

Nuclear and cytosolic calcium are regulated independently

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Nuclear calcium (Ca^{2+}) regulates a number of important cellular processes, including gene transcription, growth, and apoptosis. However, it is unclear whether Ca^{2+} signaling is regulated differently in the nucleus and cytosol. To investigate this possibility, we examined subcellular mechanisms of Ca^{2+} release in the HepG2 liver cell line. The type II isoform of the inositol 1,4,5-trisphosphate (InsP_3) receptor (InsP_3R) was expressed to a similar extent in the endoplasmic reticulum and nucleus, whereas the type III InsP_3R was concentrated in the endoplasmic reticulum, and the type I isoform was not expressed. Ca^{2+} signals induced by low InsP_3 concentrations started earlier or were larger in the nucleus than in the cytosol, indicating higher sensitivity of nuclear Ca^{2+} stores for InsP_3 . Nuclear InsP_3R channels were active at lower InsP_3 concentrations than InsP_3R from cytosol. Enriched expression of type II InsP_3R in the nucleus results in greater sensitivity of the nucleus to InsP_3 , thus providing a mechanism for independent regulation of Ca^{2+} -dependent processes in this cellular compartment.

hepatocytes | liver | inositol 1,4,5-trisphosphate | intracellular calcium signals | HepG2 cells

Calcium (Ca^{2+}) is a ubiquitous second messenger that regulates a wide range of activities in every type of cell. Its range includes such varied processes as secretion, contraction, metabolism, gene transcription, and apoptosis (1, 2). It is not completely established how a single second messenger can coordinate such diverse effects within a single cell, yet there is increasing evidence that the spatial patterns of Ca^{2+} signals may determine the specificity of these signals. Moreover, several lines of evidence (3–6) suggest that increases in Ca^{2+} in the nucleus have specific biological effects that differ from the effects of increases in cytosolic Ca^{2+} . Studies in rat basophilic leukemia and HeLa cell lines suggest that nuclear Ca^{2+} passively follows cytosolic Ca^{2+} (7, 8), although studies in *Xenopus* oocytes and in isolated hepatocyte nuclei suggest instead that nuclei are competent to generate their own Ca^{2+} signals (9, 10). Thus, it remains unclear whether or how Ca^{2+} signaling within the nucleus is regulated.

Inositol 1,4,5-trisphosphate (InsP_3) receptors (InsP_3R) are Ca^{2+} -permeable channels in the endoplasmic reticulum (ER) that transduce a number of extracellular stimuli into cytosolic Ca^{2+} (Ca_i^{2+}) signals (2, 11–14). Three isoforms of the InsP_3R have been described (13, 15), each of which differs in its amino acid sequence, affinity for InsP_3 , and modulation by Ca_i^{2+} (16–19). It is postulated that differences in the subcellular distribution of these intracellular Ca^{2+} channels shape the amplitude, duration, and wave patterns of Ca_i^{2+} transients in the cytosol (16, 20–24).

Components necessary for InsP_3 -mediated Ca^{2+} signaling are found not only in the cytosol, but in the nuclear membrane as well. The nuclear membrane contains phosphatidylinositol 4,5 biphosphate (PIP_2), the precursor lipid of InsP_3 (25), phospholipase C, the enzyme that hydrolyzes PIP_2 to InsP_3 and diacylglycerol (26), InsP_3 3-kinase, the enzyme that phosphorylates InsP_3 to form InsP_4 (27), and the InsP_3R (28–31). Both the nuclear membrane facing the cytoplasm and the surface facing into the nucleus contain functional InsP_3R (30, 32), suggesting

that production of InsP_3 in the nucleus could lead to increases in Ca^{2+} in the nucleus, independent of cytoplasmic Ca^{2+} levels. Here, we examine whether Ca^{2+} signaling in the nucleus and cytosol are differentially regulated, and whether such differences are related to the subcellular distribution of InsP_3R isoforms.

Materials and Methods

Immunoblotting. An affinity-purified-specific rabbit polyclonal antiserum against the mouse type I InsP_3R (directed against the 19 C-terminal residues; custom produced by Research Genetics, Huntsville, AL; characterized as described in ref. 33; diluted 1:500), an affinity-purified specific rabbit polyclonal antiserum against the rat type II InsP_3R (directed against the C-terminal residues CG-FLGSNTPHENHHMPPH; characterized as described in ref. 34; diluted 1:200), and a monoclonal antibody against the human type III InsP_3R (Transduction Laboratories, Lexington, KY; diluted 1:100; ref. 16) was used in this study. The expression of InsP_3R isoforms in membrane fractions of the nucleus, ER, and in whole cells was monitored with immunoblotting with a 5% polyacrylamide gel and standard Western blotting techniques (16, 24, 35).

Immunocytochemistry. Immunocytochemistry was performed as described (20, 36). Cells were immersion-fixed on coverslips for 5 min in 4% (wt/vol) paraformaldehyde in phosphate buffer (PB; 0.1 M, pH 7.4). Immunocytochemical labeling was carried out by using the indirect fluorescence method. After blocking in PBS (0.01 M, pH 7.4) containing 10% (vol/vol) normal goat serum, 1% (vol/vol) BSA, and 0.05% Triton X-100, the binding sites of the primary antibodies were revealed by secondary antibodies: goat anti-rabbit or goat anti-mouse IgG coupled to Alexa 488 (Molecular Probes; diluted 1:100). Primary and secondary antibodies were diluted in PBS containing 3% (vol/vol) normal goat serum, 1% (vol/vol) BSA, and 0.05% Triton X-100. Controls in which the primary antibodies were omitted showed no specific staining. Cells were counterstained with propidium iodide (Sigma). Immunofluorescently labeled sections were examined and recorded by using a Zeiss LSM 510 laser scanning confocal microscope.

Detection of Subcellular Ca^{2+} Signals with Confocal Microscopy.

Nuclear and cytosolic Ca^{2+} were monitored in individual cells by using time-lapse confocal microscopy, as described (37, 38). HepG2 cells (American Type Culture Collection) were cultured on glass coverslips and kept in a Hepes-buffered solution during experiments. Cells were incubated with 4 μM cell permeant fluo-3 (fluo-3 acetoxymethyl ester; Molecular Probes); fluo-3 fluorescence was measured with a Bio-Rad MRC-1024 Confocal

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Abbreviations: InsP_3 , inositol 1,4,5-trisphosphate; InsP_3R , InsP_3 receptors; ER, endoplasmic reticulum; NPE, nitrophenylethyl ester.

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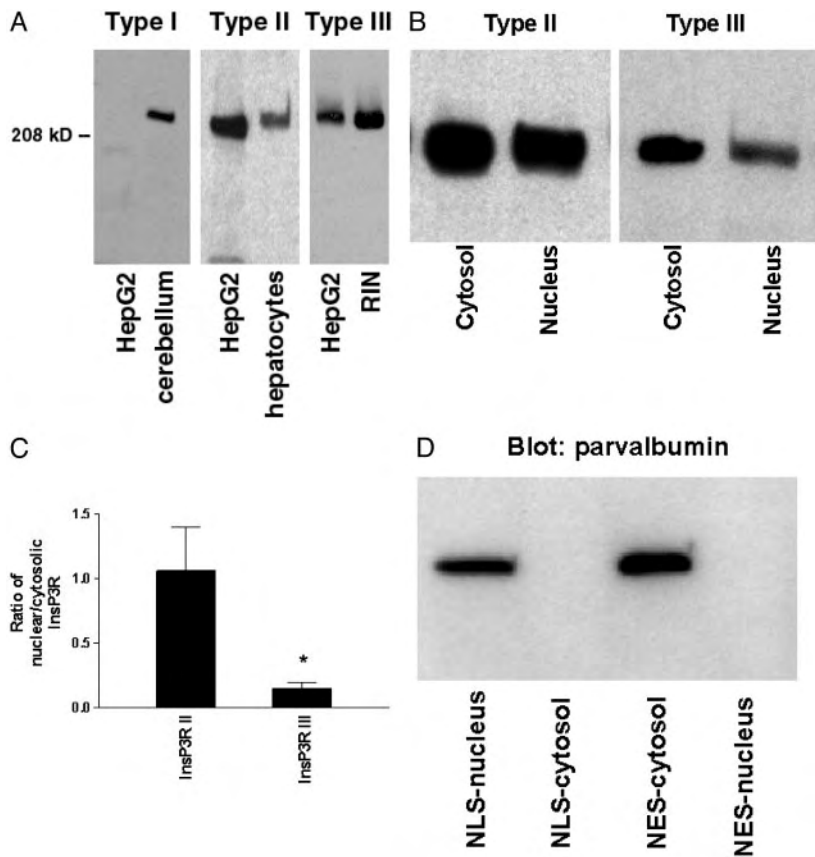


Fig. 1. Expression and subcellular distribution of InsP₃R isoforms in HepG2 cells. (A) Immunoblot of whole-cell protein demonstrates that HepG2 cells express types II and III but not type I InsP₃Rs. Cerebellum, hepatocytes, and RIN-5F cells are used as isoform-specific controls for type I, II, and III InsP₃Rs, respectively. The entire lane of each blot is shown. (B) Immunoblot comparing cytosolic and nuclear membrane proteins demonstrates that the type II InsP₃R is expressed to a similar extent in both compartments, but the type III InsP₃R is relatively decreased in nucleus. Immunoreactive bands were detected at a molecular mass of 240–260 kDa. (C) Densitometry confirms that the type II InsP₃R is enriched in the nucleus relative to the type III isoform (*, $P < 0.025$ based on $n = 4$ blots). (D) Demonstration of the purity of nuclear and cytosolic preparations. Parvalbumin was either targeted to the nucleus with an NLS sequence or excluded from the nucleus by using an NES sequence. NLS-parvalbumin was detected only in the nuclear fraction of HepG2 cells, whereas NES-parvalbumin was detected only in the cytosolic (nonnuclear) fraction.

Imaging System. Changes in fluorescence intensity were calculated by dividing the measured fluorescence intensity during application of nucleotide or photorelease of InsP₃ (F) by the measured average baseline fluorescence intensity (F₀). Images were acquired every 150–600 msec. InsP₃ production through stimulation of P2Y ATP receptors was induced by bath-application of ATP (1–100 μM). Alternatively, some cells were microinjected with 1 mM nitrophenylethyl ester (NPE)-caged InsP₃ (Calbiochem). In these experiments, microinjection pipettes were loaded with the cell-impermeant form of fluo-3 (1 mM) along with caged InsP₃ to detect Ca²⁺ and to verify successful injection. After injection, cells were allowed to recover for 5–10 min before flash photolysis studies were performed. Fluo-3 has a greater molecular weight than NPE-InsP₃, so only those cells with uniform fluo-3 fluorescence in the nucleus and cytosol were used. NPE-InsP₃ was photolyzed by using a mercury arc lamp (50 W) coupled to a 1-mm quartz fiberoptic cable through a high-speed shutter and filter wheel (16). Because the microinjection volume was <10% of the total cell volume, the maximum intracellular concentration of NPE-InsP₃ was 100 μM. Maximal uncaging occurred with flashes >300 msec in duration, so the concentration of free InsP₃ after an initial flash was estimated by $(100 \mu\text{M}) \times (\text{flash duration}) / (300 \text{ msec})$, up to a maximum of 100 μM. The amount of InsP₃ liberated by subsequent flashes was determined by the same formula, but reducing the maximum releasable InsP₃ by the amount that already had been released. These estimates are similar to those described by others using similar techniques (39).

Bilayer Experiments. Nuclei were separated from HepG2 cells by using a kit from Pierce. Briefly, nuclear and ER membrane vesicles were isolated from the nuclear and cytoplasmic fractions, respectively, in the presence of protease inhibitors by

differential centrifugation (40). Vesicles were aliquoted and stored at –80°C until needed for experiments. InsP₃Rs from nuclear or ER vesicles were incorporated into planar lipid bilayers containing phosphatidylethanolamine and phosphatidylserine (3:1 wt/wt; Avanti Polar Lipids) dissolved in decane (40 mg of lipid per ml). The experiments were performed with

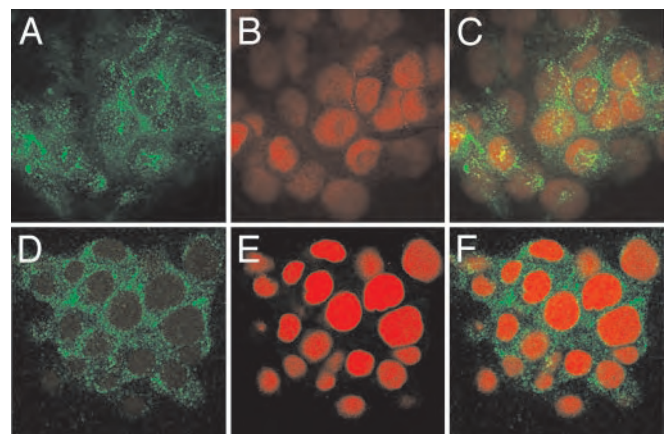


Fig. 2. Subcellular localization of InsP₃R isoforms in HepG2 cells. Type II and III InsP₃Rs were localized by confocal immunofluorescence. Cells were double-labeled with isoform-specific InsP₃R antibodies (green) or with added propidium iodide (red) to identify the nucleus. (A) Type II InsP₃R labeling. (B) Propidium iodide. (C) Merged images demonstrate that type II InsP₃R is in both cytosol and nucleus. Notice the reticular pattern of nuclear staining. (D) Type III InsP₃R labeling. (E) Propidium iodide. (F) Merged images demonstrate that type III InsP₃R is in the cytosol but not in the nucleus of HepG2 cells. Labeling with secondary but not primary antibody plus propidium iodide reveals no nonspecific staining (not shown).

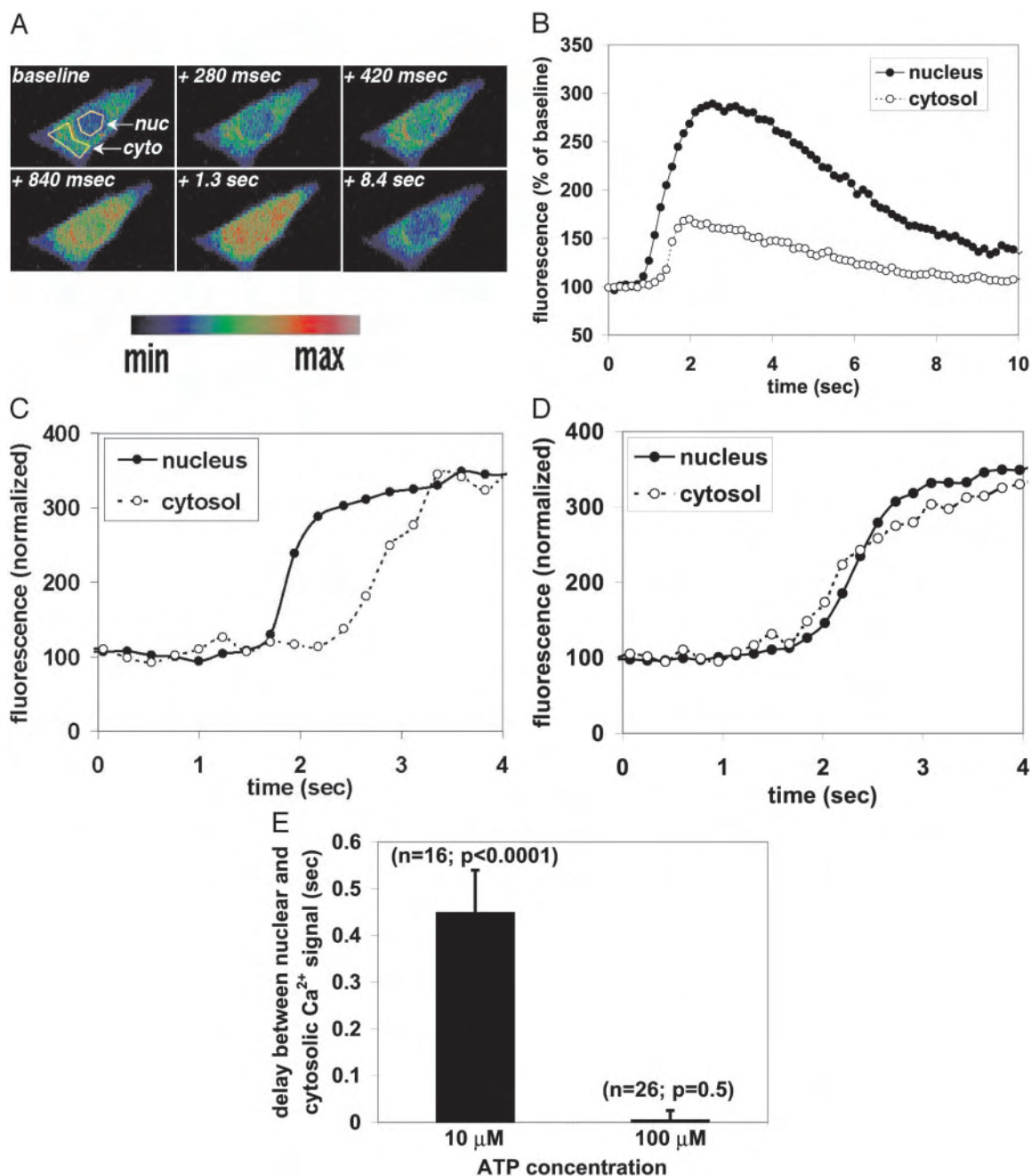


Fig. 3. Nuclear and cytosolic Ca^{2+} signals in HepG2 cells stimulated with extracellular ATP. (A) Serial confocal images of a HepG2 cell stimulated with ATP (10 μ M). The cell is loaded with fluo-3, and the images are pseudocolored by using the scale that is shown. A fluorescence increase in the nucleus precedes the increase in the cytosol; the increase in the nucleus is first detectable in part of the nucleus after 280 msec and occurs throughout the nucleus within 420 msec, while the cytosolic increase is not detected until 840 msec have elapsed. (B) Tracing shows nuclear and cytosolic fluorescence in the regions outlined in yellow in the cell shown in A. The increase in nuclear fluorescence slightly precedes the increase in the cytosol, then both increases return toward baseline. (C) Expanded time scale in a similar experiment reveals that Ca^{2+} increases in the nucleus almost 0.5 sec before Ca^{2+} increases in the cytosol of another HepG2 cell stimulated with 10 μ M ATP. Nuclear and cytosolic Ca^{2+} signals in C and D are normalized to the same baseline and peak values to facilitate comparison of the two curves. The result is representative of that seen in $n = 16$ cells. (D) Ca^{2+} increases simultaneously in the nucleus and cytosol of a HepG2 cell stimulated with 100 μ M ATP. The result is representative of that seen in $n = 26$ cells. (E) Summary of results. Values are mean \pm SEM.

a 250 mM Hepes-Tris solution, pH 7.35, on the cis and a 250 mM Hepes/55 mM $\text{Ba}(\text{OH})_2$ solution, pH 7.35, on the trans side of the bilayer. Ba^{2+} was used as the current carrier because it has been shown for InsP_3 Rs not to influence channel activity and to produce larger current amplitudes than Ca^{2+} (41). Channels were activated by the addition of Ca^{2+} (300 nM, because this is the optimal concentration for InsP_3 R activity)

and InsP_3 to the cis side. Demonstration of InsP_3 -dependent channel activity was taken as evidence for the presence of InsP_3 Rs in the bilayer. Channel activity was monitored over a range of InsP_3 concentrations on the cytosolic side of the channels. Experiments were recorded under voltage-clamp conditions, filtered at 1 kHz, digitized at 3 kHz, and transferred directly to a computer. The data were acquired, ana-

lyzed, and absolute open probabilities were calculated with PCLAMP V.6.0.3 (Axon Instruments, Foster City, CA). The data shown in the present study were obtained from three or more independent trials for each experimental condition.

Results and Discussion

InsP₃R Subtypes Are Differentially Expressed in Nucleus and Cytosol.

We examined expression and subcellular localization of InsP₃R isoforms in HepG2 cells by immunoblotting and confocal immunofluorescence. Immunoblotting of intracellular membrane preparations detected type II and III but not type I InsP₃R (Fig. 1A). Similar concentrations of the type II InsP₃R were detected in HepG2 nuclei and ER, whereas the type III isoform was enriched 10-fold in ER relative to nuclei ($P < 0.025$; Fig. 1B and C). The purity of the nuclear and cytosolic fractions was confirmed by using cells transfected with a construct that targets parvalbumin to either the nucleus (NLS-PV) or cytosol (NES-PV; ref. 6). Targeted constructs were used because ER markers can be found in both cytosol and nucleus (42). Parvalbumin immunoblots detected the protein in the nuclear but not the cytosolic fraction of NLS-PV-transfected cells, and in the cytosolic but not nuclear fraction of NES-PV-transfected cells (Fig. 1D). Consistent with immunoblots, immunocytochemistry demonstrated strong type II InsP₃R immunoreactivity in both the nuclear and cytosolic regions (Fig. 2A–C). Type II InsP₃R antibodies occasionally decorated not only the cytosol and nuclear envelope (NE), but also other portions of the nuclear interior. These findings are consistent with previous observations that protrusions from the ER and NE extend into the nuclear interior in most cell types (42). In contrast to the distribution of the type II InsP₃R, type III InsP₃R immunoreactivity was minimal in the nucleus (Fig. 2D–F). These findings are in agree-

ment with studies that report differentially distributed InsP₃R isoforms within substructures of the ER (20, 24, 35, 43, 44) and studies that describe the expression of specific InsP₃R subtypes within the nuclear envelope (28, 45, 46). In separate experiments, RT-PCR was performed by using primers to amplify a region common to all three isoforms of the ryanodine receptor (RyR) isoforms (47). These experiments suggested that HepG2 cells do not express RyR (not shown), similar to liver (35, 48) and other hepatic cell lines (49, 50). Thus, it would be expected that intracellular Ca²⁺ release in HepG2 cells should be mediated entirely through InsP₃Rs, and differences in the function of type II InsP₃R relative to type III InsP₃R should result in differences in Ca²⁺ signaling in the nucleus relative to the cytosol.

The Nucleus Is More Sensitive than the Cytosol to InsP₃. The subcellular distribution of InsP₃R isoforms in HepG2 cells suggests that InsP₃-mediated Ca²⁺ signaling may differ in nucleus and cytosol. To investigate this possibility, InsP₃-induced Ca²⁺ transients in the nucleus and cytosol were compared. Cells were stimulated with extracellular ATP which binds to P2Y nucleotide receptors expressed on the plasma membrane of HepG2 cells (51–54). Activation of these receptors initiates the InsP₃-induced Ca²⁺-signaling cascade (51, 52). Nuclear and cytosolic Ca²⁺ were monitored in individual HepG2 cells by using time-lapse confocal microscopy (Fig. 3A and B; refs. 37 and 38). This approach accurately reflects the temporal pattern of Ca²⁺ signals in both of these subcellular regions, which permits reliable comparisons of the timing of increases in nuclear and cytosolic Ca²⁺ (55–57). However, factors such as dye compartmentalization and local differences in Ca²⁺-binding affinity complicate comparisons of the magnitude of

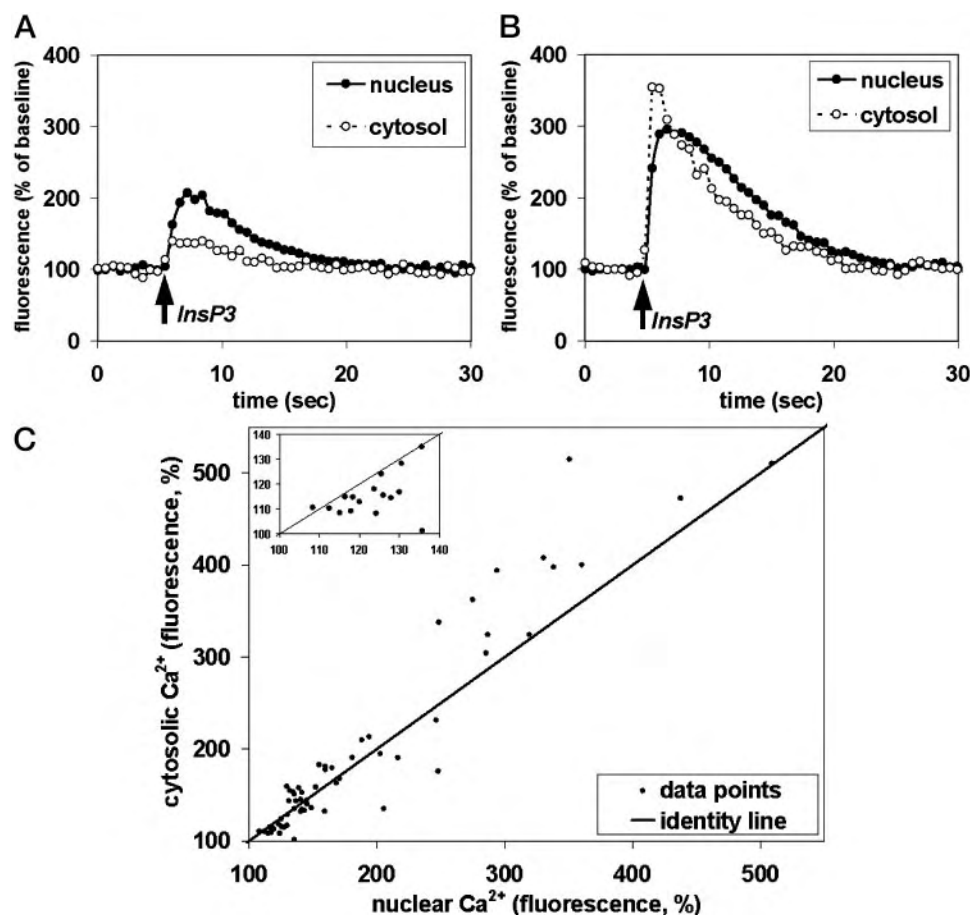
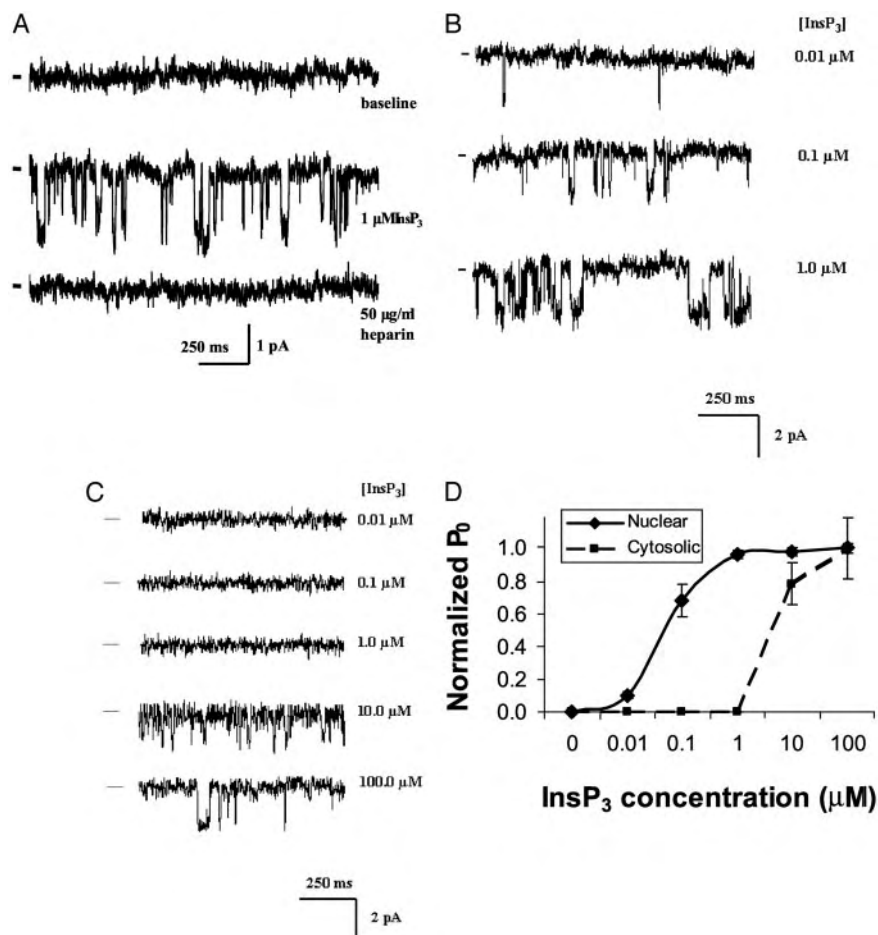


Fig. 4. Nuclear and cytosolic Ca²⁺ signals induced by photorelease of caged InsP₃. Cells were microinjected with NPE-InsP₃ plus fluo-3, then InsP₃ was liberated in a controlled fashion by flash photolysis as cells were monitored by time-lapse confocal microscopy. (A) Liberation of 0.2 μM InsP₃ results in a small increase in nuclear Ca²⁺, and an even smaller increase in cytosolic Ca²⁺. (B) Liberation of 9 μM InsP₃ results in much greater increases in nuclear and cytosolic Ca²⁺, where the magnitude of the signal is greater in the cytosol than in the nucleus. (C) Summary of flash photolysis studies. Individual data points show the peak nuclear vs. cytosolic fluorescence (relative to baseline) in each of 60 observations. The identity line is shown for reference, to illustrate where data points would lie if nuclear and cytosolic fluorescence were equal. The regression curve for these data is: (% increase in cytosol) = 1.27 × (% increase in nucleus) – 39.2 ($R^2 = 0.92$; $P < 0.00001$), which demonstrates that nuclear Ca²⁺ increases more than cytosolic Ca²⁺ for small Ca²⁺ signals, but that cytosolic Ca²⁺ increases more than nuclear Ca²⁺ for larger Ca²⁺ signals (main graph). Note that nuclear Ca²⁺ signals predominate when the Ca²⁺ transient is <140% of baseline (see *Inset*).

Fig. 5. InsP₃ dependence of InsP₃R channels from HepG2 nuclear and ER membranes. (A) Single-channel recordings of InsP₃Rs from HepG2 nuclear membranes. (Top) After fusion of vesicles from HepG2 cell nuclear membranes, no channel activity was observed in the presence of 4 μ M ruthenium red (to inhibit non-InsP₃-dependent Ca²⁺ channels), 0.5 mM ATP (which is an InsP₃R coagonist), and 10 μ M Ca²⁺ at the cytosolic side with Ba²⁺ as the current carrier. Addition of 1 μ M InsP₃ to the cytosolic side induced channel activity. (Middle) Channel openings can be seen here and in subsequent tracings as downward deflections from the baseline. The bar to the left of each tracing here and subsequently indicates the baseline. (Bottom) Addition of 50 μ g/ml heparin blocked InsP₃R channel activity specifically (representative of $n = 4$ experiments). Similar results were obtained with vesicles from cytosolic HepG2 membrane preparations (not shown). (B) Increasing amounts of InsP₃ added to the cytosolic (nucleoplasmic) side of the InsP₃R channel from HepG2 cell nuclear membranes increase channel activity. Shown are traces with InsP₃ concentrations of 10 nM (Top), 100 nM (Middle), and 1 μ M (Bottom). (C) Increasing amounts of InsP₃ added to the cytosolic side of the InsP₃R channel from HepG2 cell ER membranes increase channel activity. Shown are traces with InsP₃ concentrations of 10 nM, 100 nM, 1 μ M, 10 μ M, and 100 μ M (Top to Bottom). (D) InsP₃R from HepG2 cell nuclei are more sensitive to InsP₃. Membranes from both cell compartments show increasing activity with increased concentrations of InsP₃ at the cytosolic/nucleoplasmic side. Nuclear channels are already active at 10 nM InsP₃ and reach a plateau at 1 μ M InsP₃. Regression analysis of nuclear InsP₃R activity dependence on the InsP₃ concentration shows half-maximal activity at 64 nM InsP₃. In contrast, ER channels are not active until they are above 1 μ M InsP₃, when they also reach a plateau. Regression analysis of ER InsP₃R activity dependence on the InsP₃ concentration shows half-maximal activity at 3.7 μ M InsP₃. This figure shows the normalized open probability (P_0) for ER and nuclear InsP₃Rs; the absolute P_0 using 100 μ M InsP₃ is 7.4% and 2.9% for ER and nuclear receptors, respectively. The averages of at least three single-channel experiments and their SEs are shown for each data point.



fluorescence intensity in the nucleus and cytosol (55, 57, 58). Therefore, for these studies, nuclear and cytosolic Ca²⁺ signals were normalized to the same baseline and peak value to focus on the temporal differences between the two tracings (Fig. 3 C and D). All cells tested ($n = 60$ cells) responded to stimulation with 100 μ M ATP, whereas most (56 of 60 cells, or 93%) responded to 10 μ M ATP, and very few (13 of 70 cells, or 19%) responded to 1 μ M ATP. Therefore, we compared Ca²⁺ signals in the nucleus and cytosol in cells stimulated with 10 or 100 μ M ATP (Fig. 3 C–E). In cells stimulated with 10 μ M ATP (Fig. 3C), nuclear Ca²⁺ signals preceded cytosolic Ca²⁺ signals by 449 ± 91 msec (mean \pm SEM, $n = 16$; $P < 0.0001$ by paired t test). In contrast, nuclear and cytosolic Ca²⁺ signals began nearly simultaneously in cells stimulated with 100 μ M ATP (time difference, 0.0 ± 0.8 msec; $n = 26$; $P = 0.50$ by paired t test; Fig. 3D). This finding is similar to the observation that nuclear and cytosolic Ca²⁺ signals begin simultaneously in primary hepatocytes stimulated with a maximal concentration of vasopressin (56), which also increases Ca²⁺ via InsP₃ (26). Thus, although nuclear and cytosolic Ca²⁺ signals are similar in maximally stimulated cells, differences during submaximal stimulation suggest that the threshold for InsP₃-induced Ca²⁺ signaling is lower in the nucleus than in the cytosol. To investigate this finding more directly, controlled amounts of InsP₃ were released in HepG2 cells by flash photolysis of microinjected, caged InsP₃ while cells were observed with time-lapse confocal microscopy (Fig. 4). Photorelease of smaller amounts of InsP₃ resulted in Ca²⁺ signals that were

greater in the nucleus than in cytosol (Fig. 4 A and C). Photorelease of larger amounts of InsP₃ resulted in Ca²⁺ signals that were greater in the cytosol (Fig. 4 B and C). Together, these findings suggest that the nucleus has a lower threshold than the cytosol for InsP₃-induced Ca²⁺ signaling, which results in preferential signaling in the nucleus when cells are stimulated with submaximal concentrations of agonist.

The InsP₃R of Nuclear and ER Membranes Behave Differently. To determine whether the observed differences between nuclear and cytosolic Ca²⁺ signals could be attributed to differences in InsP₃R isoforms, biophysical properties of InsP₃Rs expressed in HepG2 nucleus and ER membranes were determined. Vesicles were prepared from nuclear and ER membranes, and single-channel recordings of InsP₃Rs present in these vesicles were performed. InsP₃R channels from both nuclei and ER could be activated by the addition of physiological concentrations of InsP₃ to their cytosolic side (Fig. 5A Middle), similar to the type I (59), type II (18, 60), and III InsP₃Rs (16). Channel inactivation by heparin was used to verify that HepG2 Ca²⁺ channels had typical InsP₃R pharmacology (Fig. 5A Bottom). Nuclear Ca²⁺ channel activity was initiated by InsP₃ concentrations of 10 nM and greater, and increasing InsP₃ concentrations stimulated higher channel activity (Fig. 5B). Half-maximal activation occurred at 64 nM InsP₃, a value similar to the EC₅₀ value of 58 nM determined for recombinant type II InsP₃Rs (18) and significantly lower than the value of 3.2 μ M described for native type III InsP₃Rs (17). In contrast, ER Ca²⁺ channel activity was not

initiated unless cytosolic InsP_3 concentrations exceeded $1 \mu\text{M}$ (Fig. 5 C and D). Half-maximal activation occurred at $3.7 \mu\text{M}$ InsP_3 , a value significantly higher than that described for type II InsP_3Rs but similar to EC_{50} values determined for native type III InsP_3Rs . Although HepG2 cells express both the type II and type III InsP_3R in their cytosol, there are several possible reasons why the behavior of the type III isoform appears to predominate. First, the type III isoform may be expressed to a greater extent than the type II InsP_3R in cytosol. Although this may not be determined from the immunoblots in Fig. 1, this explanation is consistent with the observation that single-channel activity was observed in only 1 of 20 experiments using ER membranes and submicromolar concentrations of InsP_3 . Second, different isoforms of the InsP_3R can form heterotetramers (61, 62), and heterotetramers consisting of both type II and III InsP_3Rs may have properties that differ from those of homotetrameric type II InsP_3Rs . Finally, because InsP_3Rs have multiple InsP_3 -binding sites (63–65), the presence of type III InsP_3Rs can diminish net binding of InsP_3 to type II InsP_3Rs , even though the type II isoform has a greater affinity for InsP_3 (18, 60). Together, these findings suggest that nuclear expression of the type II InsP_3R isoform may be responsible for the preferential Ca^{2+} signaling that occurs in the nucleus of HepG2 cells.

The subcellular pattern of Ca^{2+} signals is an important component of how this second messenger regulates cell function. For example, presynaptic increases in Ca^{2+} regulate neurotrans-

mitter release (66), Ca^{2+} gradients direct cell movement (67, 68), and Ca^{2+} waves may direct morphogenesis (69). The three InsP_3R isoforms each behave in a distinct fashion, both at the single-channel level (16, 60) and in intact cells (21). Therefore, it has been proposed that the subcellular distribution of each isoform helps shape the subcellular pattern of Ca^{2+} signals in the cytosol (16, 24, 44). However, elevations in nuclear rather than cytosolic Ca^{2+} are required for some processes, such as transcription of certain genes (4–6). In addition, the nucleus contains its own InsP_3 -sensitive Ca^{2+} stores, within the nuclear envelope (30). The current work provides a mechanism for regulation of the Ca^{2+} concentration in the nucleus independent of changes in cytosolic Ca^{2+} (Figs. 3 and 4; refs. 9 and 70). The findings of the present study support the idea of an independent regulation of intracellular Ca^{2+} changes based on compartment-specific, differential distribution of intracellular Ca^{2+} channels with different biophysical properties. Specificity in the subcellular distribution and modulation of the InsP_3R isoforms in nucleus and cytosol may be the mechanism used by many cell types to coordinate and independently regulate nuclear and cytosolic processes.

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