Robust G2 pausing of adult stem cells in Hydra

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Abstract

The importance of this study is at two levels, first it provides a fast and reliable procedure to analyze the cycling profile of small amounts of tissues obtained in multiple contexts. Second, it provides a framework to study the impact of G2 pausing on regenerative processes. Indeed, Hydra adult stem cells share similarities with the mammalian embryonic stem cells, characterized by a lack of G1 phase and an extended G2 phase. Cyclin-E is overexpressed in these cells, supporting the fact that stem cells do not stop at the G1/S checkpoint. A correlation between G2 pausing and regeneration is not only observed in Hydra, but also in Axolotl, in mice. Therefore it is important to understand how G2 pausing might positively impact the initiation of tissue repair and regeneration. We propose four types of “pro-regenerative” benefits linked to an extended G2 phase (Figure 2): (1) an extended G2 phase might enhance the DNA repair program, as homologous recombination, one of the well conserved mechanisms involved in the repair of double strand DNA is activated during late S-phase and G2, (2) G2 paused cells show an enhanced [...]

Robust G2 pausing of adult stem cells in *Hydra*

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Abstract

*Hydra* is a freshwater hydrozoan polyp that constantly renews its two tissue layers thanks to three distinct stem cell populations that cannot replace each other, epithelial ectodermal, epithelial endodermal, and multipotent interstitial. These adult stem cells, located in the central body column, exhibit different cycling paces, slow for the epithelial, fast for the interstitial. To monitor the changes in cell cycling in *Hydra*, we established a fast and efficient flow cytometry procedure, which we validated by confirming previous findings, as the Nocodazole-induced reversible arrest of cell cycling in G2/M, and the mitogenic signal provided by feeding. Then to dissect the cycling and differentiation behaviors of the interstitial stem cells, we used the AEP_cnnos1 and AEP_Icy1 transgenic lines that constitutively express GFP in this lineage. For the epithelial lineages we used the sf-1 strain that rapidly eliminates the fast cycling cells upon heat-shock and progressively becomes epithelial. This study evidences similar cycling patterns for the interstitial and epithelial stem cells, which all alternate between the G2 and S-phases traversing a minimal G1-phase. We also found interstitial progenitors with a shorter G2 that pause in G1/G0. At the animal extremities, most cells no longer cycle, the epithelial cells terminally differentiate in G2 and the interstitial progenitors in G1/G0. At the apical pole ~80% cells are post-mitotic differentiated cells, reflecting the higher density of neurons and nematocytes in this region. We discuss how the robust G2 pausing of stem cells, maintained over weeks of starvation, may contribute to regeneration.

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Supplement

Adult Stem Cells (ASCs) play an important role in regenerative processes, however their presence in a given tissue does not suffice to promote organ regeneration, as observed in the mammalian intestine that self-renew but does not regenerate. Here we investigated the properties of ASCs that might help promote regeneration. For this, we use the freshwater cnidarian *Hydra* polyp that regenerates any missing part after amputation of its body column. Since this discovery by Abraham Trembley 275 years ago, *Hydra* emerged as a unique model to study the mechanisms that underlie stem cell regulation, tissue homeostasis and reactivation of complete developmental programs in an adult organism [1]. *Hydra* exhibits a simple tube shape terminated by a mouth/anus opening surrounded by tentacles at the apical pole, and a basal disk at the other extremity (Figure 1). Its bilayered anatomy is made of three stem cell populations, two unipotent epithelial ones that build the ectodermal and endodermal layers respectively, and the multipotent interstitial stem cells (ISCs) that give rise to neurons, mechano-sensory cells, secretory gland cells and germ cells when the animals become sexual. The three stem cell contingents reside exclusively in the central part of the body column, whereas the differentiated cells are predominantly found at the extremities, in the apical and basal regions.
ASCs in *Hydra* are known to pause in the G2 cell cycle phase rather than in G1. Also these three stem cell populations display distinct cycling properties, the epithelial cells cycle slowly, every 3-5 days, while the ISCs cycle faster, about once a day. These properties were identified decades ago by classical methods such as tritiated thymidine radiolabeling, microfluorimetry or BrdU incorporation. In the present study, we performed a precise monitoring of cell cycling to test how cell cycling varies along the *Hydra* body axis, how environmental conditions affecting the nutritional status modify cell cycling, how cell cycling varies between stem cells and progenitors, and finally how the properties of cell cycling might support a high regenerative potential. To address these questions, we established a fast and efficient flow cytometry procedure based on nuclear propidium iodide staining of small amounts of tissues.

To investigate the cycling behavior of the epithelial cells, we used the thermosensitive strain *Sf-I* that upon a two-day heat-shock, eliminates all interstitial cycling cells, leaving the animals quasi “epithelial”. To investigate the cycling and differentiation dynamics in the interstitial lineage, we used two transgenic strains, one, the *AEP_Icy1* strain, which constitutively expresses GFP in interstitial progenitors and interstitial differentiated cells [2], and the other, the *AEP_cnos1:GFP* strain, which is strongly GFP fluorescent in the ISCs thanks to the ISC-specific Nanos1 promoter, a fluorescence that persists at lower levels in interstitial progenitors [3]. Interestingly, in *Drosophila* and in mammals, the RNA-binding protein Nanos regulates protein translation in germ cells.

When FACS profiling was performed on cells from “epithelial” animals, epithelial cells were predominantly found in G2 phase in the central region (60%), but also at the extremities where epithelial cells can differentiate directly in G2, without undergoing a final division, a property previously identified [4]. The cell cycle analysis of the *cnos1:eGFP* cells sorted thanks to their high GFP fluorescence found 95% cells in S or G2 phases, indicating that, as the epithelial stem cells, the ISCs lack the G1 phase. By contrast, we found among the *cnos1:eGFP* cells with low GFP intensity 23% cells in G0/G1 phase, suggesting that the interstitial progenitors, mostly mechano-sensory precursors (named nematoblasts), rather pause in G1, before getting arrested to terminally differentiate in G0. In agreement with this view, one observes in heat-shocked *sf-I* animals a drastic and rapid reduction in G1/Go cells of the central region, which parallels the loss of ISCs and interstitial progenitors, and in the same time period, a slow disappearance of the terminally differentiated interstitial cells in Go/G1 at the apex. Thus, ISCs and interstitial progenitors show distinct cell cycle profiles, ISCs pause in G2 and lack G1, whereas interstitial progenitors likely have a more classical cycle, with a shortened G2 and an extended G1 phase. However we cannot exclude that a subset of these interstitial progenitors correspond to post-mitotic cells that just started to differentiate.

The importance of this study is at two levels, first it provides a fast and reliable procedure to analyze the cycling profile of small amounts of tissues obtained in multiple contexts. Second, it provides a framework to study the impact of G2 pausing on regenerative processes. Indeed *Hydra* adult stem cells share similarities with the mammalian embryonic stem cells, characterized by a lack of G1 phase and an extended G2 phase. Cyclin-E is overexpressed in these cells,
supporting the fact that stem cells do not stop at the G1/S checkpoint. A correlation between G2 pausing and regeneration is not only observed in Hydra, but also in Axolotl, in mice. Therefore it is important to understand how G2 pausing might positively impact the initiation of tissue repair and regeneration. We propose four types of "pro-regenerative" benefits linked to an extended G2 phase (Figure 2): (1) an extended G2 phase might enhance the DNA repair program, as homologous recombination, one of the well conserved mechanisms involved in the repair of double strand DNA is activated during late S-phase and G2, (2) G2 paused cells show an enhanced resistance to cell death, possibly linked to G2 check-point proteins as observed in mammalian epithelial stem cells [5], (3) G2 pausing favors an immediate entry into mitosis upon injury as shown by Hydra ISCs, (4) in the framework of a remodeling process that characterizes Hydra regeneration, epithelial cells can directly differentiate in G2 in response to injury.

Figure 2: Summary scheme indicating the four biological processes linked to an extended G2 phase (written black), which likely contribute to promote the competence of tissues for regeneration.

References

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