ILE MULTIDISCIPLINARY JOURNAL

VOLUME 4 AND ISSUE 2 OF 2025

INSTITUTE OF LEGAL EDUCATION

ILE MULTIDISCIPLINARY JOURNAL

APIS – 3920 – 0007 | ISSN - 2583-7230

(OPEN ACCESS JOURNAL)

Journal's Home Page – https://mj.iledu.in/

Journal's Editorial Page - https://mj.iledu.in/editorial-board/

Volume 4 and Issue 2 (Access Full Issue on - https://mj.iledu.in/category/volume-4-and-issue-2-of-2025/)

Publisher

Prasanna S,

Chairman of Institute of Legal Education

No. 08, Arul Nagar, Seera Thoppu,

Maudhanda Kurichi, Srirangam,

Tiruchirappalli – 620102

Phone : +91 94896 71437 - info@iledu.in / Chairman@iledu.in

ILE MULTIDISCIPLINARY JOURNAL [IF SCORE – 7.58]
VOLUME 4 AND ISSUE 2 OF 2025
APIS – 3920 – 0007 | ISSN - 2583-7230
Published by
Institute of Legal Education
https://iledu.in

POLYPS REGENERATE, WE DON'T: HYDRA'S CELLULAR AND MOLECULAR REGENERATION SYSTEM

AUTHOR - Y. O. KOROLOV, CANDIDATE OF LAW SCIENCE, DOCTORAL STUDENT OF THE DEPARTMENT OF LAW, PRIVATE HIGHER EDUCATIONAL ESTABLISHMENT, «EUROPEAN UNIVERSITY», SPACE INTERNATIONAL COMPANY, INVIDUAL BUSINESS Y.O.KOROLOV. EMAIL: SOAHCIT@GMAIL.COM. ORCID ID: 0009-0000-2037-3419

BEST CITATION - Y. O. KOROLOV, POLYPS REGENERATE, WE DON'T: HYDRA'S CELLULAR AND MOLECULAR REGENERATION SYSTEM, ILE MULTIDISCIPLINARY JOURNAL, 4 (2) OF 2025, PG. 167-174, APIS – 3920-0007 | ISSN - 2583-7230

Abstract

The basis of Hydra's tremendous regenerative capacity is the "stemness" of its epithelium, which continuously undergoes self-renewing mitotic divisions and has the ability to follow the pathways of differentiation and youthfulness of the organism. Now, new molecular tools have shed light on the molecular processes that control these pathways and may create rejuvenation. In this review, I discuss how a modular tissue architecture may enable continuous cell turnover in Hydra. I also describe the discovery and regulation of factors that control the transition from self-renewing epithelial stem cells to differentiated cells.

Keywords: RNA-binding domains; RNA-binding proteins; machine learning; protein-protein interaction network.

Introduction

If we lose a limb, we cannot regenerate it. If, however, we divide an intact Hydra into individual cells, a perfect polyp will regenerate from the sediment of centrifuged cells over the next few days. Why shouldn't we also do the same for ourselves? Why do some animals have a remarkable capacity for self-renewal and others don't? Hydras have had regeneration for over 200 years. In the 1740s, the Swiss scientist Abraham Tremblay (1744) discovered that freshwater polyps could regenerate their heads and legs, and – if cut into several pieces – they would all regenerate to form new individuals (Lenhoff and Lenhoff, 1988). Hydra also provides an excellent opportunity to understand how morphogen gradients can be established and maintained to control local developmental processes (Wolpert et al., 1972, 1974). In Hydra, regenerative tissue has been reorganized, how positional information is encoded at the molecular level, and how cells respond to diffuse positional cues. The impressive accumulation of gene sequences, new tools, and the development of genomic resources over the past few years have brought new insights into the ability of Hydra to regenerate. Hydra has also become capable of modifying its genetics through RNAi experiments, further expanding the capabilities of this model organism (Lohmann et al., 1999; Takahashi et al., 2005; Cardenas and Salgado, 2003; Chera et al., 2006; Amimoto et al., 2006). Finally, transgenic Hydra (Wittlieb et al., 2006; Steele, 2006) are paving the way for many important scientific and technological applications by making available the resources and methods to fully explore the biological capabilities of the polyp. Since epithelial cells are key players in regeneration in Hydra, I will focus on epithelial stem cells. We will first observe them at the site of regeneration and then discuss the mechanisms by which they are thought to become new cells.

Regeneration in Hydra occurs by morphalaxis

Hydra consists of two cell layers, the ectoderm and the endoderm, separated by a thin extracellular matrix (ECM) called the mesoglea. The polar body plan has a head and

tentacles at one end and a foot at the opposite end of a hollow column. The cells belong to either the ectodermal or endodermal epithelial cell lineage or the interstitial cell lineage. Epithelial cells are epithelial-muscle cells that cover the animal externally or line the stomach cavity. Interstitial cells are mostly localized in the interstitial space between ectodermal epithelial cells and differentiate into nerve cells, cnidocytes, gland cells, and during sexual differentiation, into gametes.

Any isolated fragment of the Hydra body larger than a few hundred epithelial cells can regenerate into a miniature version of the animal. This ability to self-organize is due to the continuous production of cells and signaling factors in the adult tissue. Pieces of regenerating tissue cut from sections of the stomach exhibit a directional property called polarity. Such pieces regenerate the head at the apical end of the isolated fragment. The foot always regenerates at the basal end of such a piece. Polarity is thought to be based on gradients of molecules whose concentrations provide positional information (Wolpert et al., 1974; MacWilliams, 1983a,b). Regeneration in Hydra provides an excellent experimental system for studying de novo pattern formation and highlights an important process of pattern formation in multicellular organisms: visible patterns are preceded by pre-patterns or morphogenetic fields. Morphallaxis refers to a type of regeneration that occurs in the absence of cell proliferation and involves the transformation of existing body parts or tissues into newly organized structures. Epimorphosis refers to regeneration that requires active cell proliferation. In planarians, as in some vertebrates such as salamanders, both the generation of new tissue at the wound site through cell proliferation (blastema formation) and morphallaxis are required for complete regeneration (Brockes et al., 2001; Agata, 2003; Reddien and Sánchez Alvarado, 2004; Sanchez Alvarado, 2006). There, cells near the site of injury lose their specialized properties and revert to their original state in a process called dedifferentiation. These stem cells are then thought to rapidly proliferate and redifferentiate, forming the tissue needed to repair the limb or organ (Brockes and Kumar, 2005; Slack, 2006).

In the marine hydrozoan Podocoryne, some cells can dedifferentiate or transdifferentiate under certain conditions (Schmid and Reber-Muller, 1995; Reber-Muller et al., 2006). However, early regenerative processes in Hydra always occur in the absence of DNA synthesis as a morpholactic process in which cells of the gastric region differentiate into cells specific for the head or foot (Cummings and Bode, 1984). My laboratory has re-examined the question of cell regeneration at the site of injury using transgenic polyps and in vivo tracking of GFP-expressing endodermal epithelial cells in the regenerating tissue. We have shown that at the tip of the regenerating tissue there is localized cell proliferation of endodermal epithelial cells.

Thus, the observation that regeneration in Hydra occurs almost exclusively by morpholaxy. Regeneration in Hydra has also been described at the ultrastructural level as a rapid wound healing process initiated by the endoderm (Bibb and Campbell, 1973). Elegant in vitro studies (Takaku et al., 2005) with isolated ectodermal and endodermal epithelial cells have led to the conclusion that during the reorganization of the epithelial layers, endodermal epithelial cells display unexpected motility. Cells at the regenerating tip become activated within 2–3 hours of amputation (Technau and Bode, 1999) and undergo phenotypic changes in cellular, biochemical, and functional properties, leading to the expression of novel cell surface antigens (e.g., Bode et al., 1988) and genes (e.g., Takahashi et al., 2005; Amimoto et al., 2006; see Fig. 2D and E). The events during regeneration resemble normal morphogenesis and involve the interaction of multiple cell types, signaling pathways, extracellular matrix components, and soluble factors. Regeneration always begins with a wound; and wound healing may require

some specialized activities that are not required during normal morphogenesis. The function of the evolutionarily conserved Kazal1 gene, which is expressed in endodermal gland cells and is activated during regeneration, has been investigated. Silencing Kazal1 by RNA interference resulted in severe tissue disorganization, followed by massive cell death of the gland and accumulation of autophagosomes in the cytoplasm of digestive cells.

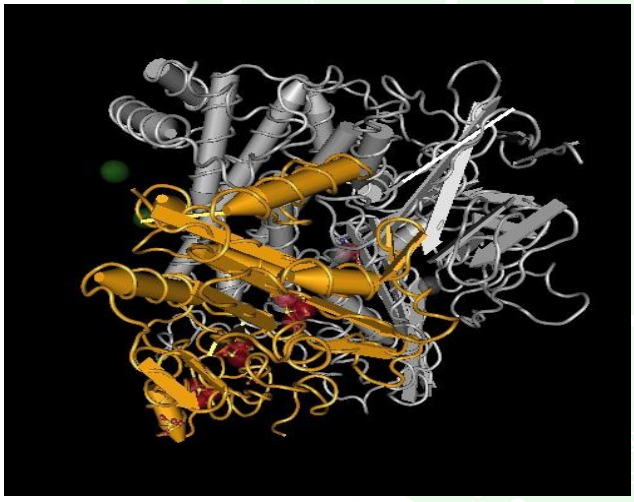
Only epithelial cells can regenerate in Hydra. This has been confirmed by experiments in which cell types were selectively removed from Hydra and the developmental potential of the resulting animals was studied. Furthermore, a chimeric Hydra containing epithelial cells from normal Hydra and interstitial cells from the reg16 mutant revealed that the defect responsible for the low head regeneration potential of this mutant was located in epithelial cells (Nishimiya et al., 1986). First, an intact ECM (mesoglea) separating the two cell layers is required. Mature mesoglea contains macromolecules such as laminins, collagens, heparan sulfate proteoglycans, and fibronectin-like molecules (reviewed in Sarras and Deutzmann, 2001). Regeneration begins with the immediate retraction of the mesoglea, which must subsequently be reestablished (Shimizu et al. 2002). The key role of mesoglea in Hydra epithelial homeostasis is also highlighted by the discovery (Kuznetsov et al. 2002) that the survival of Hydra epithelial cells depends on their attachment to extracellular matrix molecules. In the 3634 sequences analyzed, we identified not only previously known protease-encoding genes but also as yet undescribed proteases, including the Hydra homolog of the sea urchin metalloprotease SpAN, the Hydra gene related to the C. elegans metalloprotease ADAM,

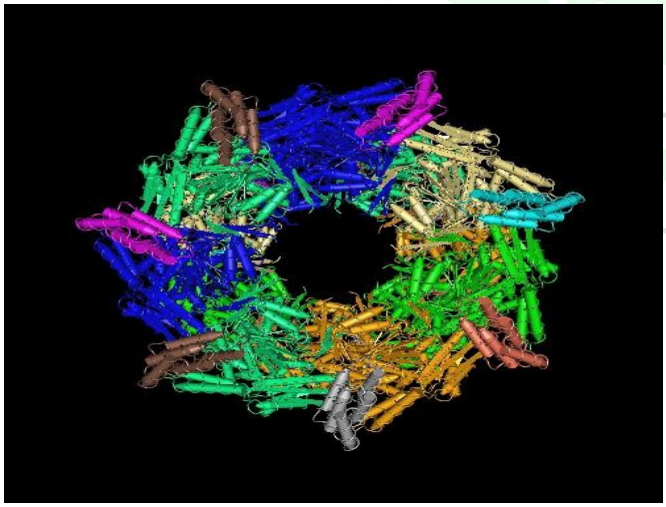
Hydra has several components of the Wnt cascade (Hobmayer et al., 2000; Broun et al., 2005; see Lee et al., 2006, for review). During head regeneration, HyWnt appears as a bottleneck at the end of the regenerating body axis. B-catenin and TCF are also upregulated, but in a larger part of the head (Hobmayer et al., 2000). Treatment of Hydra with alsterpaulon (Broun et al., 2005), which specifically blocks GSK-3β activity, increases β-catenin levels in the nuclei of body column cells, confers head organizer characteristics to the body column, and induces expression of Wnt pathway genes in the body column (Broun et al., 2005). These results provide direct evidence for a role for the canonical Wnt pathway in the formation and maintenance of the head organizer in Hydra.

The mechanisms underlying the regulation of Wnt signaling in Hydra are not yet understood. Well-known antagonists of Wnt signaling are the Dickkopf proteins 1, 2, and 4 (Bejsovec, 2005). During a screening of yeast signal peptide secretion for regeneration-specific genes in Hydra, the Holstein team (Guder et al., 2006) isolated a small 95-amino acid protein with high sequence similarity to a conserved cysteine motif in Dickkopf proteins. Guder et al. (2006) therefore named the Hydra gene hydkk1/2/4. Unlike the usual Dickkopf family members, which have two cysteine-rich domains, hydkk1/2/4 contains only one cysteine-rich domain, similar to Dickkopf 2. The gene is expressed in endodermal gland cells and is also an early regeneration gene in foot and head regeneration. Gooder et al. (2006) found a rapid and dramatic increase in hydkk1/2/4 expression on the side of injury within 30 minutes of head removal. This early increase was clearly related to the injury stimulus, as it was also induced by simply cutting the animal on both sides of the body column. Gooder et al. (2006) suggest that the early release of Dickkopf proteins from the cut side is an important trigger for head regeneration. Does HyDkk1/2/4 act as a Wnt antagonist? When HyDkk1/2/4 mRNA was injected into Xenopus embryos, HyDkk1/2/4 had similar Wnt antagonistic activity as XDkk1 in Xenopus embryos. Furthermore, in Hydra, expression of hydkk1/2/4 is complementary to that of hywnt3a, brachyury, and other head-specific genes (Hobmayer et al., 2000; Technau and Bode, 1999). Finally, experimental activation

ILE MULTIDISCIPLINARY JOURNAL [IF SCORE – 7.58]
VOLUME 4 AND ISSUE 2 OF 2025
APIS – 3920 – 0007 | ISSN - 2583-7230
Published by
Institute of Legal Education
https://iledu.in

of Wnt/β-Catenin signaling results in complete downregulation of hydkk1/2/4 transcripts. Although these observations may indicate a role in Wnt signaling, this may not be the whole story for several reasons. First, early in regeneration, hydkk1/2/4 and hywnt3a are coexpressed, making a direct role for HyDkk1/2/4 as a Wnt antagonist difficult. Second, preliminary data from H. Shimizu (personal communication) and our own laboratory show that in the A10 strain of H. Magnipapillata, in the complete absence of all hydkk1/2/4-expressing cells, animals exhibit normal morphogenesis, indicating that at least in this strain hydkk1/2/4 does not play a significant role in morphogenesis. Third, my laboratory has recently shown (Augustin et al., 2006) that there is a closely related gene for hydkk1/2/4 (named HyDkk1/2/4-A in the study by Augustin et al.), hydkk1/2/4-C, which does not





respond to regeneration signals at all. The co-expression of both genes may be functionally relevant, as preliminary data based on the yeast two-hybrid system suggest that HyDkk1/2/4-A and HyDkk1/2/4-C interact with the same putative receptor (R. Kiko and T.C.G. Bosch, unpublished). Additional experimental data are needed, but at this point I believe that these observations make a direct role for Dickkopf-related small molecules as regulators of Wnt signaling in Hydra less likely.

One important class of proteins that has been shown to be involved in cell fate and terminal differentiation processes in many vertebrates and invertebrates are the Notch proteins. Therefore, to complete our understanding of cellular communication during regeneration in Hydra, future efforts will need to address issues related to Notch signaling and crosstalk between Notch and other pathways. Interestingly, Hydra expressing the Notch-GFP fusion protein has nuclear localization

There is also experimental evidence that spatially restricted gene expression in Hydra is controlled by transcriptional repressors. Examples include Cnox-2, an ortholog of the ParaHox gene Gsx, which prevents head formation from body column tissue. Cnox-2 is expressed in the body column but not in the head region, and protein levels are reduced after removal of the head (Shenk et al., 1993a,b). By analyzing protein binding sites of the ks-1 promoter, we have shown (Endl et al., 1999) that Cnox-2 binds to the ks-1 promoter in the body column but not in the head tissue, where the ks-1 gene is actually expressed. Thus, Cnox-2 and possibly other repressors may prevent transcription of ks-1 and other head-specific genes in body column cells. This may imply that the default state of at least some of the spatially restricted developmental genes is “on” and that locally active transcriptional repressors drive differential expression patterns. Gene expression profiles are a consequence of the activity of transcription factors, which in turn are controlled by extracellular signals.

ILE MULTIDISCIPLINARY JOURNAL [IF SCORE – 7.58]
VOLUME 4 AND ISSUE 2 OF 2025
APIS – 3920 – 0007 | ISSN - 2583-7230
Published by
Institute of Legal Education
https://iledu.in

The relationships between all of these regulators form a genetic regulatory network that can be used to predict cell behavior in changing environments. Although we are far from understanding the genetic regulatory networks for any of the Hydra cell types, new genomic technologies and promoter analysis of the foot homeobox gene, CnNK-2, have inspired us to delineate a genetic regulatory network for foot regeneration (Thomsen et al., 2004; Siebert et al., 2005; Thomsen and Bosch, 2006). As noted above, the differentiation of cells at the basal end of the Hydra axis into specific stem and foot cells depends on two important signaling factors, pedibin. The homeodomain factor CnNK-2 is sensitive to these peptides and is involved in translating the positional significance gradient into changes in cell behavior and foot-specific differentiation (Grens et al., 1996, 1999). CnNK-2 and pedibin are coexpressed in endodermal epithelial cells located at the basal end of the body column. In polyps treated with pedibin, CnNK-2 expression is significantly extended towards the gastric region (Grens et al., 1999). Thus, the peptide appears to cause a decrease in the positional significance of the gastric tissue, which leads to an increase in the spatial domain of expression of the homeobox gene CnNK-2. In an attempt to unravel the transcriptional regulatory network controlling the expression of a specific foot gene, we analyzed the 5′-flanking sequence of CnNK-2 using phylogenetic fingerprinting (Siebert et al., 2005). Unexpectedly, budhead, a nuclear factor involved in head and bud formation (Martinez et al., 1997), was found to specifically bind to the regulatory region of CnNK-2 (Siebert et al., 2005). Since budhead is expressed opposite CnNK-2 in the head region (Fig. 4; Martinez et al., 1997), our results indicate molecular crosstalk between the head, bud, and foot patterning systems during axis formation in Hydra. As schematically shown in Fig. 4, members of the signaling network controlling foot formation include the peptide pedibin, which is upstream and controls the expression of CnNK-2. CnNK-2, in turn, controls the localized expression of pedibin and, presumably, also genes located downstream, the products of which are directly involved in foot differentiation. In addition, we have obtained experimental evidence that CnNK-2 regulates its own expression through autocatalytic feedback (Thomsen et al., 2004). Budhead (Siebert et al., 2005) is believed to be a transcriptional regulator of CnNK-2.

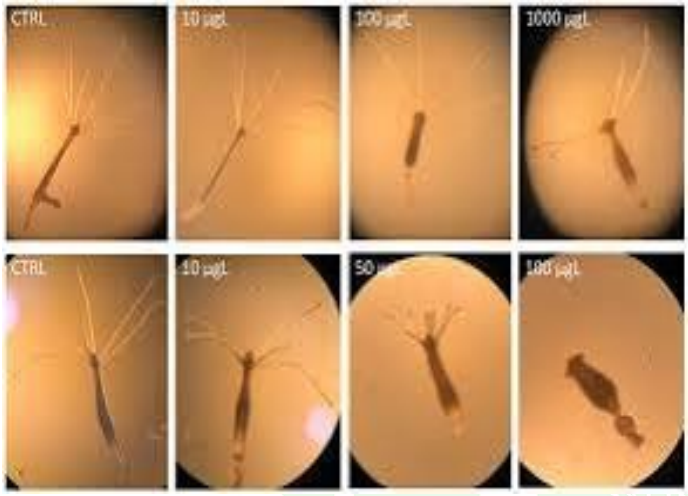
In summary, key elements of the mechanisms that control self-renewal and differentiation of epithelial cells in Hydra.

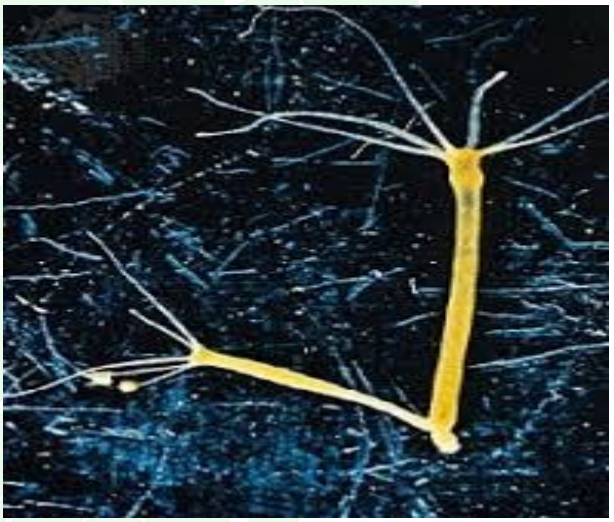
Hydra Epithelium—A Recipe for Successful Regeneration.

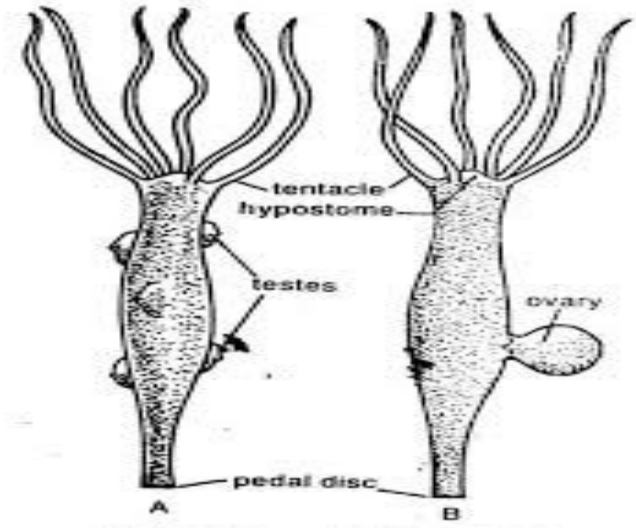
Hydra has chosen a life cycle in which reproduction occurs predominantly asexually by budding. This requires that each bud receive a full cellular repertoire from the mother polyp. By endowing all epithelial cells in the budding region with stem cell properties and by filling the interstitial space with multipotent interstitial stem cells capable of differentiating not only into somatic cells but also into gametes, the buds receive everything they need. Thus, it is the stem cellularity of the tissue that provides the unique life cycle of Hydra. This property alone seems sufficient to explain Hydra's unprecedented capacity for regeneration. Does this reflect a particularly simple or even "primitive" molecular and cellular tissue architecture? I think not, for two reasons. First, at the molecular level, Hydra, like all other members of the phylum Cnidaria, is extremely complex. The genomes of different species of Hydra vary, but are generally large, with Hydra vulgaris having a genome of 1250 Mbp (Zacharias et al., 2004). This is about half the size of the haploid human genome. Furthermore, not only do Cnidaria have about the same number of genes as humans, and most of their genes are shared with humans (Miller et al., 2005), but their protein sequences, surprisingly, are often more similar to human sequences than to those of flies and worms (Kortschak et al., 2003). Thus, at the level of genomic complexity and gene complement, Hydra is much more complex than previously thought. Given its morphological simplicity, such complexity is surprising.

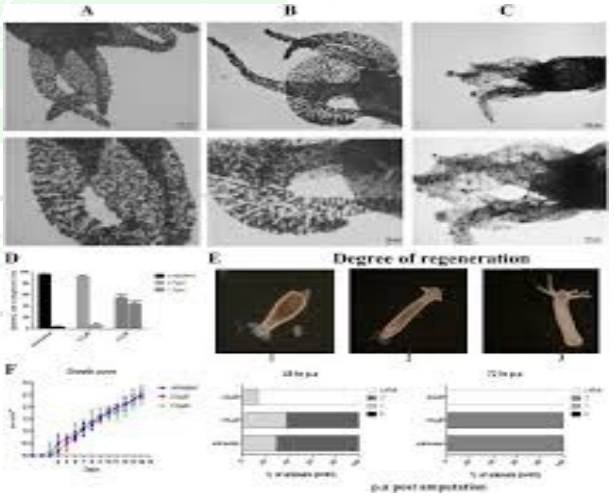
ILE MULTIDISCIPLINARY JOURNAL [IF SCORE – 7.58]
VOLUME 4 AND ISSUE 2 OF 2025
APIS – 3920 – 0007 | ISSN - 2583-7230
Published by
Institute of Legal Education
https://iledu.in

However, it does indicate that the difference between "them" and "us" in terms of regeneration is unlikely to be based on the available gene pool. Second, there is also no evidence that Hydra cells are fundamentally different from zebrafish or human cells. However, there may be a profound difference in the differentiation potential and plasticity of Hydra and vertebrate cells. Vertebrates, including humans, depend on specialized cells with limited differentiation potential to perform complex functions. Hydra cells, in contrast, are able to produce and receive positional signals continuously even in adult tissue and thus possess features that most vertebrate cells possess only during a short period of embryogenesis.









Analysis of one of the most studied mammalian epithelial stem cell systems, the small intestinal crypts, has shown that stem cells are present in vertebrates only in very low numbers.

(Moore and Lemischka, 2006; see Fig. 5C) and – due to the complexity of the niche microenvironment – are difficult to study directly. In Hydra, by contrast, most epithelial cells have a high capacity for self-renewal and high phenotypic plasticity. As I have attempted to outline above, in Hydra all the raw materials for regeneration come from just three lineages of stem cells. With the right instructions, these stem cells have the potential to enter all possible differentiation pathways, can be directly visualized, and can be manipulated experimentally (Figs. 2C and 3). Since fundamental regulatory mechanisms are expected to be conserved across the animal kingdom, and since most vertebrate gene families have deep evolutionary roots (Kortschak et al., 2003; Miller et al., 2005; Tehnau

et al., 2005), these instructions are the same for Hydra stem cells and for human cells.

Reference:

1. Castello, A., Fischer, B., Frese, C.K., Horos, R., Alleaume, A.M., Foehr, S.,Curk, T., Krijgsveld, J., and Hentze, M.W. (2016). Comprehensive identification of RNA-binding domains in human cells. Mol. Cell 63, 696–710. https://doi.org/10.1016/J.MOLCEL.2016.06.029

2. Beckmann, B.M., Horos, R., Fischer, B., Castello, A., Eichelbaum, K., Alleaume, A.M., Schwarzl, T., Curk, T., Foehr, S., Huber, W., et al. (2015). The RNA-binding proteomes from yeast to man harbour conserved enigmRBPs. Nat. Commun.6, 10127.https://doi.org/10.1038/ncomms10127

3. Baltz, A.G., Munschauer, M., Schwanh €ausser, B., Vasile, A., Murakawa, Y., Schueler, M., Youngs, N., Penfold-Brown, D., Drew, K., Milek, M., et al. (2012). The mRNA-bound proteome and its global occupancy profile on protein-coding transcripts. Mol. Cell 46, 674–690. https://doi.org/10.1016/j.molcel.2012.05.021

4. Conrad, T., Albrecht, A.-S., de Melo Costa, V.R., Sauer, S., Meierhofer, D.,and Ørom, U.A. (2016). Serial interactome capture of the human cell nucleus. Nat. Commun. 7, 11212. https://doi.org/10.1038/ncomms11212

5. Queiroz, R.M.L., Smith, T., Villanueva, E., Marti-Solano, M., Monti, M., Pizzinga, M., Mirea, D.-M., Ramakrishna, M., Harvey, R.F., Dezi, V., et al. (2019). Comprehensive identification of RNA–protein interactions in any organism using orthogonal organic phase separation (OOPS). Nat. Biotechnol. 37, 169–178. https://doi.org/10.1038/s41587-018-0001-2

6. Trendel, J., Schwarzl, T., Horos, R., Prakash, A., Bateman, A., Hentze, M.W., and Krijgsveld, J. (2019). The human RNA-binding proteome and its dynamics during translational arrest. Cell 176, 391–403.e19. https://doi.org/10.1016/j.cell.2018.11.004

7. Kumar, M., Gromiha, M.M., and Raghava, G.P.S. (2011). SVM based prediction of RNA-binding proteins using binding residues and evolutionary information. J. Mol. Recognit. 24, 303–313. https://doi.org/10.1002/jmr.1061

8. Zhao, H., Yang, Y., Janga, S.C., Kao, C.C., and Zhou, Y. (2014). Prediction and validation of the unexplored RNA-binding protein atlas of the human proteome. Proteins 82, 640–647. https://doi.org/10.1002/prot.24441

9. Livi, C.M., Klus, P., Delli Ponti, R., and Tartaglia, G.G. (2016). catRAPID signature: identification of ribonucleoproteins and RNA-binding regions. Bioinformatics 32, 773–775. https://doi.org/10.1093/bioinformatics/btv629

10. Zhang, X., and Liu, S. (2017). RBPPred: predicting RNA-binding proteins from sequence using SVM. Bioinformatics 33, 854–862. https://doi.org/10.1093/bioinformatics/btw730

11. Bressin, A., Schulte-Sasse, R., Figini, D., Urdaneta, E.C., Beckmann, B.M., and Marsico, A. (2019). TriPepSVM: de novo prediction of RNAbinding proteins based on short amino acid motifs. Nucleic Acids Res. 47, 4406–4417. https://doi.org/10.1093/nar/gkz203

12. Brannan, K.W., Jin, W., Huelga, S.C., Banks, C.A.S., Gilmore, J.M., Florens, L., Washburn, M.P., Van Nostrand, E.L., Pratt, G.A., Schwinn, M.K., et al. (2016). SONAR discovers RNA-binding proteins from analysis of large-scale protein-protein interactomes. Mol. Cell 64, 282–293. https://doi.org/10.1016/j.molcel.2016.09.003

13. Nambiar, A., Heflin, M., Liu, S., Maslov, S., Hopkins, M., and Ritz, A. (2020). Transforming the language of life: transformer neural networks for protein prediction tasks ACM reference format. In Proceedings of the 11th ACM International Conference on Bioinformatics (Computational Biology and Health Informatics). https://doi.org/10.1145/3388440

14. Yang, X., He, X., Liang, Y., Yang, Y., Zhang, S., and Xie, P. (2020). Transfer learning or self-supervised learning? A tale of two pretraining paradigms. https://doi.org/10.36227/techrxiv.12502298

15. Brandes, N., Ofer, D., Peleg, Y., Rappoport, N., and Linial, M. (2022). ProteinBERT: a universal deep-learning model of protein sequence and function. Bioinformatics 38, 2102–

2110.
https://doi.org/10.1093/BIOINFORMATICS/BTAC020