Multi-functionality and plasticity characterize epithelial cells in Hydra

BUZGARIU, Wanda Christa, et al.

Abstract

Epithelial sheets, a synapomorphy of all metazoans but porifers, are present as two layers in cnidarians, ectoderm and endoderm, joined at their basal side by an extra-cellular matrix named mesoglea. In the Hydra polyp, epithelial cells of the body column are unipotent stem cells that continuously self-renew and concomitantly express their epitheliomuscular features. These multifunctional contractile cells maintain homeostasis by providing a protective physical barrier, by digesting nutrients, by selecting a stable microbiota, and by rapidly closing wounds. In addition, epithelial cells are highly plastic, supporting the adaptation of Hydra to physiological and environmental changes, such as long starvation periods where survival relies on a highly dynamic autophagy flux. Epithelial cells also play key roles in developmental processes as evidenced by the organizer activity they develop to promote budding and regeneration. We propose here an integrative view of the homeostatic and developmental aspects of epithelial plasticity in Hydra.

Reference

BUZGARIU, Wanda Christa, et al. Multi-functionality and plasticity characterize epithelial cells in Hydra. Tissue Barriers, 2015, vol. 3, no. 4

DOI: 10.1080/21688370.2015.1068908
Multi-functionality and plasticity characterize epithelial cells in Hydra

W Buzgariu, S Al Haddad, S Tomczyk, Y Wenger & B Galliot

Department of Genetics and Evolution; Institute of Genetics and Genomics in Geneva (IGe3); Faculty of Sciences; University of Geneva; Geneva, Switzerland

Accepted author version posted online: 15 Jul 2015. Published online: 15 Jul 2015.

To cite this article: W Buzgariu, S Al Haddad, S Tomczyk, Y Wenger & B Galliot (2015): Multi-functionality and plasticity characterize epithelial cells in Hydra, Tissue Barriers, DOI: 10.1080/21688370.2015.1068908

To link to this article: http://dx.doi.org/10.1080/21688370.2015.1068908

PLEASE SCROLL DOWN FOR ARTICLE
Multi-functionality and plasticity characterize epithelial cells in *Hydra*

W Buzgariu, S Al Haddad, S Tomczyk, Y Wenger*, and B Galliot*

Department of Genetics and Evolution; Institute of Genetics and Genomics in Geneva (IGe3); Faculty of Sciences; University of Geneva; Geneva, Switzerland

Keywords: autophagy, epithelial plasticity, evolution, *Hydra* epitheliomuscular layers, injury-induced response, neuromuscular transmission, regeneration and organizer activity

Epithelial sheets, a synapomorphy of all metazoans but porifers, are present as 2 layers in cnidarians, ectoderm and endoderm, joined at their basal side by an extra-cellular matrix named mesoglea. In the *Hydra* polyp, epithelial cells of the body column are unipotent stem cells that continuously self-renew and concomitantly express their epitheliomuscular features. These multifunctional contractile cells maintain homeostasis by providing a protective physical barrier, by digesting nutrients, by selecting a stable microbiota, and by rapidly closing wounds. In addition, epithelial cells are highly plastic, supporting the adaptation of *Hydra* to physiological and environmental changes, such as long starvation periods where survival relies on a highly dynamic autophagy flux. Epithelial cells also play key roles in developmental processes as evidenced by the organizer activity they develop to promote budding and regeneration. We propose here an integrative view of the homeostatic and developmental aspects of epithelial plasticity in *Hydra*.

**Hydra**, a Classical Model for Studying the Multiple Functions of Epithelial Layers

Eumetazoans, defined as the large cohort of “true” animals formed by cnidarians and bilaterians (Fig. 1A), are multicellular organisms whose organization relies on epithelial cells. Epithelial cells are characterized by a typical apical to basal polarity and by a variety of junction and adhesive properties that allow them to form epithelial sheets. All cnidarians share a bi-layered body wall made of an external layer named ectoderm, and an internal layer named endoderm, which are tightly connected through an extra-cellular matrix called mesoglea (Fig. 1B-D). The ectoderm provides a protective function analogous to the one of epidermis whereas the endoderm, also named gastrodermis as it lines the surface of the gastric cavity, is involved in food uptake and digestion. *Hydra* makes use of a third stem cell population, the multi-potent interstitial stem cells (i-cells) that are predominantly distributed in the central body column, intermingled between the ectodermal epithelial cells (see in1). These i-cells provide migratory progenitors that after one or several rounds of divisions differentiate into nerve cells, nematocytes (mechano-sensory cells) and gland cells. Indeed some of these interstitial progenitors traverse the mesoglea to reach the gastrodermis where they differentiate as secretory gland cells. In summary, the endodermal layer contains myoepithelial digestive cells, gland cells, and a few neurons. In contrast, the ectodermal layer contains a different population of myoepithelial cells, a large fraction of proliferating stem cells and progenitors of the i-cell lineage, which differentiate into neurons and nematocytes in asexual animals.

The freshwater *Hydra* cnidarian polyps, a classical model system in cell and developmental biology over the past centuries,2 greatly contributed to the identification of the typical features of epithelia. The behavior of the ciliated endodermal cells during digestive processes was described in *Hydra* in the late XIXe century.3 Seventy years later, the discovery and visualization of septate junctions (SJs) in *Hydra* epithelia by electronic microscopy provided the basis to apprehend cell-cell communication,4 completed a few years later by the comparative analysis of SJs and gap junctions (GJs) in the same animal.5 More recently, the analysis of the *Hydra* genome indicated that the molecular toolkit for establishing apical basal polarity, for differentiating SJs, GJs but also adherens junctions (AJs) and hemidesmosome-like structures is shared between cnidarians and bilaterians.6

Beside the analysis of the *Hydra* genome, efforts were made over the last decades to systematically identify the molecular signatures of the different *Hydra* cell types, first through peptidomic approaches that led to the discovery of epitheliopeptides and neuuropeptides,7,8 then through cDNA microarrays,9 and more recently through strategies that combine transgenesis, cell sorting and RNA-seq.10 *Hydra* transgenesis was established in 200611 and led to the production of transgenic strains that constitutively express eGFP in one or the other cell lineage, offering the possibility to FACS-sort GFP expressing cells and to analyze their cell-type specific transcriptomes.10 To complement the transcriptomic profiles of stem cells in *Hydra*, we recently applied this latter approach. We dissected the central body column of animals from AEP transgenic strains produced by the

---

© W Buzgariu, S Al Haddad, S Tomczyk, Y Wenger, and B Galliot
*Correspondence to: Y Wenger; Email: yvan.wenger@unige.ch; B Galliot; Email: brigitte.galliot@unige.ch
Submitted: 05/05/2015; Revised: 06/23/2015; Accepted: 06/27/2015
http://dx.doi.org/10.1080/21688370.2015.1068908

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.
Bosch laboratory (which constitutively express GFP, either in endodermal epithelial cells\textsuperscript{11} or in the interstitial stem cells\textsuperscript{10}), dissociated the tissues carefully observed in electron-microscopic studies in the 60s,\textsuperscript{13,15} the fine structure and the components of the \textit{Hydra} glycocalyx were only recently identified.\textsuperscript{16} This fibrous cuticle, up to

to sort the GFP-expressing cells by flow cytometry,\textsuperscript{13} and quantified the level of expression of each gene by RNA-seq (Fig. 2A) (for details, see \textsuperscript{14}). Hence, detailed expression levels of transcripts in endodermal and/or ectodermal epithelial cells were obtained (Table 1).

In this review, we highlight the recent progress made in our understanding of the multiple functions carried out by \textit{Hydra} epithelia, such as protection to the environment, nutrient adsorption, cell-cell communication, contractility, resistance to starvation, resistance to pathogens, wound healing, reactivation of developmental programs. Given the evolutionary conservation of epithelial functions among ctenophorans, we assume that tracing back in \textit{Hydra} epithelia the mechanisms that support these functions will provide new concepts and possibly new tools to face the physiological and pathological consequences of epithelial alterations in mammals.

**The Cuticle Provides a Protective Physical Barrier to the Environment**

In \textit{Hydra}, the ectodermal epithelial layer, which delimits the outlines of the animal protects the animal from constant environmental challenges: physical interactions, osmotic pressure or invading pathogens. Similarly to the mammalian epidermis, the ectoderm synthesizes a fibrous assembly called cuticle, which resembles the glycocalix that surrounds many epithelial cells and shields the external surface of the animal (Fig. 1D). Although

- Figure 1. \textit{Hydra} epithelial cells in homeostatic and stressed conditions. (A) Phylogenetic position of \textit{Hydra} among metazoans. Note the sister group position of cnidarians that include anthozoans and medusozoa (orange background) to bilaterians (blue background). Among the early-diverged metazoan phyla (Porifera, Placozoa, Ctenophora), only Porifera do not differentiate epithelia. (B) Anatomy and tissue dynamics in \textit{Hydra}. \textit{Hydra} polyps have a cylindrical tube shape, terminated at the oral pole by a dome named hypostome and a single opening, the mouth, encircled by tentacles. At the basal pole, the basal disk or foot secretes mucus that helps animals to attach to substrates. Upon regular feeding, polyps reproduce asexually through budding, however when the environment becomes critical for survival, the animals shift to gametogenesis and sexual reproduction (not shown). Epithelial and interstitial stem cells continuously cycle along the body column. Arrows indicate the displacement in time of the epithelial cells toward the bud and the extremities.\textsuperscript{90} When reaching the poles, epithelial cells stop cycling to undergo terminal differentiation as head- or foot-speciﬁc cells (ic), nematoblasts (nb), nematocytes (nc). (C) Schematic view of the bilayered tissue organization (framed region in B) with endodermal (brown) and ectodermal (mauve) epithelial cells (ep), gland cells (gl), ganglia nerve cell (ggl), a pair of interstitial stem cells (ic), nematoblasts (nb), nematocytes (nc). (D) Low magnification electron micrograph of a segment of body wall of \textit{Chlorohydra viridissima} reproduced from\textsuperscript{4} (Fig. 1). Note the acellular mesoglea (me) that separates the thinner epidermis from the gastrodermis, which, in this species, contains intracellular symbiotic green algae (z); the myoﬁbrils (m) in the epidermis (cross-section) and in the gastrodermis (longitudinal section); in the gut lumen the flagellae (fl) of endodermal epithelial cells; the intracellular vacuoles (v) in both layers; the thin cuticle (c) covering the epidermis; a nematocyst within a nematocyte (ne); regions of increased density (a), which correspond to the attachment areas. Scale bar: 5 \textmu m. (E) Immunodetection of the ectodermal epithelial cell membranes with the anti-FGF2 antibody (Santa Cruz sc7911) in untreated animals and Colchicine-treated animals fixed 10 days after an 8 hour colchicine exposure. Note the elimination of the interstitial cells and their derivatives as evidenced by the absence of small DAPI-stained nuclei in colchicine-treated animals. Scale bar: 20 \textmu m. (F) Starvation induces autophagy in \textit{Hydra} epithelial cells as evidenced here by the dramatic increase in autophagosomes (arrowhead) immunodetected after 21 days of starvation with the anti-LC3 antibody (Novus Biological NB100-2220, green).\textsuperscript{32,56} Note the presence of numerous mitochondria inside the autophagic vacuoles detected with Mitotracker (red, arrowheads). Scale bar: 10 \textmu m. (G) Engagement of apoptotic bodies and loss of epithelial polarity in head-regenerating tips (ref. 62, Suppl S2). Efferocytosis by the epithelial endodermal cells (digestive cells) is detected here with Hoechst staining (blue) and anti-CREB (red) and anti-RSK (green) immunodetection. At stage 0 cells display the usual apical to basal hourglass morphology; at stage 1 their apical part gradually detaches (red arrows); at stage 2 they shape ovoid and come into contact with apoptotic bodies, thus named “early engulfing cells”; at stage 3, the “mature engulfing cells” include phagosomes that are large vesicles containing strongly condensed DNA surrounded by a rim of RSK-positive cytoplasm; at stage 4 cells contain phagosomes (blue, arrowheads) but have regained their epithelial cell shape.
1.5 μm thick, is formed of 5 distinct layers that contain 3 main components: (i) glycosaminoglycans, namely unsulfated chondroitin and chondroitin-6-sulfate disaccharides, (ii) several SWT “sweet tooth” proteins, and (iii) 3 distinct PPOD (Putative PerOxiDase) proteins (Table 1). These proteins, stored in vesicles close to the apical side, are secreted by the ectodermal cells. Thanks to their β-trefoil structure and their haemagglutinin activity, these proteins can bind to chondroitin sulfate and thus contribute to the cuticle organization. Interestingly, the family of PPODs found in Hydra seems to be absent in plant or animal species, suggesting that this Hydra specificity was acquired by horizontal gene transfer from bacteria.16,17,18

Epithelial Polarity and Epithelial Junctions

Hydra epithelial cells exhibit a typical apico-basal polarity, possibly resulting from the activity of the 3 complexes that set up the epithelial polarity in bilaterians19: the sub-apical Crumbs complex (Crbs, MPP5/Pals1, InaD/PatJ), the apico-lateral Par complex (Par3, Par6, aPKC, cdc42) and the lateral Scribble complex (Scrbl, Lgl, DLG). Whether the function of the Hydra Crumbs-like protein in the sub-apical complex is conserved remains to be tested. As expected, epithelial cells also express a full set of proteins that establish permeability barriers, the septate junctions (SJs), the anchoring junctions as baseo-lateral adherens junctions (AJs) and the basal hemidesmosome-like structures (see Table 1). Important components of the AJs are the classical cadherins. These are present in Nematostella vectensis20; in Hydra we found a single classical cadherin protein, which encodes a series of cadherin tandem repeat domains and 2 laminin domains as extra-cellular domains, as well as a conserved cadherin cytoplasmic domain (see Table 1).

SJs are shared by all metazoans, but vertebrates also evolved tight junctions (TJs), characterized by the presence of “stricto sensu” claudins proteins, which are not found in invertebrates. Those rather express claudin-like proteins.21 Hydra expresses 14 claudin-like (CLDN-l) genes: 3 exclusively in the endodermal epithelial cells (CLDN-l2, CLDN-l9, CLDN-l11), 3 at similar levels in both epithelial layers (CLDN-l3, CLDN-l5), and 4 in both layers although at higher levels in the ectoderm (CLDN-l1, CLDN-l7, CLDN-l10, CLDN-l12) (Fig. 2B, Table 1). Finally, 4 are not detected in the body column or at very low levels (CLDN-l6, CLDN-l8, CLDN-l14, CLDN-l15).

Gap junctions (GJs) play a major role in cell-cell communication in Hydra and epithelial cells communicate by electric conduction through GJs.22 GJs in deuterostomes (including vertebrates) are formed by connexins/pannexins, whereas in protostomes, GJs are formed by proteins from the innexin (Inx) family, similarly to what is observed in Hydra.6–23 Hydra innexins can be expressed either at similar levels in the 2 epithelial layers (Inx1, Inx3, Inx13), or predominantly in the ectoderm (Inx4, Inx5, Inx6, Inx7, Inx10) or in the endoderm (Inx12)14 (Table 1). Surprisingly, innexins were not found so far in other cnidarian species.

Beside the general conservation of the epithelial toolkit in the ectodermal and the endodermal epithelial cells, this analysis also shows that the 2 epithelial cell layers are structurally different as

Figure 2. Molecular patterns of the ectodermal and endodermal epithelial cells as deduced from RNA-seq transcriptomic analyses. (A). Scheme depicting the procedure to produce RNAs from each stem cell population by dissecting the body columns of 3 transgenic AEP strains that constitutively express GFP either in the endodermal epithelial cells (ECTO actin::eGFP12), or in the endodermal epithelial cells (ENDO actin::eGFP11), or in the interstitial stem cells.39 The quantitative RNA-seq analysis was performed on FACS-sorted cells.13,14 (Fig. 2B–D). Ternary plots showing the cellular distribution of gene transcripts encoding epithelial junction - cell adhesion proteins (B), injury-induced immune proteins (C) and autophagy proteins (D). Each dot represents the expression of a unique gene as the computation of the median values of 4 biological replicates in each cell type. Maximal endodermal expression is at the top (endo), ectodermal at the bottom left (ecto) and interstitial at the bottom right (cnnos1). The position of each dot results from the relative transcript abundance in these 3 cell types, with genes similarly expressed in the 3 cell types located in the gray central zone. The dot size is proportional to the number of log10(reads) reads as indicated on the scale.
Table 1. Cell-type specific expression of epithelial cell markers in Hydra (for protein sequences and expression levels see Supplemental Data). Table showing the relative level of expression of several classes of epithelial markers in the ectodermal epithelial cells (ecto), endodermal epithelial cells (endo) or interstitial cells (i-cells) of the body column of AEP Hydra as deduced from quantitative RNA-seq applied to GFP-sorted cells (see Fig. 2A). “Expressing cells” column: > > > or > < < indicate a minimal 10× difference, > > or < < a minimal 2× difference, uppercase writing indicates over 1,000 reads. Hydra protein sequences are available on Uniprot.org either as individual sequences or as sequences from RNA-seq transcriptomes designed to identify cell-type specific proteins,10 *Hydra vulgaris*/human orthologs.91,92 GO-annotated immune proteins,82 neuromuscular transmission proteins,14 epithelial markers (this work, all annotated protein sequences are accessible from UniProt release 2015_10. For the nomenclature of claudin-like proteins, see Ganot et al.2015 (ref. 21).

| Predicted FUNCTIONS | PROTEIN NAMES | Gene families | EXPRESSION in GFP Hv-AEP CELLS | Protein ACCESSION (Hv-Basel, Zurich, Jussy) |
|---------------------|---------------|---------------|-------------------------------|---------------------------------------------|
| Sub-apical complex  | LINC7 Protein lin-7 homolog C | ECTO > Endo >> i-cells | T2M9I_3_HYDVU |
|                     | MPP5 MAGUK p55 (Stardust, Pals1) | Ecto, Endo > i-cells | T2M5S67_HYDVU |
|                     | Notch2 (Crumbs-like) | Ecto > Endo >> i-cells | T2M9J3_HYDVU |
|                     | CDC42 Cell division control protein 42 homolog | ENDO >> ECTO > i-CELLS | T2MDK9_HYDVU |
|                     | PARP3 Partitioning defective 3 homolog | ECTO > ENDO > i-cells | T2MEG1_HYDVU |
|                     | PARP6G Partitioning defective 6 homolog gamma | Ecto > Endo >> i-cells | T2M6I3_HYDVU |
|                     | PRKCI Protein kinase C | ECTO > ENDO >> i-cells | T2MGBA_HYDVU |
| LAT complex         | DLG1 Disks large homolog 1 | ECTO > ENDO > > i-CELLS | T2ME64_HYDVU |
|                     | DLG5 Disks large homolog 5 | Ecto >> Endo, i-cells | T2MB88_HYDVU |
|                     | LGLL1 Lethal(2) giant larvae prot. homolog 1 | ECTO > ENDO > > i-CELLS | T2MCV2_HYDVU |
|                     | SCRIB Protein scribble homolog | ECTO > ENDO > > i-CELLS | T2MDC2_HYDVU |
| Structural           | CLDN-11, 7, 12 Claudin-like 1,7, 12 | ECTO > ENDO >> i-cells | CRX73236, CRX73250, CRX73241 |
| Septate Junctions   | CLDN-12, 9, 11 Claudin-like 2,9,11 | ECTO > ENDO > > i-cells | CRX73238, |
| (St SJs)            | CLDN-13, CLDN-14, Claudin-like 3, 4 | Ecto, Endo | CRX73242, CRX73253, CRX73239 |
|                     | CLDN-15 Claudin-like 5 | Ecto > Endo >> i-cells | CRX73247, T2MF59_HYDVU |
|                     | CLDN-16, 8, 14, 15 Claudin-like 6,8,14,15 | No or very low expression | CRX73249, CRX73252, CRX73244, CRX73246 |
|                     | CNTN2 Contactin 2 | ENDO > ECTO > > i-cells | T2MEK3_HYDVU |
|                     | CNTN4 Contactin 4 | ECTO > ENDO >> i-cells | CRX73254 |
|                     | CNTNAP2 Contactin assoc. prot 2 | ECTO > ENDO >> i-cells | T2M432_HYDVU |
|                     | CNTNAP2I Contactin assoc. prot 2 like | Ecto > i-cells > Endo | CRX73256 |
|                     | CNTNAP4 Contactin assoc. prot 4 | Ecto >> Endo | CRX73257 |
|                     | CNTNAP4I Contactin assoc. prot 1 like | Ecto >> Endo | CRX73258 |
|                     | CNTNAP5 Contactin assoc. prot 5 | ECTO > ENDO > > i-CELLS | T2MB81_HYDVU |
|                     | CNTNAP53I Contactin assoc. prot. like 5-3 | Ecto >> endo | CRX73259 |
|                     | DSCAM Down syndrome cell adhesion mol. | Endo < Ecto << i-cells | T2MIF2_HYDVU |
|                     | NRNX1 Neurexin-1α like | Apical expression only | CRX73281 |
|                     | NRNX3 Neurexin-3α like | ECTO >> ENDO >> i-cells | T2M365_HYDVU |
| Scaffold             | ATP1A1 NaK ATPase-α 1 | ECTO >> ENDO >> i-cells | CRX73229 |
| Septate Junctions   | ATP1A2 NaK ATPase-α2 | ECTO >> ENDO >> i-cells | CRX73230 |
| (Sc SJs)            | ATP1A3 / AT1A NaK ATPase-α3 | ECTO < ENDO < i-CELLS | AT1A_HYDVU, T2MGY6_HYDVU |
|                     | ATP1A4 NaK ATPase-α4 | Ecto | CRX73232 |
|                     | ATP1A5 NaK ATPase-α5 | Ecto > Endo > i-cells | CRX73233 |
|                     | ATP1B1 NaK ATPase-β2 (NRV Nervana) | ECTO > I-CELLS > ENDO | T2MHY2_HYDVU |
|                     | EPB41L4A Band 4.1 14 (Coracle) | Endo > Ecto > i-cells | T2MS72_HYDVU |
|                     | EPB41L5 Band 4.1 15 (Yurt) | ECTO >> Endo > > i-cells | T2MS19_HYDVU |
|                     | ZO-1 Zonula Occludens 1 (TJP1) | ECTO >> ENDO > > i-cells | T2MDH6_HYDVU |
| Adherens Junctions  | ACTN1 α-actinin | ECTO >> ENDO > > i-cells | T2MH15_HYDVU |
| (AJs)               | CDH Classical cadherin | ECTO >> i-cells >> Endo | CRX73223 |
|                     | CELSR2 Cadherin EGF LAG 7pass | ECTO > i-cells > Endo | T2MS06_HYDVU |
|                     | CTNNA1 α-catenin | ECTO > > i-cells, Endo | T2MZ5_HYDVU |
|                     | CTNNB1 β-catenin | ENDO > I-CELLS > ECTO, | T2MGP6_HYDVU |
|                     | CTNND2 δ-catenin | Ecto >> i-cells > Endo | T2M3M0_HYDVU |
|                     | DAG1 Dystroglycan | Ecto | T2MD1Z_HYDVU |
|                     | DCHS1 Proteocadherin 16 | Ecto > Endo > i-cells | T2MD72_HYDVU |
|                     | FAT1 Proteocadherin 1 | ENDO > ECTO > > i-cells | T2MDR9_HYDVU |
|                     | FAT4I Proteocadherin Fat4-like | ENDO >> ECTO > > i-cells | CRX73260 |
|                     | MICAL2 MICAL like protein 2 | ECTO >> ENDO > > i-cells | T2MAH1_HYDVU |
|                     | MLLT4 (Araf) | ECTO >> ENDO > > i-cells | T2MF28_HYDVU |
|                     | SGCE Sarcoglycan | Endo > Ecto > i-cells | T2MJS5_HYDVU |
|                     | VCL Vinculin | ECTO >> ENDO > > i-CELLS | T2MH95_HYDVU |

(Continued on next page)
Table 1. Cell-type specific expression of epithelial cell markers in Hydra (for protein sequences and expression levels see Supplemental Data). Table showing the relative level of expression of several classes of epithelial markers in the ectodermal epithelial cells (ecto), endodermal epithelial cells (endo) or interstitial cells (i-cells) of the body column of AEP Hydra as deduced from quantitative RNA-seq applied to GFP-sorted cells (see Fig. 2A). "Expressing cells" column: >>> or <<< indicate a minimal 10× difference, >>> or <<< a minimal 2× difference, uppercase writing indicates over 1’000 reads. Hydra protein sequences are available on Uniprot.org either as individual sequences or as sequences from RNA-seq transcriptomes designed to identify cell-type specific proteins, GO-annotated immune proteins, neuromuscular transmission proteins, epithelial markers (this work, all annotated protein sequences are accessible from UniProt release 2015_10. For the nomenclature of claudin-like proteins, see Ganot et al. 2015 (ref. 21).)

| Predicted FUNCTIONS | PROTEIN NAMES Gene families | EXPRESSION in GFP Hv-AEP CELLS | Protein ACCESSION (Hy-Basel, Zurich, Jussy) |
|---------------------|-----------------------------|---------------------------------|------------------------------------------|
| Gap junctions (GJs) | Inx1, Inx1n1                 | ENDO >> ETTO >> i-cells         | Q2EM6_HYDVU, seq57378, seq46622 (pending), CRX73266, seq57910, seq50316, seq46223 (pending) |
|                     | Inx2, Inx9, Inx10, Inx11, Inx14, Inx15 | No or very low expression in body column | CRX73272, CRX73275, CRX73274, CRX73277, CRX73268, Seq57322 (pending) |
|                     | Inx3, Inx13 Inx1n3, 13       | ECTO > ENDO >> i-cells          | CRX73269, CRX73271 |
|                     | Inx4, Inx5, Inx6, Inx7,      | ECTO or Ecto                    | CRX73268 |
|                     | Inx8 Inx1n8                  | i-cells                         | CRX73268 |
|                     | Inx12 Inx1n12                | Ecto                            | CRX73268 |
| Hemi-desmosomes     | ADAM10                      | ECTO > ENDO >> i-cells          | T2MJ41_HYDVU, T2MIA5_HYDVU |
|                     | ADAM12                      | Endo, i-cells                   | T2MBF8_HYDVU, CRX73278 |
|                     | ADAM17                      | ECTO > ENDO < i-cells           | T2MFIQ_HYDVU, T2ME2_HYDVU |
|                     | ADAM33                      | Ecto < i-cells                  | T2ME15_HYDVU, T2M6HG_HYDVU |
|                     | ADAMTS9 Disintegrin MP thrombospondin | Endo > i-cells > Ecto           | T2M4C5_HYDVU |
|                     | CB1 Calcium and integrin-binding protein 1 | Endo > Ecto >> i-cells         | T2M774_HYDVU |
|                     | FAK1 Focal adhesion kinase   | ECTO, ENDO << i-cells           | T2MDJ8_HYDVU |
|                     | ILK Integrin linked kinase   | ECTO, ENDO >> i-cells           | T2M6A7_HYDVU |
|                     | ILKAP ILK-associated protein | ECTO < ENDO < i-cells           | T2MDM8_HYDVU |
|                     | ITGF2 Integrin-a FG-GAP      | Endo, i-cells > Ecto            | T2M8F8_HYDVU |
|                     | ITG4A integrin-alpha4        | ECTO > ENDO >> i-cells          | CRX73278 |
|                     | ITG4B integrin-alpha8        | ECTO > ENDO >> i-cells          | T2M6E0_HYDVU |
|                     | ITG9A Integrin-alpha9        | ECTO > ENDO >> i-cells          | T2ME15_HYDVU, T2M6HG_HYDVU |
|                     | ITG81 Integrin-beta1         | ECTO > ENDO < i-cells           | T2M4C5_HYDVU, T2M6HG_HYDVU, T2M6E0_HYDVU |
|                     | ITG82 Integrin-beta2         | ECTO > ENDO < i-cells           | T2M774_HYDVU, T2M6HG_HYDVU, T2M6E0_HYDVU |
|                     | ITG3 Integrin-beta3          | Ecto < ENDO >> i-cells          | CRX73268 |
|                     | PXN Paxillin                 | ENDO < ECTO < i-cells           | T2MG50_HYDVU |
|                     | TLN2 Talin2                  | ENDO < ECTO < i-cells           | T2MG50_HYDVU |
|                     | TNS1 Tensin1                 | ENDO < ECTO < i-cells           | T2MG50_HYDVU |
|                     | Cell adhesion                | ENDO < ECTO < i-cells           | T2MG50_HYDVU |
|                     | ANX12 Annexin XII            | ENDO < ECTO < i-cells           | T2MG50_HYDVU |
|                     | ANX17 Annexin                | ENDO < ECTO < i-cells           | T2MG50_HYDVU |
|                     | CASK Peripheral plasma mbrane protein CASK | Ecto >> ENDO >> i-cells         | CRX73235 |
|                     | DSCAM Down syndrome cell adhesion mol | Endo < Ecto < i-cells           | T2MF2_HYDVU, T2M666_HYDVU |
|                     | EpH1 Ephrin receptor 1       | ENDO >> i-cells, Ecto           | AG006063.1 |
|                     | EpH2 / EPH7 Ephrin receptor 2 | ECTO < i-cells                  | AG006064.1, T2M6F6_HYDVU |
|                     | EpH3 / EPHAS Ephrin receptor 3/5 | ECTO < i-cells                  | AG006066.1, T2MF36_HYDVU |
|                     | EpH4 / EPHA4 Ephrin receptor 4 | Ecto > Ecto < i-cells           | AG006065.1, T2M7M7_HYDVU |
|                     | EpH1B Ephrin ligand B1       | Ecto >> i-cells, Ecto           | AG006067.1, R9W58_HYDVU |
|                     | EpH3B Ephrin ligand B3       | Ecto, Endo < i-cells            | AG006068.1, R9W59C9_HYDVU |
|                     | FARP2 I FERM RhoGEF pleckstrin domain | Ecto >> ENDO >> i-cells         | AG006069.1, R9X0X4_HYDVU |
|                     | GUK1 like Guanylate Kinase 1 | Ecto >> ENDO >> i-cells         | AG006069.1, R9X0X4_HYDVU |
|                     | IQGAP / IQGAP1 GTPase-activating like prot | ENDO < ECTO < i-cells           | AG006069.1, R9X0X4_HYDVU |
|                     | LRG3 Leu Rich Repeats Ig-like prot 3 | ENDO < ECTO < i-cells           | AG006069.1, R9X0X4_HYDVU |
|                     | Trap1 Tropomyosin            | ENDO < ECTO < i-cells           | AG006069.1, R9X0X4_HYDVU |
| Cuticule structure   | Sweet Tooth proteins         | 22 proteins                     | See Bottger et al. 2012 (ref. 16) |
|                     | PP0D1 Putative Peroxidase 1  | ECTO >> ENDO >> i-cells          | Q2F8K4_HYDVU, Q2FBK7_HYDVU |
|                     | PP0D2 Putative Peroxidase 2  | No PP0D2 in Hv-AEP              | Q2F62G1_HYDVU, Q2FBK2_HYDVU |
|                     | PP0D2-like Putative Peroxidase 2-like | ECTO < ENDO < i-cells           | Q2FB9_HYDVU |
|                     | ANKFN1 Ankyrin repeat fibronectin III | ENDO << ENDO >> i-cells         | T2M9C4_HYDVU |
|                     | COL4A1 / COL4A5 collagen-alpha5 (IV) | ENDO >> i-cells, Ecto           | T2M6C9_HYDVU |
|                     | FARM1 secreted astacin       | ECTO, ENDO >> i-cells           | T2M6C9_HYDVU |
|                     | FICol fibrillar collagen     | ENDO >> i-cells, Ecto           | T2M6C9_HYDVU |
|                     | FND3CBF FN type III containing protein 3A | ENDO >> i-cells, Ecto           | T2M6C9_HYDVU |
|                     | HMCN1II Hemicentin1 like1    | ECTO                             | CRX73261 |

(Continued on next page)
for example, Contactin 4 (CNTN4), CNTNAP53l, Neurexin-3a like, Zonula Occludens 1 (ZO-1), α-catenin (CTNNA1), Inx4, Inx5, Inx6, Inx7, Inx10 genes that are strictly or predominantly expressed in the ectodermal cells, whereas Crumbs-like, Claudin-like 2, 9, 11, Protocadherin Fat4-like, Inx12 are strictly or predominantly expressed in the endodermal ones (Fig. 2B, Table 1). If confirmed at the protein level, this implies that the epithelial organization is largely similar in the epidermis and the gastrodermis although not identical. This difference, previously noted by Hemmrich et al.,10 is not so surprising as the corresponding epithelial cell types have different anatomies, carry functions specific to the layer they belong to, and cannot replace each other.

### Extracellular Matrix Production and Regulation of Developmental Processes

The extracellular matrix (ECM) deposit named mesoglea, which separates the 2 epithelial layers in Hydra, contributes to the adhesion and the anchoring of epithelial cells, keeping the 2 layers tightly connected. The mesoglea consists in fine fibrils of different diameters organized as 2 basal lamina matrix with a central fibrous area (see in Fig. 2B, Table 1). Ultrastructural, histochemical and biochemical studies showed that the structural components of Hydra ECM are highly similar to those found in the basement membrane of vertebrates i.e. type IV and fibrillar collagens, laminins, fibronectin and proteoglycan-like molecules, as well as several types of fibrillar collagens, and confirmed the lax and porous structure of the mesoglea, with pores of 0.5-1 μm in diameter, which facilitate the communication between ectoderm and endoderm. In situ hybridization and cell type specific transcriptomes showed that both epithelial layers produce the ECM components although with specific roles, the ectodermal cells synthesising fibronectin and the α/β integrins, and the endodermal cells synthesising all types of collagens, the laminins (α1, β1) and the matrix metalloproteinases (HMP1, HMP2, HMPM) (see in Table 1, refs 10,24). All these components, assembled together in the extracellular space, also play an important role in

**Table 1.** Cell-type specific expression of epithelial cell markers in *Hydra* (for protein sequences and expression levels see Supplemental Data). Table showing the relative level of expression of several classes of epithelial markers in the ectodermal epithelial cells (ecto), endodermal epithelial cells (endo) or interstitial cells (i-cells) of the body column of AEP Hydra as deduced from quantitative RNA-seq applied to GFP-sorted cells (see Fig. 2A). "Expressing cells" column: >>> or <<< indicate a minimal 10× difference, >>> or <<< a minimal 2× difference, uppercase writing indicates over 1,000 reads. *Hydra vulgaris* protein sequences are available on Uniprot.org either as individual sequences or as sequences from RNA-seq transcriptomes designed to identify cell-type specific proteins,10 *Hydra vulgaris* human orthologs,91,92 GO-annotated immune proteins,82 neuromuscular transmission proteins,14 epithelial markers (this work, all annotated protein sequences are accessible from Uniprot release 2013_10). For the nomenclature of claudin-like proteins, see Ganot et al. 2015 (ref. 21). (Continued)
morphogenetic processes as regeneration and budding.²⁴,²⁵ As an example, the strength of the adhesion of the epithelial cells to the ECM varies with morphogenetic displacements along the body column and in the region where the bud develops.²⁶

**Epithelial Cells in the Hydra Body Column are Both Differentiated and Stem Cells**

All epithelial cells in *Hydra* are epithelial-muscular cells that, in the central body column, continuously proliferate and self-renew, displaying thus stem cell properties and differentiated features concomitantly.²⁷ Both ectodermal and endodermal populations exhibit a rather unusual cycling pattern, characterized by the lack of G1-phase and an extended G2-phase, which is reminiscent of the cell cycle properties of embryonic stem cells.²⁸,²⁹,³⁰ A recent flow cytometry analysis confirmed that 85% epithelial stem cells distribute between the S and G2 phases.¹³ Given the fixed S phase length (about 12 hours), the total length of the epithelial cell cycle is imposed by the length of the G2 phase, which varies according to the feeding regime: An epithelial cell cycle takes 3-4 days to complete in well-fed animals versus up to 10-12 days in starving animals.³³,³⁴ *Hydra* epithelial cells are not migratory, but as a result of their rapid proliferation in the body column, they get progressively displaced laterally into newly developing buds or pushed toward the extremities of the animal (Fig. 1B). When reaching extremities, epithelial cells stop cycling and terminally differentiate in G2 phase, giving rise to foot-, head-, or tentacle-specific cells.¹³,³⁰

So far, our knowledge concerning the genetic circuitry regulating stemness in *Hydra* is limited (see Table 1). The famous “Yamanaka OKSN factors” are not well conserved in cnidarians,³¹ either completely missing as Nanog (N), or distantly related as Sox2 (S) and Oct4 (O). However, in *Hydra* the Oct4-like transcription factor named “Polynem” promotes self-renewal³² and in *Hydra*, the related POU4F2 transcription factor, predominantly expressed in ectodermal and interstitial stem cells, might play a similar role. Several Krüppel-like factors (Klf) are expressed in *Hydra*, 2 of them exclusively in the epithelial cells (KLF3, KLF8) and a third one, KLF11, predominantly but not exclusively in the epithelial cells. Although not a clear vertebrate Sox2 ortholog, the *Hydra* Sox-2 like gene is a potential regulator of self-renewal.¹⁰ As additional stem cell transcription factors, the proto-oncogene *Myc* is present as 4 copies in the *Hydra* genome; *HyMyc1* and *HyMyc2* contain a typical bHLH-ZIP DNA-binding box and several Myc domains, whereas *HyMyc3* and *HyMyc4* contain only the DNA-binding domain.³²,³³ *HyMyc1* is predominantly expressed in the interstitial stem cells, likely controlling their proliferation.³⁶ By contrast, *HyMyc2* is expressed at high levels in all 3 stem cell populations, suggesting that paralogs of an ancestral Myc gene also control epithelial proliferation.³⁵ Among candidate regulators of stem cells, one also finds the Ets transcription factors that in vertebrates regulate proliferation, inhibit apoptosis and promote neuronal specification.³⁷ Two of them (Ets1, Ets2) are specifically expressed in the epithelial cells.¹⁰

The role of all these genes on the behavior of epithelial stem cells remains to be tested in *Hydra*.

The FoxO gene that encodes a forkhead transcription factor, was initially identified for its role in stress response.³⁸ Subsequently, it was selected together with Tef, PIWI and *vasa* for its high level of expression in the 3 stem cell populations, providing thus candidate regulators of stem cell behavior in *Hydra*.¹⁰ Indeed FoxO down-regulation in epithelial cells leads to a reduced growth and to an enhanced differentiation of foot and head epithelial cells, supporting a role for FoxO in the control of stem cells.³⁹ Surprisingly, FoxO silencing also affects the innate immune response, enhancing the expression of antimicrobial peptides, suggesting a role in host defense mechanisms.

*Hydra* expresses 2 PIWI genes, PIWIL1 named *Hywi* or *Cnwi*, and PIWIL2 named *Hyli*, both expressed in the 3 stem cell populations.⁴⁰,⁴¹ The mapping of piRNAs on cell-type specific transcriptomes revealed non-transposon putative PIWI targets in epithelial cells, pointing to adhesion and ECM protein genes in the ectoderm, and to proteolytic and ECM genes in the endoderm.⁴⁰ The role of PIWI proteins in epithelial cells is largely supported by *Hyli*, as shown in *hyl1*-RNAi transgenic lines where the epithelial integrity of F1 hatchlings is altered, leading to tissue disintegration and death. In i-cells, the PIWI-piRNA pathway is associated with transposon silencing.⁴¹

**Pacemaker Contractile Activity of the Epitheliomuscular Cells**

The two distinct epithelial cell lineages that build up the body wall of *Hydra* are actually myoepithelial, i.e. contain at their basal side myofibrils, oriented perpendicular to each other, i.e., circular in the endoderm, longitudinal in the ectoderm, acting thus as circular or longitudinal muscles⁴,⁴² (Fig. 1C). Electrophysiological studies have shown that well-fed animals contract on average once every 5 to 10 minutes with periodic bursts of contractions, each layer exhibiting an autonomous pacemaker activity.³³,³⁴ Indeed these myoepithelial pacemakers function autonomously as their activity persists, although at a slower pace, in nerve-free animals.⁴⁵,⁴⁶ This autonomous contractile behavior possibly reflects the proto-neuronal status of the epithelial cells.⁴⁷ It occurs thanks to electrical synapses such as gap junctions, which connect epithelial cells⁴⁸ via innexins³³ (Table 1). In fully-equipped animals, neurons control this activity through *Inx2*: *Inx2* is expressed in a small subgroup of nerve cells in the peduncle of the animal, and initiates the epithelial pacemaker activity in this region.⁴⁹ By contrast the complex feeding response that involves tentacle swirling and mouth opening requires a coordinated neuronal network.⁵⁰ At the base of the tentacles, the myoepithelial cells express sodium channel receptors (NaC) that are directly activated by the RFamide neuropeptides, implying that peptide-gated ion channels are involved in neuromuscular transmission in *Hydra*.⁵¹ Thus cnidarians, and so far only cnidarians, have independently recruited peptides as fast transmitters for neuromuscular transmission.
Digestive Functions

An important function of the gastrodermis is to digest nutrients and to perform exchanges with the content of the lumen. In its natural environment, i.e. wild ponds, Hydra eat small swimming crustaceans (Daphnia nauplii), whereas in laboratory, the animals feed on desalted Artemia nauplii (brine shrimps larvae). Polyps paralyze preys thanks to a touch-induced discharge of venom contained in the capsules (named nematocysts or cnidocysts) embedded in their nematocytes.52 Then, preys are progressively introduced through the mouth opening inside the gastric cavity by coordinated tentacle movements. Once inside the gastric cavity, the food is partially degraded by the proteolytic enzymes released by the gland cells, and absorption by digestive cells occurs through phagocytosis and pinocytosis. The whole digestive process is highly dynamic, with peristalsis, segmentation movements and defection reflex, the latter ejecting feces through the mouth opening 6 to 9 hours after feeding.46

The epithelial endodermal cells display a typical columnar shape with short processes at the basal pole, extending microvilli and flagella into the gastric cavity. Early electron-microscopic studies of digestive cells evidenced a very heterogeneous cytoplasmic content, with diverse vesicle types, lipid droplets and glycogen granules that serve as nutrients for the surrounding cells.32,55 Based on precise ultrastructural and immuno-histochemical criteria (Lysotracker red-LTR, MitoFluor 589, LBPA, DAPI, LC3), three distinct types of vacuoles were identified in the digestive cells: digestive vacuoles, autophagic vacuoles and apoptotic bodies.54-56 This diversity of vesicles actually reflects the multiple functions of epithelial cells, which, besides their digestive role, contribute to the elimination of cell debris, or can activate cyto-protective or pro-survival mechanisms.

Autophagy and Maintenance of Fitness

Hydra polyps readily adjust to caloric restriction by activating the autophagy process.55,56 This evolutionarily conserved survival strategy affects both epithelial cell populations that display autophagic vacuoles already 3 days after the onset of starvation.56 After 3 weeks of starvation, epithelial cells contain numerous autophagosomes that can be easily immunodetected with the universal autophagy marker LC3/ATG8 (Fig. 1F). In fact, autophagy activation was first recorded in endodermal epithelial cells of animals knocked-down for Kazal1, a gene that encodes a serine protease inhibitor (SPINK) expressed by the gland cells.57 The phenotype, which mimics the SPINK1/SPINK3 mammalian phenotype, consists in a progressive autophagy of all endodermal cells linked to a progressive loss of fitness, a parallel loss of budding, and in head-regenerating tips, an immediate excessive autophagy after bisection, which in few hours leads to cell death. Hence, autophagy has a double role in Hydra: survival in case of starvation, and cytoprotection in stressed or damaged tissues.58

Orthologs of most components of the autophagy and TOR pathways were identified in Hydra and Nematostella, indicating that the machinery is well conserved in cnidarians.56 As anticipated the drugs rapamycin, wortmannin and bafilomycin similarly modulate autophagy in Hydra and mammals, as the mTOR inhibitor rapamycin that enhances autophagy in all Hydra epithelial cells.55,56 A cell-type specific RNA-seq analysis shows that all members of the autophagy pathway examined here but ATG7, are expressed in epithelial as well as in i-cells (Fig. 2C). However the Ubiquitin-like inhibitor of autophagy enzyme ATG7 is almost exclusively expressed in the endodermal epithelial cells. In addition the mTOR kinase that acts as a central regulator of cellular metabolism, the kinase Ulk1 that responds to starvation, the positive regulator of autophagy UVRAG are predominantly expressed in epithelial cells, likely reflecting the distinct regulations of autophagy between epithelial and interstitial cell types.

Resistance to Cell Death and Efferocytosis

Epithelial cells are extremely resistant to cell death59 and are in charge of engulfing the apoptotic bodies, a process named efferocytosis. Epithelial efferocytosis was first reported by Campbell who observed apoptotic bodies in both the ectodermal and the endodermal epithelial cells of polyps exposed to colchicine.60

Since then, numerous studies confirmed the active role of the epithelial cells in apoptotic cell clearance by engulfment, whatever the pro-apoptotic agent, pharmacological, heat-shock, starvation, gametogenesis, wounding, head regeneration or histocompatibility reaction (see in59). The epithelial cells recognize the dying cells, which in most circumstances are of interstitial origin, probably thanks to “eat-me” signals present on apoptotic membranes. In mammalian cells, phosphatidylserine translocation to the outer cellular membrane provides a typical signal for engulfment, and this classical marker of apoptosis was also identified in Hydra.61,62 However the phagocytic receptors recognizing eat-me signals in Hydra have not been identified yet, but similarly to biliary epithelial cells, receptor tyrosine kinases expressed in epithelial cells might play an important role in this recognition process.9,10

In case of head regeneration, an immediate and massive wave of efferocytosis can be observed in the endodermal epithelial cells located below the bisection plane.62 Interestingly these cells transiently lose their apico-basal polarity during the first hours (Fig. 1G). A similar transient loss of the polarity of the endodermal epithelial cells was previously observed during early reaggregation.63 Both observations suggest that the maintenance of the endoderm as an epithelial layer requires dynamic interactions with the sus-jacent ectodermal layer. The impact of efferocytosis in head-regenerating tips on the regenerative process was not tested so far, it might be limited to a scavenging function, but it might also trigger the developmental function of the endodermal cells, which at that time start developing an organizer activity.

Antimicrobial Host Defense Role of Hydra Epithelium

As an aquatic species living in an open environment and thus exposed to a multitude of potential pathogens such as protists,
bacteria or viruses, *Hydra* developed host defense strategies that integrate innate immunity tools located in the epithelial layers. As output, 3 classes of AMPs are synthesized in various interactions between the host epithelial cells and the microbial arminins, which show efficient bactericidal activity. Moreover, under a massive pathogenic aggression, ectodermal cells are able to emit pseudopods and engulf bacteria, providing another protective defense response. Thus, both epithelial layers are well equipped with potent defense molecules and mechanisms showing the adaptability of this simple animal to develop defending strategies against external attacks, but also against internal invasion by ingestion of bacteria into the gastric cavity.

**Microbiota Formation and Epithelial Cells - Bacteria Colonization**

Like in most animal species, the interactions with commensal bacterial populations that form the microbiota are important for *Hydra* homeostasis. In fact, polyps cultured in sterile conditions cease to reproduce asexually through budding. More recent systematic studies reveal that different *Hydra* species develop particular preferences for certain bacterial phylotypes. This process encompasses several steps: the initial colonization of juvenile animals with highly variable groups of bacteria, then the transient selection and extension of a bacterial type that will become the principal species of the colonizing group. The severe reduction in variability is thus associated with a stable species-specific microbiota interaction: bacteroidetes and β-proteobacteria are predominant in *H. vulgaris*, α-proteobacteria (rickettsiales) and endosymbionts in *H. oligactis*. Hence the bacterial community is modeled by continuous interactions between the host epithelial cells and the microbial populations, with host-related components playing a crucial role.

Ultimately these interactions are beneficial for the host as the microbiota protects it from pathogens.

These interactions imply several levels of regulation. The analysis of the colonization process in arminin-deficient *Hydra* showed that these animals do not select properly their bacterial partners, implying that AMPs control the selection of bacterial phylotypes populating the microbiota. Also "epithelial" *Hydra* lacking nerve and gland cells, show a different composition of their colonizing microbiota. However the elimination of the interstitial cells is not sufficient to alter the microbiota, indicating that nerve cells and gland cells play an important role in setting the microbiota. Hence in *Hydra*, the highly dynamic host-microbiota interactions are modulated by the cellular composition of the epithelial layers.

**Immune Response of Epithelial Cells to Stress and Injury**

A series of studies investigating the events taking place in head-regenerating tips after bisection, point to an essential role of the MAPK/CREB pathway. Immediately after mid-gastric amputation, a massive wave of cell death is observed at the head-regenerating edge, affecting interstitial progenitors and interstitial derivatives. The resulting apoptotic bodies are engulfed by the endodermal epithelial cells, which transiently change their columnar phenotype, lose their apical to basal polarity and become spherical. Dying cells release Wnt3, which promotes the division of the surrounding progenitors and is necessary for a later Wnt3 up-regulation in the endodermal epithelial cells. By contrast, cell death remains limited and cell proliferation does not increase in foot-regenerating tips, indicating that head and foot regeneration processes are immediately different.

In an attempt to characterize the genes immediately up-regulated upon injury, we recently applied a transcriptomic approach, which led to the identification of 43 immune-associated genes similarly regulated whatever the regenerative context. Among them, we identified components of the ROS signaling pathway, TNFR and TLR signaling related transcription factors like jun, fos, ATF1/CREB, SIK2, all possibly modulating the NF-kB pathway. This study suggests that the response to injury involves the innate immune system, and raises the question of the developmental impact of this stress-induced immune response on the regenerative processes, and on the potential of epithelial cells to set up an organizer activity.

**Developmental Functions of Epithelial Cells**

Thanks to its remarkable competence for regeneration and sexual reproduction through budding, the *Hydra* polyp provides a unique model for deciphering the mechanisms leading to the reactivation of developmental processes in an adult organism. Except extremities, each piece of *Hydra* tissue is able to undergo a perfect regeneration process and give rise to a complete animal
Within few days. Transplantation experiments performed at various time-points after bisection showed that the head- or foot-regenerating tips acquire organizer activity in few hours i.e., become able to instruct and recruit host tissues to rebuild the missing head and/or foot regions. For head regeneration, activation of the MAPK/CREB pathway and induction of the canonical Wnt pathway play essential roles.

In this developmental transition, the epithelial cells play the key role, as first, chimeric animals resulting from recombination of strains with different morphologic properties, preserve the morphogenic properties of the parental epithelial cells and not that of the interstitial cells (see in ). Second, Hydra depleted of their interstitial cells, the so-called “epithelial” Hydra (Fig. 1E), are able to regenerate, although at a slower pace. If manually fed, they can also reproduce asexually through budding, which indicates that the interstitial cells can be dispensable for developmental processes. In fact, the genetic circuitry launched upon amputation is sequentially activated and relies preponderantly on epithelial specific genes in the immediate and immediate-early phase.

We view the plasticity of Hydra epithelial cells as an intrinsic property that has multiple facets, quite distinct when regulated in acute or chronic contexts. In fully-equipped animals, signals received from the interstitial cells immediately after amputation (as signals released by the dying cells – see above) speed up the transition phase whereby epithelial cells quickly adopt a developmental role, which is absent before amputation. In epithelial animals, we suspect that epithelial cells adapt to the loss of interstitial cells by “slowly” reprogramming a large series of genetic programs already in homeostatic conditions, i.e. in the absence of injury signals (ref. 14 and unpublished). Our hypothesis is that in such “reprogrammed” Hydra, the response to injury is still efficient, although different from that observed in fully equipped Hydra. Nevertheless the reprogramming potential of the epithelial cells remains limited as epithelial cells never transform into cells of the interstitial lineage. In summary, the ability of the epithelial cells to adapt to the loss of the nervous system and the potential of digestive cells to develop at any time an organizer activity are amazing, reflecting distinct roles, to control tissue homeostasis, and to maintain fitness of the organism through repair and regeneration.

**Conclusions and Perspectives**

As reported above, multiple properties characterize the epithelial cells of the Hydra body column, with some significant quantitative and qualitative differences between the epithelial cells of the outer layer, which form an epidermis, and the epithelial cells of the inner layer, which form a gastrodermis (Fig. 3). However, the cells of a given layer do not express the full repertoire of their properties at the same time. Rather, they provide the animal with the abilities to react and to adapt to stress, infection, starvation, amputation, so that homeostasis is reestablished and maintained over weeks, months and, in favorable environment, over years. Therefore, Hydra offers a unique model system to test the multiple facets of cellular plasticity. Our view of the molecular signaling supporting epithelial plasticity in Hydra is currently limited, but available data point to evolutionarily-conserved signaling pathways, such as (i) a ROS signaling pathway for the immediate response to stress, heatshock and injury, which efficiently contributes to the wound healing process, (ii) a highly diversified innate immune system for a sustained response to stress, infection and injury, (iii) autophagy and TOR signaling pathways to efficiently respond to starvation and thus support animal survival for weeks, (iv) evolutionarily-conserved developmental pathways involving Wnts, FGF, BMP, Notch and Nodal signaling for the full reactivation of developmental processes in an adult organism.

A series of puzzling questions remain pending: Which of these pathways respond to taxon-specific signals such as epitheliopeptides that are numerous in Hydra? How do these pathways cross-talk? How do the epithelial cells prioritize the different tasks they have to execute? Can we establish hierarchies in the meta-signaling network linking the specific environmental contexts and thus identify master components of environmental-dependent regulators of plasticity? Deciphering the molecular networks supporting epithelial plasticity in Hydra, should highlight the mechanisms...
that support specific biological competences as the maintenance of fitness to face stressful environmental conditions, the ability to repair tissues and appendages, the ability to reproduce asexually and thus bypass the costs of sexual reproduction, and the ability to resist to aging. No doubt that the most robust molecular regulators of these competences in Hydra should be tested in mammalian contexts, potentially offering new tools for regenerative medicines.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Funding
This work was supported by the State of Geneva, the Swiss National Funds for Research (snf-31003A-149630), the NIH (grant 7R01AG037962), the HFSP (grant RHP0016-2010) and the Claraz donation.

References
1. David CN. Intestinal stem cells in hydra: multipotency and decision-making. Int J Dev Biol 2012; 56:489-97; PMID:22689367; http://dx.doi.org/10.1387/ijdb.113476cd
2. Gallot B. Hydra, a fruitful model system for 270 years. Int J Dev Biol 2012; 56:411-23; PMID:22855328; http://dx.doi.org/10.1387/ijdb.120088bg
3. Greenwood M. On digestion in hydra, with some observations on the structure of the endoderm. J Physiol 1888; 9:317-436; PMID:16991502; http://dx.doi.org/10.1113/jphysiol.1888.sp002090
4. Wood RL. Intercellular attachment in the epithelium of hydra as revealed by electron microscopy. J Biophys Biochem Cytol 1959; 6:343-52; PMID:13848583; http://dx.doi.org/10.1083/jcb.6.3.343
5. Hand AR, Gobel S. The structural organization of the septate and gap junctions of Hydra. Cell Biol 1972; 52:397-408; PMID:10833; http://dx.doi.org/10.1083/jcb.52.3.397
6. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mittos T, Weinmaier T, Rattee T, Balasubramanian PG, Borman J, Busan D, et al. The dynamic genome of hydra. Nature 2010; 466:592-6; PMID:22288792; http://dx.doi.org/10.1038/nature09083
7. Takahashi T, Muneoka Y, Lohmann J, Lopez de Haro R. Differentiation 2014; 52:109-24; PMID:2409925; http://dx.doi.org/10.1007/s00773-014-2489-z
8. Thomasen S, Bosch T. Foot formation and geno- plasticity in Hydra: lessons from the pod gene family. Dev Genes Evol 2006; 216:57-68; PMID:16402271; http://dx.doi.org/10.1007/s00727-006-0035-9
9. Martin-Belmonte F, Perez-Moreno M. Epithelial cell polarity, stem cells and cancer. Nat Rev Cancer 2012; 12:23-38; PMID:22169794; http://dx.doi.org/10.1038/nrncancer.2011.369
10. Hulpiau P, Roy F. New insights into the evolution of metazoan cadsynins. Mol Biol Evol 2011; 28:647-57; PMID:20817718; http://dx.doi.org/10.1093/molbev/mmq033
11. Kantor E, Zoccola D, Tambutte E, Voolstra CR, Aragno M, Allemand D, Tambutte S. Structural molecular components of septate junctions in cnidarians point to the origin of epithelial junctions in eukaryotes. Mol Biol Evol 2015; 32:44-62; PMID:25246700; http://dx.doi.org/10.1093/molbev/msu265.
12. Fraser SE, Green CR, Bode HR, Gilula NB. Selective components and matrix metalloproteinases. Development 2012; 139:475-85; PMID:22664437; http://dx.doi.org/10.1242/dev.073114
13. Alegopoulos H, Böttger A, Fischer S, Lein A, Wolf A, Fujisawa T, Hayakawa S, Gojobori T, Davies JA, David CN, et al. Evolution of gap junctions: the missing link? Curr Biol 2004; 14:879-84; PMID:15098476; http://dx.doi.org/10.1016/j.cub.2004.09.067
14. Sarris MPJ. Components, structure, biogenesis and function of the hydra extracellular matrix in regeneration, pattern formation and cell differentiation. Int J Dev Biol 2012; 56:567-76; PMID:22689358; http://dx.doi.org/10.1387/ijdb.113445ms
15. Shimizu H, Zhang X, Zhang J, Leontovich A, Fei K, Yan L, Sarris MPJ. Epithelial morphogenesis in hydra requires de novo expression of extracellular matrix components and matrix metalloproteinases. Development 2002; 129:1521-32; PMID:1188360
16. Aufschnaiter R, Zamir EA, Little CD, Ozbek S, Münder S, David CN, Li L, Sarris MPJ, Zhan X. In vivo imaging of basement membrane movement: ECM patterning shapes hydra polyps. J Cell Sci 2011; 124:4027-38; PMID:21294305; http://dx.doi.org/10.1242/jcs.075563
17. David CN, Campbell RD. Cell cycle kinetics and development of hydra attenuata. I. Epithelial cells. J Cell Sci 1972; 11:557-68; PMID:5076361
18. Flackeck AC, Mary G, Marchand M, Nègre D, Geset FL, Miralajo S, Wolf D, Savatier P, Delye C. Cell cycle features of prismatic embryonic stem cells. Stem Cells 2006; 24:547-56; PMID:16239321; http://dx.doi.org/10.1667/scm.2005-0194
19. Bosch TC, David CN. Growth regulation in hydra: relationship between epithelial cell cycle length and growth rate. Dev Biol 1984; 104:161-71; PMID:6759433; http://dx.doi.org/10.1016/0012-1606(84)90045-9
20. Dubel S, Schaller HC. Terminal differentiation of ectodermal epithelial stem cells of hydra can occur in g2 without requiring mitosis or s phase. J Cell Biol 1990; 110:399-45; PMID:2108971; http://dx.doi.org/10.1083/jcb.110.4.939
21. Okita K, Yamanaka S. Induction of pluripotency by defined factors. Exp Cell Res 2010; 316:2565-70; PMID:20420827; http://dx.doi.org/10.1016/j.yexcr.2010.04.023
22. Millane RC, Kaniska J, Duffy DJ, Sengie C, Cunningham S, Pilkkrig F, Frank U. Induced stem cell neoplasia in a cnidian by ectopic expression of a pou domain transcription factor. Development 2011; 138:2429-39; PMID:21610024; http://dx.doi.org/10.1242/dev.064931
23. Hofmayr B, Jenewein M, Eder D, Eder MK, Glaußar M, Güller S, Harrl M, Salvenmoser W. Stemness in hydra - a current perspective. Int J Dev Biol 2012; 56:509-17; PMID:22689357; http://dx.doi.org/10.1387/ijdb.113426bb
24. Hartl M, Mitteteller AM, Valotka T, Breuker K, Hofmayr B, Bister K. Stem cell-specific activation of an ancestral myc proto-oncogene with conserved basic functions in the early metazoan hydra. Proc Natl Acad Sci U S A 2010; 107:4051-6; PMID:20435077; http://dx.doi.org/10.1073/pnas.0910616107
25. Harl M, Glaußar M, Valotka T, Breuker K, Hofmayr B, Bister K. Hydra myc2, a unique pre-bilaterian member of the myc gene family, is activated in cell proliferation and gametogenesis. Biol Open 2014; 3:397-407; PMID:24771621; http://dx.doi.org/10.1242/bio.20147005
26. Ambrosone A, Marchesano V, Tino A, Hofmayr B, Tortiglione C. Hymyc1 downregulation promotes stem cell proliferation in hydra vulgaris. PLoS One 2012; 7:e30660; PMID:22290212; http://dx.doi.org/10.1371/journal.pone.0011686
27. Amoroso I, Margulies D. North-Holland Pub-
Juliano CE, Reich A, Liu N, Ginzfried J, Zheng M, Uman S, Rennan RA, Wessel GM, Steele RE, Lin H. Pwio proteins and pwio-interacting nuu function in hydro somatic stem cells. Proc Natl Acad Sci U S A 2012; 109:19607-702; PMID:23150652; http://dx.doi.org/10.1073/pnas.1207647109.

51. Grunder S, Assmann M. Peptide-gated ion channels. J Exp Biol 2014; 217:237-51; PMID:24355748; http://dx.doi.org/10.1242/jeb.13096111.

52. Campbell RD, Josephson RK, Schwab WE, Rushforth IT. Reactivation of developmental programs: the cAMP-stabilized cytoskeleton. Dev Cell 2011; 20:489-500; PMID:21338345; http://dx.doi.org/10.1016/j.devcel.2011.04.008.

Encyclopedia of stem cell and regenerative medicine 2011. 2011; 810:937-42; PMID:21576458; http://dx.doi.org/10.1016/j.devcel.2011.04.008.

53. Miller DJ, Hemmrich G, Ball EE, Hayward DC, Kuhlthun K, Funaya N, Agata K, Bosch TC. The innate immune repertoire in cidaria-ancestral complexity and stochastic gene loss. Genome Biology 2007; 8:R93; PMID:17547634; http://dx.doi.org/10.1186/gb-2007-8-4-r59.

54. Augustin R, Anton-Erlebahren F, Jungnickel S, Hemmrich G, Spudich A, Balat R, Grunder S, Assmann M, et al. Uncovering the evolutionary history of innate immunity: the simple metazoan cidaria uses epithelial cells for host defence. Dev Comp Immunol 2009; 33:559-69; PMID:19013190; http://dx.doi.org/10.1016/j.devimm.2008.12.005.

55. Fraune S, Buzgariu W, Reiter S, Dobretz K, Miljkovic-Licina M, Galliot B. Unusual spicules in the basal plate of cidaria and the mechanism of hydra head regeneration. Ii. Pacemaker system and the simple nervous system of hydra. J Exp Biol 1965; 42:205-31; PMID:14328679; http://dx.doi.org/10.1016/0012-1606(83)90325-1.

56. Franzenburg S, Walter J, Kunzel S, Wang J, Baines JF, Gruener SK, Looso M, Lengfeld T, Kuhn €O, Hoffmeister S, Schaller HC. The cAMP response element binding protein is involved in hydra regeneration. Development 1995; 121:1205-16; PMID:7734932.

57. Chera S, Ghia L, Dobretz K, Wengeler C, Buzgariu W, Galliot B. Silencing of the hydro serine protease inhibitor kazalin gene mimics the human spink1 pancreatic phenotype. J Cell Sci 2006; 119:846-
regeneration. Mol Biol Evol 2015; PMID:25841488; http://dx.doi.org/10.1093/molbev/msv079
89. Gierer A. The Hydra model - a model for what? Int J Dev Biol 2012; 56:437-45; PMID:22451043; http://dx.doi.org/10.1387/ijdb.113458ag
90. Campbell RD. Tissue dynamics of steady state growth in hydra littoralis. II. Patterns of tissue movement. J Morphol 1967; 121:19-28; PMID:4166265; http://dx.doi.org/10.1002/jmor.1051210103
91. Wenger Y, Galliot B. Punctuated emergences of genetic and phenotypic innovations in eumetazoa, bilaterian, euteleostome, and hominidae ancestors. Genome Biol Evol 2013a; 5:1949-68; http://dx.doi.org/10.1093/gbe/evt142
92. Wenger Y, Galliot B. RNAseq versus genome-predicted transcriptomes: a large population of novel transcripts identified in an illumina-454 hydra transcriptome. BMC Genomics 2013b; 14:204; http://dx.doi.org/10.1186/1471-2164-14-204