

Review

## Regeneration in Echinoderms: repair, regrowth, cloning

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Accepted June 16, 2006

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### Abstract

Regenerative potential is expressed to a maximum extent in echinoderms. It is a common phenomenon in all the classes, extensively employed to reconstruct external appendages and internal organs often subjected to amputation, self-induced or traumatic, rapidly followed by complete successful re-growth of the lost parts. Regeneration has been studied in adult individuals as well as in larvae. In armed echinoderms, regeneration of arms is obviously frequent: in many cases, the detached body fragments can undergo phenomena of partial or total regeneration independently of the *donor* animal, and, in a few cases (asteroids), the individual autotomised arms can even regenerate to produce new complete adults, offering superb examples of cloning strategies. In the species examined so far most results throw light on aspects related to wound healing, growth, morphogenesis and differentiation, even though in most cases many crucial questions remain unanswered. The present paper provides an overview of the current understanding of the phenomenon and covers the main biological aspects of regeneration giving an idea of the "state of the art" across the phylum in terms of experimental approaches and representative models.

**Key words:** regenerative development; repair; regrowth; cloning

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### Introduction to regeneration

Development is a broad-spectrum process intrinsic of life which does not always start from the egg, fertilized or not, but can involve significantly all the stages of the life cycle, including not only the embryonic and post-embryonic periods, but also the adult phase.

Regeneration is in fact a distinct type of developmental process typically occurring in adults or larvae: it can involve limited processes of cell turnover and tissue repair, replacement of lost parts or organs, cast off following self-induced or traumatic mutilations, and even complete regrowth of whole individuals from body fragments (thus contributing to typical asexual reproductive processes).

In terms of general significance, regeneration is an important regulatory phenomenon with wide implications on reproductive biology, as previously suggested by Spallanzani in his historical studies on head regeneration in snails (1768). It does not represent the first expression of new combination of genes resulting from sexual processes, but starts from cells, which have an already tested genetic program. It is fundamentally a conservative asexual process. In fact, thanks to its evident close relation with fission phenomena and cloning processes, regeneration can be regarded as the necessary and specific developmental complement to asexual reproduction, in analogy, and in parallel, to what happens for embryogenesis which is the established developmental strategy complementary to sexual gametic reproduction. Therefore, although regeneration unavoidably involves analogous problems in terms of basic mechanisms and often superficially resembles embryogenesis, fundamental differences in its intrinsic asexual start and meaning makes regeneration a significantly diverse biological process. In embryogenesis, the whole organismic structure is totally created *de novo*; in contrast, in regeneration an anatomically defined part of the

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organism, small or large, is reformed after its loss or severe injury and the new cells develop in an established context of mature tissues and differentiated cells in individuals (adult or larval) well characterized in terms of morphology and functions. Therefore, in all animals, the regenerative processes related to different organs and structures should be regarded as fundamentally distinct developmental processes, which can not be considered an accelerated version of ontogenetic processes (Candia Carnevali and Bonasoro, 2001a; Reik and Dean, 2002).

Although a response to injury is evoked in all animals, there is a remarkable variability in terms of degree of morphological and functional recovery, not only between unrelated groups, but also between closely related species, and even between organs and parts of the same individual. In contrast to old traditional views regarding the regenerative potential as a unique prerogative of the simplest and most primitive animals, regeneration is actually a common and widespread phenomenon through phylogeny, and its quite heterogeneous distribution from the lowest to the highest phyla appears to be independent of their organization and complexity level (Ferretti and Géraudie, 1997; Thouveny and Tassava, 1997; Candia Carnevali and Bonasoro, 2001a). In fact, the regenerative capabilities appear to depend upon the individual potential for histogenetic and morphogenetic plasticity expressed in terms of recruitment of stem cells and/or dedifferentiated cells, cell proliferation and migration, supply of specific regulatory/trophic factors, and finally expression, or re-expression, of a specific developmental program (Weissman, 2000; Wadman, 2005). Despite the differences between the species, repair and regrowth are common to all organisms, and differentiated tissues (liver, muscles) can be replaced *de novo* even in adult mammals, at least to a certain degree.

Obviously the regenerative potential can vary significantly with the stage of the life cycle (embryonic, larval, adult) and the age of the individual, the regenerative capabilities being higher in larval tissues and organs in comparison with those of adults, and in many cases present only in the early embryonic stages (mammals). It is not a case that traditional studies of regeneration in vertebrates have often employed larval stages of amphibians.

If a close correlation between the regenerative potential of the individual and its possibility of survival can be inferred easily, self-repair abilities appear not only an obvious advantage for the individual, but gives a fundamental contribution to the adaptive capacities of the species and its fitness, since they increase the individual's chances of reproducing, sexually or asexually, even when its body integrity is dramatically compromised.

The universal character of regeneration is expressed in the famous aphorism by Goss (1969): "If there were no regeneration there could be no life, if everything regenerated there would be no death. All organisms exist between these two extremes ". On the other hand, there are still unsolved fundamental questions concerning *why* regenerative potential is expressed to such different extents in different organisms and *whether* this phenomenon has been selected for or against in the course of evolution.

According to a modern authoritative hypothesis (Goss, 1988, 1992; Thouveny and Tassava, 1997) which takes into account the evolution of regeneration abilities in the organisms, regeneration should be regarded as a primary attribute of life: all organisms in principle possess a latent potential for regeneration, that could have been suppressed in response to specific selection pressures, i.e. inhibited/eliminated wherever its expression does not help survival and reproduction and does not counterbalance the disadvantages of its high metabolic cost. In other words, as far as the animal kingdom is concerned, the suppression of regeneration should not be regarded as a negative adaptation but rather as a pleiotropic epiphenomenon related to specific constraints and linked to more useful but incompatible adaptations (Goss, 1988, 1992).

In spite of the wide choice of potential models for studying regeneration, this phenomenon has been appropriately explored only in a few animal models traditionally and successfully employed by developmental biologists (hydrozoans and planarians among invertebrates and amphibian urodeles among vertebrates). Surprisingly, with regard to many other animal groups well known for their spectacular regenerative capabilities, the actual knowledge is very poor not only in terms of cellular and molecular aspects of regeneration, but also in terms of basic mechanisms (Candia Carnevali and Bonasoro, 2001a).

With regard to these latter, according to a traditional view, regeneration involves two alternative basic mechanisms, epimorphosis and morphallaxis (following the terminology introduced by Morgan, 1901). In epimorphosis, new tissues arise from undifferentiated cells (stem cells or de-differentiated cells), which are recruited to develop a typical blastema. This pool of new cells represents a discrete centre of proliferation activity from which all the regenerated structures are derived. In morphallaxis extensive phenomena of rearrangement/recycling from differentiated tissues take place and no blastema is formed: only limited and localized proliferation activity involves cells derived from existing tissues by reversal of differentiation and/or migration. In spite of this schematic and apparently well-established difference between epimorphosis and morphallaxis, there is recent experimental evidence (Candia Carnevali and Bonasoro, 2001b) that the mechanisms at the tissue/cellular level can be much more flexible and largely overlapped to each other, and that the dichotomic view of these two alternative processes is too reductive.

### **The regenerative potential in echinoderms**

Regeneration is a physiological phenomenon in echinoderms, common in all classes (Hyman, 1955). Thanks to their spectacular regenerative capabilities, echinoderms were favourite models for the pioneer *regenerationists* of the 19<sup>th</sup> and early 20<sup>th</sup> centuries. After an unexplainable long scientific oblivion, they were recently re-proposed to the attention by a series of papers (for review see Candia Carnevali, 2005; Candia Carnevali and Bonasoro, 2001b; Thorndyke and Candia Carneval, 2001) exploring the basic

mechanisms of the regenerative phenomenon, its cellular and molecular aspects and also its potential for applied research. Regeneration is extensively employed to reconstruct external parts (arms or appendages such as spines or pedicellariae) and internal organs (gonads, gut, whole visceral mass) often subjected to predation or amputation, self-induced or traumatic, rapidly followed by complete regrowth of the lost parts; it is also part of the life cycle as an indispensable complement of the programme of asexual reproduction and is extensively employed in cloning processes. Repair, regrowth and cloning processes can involve both the adult and the larval stages (Eaves and Palmer, 2003).

Regenerative potential in echinoderms is developed to a very broad extent: contradicting Goss's paradigm about the "non-regenerability" of the truly vital structures (1965, 1969), it enables vital organs such as the whole visceral mass to be regenerated completely after visceration. It is largely a predicted phenomenon and in most cases follows autotomic self-induced mutilations, which can be considered the most important proximate cause of structural loss and depends on the presence and unique properties of "mutable collagenous tissues" (MCTs, see for a review Wilkie, 2001, 2005) at the level of the autotomy plane. It is relevant that in physiological conditions regeneration is always prompted by autotomy and proceeds from the retained side of a fractured autotomy plane.

**Adults.** The capabilities to regenerate body parts represent an obvious advantage for echinoderms. Firstly, for the replacement of tissues following predation, an ability that echinoderms show to a greater extent than other invertebrates. Reconstitutive regeneration is particularly frequent and extensive in crinoids and ophiuroids which both have long, fragile arms often subjected to predation or mutilations, self-induced or traumatic, rapidly followed by complete regrowth of the lost parts. These regenerative phenomena are so frequent that specimens collected in natural environments always have two or more regenerating arms at different regrowth stage. In many cases, the detached body fragments can survive for a long time and undergo phenomena of partial or total regeneration independently of the *donor* animal (Candia Carnevali *et al.*, 1998). These phenomena, quite common also in asteroids, provide clear evidence for the wide exploitation and implications of regenerative potential in echinoderms. In particular in asteroids, besides the extensive application in common repair mechanisms, arm regeneration offers in fact the most complete examples of cloning strategies carried out by adult specimens. As well known, in some starfish (*Linkia sp.*, *Coscinasterias sp.*), new complete adults can be regenerated from individual autotomised arms. This extreme case clearly shows that, in echinoderms, regeneration is employed as part of a programme of asexual reproduction leading to the development of new individuals through specific fission mechanisms (Emson and Wilkie, 1980; Mladenov and Burke, 1994). Besides asteroids, also many ophiuroids and holothuroids undergo asexual propagation involving the splitting of adults into two or three pieces with subsequent regenerative development of new

complete individuals from each isolated portion. Fission phenomena appear to be correlated with seasonal and metabolic factors as well as to ecological factors, and also depend on the age and size of the individuals: they are particularly frequent in small specimens, whereas the larger ones appear to prefer sexual processes.

In terms of epimorphosis versus morphallaxis, echinoderms appear to employ in regeneration one or the other of these two processes according to apparently established criteria which depend on the specific start-conditions and requirements; however, just in echinoderms there is the clearest evidence that epimorphic regeneration often involves a significant contribution of morphallactic processes and that the borderline between these two processes is not so defined (Candia Carnevali and Bonasoro, 2001a, b). If we consider the distribution of typical epimorphic or morphallactic processes in the different echinoderm groups (Bonasoro *et al.*, 1998, Candia Carnevali and Bonasoro, 2001a; Thorndyke *et al.*, 1999), the emerging pattern can be summarized as follow. Typical epimorphic processes with blastema formation appear to be employed in those situations where regeneration is a widely predicted, rapid and effective phenomenon, which takes place following autotomy (for instance in crinoids and ophiuroids, Candia Carnevali and Bonasoro, 2001a, b; Thorndyke *et al.*, 2001b). In contrast, morphallactic regeneration seems to be a more complicate and slower process, which tends to follow traumatic mutilations (for instance in arm tip regeneration of asteroids, Mladenov *et al.*, 1989; Moss *et al.*, 1998): in this case amputation is not a predictable event and the regenerative mechanisms imply phenomena of substantial rearrangement of the old structures. In spite of their apparently different meaning and alternative employment in echinoderm regeneration processes, recent results (see crinoid regeneration in different experimental conditions, Candia Carnevali and Bonasoro, 2001b) show that the same individual can employ both epimorphic and morphallactic mechanisms, modulating their different contributions according to its specific needs. This suggests that previous interpretations of the mechanisms at the tissue/cellular level need to be revised substantially.

Although occurring in all echinoderm classes, regeneration was investigated by a modern approach only in a few echinoderm models. Rather detailed investigations are related to crinoids, ophiuroids, asteroids, and recently also holothuroids, which were explored both in terms of mechanisms at the tissue/cellular level and ecological significance (Thorndyke *et al.*, 1999; Candia Carnevali and Bonasoro, 2001b; Thorndyke and Candia Carnevali, 2001; Dolmatov and Ginanova, 2001; Garcia-Ararras and Greenberg, 2001). Regeneration also occurs in echinoids, but is less spectacular in terms of extent and degree of capabilities and only a few examples have been investigated so far (Bonasoro *et al.*, 2004; Dubois and Ameye, 2001).

**Larvae.** It is well known that traditional studies of regeneration in vertebrates have often employed larval stages of amphibians. This interest in larval stages is justified by the much higher regenerative potential of larval tissues and organs in comparison with those of

adults. As a general rule, any regeneration, which begins during larval life, appears to be inhibited or retarded after metamorphosis (Wallace, 1981). In spite of the many obvious reasons for exploring the problem of the regenerative potential of echinoderm larvae, and apart from some fascinating descriptions provided by historical reports (Mortensen, 1921; Hörstadius, 1925a, b, c), this aspect of echinoderm regeneration has been neglected until recent times, when some exciting results have renewed interest in this phenomenon and in particular have focused attention on its possible implications for evolution, phylogeny and clonal populations. Current research has been specifically addressed to regeneration of larvae: the comparative analysis of the different potential exploited by the diverse groups shows that the phenomenon of regeneration has an unexpected plasticity in the larvae and indicates its direct and close relationship to asexual reproduction and cloning (Vickery *et al.*, 2001).

As is the case for all other regenerating systems, the basic goal in echinoderm regeneration research is to answer a few crucial questions regarding how regeneration processes are initiated, which sets of genes are activated (or reactivated), what is the origin of the cells involved in reconstruction or repair of the damaged or lost structure, and which factors (morphogens and/or mitogens) regulate growth, morphogenesis and differentiation at the right time and at the correct place to ensure a complete re-establishment of anatomical pattern and functional integrity (Carlson, 1998; Ferretti and Géraudie, 1998; Thouveny and Tassava, 1998). In the species examined so far most results throw light on aspects related to wound healing, growth, morphogenesis and differentiation, but in most cases many fundamental questions remain unanswered, especially those related to specific cellular and molecular aspects and much work is still requested. In spite of the widespread and successful employment of echinoderms for molecular studies based on embryonic or larval development, there is at present a large gap in our knowledge of regeneration and only a few recent data are available.

The following synthetic overview, although far to be exhaustive, offers an account of the phenomenological aspects of regeneration through the echinoderm phylum, presenting a summary of results obtained by diverse perspectives and different experimental approaches in representative models, and highlighting the biological relevance and the evolutionary implications of the phenomenon.

### **Crinoids**

#### Adult:

- regeneration of body parts (crown and stalk) following self-induced or traumatic amputation in sea-lilies;
  - regeneration of appendices (arms, pinnulae, cirri) or body parts following self-induced or traumatic amputation in feather stars;
  - regeneration of individual internal organs (gonads, gut) or whole visceral mass following self-induced or traumatic mutilation/evisceration in feather stars.
- The main cellular and molecular aspects of the regenerative processes are summarized in Table 1.

Crinoids are well known for their spectacular regenerative potential. Feather stars extensively employ regeneration to reconstruct both external parts, namely arms, pinnules and cirri, and internal organs, such as digestive apparatus, gonads, and even complete visceral mass, which can be frequently lost following traumatic injury, predation or spontaneous autotomy (Perrier, 1873; Minckert, 1905; Reichensperger, 1912). Specimens collected in nature always show regenerating arms at different stages of growth. These regenerative phenomena can be easily reproduced in the lab mimicking the autotomy conditions and amputating the arms at the level of the autotomy plane (sutures). The overall regenerative potential of comatulids was accurately tested by Przibram (1901) in experiments of severe mutilations. According to these studies, the animal appears to be able to survive and subsequently regenerate the lost parts in a number of traumatic conditions: for instance, when the body is halved and even when reduced to only one fifth. Crinoid sea lilies (*Metacrinus rotundus*) are also spectacular in their regeneration powers, and not only a new stalk can be regenerated following partial or complete removal (Nakano *et al.*, 2004), but also a completely new and functional crown can be regenerated following total ablation (Amemiya and Oji 1992). Regeneration is also documented in fossil crinoids, particularly in extinct crinoids of the Paleozoic, Mesozoic and Cenozoic, which provide evidence of additional branchings in regenerated arms, underlining the biological, ecological and evolutionary implications of the regenerative phenomenon in echinoderm phylogeny (Oji, 2001).

Arm regeneration in feather stars represents the most thoroughly explored model in echinoderm regeneration studies (Candia Carnevali and Bonasoro, 2001b). In these recent years a comprehensive study of the overall process of arm regeneration was carried out in *Antedon mediterranea*, a flexible experimental model previously successfully employed in old classical studies (Minckert 1905; Perrier 1873; Reichensperger 1912), which was re-explored in all its aspects from the macroscopic to the molecular level. This phenomenon can be described on the whole as a typical blastemal regeneration in which new structures develop from migratory pluripotent, actively proliferating cells in the presence of presumptive regulatory factors, which are responsible for both repair and regenerative phenomena. The overall process can be subdivided into three main phases: a repair phase, an early regenerative phase and an advanced regenerative phase, whose crucial aspects are related to common fundamental mechanisms such as 1) intervention of stem cells and/or dedifferentiated cells; 2) cell migration and proliferation; 3) contribution of putative growth factors, particularly in terms of specific neurally derived factors; 4) mechanisms of pattern formation. The data obtained so far are derived from an integrated approach, which utilizes different methods on experimentally induced arm regenerations (standard or abnormal) obtained in significantly different experimental conditions, including extreme mutilations (*explants*). The regenerative response was also employed in applied research as a new valuable model for ecotoxicological studies addressed to the effects of the exposure to specific classes of

**Table 1.** Crinoids. Summary of main cellular and molecular aspects of regeneration

Mechanisms	Responsible cells	Recruitment	Growth factors	Genes involved
-epimorphic process with main contribution of undifferentiated cells	-stem cells (amoebocytes, coelomocytes)	-differentiation -dedifferentiation	-neurotransmitters (dopamine, serotonin)	<i>Anbmp2/4</i> (TGF- $\beta$ superfamily)
-blastema	-differentiated cells (phagocytes, granulocytes)	-(re)differentiation -transdifferentiation	-neuropeptides (S1, S2, Substance-P)	
-nerve-dependent regeneration	-dedifferentiated cells (myocytes)	-extensive migration -extensive proliferation	- neural growth factors (TGF- $\beta$ , BMP, NGF, FGF-2)	

environmental contaminants (Candia Carnevali, 2005). In particular, the normal mechanisms and pattern of the regenerative processes in standard conditions have been established in a series of experiments on regeneration at different stages following pseudo-autotomy amputations (Candia Carnevali *et al.* 1993; 1995; 1997). A parallel analysis has been carried out on the regenerative processes of both the normal regenerating arms and the respective amputated arm segments (*explants*) (Candia Carnevali *et al.*, 1998; Bonasoro *et al.*, 1999; Candia Carnevali and Bonasoro, 2001b): the explants can be maintained in good living conditions for weeks and represent excellent models for testing the arm regenerative potential in terms of autonomy of resources and control and for comparing regenerative mechanisms in a same individual. Different types of isolated *explants* have been successfully employed: during the culture period they are able to undergo extensive repair and regenerative processes in parallel with their donor arms. Comparison between the regenerative processes of arm *explants* and normal regenerating arms of corresponding stages highlights that beside general similarities in the basic regenerative processes there are some meaningful differences in terms of mechanisms employed and cellular/tissue elements involved. In terms of mechanisms, there is clear evidence that the epimorphic blastemal regeneration can involve a significant contribution of morphallactic processes (Candia Carnevali and Bonasoro, 2001a, b). The regenerative potential, mechanisms and pattern have been also explored and compared with regard to aberrant regenerations resulting from arms deliberately subjected to traumatic mutilations which do not reproduce autotomy (Candia Carnevali and Bonasoro, 2001b). The bulk of the results obtained so far in crinoids not only contribute to throw light on the most relevant aspects related to wound healing, morphogenesis, differentiation and growth in echinoderm regeneration, but also strongly suggest to employ this fascinating and promising experimental model for a successful applied approach. In terms of the specific cellular contribution, most of the cell types involved in regeneration are morphologically undifferentiated migratory elements, which are produced at the level of a) the brachial nerve (amoebocytes) and b) the coelomic epithelium

(coelomocytes) respectively. Available evidence suggests that the migratory amoebocytes produce the blastemal cells and all the blastema-derived differentiated cells, whereas the coelomocytes give rise to all the differentiated elements related to coelomic tissues. Other types of migratory cell involved include phagocytes and granulocytes ("wanderzellen", Reichensperger, 1912). These latter are considered to be a source of putative growth factors. The growth processes are supported by extensive cell cycle activity as evidenced by BrdU incorporation studies showing sites of extensive cell proliferation in the blastema and in the coelomic epithelium (Candia Carnevali *et al.*, 1995, 1997, 1998).

The nervous system with its differentiated components (ectoneural, entoneural, hyponeural) plays a crucial role in regeneration: this is related to its striking capacity to regenerate itself, to its pilot-action as a promoter/inductor of the overall regenerative processes, particularly for the development of the musculo-skeletal components, and finally to its contribution in terms of release of regulatory factors (Candia Carnevali *et al.*, 1989, 1996; Thorndyke and Candia Carnevali, 2001; Patruno *et al.*, 2002, 2003). A number of *neurohumoral factors* with *paracrine* or *autocrine* action are involved in regenerative development: neurotransmitters, particularly monoamines such as dopamine and serotonin; neuropeptides, such as Substance-P, SALMFamide 1 (S1), SALMFamide 2 (S2), nerve-derived growth factors, particularly TGF- $\beta$  and related peptides (BMP), NGF, FGF-2. Recent specific results are related to differential localization and levels of TGF- $\beta$ 1 and TGF- $\beta$ -type II receptor expression during regeneration in *A. mediterranea* (Patruno *et al.*, 2002), and to the cloning of native growth factors in crinoids and their implications for regenerative processes (Patruno *et al.*, 2003). In particular, the gene identified so far in *Antedon bifida*, is a new member of the TGF- $\beta$  superfamily, *AnBMP2/4*, which shows a sequence similarity with other echinoderm and human BMPs. According to the expression pattern of this gene a plausible role can be suggested in specification of migratory stem cells, blastemal growth and tissue differentiation, particularly skeletogenesis (Patruno *et al.*, 2003). It is relevant to remind that, in general, the active gradient established by BMP ligands is considered as one of the main factors responsible for

**Table 2.** Asteroids. Summary of main cellular and molecular aspects of regeneration

Mechanisms	Responsible cells	Recruitment	Growth factors	Genes involved
-morphallactic processes: substantial contribution of old tissues  -no blastema  - nerve-dependent regeneration	-dedifferentiated cells  -stem cells (coelomocytes?)	-dedifferentiation  -(re)differentiation  -transdifferentiation  -extensive migration  -limited proliferation	-neurotransmitters (dopamine, serotonin) -neuropeptides (S1, S2)	- <i>ArHox1</i> (homeobox-containing gene)

generating the positional information during development, particularly in regeneration.

Visceral regeneration. If arm regeneration in crinoids can be considered the representative model of complete regeneration of a complex body part, the regenerative potential of the internal organs is well illustrated by the visceral regeneration process. In all crinoids, the loss of the visceral mass does not seem to be a very traumatic event and, in spite of the apparent complexity of the organs and tissues involved, can be easily repaired by prompt regeneration (Dendy, 1886; Clark, 1921; Hyman, 1955). Current research (Dolmatov *et al.*, 2003; Mozzi *et al.*, 2004) is actually focusing on the overall process of visceral regeneration (involving gut and associated tissues and organs) in *A. mediterranea*, a phenomenon which was explored in the past by the historical study by Dendy (1886). The preliminary results collected so far show that visceral regeneration is a very rapid and effective process during which a small gut, functionally and anatomically complete, is reformed *de novo* in a loose context of new tissues (mainly coelomic cavities and hemal lacunae). In terms of responsible cellular elements, the migratory cells usually employed in regeneration (see arm regeneration) are involved (amoebocytes, coelomocytes, granulocytes, phagocytes). In addition, recent experiments on transplantation of the tegmen and related viscera also show an unexpected plasticity and adaptability of tissues and organs to extremely traumatic conditions. In terms of repair and reconstitutive processes at the tissue level, the mechanisms involved appear to be only partly comparable to those described in visceral regeneration: all the migratory cells seen above contribute to regeneration and are involved in mutual cell exchange between donor and acceptor tissues (Mozzi *et al.*, 2004). These results show a striking potential of cell plasticity and tissue histocompatibility in crinoids and confirm their remarkable repair/regenerative capabilities. On the whole, regeneration appears to be a quick and effective process when the mutilation involves a vital organ or the injury is particularly traumatic: in contrast, it seems to be a slower and less effective phenomenon when the part involved is not so indispensable for survival (Reichensperger, 1912). Interestingly, there is no

apparent correlation between availability/assimilation of food and regenerative capabilities which seem to be comparably rapid and effective in both well-fed and starved animals.

Larvae:

- partial regeneration of body parts following traumatic amputations.

Surprisingly, larval regeneration was only rarely recorded in crinoids. The available data refer to phenomena of partial regeneration of body parts following traumatic amputations in both swimming and sessile larval stages (Runnström, 1915, 1925), which have been re-explored also in recent preliminary studies (Barbaglio *et al.*, unpublished). Nothing is known at the moment about possible phenomena of larval cloning, which is actually a field of topical interest and of expanding potential for future research.

**Asteroids**

Adults:

- regeneration of body parts ( arms) following self-induced or traumatic amputation;
- regeneration of internal organs (pyloric caeca, cardiac stomach) following self-induced or traumatic mutilation;
- fission processes.

The main cellular and molecular aspects of the regenerative processes are summarized in Table 2.

In asteroid arm regeneration (for instance in *Leptasterias hexactis* and *Asterias rubens*), no discrete blastema as a centre of cell proliferation is evident. This appears to apply to both post-traumatic and post-autotomic regenerations (Mladenov *et al.*, 1989; Moss *et al.*, 1998). Typical morphallactic processes seem to be employed and most cell cycle activity is concentrated in the epidermal layer and in the epithelium of the coelomic canals, the two different populations of cells, epidermal and coelomic-mesothelial, providing a significant contribution in terms of development/regrowth of the tissues. Migratory cells (coelomocytes or others) recruited at distance contribute to a minor extent to tissue

regeneration. As noted above, the morphallactic process in asteroids is rather slow when compared to the epimorphic processes of ophiuroids and crinoids; in particular, the initial repair phase may last for a week or more depending on temperature and species, the cell cycle activity being rather low at this stage (Moss *et al.*, 1998) and more effective at more advanced stages of arm regrowth. It is also at this period that the regenerating tip begins to display the organization and features of an adult arm complete with distal optic cushion and terminal tentacle. The results obtained so far suggest: a) reversal of differentiation, proliferation and redifferentiation and/or b) direct transdifferentiation of committed cells which can switch from their 'default' pathway to another and generate all the other cell types (Moss *et al.*, 1998). Asteroid arm regeneration is a nerve-dependent process. In model species (*Asterina gibbosa*), arm regeneration can not occur if the radial nerve has been removed and the neurotrophic action of the nervous system is needed throughout the whole course of regeneration (Huet, 1975; Huet and Franquinet, 1981; Thorndyke and Candia Carnevali, 2001).

In some species, as stated above, following autoamputation isolated arms (comets) can regenerate to produce completely new adults. In *Coscinastris muricata* the phenomenon is reproducible in the lab: two isolated arms, including a minimum portion of the original central disk, are able to regenerate two complete new starfishes following a process which is perfectly simultaneous in both uniparental comets and similar in terms of regeneration rate and histological aspects (Ducati *et al.*, 2004). This phenomenon is regulated by both exogenous (seasonal changes, environmental stimuli) and endogenous factors (body size, humoral and nervous factors).

Recent results in *Asterias rubens* (Thorndyke *et al.*, 2001a) give a further insight in the promising field of the molecular approach to echinoderm regeneration, with particular reference to the characterization and implication of homeobox-containing genes (*ArHox1*) and their spatial and temporal expression patterns during starfish arm regeneration.

#### Larvae (all stages):

- regeneration of body parts following traumatic amputation;
- processes of larval cloning .

In asteroid larvae, post-traumatic regeneration was explored in several past and recent studies (Hörstadius, 1925a, b, c, 1973; Vickery and McClintock, 2000; Vickery *et al.*, 2001). In some species (*Luidia foliolata*), more substantial regeneration can take place whereby two new, completely independent larvae can be produced following surgical bisection of the original (Vickery and McClintock, 1998). In planktonic asteroid larvae, there is also convincing evidence for regeneration in terms of true asexual reproduction and potential for clonal growth (Bosch, 1988; Vickery *et al.*, 2001; Eaves and Palmer, 2003). Planktotrophic bipinnaria larvae of some starfish species have been shown to undergo clonal reproduction whereby posterolateral arms

transform into new larvae and are released by fission, leaving the arm stump to regenerate in its entirety (Bosch *et al.* 1989). In optimized laboratory conditions, up to 24% bipinnaria larvae of *Pisaster ochraceus* undergo asexual reproduction (Vickery and McClintock, 2000); in samples of field collected larvae of other species up to 90% cloning was recorded (Eaves and Palmer, 2003).

## **Ophiuroids**

### Adults:

- regeneration of body parts (arms) following self-induced or traumatic amputation;
- regeneration of internal organs (pyloric caeca, cardiac stomach) following self-induced or traumatic mutilation;
- fission processes.

The main cellular and molecular aspects of the regenerative processes are summarized in Table 3.

Ophiuroids also show a great capacity for regeneration, since they frequently cast off their arms at all levels, with breakage occurring at all the autotomy planes after damage, predation, or handling. Regeneration is usually rather rapid and can involve several arms. The cellular aspects of the regeneration process in ophiuroids are poorly understood. Most recent work has focused on the ecological benefits and impacts of the extensive regeneration seen in many species (Stancyk *et al.*, 1994; Thorndyke *et al.*, 2001b). These animals are certainly ideal subjects for studying the implications of regrowth on other physiological processes. They are also valuable models for experiments concerning the cellular basis of regeneration: present evidence suggests that a combination of morphallactic and epimorphic processes is responsible for regeneration in ophiuroids. Thus morphallactic wound-healing processes involving initial expansion and migration of epidermal cells are followed by the rapid formation of an epimorphic blastemal structure due to a local accumulation of coelomocytes (Dobson, 1988, Thorndyke *et al.*, 2001b; Bannister *et al.*, 2005). Several specific studies on cell cycle activity (Dobson 1988; Thorndyke *et al.*, 2001b) clearly indicate the importance of cell division in this regenerative process. Moreover, the primary location of the blastema at the end of the severed nerve cord provides clear support for the idea of an important role for the nervous system in regrowth, as previously suggested by early studies (Zeleny, 1903; Morgulis, 1909;).

Recent molecular studies in *Amphiura filiformis* (Bannister *et al.*, 2005) has led to identify and clone *Afuni*, a novel TGF- $\beta$  gene, expressed in regenerating tissues and showing a sequence similarity with sea urchin *univin* (85 % identity). On the basis of the differential spatiotemporal expression of this gene in regenerating arm of advanced stages, a presumptive role in arm growth and segmentation, as well as in neurogenesis and skeletogenesis was suggested.

### Larvae (pre-metamorphic stages):

- regeneration of body parts following traumatic amputation;

**Table 3.** Ophiuroids. Summary of main cellular and molecular aspects of regeneration

Mechanisms	Responsible cells	Recruitment	Growth factors	Genes involved
-epimorphic regeneration with main contribution of undifferentiated cells  -blastema formation  - nerve-dependent regeneration	-stem cells (coelomocytes)  -dedifferentiated cells ( ? )	-dedifferentiation  -(re)differentiation  -transdifferentiation  -extensive migration  -extensive proliferation	-neural factors (neuropeptides S1, S2)	<i>Afuni</i> (novel TGF- $\beta$ gene cloned)

**Table 4.** Holothuroids. Summary of main cellular and molecular aspects of regeneration

Mechanisms	Responsible cells	Recruitment	Growth factors	Genes involved
-epimorphic-like regeneration with presumptive contribution of undifferentiated cells  - pseudo-blastema formation  - nerve-dependent regeneration	-dedifferentiated cells (myocytes)  - stem cells (coelomocytes)	-dedifferentiation  -(re)differentiation  -transdifferentiation  -extensive migration  -limited local proliferation	-neural factors (?) -ependimin-related protein (?)	<i>EpenHg</i> (ependymin-related gene)

- processes of larval cloning (secondary larvae) from larval posterolateral arms detached from primary ophiopluteus.

In ophiuroids, larval regeneration has been described in pre-metamorphic stages. The most frequent phenomenon is the regeneration of secondary larvae from the posterolateral arms which are lost or released on settlement of the metamorphosed juvenile (Mortensen, 1921; Balsler, 1996, 1998; Eaves and Palmer, 2003), which can even involve the continuous and progressive development of serial clones of regenerating larvae (tertiary larvae, etc.) in a sort of strobilation process. The overall phenomenon appears to involve also pseudo-gastrulation morphogenetic events, whereby cell proliferation, de-differentiation, migration and differentiation take place to produce larval clones (Balsler, 1998).

### **Holothuroids**

#### Adults:

- regeneration of body parts and appendages (tentacles) following self-induced or traumatic amputation;  
- regeneration of whole visceral mass or individual internal organs (gut, gonads, haemal system, respiratory trees, Cuvierian tubules) following self-

induced or traumatic amputation;  
- asexual fission processes.

The main cellular and molecular aspects of the regenerative processes are summarized in Table 4.

In holothurians the regenerative potential can differ a lot between different groups (Dendrochirota, Aspidochirota, Apoda) and also vary with the age of the individuals. Since the first half of the 20<sup>th</sup> century, much attention has been focused on visceral regeneration (gut and related structures, Bertolini, 1930; Dolmatov, 1992; Garcia-Arraras *et al.*, 1998; 1999; Garcia-Arraras and Greenberg, 2001) and on muscle regeneration (Dolmatov *et al.*, 1996).

Visceral regeneration. Recent work (Garcia-Arraras *et al.*, 1998, 2001; Garcia-Arraras and Greenberg, 2001; Dolmatov, 1992, 1996) has indicated that in *Holothuria glaberrima*, following evisceration the new digestive tube develops from the mesenterial lamina, which anchored the original gut to the body wall. This phenomenon is apparently a typical epimorphic process, where a blastema-like structure is formed as a thickening of the mesenterial edge of the lamina. In the following stages the regrowth of the intestinal tract can imply two alternative mechanisms of cell recruitment (Garcia-Arraras and Greenberg, 2001): a) from the remnants of the oesophagus and cloaca, through morphallactic mechanisms of tissue



rearrangement and migration/proliferation of endodermally derived cells (Garcia-Arraras *et al.*, 1998; Mashanov and Dolmatov, 2001); b) from coelomic epithelium, through direct and exclusive proliferation/migration of new mesodermally derived progenitor cells, this phenomenon typically occurring when endodermally derived tissues are completely lost by evisceration (Mosher, 1956; Mashanov *et al.*, 2004). On the basis of indirect evidence, the overall process is considered to be a nerve-dependent regeneration. The employment of immunocytochemical methods underpin specific activities in the mesenterial septum: at early stages, extensive ECM (extracellular matrix) degradation (collagen, fibronectin, laminin) and metalloproteinase (MMPs) activity or effects of their inhibitors; at advanced stages, extensive rearrangement of the ECM (collagen, fibronectin, laminin) and myocyte dedifferentiation (Quinones *et al.*, 2002; Garcia-Arraras *et al.*, 2001). Although more specific mechanisms at cellular level have still to be explored, particularly by developing appropriate methods for cell culture (Odintsova *et al.*, 2005), a plausible interpretation about elements involved and their possible roles has been proposed, with particular reference to the fundamental role of the mesothelium and the organogenetic potential of the coelomic epithelium, which also implies a presumptive mesodermal derivation of the luminal epithelium of the new gut.

Recent work was carried out on the molecular aspects of regeneration and has allowed identification of at least one gene, *EpenHg* (ependymin-related gene), which is expressed in many tissues and overexpressed during regeneration. This gene shows a sequence similarity with holothurian, sea-urchin and vertebrate (frog and mammalian) ependymin-related sequences. In terms of specific roles, its presumptive involvement as promotor of proliferation and differentiation, neurotrophic factor and inductor of axonal regrowth was proposed (Suarez-Castillo *et al.*, 2004)

Muscle regeneration. A specific aspect of regeneration which is extremely interesting *per se* in terms of morphogenetic and histogenetic processes and particularly relevant for the functional recovery of the injured individual, is the regenerative development of the muscles. This problem has been explored in holothurians, the echinoderm group in which muscles are most developed, as far as myogenesis of somatic and visceral muscles is concerned (Dolmatov *et al.*, 1996; Dolmatov and Ginanova, 2001). Here the regeneration process appears to be due to the coelomic epithelial cells which apparently de-differentiate, migrate and invade the muscle bands where they differentiate into muscle bundle rudiments, finally giving rise to new muscle cells. The myogenesis appears to start from two different cell precursors: with regard to somatic muscles, myogenesis starts from undifferentiated coelomocytes, in their turn derived from epithelial cells of the coelothelium (peritoneocytes) via migration, proliferation, differentiation; with regard to visceral muscles, from myocytes of the coelothelium, via partial dedifferentiation/transdifferentiation and migration (Dolmatov and Ginanova, 2001; Murray and Garcia-

Arraras, 2004). On the basis of the resulting pattern in terms of occurrence and distribution of dedifferentiation, migration, proliferation and redifferentiation at tissue and cellular level, a basic mechanism for holothurian muscle regeneration is proposed which excludes a significant role for cell division but is based fundamentally on the recycling and transdifferentiation of cells from the coelomic epithelium.

#### Larvae (different stages):

- regeneration of body parts following traumatic amputations;
- processes of larval cloning.

Larval regeneration after surgical transection was previously described in holothurians in earlier and recent papers (Hörstadius, 1925a, b, c, 1973; Dolmatov, 1991, 1996). In *Eupentacta fraudatrix*, the pentactula juvenile stage, after bisection, can regenerate the posterior part including the intestinal tract (Mashanov and Dolmatov, 2001). Besides these processes of post-traumatic regeneration, frequent phenomena of larval cloning have been also observed (Eaves and Palmer, 2003), particularly development of secondary auricularia larvae (*Parastichopus californicus*). In addition, asexual budding is also frequently observed in early pentactulae.

#### **Echinoids**

##### Adult:

- regeneration of external appendages (spines, pedicellariae) post-fracture, removal or self-induced release;
- regeneration of test after surgical treatment or traumatic mutilations.

The main cellular and molecular aspects of the regenerative processes are summarized in Table 5.

Echinoids show an apparent reduced requirement for regeneration as they do not reproduce by fission and do not show large exposed appendages vulnerable to predation. Nevertheless, if arm regeneration is a typical feature of long-armed echinoderms, traumatic or self-induced loss and subsequent regeneration of external appendages, such as spines and pedicellariae, are frequently occurring in sea urchins and recent studies have concentrated on regeneration of spines and pedicellariae in the common sea urchin *Paracentrotus lividus* (Carpenter, 1847; Dubois and Ameye, 2001) after fracture or total removal. Interestingly, a pattern emerges from the detailed comparative analysis of diverse situations, which suggests that the regenerative processes in these external appendages can vary significantly in terms of basic mechanisms and processes and can be modified in response to different types of injury. These results are strongly consistent with the idea of a fundamentally different significance of post-traumatic and post-autotomic regeneration and confirm the wide plasticity/adaptability of the repair and regenerative processes in echinoderms in relation to the specific conditions of local damage.

**Table 5.** Echinoids. Summary of main cellular and molecular aspects of regeneration

Mechanisms	Responsible cells	Recruitment	Growth factors	Genes involved
-morphallactic processes with substantial contribution of migratory cells  -annular blastema, centripetal growth	-dedifferentiated cells  -stem cells (coelomocytes?)	-dedifferentiation  -(re)differentiation  -transdifferentiation  -extensive migration  -limited local proliferation		

Only a few reports have been focused on test regeneration after injury (Bonasoro *et al.*, 2004; Ameye and Dubois, 1995; Shimizu *et al.*, 1994) and the process has still to be explored in its detail. In terms of timing and modalities, sea-urchin test regeneration appears to vary a lot, depending on many intrinsic and extrinsic factors. Nevertheless, the main events tend to follow a constant sequence: in *Paracentrotus*, after surgical removal of a small test portion (one or two skeletal plates), the experimental animals are able to repair their wounds by employing regenerative mechanisms which involve three main stages (repair, early and advanced regeneration) and lead to the complete closure of the wound. The overall regeneration process implies the formation of an annular blastema and follows a concentric centripetal regrowth (Bonasoro *et al.*, 2004): the new tissues are progressively reformed due to the substantial contribution of migratory cells presumably derived through a morphallactic rearrangement of old tissues, with only a minor local employment of cell proliferation.

Larvae:

- regeneration of body parts after surgical division or amputation;
- larval cloning.

The regenerative potential of sea-urchin larvae after surgical or traumatic amputation was described in historical and recent papers (Runnström, 1915; 1925; Hörstadius, 1925a, b, c, 1973; Vickery *et al.*, 1999, 2001). In *Lytechinus variegates*, after bisection, different larval stages, particularly echinoplutei, can completely regenerate the anterior or posterior body portion (Vickery *et al.*, 2001). In addition, in different larval stages frequent phenomena of larval cloning by budding have been also described (for instance in *Strongylocentrotus purpuratus*, Eaves and Palmer, 2003), leading also in echinoids to the regular formation of secondary larvae.

**Concluding remarks**

The extensive and strategic employment of regenerative phenomena throughout the phylum indicates that in echinoderms regeneration actually

represents an essential component of the life-cycle and has a wide range of relevant biological implications: not only it increases the potential of survival of the individuals, but it can also be considered the indispensable requisite for performing fissiparous reproduction, thus allowing the rapid colonization of new environments through the production of multiple copies of genotypes (clones) well adapted to local conditions. On the basis of its evident contribution to the adaptive capacities of the species and of its biological and ecological implications for echinoderm phylogeny the regeneration phenomenon must be considered one of the most important responsible factor for the evolutionary success of the groups and for the striking success of the whole echinoderm phylum throughout the marine ecosystem.

Unfortunately what we actually know about the biology of echinoderm regeneration is far to be exhaustive. Much work is requested and many questions are still to be answered. Results obtained so far strongly encourage a wider employment of molecular approaches in regeneration research (Odelberg, 2004) and promote the hope that, with the powerful tools provided by molecular biology, the key of the striking regenerative performances of echinoderms can be soon appropriately studied and completely explained. We should not forget that what we learn from echinoderms can be relevant in applied research: regenerative medicine can in fact receive a significant improvement if echinoderm models are extensively studied in parallel with traditional mammal models, in the reasonable hope that what echinoderms can do so easily may eventually become easy also for other animals, humans included (Lagasse *et al.*, 2001).

**Acknowledgements**

This work was carried out thanks to the support of a number of specific projects financed through the years by CNR, MURST (COFIN) and University of Milano (FIRST) programs. The author is particularly grateful to the following colleagues for their indispensable help and valuable collaboration during the research work: A Barbaglio, A Biressi, F Bonasoro, C Di Benedetto, D Mozzi, L Parma, M Patruno and M Sugni.

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