Biochemical Evolution and the Origin of Life, ed. E. Schoffeniels

ON SOME BIOCHEMICAL ASPECTS OF REGRESSIVE EVOLUTION IN ANIMALS

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In the field of the classical theory of evolution based at first on the consideration of morphological characteristics, it clearly appears that evolution is not only progressive, but in many cases also regressive. It is well-known indeed that considerable simplification or even loss of one or some organs (or systems of organs) has occurred as a response to profound adaptation to a particular way of life, such as parasitism, benthic or sessile life, etc.

From the point of view of comparative morphology, it seems therefore that such a regressive evolution is irreversible, as pointed out by the Belgian paleontologist Louis Dollo, in his "law of irreversibility of evolution".

The purpose of the present communication is to introduce and discuss some instances of regressive evolution at the level of the biochemical organisation of animals. This paper will be restricted to the consideration of some enzymatic systems, implicated in the biosynthesis and in the hydrolysis of polysaccharides playing a fundamental role in the edification of skeletal structures in animals and plants.

Evolution of chitin biosynthesis

Chitin is a high polymer of N-acetyl-D-glucosamine (or 2-acetamido-2-deoxy-D-glucose). The chitin chain is linear, and the units of N-acetyl-D-glucosamine are linked through β-glycosidic bonds between C–1 and C–4, as are the glucose units in cellulose. Chitin may however depart perhaps from an idealized poly-N-acetylglucosamine structure in having one residue deacetylated for every six or seven residues [8, 19]. Nevertheless, as far as we know, from the point of view of the chemist, there is only one kind of chitin. Crystallographic differences between α-, β- and γ-chitin are known, but they are due to the number and arrangement of the chitin chains within the unit

cell, and not to different chemical compositions [18, 19]. Moreover, a given preparation of purified chitinase, obtained from a culture of *Streptomyces* reared on purified shrimp chitin, is able to hydrolyse every preparation of chitin, whatever its origin, and to produce the same hydrolytic products, mainly chitobiose and chitotriose [1, 13].

Thus, the different chitins isolated from different animals or molds, show a high degree of isology.

The biosynthesis of chitin is realized, in all the cases so far studied, by a system in which UDPAG plays the role of donor; the transfer of the acetylglucosamine units on a chitodextrin is catalysed by a chitin–UDP acetylglucosaminyltransferase (EC 2.4.1.16). This enzyme has been detected in the cells or tissues elaborating chitinuous structures, in several insects or crustaceans, as well as in larvae as in adults, and in molds [3, 4, 9, 10, 17]. The properties of the chitin–UDP acetylglucosaminyltransferases so far studied seem to be identical. Thus, despite the lack of information concerning the amino acid sequences of the enzymes, we can reasonably admit that they are homologous, and that, as a consequence, the isologous chitins identified in different animals are themselves homologous (an "indirect homology", in the meaning of Florkin [6], or "episemantides" in that of Zuckerkandl and Pauling [22]). We thus can consider the distribution of chitin in animals in order to discuss the evolution of chitin biosynthesis. According to the fact that the classical histochemical methods for chitin detection are often non reliable, the location of chitin in cuticular and skeletal structures of animals has been entirely re-examined, using a highly specific method, involving the use of a purified chitinase [13, 14].

In figs. 1 and 2, the distribution of chitin in the different taxa of the animal kingdom is superimposed upon the phylogenetic trees proposed by two zoologists. The following conclusions can be drawn.

(1) Chitin biosynthesis is a primitive property of the animal cell, already present in protozoa. Many Ciliata and some Rhizopoda indeed utilize chitin in the cyst-walls or shells [2]; the envelopes of the spores of Cnidosporidia are also made of chitin [20].

(2) Diblastic Metazoa (not only all the Hydrozoa, but also other Cnidaria) are equipped with a biosynthetic chitin-system, located at the level of ectodermal tissues.

(3) In triblastic Metazoa, chitin is synthesized by most of the animals belonging to the phyla and classes of protostomian invertebrates. At the top of this lineage, i.e., in Arthropoda, chitin is intensively used to build the remarkable chitino-proteic cuticle which wraps and protects the whole animal.

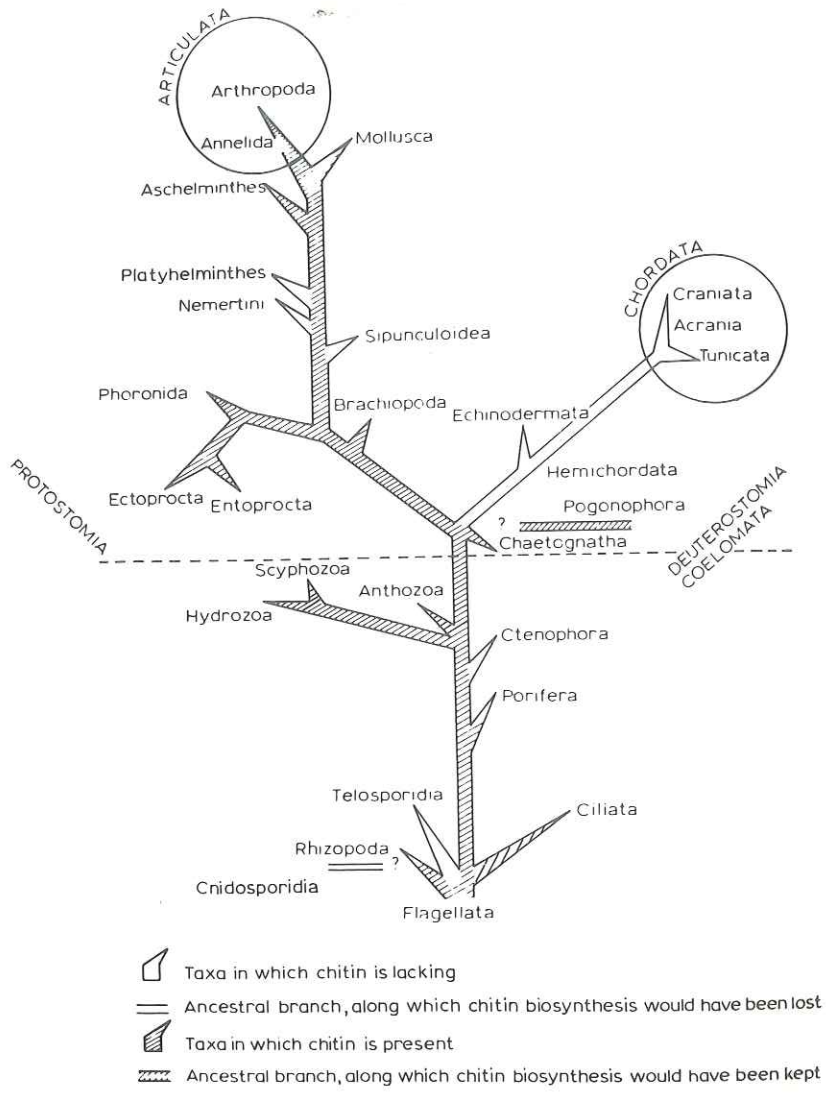

Fig. 1. Distribution of chitin, and phylogenic relationships proposed by Marcus in 1958. (Modified after Jeuniaux [13].)

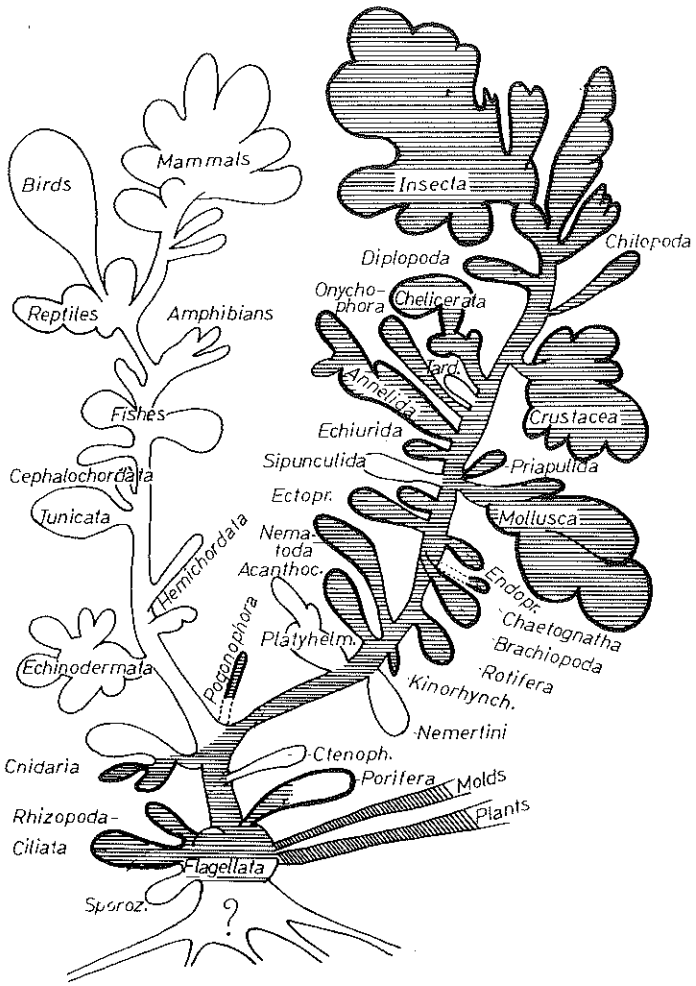

Fig. 2. Distribution of chitin (striped areas) and phylogenic relationships proposed by Cuénot in 1952. (Modified after Jeuniaux [13].)

(4) In contrast with the wide distribution and utilization of chitin biosynthesis in most protostomian phyla, the absence of chitinous structures in the acoelomatous protostomian phyla (Platyhelminthes and Nemertini) appears as being the result of the loss (or deletion) of the property of synthesizing this polysaccharide.

(5) The same hypothesis of a loss of chitin-biosynthesis during evolution is also proposed in the case of the important lineage of Deuterostomia. In this branch of the phylogenetic tree, the presence of chitinous structures has only been detected in two very primitive and highly specialized phyla: Pogonophora and Chaetognatha *. In all the other phyla of the deuterostomian lineage, all the skeletal or cuticular structures so far studied are devoid of chitin. It thus seems that the biosynthesis of chitin, or more precisely the synthesis of a chitin–UDP acetylglucosaminyltransferase, has been lost shortly after the starting point of the deuterostomian branch.
(6) The evolution of chitin biosynthesis in animals thus appears as an example of enzymapheresis, a word proposed by Florkin [5] to characterize such cases of regressive evolution by deletion of enzyme biosynthesis.

Evolution of chitinolytic enzymes

The complete hydrolysis of chitin into free acetylglucosamine is obtained by the action of two hydrolases: a polysaccharidase, the chitinase (EC 3.2.1.14) and an oligosaccharidase, the chitobiase (EC 3.2.1.29).

On the basis of an exhaustive study of the distribution, tissular location and properties of these chitinolytic enzymes in animals [11,12,13], the evolution of the biosynthesis of these enzymes can be drawn as follows.
(1) The widespread distribution of chitinases and chitobiases in bacteria, molds and Protozoa firmly suggests that the synthesis of both chitinolytic enzymes was a property appertaining already to the primitive unicellular animals, among which the origin of Metazoa is to be sought.
(2) In diblastic Metazoa, more precisely at least in the sea-anemones, chitinases and chitobiases are synthesized by both ectodermal and endodermal tissues. From this situation, we can observe some parallel evolutionary tendencies, along which the synthesis of one or both enzymes of the chitinolytic system is apparently lost by one or both embryonic layers.
(3) The synthesis of chitinase is generally lost by the ectodermis of Protostomia, while the synthesis of chitobiase is always retained by these cells. Two remarkable exceptions must be emphasized: the synthesis of chitinase at the level of epidermis is retained by the embryos of Nematoda and during the whole life in Arthropoda (except in the adults of insects). The preservation of

* Chaetognatha are placed in the lineage of Protostomia by Cuénot (fig. 1), but Marcus has adopted the more recent views concerning the phyletic position of this enigmatic group.

these biosynthetic abilities by these animals is related to the molting phenomenon in Arthropoda, and to the hatching process in Nematoda.

(4) On the contrary, the biosynthesis of chitinase is generally retained by tissues and organs of endodermic origin (digestive tract and glands) in most invertebrates, except in some species which are strongly adapted to a highly specialized diet, entirely devoid of chitin. This is the case, for instance, with strictly phytophagous or xylophagous insects such as the stick-insect (*Carausius morosus*), the silk-worm (*Bombyx mori*) or the wood-boring larvae of Longhorn-beetles (*Ergates faber*).

(5) In deuterostomian animals, and mainly in vertebrates, a marked tendency to the deletion of the biosynthesis of chitinase and chitobiase is evident. The distribution of chitinases in the digestive system of vertebrates, presented in fig. 3, appears as being a typical example of regressive evolution by the loss of a biosynthetic property obviously present in the ancestral lineage.

In fishes, indeed, which are generally chitin-eating animals, the biosynthesis of chitinase is very widely distributed, with only a few exceptions [15, 16]. In more evolved vertebrates, besides a more restricted location of chitinase elaboration in the digestive tract, one can observe the absence of chitinase secretion in those species which are adapted to a specialized diet devoid of chitin, such as terrestrial tortoise, pigeon, rabbit, sheep, guinea-pig, sloth, or cat. This is also the case for man.

Before interpreting these facts as being the result of a loss of the gene corresponding to chitinase biosynthesis, we must take into account the results of experiments concerning the effects of change of diet on the chitinase secretion [7, 13]. It has been shown that animals, which are able to secrete chitinase when they receive their normal food (mice, or rats for instance), continue to secrete this enzyme when reared on a diet devoid of chitin. On the other hand, animals which do not secrete chitinase when they eat their usual foods (guinea-pig for instance) do not acquire this property when chitin is added to their food, or when they receive a chitin-containing diet, whatever the duration of the experiment. It thus appears that chitinase is not an inducible enzyme in vertebrates; the lack of chitinase secretion in an animal is not the direct consequence of the nature of the current food of the individual, but the consequence of the genetic adaptation of the species (or the genus, or the family) to this diet, by loss of the gene corresponding to a "useless" enzyme.

(6) Considering the persistence of chitinase secretion in endodermic digestive tissues in most lower vertebrates, we can believe (and this hypothesis is in good agreement with the opinion of morphologists) that *the ancestors of the lower vertebrates were necessarily chitin-eating animals, or at least secondary*

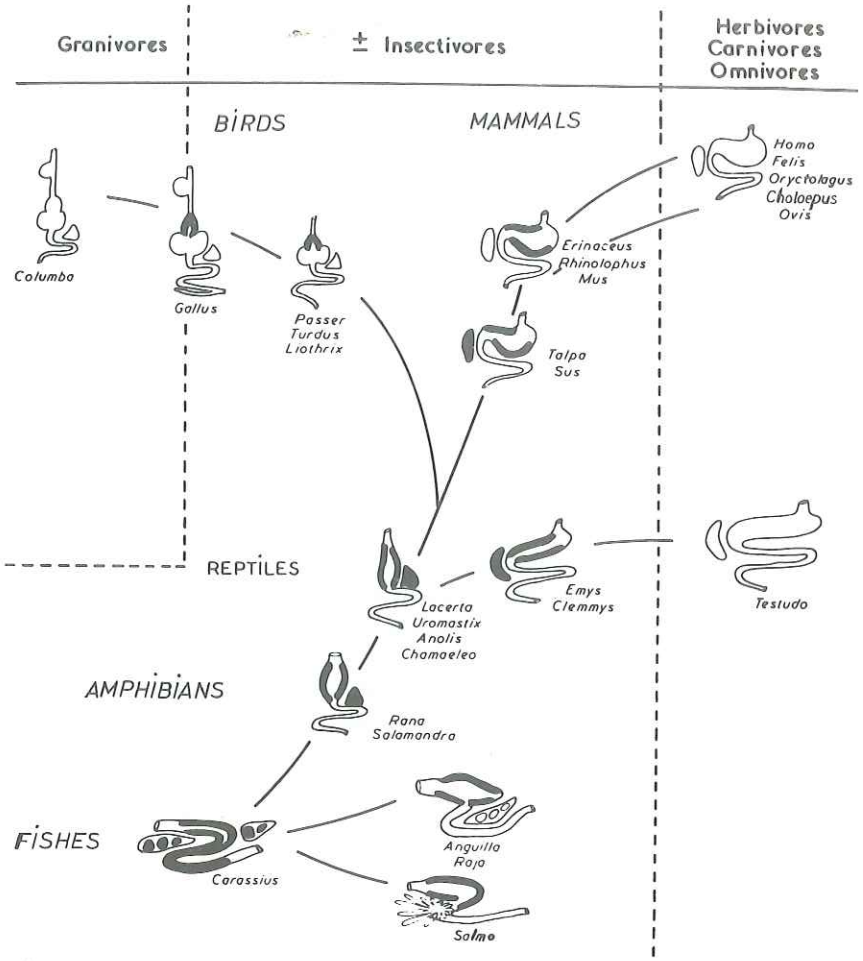

Fig. 3. Location of chitinase biosynthesis and secretion in the digestive system of vertebrates (after Jeuniaux [13]). (Glandular tissues in which chitinase secretion has been identified are marked by black areas.)

consumers eating chitin-covered animals (zooplancton or bigger preys) but not plants.

Evolution of cellulase secretion

The distribution of another type of polysaccharidase, cellulase (EC 3.2.1.4), is another example of enzymapheresis, and seems to support the former hypothesis concerning the feeding habits of ancestral vertebrates.

The distribution of cellulase in the digestive glands and tissues has been studied by many workers, and especially by Yokoe and Yasumasu [21]. The available data are summarized in table 1. It can be seen that, like chitinases, cellulases are constituents of the ancestral equipment of digestive hydrolases of the animal cell: they are found in 8 different species of Protozoa, in one Coelenterata, in 23 species of polychaetous annelids (against 4 species lacking this enzyme), in all the 57 species of Mollusca so far studied, with the exception of only 3 species, in most crustaceans and insects, etc. Cellulases have also been found in echinoderms, but only occasionally. But they have never been found in tunicates or in vertebrates.

This lack of cellulase biosynthesis in vertebrates cannot be interpreted otherwise than as being the result of the deletion of the corresponding gene. It is well known, indeed, that many higher vertebrates are adapted to a diet consisting only of green plants, or even of wood. Such cellulose-eating

Table 1
Distribution of cellulase in animals, according to Yokoe and Yasumasu [21].

Taxa	Number of species in which cellulase secretion has been observed	Number of species in which cellulase secretion is lacking
Protozoa	8	1
Coelenterata	1	2
Annelida	23	4
Echiurida	1	0
Brachiopoda	1	0
Mollusca	54	3
Onychophora	0	4
Crustacea	19	2
Arthropoda	19	12
Echinodermata	3	11
Hemichordata	0	1
Urochordata	0	5
Vertebrata	0	9

vertebrates evolved in different lineages: the tortoise in reptiles; the kangaroo in marsupials; sloths, rodents and of course ruminants in mammals. Now, it is well known that, in all these animals, the digestion of cellulose is realized thanks to cellulases which are not secreted by the animal itself, but by symbiotic bacteria living in the digestive tract. The adaptation of vertebrates to this cellulose-rich food is the result of convergent modifications, both at the morphological level (development of fermentation pouches) and at the physiological level (involving the rapid absorption by the mucosa of the volatile fatty acids resulting from the fermentation of the hydrolytic products of cellulose). But the re-appearance of cellulase biosynthesis by the animal tissues could never be observed. It thus seems that, in this case, the law of irreversibility of evolution has obviously been kept.

Conclusions

The evolution of the cellulolytic and chitinolytic systems, as well as that of chitin biosynthesis, can be interpreted as being examples of *regressive evolution*, or *"enzymapheresis"*. Other examples, for instance the evolution of the enzymes of the urea cycle, or that of the uricolytic system, have been treated elsewhere by Florkin [6]. Enzymapheresis is one of the evolutionary processes which explains the adaptative correlations that we can observe between the ecology of an animal and its biochemical equipment, without introduction of new molecules.

Summary

The consideration of the zoological distribution of some high polymers, such as chitin, or of some enzymes such as chitinase, chitobiase and cellulase, leads to the conclusion that biochemical evolution can be a regressive process, in which the genes corresponding to the synthesis of a given enzyme are deleted. This regressive evolution, also called "enzymapheresis", seems to be irreversible. As is the case for morphological characteristics, regressive evolution of hydrolases appears to be consecutive to a profound adaptation of a given species or taxon to a specialized diet.

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