Communication between the cell surface and the nucleus is essential for regulated gene expression. In neurons, Ca\textsuperscript{2+}-dependent gene transcription is sensitive to local Ca\textsuperscript{2+} entry. In immune cells, excitation-transcription coupling is thought to involve global Ca\textsuperscript{2+} signals. Here, we show that in mast cells, Ca\textsuperscript{2+} microdomains from store-operated Ca\textsuperscript{2+} release-activated Ca\textsuperscript{2+} channels activate expression of the transcription factor c-fos. Local Ca\textsuperscript{2+} entry is sensed by the tyrosine kinase Syk, which signals to the nucleus through the transcription factor STAT5. Ca\textsuperscript{2+} microdomains also promote secretion of proinflammatory messengers, which, like gene expression, require Syk. Syk therefore couples Ca\textsuperscript{2+} microdomains to the activation of two spatially and temporally distinct cellular responses, revealing the versatility of local Ca\textsuperscript{2+} signals in driving cell activation.

Ca\textsuperscript{2+} is a universal intracellular messenger, which activates a wide array of important cellular responses, including secretion, mitochondrial metabolism, gene expression, and cell growth and differentiation (1). Because cells can respond to Ca\textsuperscript{2+} by generating more than one type of Ca\textsuperscript{2+}-dependent response, a fundamental question concerns how specificity can occur to such a multifarious signal (2, 3). Growing evidence points to a major role for local Ca\textsuperscript{2+} signals in activating specific cellular targets (4). The simplest form of a local Ca\textsuperscript{2+} signal is a Ca\textsuperscript{2+} microdomain, which occurs following the opening of a Ca\textsuperscript{2+}-permeable channel in either the plasma membrane or intracellular organelles (5). Because the volume a microdomain occupies is extremely small, the Ca\textsuperscript{2+} concentration can rise to reach levels that are orders of magnitude greater than the bulk cytoplasmic Ca\textsuperscript{2+} rise (3, 5).

Store-operated Ca\textsuperscript{2+} channels are the major route for agonist-evoked Ca\textsuperscript{2+} entry in non-excitable cells and open following stimulation of phospholipase C-coupled receptors (6). These receptors generate inositol 1,4,5-trisphosphate, which releases Ca\textsuperscript{2+} from the endoplasmic reticulum (ER) (7). The fall in Ca\textsuperscript{2+} content within the store is detected by the ER Ca\textsuperscript{2+} sensor STIM1, which migrates to specialized ER-plasma membrane junctions, where it opens the store-operated channels (8). The best characterized store-operated channel is the Ca\textsuperscript{2+} release-activated Ca\textsuperscript{2+} (CRAC) channel (6), the pore-forming subunit of which is composed of Orai1 (9–12). Ca\textsuperscript{2+} entry through CRAC channels regulates enzyme activity, secretion, gene expression, and cell growth and proliferation (13).

Here, we have examined whether excitation-transcription coupling is driven by Ca\textsuperscript{2+} microdomains arising from open CRAC channels in mast cells. In T cells, NFAT-dependent gene expression requires a global Ca\textsuperscript{2+} rise (14–16). We find that local Ca\textsuperscript{2+} influx signals to the nucleus much more effectively than a robust bulk Ca\textsuperscript{2+} rise. This leads to the expression of the transcription factor c-fos, a regulator of proinflammatory gene expression (17). Furthermore, the non-receptor tyrosine kinase Syk clusters at the cell periphery and couples Ca\textsuperscript{2+} microdomains to c-fos expression through recruitment of the cytoplasmic transcription factor STAT5 in a protein kinase C- and MEK/ERK-independent pathway. Ca\textsuperscript{2+} microdomains following CRAC channel activation also activate Ca\textsuperscript{2+}-dependent phospholipase A\textsubscript{2}, followed by secretion of cysteiny1 leukotrienes (18). However, unlike c-fos expression, this is mediated via the MEK/ERK pathway. Parallel processing of the Ca\textsuperscript{2+} microdomain by Syk through two distinct signaling pathways constitutes a novel mechanism to evoke spatially and temporally different cellular responses.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Rat basophilic leukemia-1 (RBL-1), an immortalized mast cell line, was bought from ATCC. Cells were cultured (37 °C, 5% CO\textsubscript{2}) in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum, 2 mM l-glutamine, and penicillin/streptomycin, as described previously (19). For Ca\textsuperscript{2+} imaging and patch clamp experiments, cells were passaged (using trypsin) onto glass coverslips and used 24–48 h after plating. All cells were used between passages 4 and 16.

**Ca\textsuperscript{2+} Imaging**—Ca\textsuperscript{2+} imaging experiments were carried out using the IMAGO CCD camera-based system from TILL Photonics, as described previously using Fura 5f (loaded in the AM form) (18). Cells were alternately excited at 356 and 380 nm (20-ms exposures; 0.5 Hz) using a polychrome monochromator. Images were analyzed offline using IGOR Pro. Cells were
**Ca^2+ Channels and Gene Expression**

**A**

![Graph showing Ca^2+ signals evoked by thapsigargin (2 μM) for 40 min at room temperature.](image)

**B**

![Image of Western Blot panels showing c-fos expression](image)

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**RESULTS AND DISCUSSION**

We previously found that activation of store-operated CRAC channels in mast cells triggered expression of the c-fos gene (20). To see whether such excitation-transcription coupling was driven by a local or global Ca^2+ signal, we stimulated a population of RBL-1 cells (a mast cell line) with thapsigargin, a Ca^{2+} ATPase inhibitor on the endoplasmic reticulum, which depletes the Ca^{2+} stores and thereby opens CRAC channels (6). Because the RBL-1 cell culture is a homogenous population and the cells respond to thapsigargin by generating Ca^{2+} signals with very similar kinetics, we were able to relate Ca^{2+} expression in cell populations to Ca^{2+} signals in individual cells. Stimulation with thapsigargin for 240 s in the absence of external Ca^{2+} produced a transient Ca^{2+} rise in Fura 5F-loaded cells (Fig. 1A), but the Ca^{2+} signal was not associated with any activation of c-fos expression (Fig. 1B; labeled 0Ca/thap.; aggregate data are summarized in the bottom panel; p > 0.5 when compared with control (non-stimulated) cells, ANOVA). On the other hand, stimulation with thapsigargin for the same time in the presence of 2 mM Ca^{2+}, which results in Ca^{2+} influx through CRAC channels, elicited only a slightly larger Ca^{2+} rise (Fig. 1A), but this nevertheless evoked robust c-fos expression (Fig. 1B; *p < 0.01; ANOVA). A major mechanism contributing to the decline of the Ca^{2+} signal in Ca^{2+}-free external solution is the plasma membrane Ca^{2+} ATPase pump (22). Block of this pump with La^{3+} increases the size and prolongs the duration of the Ca^{2+} signal in response to thapsigargin (applied in Ca^{2+}-free solution (Fig. 1A) (18)). Despite this substantial increase in cytoplasmic Ca^{2+} concentration, no c-fos expression was induced (Fig. 1B). These results demonstrate that excitation-transcription coupling is driven by local Ca^{2+} influx through CRAC channels rather than a bulk Ca^{2+} rise. A major determi-
nant of the size of a Ca\textsuperscript{2+} microdomain is the single channel current, which depends on the prevailing electrochemical gradient for Ca\textsuperscript{2+} entry (3, 5). We manipulated this gradient in two ways: first, we reduced the electrical driving force for Ca\textsuperscript{2+} entry by depolarizing the membrane potential. Second, we altered the concentration gradient for Ca\textsuperscript{2+} influx by varying the external Ca\textsuperscript{2+} concentration.

Fig. 2A summarizes the effects of membrane depolarization on the Ca\textsuperscript{2+} signal following exposure of cells to Cs\textsuperscript{+} and TEA\textsuperscript{+}. These agents block the inwardly rectifying K\textsuperscript{+} current in RBL-1 cells (23), leading to a membrane depolarization from a resting potential of ~80 to ~40 mV (24). Over the 4-min exposure to thapsigargin, bulk Ca\textsuperscript{2+} was only slightly lower in depolarized cells (Fig. 2A). However, c-fos expression, following stimulation with thapsigargin for the same time period, was substantially reduced following membrane depolarization (Fig. 2B; *, p < 0.01 when thapsigargin was compared with thapsigargin/Cs\textsuperscript{+}/TEA\textsuperscript{+}, ANOVA). Stimulation with thapsigargin in 0.5 mM Ca\textsuperscript{2+} evoked a bulk Ca\textsuperscript{2+} signal that was very similar to that seen in 2 mM Ca\textsuperscript{2+} (Fig. 2C). However, c-fos expression was significantly lower when cells were challenged in 0.5 mM Ca\textsuperscript{2+} (*, p < 0.01 for 2 mM versus 0.5 mM Ca\textsuperscript{2+}, ANOVA; Fig. 2D). Stimulation in the presence of a lower Ca\textsuperscript{2+} concentration (0.25 mM) resulted in a smaller bulk Ca\textsuperscript{2+} rise and only modest gene expression (Fig. 2, C and D). Collectively, these results reveal that maneuvers that alter local Ca\textsuperscript{2+} entry through CRAC channels but have little effect on the bulk Ca\textsuperscript{2+} rise impact strongly on c-fos expression.

Consistent with this, we found that loading cells with the slow Ca\textsuperscript{2+} chelator EGTA failed to reduce gene expression following CRAC channel activation (Fig. 3A; *p < 0.01 when thapsigargin and thapsigargin/EGTA groups were compared with the control (non-stimulated) group, ANOVA; p > 0.2 when thapsigargin was compared with thapsigargin/EGTA, ANOVA), despite substantially slowing the rate of development of the bulk Ca\textsuperscript{2+} signal (Fig. 3B) (see Ref. 18). Because EGTA is too slow to buffer incoming Ca\textsuperscript{2+} through Ca\textsuperscript{2+} channels, it does not impact local Ca\textsuperscript{2+} signals (3, 25). On the other hand, the fast Ca\textsuperscript{2+} chelator BAPTA can reduce the extent of Ca\textsuperscript{2+} microdomains (3, 5), and it impaired the ability of CRAC channels to trigger gene expression (Fig. 3A; *p > 0.3 for thapsigargin and BAPTA group compared with the control (non-stimulated) group, ANOVA; p < 0.01 for thapsigargin/BAPTA versus thapsigargin/EGTA, ANOVA), despite reducing bulk Ca\textsuperscript{2+} to a similar extent as that seen in EGTA (Fig. 3B).

What senses this local Ca\textsuperscript{2+} entry? We recently found that the non-receptor tyrosine kinase Syk can be activated by Ca\textsuperscript{2+} microdomains arising from open CRAC channels (21), prompting us to consider whether it translates local Ca\textsuperscript{2+} entry into gene expression. We tested this possibility using two different approaches. First, we exposed cells acutely to the Syk inhibitor 3-(1-methyl-1H-indoyl-3-yl-methylene)-2-oxo-2,3-dihydro-1H-indole-5-sulfonamide (10 \textmu M for 10 min) and then stimulated cells with thapsigargin. c-fos expression was suppressed (Fig. 4A; aggregate data from four experiments are summarized in the bottom panel). There was no significant difference between the thapsigargin and Syk inhibitor group versus the control group (ANOVA), whereas thapsigargin versus control was significantly different (p < 0.01, ANOVA; the difference between thapsigargin versus thapsigargin/Syk was also significant, p < 0.01, ANOVA). Importantly, Ca\textsuperscript{2+} flux through CRAC channels was unaffected by the inhibitor (Fig. 4, B and C) as was the membrane potential, measured in current clamp recordings (~78 ± 3 mV control and ~74 ± 5 mV after Syk inhibitor; data not shown). Hence, the loss of c-fos expression is not due to a change in the CRAC channels themselves nor to the driving force for Ca\textsuperscript{2+} influx. Second, we knocked down expression of

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**FIGURE 2.** Changes in local Ca\textsuperscript{2+} influx impact upon c-fos expression. A, Ca\textsuperscript{2+} signals in response to 2 \textmu M thapsigargin in 2 mM Ca\textsuperscript{2+} (normal) are compared with those seen in the presence of 10 mM Cs\textsuperscript{+} and 10 mM TEA\textsuperscript{+}, to block inwardly rectifying K\textsuperscript{+} channels. B, c-fos expression was significantly reduced, compared with thapsigargin (Thap.) stimulation, when cells were stimulated with thapsigargin and Cs\textsuperscript{+}/TEA\textsuperscript{+}. Aggregate data from three independent experiments are shown in the bottom panel. Both thapsigargin groups were significantly different from the resting group and from each other (p < 0.01, ANOVA). C, Ca\textsuperscript{2+} signals following stimulation with thapsigargin in different external Ca\textsuperscript{2+} concentrations (0.25, 0.5, and 2 mM) are compared. D, c-fos expression is compared for the different conditions. Aggregate data from four independent experiments are depicted in the histogram. Data have been normalized to control (non-stimulated levels). Differences between each data set were significant (p < 0.01, ANOVA). There was a significant difference between thapsigargin/2 mM Ca\textsuperscript{2+} and thapsigargin/0.5 mM Ca\textsuperscript{2+} (p < 0.01, ANOVA).
Syk using an RNAi approach. Whereas Syk was clearly expressed in control cells (measured in Western blots; Fig. 4D), transfection with Syk RNAi reduced protein expression by ~60% (Fig. 4D; *, p < 0.01, Student’s t test). This was associated with a significant reduction in c-fos gene activation following stimulation with thapsigargin (Fig. 4E; aggregate data from four experiments are summarized in the bottom panel; *, p < 0.01 between control (non-stimulated) and thapsigargin groups, ANOVA; p < 0.01 between thapsigargin and thapsigargin/Syk RNAi groups, ANOVA). Collectively, these results reveal that Syk couples CRAC channel activity to nuclear events.

If Syk detects local Ca\(^{2+}\) entry, it should be located at the cell periphery. Immunocytochemical studies revealed that this was indeed the case (Fig. 5A). We plotted the lateral profile of Syk across the cell and found two peaks, corresponding to the two plasma membrane sections, with a substantial dip between them (reflecting bulk cytoplasm; Fig. 5B). Enhanced green fluorescence protein is widely used to measure the cytoplasmic distribution (26). In marked contrast to Syk, a relatively stable and elevated profile was found when the cytoplasmic profile of enhanced green fluorescence protein was analyzed in the same way (Fig. 5, B and C) with no peaks near the plasma membrane. Does Syk migrate to the nucleus after Ca\(^{2+}\) influx? To examine this possibility, we stimulated cells with thapsigargin and measured the spatial profile of Syk. Syk remained at the cell periphery with no detectable translocation into the cytoplasm (Fig. 5D). An intermediary signal is therefore needed to couple Syk to the nucleus. We tested for the involvement of a range of downstream cascades including protein kinase C, calmodulin, calcineurin, MEK/ERK, and JNK pathways. Inhibition of each of these pathways had no effect on CRAC channel-transcription coupling. Pretreatment for 20−30 min with 1 \(\mu M\) GO-6983 (to block protein kinase C), 10 \(\mu M\) calmidazolium (to block calmodulin), 5 \(\mu M\) cyclosporine (to block calcineurin), and 50 \(\mu M\) AG490 (to block the JNK pathway) all failed to interfere with thapsigargin-evoked c-fos expression (data not shown). In agreement with our previous work, we found that block of the MEK/ERK pathway with U0126 (10 \(\mu M\); 20 min pretreatment) failed to interfere with c-fos expression following stimulation with thapsigargin (Fig. 5E). We also failed to see any phosphorylation of CREB following CRAC channel activation, suggesting this transcription factor likewise is not involved (data not shown). Signal transducers and activators of transcription
(STAT), is a family of cytoplasmic transcription factors (27) that are widely expressed in immune cells. STATs can be activated following tyrosine phosphorylation by non-receptor tyrosine kinases (28). Phosphorylated STATs dimerize and then rapidly translocate to the nucleus, where they bind to enhancer elements and regulate gene expression (28). Using an antiphospho-specific STAT5 antibody, we found that stimulation with thapsigargin resulted in STAT5 activation within 240 s of stimulation (Fig. 5F; aggregate data from three independent experiments are summarized in Fig. 5G), and this was fully prevented by pretreating cells with the Syk inhibitor (Fig. 5G; *, p < 0.01 between control and thapsigargin groups, ANOVA; p > 0.4 between control and thapsigargin/Syk inhibitor groups, ANOVA). An anti-phospho-specific STAT3 antibody failed to detect activation of STAT3 following opening of CRAC channels (data not shown). Hence, Syk recruits the transcription factor STAT5.

We have recently established that Ca2+ microdomains near CRAC channels activate the cytoplasmic enzymes Ca2+-dependent phospholipase A2 and 5-lipoxygenase via recruitment of the MEK/ERK pathway (20). This results in the generation of the intracellular messenger arachidonic acid, which is rapidly metabolized by 5-lipoxygenase to the proinflammatory paracrine signal leukotriene C4 (18, 20). Do the Ca2+ microdomains couple to gene expression and ERK/cPLA2/leukotrienes by the same mechanism?

Pharmacological data implicated Syk in coupling Ca2+ microdomains to ERK and cPLA2 activation (18). To strengthen this, we measured ERK activation, before and then after knockdown of Syk. Whereas thapsigargin evoked robust stimulation of ERK in control cells (measured as ERK phosphorylation (18) after 4-min stimulation), the extent of ERK activation was significantly reduced following pretreatment of the cells with siRNA against Syk (Fig. 6, A and B). Hence ERK activation and gene expression, which occur over very different time frames, are both triggered by local Ca2+ influx-mediated stimulation of Syk. However, the signaling mechanisms then diverge in that Syk activates gene expression independent of the MEK/ERK pathway (Fig. 5E). These findings reveal the remarkable versatility of Ca2+ microdomains in activating key cell responses. Local Ca2+ influx can simultaneously activate two spatially and temporally distinct processes via Syk (Fig. 7). Syk might detect the local Ca2+ signal directly, or a Ca2+-dependent tyrosine kinase like Pyk-2 (29) could act as an intermediary, linking the Ca2+ microdomain to Syk. Parallel processing of the Ca2+ microdomain by Syk activates cytoplasmic enzymes as

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**FIGURE 5.** Syk recruits the transcription factor STAT5. A, distribution of endogenous Syk in resting RBL-1 cells, observed using immunocytochemistry. B, aggregate data measuring the intensity of Syk fluorescence across the lateral profile of cells is shown, measured in line scan mode (nine cells for Syk and 11 for green fluorescent protein). C, distribution of green fluorescent protein (GFP), used as a cytoplasmic marker, in an RBL-1 cell. D, Syk remains at the plasma membrane following stimulation with thapsigargin. E, thapsigargin (Thap)-evoked c-fos expression is unaffected by blocking the MEK/ERK pathway with U0126. F, thapsigargin promotes phosphorylation of the transcription factor STAT5, and this is prevented by inhibition of Syk. G, aggregate data from three experiments (as in F) are shown. The thapsigargin-stimulated group differs significantly from control (non-stimulated) and thapsigargin/Syk inhibitor groups (*, p < 0.01, ANOVA). Thapsigargin/Syk inhibitor group was not significantly different from the control (non-stimulated) one (p > 0.5).

**FIGURE 6.** Syk knockdown reduces ERK activation. A, Western blot showing that ERK phosphorylation evoked by thapsigargin (Thap.) (in scrambled siRNA-treated cells) is reduced by siRNA knockdown of Syk. B, aggregate data from three independent gels are compared. *, p < 0.01, Student’s t test.
well as gene expression in the nucleus, several μm away. Such an intimate interaction between the Ca$^{2+}$ channel and signal transduction to the nucleus would greatly increase the fidelity, speed, and selectivity of excitation-transcription coupling.

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