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Multi-functionality and plasticity characterize epithelial cells in *Hydra*

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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 multipotent interstitial stem cells (i-cells) that are predominantly distributed in the central body column, intermingled between the ectodermal epithelial cells (see in[1]). 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 neuropeptides,[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 2006[11] 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

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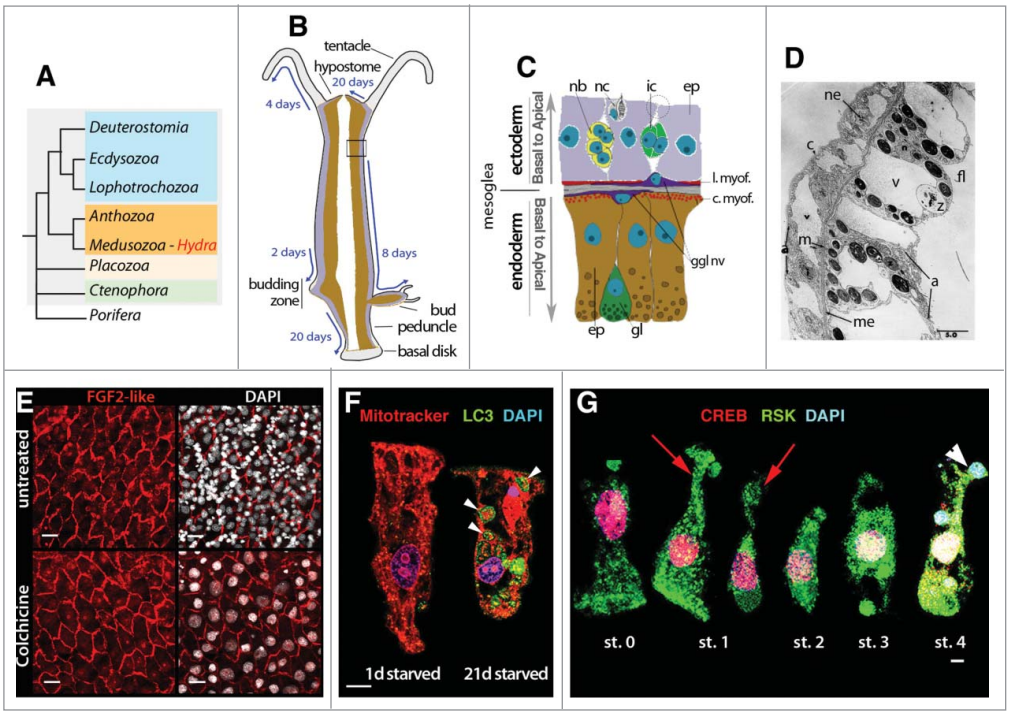

Figure 1. *Hydra* epithelial cells in homeostatic and stressed conditions. (**A**) Phylogenetic position of *Hydra* among metazoans. Note the sister group position of cnidarians that include anthozoans and medusozoans (orange background) to bilaterians (blue background). Among the early-diverged metazoan phyla (Porifera, Placozoa, Ctenophora), only Porifera do not differentiate epithelia. (**B**) Anatomy and tissue dynamics in *Hydra*. *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.[90] When reaching the poles, epithelial cells stop cycling to undergo terminal differentiation as head- or foot-specific cells (gray zones). (**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 *Chlorohydra viridissima* reproduced from[4] (Fig. 1). Note the acellular mesoglea (me) that separates the thinner epidermis on the left 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 μm. (**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 μm. (**F**) Starvation induces autophagy in *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).[55,56] Note the presence of numerous mitochondria inside the autophagic vacuoles detected with Mitotracker (red, arrowheads). Scale bar: 10 μm. (**G**) Engulfment of apoptotic bodies and loss of epithelial polarity in head-regenerating tips (ref. 62, Supplt 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.

applied this latter approach. We dissected the central body column of animals from AEP transgenic strains produced by the Bosch laboratory (which constitutively express GFP, either in the endodermal epithelial cells[11] or in the ectodermal epithelial cells,[12] or in the interstitial stem cells[10]), dissociated the tissues to sort the GFP-expressing cells by flow cytometry,[13] and quantified the level of expression of each gene by RNA-seq (**Fig. 2A**) (for details, see[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 *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 eumetazoans, we assume that tracing back in *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 *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 carefully observed in electron-microscopic studies in the 60s,[4,15]

the fine structure and the components of the *Hydra* glycocalyx were only recently identified.[16] This fibrous cuticle, up to 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]

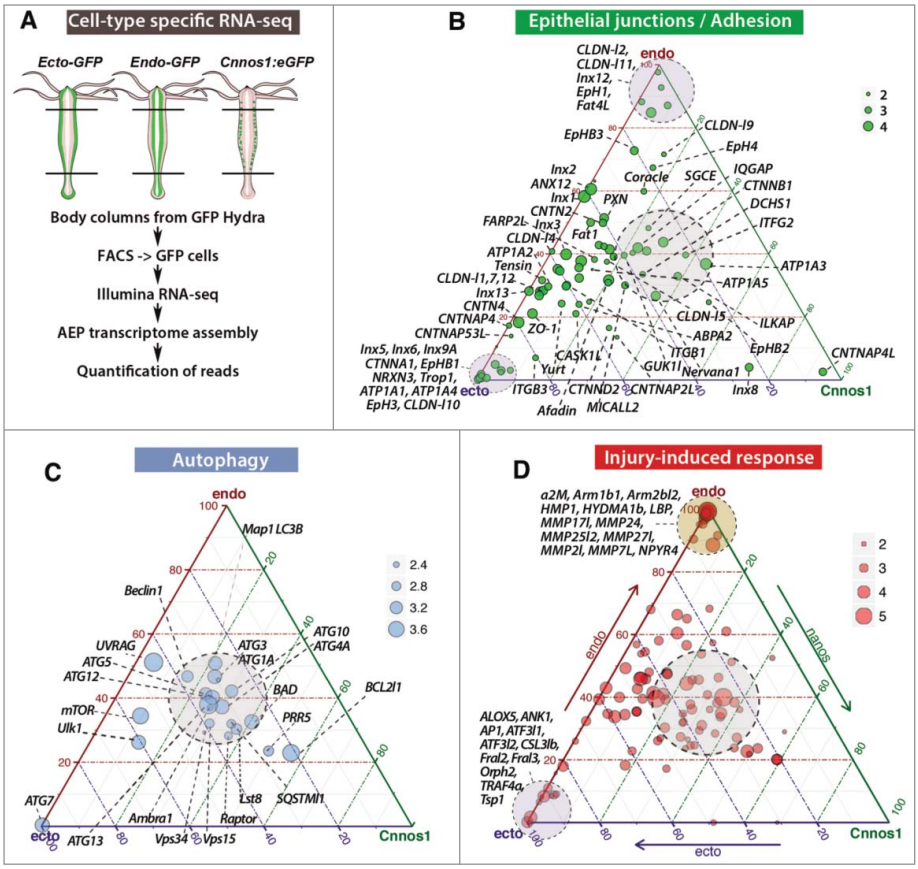

Figure 2. Molecular patterns of the ectodermal and endodermal epithelial cells as deduced from RNA-seq transcriptomic analyses. (**A**). Scheme depicting the procedure to produce RNAs from each stem cell population by dissecting the body columns of 3 transgenic AEP strains that constitutively express GFP either in the ectodermal epithelial cells (ECTO actin::eGFP[12]), or in the endodermal epithelial cells (ENDO actin::eGFP[11]), or in the interstitial stem cells.[39] The quantitative RNA-seq analysis was performed on FACS-sorted cells.[13,14] (**B–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.

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 bilaterians[19]: 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 baso-lateral adherens junctions (AJs) and the basal hemi-desmosome-like structures (see **Table 1**). Important components of the AJs are the classical cadherins. These are present in *Nematostella vectensis*[20]; 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" claudin 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 (*CLDNl2, 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

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, Zürich, Jussy*)
Sub-apical complex	INADL InaD-like protein (PatJ)	ECTO > Endo >> i-cells	T2M9I3_HYDVU
	LIN7C Protein lin-7 homolog C	Ecto, Endo > i-cells	T2M567_HYDVU
	MPP5 MAGUK p55 (Stardust, Pals1)	Ecto > Endo >> i-cells	T2M9J3_HYDVU
	Notch2 (Crumbs-like)	ENDO >>> Endo >> i-cells	T2MDK9_HYDVU
Apico-lateral complex	CDC42 Cell division control protein 42 homolog	ENDO > ECTO > I-CELLS	T2MEG1_HYDVU
	PARD3 Partitioning defective 3 homolog	ECTO > ENDO > i-cells	T2M994_HYDVU
	PARD6G Partitioning defective 6 homolog gamma	Ecto > Endo >> i-cells	T2M6J3_HYDVU
	PRKCI Protein kinase C	ECTO > Endo >> i-cells	T2MGA0_HYDVU
Lateral complex	DLG1 Disks large homolog 1	ECTO > ENDO >> I-CELLS	T2ME64_HYDVU
	DLG5 Disks large homolog 5	Ecto >> Endo, i-cells	T2M8B5_HYDVU
	LLGL1 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-l1, 7, 12 Claudin-like 1,7, 12	ECTO > ENDO >> i-cells	CRX73236, CRX73250, CRX73241
Septate Junctions	CLDN-l10 Claudin-like 10	ECTO >> Endo >> i-cells	CRX73238,
(St SJs)	CLDN-l2, 9, 11 Claudin-like 2,9,11	Ecto << Endo >> i-cells	CRX73242, CRX73253, CRX73239
	CLDN-l3, CLDN-l4, Claudin-like 3, 4	Ecto, Endo	CRX73247, T2MFM9_HYDVU
	CLDN-l5 Claudin-like 5	Ecto > Endo >> i-cells	T2MBI9_HDYVU
	CLDN-l6, 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
	CNTNAP2l Contactin assoc. prot 2like	Ecto > i-cells > Endo	CRX73256
	CNTNAP4 Contactin assoc. prot 4	Ecto >> Endo	CRX73257
	CNTNAP4l Contactin assoc. prot 4like	Ecto, Endo << I-CELLS	CRX73258
	CNTNAP5 Contactin assoc. prot.-like 5	ECTO > ENDO >> i-cells	T2M8X1_HYDVU
	CNTNAP53l Contactin assoc. prot. like 5-3	Ecto >> endo	CRX73259
	DSCAM Down syndrome cell adhesion mol.	Endo < Ecto < i-cells	T2MIF2_HYDVU
	NRXN1 Neurexin-1a like	Apical expression only	CRX73281
	NRXN3 Neurexin-3a like	ECTO >> Endo > i-cells	T2M365_HYDVU
Scaffold	ATP1A1 NaK ATPase-α1	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 l4 (Coracle)	Endo > Ecto > i-cells	T2M572_HYDVU
	EPB41L5 Band 4.1 l5 (Yurt)	ECTO >> Endo >> i-cells	T2M5L9_HYDVU
	ZO-1 Zonula Occludens 1 (TJP1)	ECTO >> ENDO >> i-cells	T2MDH6_HYDVU
Adherens Junctions	ACTN1 α-actinin	ECTO > ENDO >> i-cells	T2MHI5_HYDVU
(AJs)	CDH Classical cadherin	ECTO >> i-cells >> Endo	CRX73223
	CELSR2 Cadherin EGF LAG 7pass	ECTO > Endo > i-cells	T2M506_HYDVU
	CTNNA1 α-catenin	ECTO >> i-cells, Endo	T2M3Z5_HYDVU
	CTNNB1 β-catenin	ENDO > I-CELLS> ECTO,	T2MGP6_HYDVU
	CTNND2 δ-catenin	Ecto > i-cells > Endo	T2M3M0_HYDVU
	DAG1 Dystroglycan	Ecto	T2MDZ1_HYDVU
	DCHS1 Protocadherin 16	Ecto > Endo> i-cells	T2M7D2_HYDVU
	FAT1 Protocadherin 1	ENDO > ECTO >> i-cells	T2MDR8_HYDVU
	FAT4l Protocadherin Fat4-like	ENDO >> ECTO > i-cells	CRX73260
	MICALl2 MICAL like protein 2	ECTO > Endo > i-cells	T2MAH1_HYDVU
	MLLT4 (Afadin)	ECTO >> ENDO >> i-cells	T2MF28_HYDVU
	SGCE Sarcoglycan	Endo > Ecto > i-cells	T2MJ55_HYDVU
	VCL Vinculin	ECTO > ENDO >> I-CELLS	T2MH95_HYDVU

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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).) *(Continued)*

Predicted FUNCTIONS	PROTEIN NAMES Gene families	EXPRESSION *in GFP Hv-AEP* CELLS	Protein ACCESSION (*Hv-Basel, Zürich, Jussy*)
Gap junctions (GJs)	Inx1, Innexin 1	ENDO > ECTO >>> i-cells	Q2EMV6_HYDVU,
	Inx2, Inx9, Inx10, Inx11, Inx14, Inx15	No or very low expression in body column	seq57378, seq46622 (pending), CRX73266, seq79106, seq05316, seq64623 (pending)
	Inx3, Inx13 Innexin 3, 13	ECTO > ENDO >> i-cells	CRX73271, CRX73269
	Inx4, Inx5, Inx6, Inx7,	ECTO or Ecto	CRX73272, CRX73275, CRX73274, CRX73277,
	Inx8 Innexin 8	I-CELLS >> Ecto >> Endo	Seq55322 (pending)
	Inx12 Innexin12	Endo >> Ecto >>> i-cells	CRX73268
Hemi-desmosomes (HDs)	ADAM10	ECTO > ENDO >> i-cells	T2MJ41_HYDVU
	ADAM12	Endo > Ecto > i-cells	T2MIA5_HYDVU
	ADAM17	ECTO < ENDO < I-CELLS	T2MEE2_HYDVU
	ADAM33	Ecto < Endo < i-cells	T2M6H9_HYDVU
	ADAMTS9 Disintegrin MP thrombospondin	Endo > i-cells > Ecto	T2M4C5_HYDVU
	CIB1 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	T2ME09_HYDVU
	ILKAP ILK-associated protein	ECTO < ENDO < I-CELLS	T2M6A7_HYDVU
	ITFG2 Integrin-a FG-GAP	Endo, i-cells > Ecto	T2M8F8_HYDVU
	ITGA4 integrin-alpha4	ECTO > ENDO >> i-cells	CRX73278
	ITGA8 Integrin-alpha8	ENDO > ECTO > I-CELLS	T2MFQ0_HYDVU
	ITGA9 Integrin-alpha9	ECTO > ENDO >>i-cells	T2ME15_HYDVU
	ITGB1 Integrin-beta1	ECTO >> ENDO >> i-cells	T2MHW4_HYDVU
	ITGB2 Integrin-beta2	ECTO > ENDO >> I-CELLS	T2MGW7_HYDVU
	ITGB3 Integrin-beta3	Ecto >>> Endo < i-cells	CRX73280
	PXN Paxillin	ENDO > ECTO >> I-CELLS	T2MG05_HYDVU
	TLN2 Talin2	ECTO > ENDO > I-CELLS	T2M2W2_HYDVU
	TNS1 Tensin1	ECTO > Endo >>> i-cells	T2M5L6_HYDVU
Cell adhesion	ANX12 Annexin XII / Annexin –B12	ENDO > ECTO >>> i-cells	P26256_HYDVU
Scaffolding proteins	ANXA7 Annexin	ECTO > ENDO >> i-cells	T2MGP1_HYDVU
	CASK Peripheral plasma mbne protein CASK	Ecto >> Endo, i-cells	CRX73235
	DSCAM Down syndrome cell adhesion mol	Endo < Ecto < i-cells	T2MIF2_HYDVU
	EpH1 Ephrin receptor 1	ENDO >>> i-cells, Ecto	AGO06063.1
	EpH2 / EPHA7 Ephrin receptor 2/7	ECTO, Endo >> i-cells	AGO06064.1, T2MDF6_HYDVU
	EpH3 / EPHA5 Ephrin receptor 3/5	ECTO >>> i-cells, endo	AGO06066.1, T2MF36_HYDVU
	EpH4 / EPHA4 Ephrin receptor 4	Endo >> Ecto > i-cells	AGO06065.1, T2MEM7_HYDVU
	EpHB1 Ephrin ligand B1	Ecto >>> i-cells, Endo	AGO06067.1, R9WY58_HYDVU
	EpHB2 Ephrin ligand B2	Ecto, Endo << i-cells	AGO06068.1, R9WWC9_HYDVU
	EpHB3 Ephrin ligand B3	ENDO >> Ecto >> i-cells	AGO06069.1, R9X0X4_HYDVU
	FARP2 I FERM RhoGEF pleckstrin domain	Ecto > Endo >> i-cells	T2MID3_HYDVU
	GUK1 like Guanylate Kinase 1	Ecto, Endo > i-cells	T2MD66_HYDVU
	IQGAP / IQGAP1 GTPase-activating like prot	ENDO > ECTO > I-CELLS	Q9XZE9_HYDVU, T2MFN7_HYDVU
	LRIG3 Leu Rich Repeats Ig-like prot 3	Ecto > Endo > i-cells	T2MAL0_HYDVU
	Trop1 Tropomyosin	ECTO >>> Endo	TPM1_HYDVU
Cuticle structure	Sweet Tooth proteins	22 proteins	See Böttger et al. 2012 (ref. 16)
	PPOD1 Putative Peroxidase 1	ECTO >> ENDO >>> i-cells	Q2FBK4_HYDVU, Q2FBK7_HYDVU
	PPOD2 Putative Peroxidase 2	No PPOD2 in *Hv-AEP*	Q962G1_HYDVU, Q2FBK2_HYDVU
	PPOD2-like Putative Peroxidase 2-like	No PPOD2l in *Hv-AEP*	Q2FBJ9_HYDVU
Extra-Cellular Matrix (ECM)	ANKFN1 Ankyrin repeat fibronectin III	Ecto > Endo >> i-cells	T2M9C4_HYDVU
	COL4A1 / COL4A5 collagen-alpha5 (IV)	ENDO >>> Endo, i-cells	Q9GQB1, T2MFW7_HYDVU
	FARM1 secreted astacin	Endo >>> Ecto	Q9U4X9_HYDVU
	FiCol fibrillar collagen	ENDO >>>> Ecto, i-cells	Q8MUF5_HYDVU
	FNDC3B FN type III containing protein 3A	ECTO, ENDO >> i-cells	T2MCC9_HYDVU
	HMCN1l1 Hemicentin1 like1	ENDO >>> i-cells > Ecto	CRX73261

(*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,[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).) *(Continued)*

Predicted FUNCTIONS	PROTEIN NAMES Gene families	EXPRESSION *in GFP Hv-AEP* CELLS	Protein ACCESSION (*Hv-Basel, Zürich, Jussy*)
	HMCN1l2 Hemicentin1 like2	ENDO >> i-cells > Ecto	CRX73263
	HMCN2l1 Hemicentin2 like1	ECTO >>> i-cells, Endo	CRX73264
	HMP1 Metalloendopeptidase	ENDO >> Ecto > i-cells	Q25174_HYDVU
	HSPG2 basement membrane-specific heparan sulfate proteoglycan protein	ENDO >> i-cells > Ecto	T2MDT4_HYDVU
	LAMA5 Laminin subunit alpha-5	ENDO >> Ecto > i-cells	T2MIW4_HYDVU
	LAMB1 Laminin subunit beta-1	ENDO >>> i-cells > Ecto	LAMB1_HYDVU
	MMP matrix metalloproteinase	ENDO >>>> Ecto > i-cells	Q9U9P0_HYDVU
	MP2 Metalloendopeptidase (meprin-like)	Endo >> i-cells > Ecto	Q9XZG0_HYDVU
Stem Cell Behavior	Ets1 / ERG	ECTO > ENDO >>> i-cells	I3V7X0_HYDVU, T2MHK5_HYDVU
& Stemness	Ets2 / GABPA	Endo > Ecto >> i-cells	T2MDI3_HYDVU
	FoxO	ECTO > I-CELLS > ENDO	J7HWF0_HYDVU
	Klf1 Krueppel like factor 1	ECTO > ENDO >>> i-cells	T2MDQ7_Hydvu
	Klf3 Krueppel like factor 3	ECTO > Endo	I3V7X3_HYDVU
	Klf7 Krueppel like factor 7	ECTO > ENDO >> i-cells	T2MIK5_Hydvu
	Klf8 Krueppel like factor 8	ECTO > ENDO	I3V7V7_HYDVU
	Klf11 Krueppel like factor 11	ECTO > ENDO >> i-cells	I3V7X4_HYDVU, T2MJ10_HYDVU
	Klf13 Krueppel like factor 13	Endo < Ecto << I-CELLS	I3V7W6_HYDVU, T2M360_HYDVU
	MAX	ECTO < ENDO < I-CELLS	D0EM50_HYDVU
	Myc-1	Endo < Ecto << i-cells	D0EM49_HYDVU
	Myc-2	Ecto < ENDO < I-CELLS	D2KBP8_HYDVU, T2MH01_HYDVU
	Myc-3	Endo < Ecto <<< i-cells	CRX73227
	PIWIL1 /Hywi /Cniwi Piwi-like protein 1	ENDO < ECTO < I-CELLS	T2M7W7, J7HWM3, T2HRA5
	PIWIL2 / Hyli Piwi-like protein 2	ENDO < ECTO << I-CELLS	T2M9F7, U5XHW4, T2HRQ9
	PL10	ENDO < ECTO < I-CELLS	Q9GV14_HYDVU
	POU4F2	Endo << Ecto < i-cells	T2MDR7_HYDVU
	SOX2	ECTO > Endo, i-cells	T2MFM3_HYDVU
	TCF Ternary Complex Factor	ENDO > I-CELLS > ECTO	Q9GTK1_HYDVU
	TCTP (p23)	ENDO > ECTO > I-CELLS	TCTP_HYDVU
	TP53BP2	ECTO >> ENDO > i-cells	T2MDM1_HYDVU
	TP73	Endo >> Ecto > i-cells	T2MIU9_HYDVU
	Vasa1 / Cnvas1	ECTO < ENDO < I-CELLS	Q9GV13_HYDVU
	Vasa2 / Cnvas2	ECTO < ENDO < I-CELLS	Q9GV12_HYDVU

the 2 epithelial cell layers are structurally different as 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[24]). 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, HMMP) (see in **Table 1**, refs[10,24]). All these components, assembled together in the extracellular space, also play an important role in 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.[13,29] *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 in **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 *Hydractinia* the Oct4-like transcription factor named "Polynem" promotes self-renewal[32] 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[33]; *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.[34,35] *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 *Tcf, PIWI* and *vasa1* 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 *Cniwi*, 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 hyli-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 muscles[4,42] (**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.[43,44] 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 cells[48] via innexins[23] (**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 defecation 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.[42,53] 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 modifier-activating enzyme ATG7 is almost exclusively expressed in the ectodermal 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 death[59] 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 in[59]). 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 bilaterian 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.[64] These immune responses, also present in porifers, were deeply dissected in *Hydra*, which makes use of Toll-like receptors (TLR), NOD-like pattern recognition receptors (NLR) and the cytoplasmic cascades that mediate the production of antimicrobial peptides (AMPs).[65-68] TLR signaling in *Hydra* was revealed by silencing the universal transducer protein Myd88.[67] Unlike *Nematostella*,[69] where the TLR function is achieved by receptors that contain both the LRR (leucine rich repeat) and the TIR (Toll/Interleukin-1 receptor) domains, *Hydra* possesses 2 distinct proteins that functionally interact, one harboring the LRR, the other the TIR domains.[65] The activation of the TLR transduction pathway elicits an antimicrobial response, as the production of the periculin peptide by the endodermal epithelial cells and the interstitial cells.

The second line of defense includes the surprisingly complex inventory of NLR family receptors. Although the function of this family of receptors in the innate defense is well established, the interacting partners and the members of signaling pathway are not completely understood in *Hydra*. So far, *in vitro* studies identified one caspase containing a DEATH domain that interacts with *hy*NLR type 1 protein, suggesting that NLR induction triggers caspase activation.[66] As output, 3 classes of AMPs are synthetized by the endodermal epithelial cells, periculin, hydramacin and arminins, which show efficient bactericidal activity.[65,70,71] As a third line of defense, the gland cells produce serine protease inhibitors, among them *Kazal2* that exhibits a powerful activity against *Staphylococcus aureus*.[72] Moreover, under a massive pathogenic aggression, ectodermal cells are able to emit pseudopods and engulf bacteria, providing another protective defense response.[65] 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.[73] More recent systematic studies reveal that different *Hydra* species develop particular preferences for certain bacterial phylotypes.[74] This process encompass 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.[68] 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*.[74] 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.[75]

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.[68] Also "epithelial" *Hydra* lacking nerve and gland cells, show a different composition of their colonizing microbiota.[76] 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.[70,72,77] 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.[62,78,79,80] 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, loose their apical to basal polarity and become spherical (**Fig. 1G**). 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[62,81]. 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.[62,80]

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 (**Fig. 2D**).[82] 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 asexual 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

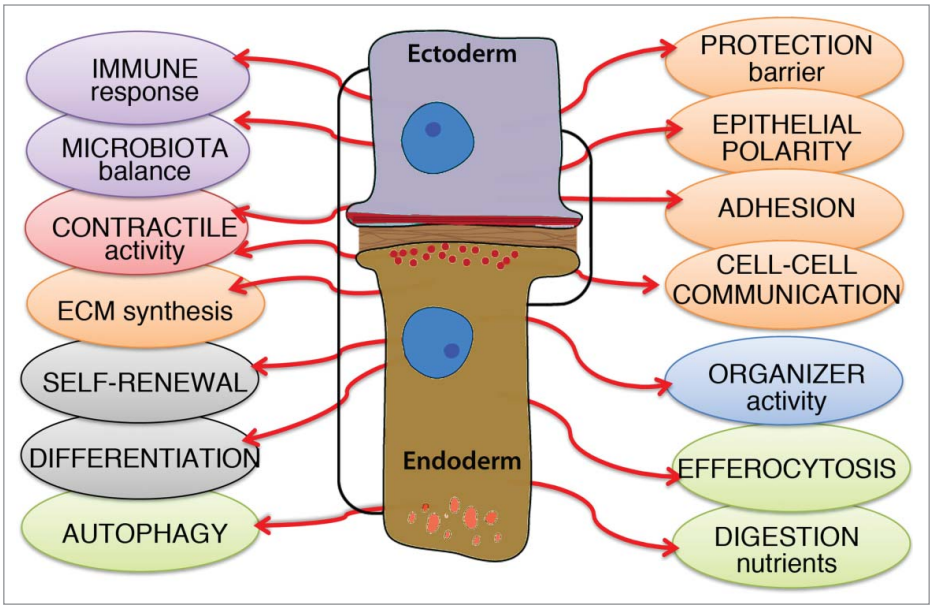

Figure 3. Summary scheme depicting the multiple functions of endodermal and ectodermal epithelial cells in *Hydra*. Note the functions that are common to both epithelial cell types (brackets).

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.[83,84] For head regeneration, activation of the MAPK/CREB pathway and induction of the canonical Wnt pathway play essential roles.[62,80,81,85]

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 [86]). 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.[82,87,88]

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.[89] 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.

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Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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