Air bubble contact with endothelial cells in vitro induces calcium influx and IP3-dependent release of calcium stores

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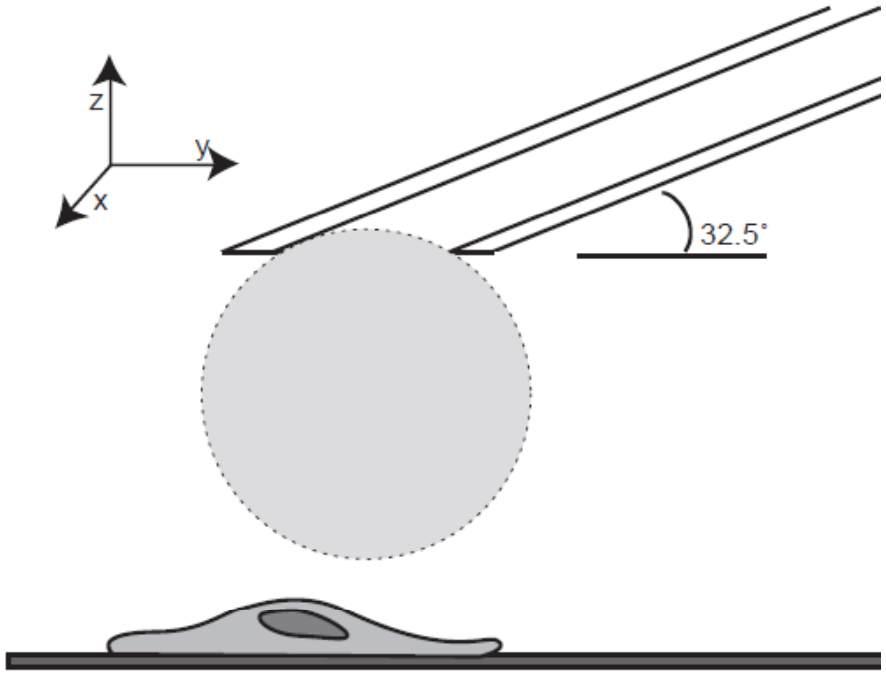
Introduction

- Decompression carries risk of gas embolism
  - Limited treatment options
    - Hyperbaric oxygen therapy
    - 30 years of research, no drug translated to the clinic
- Gas microemboli can cause changes in blood flow and organ function in the absence of vessel obstruction
- Air bubble contact with endothelial cells causes a transient increase in intracellular calcium which is associated with cell injury/death
- Determining the mechanism responsible for the generation of the intracellular calcium transients may offer targets for novel therapies

Revolutionary Research . . . Relevant Results

Methodology

- *In vitro* cell culture
  - human umbilical vein endothelial cells (HUVEC)
- Load with Fluo-4
  - Calcium sensitive, fluorescent dye
- Blow and manipulate 50-150 μm air bubbles



- Examine using epifluorescence microscopy
- Focus on non-lethal air bubble contact

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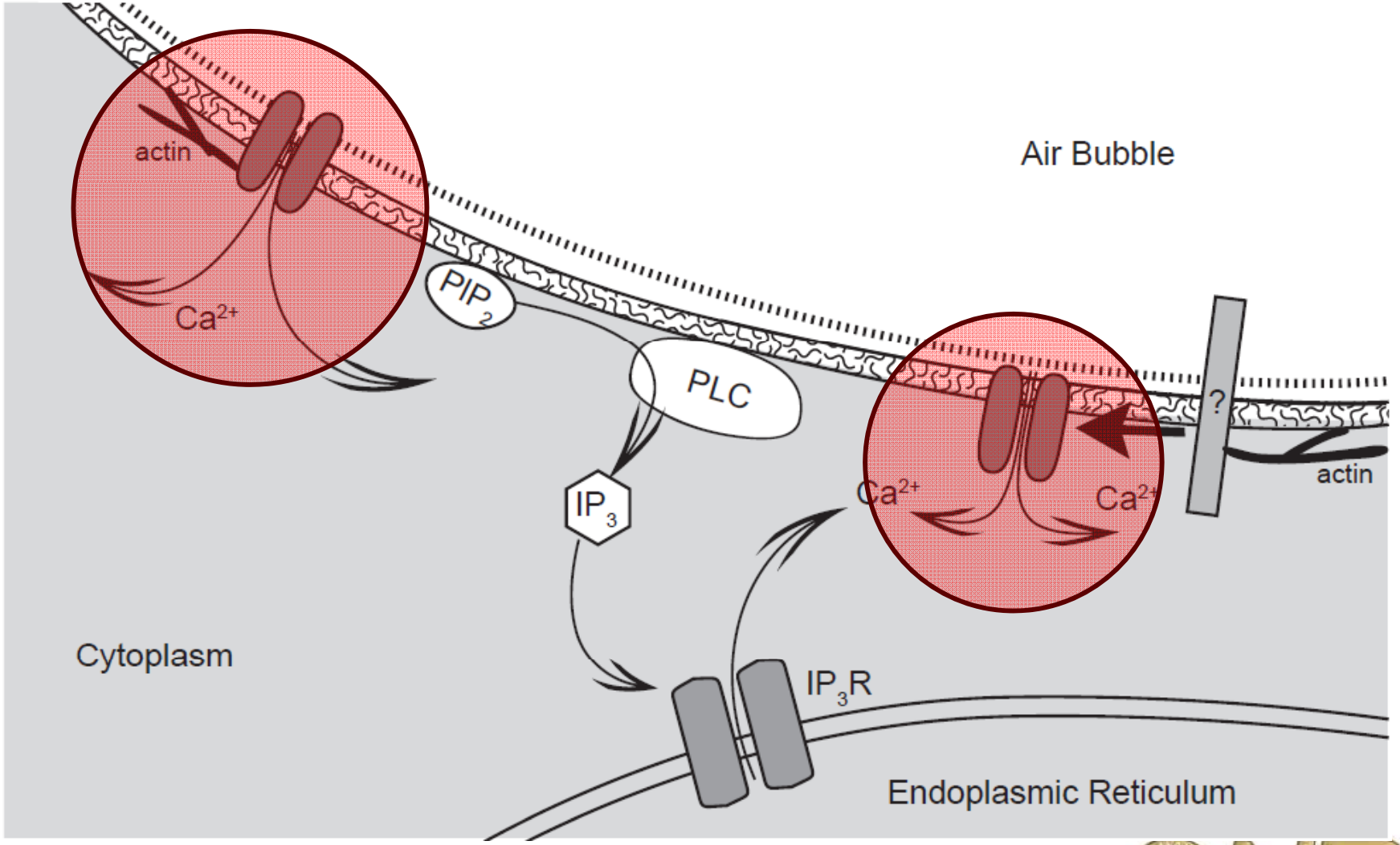
Calcium influx via channels



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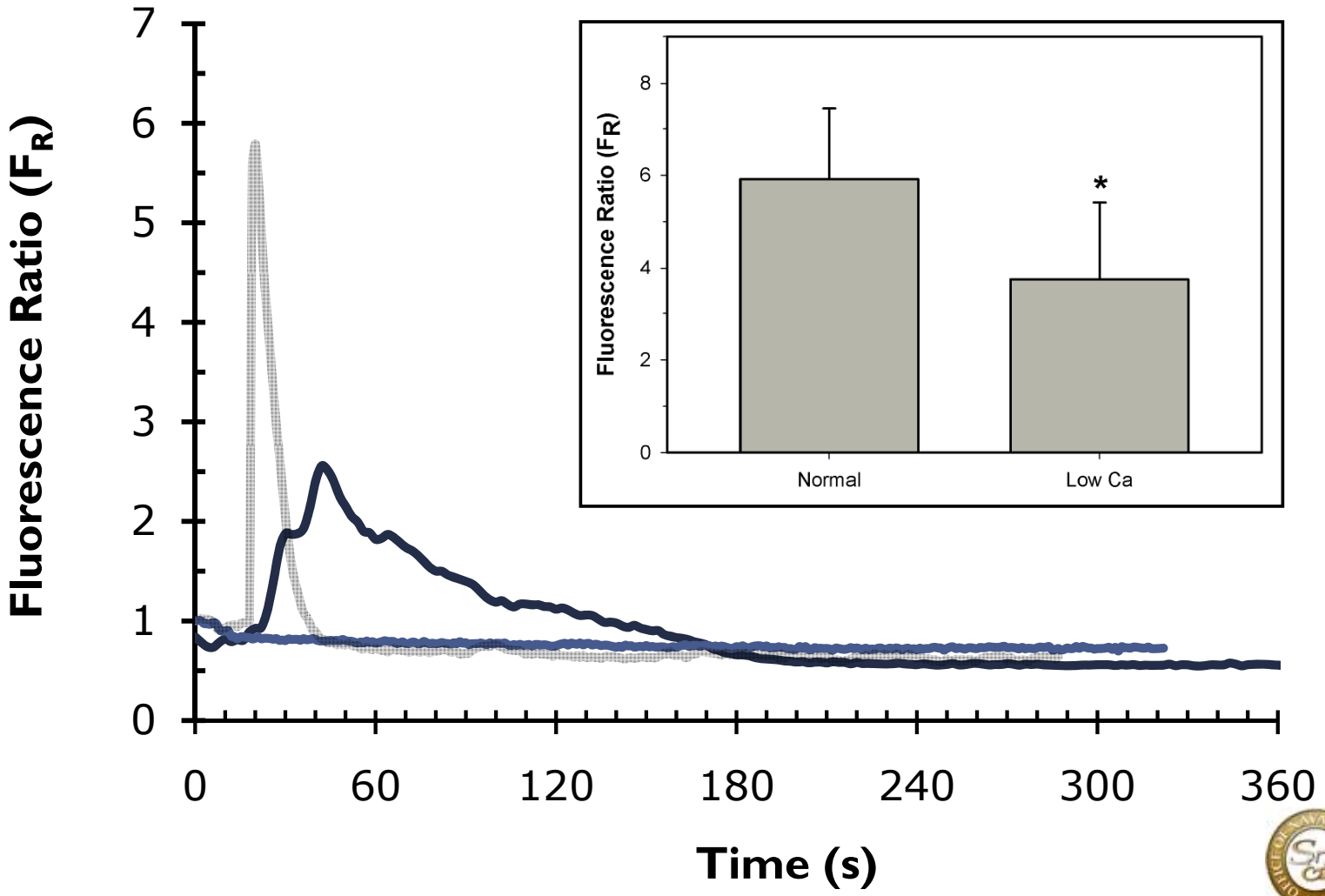
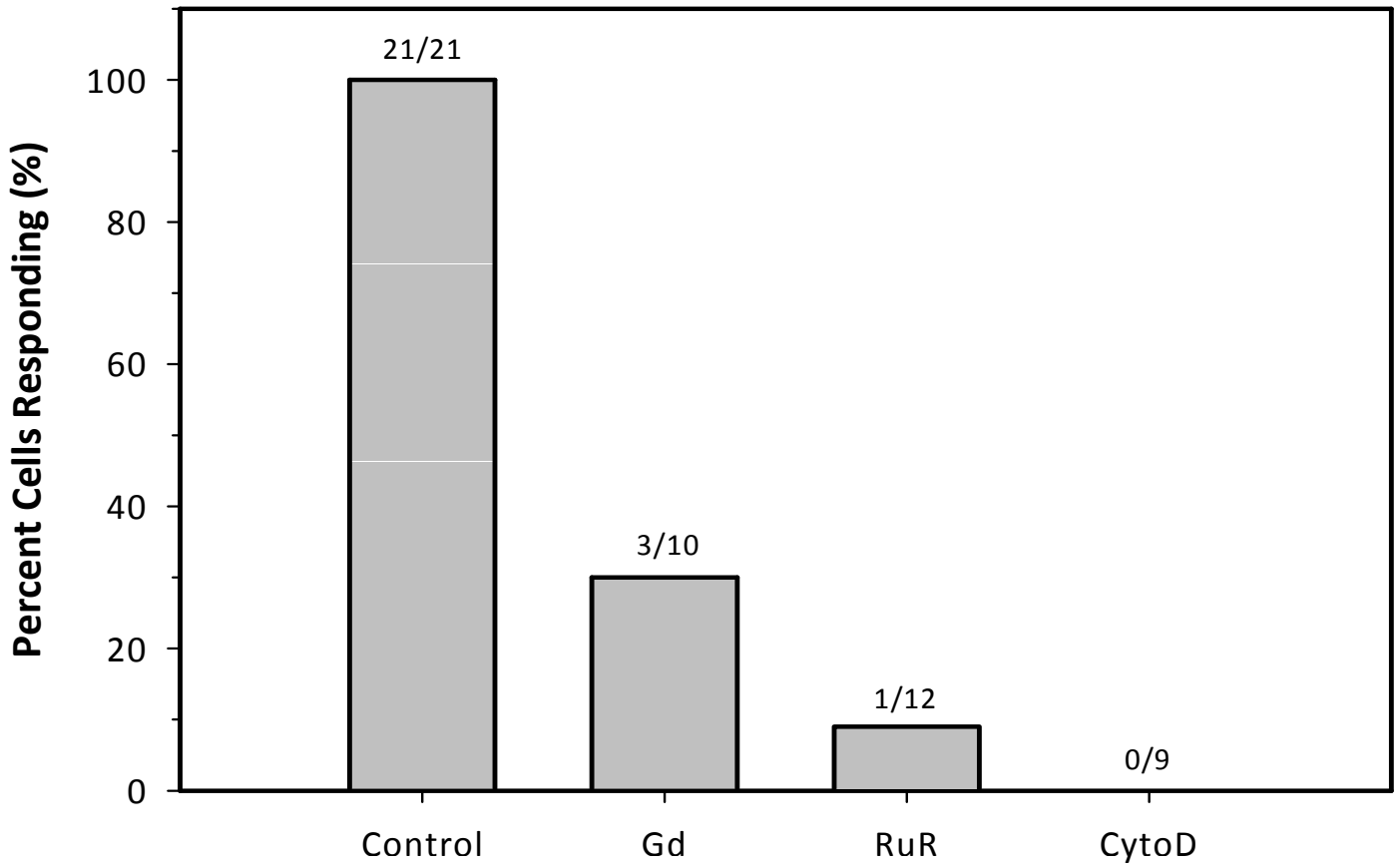
Extracellular calcium is necessary

- Low extracellular calcium (1:50, ~26µM):
  n=13: 6 non-responders, 7 responders



Calcium enters via channels

- Channel inhibitors Gadolinium (Gd) and Ruthenium Red (RuR) markedly inhibit air bubble-induced calcium transients



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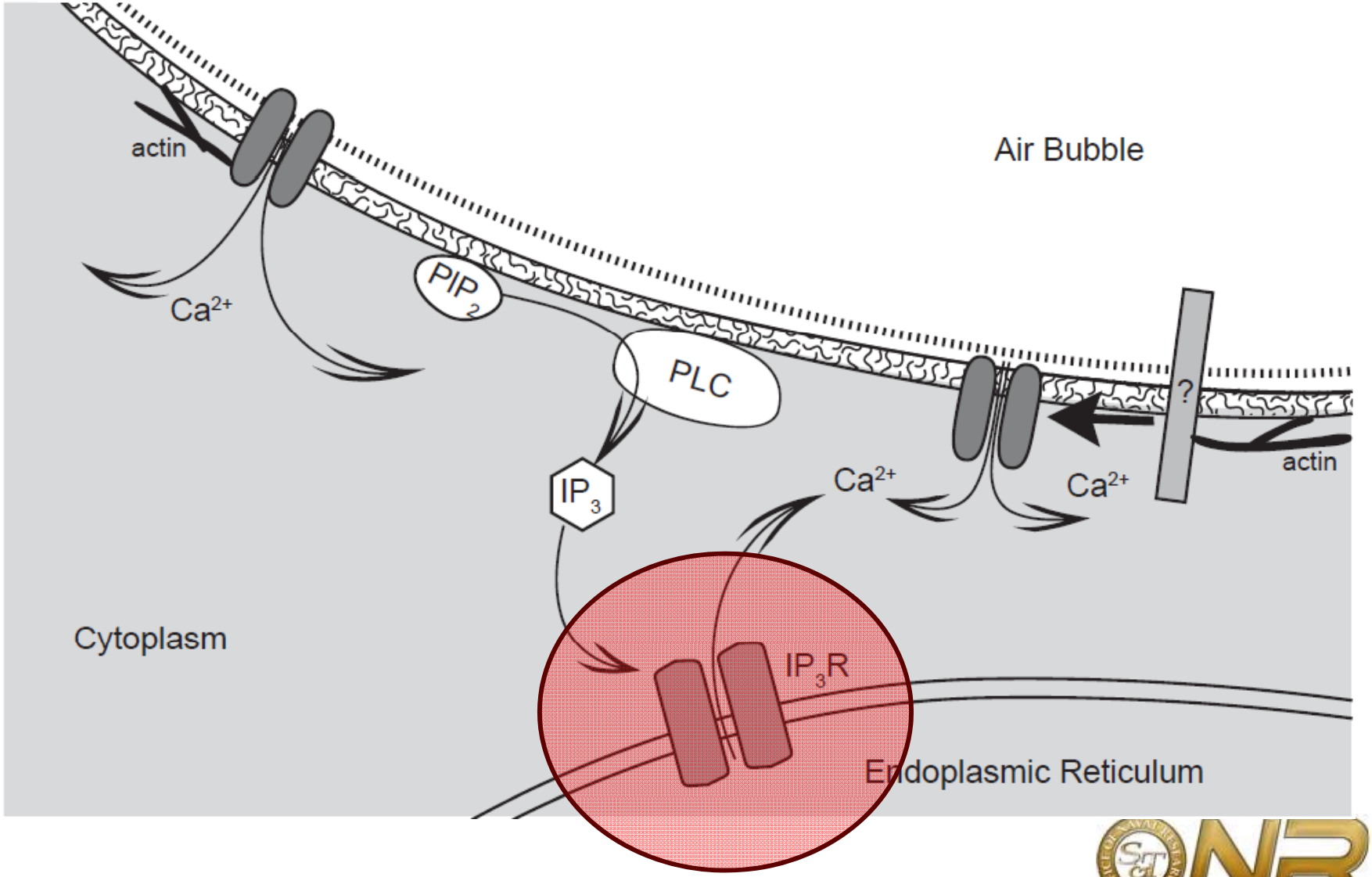
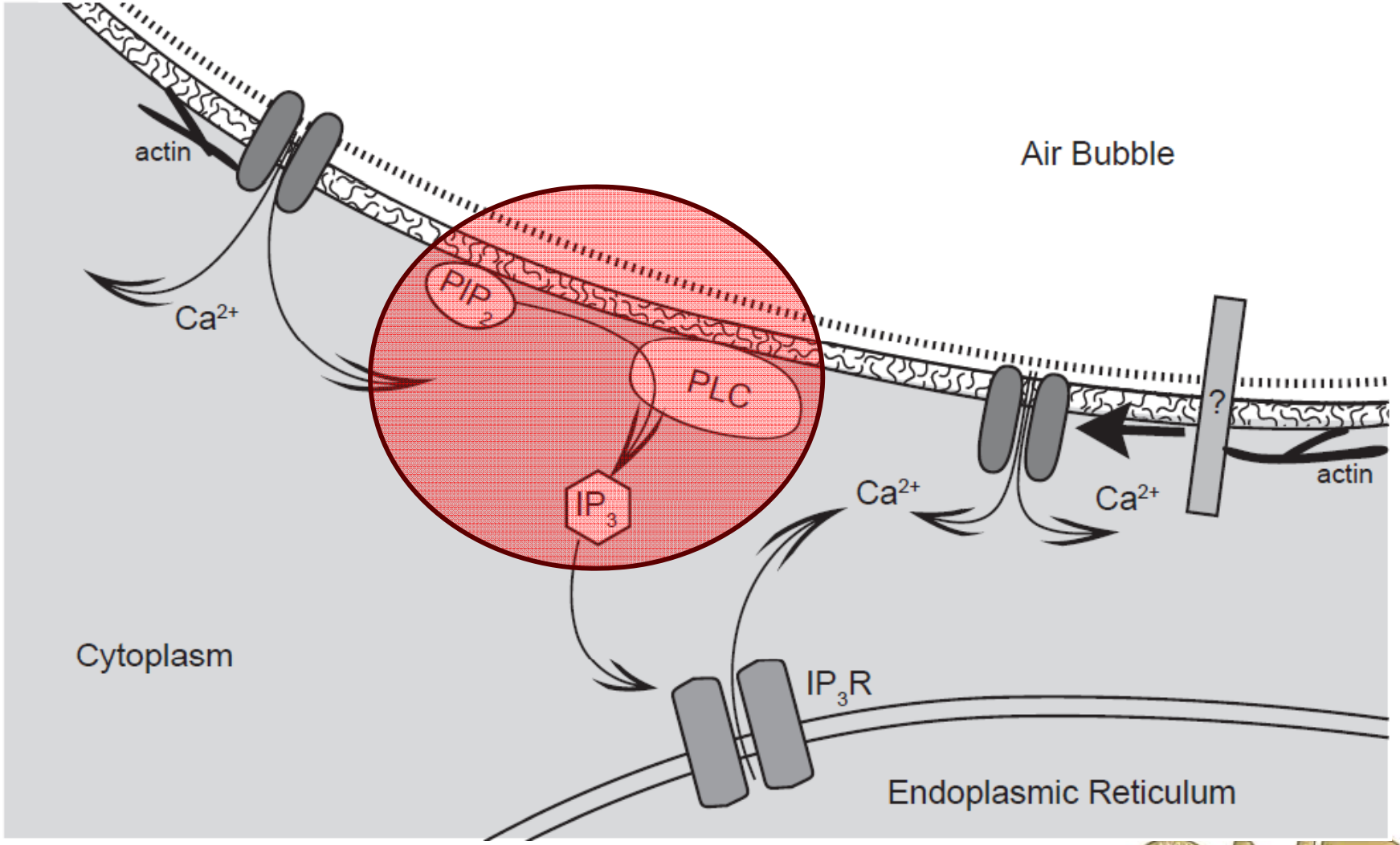
IP3-dependent release of ER calcium

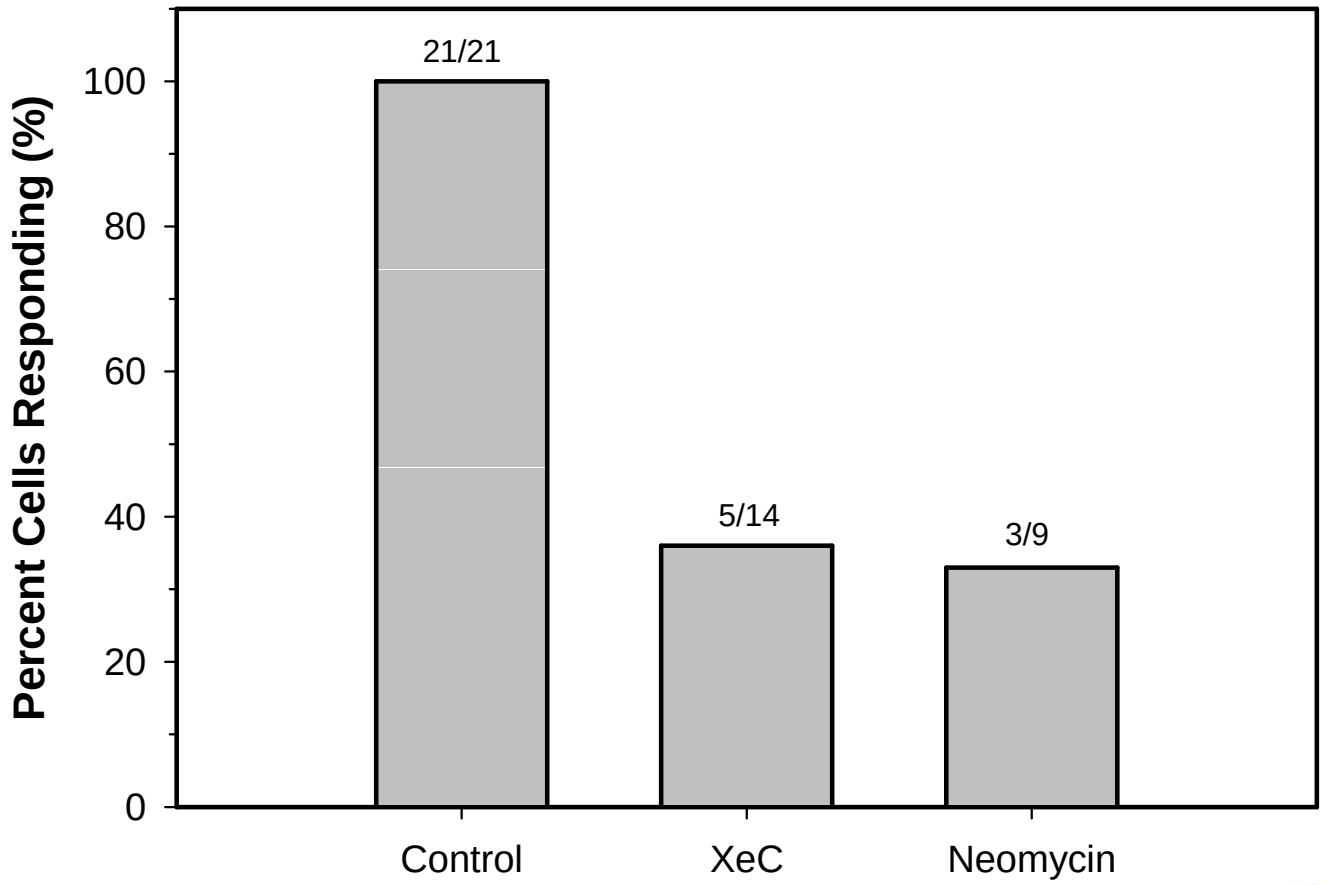


IP3 generation via phospholipase C



IP3-dependent release of ER calcium

- Inhibition of IP3 Receptor via Xestospongin C, or PLC via Neomycin, ameliorates the cellular response to air bubble contact



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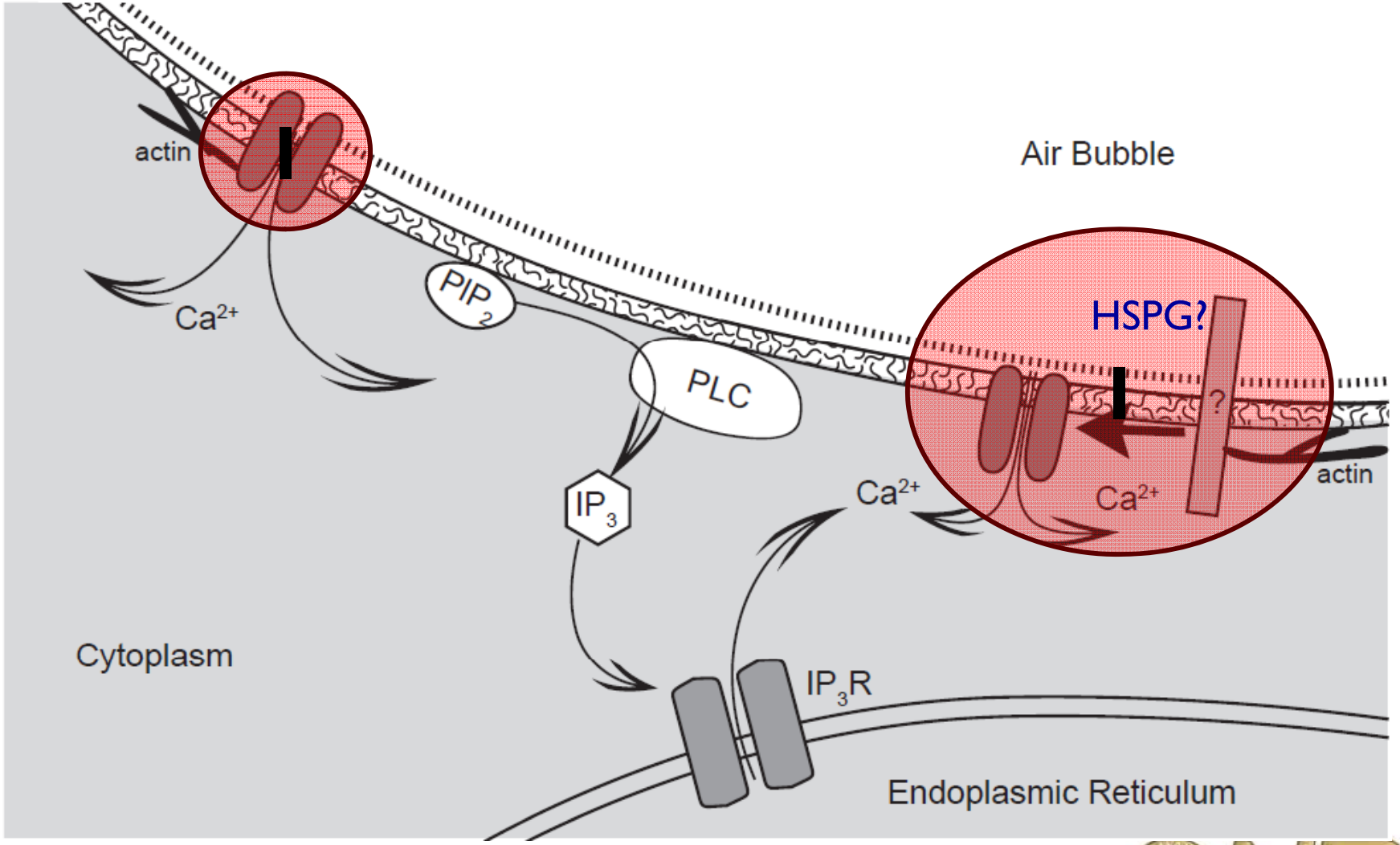
Summary



Revolutionary Research . . . Relevant Results

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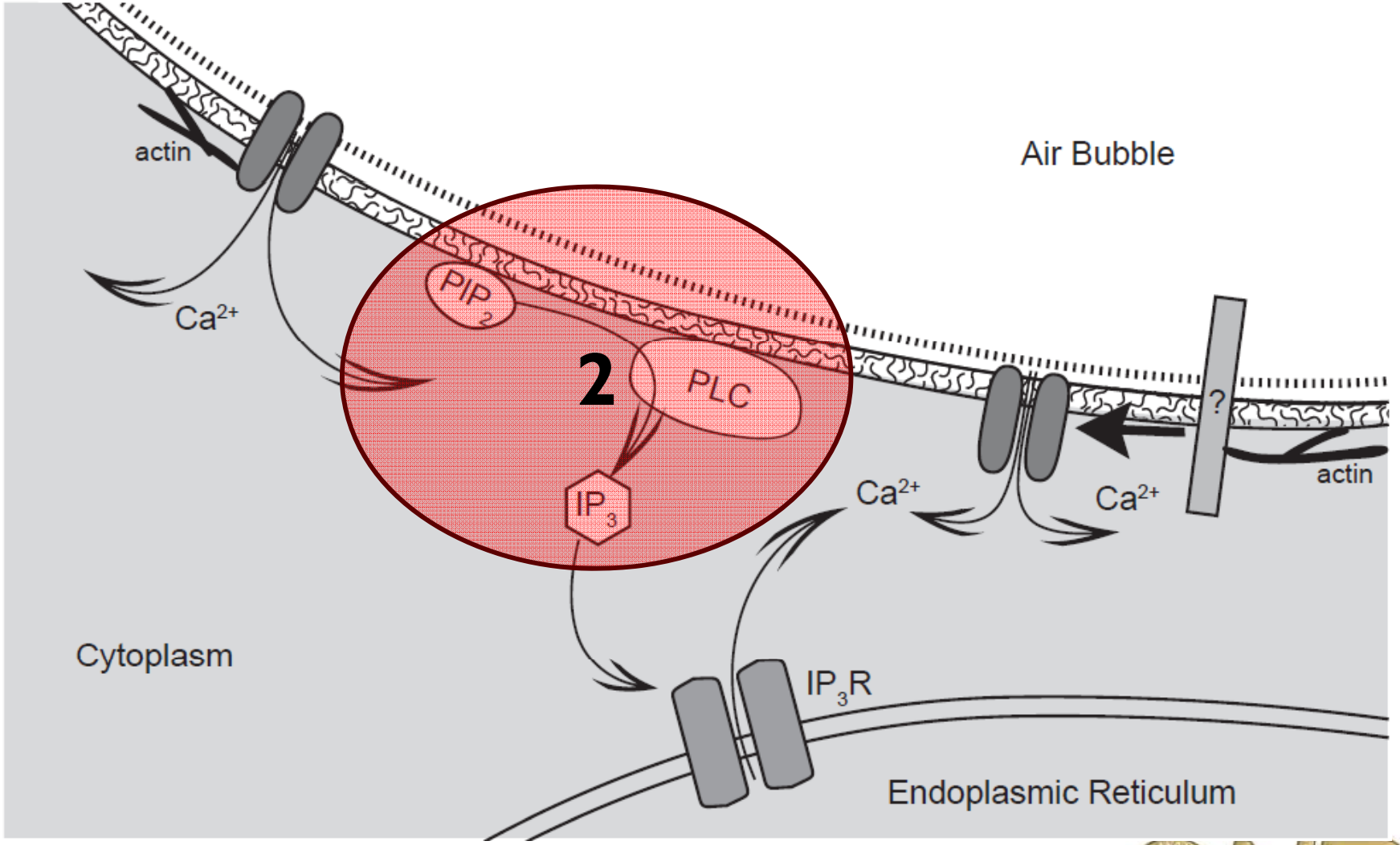

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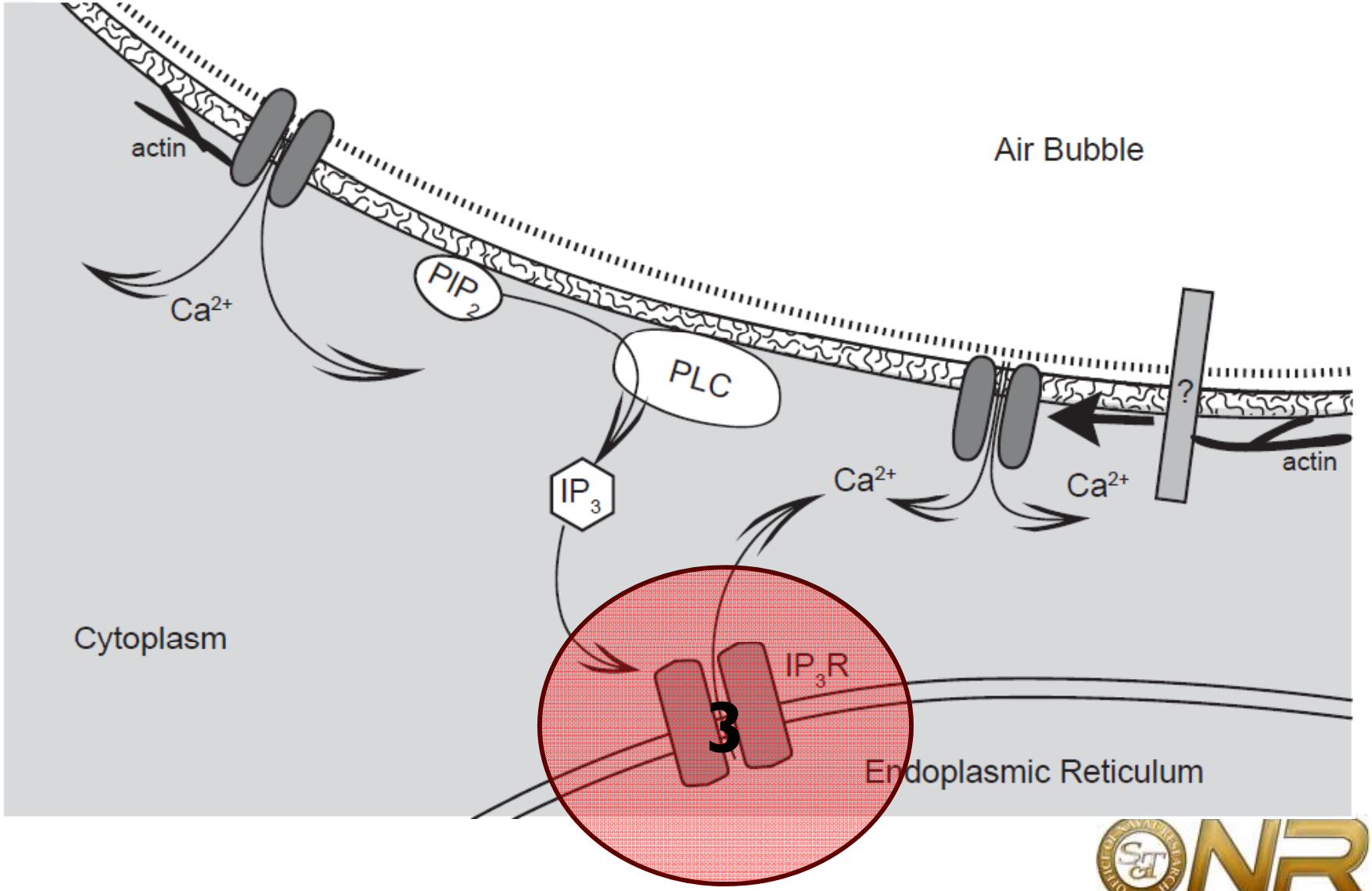

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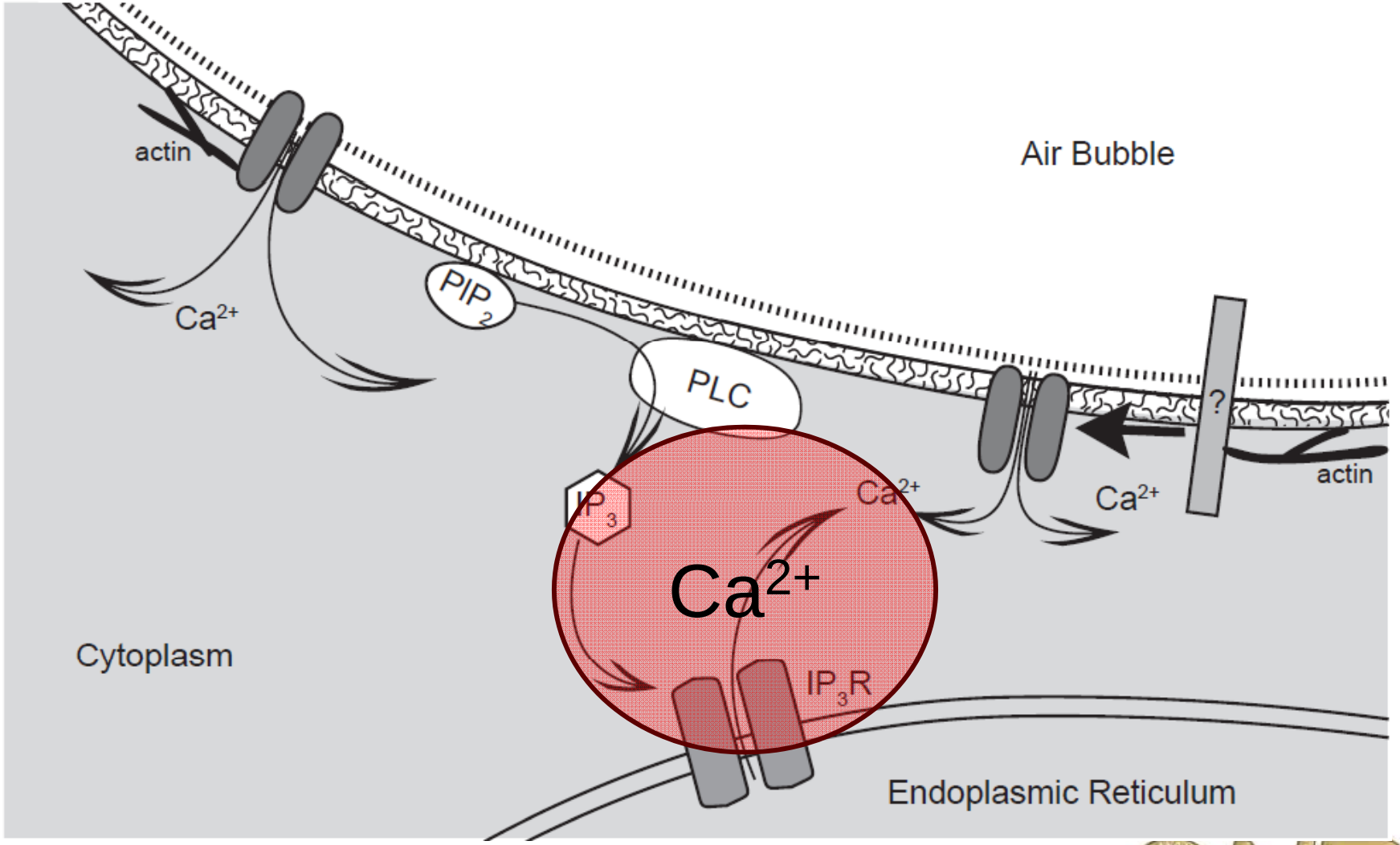
Summary



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Conclusions

- Air-liquid interface interaction with endothelial cells causes an intracellular calcium transient
- The transient is dependent on extracellular calcium influx via a calcium channel, such as TRPV family member
- Calcium influx activates PLC, resulting in IP3-dependent release of calcium from the ER
- The interfacial interaction, which acts as a trigger, offers an attractive target for preventative and therapeutic pharmacological interventions, such as surfactants

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