
E-mail: putney@niehs.nih.gov

DOI: 10.1016/j.cub.2011.12.030