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Impact of cycling cells and cell cycle regulation on *Hydra* regeneration

Wanda Buzgariu, Yvan Wenger, Nina Tcaciuc, Ana-Paula Catunda-Lemos, Brigitte Galliot*

Department of Genetics and Evolution, Institute of Genetics and Genomics in Geneva (iGE3), University of Geneva, Geneva, Switzerland

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ABSTRACT

Hydra tissues are made from three distinct populations of stem cells that continuously cycle and pause in G2 instead of G1. To characterize the role of cell proliferation after mid-gastric bisection, we have (i) used flow cytometry and classical markers to monitor cell cycle modulations, (ii) quantified the transcriptomic regulations of 202 genes associated with cell proliferation during head and foot regeneration, and (iii) compared the impact of anti-proliferative treatments on regeneration efficiency. We confirm two previously reported events: an early mitotic wave in head-regenerating tips, when few cell cycle genes are up-regulated, and an early-late wave of proliferation on the second day, preceded by the up-regulation of 17 cell cycle genes. These regulations appear more intense after mid-gastric bisection than after decapitation, suggesting a position-dependent regulation of cell proliferation during head regeneration. Hydroxyurea, which blocks S-phase progression, delays head regeneration when applied before but not after bisection. This result is consistent with the fact that the *Hydra* central region is enriched in G2-paused adult stem cells, poised to divide upon injury, thus forming a necessary constitutive pro-blastema. However a prolonged exposure to hydroxyurea does not block regeneration as cells can differentiate apical structures without traversing S-phase, and also escape in few days the hydroxyurea-induced S-phase blockade. Thus *Hydra* head regeneration, which is a fast event, is highly plastic, relying on large stocks of adult stem cells paused in G2 at amputation time, which immediately divide to proliferate and/or differentiate apical structures even when S-phase is blocked.

1. Introduction

The freshwater *Hydra* polyp, which belongs to the Hydrozoa class in Cnidaria, a sister phylum to Bilateria, is famous for its amazing ability to regenerate any missing body part whatever its age. Since the discovery of this phenomenon in the 18th century, *Hydra* has remained a fruitful experimental model system to study regeneration (Galliot, 2012). An interesting observation is that, in contrast to the gastric column, pieces of tissue originating from the extremities, such as tentacles or basal disk do not regenerate. Given the restricted distribution of stem cells along the body column (Fig. 1A), this suggests that their presence is required for regeneration. *Hydra* polyps are populated with three distinct adult stem cell (ASC) populations, the unipotent epidermal and gastrodermal epithelial stem cells (eESCs, gESCs) present along the body column, and the multipotent interstitial cells (ISCs) that populate the central gastric region (David and Plotnick, 1980). All these adult stem cells are characterized by a highly proliferative status, ESCs dividing every 3–4 days, ISCs every 24–30 h (David and Campbell, 1972; Campbell and David, 1974). Similarly to mammalian embryonic stem cells (White and Dalton, 2005) and epithelial stem cells (Harper et al., 2010), *Hydra* ASCs exhibit a very short G1 phase and an extended G2 phase (David and Campbell, 1972; Campbell and David, 1974; Holstein and David, 1990; Holstein et al., 1991; Buzgariu et al., 2014) (Fig. 1B). A recent study suggests that a small subpopulation of *Hydra* ASCs remain quiescent in G2 (Govindasamy et al., 2014).

In the central body column, ISCs divide and give rise to interstitial progenitors (IPs), which for a large fraction, migrate towards the apical and basal poles and differentiate post-mitotically (Holstein and David, 1990; Technau and Holstein, 1996; Hager and David, 1997). By contrast, ESCs continuously divide along the body column and abruptly stop before reaching the extremities to terminally differentiate and finally get sloughed off. Surprisingly epithelial cells differentiate without traversing mitosis, thus remaining in G2 phase (Dubel et al., 1987; Dubel and Schaller, 1990; Buzgariu et al., 2014). As a result of these cell dynamics, the central body column of the animal is enriched in dividing stem cells, while the apical and basal poles are predominantly composed of terminally differentiated cells.

The relevance of cell proliferation in the regenerative strategy(-ies) adopted by *Hydra* is a longstanding question. The classical view is that *Hydra* regeneration is *morphallactic*, i.e. relying only on the remodeling of the pre-existing tissues and not on the formation of new tissue

* Corresponding author.
E-mail address: brigitte.galliot@unige.ch (B. Galliot).

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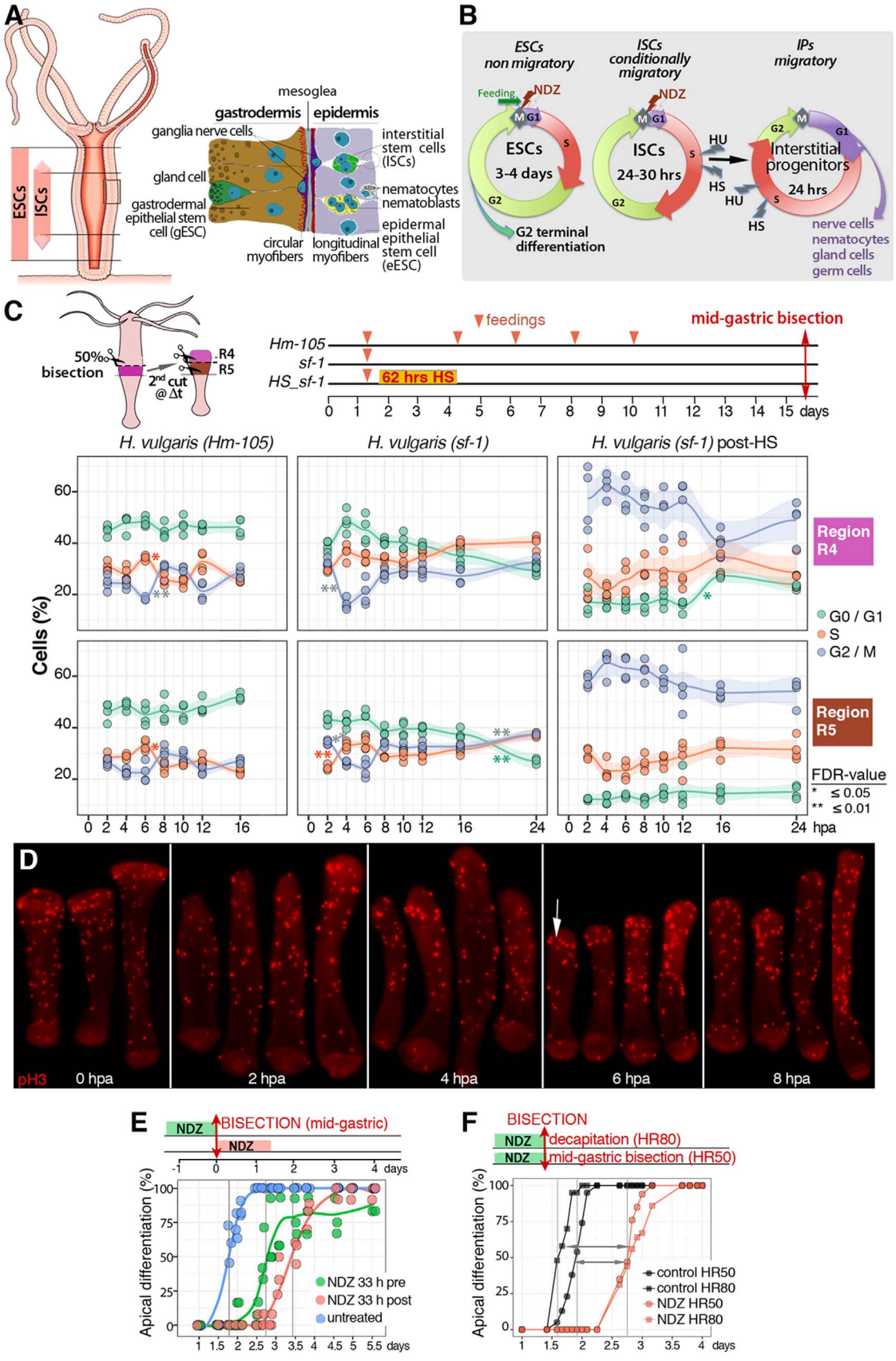
A
ESCs
ISCs
mesoglea
gastrodermis
epidermis
ganglia nerve cells
gland cell
gastrodermal epithelial stem cell (gESC)
interstitial stem cells (ISCs)
nematocytes
nematoblasts
epidermal epithelial stem cell (eESC)
circular myofibers
longitudinal myofibers
B
ESCs non migratory
ISCs conditionally migratory
IPs migratory
Feeding
NDZ
ESCs 3-4 days
ISCs 24-30 hrs
HU
HS
Interstitial progenitors 24 hrs
G2 terminal differentiation
nerve cells
nematocytes
gland cells
germ cells
C
50% bisection
2nd cut @ Δt
R4
R5
feedings
mid-gastric bisection
Hm-105
sf-1
HS_sf-1
62 hrs HS
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 days
H. vulgaris (Hm-105)
H. vulgaris (sf-1)
H. vulgaris (sf-1) post-HS
Cells (%)
Region R4
Region R5
G0 / G1
S
G2 / M
FDR-value
* ≤ 0.05
** ≤ 0.01
hpa
D
pH3
0 hpa
2 hpa
4 hpa
6 hpa
8 hpa
E
NDZ
BISECTION (mid-gastric)
NDZ
Apical differentiation (%)
NDZ 33 h pre
NDZ 33 h post
untreated
days
F
BISECTION
NDZ
decapitation (HR80)
NDZ
mid-gastric bisection (HR50)
Apical differentiation (%)
control HR50
control HR80
NDZ HR50
NDZ HR80
days