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Towards a parsimonious analysis of regeneration and self-repair in animal evolution

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ABSTRACT

All metazoan phyla contain species that undergo regeneration after amputation. Simple organisms as *Hydra* or planarians regenerate their main body axes, while salamanders and fish regenerate complex structures such as limbs, fins, tails but also heart, retina, lens. Hence, in these species the developmental programs remain accessible to reactivation throughout life, whereas in frogs they become locked after metamorphosis and in most mammals, adult regeneration is restricted to tissues as bone, liver. The consensus view is that regeneration is an ancestral character, randomly maintained or lost along evolution. To identify the possible homologies among the various regenerative processes, we selected 30 regenerative contexts where the cellular processes driving blastema formation as dedifferentiation, transdifferentiation, stem cell recruitment or proliferation of differentiated cells, were characterized. Moreover the complexity of the regenerative process and three developmental criteria including the developmental status when regeneration occurs, the presence of metamorphosis or asexual reproduction in adulthood were considered. These features were assembled to form a "*regenerative code*" that defines each regenerative context. These regenerative codes were then analysed under the maximum parsimony criteria to define groups of processes sharing common features. A majority-rule consensus tree shows seven unambiguous groupings that support the validity of this approach.

INTRODUCTION

In adult organisms, cells, tissues and organs undergo under normal conditions a permanent remodeling of their components to maintain their functionality, this remodeling being named *homeostatic maintenance*. In addition to homeostatic maintenance, different programs can be activated when the reconstruction of portions of tissues or organs that have been seriously injured or lost is needed. These programs that lead to the replacement of the missing part, are collectively named *regeneration*. However, whereas the capability to heal wounds to guaranty the surviving of the animal is common to multicellular organisms, the ability to regenerate tissues or complete organs although widely spread across the animal phyla (Figure 1), dramatically varies between species in a given phylum. In contrast to embryonic and fetal development where the cells migrate and differentiate in specific tissues that form structures following strict temporal-spatial rules, the cells have to orchestrate and harmonize their organization in the presence of stress (usually that of amputation) and within the preexisting tissues in the case of the regeneration. Therefore patterning during development and regeneration occur neither in the same context nor at the same scale Brockes and Kumar (2005).

Hence understanding the differences between regeneration and development is an old and fundamental question that still needs to be elucidated. Many difficulties have hampered this understanding: firstly, many animal species that display strong regenerative abilities, actually do not display the full characteristics of adulthood suggesting that regulative and regenerative processes are intermingled in those contexts. Secondly, in contrast to the developmental processes that were successfully deciphered by the combination of genetic and molecular tools in the last thirty years, the lack of genetic tools in regenerating model systems have delayed the analysis of the mechanisms that underlie the regenerative processes. Therefore the mechanisms at work during regeneration and development in each of these model systems could not be compared and mechanisms driving

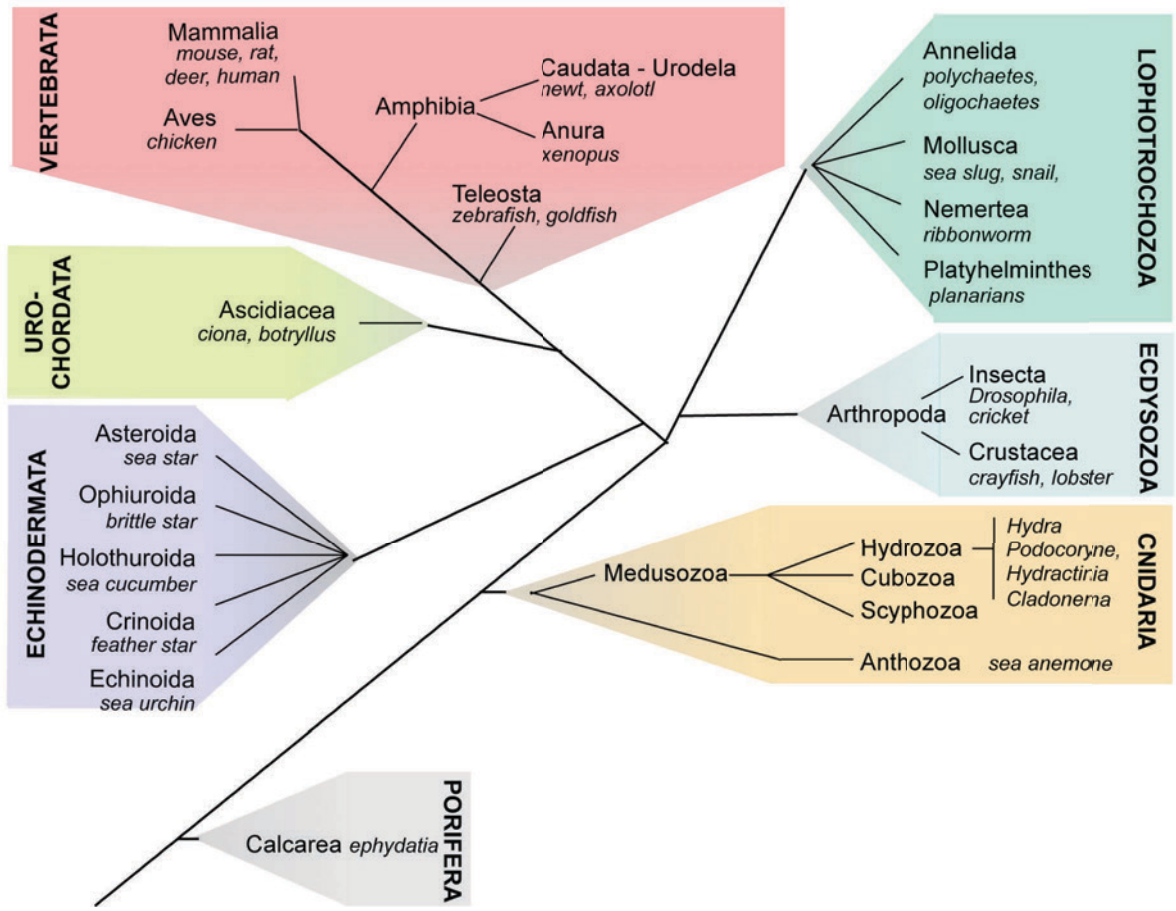


Figure 1: Animal phylogenetic tree representing species with variable regenerative potential investigated at the cellular and molecular levels. Phyla are written uppercase, species italic.

regeneration that would be conserved across the different model systems remain to be characterized. Thanks to recent technological advances, our understanding of vertebrate as well as invertebrate regeneration tremendously increased in the recent years. In this paper, we propose a systematic and parsimonious approach of regeneration and self-repair across animal evolution, searching for relationships between regeneration systems either in the most classical organisms or in the novel, rising model organisms.

For this, a first concern is that embryos easily replace missing parts and heal their wounds without scarring whereas appendage or full body regeneration often correlates with larval or neotenic contexts, when adult organisms display limited regeneration. This suggests that reactivation of developmental programs is enhanced when developmental processes are still going on whereas the adult status would impede regeneration and tissue repair processes. To clarify this issue, we took into account several developmental criteria.

Moreover, for each of these various regenerative systems, we recapitulated the sequence of the cellular processes that support blastema formation after wounding, amputation or toxic stress. That way a "*regenerative code*" that includes the developmental status of the regenerating organism as well as the cellular components underlying blastema formation, was applied to each regenerative context. After alignment, these various regenerative codes that are supposed to reflect the different regenerative modes were tested in maximum parsimony (MP) analyses where an equal weight was given to each criteria. As a first result, these analyses show unambiguously seven groupings where regeneration processes appear as sharing some homologous components, indicating a putative shared ancestral process.

RESULTS

Class I criteria : The developmental criteria

Once reached the adulthood, cnidarian polyps as *Hydra*, or planarians can fully regenerate the missing part after amputation Sanchez Alvarado and Tsonis (2006). Similarly, echinoderms will maintain the ability to restore a wide plethora of cells and tissues as limbs, spines, and intestines. The sea stars can even reproduce asexually by breaking a ray or arm or by deliberately dividing portions of the body, a defence behavior named autotomy Garcia-Arraras and Greenberg (2001). Among vertebrates, the fish and urodeles (one class of amphibians) seem to be highly privileged

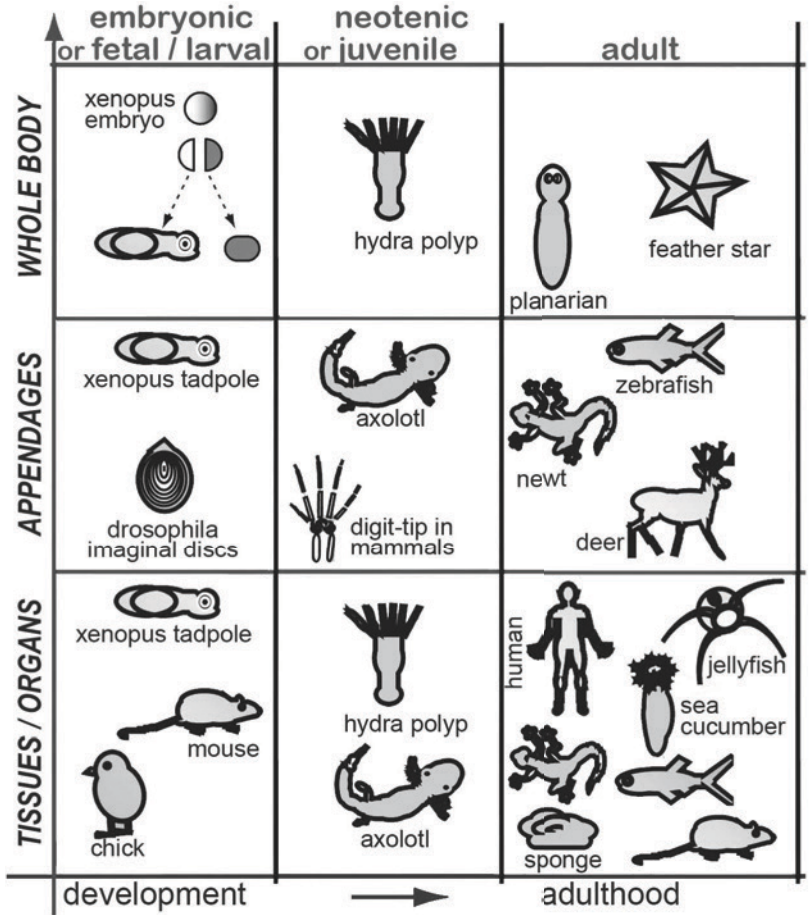


Figure 2: Developmental features of various regenerating model systems. The organisms are sorted horizontally following their developmental status at the time the regenerative process takes place: restricted to embryonic or fetal/larval stages (left) or extended throughout adulthood (right column). Neotenic animals and regeneration at juvenile stages are listed in the middle column. The same model systems are categorized vertically according to the extent of the regeneration process: body parts (upper), appendages (middle), tissue repair or organ reconstruction (bottom).

as the former regenerate fins, heart and retina, the latter limbs, jaws, tail, heart and in case of the newt, even lens and retina. In contrast, *Xenopus*, mouse and human display very limited capability of regeneration once they have reached adulthood. Therefore the evolution of the universal totipotency observed at the earliest stages of development, into multipotency at later stages can either be sustained during adulthood as in *Hydra*, planarians, echinoderms, fish or salamanders or become highly restricted to few cell lineages as in most mammals (Figure 2, Table 1).

To perform a systematic comparative analysis of the different regenerative contexts, we selected as a first criteria the developmental status when regeneration occurs in a given species. Three distinct stages were considered i) *fetal or larval* (L) when regeneration occurs at a time when development is not achieved, the animal being in the last phase of development, ii) *neotenic* (N) when animals have gained their sexual maturity before reaching their full development, the typical example being the axolotl (amphibian) that becomes sexually mature before the onset of metamorphosis and therefore never goes through metamorphosis, iii) *adult* (A) when regeneration occurs in sexually mature animals, after the juvenile stage (Figure 2). Embryos depicted in Figure 2 were not entered in this comparative analysis as the regulative processes observed at early developmental stages (prior to organogenesis) do not fulfill the definition of regeneration, i.e *"reactivation of a developmental program upon injury in a previously developed organism"*.

Concerning the neotenic status, the axolotl is indeed regarded as an animal that acquires its sexual maturity before reaching full development, fulfilling thus the "neotenic" definition. But hydrozoan polyps as *Hydra* can also be considered as neotenic. In fact, medusozoans (hydrozoans, cubozoans, scyphozoans) share a complex life cycle where they alternate between two stages, benthic as a polyp and pelagic as a medusa. In those species with a full life cycle, the polyp stage represents the juvenile form whereas the medusa stage corresponds to the sexually mature form. However, some hydrozoans as *Hydra* or *Hydractinia equinata* have lost the medusa stage and the polyp then exhibits the capacity to produce mature gonads without any decrease of its regenerative potential Frank et al. (2001); Galliot and Schmid (2002). As a consequence, medusozoan species that do not include the medusa stage in their life cycle can be regarded as neotenic animals.

Finally, two additional developmental criteria were included in this study. Firstly species were sorted according to the presence or not of a metamorphosis stage during their sexual development. The species that include a metamorphosis stage to reach adulthood (M) follow an indirect mode of development whereas those that develop without traversing any larval and metamorphosis stage, follow a direct mode of development (G). Indeed in some contexts, the same proteins and the same cellular processes as apoptosis, regulate metamorphosis and

regeneration Ohtsuka et al. (2001); Seipp et al. (2001); Chambon et al. (2007); Tseng et al. (2007), suggesting that regeneration at the post-metamorphosis stage involves a possible reactivation of the metamorphosis process. Secondly as asexual reproduction during adulthood and regeneration in adult organisms were proposed to share some mechanisms, as budding in *Hydra* Galliot (2000); Holstein et al. (2003), fission in planarians, fragmentation and neural morphallaxis in annelids Myohara et al. (1999); Martinez et al. (2005), colonial growth in the botryllus urochordate Lauzon et al. (2002), we included the asexual reproduction as a criteria with two possible states, present (Y) or absent (F). The combination of these three developmental criteria provides 12 possible codes (3 x 2 x 2).

Class II criteria : The complexity of the regenerated structure as a hint towards the complexity of the regenerative process

As a second class of criteria, we focused on the complexity of the regenerated structure, which we assumed, reflects the complexity of the regeneration process. Four distinct levels are classically considered, i) regeneration of tissues (also named tissue repair), ii) regeneration of organs, iii) regeneration of appendages, iii) full body regeneration (Figure 2, Table 1). Nevertheless, we found these four levels of regenerative complexity rather ambiguous. For example, lens regeneration in newt can occur after complete ablation of the lens, but involves two cell lineages that participate in the transdifferentiation of the pigmented epithelial cells of the dorsal iris into new lens fibers Del Rio-Tsonis and Tsonis (2003). In that case full organ regeneration is achieved with the participation of a limited number of cell lineages and a unique cellular process. In contrast, heart regeneration, which takes place when 20% to 30% of the heart tissue is dissected in zebrafish and newt, relies on several cellular processes (see below) and involves at least four distinct cell lineages (Table 1). Therefore, giving an identical definition to these two processes ("organ regeneration") would certainly not reflect the complexity underlying the respective regeneration processes. Similarly, regeneration of body parts after amputation as in *Hydra* or planarian is impressive as it demonstrates a unique potential for surviving in the absence of the head region that contains the highly organized apical/central nervous system Sanchez Alvarado and Tsonis (2006); Miljkovic-Licina et al. (2007). Nevertheless, if we consider the number of cell lineages involved in *Hydra* or planarian body part regeneration on the one hand and those involved in vertebrate appendage regeneration on the other hand, their respective numbers are high and close whatever the context: 6 in *Hydra* and planarian body regeneration, 6 in zebrafish fin, 7 in newt or axolotl limb and tail regeneration. This comparative analysis suggests that these regenerative processes cannot be regarded as highly different in terms of their respective complexities. As a consequence, to assess the complexity of the regenerative process, we decided to systematically refer to the number of cell types or cell lineages that participate in the regeneration process and/or become part of the final structure that is regenerated. This number was carefully evaluated for each regenerative context from published data and found to range from 1 to 7. Then the regenerative complexity was encoded as 5 distinct states as indicated in Table 1, corresponding to one, two, three or four, five, six or seven cell lineages involved in the regenerative process. Hence, the number of possible regenerative codes reached 60: 12 possible states related to developmental criteria independently combined to 5 possible states related to the complexity of the regenerated structure.

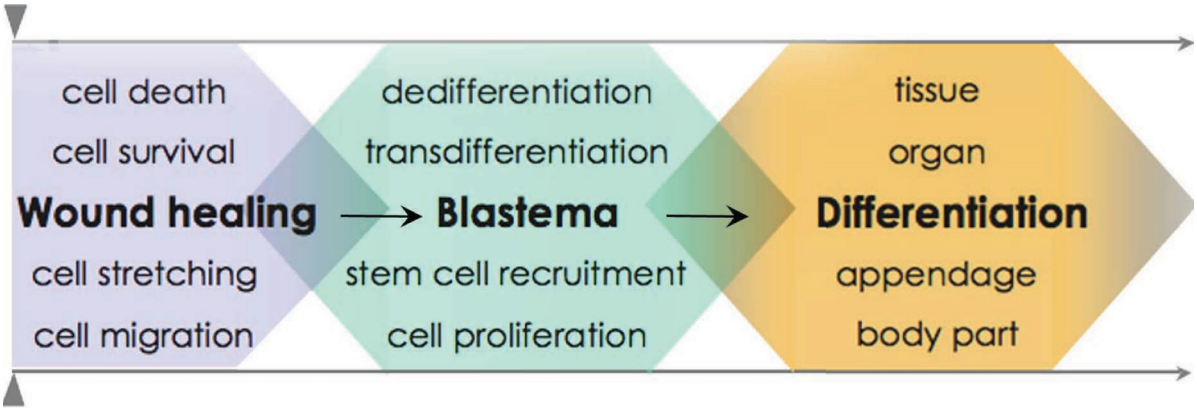


Figure 3: Scheme depicting the three successive phases that lead to regeneration in vertebrates and invertebrates, first wound healing, then blastema formation and finally differentiation of the de novo formed tissue or structure. Arrowheads represent the precise time point when amputation induces the regeneration process, long grey horizontal arrows time after induction. Four main cellular mechanisms can potentially drive blastema formation, i) *dedifferentiation* when differentiated cells loose their specific features, re-enter the cell cycle and provide proliferating progenitors (e.g. myotubes); ii) *transdifferentiation* when differentiated cells change their identity with or without re-entering the cell cycle (e.g. iris cells provide lens cells in the newt); iii) *stem cell recruitment* when stem cells or multipotent progenitor cells that stay quiescent in homeostatic conditions re-enter the cell cycle, proliferate and differentiate (e.g. satellite cells in the muscle); iv) *direct cell proliferation* when differentiated cells re-enter the cell cycle without loosing their specific features (e.g. cardiomyocytes in the zebrafish). From the blastema, i.e. a transient mass of proliferating cells in the vicinity of the amputation plane,

Class III criteria: the cellular processes involved in the organization and the growth of the regenerative blastema

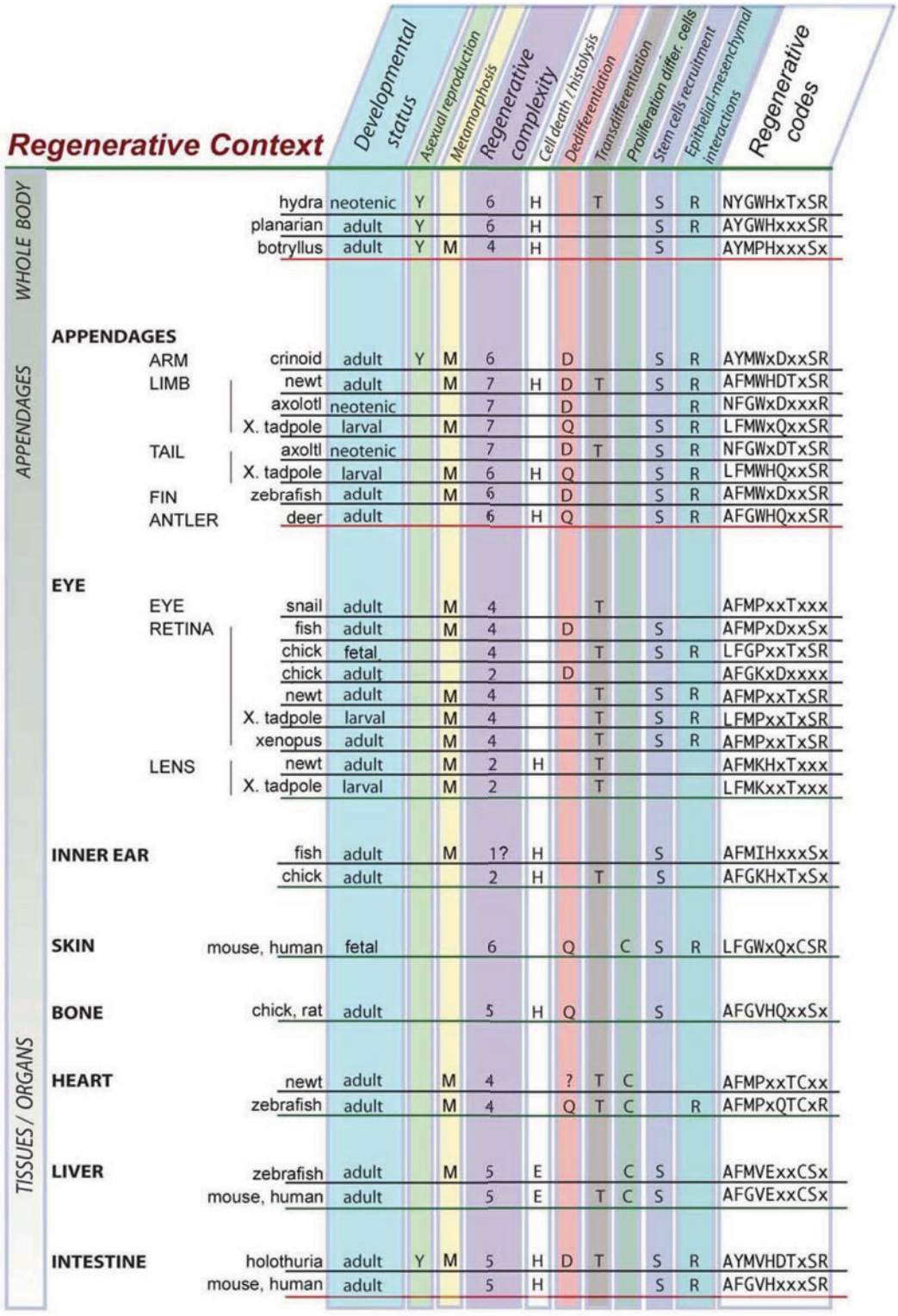
In organ, appendage and body reconstruction, regeneration can be schematically divided into three successive phases: a first wound healing phase, where surviving cells will cover the wound, a second blastema phase, characterized by the formation of a mesenchymal growth zone named blastema, and finally a third phase of differentiation that leads to the regenerated structure or tissue (Figure 3). The blastema phase is essential and unique to the regeneration process in all types of regeneration, and, at least in vertebrates has no counterpart during development Brockes and Kumar (2005). The blastema is a highly proliferative transient structure, which is induced upon injury and requires cell plasticity that involves several distinct cellular processes Pomerantz and Blau (2004). One mode of blastema formation involves the recruitment of multipotent cells present in the adult tissue in a quiescent state prior to any injury. Those cells are maintained undifferentiated into specific cellular niches until they respond to the injury stimulus by re-entering the cell cycle, proliferating in the blastema and subsequently differentiating a *de novo* structure. This process takes place in planarian body regeneration Reddien et al. (2005); Rossi et al. (2007), but also in salamander limb regeneration Morrison et al. (2006), mammal liver regeneration Oh et al. (2002) and mammal muscle repair Buckingham (2006).

A second and third types of blastema formation rely on the cell plasticity of differentiated cells that may undergo either dedifferentiation and/or transdifferentiation upon injury. Differentiated cells located at the site of amputation can dedifferentiate to re-enter the cell cycle, proliferate and as such participate in blastema formation until they differentiate again to participate in the formation of the new structure. This process takes place in myofibers and fibroblasts during limb and tail regeneration in salamanders Brockes and Kumar (2002); Tanaka (2003), in glia cells during retina regeneration in fish and chick Fischer (2005), but also in mesenterial muscle cells during intestine regeneration in sea cucumber Candelaria et al. (2006). In contrast, dedifferentiation is clearly not used in contexts as tail or limb regeneration in the *Xenopus* tadpole Slack et al. (2007), deer antler regeneration Price et al. (2005), heart regeneration in zebrafish skin or bone regeneration in mammals

As an alternative to dedifferentiation, the cells in the vicinity of the amputation site can also transdifferentiate to another differentiated cell type, with or without traversing a proliferative phase. Transdifferentiation initially appeared less common than the two previously reported processes. It was first identified in the eye where the lens can regenerate either from iris transdifferentiation as in the newt Henry and Elkins (2001), or from cornea transdifferentiation as in the *Xenopus* Del Rio-Tsonis and Tsonis (2003). Similarly the retinal pigmented cells transdifferentiate in neural retina in developing chick Fischer (2005) or adult amphibians Araki (2007). In the inner ear, the supporting cells provide new hair cells in adult chick Cafaro et al. (2007). Moreover regeneration of intestine in holothuria Mashanov et al. (2005), tail in axolotl Echeverri and Tanaka (2002) and heart in zebrafish Lepilina et al. (2006) also involve transdifferentiation.

A fourth possibility is the proliferation of non-terminally differentiated cells that upon injury re-enter the cell cycle without dedifferentiating, as observed in the case of liver regeneration with the proliferation of hepatocytes Zimmermann (2002), or heart regeneration in zebrafish or newt with the proliferation of cardiomyocytes Poss et al. (2002); Bettencourt-Dias et al. (2003). A last possibility consists in the formation of *heterokaryons* through the fusion of a stem cell together with a differentiated cell. These heterokaryons, although not entering the cell cycle, would participate in the tissue repair process through reprogramming the differentiated cells. This latter process appears as very rare in homeostatic conditions, i.e. intact adult tissues, but dramatically increased upon injury Pomerantz and Blau (2004); Dorrell and Grompe (2005). In the absence of systematic data concerning the formation of heterokaryons during spontaneous regeneration, this process could not be recorded in this study.

Two additional cellular criteria were considered. One deals with the roles played by programmed cell death (apoptosis) or histolysis in regeneration. In some contexts, this role can be positive (H) when apoptosis or histolysis is required for processes as bone remodeling, blastema formation as in deer antler regeneration Colitti et al. (2005), *Xenopus* tail regeneration Tseng et al. (2007), intestine regeneration Thompson et al. (2000), *Hydra* body regeneration (S. Chera et al., submitted), or suspected to be important as in planarian body regeneration Hwang et al. (2004). In few other contexts, suppression of apoptosis (E) is necessary to promote cell survival as in liver regeneration Steiling et al. (2004); Hata et al. (2007). Another criteria relates to the necessity of mutual interaction between two cell layers, usually the blastema and the sus-jacent epidermal layer. This interaction, named epithelial-mesenchymal interaction (EMI, encoded here as R), was shown to be critical for limb regeneration in *Xenopus* Yokoyama et al. (2000), fin regeneration in zebrafish Akimenko et al. (2003), intestine regen-



Regenerative Context			Developmental status	Asexual reproduction	Metamorphosis	Regenerative complexity	Cell death / histolysis	Dedifferentiation	Transdifferentiation	Proliferation differ. cells	Stem cells recruitment	Epithelial-mesenchymal interactions	Regenerative codes
WHOLE BODY		hydra	neotenic	Y		6	H		T		S	R	NYGWHxTxSR
		planarian	adult	Y		6	H				S	R	AYGWHxxxSR
		botryllus	adult	Y	M	4	H				S		AYMPHxxxSx
APPENDAGES													
	ARM	crinoid	adult	Y	M	6		D			S	R	AYMWxDxxSR
	LIMB	newt	adult		M	7	H	D	T		S	R	AFMWHDTxSR
		axolotl	neotenic			7		D				R	NFGWxDxxxR
		X. tadpole	larval		M	7		Q			S	R	LFMWxQxxSR
	TAIL	axoltl	neotenic			7		D	T		S	R	NFGWxDTxSR
		X. tadpole	larval		M	6	H	Q			S	R	LFMWHQxxSR
	FIN	zebrafish	adult		M	6		D			S	R	AFMWxDxxSR
	ANTLER	deer	adult			6	H	Q			S	R	AFGWHQxxSR
EYE													
	EYE	snail	adult		M	4			T				AFMPxxTxxx
	RETINA	fish	adult		M	4		D			S		AFMPxDxxSx
		chick	fetal			4			T		S	R	LFGPxxTxSR
		chick	adult			2		D					AFGKxDxxxx
		newt	adult		M	4			T		S	R	AFMPxxTxSR
		X. tadpole	larval		M	4			T		S	R	LFMPxxTxSR
		xenopus	adult		M	4			T		S	R	AFMPxxTxSR
	LENS	newt	adult		M	2	H		T				AFMKHxTxxx
		X. tadpole	larval		M	2			T				LFMKxxTxxx
INNER EAR		fish	adult		M	1?	H				S		AFMIHxxxSx
		chick	adult			2	H		T		S		AFGKHxTxSx
SKIN		mouse, human	fetal			6		Q		C	S	R	LFGWxQxCSR
BONE		chick, rat	adult			5	H	Q			S		AFGVHQxxSx
HEART		newt	adult		M	4		?	T	C			AFMPxxTCxx
		zebrafish	adult		M	4		Q	T	C		R	AFMPxQTCxR
LIVER		zebrafish	adult		M	5	E			C	S		AFMVExxCSx
		mouse, human	adult			5	E		T	C	S		AFGVExxCSx
INTESTINE		holothuria	adult	Y	M	5	H	D	T		S	R	AYMVHDTxSR
		mouse, human	adult			5	H				S	R	AFGVHxxxSR

Table 1- Table enlisting the values given to the ten criteria used to deduce the regenerative code in 30 distinct regenerative contexts. From left to right: *class I criteria: developmental status of the regenerating organism:* **A**: adult, **L**: larval or fetal, **N**: neotenic, **Y**: asexual reproduction, **F**: no asexual reproduction, **M**: metamorphosis**, G**: no metamorphosis during sexual development; *class II criteria: regenerative complexity* assessed from the number of cell lineages involved in the regenerative process; **I:** 1, **K:** 2; **P:** 3 or 4, **V:** 5, **W:** 6 or 7; *class III criteria: cellular processes* involved in early regeneration: **H:** programmed cell death (apoptosis) or histolysis; **E:** suppression of apoptosis; **D:** dedifferentiation; **Q**: no dedifferentiation; **T:** transdifferentiation; **C:** proliferation of differentiated cells; **S:** recruitment of stem cells or multipotent progenitors; **R:** epithelial-mesenchymal interaction; **x:** unknown state. Question marks were introduced when reported data are controversial.

eration in mammals Thompson et al. (2000), heart regeneration in zebrafish Lepilina et al. (2006), to provide positional information during body regeneration in planarians Kato et al. (2001) and limb regeneration in urodeles Campbell and Crews (2007).

Maximum Parsimony (MP) analysis of the regenerative codes

Hence for each regenerative context, we have recapitulated the currently available data that characterize the regenerative process at the cellular level, namely recruitment of stem cells or progenitors (S), presence or absence of dedifferentiation (D, Q), transdifferentiation (T), proliferation of differentiated cells (C), activation or repression of apoptosis / histolysis (H, E), epithelial-mesenchymal interactions (R) (Table 1). As these cellular processes are not mutually exclusive, they were entered as six independent entities. In the absence of data concerning one or the other process, the letter X was used. The combination of these different values represents 144 distinct possibilities if one takes into account the undetermined values corresponding to the absence of experimental evidence for a given criteria (2 x 3 x 2 x 2 x 3 x 2). However, it should be noted that these cellular regenerative codes correspond to regenerative processes occurring spontaneously after amputation or injury in the absence of any molecular, cellular or tissue manipulation. Moreover, these cellular codes are only qualitative. In fact, when several cellular mechanisms were shown to drive regeneration, their respective contribution in the process is presently not evaluated and could not be integrated in these regenerative codes. Finally each regenerative context received a regenerative code of ten criteria (Table 1), corresponding to the assembling of the six cellular criteria together with the three developmental criteria (representing 12 possibilities) and the regenerative complexity criteria (5 possible values). The combination of these values represented 8'640 putative regenerative codes.

The method of Maximum Parsimony (MP) seeks to find the evolutionary scenario (or tree topology) that requires the fewest character state changes to explain the states observed today (data analysed). We thus performed an unweighted MP analysis to identify the groups of regenerative contexts that share particular characters, possibly reflecting common mechanisms. The MP analysis resulted in 171'221 equally most parsimonious trees from which a 65% majority-rule consensus tree was derived, which shows only the relationships found in more than 65% of the tree (Figure 4). Seven groups were retrieved on 100% of the trees that are organized in three super-groups. The first of those gathers regenerative contexts that share the absence of metamorphosis as the planarian body regeneration and all the neotenic regenerative contexts included in this study, i.e. *Hydra* head regeneration and axolotl appendage reconstruction (group A). In fact, within that super-group, tail and limb regenerations in axolotl form a sub-group to which *Hydra* head regeneration systematically clusters. The neotenic character of *Hydra* polyps is usually not mentioned, those being refered as "adult" animals. However the loss of the medusa stage in this atypical hydrozoan is well admitted. *Hydra*, axolotl, and planarian also share in their respective codes cellular processes as recruitment of stem cells to form the blastema and/or proliferation of multipotent progenitors, whereas transdifferentiation/metaplasia was reported only in axolotl and *Hydra*. Cell proliferation is classically not considered as a driving force in *Hydra* regeneration as, in this species, regeneration can occur in the absence of cell proliferation and is therefore regarded as "morphallactic", i.e. relying on cell differentiation and/or transdifferentiation Bode (2003). Nevertheless, we have recently shown that in wild-type conditions and after mid-gastric amputation, a massive cell proliferation leading to the formation of a proliferative zone is indeed observed early after amputation Chera et al. (submitted). At the early-late stage, the apical tip is the site of an intense proliferation of neuronal progenitors, whose differentiation precedes head morphogenesis Galliot et al. (2006); Miljkovic-Licina et al. (2007). These results suggest that morphallaxis is either limited in normal conditions or rather used as an emergency lane when proliferation is inhibited.

This first super-group that was never suspected in previous considerations about evolution of regeneration, is opposed to the metamorphosing model systems (M<->G), which include adult as well as fetal or larval contexts (Figure 4). However these four contexts differ by developmental and cellular criteria, as asexual reproduction and apoptosis found in *Hydra* and planarian but not in axolotl, dedifferentiation involved in axolotl regeneration but not in that of *Hydra* or planarian. This puzzling result obtained by MP analysis should now be tested when additional variations of the dataset are introduced, as for instance introducing the regenerative codes of other neotenic model systems as *Hydractinia* Frank et al (2001), adding molecular criteria, testing those enlarged datasets in the absence of the neoteny criteria.

The second grouping observed in 100% of the trees includes regeneration of the retina and regeneration of the inner ear both in the adult chick (group B). This grouping that is supported by the shared criteria "absence of metamorphosis" (M<->G), arises from the part of the tree where "adult" post-metamorphosis regenerative contexts group together. Group B actually unifies "adult" regenerative modes that involve a limited number of cell types, only two in each case. The fact that it does not include "adult" regenerative processes of the same tissue, the retina in fish and *Xenopus*, can be explained by the fact that retina regeneration in

adult chick is partial, whereas in fish and *Xenopus* it is far more complete, leading to the full regeneration of the multilayered retina. Moreover, it is well established that retina regeneration follows distinct cellular processes in chick, fish and *Xenopus* Moshiri et al. (2004); Hitchcock et al. (2004); Raymond et al. (2006); Yoshii et al. (2007). Nevertheless even though those regenerated tissues of group B are both of neuro-ectodermal origin, their signatures differ by four criteria, questioning the possibility of an underlying shared mechanism.

The third unambiguous group identified within this study, group C, also contains exclusively adult regenerative contexts but emerging now from the "larval" part of the tree (L<->A). It includes a derived sub-group detected in 100% of the trees: zebrafish and mice liver regeneration that share the repression of apoptosis as a common criteria (H<->E). This group clusters together with mouse intestine and chick bone regeneration, this last sub-group forming with deer antler regeneration the complete group C (Figure 4). Except deer antler, the other four contexts regenerate a structure formed of 5 cell lineages (W<->V). The mouse and zebrafish liver regenerative codes differ by a single criteria out of seven (metamorphosis in zebrafish), and, as anticipated, liver regeneration appears as an homologous process among vertebrates. However mouse intestine and vertebrate liver regeneration, although regenerating both tissues of endodermal origin, actually share a single cellular criteria (S). Moreover the second type of adult intestine regeneration included in this analysis, that of holothuria, does not fall into this grouping, given the holothuria-specific developmental and cellular criteria as metamorphosis, asexual reproduction, dedifferentiation and transdifferentiation. In contrast, bone and deer antler regeneration share developmental (A,F,G) and cellular criteria including the absence of dedifferentiation (H,Q,S) but slightly differ by the complexity of the regenerated structure (5 cell lineages in case of the bone, 6 in case of the deer antler) and the absence of epithelial-mesenchymal interaction in case of bone regeneration. In conclusion, group C includes two sub-groups that appear to include

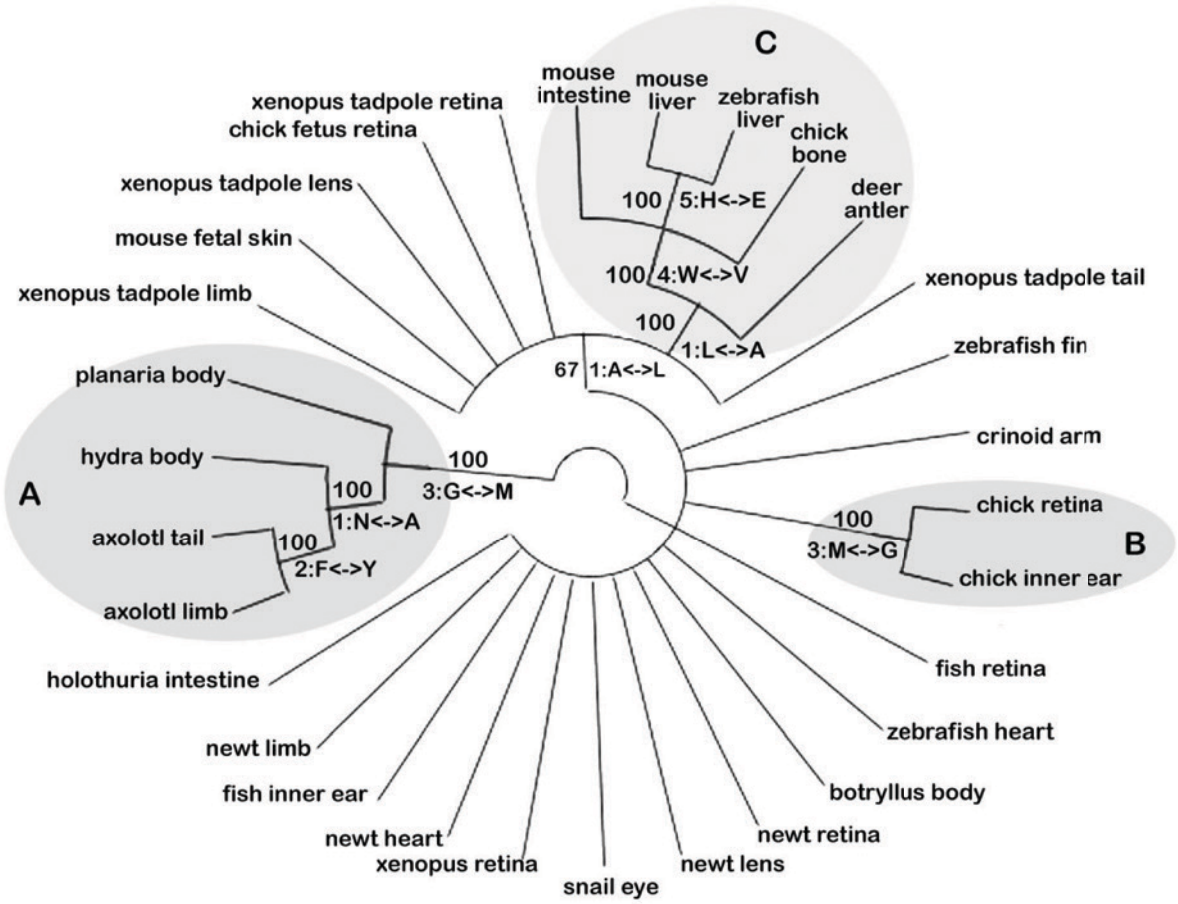


Figure 4: Maximum parsimony consensus circular cladogram depicting the relationships among 30 regenerative contexts. The MP analysis was performed using the PAUP* sofware Swofford (2003). Characters have been equally weighted and tree search was performed with 10 starting tree replicates obtained by random addition of taxa and TBR branch swapping algorithm. The tree search was limited to 50 million rearrangements and resulted in 171'221 equally most parsimonious trees of length = 20 and consistency index = 0.5. The tree shown is the 65% majority rule consensus tree. Values near branches indicate the percentage of the equally most parsimonious trees displaying the branch. Characters and state changes supporting the groups are indicated by their corresponding codes (see Table 1).

homologous regenerative contexts (liver, bone-antler) but additional criteria are required to better define the adult ancestral mode of regeneration of that group.

CONCLUSIONS & PERSPECTIVES

Here we present the first attempt at classifying regeneration modes by analyzing in a systematic manner their similarities. This new approach that provides a systematic sorting of the regenerative processes that support tissue repair, organ and appendage regeneration in the animal kingdom according to developmental, regenerative complexity and cellular contexts, is aimed at classifying in a logic manner the various modes of regeneration, at uncovering the homologies and the convergences between those numerous and highly variable regenerative modes. As a first result, this MP approach showed seven unambiguous groupings. The first group contains body / appendage regeneration in planarian, *Hydra* and axolotl being considered as neotenic; the second group contains regeneration of neuroectodermal derivatives in adult chick (inner ear and retina), and the third one, regeneration of endodermal (intestine, liver) and endo-mesodermal derivatives (bone, antler) in adult vertebrates (fish, chick and mammals). Moreover, fetal / larval regenerative contexts also group together with a significant support value. This attempt to quantitatively evaluate this amazing biological process is still rather hazy but should help us predict the homologies or convergences between the different regenerative processes at work in the animal kingdom.

Nevertheless, several aspects of this approach need be strengthened. The regenerative codes we established are still incomplete in many contexts, as to an example the eye regeneration in snail. In fact numerous regenerative contexts could not be taken into consideration for this study given the paucity of the cellular data. Another difficulty of the present approach concerns the weight given to each of the criteria. Arbitrarily we decided to give the same weight to every criteria used in this study. Nevertheless the validity of each criteria should be tested independently by removing one or the other criteria from the dataset, as some criteria might not be relevant to the type of regenerative mode Sidall (1995). If not relevant, we expect the grouping of the regenerative modes to remain stable when this criteria is removed. In addition, the quantitative aspect of each of the cellular criteria is not addressed in this study. We expect that, as the different cellular processes are not mutually exclusive, the quantitative combination of the same set of cellular processes might be quite different form one context to the other. This means that within a given context, for a given set of cellular processes, each cellular process should receive a weight that reflects the importance of its contribution to the regeneration process. As an example, retina regeneration in fish is supposed to rely mostly on the recruitment of stem cells or multipotent progenitors. Therefore the other cellular processes, that might be dispensable, should be either ignored or given a very low weight.

An additional level of complexity that should be considered in this analysis concerns the interactions that take place between the different cellular processes. The blastema is a very dynamic structure where blastema cells in fact need to receive and give positional information about the state of the regeneration process, where the balance between each of the cellular processes is tightly tuned in time and space. However, the main drawback of this approach is the heterogeneity in the functional significance of the collected data: the number of regenerative contexts where the genetic networks involved in the regenerative process were tested in loss-of-function or gain-of-function assays, are currently limited, preventing us to include molecular criteria in the present study. In the future, we expect that systematic functional analyses of evolutionarily-conserved signaling pathways will help fill in the missing data of this matrix and increase the number of characters to be analyzed. Ultimately we expect that the systematic integration of molecular criteria to related cellular processes, including the chromatin modifications required for blastema formation, will help understand the regulation of a transient regeneration-specific structure, the regenerative blastema. For instance, a suggestive hypothesis postulates the capability of the blastema to supply positional information to the dividing stem cells Brockes and Kumar (2005). All these informations are required in order to organize the growth of a stump inserted into the pre-existing tissues, to induce through cell layer interactions the patterning of missing tissues and to prevent overgrow of the re-built structures. The understanding of these interactions will allow the design of integrated models. Such models are expected to predict the behavior of cells and tissues in regenerative contexts, and to identify the parameters that in mammals restrict the cellular plasticity and the regeneration potential to few specific tissues.

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