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# ATP-Induced Nuclear and Cytosolic Ca$^{2+}$ Signal in Various Stages of Interphase HeLa/Fucci2 Cell

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**Abstract**

Cell cycle progression involves the coordination of growth, DNA replication and division in proliferating cells. These intracellular activities are linked with calcium signaling in which calcium ion (Ca$^{2+}$) act as a second messenger or as an agent for phosphorylation processes. Previous studies have examined the Ca$^{2+}$ in the dividing cells; however, none of these gave the detailed explanation on Ca$^{2+}$ dynamics in an interphase cell. This study addresses here the issue of whether the nuclear and cytosolic Ca$^{2+}$ concentrations ($[Ca^{2+}]_n$ and $[Ca^{2+}]_c$) changes are related or not in the dividing interphase cells. Current approach used interphase HeLa/Fucci2 cells to investigate the intracellular Ca$^{2+}$ dynamics between the nucleoplasm and cytoplasm following stimulation with extracellular adenosine triphosphate (extracellular purinergic-2 receptor agonists). The results revealed that $[Ca^{2+}]_n$ and $[Ca^{2+}]_c$ signals differed both between and within interphase stages.

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These observations suggested that the expressions of intracellular $Ca^{2+}$ signal in a dividing cell is very much dependent on the nuclear and cytoplasmic activities during a particular interphase stage and whether it is early and/or late stages of that interphase stage. Implications of the results and future research directions are also presented.

*Keywords:* cell cycle; interphase HeLa/Fucci2 cell; intracellular calcium ion; dynamics

## 1. Introduction

Calcium ion ($Ca^{2+}$) is well regarded as a ubiquitous second messenger found in both nucleoplasm and cytoplasm, which regulates wide range of cellular activities including gene transcription and cell proliferation [1,2,3]. Intracellular $Ca^{2+}$ concentration depends on the $Ca^{2+}$ release from the intracellular stores as well as $Ca^{2+}$ entry from the extracellular environment, as a result of cellular response to various stimuli such as growth factors. Expression of the intracellular $Ca^{2+}$ signals carries a variety of information in relation to its diverse effects on a single cell. It has been suggested that increase in $Ca^{2+}$ concentration in the nucleoplasm have specific biological effects that differ from the effects of increases observed in cytoplasm [4] and it is considered to be regulated independently [5,6] as well as the $Ca^{2+}$ entry into the two cellular compartments.

It is known that ATP (an extracellular P2 receptor agonist) is involved in specific extracellular signaling actions that lead to mobilization of $Ca^{2+}$ from intracellular stores such as endoplasmic reticulum and nuclear envelope, hence elevation of the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$ ) through the $IP_3$-mediated $Ca^{2+}$ signaling pathway, thus, playing a role in the regulation of a variety of intracellular activities such as cell cycle progression. The necessary components for $IP_3$ –pathway are found not only in the cytosol, but in the nuclear membrane as well.

Although studies have been conducted previously on the mammalian somatic cell describing the $Ca^{2+}$ dynamics on the interphase cell, most of these are only reporting the general observations either between interphase and mitotic [7] or with reference to $G_1S$ transition [8] or $G_2M$ transition [9]. To date, however, there is no documented information comparing the relationship between phases or relation between nucleoplasmic and cytoplasmic calcium dynamics in the interphase cell cycle stages. These observations raised some questions regarding the intracellular $Ca^{2+}$ dynamics, whether nucleoplasmic $Ca^{2+}$ ($[Ca^{2+}]_n$ ) and cytoplasmic $Ca^{2+}$ ($[Ca^{2+}]_c$ ) transients are related or not in the dividing interphase cell.

Consequently, we decided that the best approach would be to use the HeLa/Fucci2 cells, a non-excitable epithelial cell line, using the real-time confocal microscopy to study the intracellular $Ca^{2+}$ dynamics in an interphase cell between the two cellular compartments. HeLa/Fucci2 cells uses expression of *Cdt1* and *Geminin* proteins to indicates the various stages of the cell cycle. Previous studies in cell culture have demonstrated that *mCherry-hCdt1* (red) signal occurs in nuclei at early $G_1$ phase and decrease with transition to S phase, and disappears at S phase. Likewise, *mVenus-hGeminin* (green) signal occurs in nuclei at early S phase, increases towards $G_2M$ and M phase [10] of the cell cycle. In the present study, blue-green (BG) excitation under a laser scanning microscopy was used to determine these nuclei expressions indicating various stages of the cell cycle,

whereas calcium dynamics were measured by a $Ca^{2+}$ sensitive fluorescent dye excited by ultraviolet (UV) beam. We aimed to investigate the intracellular $Ca^{2+}$ mobilization from intracellular $Ca^{2+}$ stores induced by ATP, occurring in the nucleoplasm and cytoplasm during various stages of interphase cell. The results demonstrated that, the intracellular $Ca^{2+}$ dynamics in an interphase cell differs between stages and within a particular interphase stage.

## 2. Material and Method

### 2.1. Cell lines and Culture Conditions

HeLa/Fucci2 cells expressing cell cycle stages indicators were obtained as a gift from Prof. Chihaya Maesawa Laboratory in the Department of Histopathology, Iwate Medical University, Japan and maintained in DMEM (Sigma Aldrich) supplemented with 5% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a 5% $CO_2$ humidified atmosphere. Cells between passages 2 and 8 were used throughout the experiment.

### 2.2. Cell Treatments

The cells were sub-cultured on cover slips in full growth media and incubated at 37 °C, 5% $CO_2$ for 24 -36 hours to allow adherence on the culture plate. Then serum deprived DMEM were added and incubated for 24 hours to synchronize cells into $G_0/G_1$ phase. Thereafter, cells were grouped into three cohorts: full growth media; full growth media with 3μM Aphidicolin (a reversible inhibitor of DNA replication, Sigma-Aldrich); full growth media with 10μM Etoposide (Topoisomerase II inhibitor, Sigma-Aldrich); and incubated for 60 hours. Growth media were replaced every 24 hours. All cohorts were treated under similar experimental conditions.

### 2.3. Measurement of Intracellular $Ca^{2+}$ Concentration Dynamics

The cover slips (cells) were placed into modified Sykes-Moore chambers. For exclusive intracellular $Ca^{2+}$ dynamics, cells were gently washed twice with $Ca^{2+}$-free HEPES-buffered Ringer's solution (HR) containing 118 mM NaCl, 5.5 mM D-Glucose, MEM amino acids solution (Gibco Grand Island, NY, USA), 4.7 mM KCl, 1.13 mM $MgCl_2$, 1 mM $NaH_2PO_4$, 10 mM Hepes-NaOH, 2 mM Sodium L-glutamate, 0.2% bovine serum albumin (Sigma, St. Louis, MO, USA), and 0.5M EGTA, and the pH was adjusted to 7.4 with 4 M NaOH. Cells were loaded with the calcium-sensitive dye, Indol-1/AM (2μM, a ratiometric fluorescence dye; Dojindo, Kumamoto, Japan) in HR, and incubated for 30 minutes at 37 °C, 5% $CO_2$ humidified atmosphere. Excessive dye was removed by gently washing the cells twice with standard HR.

The loaded cells (perfusion chambers) were mounted and observed on real time confocal microscope (RCM/Ab; a modified version of a Nikon model RCM-8000, Tokyo, Japan) with an Inverted microscope equipped with an argon-ion laser (TE-300, Nikon). The fluorescence emission was passed to a pinhole diaphragm through a $H_2O$-immersion objective lens (Nikon C Apo 40×, N.A. 1.15). The specimens were exposed to blue-green (BG; 488 nm) excitation beam to determine the cell cycle stage of individual cells whereby the nuclei in $G_1$, $G_1S$ and $S/G_2/M$ phases displayed red, yellow and green respectively. To measure $[Ca^{2+}]_i$, cells were exposed to UV excitation beam and confocal images (512×480 pixels) with optimum spatial resolution were obtained at average of eight frames from emissions at <440 nm and >440 nm. Image ratios were computed from

fluorescence intensities of less than 440 nm ($F_{<440}$) to that greater than 440 nm ($F_{>440}$). A higher ratio is indicative of a higher $[Ca^{2+}]_i$. Preliminary examination with UV excitation revealed that, fucci-fluorescence did not interfere the $[Ca^{2+}]_i$.

We measured $[Ca^{2+}]_i$ in nuclear and cytoplasm by ratiometry and separately analyze by spot analysis methodology in individual cells. During imaging, the cells were continuously perfused with standard HR $Ca^{2+}$-free with 0.5M EGTA containing selected agonists and/or antagonists at rate of 1 ml per minute. The experiments were conducted both at room temperature and controlled temperature (Warner Bipolar temperature controller; Warner Instruments-model CL-100, CT, USA).

### 2.4. Cell Perfusion

The intracellular $Ca^{2+}$ concentration changes were examined following continuous perfusion with standard HR containing the following agonists and/or antagonists: 10µM - 100µM adenosine triphosphate (ATP, an extracellular purinergic-2 receptor agonist; Kohjin, Tokyo, Japan), 2-aminoethyl diphenylborinate (2-APB, an $IP_3R$ antagonist, 100µM; Tocris Bioscience, MO, USA), U73122 (a phospholipase C inhibitor, 5-10µM; Sigma-Aldrich), or reactive blue-2 (RB-2, a P2Y receptor antagonist, 100µM; Sigma-Aldrich).

### 3. Results

### 3.1. General Characteristics of ATP-Induced Intracellular $Ca^{2+}$ Wave Expressed in the Nuclear and Cytoplasm of an Interphase HeLa/Fucci2 cell

To examine intracellular $Ca^{2+}$ signal as a result of $Ca^{2+}$ release from the intracellular stores, we transiently transfected the HeLa cells with the ratiometric fluorescence calcium indicator, Indol-1/AM, and monitored $Ca^{2+}$ signal in the presence of EGTA. To understand how difference the Fucci-fluorescence influence $Ca^{2+}$ signaling, we examined $Ca^{2+}$ transients expressed in non-Fucci HeLa and HeLa/Fucci2 cells treated with aphidicolin or etoposide and compared the emitted fluorescence intensities both at baseline and following stimulation by ATP. We confirmed that Fucci-fluorescence did not interfere the calcium signal.  To examine the $Ca^{2+}$ signal in various interphase stages following agonist stimulation, we exposed Indol-1/AM transfected $G_1$, $G_1S$, S and $G_2M$ HeLa/Fucci2 cells to extracellular ATP, this resulted to a transient and reproducible increase in intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) in both cellular compartments. Frequently the responses commenced in the cytoplasm followed with the nucleoplasmic responses (Figure 1). The observed responses had sharp spikes with clear peak followed by a gradual decrease with clear repetitive short spikes. ATP concentrations more than 10µM did not led to an increase or prolong the cellular responses.

### 3.2. Properties of ATP-Induced $Ca^{2+}$ Responses in Various Stages of the Interphase Cell

In order to compare the effects of ATP on nuclear and cytosolic $Ca^{2+}$, interphase HeLa/Fucci2 cells expressing either red-FP (*mCherry*) or yellow (intermediate *mVenus/mCherry*) or green-FP (*mVenus*), for $G_1$, $G_1S$, and S/$G_2$/M stages respectively, were stimulated with 10µM ATP. At baseline the $[Ca^{2+}]_c$ was higher than $[Ca^{2+}]_n$. Most cells, spatial analysis indicated that ATP stimulation causes increase in $[Ca^{2+}]_i$ that started in the cytoplasm and subsequently followed by nucleoplasm. Interestingly, we found that the characteristic increment of the

evoked responses in $[Ca^{2+}]_i$ do differ between the stages and within a particular stage of an interphase cell. In most experiments (n) with exception of few cells the nucleoplasmic changes were observed to be much stronger with some rising higher and overlap the cytoplasmic amplitude peaks. The transient was generally characterized by a spike with apparent peak followed with a gradual decrease (Figures 2-5).
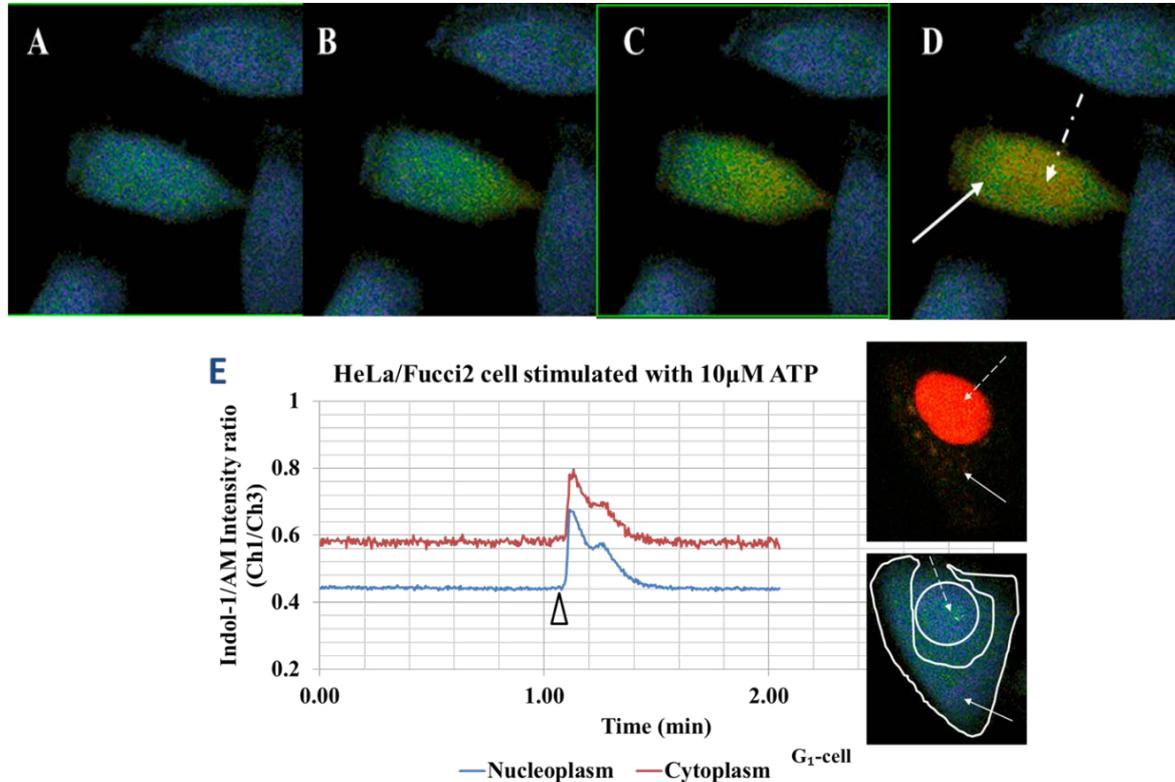


**Figure 1.** Fluorescence micrographs of interphase HeLa/Fucci2 cell showing the general expression of intracellular $Ca^{2+}$ concentration induced by ATP (**A-D**). The $Ca^{2+}$ wave commenced in the cytoplasm (*bold arrow*) and subsequently followed by the nucleoplasm (*dashed arrow*). The data shows changes in levels of intracellular $Ca^{2+}$ as a function of time, which were measured by Indol-1/AM (a ratiometric dye; 2μM) method. After addition of exogenous 10μM ATP (**E**) induced responses had a spike with apparent peak. The *arrow head* shows the time point for starting perfusion with agonists. The insert photomicrographs on **E**, shows the nucleus and cytoplasm as depicted following BG excitation (red) and how the spot analysis were conducted between the two cellular compartments following UV excitation.

### 3.2.1. ATP-Induced $Ca^{2+}$ Responses in $G_1$-phase HeLa/Fucci2 cells

ATP stimulation caused a transient increase in $[Ca^{2+}]_i$ commencing in the cytoplasm followed by that in the nucleoplasm. The transient spike evoked had an apparent peak that followed with gradual decrease (n=30). Interestingly, the responses in the two cellular compartments were observed to be variable in some experiments: being much stronger in nucleoplasm and overlap the cytoplasmic response (n=11); or being equal (n=19) between the two compartments (Figure 2).
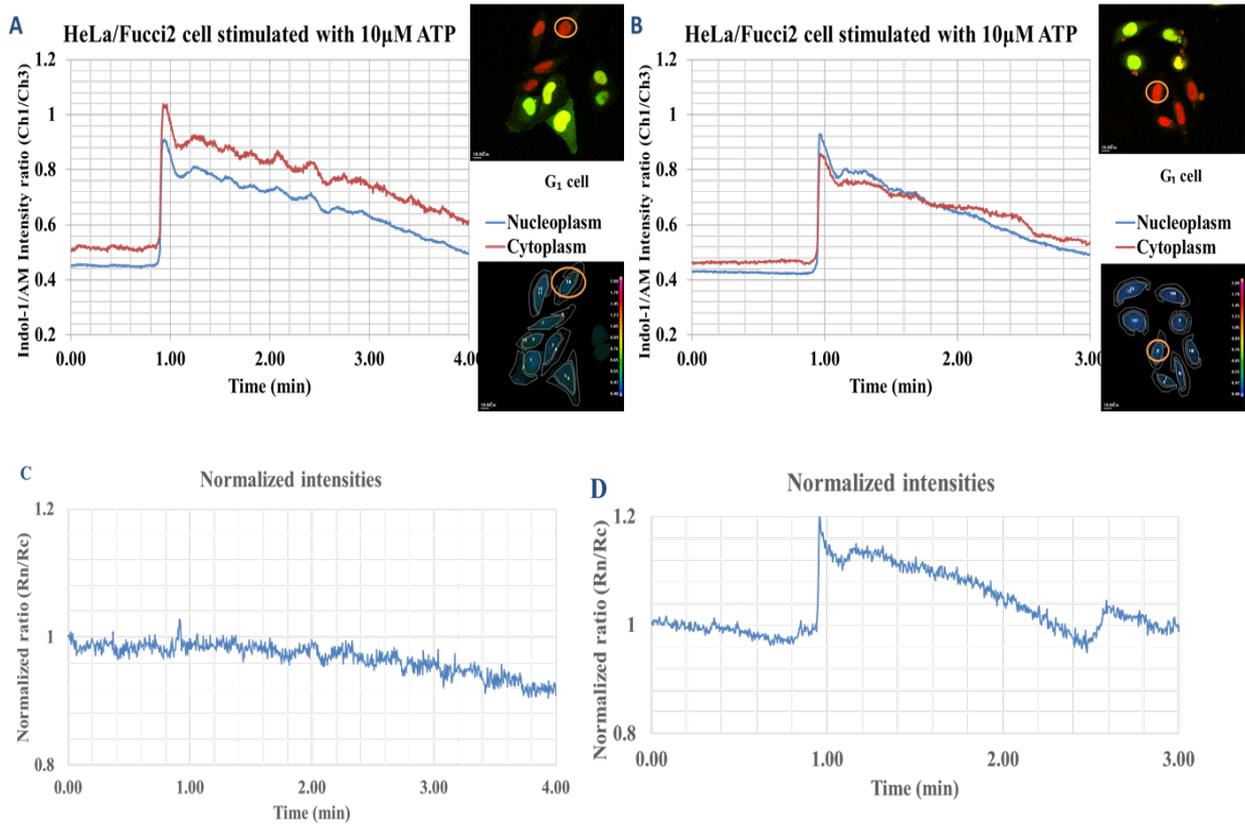
**Figure 2:** Effects of ATP on $[Ca^{2+}]_i$ transient in a $G_1$-stage of HeLa/Fucci2 cell (*n=30*). The data shows general pattern of intracellular $Ca^{2+}$ concentration as a function of time, displaying the variations in response amplitude between the nucleoplasm and cytoplasm on various $G_1$ cells. The observed responses between the two cellular compartments were of equal amplitudes in **A** (*n = 19*) but stronger in nucleoplasm than in cytoplasm in **B** (*n=11*) with their respective normalized intensities ratios, **C** and **D**. The insert shows the photomicrographs of the $G_1$ cell of which its nucleus appear *cherry red*. **Rn/Rc**: normalized fluorescence ratio. **Rn**: nucleoplasmic ratio; **Rc**: cytoplasmic ratio, where **R=Ft/Fo**. **Ft**: fluorescense intensity at time T; **Fo**: fluorescence intensity at time T=0.

### 3.2.2. ATP-Induced Ca²⁺ Responses in $G_1S$ HeLa/Fucci2 Cells

ATP stimulation, caused a transient increase in $[Ca^{2+}]_i$ commencing approximately at the same time between the cytoplasm and nucleoplasm. The evoked spike had an apparent peak that followed with a gradual decrease. Generally the response lasted longer than that observed in $G_1$ cells (Figure 3). Variations in the response amplitude were observed in both cellular compartments: nucleoplasmic responses were much stronger than that observed in the cytoplasm (n=8) while in other cells the responses were equal (n=6).

### 3.2.3. ATP-Induced Ca²⁺ Responses in S-phase HeLa/Fucci2 Cells

ATP stimulation resulted in a transient increase in $[Ca^{2+}]_i$ commencing in the cytoplasm followed by that in the

nucleoplasm. The evoked responses were stronger in the nucleoplasm than cytoplasmic (n=12) while in others it was equal (n=7) between the two cellular compartments. The response lasted relatively longer and had a spike characterized with clear peak that followed by a gradual decrease with short, sharp spikes that had a well-defined peak (Figure 4).
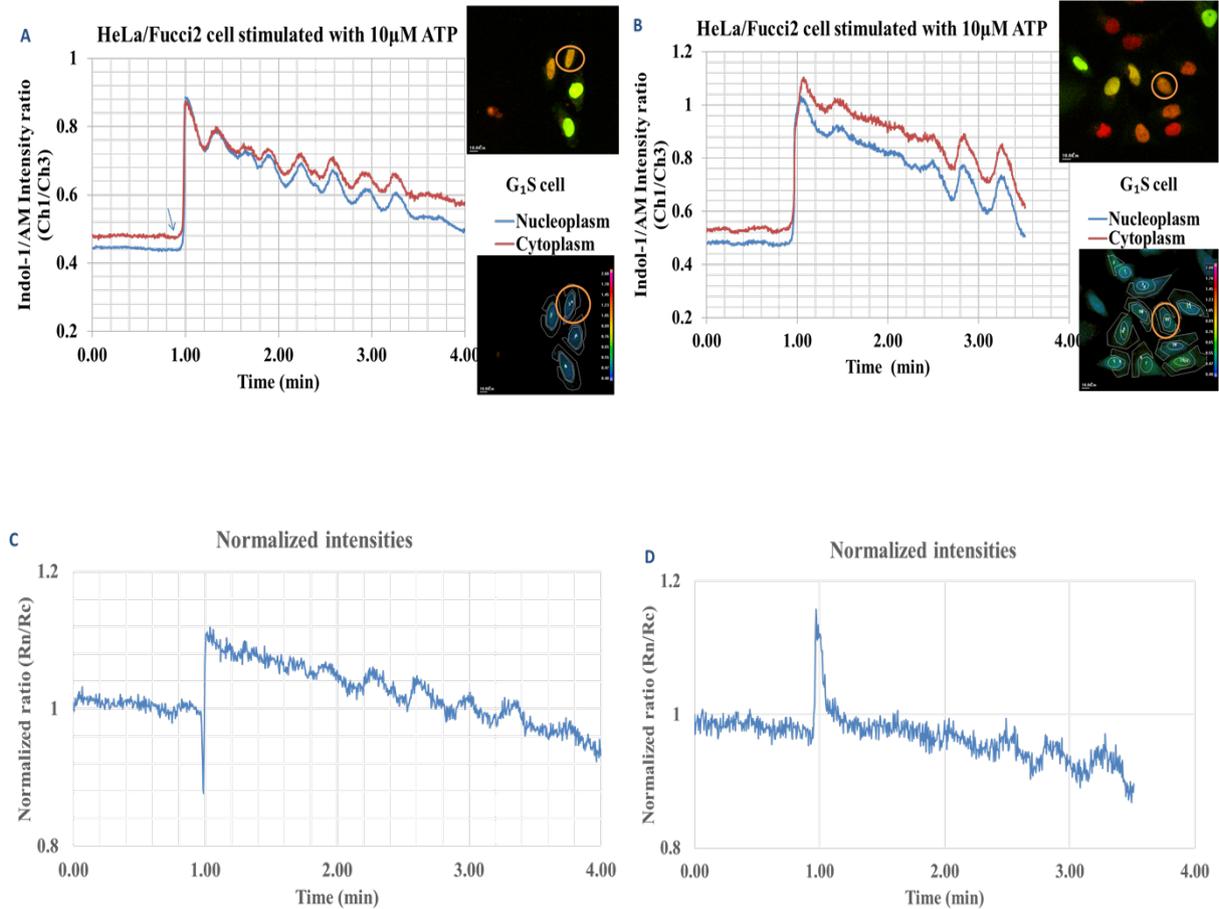


**Figure 3:** Effects of ATP on intracellular $Ca^{2+}$ concentration dynamics in a $G_1S$ HeLa/Fucci2 cell (*n=14*). The data shows changes in levels of $[Ca^{2+}]_i$ transient as a function of time, revealing the variations on response amplitude between the nucleoplasm and cytoplasm. The response started almost at the same time between two cellular compartments, however, **A:** the observed nucleoplasmic response was much stronger than that observed in the cytoplasm (*n= 8*) while in other experiments the responses were equal between the two cellular compartments (*n= 6*). **C** and **D** are the normalized intensities ratios respectively. All other conditions and parameters are the same as in Figure 1 and 2.
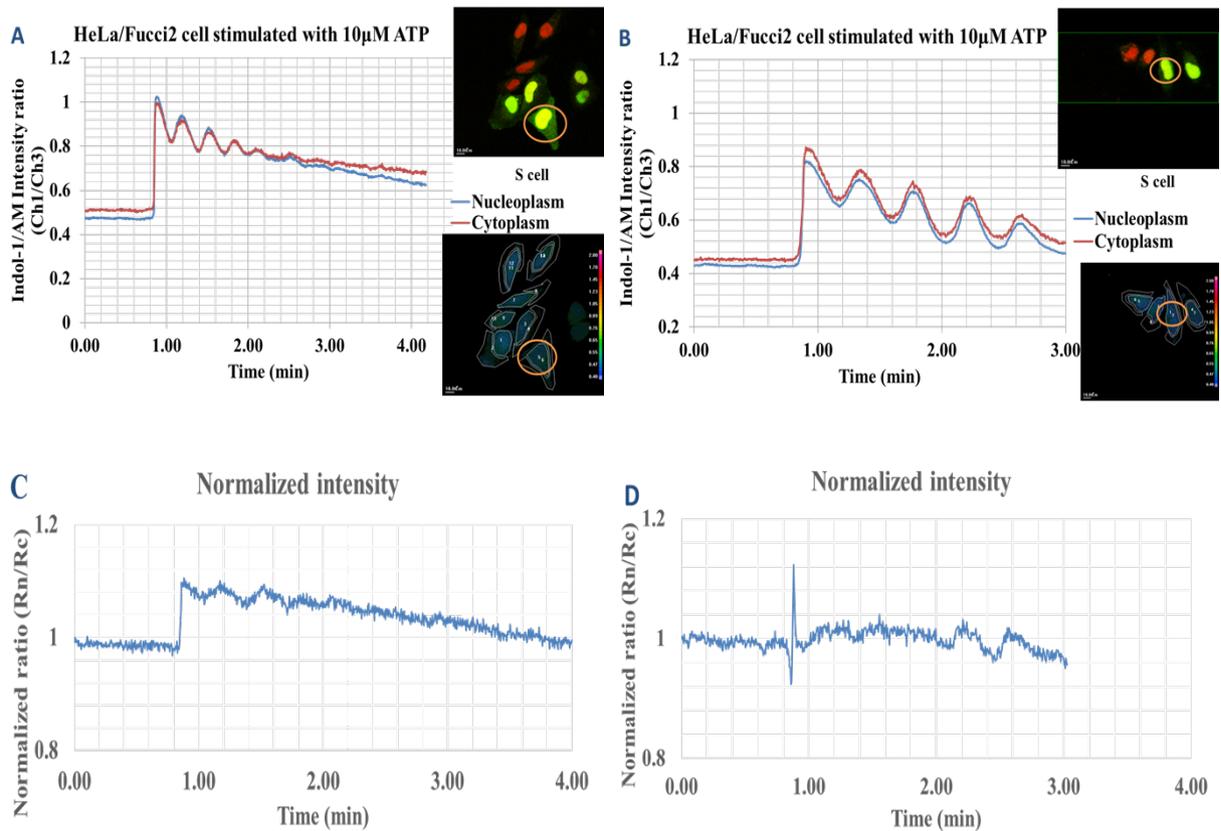
**Figure 4:** Effects of ATP on intracellular $Ca^{2+}$ concentration dynamics in S-phase HeLa/Fucci2 cell. The data shows general pattern of the $[Ca^{2+}]_i$ transient as function of time measured by Indol-1/AM fluorescence method. The observed response amplitude was; **A:** stronger in the nucleoplasm than that observed in the cytoplasm ($n=12$) or **B:** equal in other experiments ($n=7$) between the two cellular compartments. **C** and **D** are the normalized intensities ratios respectively. All other conditions and parameters are the same as in Figure 1 and 2.

### 3.2.4. ATP-Induced $Ca^{2+}$ Responses in $G_2M$ HeLa/Fucci2 Cells

ATP stimulation led to a transient increment in $[Ca^{2+}]_i$ that commenced in the cytoplasm followed by that in the nucleoplasm. Here, the evoked responses displayed an equal amplitude responses between the two cellular compartments (n=7), or much stronger in nucleoplasm (n=5) with some cells characterized with repetitive spikes (Figure 5).

### 3.3. Mechanisms Involved that Lead to Intracellular $Ca^{2+}$ Signal Changes in an Interphase HeLa/Fucci2 Cell Following Extracellular ATP Stimulation

Previous studies has demonstrated that, in non-excitable cells release of $Ca^{2+}$ from internal stores follows a ligand-receptor interaction at the cell surface that leads to activation of phospholipase C (PLCβ) due to conformational changes on the GPCR ($G_q$). To define the mechanism that lead to the rise in the intracellular $Ca^{2+}$ ions in a dividing interphase cell following mobilization of $Ca^{2+}$ from the internal stores, we examined the effects of U73122, 2-APB and RB-2 in intact HeLa/Fucci2 cells in the presence of 0.5M EGTA. U73122 (an inhibitor of PLC) inhibited the occurrence of intracellular $Ca^{2+}$ oscillations induced in a concentration dependent

effect. At 5μM U73122 did not inhibit ATP induced response, but at 10μM U73122 it successful terminated the $Ca^{2+}$ signal (Figure 6). These experiments indicated that U73122 inhibits the activity of PLCβ on cleaving the phosphatidylinositol-4,5-bisphosphate (PIP2) hence, interfere with the $IP_3$ pathway. We then examined the effects of 2-APB (an $IP_3R$ inhibitor) the results revealed that 100μM 2-APB did not abolish the ATP response. Thus, does not appear to affect the ATP stimulation on HeLa/Fucci2 cells. This finding correlates with findings of past study by Soulsby et al., [11], which demonstrated that 2-APB effects do vary between cell types. Finally, to assess the specificity of receptors involved, we examined the effects of RB-2 on the HeLa/Fucci2 cells.

Reactive Blue-2 (RB-2, a P2Y receptors antagonists; 100μM) inhibited the occurrence of intracellular $Ca^{2+}$ oscillations induced by 10μM ATP (not shown). These findings suggested that in general the intracellular $Ca^{2+}$ concentration response in sub phases of interphase HeLa/Fucci2 cell following stimulation with extracellular ATP follows the $IP_3$-pathway system mediated through P2Y receptors and PLCβ activation, which causes $Ca^{2+}$ mobilization from the intracellular stores.
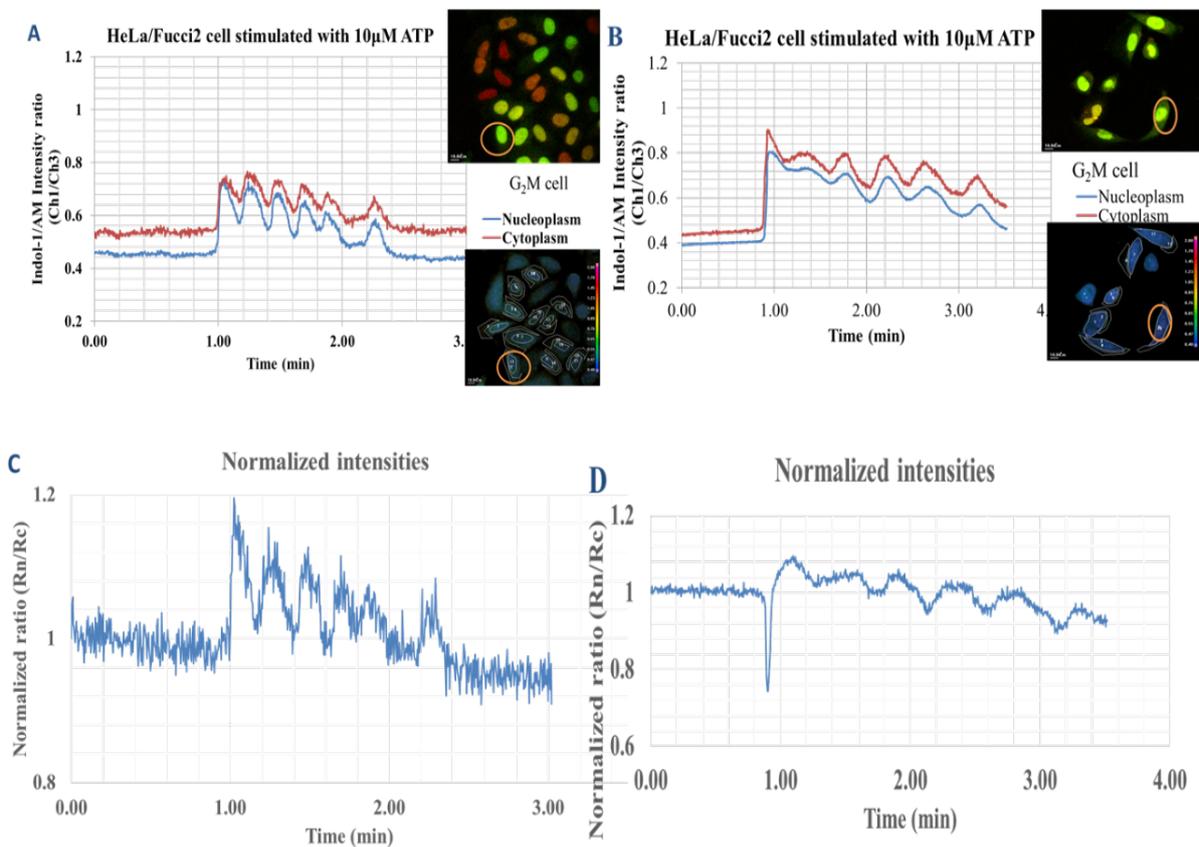


**Figure 5:** Effects of ATP on intracellular $Ca^{2+}$ concentration dynamics in a $G_2M$ phase HeLa/Fucci2 cells. The data shows changes in levels of intracellular $Ca^{2+}$ concentration as a function of time, which was measured by Indol-1/AM method. The observed response amplitude was; **A:** stronger in the nucleoplasm than that observed in the cytoplasm (*n=5*) or **B:** equal in other experiments (*n=7*) between the two cellular compartments. **C** and **D** are the normalized intensities ratios respectively. All other conditions and parameters are the same as in Figure 1 and Figure 2.
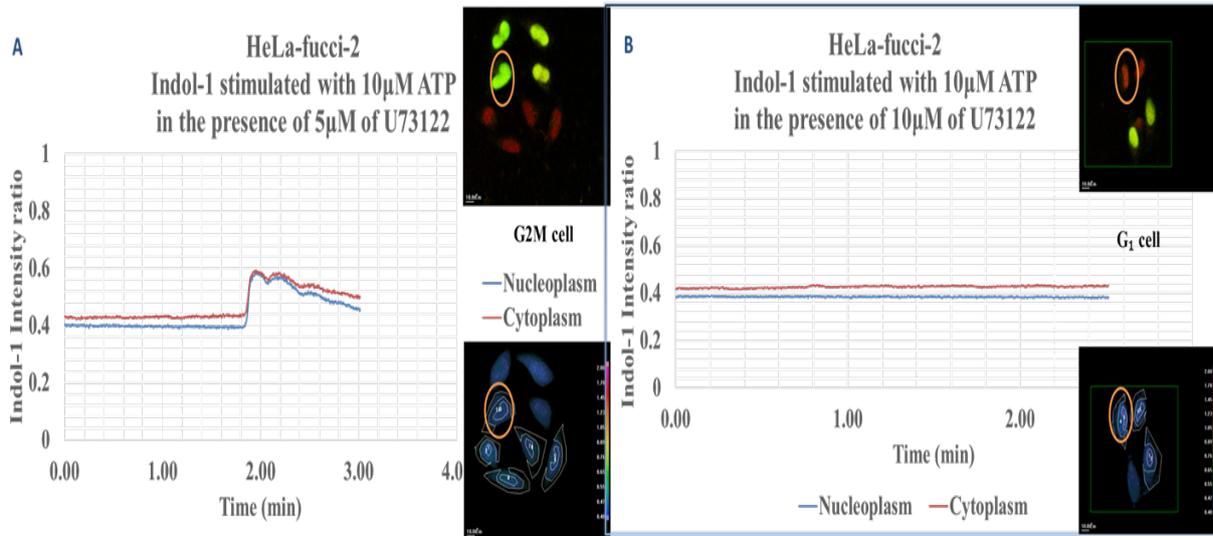
**Figure 6:** Displaying the evoked responses for $Ca^{2+}$ mobilization following ATP (10μM) stimulation in the presence of U73122. **A:** Shows the response observed at 5μM and **B:** at 10μM. The events triggered by the agonist shows the response of U73122 in abolishing the PLCβ effects is in concentration dependent manner. Cells were perfused with HR containing $Ca^{2+}$-free with 0.5M EGTA and U73122.

## 4. Discussion

Changes in intracellular $Ca^{2+}$ concentrations ($[Ca^{2+}]_i$) has been earlier linked to regulate cell division, cell proliferation, cellular growth and differentiation [12,13] involving assembly and disassembly of microtubules, the structural components of the mitotic spindle fibers, and thereby control spindle dynamics and chromosomes movement [14] hence cell cycle progression. A general comparison describing $[Ca^{2+}]_i$ in various stages of the cell cycle has been reported previously [8,15]. In contrast, the present study describes the relation of $Ca^{2+}$ signal between cytoplasm and nucleoplasm in various stages of an interphase HeLa/Fucci2 cell. Interestingly, the current study found that, both, the cytosolic and nuclear $Ca^{2+}$ signals do vary between the interphase stages and also within the same stage depending whether it is early or late in that particular stage. These findings are well supported by previous study by Okuda *et al.,* [16] which suggested that the expression of receptors (P2Y) fluctuates periodically in correlation with cell proliferation. Hence intracellular $Ca^{2+}$ signaling maybe altered in a dividing cell since it direct dependent on the expression of the signaling receptors. Based on these it is important to do further investigations on the receptor subtypes on each stages of the interphase cell.

It has been reported that $[Ca^{2+}]_n$ peaks were smaller than $[Ca^{2+}]_c$ peaks in HeLa cells [5,17]. However, this is not always the case as what have been observed in the interphase HeLa/Fucci2 cell in the present study. The variation of $[Ca^{2+}]_i$ peaks differ very much in different stages between the two cellular compartments, for example in a $G_1$ cell, we have seen at baseline $[Ca^{2+}]_n$ was lower than $[Ca^{2+}]_c$, however, following stimulation with ATP, $[Ca^{2+}]_n$ was elevated and had peaks larger than $[Ca^{2+}]_c$. Other observations showed concentrations being equal or $[Ca^{2+}]_c$ higher than $[Ca^{2+}]_n$. This strongly suggests that, the $Ca^{2+}$ signal amplification is very much dependent on the physiological or biochemical activity of a cell in a particular stage of cell division. The stronger responses observed in the nucleoplasm could be as a result of a potentiated $Ca^{2+}$ release from the

extremely small intranuclear vesicles that accumulate calcium and are sensitive to $IP_3$ as reported by Huh *et al.*, [18], and linked with intranuclear $Ca^{2+}$ release [5]. Our findings propose that, during the early $G_1$ phase, which is a growth phase, these vesicles and/or nuclear envelope (NE)-calcium stores are not yet fully matured or formed, and expression of the receptors and/or NE- PLCβ activities are not yet fully functional, while at the late $G_1$ phase they are fully matured and functional. Hence, the $IP_3R$-release $Ca^{2+}$ responses through the $IP_3$ pathways from the nuclear stores would differ between late and early stages as a result the $[Ca^{2+}]_n$ signal differ within the same stage of interphase cell.

The current study also found that, $Ca^{2+}$ signal differ in $G_1S$ check point and S phase. In a $G_1S$ the $Ca^{2+}$ signal lasted longer than that in $G_1$ phase. These findings are consistent with findings of previous study by Russa *et al.* [8] which reported longer spontaneous $[Ca^{2+}]_i$ oscillations in synchronized $G_1S$ cells. However, in their study they did not correlate between the $[Ca^{2+}]_n$ and $[Ca^{2+}]_c$, instead only reported general observation on $G_1S$ cells. Here we do report variability on the nucleoplasmic responses of which can be linked with nuclear activities during the $G_1S$ checkpoint. The most striking result in the current study is that both $[Ca^{2+}]_n$ and $[Ca^{2+}]_c$ transient increase commenced approximately at the same time. This strongly suggests that the $[Ca^{2+}]_i$ signal is very much active in both cellular compartment and more sensitive in the nucleus than in the cytoplasm at $G_1S$ stage as the cell check itself for readiness before entering the DNA replication phase.

It is apparent from this study that in the S phase the transient increase in intracellular $Ca^{2+}$ signals were also variable between the two cellular compartments. Among plausible explanations for these findings is that during the early S phase, there DNA replication in the nucleus which involve a lot of biochemical reactions of which $Ca^{2+}$ is an important second messenger or an agent for phosphorylation processes. These activities resulted in an increased intranuclear $Ca^{2+}$ signal following release from the minute intranuclear vesicles [5, 18] and/or nucleoplasmic reticulum via the $IP_3$ pathways, hence higher levels of $[Ca^{2+}]_n$ than the $[Ca^{2+}]_c$. Alternatively, the nuclear functions are much more active during the early than the late S phase.

## 5. Conclusions

Using HeLa/Fucci2 cells expressing the cell cycle stages indicators, this study has demonstrated that the intracellular calcium signal between the nucleoplasm and cytoplasm of an interphase cell is variable depending on the stages of cell division and also within the same stage reliant on whether it is in early or late phases. A plausible explanation for these variations in intracellular calcium signals is basing on the nuclear and cytoplasmic cellular activities during interphase cell cycle stage. Further investigation and experimentation into nuclear $Ca^{2+}$ signal in cell cycle sub phases is strongly recommended. It would be interesting to assess the effects of intranuclear vesicles [5, 18] and/or expression of receptors in a dividing interphase cell.

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