Intestinal Stem cells: Got Calcium?

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Summary

Calcium ions (Ca^{2+}) are well-known intracellular signalling molecules. A new study identifies local cytoplasmic Ca^{2+} as a central integrator of metabolic and proliferative signals in *Drosophila* intestinal stem cells (Figure 1).

Main Text

Adult tissue homeostasis involves tightly synchronized rates of cell production versus cell removal. Stem cells – characterised by their capacity to self-renew and produce differentiated progeny – are at the centre stage of that balancing act. The intestinal epithelium has been a long-standing paradigm for the study of tissue homeostasis by stem cells [1]. Consistent with its role as a key metabolic, immune and endocrine organ, the intestine is subject to multiple extrinsic and intrinsic stimuli, which need to be coordinated in order to adjust stem cell proliferation and differentiation to tissue demands. How these different inputs are integrated is a key open question. A new study by Deng et al. [2] in the adult *Drosophila* intestine identify local intracellular Ca^{2+} as a key node translating metabolic and growth factor signals into stem cell proliferation (Figure 1).

Following the characterization of intestinal stem cells (ISCs) in *Drosophila* [3,4] the fly gut has become an invaluable model system to study stem cell biology, host-pathogen interactions, ageing and metabolism among others [5–8]. As its mammalian counterpart, the adult *Drosophila* midgut has remarkable plasticity and can adapt its growth to multiple conditions including nutrient availability [9]. The intestine of newly hatched flies grows to its final size during the first five days of life and after feeding starts [10]. Reciprocally, the intestine enters a reversible state of quiescence during periods of starvation. While Insulin signalling is known to mediate this adaptive growth [10], the effect of individual nutrients on intestinal physiology had not been established. To assess the role of diet-derived aminoacids – an essential source of energy – in intestinal homeostasis [11,12], Deng et. al fed flies on food supplemented with L-glutamate (L-Glu) [2]. Interestingly, they found that the sole incorporation of L-Glu but not other aminoacids or sugar to the fly food was sufficient to induce ISC proliferation and tissue re-sizing. Following a comprehensive genetic and functional characterization of the pathway activated by L-Glu the authors concluded that the aminoacid absorbed by Enterocytes activates a signalling cascade including metabotropic glutamate receptor (mGluR)/Phospholipase C (PLC)/Inositol-triphosphate (IP3) within ISCs. Those sets of events are necessary and sufficient to drive stem cell proliferation [2] in the midgut (Figure 2).

The mGluR/PLC/IP3 pathway is known to signal through changes in levels of cytosolic Ca^{2+} [12]. In what represents the highlight of their work, Deng et al combine elegant live imaging with the use of transgenic Ca^{2+}-reporters to monitor cytosolic

Ca^{2+} in the intestine in vivo [2]. Strikingly, ISCs exhibited frequent, robust fluctuations in cytoplasmic calcium levels, which depended on Ca^{2+} release from the endoplasmic reticulum (ER) as well as influx through the plasma membrane. Furthermore, L-Glu signalling activation and induced proliferation of ISCs was linked to reduced Ca^{2+} oscillation frequency and elevated cytosolic ion levels [2] (Figure 1). Multiple genetic manipulations leading to elevation of cytosolic Ca^{2+} or activation of the downstream calcium-dependent serine-threonine phosphatase Calcineurin appear to be sufficient to drive ISC proliferation.

While the results presented provide a compelling detailed account for the pathway involved in glutamate-mediated ISC proliferation whether this newly discovered role of Ca^{2+} has broader implications was still an open question. To tackle this Deng et. al embarked in a 'tour de force' to address the epistatic relationship between Ca^{2+} oscillations and signalling and conditions known to drive ISC proliferation in the midgut such as Ras, JAK/Stat, JNK and InR activation as well Notch inhibition, acute damage and ageing of the intestinal epithelium [5]. In all cases ISC proliferation led to a decrease in Ca^{+2} oscillation frequency and an increase in the average level of cytosolic Ca^{+2}. Importantly, ISC proliferation within these multiple contexts was significantly reduced when Ca^{+2} signalling was impaired [2]. Therefore, far from being an exclusive translator of ISC proliferation downstream of L-Glut feeding, Ca^{+2} appears as an unsuspected integrator of multiple proliferative signals in the *Drosophila* midgut (Figures 1 and 2).

As with any new discovery, the work by Deng et al. opens up multiple questions. One unresolved issue relates to the identity of the signals that normally orchestrate Ca^{+2} oscillations within ISCs in mature unchallenged tissues. Obvious candidates are the signalling pathways required for homeostatic ISC self renewal [5]. Stem cell-specific knockdown of pathway components coupled with in vivo Ca^{+2} imaging should shed light into this issue. It is also unclear whether Ca^{+2} oscillation frequencies are uniform throughout the midgut or if they are influenced by molecular and cellular gut regionalization [13,14].

The most groundbreaking aspects of the report by Deng et al. are the identification of Ca^{+2} oscillations within ISCs and the fact that they represent a central node downstream of multiple conditions, which impact ISC proliferation. In the response to L-Glu, which involves direct activation of a G-Protein coupled receptor (GPCR), modifications to the levels of cytosolic Ca^{+2} are an expected outcome. However, the link between signalling and Ca^{2+} is more surprising –and likely indirect – in the case of the other proliferative conditions analysed. One possibility is that the activated signalling pathways impact Ca^{2+} levels indirectly through effects on L-Glu metabolism. However, that does not seem to be the case in the context damage-induced ISC proliferation upon oral infection with the Gram-negative bacterium, *Erwinia carotovora carotovora 15* (*Ecc15*) or by feeding the DNA damaging-agent Bleomycin [2]. Alternatively, heterotrimeric G-proteins might represent one link between intestinal hyperproliferation and cytosolic [Ca^2]. In

addition to GPCRs, heterotrimeric G-proteins have been shown to interact with other receptor families including receptor tyrosine kinases [15]. These receptors are also capable of directly activating PLC resulting in the IP3 signal required for ER calcium release [15].

Finally, the question that most of us working with flies get: How conserved are the presence and role of Ca^{2+} oscillations within the intestinal epithelium and in stem cells in general? Time will tell, but one can speculate that the presence of a unifying signalling molecule that coordinates multiple inputs is an essential component of the machinery required to maintain homeostatic balance in actively self-renewing tissues.

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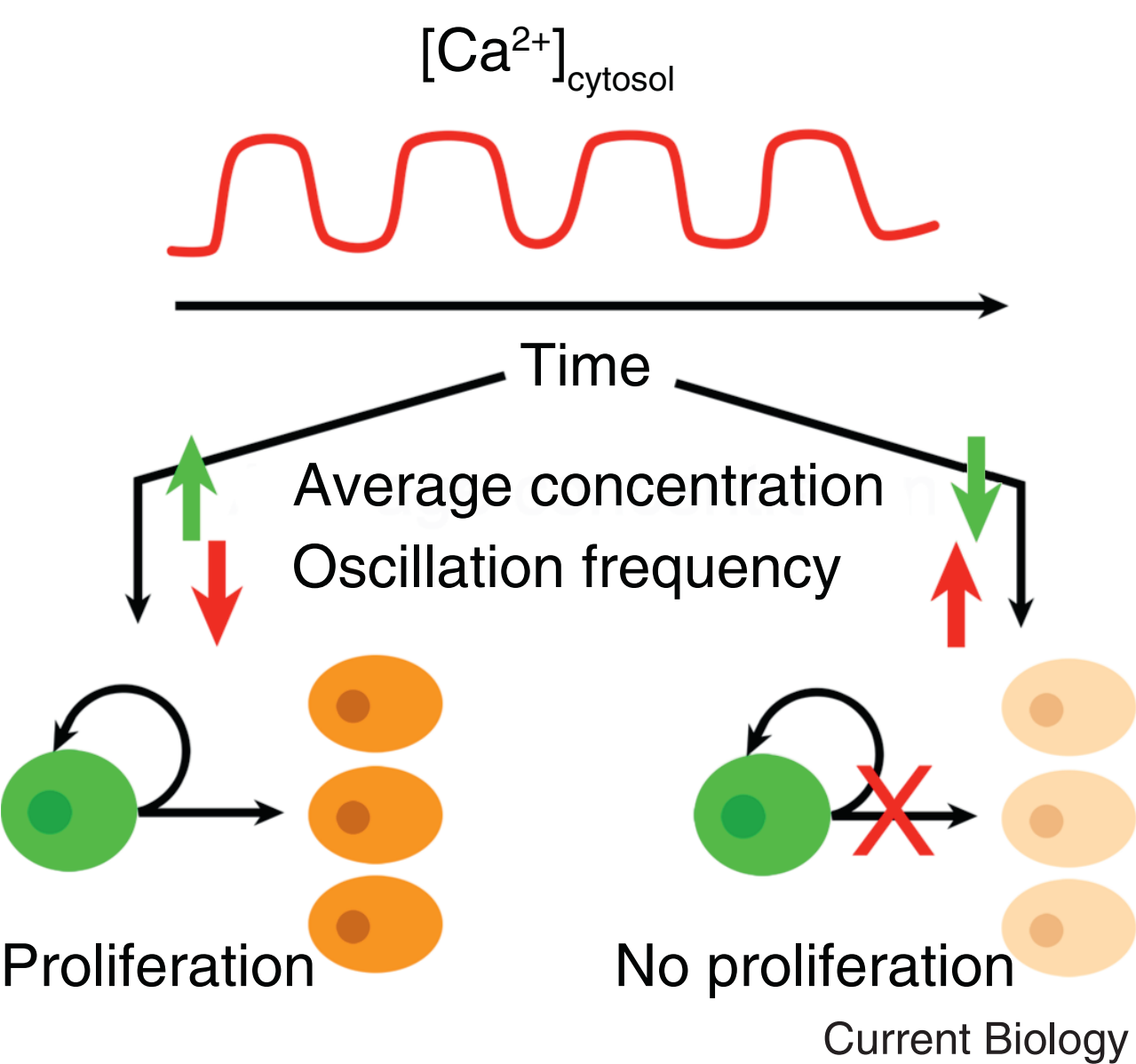
Figure Legends:

Figure 1. Ca^{2+} oscillations frequencies and stem cell proliferation. Decreased Ca^{2+} oscillation frequency and increased average Ca^{2+} concentration in the cytoplasm of *Drosophila* intestinal stem cells (green) is associated with increased rate of stem cell proliferation. Reduction in Ca^{2+} concentration and increased Ca^{2+} oscillation frequency is observed in quiescent stem cells.

Figure 2. Ca^{2+} signalling activation within intestinal stem cells in response to multiple proliferative stimuli. Diet-derived L-Glutamate (L-Glu) and multiple proliferative conditions lead to increases in cytosolic Ca^{2+} and activation of Ca^{2+}-dependent signalling. Metabotropic glutamate receptor (mGluR5); heterotrimeric G protein subunits (αq, β, γ); endoplasmic reticulum (ER); Phospholipase C (PLC); inositol-1,4,5-trisphosphate (IP3) ; inositol-1,4,5-trisphosphate receptors (InsP3R); CREB regulated transcription co-activator (CRTC); calcineurin (CaN).


[Ca2+]cytosol
Time
Average concentration
Oscillation frequency
Proliferation
No proliferation
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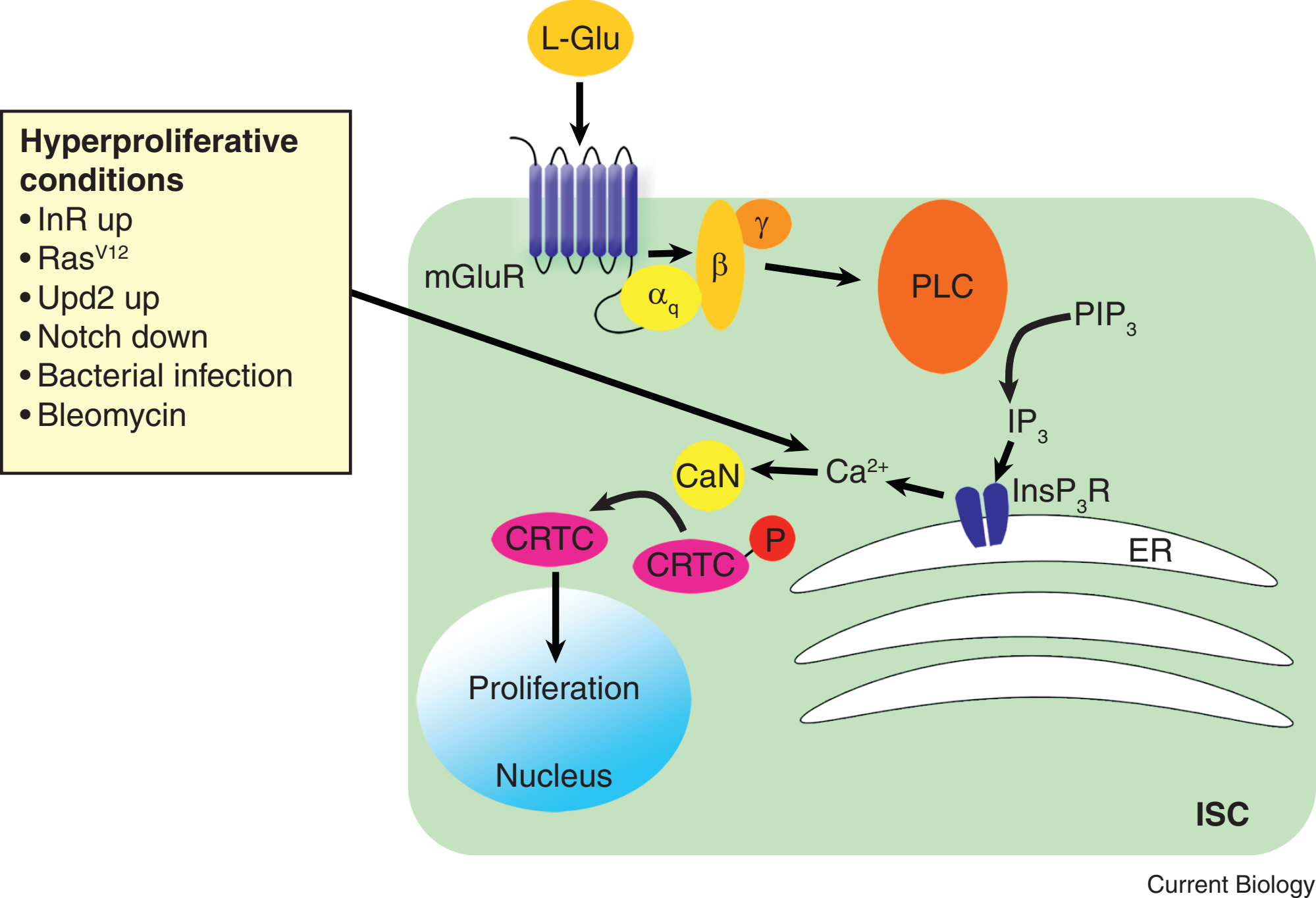

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