The role of the cell cycle in regulating intracellular calcium changes in mammalian mitosis

Abhinaya Yeddala, Mark Levasseur & Timothy R Cheek

Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences

NEWCASTLE UNIVERSITY

Introduction

Changes in intracellular free calcium concentration $[Ca^{2+}]_i$ play a central role in the regulation of a large number of cellular functions, including exocytosis, fertilisation, muscle contraction, synaptic transmission and cell division (1). Although it is accepted that Ca^{2+} is required for cell growth and proliferation, the nature of the Ca^{2+}-dependent pathways involved in this regulation are not fully understood. One possibility is that the cellular machinery responsible for generating changes in $[Ca^{2+}]_i$ is regulated in a cell cycle-dependent manner, so that Ca^{2+} signals are variable during different phases of the cell cycle (2).

Evidence in favour of alterations in Ca^{2+} signalling during the mammalian cell cycle has come from studies in which transient elevations in $[Ca^{2+}]_i$ have been detected (3). To further understand the mechanisms by which a cell controls its intracellular Ca^{2+} signals during the cell cycle, and more specifically during mitosis, we have investigated how Ca^{2+} signals alter between interphase and mitotically arrested HeLa cells (a human epithelial cell line). Specifically, we have examined cell cycle-related alterations in the store-operated Ca^{2+} entry (SOCE) pathway, which is a ubiquitous mechanism through which Ca^{2+} influx into the cell is triggered by hormones which deplete the intracellular Ca^{2+} store (the ER) via activation of the phosphoinositide signalling pathway (4).

The results reveal that in cells undergoing mitosis the SOCE pathway becomes remodelled, specifically Ca^{2+} entry becomes decoupled from the release of intracellularly stored Ca^{2+} and is inhibited.

Results

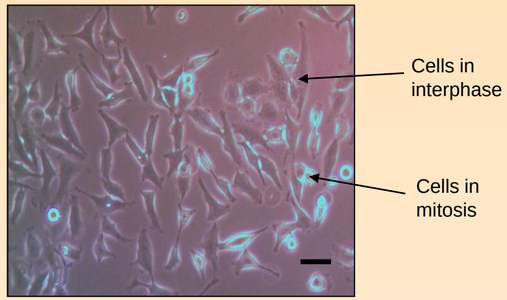

Figure 1. Differential Interference Contrast (DIC) image shows typical morphology of both interphase and mitotic cells as indicated. Cells in mitosis are typically spherical since they round-up as a prelude to cell division. Cell in interphase are typically elongated since they are strongly adherent to the substrate. Scale bar = 25 µm.

Results

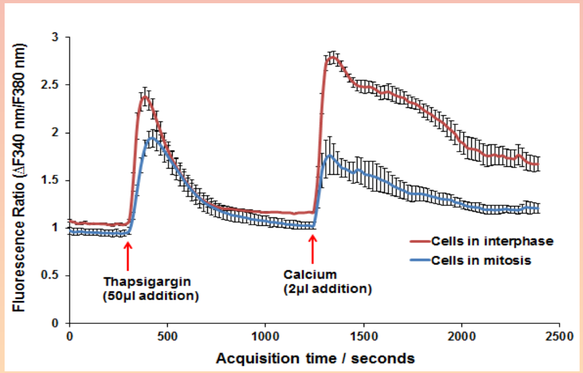

Figure 2. Ca^{2+} signals evoked by store depletion and subsequent SOCE in single HeLa cells. Traces show transient rises in $[Ca^{2+}]_i$ in response to application of 200 nM thapsigargin (to deplete the ER Ca^{2+} store) followed by application of 1 mM extracellular Ca^{2+} (to reveal SOCE). Traces show typical responses collected from 5 interphase cells (as indicated by the red traces) and 5 mitotic cells (as indicated by the blue traces). Traces plotted for each group show mean data ± S.E.M. (n = 5).

These data suggest that while the extent of store depletion is similar between interphase and mitotic cells, the extent of SOCE is reduced in mitotic cells.

Method: Cells were loaded with the fluorescent Ca^{2+}-indicator dye Fura-2/AM according to standard techniques (4). Resulting fluorescence emission was gathered at 510 nm following dual-excitation of the dye at 340 nm and 380nm. Traces show the ratio of 340/380 nm, an increase in fluorescence reflects an increase in $[Ca^{2+}]_i$. Thapsigargin inhibits the SERCA pump on the ER membrane, resulting in an increase in $[Ca^{2+}]_i$ due to depletion of the ER Ca^{2+} store. Addition of extracellular Ca^{2+} reveals Ca^{2+} entry via the SOCE pathway that is triggered by store depletion.

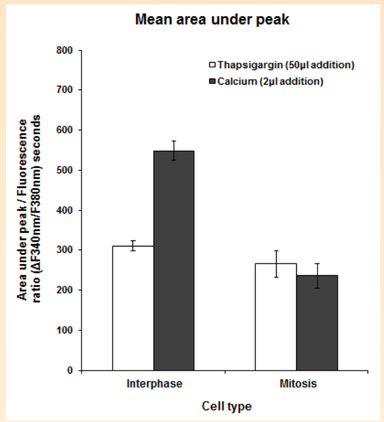

Figure 3. Quantification of Ca^{2+} signals evoked by store depletion and subsequent SOCE in single HeLa cells as shown in Figure 2. Histograms show area under peak from data such as that shown in Figure 2. Bars plotted for each group show mean data ± S.E.M. (n >17).

These data confirm that although there is no significant difference between the extent of store depletion between interphase and mitotic cells (white bars), the extent of SOCE is significantly reduced in mitotic cells (black bars).

		Control	
Parameter measured	**Peak measured**	**Interphase**	**Mitosis**
Mean height of peak / Fluorescence ratio (ΔF340nm/F380nm) units	Thapsigargin (50µl addition)	0.216	0.333
	Calcium (2µl addition)	0.515	0.243
Mean rate of rise / Fluorescence ratio (ΔF340nm/F380nm) unit seconds^{-1}	Thapsigargin (50µl addition)	0.0015	0.0023
	Calcium (2µl addition)	0.0069	0.0017

Table 1. Quantification Ca^{2+} signals evoked by the application of thapsigargin and extracellular Ca^{2+} to HeLa cells as shown in Figure 2. The table shows mean data for height of peak and rate of rise for mitotic and interphase HeLa cells from data such as that shown in Figure 2. Values for each group show mean data (n >17).

These data confirm that the mean height of peak and mean rate of rise of SOCE (Ca^{2+}) is significantly reduced in mitotic cells compared to interphase cells.

Conclusions

We used a single cell real-time protocol to investigate the effects of the cell cycle on intracellular Ca^{2+} regulation. The key finding are:

- Cells in interphase show a robust (i.e. normal) SOCE response following Ca^{2+} store depletion.
- Cells in mitosis show a significantly down-regulated SOCE response in terms of total Ca^{2+} entry, height of peak and rate of rise, despite Ca^{2+} store depletion remaining essentially unaltered.

These results demonstrate that during mitosis Ca^{2+} signalling pathways become remodelled, specifically SOCE becomes decoupled from Ca^{2+} store depletion. While the functional consequence of the remodelling of Ca^{2+} signals remains to be determined, as also does elucidation the underlying molecular mechanism, it is clear that the SOCE pathway represents a potential drug target in the control of the cell cycle.

References

1. Bootman MD *et al* (2001) Sem. Cell Dev. Biol. **12,** 3-102.
2. Berridge MJ (1995) Bioessays **17,** 491-500.
3. Ratan RR *et al* (1986) Proc. Natl. Acad. Sci. USA. **83,** 5136-5140.
4. Brown AM *et al* (2005) Biochem. J. **388,** 941-948.

Acknowledgements

We would like to thank Newcastle University for the award of a Research (Vacation) Scholarship to AY. Research in the TRC lab is supported by the MRC and the BBSRC