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Visions & Reflections (Minireview)

Hydra – ancient model with modern outfit

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Abstract. The control of growth and differentiation is a central question not only for developmental biologists but increasingly for medical research as well. The freshwater polyp hydra was one of the first organisms to be used as a model system for the study of this question. It was chosen because of its simple body plan and because it is made up of only seven to eight different cell types. Recent research has shown that despite their simple body plan, cnidarians already exhibit an impressive repertoire of molecular tools which are responsible for the control of growth and differentiation and amongst which peptides appear to play an important role.

Keywords. Regeneration, differentiation, morphogenetically active peptide, precursor, β-thymosin repeat.

Introduction

Cnidarians – corals, sea anemones, jellyfish and hydra – are an evolutionarily ancient group of soft-bodied animals. The phylum Cnidaria displays an impressive diversity of 'lifestyles'. Members of this phylum can live as sessile polyps – either as single individuals or as job-sharing members in colonies – which reproduce asexually or which can switch to a life as free-floating medusae, which reproduce sexually, thus generating a third form of organism, the planula larva. Of course, there are many exceptions, with polyps reproducing sexually and medusae that are capable of asexual reproduction, as well as organisms that do not switch between the two forms.

All cnidarians share the same simple body plan. They consist of a two-layered, radially symmetrical tube, with the polyps comprising two differentiated structures along a single body axis: head and foot. Cnidarians are more than 500 million years old and a sister group to the Bilateria [1, 2]. They are diploblastic animals lacking a mesoderm and possessing only two germ layers, an outer ectoderm and inner endoderm that are separated by an acellular mesogloea. In the animal kingdom the cnidarians were the first organisms to evolve a nervous system.

The freshwater polyp hydra is not only evolutionarily very old but is, due to its immense regeneration capability, also one of the oldest model organisms of scientific research [3]. The fact that missing parts of the body are correctly restored was found to be mainly due to the existence of continuously proliferating epithelial and interstitial cells in the body column [4, 5] resembling a sort of permanent embryo. The correctness of the regeneration events, yielding several new individual polyps maintaining the original polarity even if a polyp is cut into several pieces [6], implies that regeneration is tightly controlled. Transplantation experiments showed that tissue pieces derived from a region close to the head of a donor animal induce another head in the body column of the recipient. This 'head-activating potential' is graded along the body axis, declining towards the foot [7]. However, when head-inducing tissue pieces are implanted close to the existing head of a recipient, no second head will be induced. This demonstrates the

presence of a 'head inhibition potential', which also occurs as a gradient in the head-to-foot direction [8, 9]. Similar experiments were performed for the foot region and resulted in corresponding findings: tissue close to the foot exhibited a high 'foot-activating potential' as well as a 'foot-inhibiting potential' [10, 11]. These data suggested the existence of morphogenetically active substances occurring in the animal as gradients (Fig. 1).

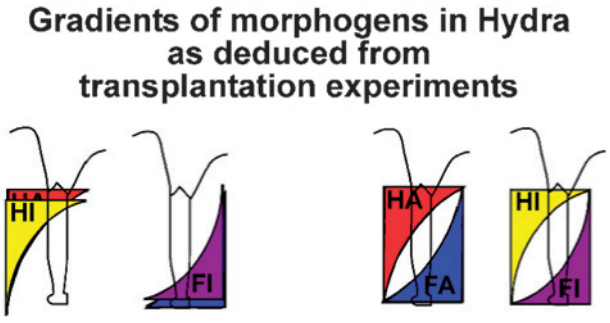

Figure 1. Shown is a schematic drawing of the distribution of morphogenetically active substances in hydra. Head activator (HA) and head inhibitor (HI) occur as gradients with the highest concentration in the head of the animal, whereas foot activator (FA) and foot inhibitor (FI) are maximally concentrated in the foot, declining in their concentration towards the head.

Search for morphogens in biological assays

The laboratory of H. C. Schaller was one of the first to apply biochemical techniques for the characterisation of morphogenetically active substances, monitoring specific effects on regeneration in quantitative assay systems [12]. Four morphogenetically active substances correlating to tissue properties as deduced from transplantation experiments have been described: head activator, foot activator, head inhibitor and foot inhibitor (Fig. 1).

The first molecule identified as a morphogen was a peptide named head activator [13]. It was purified as a factor that enhances head regeneration and stimulates stem cell proliferation, nerve cell differentiation and bud outgrowth [14]. The peptide is reported to be produced by nerve cells under normal conditions. However, in the absence of nerve cells, epithelial cells have the potential to produce head activator and other morphogenetic factors [15].

Later, for the quantitative analysis of foot-specific regeneration processes, an assay system was developed that made use of the presence of a peroxidase-like activity in the foot mucus cells in the hydra foot [16, 17]. During foot regeneration, the reappearance of the peroxidase activity coincides with the time at which the animal regains a functional foot enabling the animal to restick to a substratum. Therefore, measuring the peroxidase activity at 23 h after foot removal allows the quantification of the amount of regenerated foot-specific cells in treated compared to untreated animals.

Using this assay system for the characterisation of foot activation resulted in the isolation of two morphogenetically active molecules from hydra tissue, namely pedin and pedibin [18]. Both are short peptides with pedin consisting of 13 and pedibin of 21 amino acids, and both peptides have some properties in common with a foot activator proposed earlier [19, 20]. Whereas pedibin is more or less evenly distributed over the animal with the upper half containing slightly more of the peptide than the lower section, pedin exhibits a more 'foot-activator-like' distribution, being twice as concentrated in the lower half compared to the upper half [18]. To correlate the action of the peptides with the formerly described foot activator, the content of pedin and pedibin in a foot regeneration-deficient strain of *Hydra oligactis* was determined. This analysis showed that *H. oligactis* contains 5-fold less pedibin and 13.5-fold less pedin than *H. vulgaris*, which regenerate feet more easily, e.g. faster, thus demonstrating the relevance of these peptides for foot-specific differentiation processes.

Pedibin

Pedibin, the peptide of 21 amino acids, stimulates foot formation and is synthesised as a precursor of 49 amino acids. A putative cleavage site precedes the peptide purified from hydra tissue. The precursor, like pedibin, accelerates foot regeneration. Pedibin transcripts are concentrated in the foot region of hydra as expected, but they are also present in the head region, accumulating in the tentacle bases as well as in the buds. The early appearance of pedibin transcripts during phases of cell fate specification such as budding and regeneration (Fig. 2) implies that in hydra, pedibin plays an important role in patterning processes of foot and head. This is confirmed by the finding that pedibin also stimulates bud outgrowth [21].

As a consequence of the application of gene technology in hydra research, many genes have been cloned in homology screens. The results of such screens show that hydra is pretty well equipped with tools for the control of development known from vertebrates, like signalling molecules, receptors, extracellular matrix molecules, transcription factors and regulators of cell proliferation and apoptosis [for reviews see refs. 22, 23]. Amongst the cloned transcription factors is the homeodomain factor CnNK-2 which in hydra appears to be linked to the control of pattern formation in a foot-specific context. The expression of *CnNK-2* is

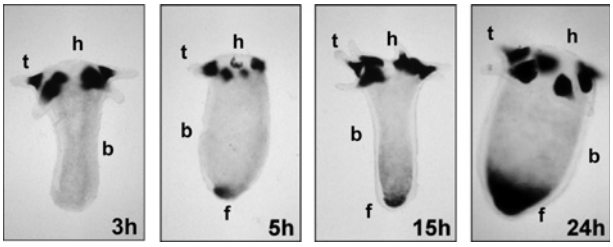

Figure 2. Whole-mount in situ hybridisation shows that the pedibin transcripts are expressed in the tentacle bases in the head (h) and in the foot (f) of hydra. After foot amputation, they start to reappear at the cut surface between 3–5 h. The concentration of transcripts increases with regeneration time, until after about 24 h the regeneration is complete. b, body column; t, tentacle.

extended towards the head region due to the application of pedibin, thus altering the positional value of the gastric tissue making it more 'footy', e.g. competent for foot-specific differentiation [24]. Experimental approaches to elucidate the transcriptional control during foot regeneration have led to a postulated scenario in which pedibin initially increases the spatial domain of *CnNK-2* expression which in turn promotes *pedibin* expression as well as its own [25].

Pedin

Pedin, the peptide of 13 amino acids, has been shown to stimulate foot formation, the proliferation of interstitial cells, which are the progenitors of nerve cells, and to stimulate the differentiation of nerve cells [18]. All these activities had been previously ascribed to a foot activator, enriched from hydra tissue [26, 27]. Recently, the cDNA coding for pedin was cloned from hydra [28]. Unlike pedibin, which is encoded as a single copy, pedin was found to be present in 13 copies in the 102-kDa precursor protein. Although this is similar to the mode of neuropeptide synthesis in cnidarians [29–32], pedin transcripts are expressed in epithelial cells [28]. Hydra was the first organism in evolution with a nervous system. Evolutionarily older animals such as the sponges show epithelial cells which are capable of simple signal conductance. Therefore, the occurrence of a neuropeptide-like precursor in epithelial cells may hint at the close relatedness of epitheliomuscular cells and nerve cells. Another finding which points in the same direction is the aforementioned ability of epithelial cells to substitute the function of nerve cells with respect to the production of morphogenetically active substances [15].

Surprisingly, the pedin precursor also comprises 27 thymosin β4-like repeats. These repeats can be placed into two different groups according to their degree of homology to thymosin β4, namely type 1 repeats with 48.6 % identity over 37 residues and type 2 repeats with 54.8 % identity over 31 residues. The type 1 repeats include the last five amino acids of the mature pedin peptide at their N terminus, while the type 2 repeats include the first six amino acids of the pedin peptide. This makes it rather unlikely that both peptides are generated simultaneously from the same precursor. Processing of the mature pedin peptide as purified from hydra tissue might occur at the arginine flanking the N terminus. This arginine probably serves as a substrate for a monobasic endopeptidase [33, 34]. The processing at the C terminus of each pedin copy must be done by an endopeptidase hydrolysing at the C-terminal side of glutamic acid. Such novel prohormone-processing enzymes had already been postulated to exist in cnidarian neurons [35].

The β-thymosins are a highly conserved family of small (5-kDa) polypeptides that act as G-actin-sequestering factors. Thymosin β4 has been found to participate in actin-dependent processes such as endocytosis, cell division, cell movement and morphogenesis. β-Thymosins are widely distributed among vertebrate classes. The thymosin β4 protein family is a distinct subfamily of a broader group of related proteins, i.e., the WH2 motif-containing proteins [Wiscott-Aldridge syndrome protein (WASp) homology domain 2] [36]. The WH2 domain is a small motif (approximately 35 amino acids) that is believed to bind to actin monomers [37]. β-Thymosins are composed of a single WH2 domain, while human WIP (WASP-interacting protein) and yeast verprolin contain two WH2 motifs [36]. In *Drosophila melanogaster,* the Ciboulot protein contains three repeats [38], the TetraThymosin beta from *Caenorhabditis elegans* contains four thymosin repeats [39, 40], and cytoskeletal protein 24 (Csp24) from the mollusc *Hermissenda crassicornis* contains five thymosin repeats [41]. The alignment of the full-length pedin precursor with the WH2 motif-containing proteins using the Clustal-X software [42] places the pedin precursor in close proximity to the thymosins, Ciboulot, Csp24 and TetraThymosin beta. Hence, the pedin precursor was named thypedin [28]. Preliminary evidence as deduced from co-localization studies and immunoprecipitation experiments performed with anti-pedin and anti-actin antiserum, respectively, implies that the WH2 motifs of thypedin are able to interact with actin [28].

Functional similarities between pedin and thymosin β4

In accordance with its distribution in the adult animal, pedin was also found to stimulate bud outgrowth. The positive effect of pedin on budding might be a

consequence of its stimulatory effect on interstitial cell proliferation and differentiation of nerve cells: since nerve cells are highly concentrated in the foot and head of hydra, nerve cell differentiation is an important process during regeneration and budding. At least as important as cell proliferation is tissue movement or cell migration for bud outgrowth [43, 44]. Therefore, pedin might also be acting as a stimulator of cell migration.

At present, nothing is known about the mode of action of pedin. Unlike the neuropeptides found in hydra, the epitheliopeptides are synthesised without an evident signal peptide. Therefore, either the peptides are released only during wounding as a result of cell rupture and enzymatic degradation or hydra has evolved its own mechanisms not only for the maturation – as outlined above – but also for the release of biologically active peptides. Since these peptides appear to function mainly during regeneration processes, release as a consequence of cell rupture may not be that unlikely. For the mode of action of pedin and its precursor, this could mean that while the peptide is used under regenerative conditions only, the precursor protein may function under steady-state conditions as well as during regeneration, provided that the interaction with actin can be proven. The related thymosin repeat-containing Ciboulot protein from *Drosophila,* for example, was shown to play an essential role during neurogenesis because of its importance for the regulation of actin assembly in neuronal outgrowth [38].

Not only does the precursor for pedin, thypedin, exhibit sequence homologies to thymosin, but the biological activities of pedin also bear striking resemblances to the multiple functions of mammalian thymosin β4 as an extracellular peptide. Like pedin, thymosin β4 is synthesised without a signal peptide, and direct evidence for its secretion is missing [45, 46]. However, it is present in human serum and may be released from cells by an unknown mechanism or as a consequence of cell death [45]. The functions of thymosin β4 are, similar to those of pedin, critical for development, regeneration and wound repair. Thymosin β4 promotes endothelial cell migration, tubule formation, angiogenesis and wound healing [47]. It was also reported to be expressed in the mammalian nervous system during pre- and postnatal development and to be required for axonal tract formation in the zebrafish brain [48, 49]. More recently, thymosin β4 was shown to be involved in cell migration and cell survival during cardiac morphogenesis as well as after cardiac infarction and to promote vessel formation and collateral growth not only during development but also in the adult [50, 51]. Therefore, concerning their respective biological activities, thypedin could be regarded as an evolutionarily ancient progenitor protein of thymosin β4.

In summary, peptides appear to play an important role in the regulation of development and regeneration. With respect to pedin and thypedin, in particular, this role appears to be well conserved in evolution.

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