SYNOPSIS. Two parallel themes emerge in the history of the investigation of the ascidian tunicate [Urochordata] embryo: the realization that the larval stage is probably a surviving example of the earliest chordate body plan from which vertebrates arose, and secondly the unusual degree of autonomous specification of cell fate involved in the development of ascidian larval parts. Such developmental autonomy in larval structures results in patterns of development referred to as “mosaic.” This paper follows the progress of these two themes from their beginnings in the second half of the nineteenth century to their status at the present time. Romer’s concept of vertebrates as a “dual animal” (somatic and visceral) stands out as a landmark perception in support of the theory of vertebrate origin by paedomorphosis through a merger of the pelagic larval and benthic adult stages of a tunicate-like animal. The present contribution attempts to unite the two themes by postulating that autonomous specification further enhanced the modular nature of the developing tunicate embryo and permitted natural selection to act differentially on the largely independent organ systems of larvae and paedomorphs, in what amounts to a mosaic selection pattern. This, in turn, favored the very rapid emergence and radiation of the chordates during the Cambrian explosion.

INTRODUCTION

During the past century, there have been two major parallel lines of inquiry related to the larval development of ascidian tunicates (phylum Chordata, subphylum Urochordata, class Asciidae). One of these has been the discovery of the tunicate larva and steady progress in uncovering particulars of ascidian larval structure. Many of these details prove to be features of chordate affinity which suggest in turn that the present day ascidian larva preserves the primitive chordate body plan from which vertebrates arose in the course of evolution (Garstang, 1928; Berrill, 1955). The other line of very long-time inquiry, recently summarized by Satoh (1994), is a progression of findings about the developmental regulation of the ascidian larval form. These observations and experiments indicate a relatively autonomous specification in the embryonic development of the strictly larval features, which later in evolution became the major chordate features of vertebrates.

This essay reviews certain highlights in these two streams of investigation and discovery and suggests how the findings may intersect. Their convergence lies in the possibility that the autonomous specification mechanisms found strongly expressed in early larval development may be one of the two major reasons, including paedomorphosis, for the rapid evolution of higher chordates, and hence of vertebrates, from a urochordate larva-like common ancestor.

A CHORDATE-LIKE LARVA

Larvae of the ascidian tunicates were known for some time before their internal anatomy was studied histologically by Alexander Kowalevsky (1866). One of the first descriptions and illustrations of an ascidian larva appeared in Henri Milne-Edward’s (1842) monograph on colonial ascidians. In it he described and depicted some of the embryonic, larval and metamorphic stages of Aplidium (Amaroucium) proliferum which he and J. V. Audouin had
first recorded in 1828. Between 1816 and 1850, at least four other investigators independently published figures depicting the ascidian larva: J. C. Savigny, Michael Sars, John Dalyell, and Louis Agassiz. Charles Darwin had studied the larvae of a colonial ascidian in 1833 during his voyage on the H.M.S. Beagle (Darwin, 1871).

The findings of Kowalevsky (1866, 1871) concerning the internal structures of the ascidian larva and their embryonic development came as a surprise to the zoological community. At the time there was no controversy or even great puzzlement over tunicate affinities. Most authorities, including Milne-Edwards and the venerable Karl von Baer, believed them to be allied most closely to the Mollusca, as suggested by the classifications of Cuvier and Lamarck. Perhaps for this reason no one of note, except for Ernst Haeckel (1868), paid any immediate attention to the 1866 publication which claimed ascidian larval similarities to vertebrates.

Kowalevsky's initial and subsequent claim was to have found the mode of development of the ascidian larva (Phallusia mammillata and Ciona intestinalis) to be closely similar to that occurring in lower vertebrates and amphioxus. The tadpole-shaped larvae of both species had a long motile tail with lateral muscle bands, and an internal rod-shaped structure which was notochord in its appearance, function and mode of development. Not until 1871 did Kowalevsky correctly describe the larval nervous system and its formation, as a dorsal tubular design formed by a vertebrate-like neurulation process, and depict the subnotochordal endodermal strand. Meanwhile Kupffer (1869, 1870), using Ciona intestinalis, had also described the formation of the anterior brain vesicle and posterior hollow nerve cord and confirmed others of Kowalevsky’s observations. The presence in the ascidian larva of an obvious notochord and a dorsal tubular nervous system, as well as its general resemblance to the vertebrate body plan was compelling evidence that ascidians were primitive relatives of the vertebrates.

After Kupffer's confirmation of Kowalevsky's findings, they became so widely accepted that by 1871 Charles Darwin was able to write in *The Descent of Man, and Selection in Relation to Sex*, “... we have at last gained a clue to the source from whence the Vertebrata have been derived. We should thus be justified in believing that at an extremely remote period a group of animals existed, resembling in many respects the larvae of our present Ascidians, which diverged into two great branches—the one retrograding in development and producing the present class of Ascidians, the other rising to the crown and summit of the animal kingdom by giving birth to the Vertebrata.”

Ascidians are, however, a dimorphic form in which a non-feeding pelagic chordate-like larva or tadpole bears essentially no resemblance to the sessile filter-feeding adult ascidian. Since deuterostomes in general have these bentho-pelagic life cycles (Jägersten, 1972), there is good reason to believe that the ascidian larva evolved from a previous larva rather than some earlier adult form (Crowther and Whittaker, 1992). The tailed larva and its nervous system has presumably evolved at a very early geologic time and become adapted to serve the adult needs of dispersion and site selection (Berrill, 1955). Nonetheless, the adult ascidian shares at least two important characters with chordates, namely the gill openings in their branchial basket which develop from prostigmata that are the likely homologues of vertebrate gill slits (Garstang, 1928), and an endostyle (ciliated groove) as part of the filter-feeding apparatus. This endostyle occurs also in the ventral pharyngeal region of Branchiostoma (=Amphioxus) and the ammocoete larva of lampreys (cyclostomes). When the ammocoete metamorphoses into the adult lamprey, some of the endostyle cells are seen clearly to become part of the vertebrate thyroid gland (Garstang, 1928).

A characteristic of the class Asciidiacea is a thick outer mantle or tunic surrounding the adult and which distinguishes the group from other invertebrate taxa; this tunic contains a unique cellulose-like substance, tunicin. The sedentary, benthic and usually attached adult is essentially a “visceral” animal engaged in efficient filter feeding, food
processing and reproduction. In many general ways the internal organs of adult tunicates serving these functions appear homologous to those of lamellibranch molluscs. Because of these organ similarities, von Baer (1873) and some others never became reconciled to tunicates as chordates, preferring instead to discount completely the significance of ascidian larval anatomy.

Chordate phylogenies based on 18S ribosomal RNA gene sequence comparisons (Turbeville et al., 1994; Wada and Satoh, 1994) and cladistic analysis of morphological and developmental features (Maisey, 1986; Schaeffer, 1987) affirm the central position which urochordates occupy in most theories of chordate evolution. The cephalochordates and vertebrates are regarded as sister groups, and the urochordates (ascidians, larvaceans, salps) are the sister group of the cephalochordate/vertebrate line. Whether the wholly pelagic larvaceans (Appendicularia) actually preceded the ascidians in origin, as suggested by Wada and Satoh (1994) and others, remains an unresolved question. There are good morphological and life cycle reasons in support of larvacean derivation from a neo-tenized ascidian larva (Nielsen, 1995). In addition, an 18S rDNA database is probably insufficient in sequence complexity to resolve cladogenetic events separated by less than about 40 million years (Myr) (Philippe et al., 1994). The several tunicate groups presumably originated within a time interval of less than 20 Myr (last section below).

CHORDATE CHARACTERS OF THE ASCIDIANS

The list of possible chordate traits in the urochordate subphylum, especially those evident in the ascidian larva, has become progressively longer in the century and a half since discovery of the larva. Comprehensive lists and discussions of these chordate characters have appeared at various times, most recently by Katz (1983), Maisey (1986), Schaeffer (1987), Cripps (1990), and Nielsen, (1995).

The ascidian larva has a bilaterally symmetrical body plan with definite head and tail ends that is notably similar to that seen in lower vertebrates. Except for lacking segmentation and an obvious coelomic body cavity, it looks much like the ammocoete larva of lampreys, and the larval Branchiostoma. Ascidian larvae are tadpole-shaped with an anterior cephalic region and a long tail-like extension, containing contractile lateral muscle bands, and a central incompressible axial skeletal rod, the notochord, running the length of posterior body. Figure 1 shows two schematic diagrams of their structural organization.

There is a tubular nervous system, with a hollow brain in the dorsal cephalic region connected to a hollow neural tube which runs dorsally along the length of the tail and above the notochord. In the central and ventral cephalic region one finds the endodermal mass of pharyngeal rudiment of the ascidi- zooid. The pharyngeal mass shows early traces of an endostyle; in larvae of some colonial species one finds "gill slit"-like prostigmatae arising there even before metamorphosis. An endodermal strand of cells connected to the main mass of the cephalic endoderm occurs subnotochordally along the length of the tail. Most authorities have generally regarded the whole larval tail as a postanal tail equivalent to that of vertebrates. In actuality, the distal fifth of the tail is composed of cells with structural characteristics and embryological origins that suggest it to be the "postanal" tail homologue (Crowther and Whittaker, 1992, 1994).

Aside from the notochord, the nervous system is perhaps the most impressive chordate character. A bipartite brain is divided into an anterior prosencephalon and a posterior deuterencephalon (Katz, 1983). The brain and posterior neural tube develop in what appears to be a completely vertebrate manner. There is formation of a neural-like stage and closure of a neural plate to form a tubular structure by inrolling of the plate of neuroectodermal cells. Even the mode of primary neural induction is topographically similar to that of amphibians. In each case cells in the roof of the archenteron induce formation of neural plate tissue in competent ectoderm although it is different cells in each taxon: notochord and endoderm in ascidians, mesoderm in amphibians.
In certain minor details the nervous system of the ascidian larva is strikingly similar to other chordate nervous systems. Ascidian larvae have tubulated bulb cell organs protruding into the brain cavity that appear to be the homologues of coronet cells in the saccus vasculosus of fishes (Svane, 1982); ascidians, as well as Branchiostoma and vertebrates, have Reissner's fibre in the canal of the posterior neural tube (Olsson, 1972). Also within the ascidian brain wall is a dorsal eye structure (the ocellus) projecting into the brain cavity; this has been homologized by Eakin (1973) and others with the pineal eye or pineal body of vertebrates.

The endodermal strand, a frequently overlooked larval structure, corresponds in position to the intestine seen in Branchiostoma and cyclostomes and may be what has given rise to the vertebrate intestine. It serves no apparent "intestinal" purpose in the ascidian larva or subsequent adult. However, the strand does retain expression of gut alkaline phosphatase activity during its developmental stages (Fig. 2), and has the same cell lineage origins as other endodermal cells which ultimately form the digestive system of the ascidiozooid (Whittaker, 1990). Ultrastructural investigation of the strand by Crowther and Whittaker (in preparation) confirms it to be a single row
of about 25 cells remaining intact throughout the whole larval period. Figure 3 illustrates the location and structure of such cells.

Two important barriers to regarding the ascidian larva as a surviving relict of the earliest chordate form have been the apparent lack of coelom (that is, a body cavity lined by mesoderm) and a segmentation/metamerism involving various parts of the body, particularly the muscle (Willmer, 1990). Berrill (1936) has claimed the pericardium to be a remnant of the coelom, and according to Ivanova-Kasas (1988), ascidians show traces of the same three coelomic compartments as found in other Deuterostomia.

Crowther and Whittaker (1994) used immunocytochemical staining with anti-tubulin antibodies to demonstrate regularly spaced cilia pairs in two rows immediately opposite each other mid-dorsally and mid-ventrally along the larval tail surface of *Ciona intestinalis*. These immotile cilia, which are embedded in the matrix of the extracellular larval test of the flattened tail fin, originate from pairs of cell bodies in the mid-dorsal and mid-ventral peripheral nerves running beneath the tail epidermis. Such serially repeated and equidistantly spaced cilia pairs (approximately ten dorsal-ventral sets) possibly indicate a primitive underlying segmentation pattern preceding chordate metamerism. The presence of functional *Hox* genes in ascidians (Katsuyama et al., 1995) is also consistent with this suggestion.

Holland and Garcia-Fernández (1996) have addressed some important evolutionary questions in reviewing their own work and that of others on *Hox* gene diversity in primitive chordates and lower vertebrates. In insects and vertebrates, the *Hox* genes are involved in controlling the specification of segment identity and the linear organization of body plan. *Branchiostoma* clearly has only a single and archetypal *Hox* gene cluster; according to recent findings of several cluster members in ascidian tunicates, so apparently do they. Duplications or multiple clusters of *Hox* genes first occur in hagfishes and lampreys; the genomes of jawed vertebrates (teleost fishes, mouse, human) each possess four *Hox* gene clusters. Hence, vertebrate evolution per se correlates with cluster duplication. Conversely, the presence of only a single *Hox* gene cluster in amphioxus and ascidians is sufficient to refute the fallacious suggestion first made by Dohrn (1875) and later by others (e.g., Jefferies, 1986) that lower chordates are not primitive but originate by the serial degeneration of vertebrates.

**The Somatic and Visceral Vertebrate Animal**

Romer (1972) described in reasoned detail how vertebrates combine the general characters and features of what appear to be two almost independent kinds of animal, a sessile visceral feeding and reproductive animal and a motile or somatic one. Others before him have understood this dichotomy, notably Garstang (1928) and Grave (1935), but the clarity of Romer's synthesis is remarkable. He reasons that the obvious source of this inherent duality is likely to be a combination of the ascidian larva and ascidian adult into one functionally integrated life cycle stage. This has been accomplished through acceleration and retar-
Textbooks and many monographs almost universally favor an explanation of vertebrate evolution that invokes neoteny or paedomorphosis of a primitive urochordate-like larva in which most of the larval characters are retained and upon which the development of the more visceral ascidian-like adult features become superimposed. This theory is attributed to Garstang (1928) and Berrill (1955). Presumably then, an amphioxus-like cephalochordate becomes one of the first stable evolutionary products of this transformation. The neoteny theory is so popular that one critic has remarked: “The suspicion that vertebrates had a tunicate-tadpole-like ancestor is well founded but neoteny of such a tadpole in the origin of vertebrates has the scientific status of a creation myth” (Jefferies, 1986, p. 350).

One of the major reasons for the popularity of the neoteny theory of vertebrate origin is that the animal kingdom abounds in examples of form changes which can be attributed reasonably to a neoteny or paedomorphosis (McKinney and McNamara, 1991). Within primitive chordates (am-

Fig. 3. Endodermal strand in the embryonic and larval tail of Ciona intestinalis. A. Light micrograph of a thin (0.5μm) sagittal section of a whole 11-h embryo. Stained with osmium tetroxide and cut in epoxy resin. Arrow shows position of the endodermal strand. Bar = 25μm. B. Transmission electron micrograph of a sagittal section along the tail of a hatched larva showing an endodermal strand cell (arrow) below the notochord. Bar = 5μm.
phioxids and lampreys), neoteny is not a rare event (Bone, 1957; Zanandrea, 1957) and there are also many recorded examples of ascidian larvae failing to metamorphose, or of their metamorphosing without resorbing their tails (e.g., Berrill, 1955).

**AUTONOMOUS SPECIFICATION**

Two decades after Kowalevsky's investigations were laying the groundwork for our understanding of the nature of the ascidian larva, a young French investigator, Laurent Chabry, began the first investigation into the so-called physiology of ascidian development. Chabry had noted in collecting ascidian embryos from the plankton that when one of the two early blastomeres within the chorionic membrane had become damaged, leading to cytolysis of that cell, a partial and incomplete embryo developed; he realized that such embryos might possess a key to understanding the mechanisms of development (Chabry, 1885). In pursuing the problem further for his Doctor of Science thesis at the Sorbonne, Chabry invented the first micromanipulator for puncturing the egg chorion with a tiny lancet to destroy selected blastomeres. He then used it to discover the effects of destroying blastomeres in two- and four-cell stages of Ascidia aspersa embryos.

Chabry (1887) first demonstrated that the earliest blastomeres, from 2- and 4-cell stages, were already specified in part for producing the particular tissues making up a region of the ascidian larva. His most famous diagrams depicting the result of destroying one of the first two blastomeres are reproduced here in Figure 4A, B. Only half-gastrulae and half-larvae resulted from such operations, a result duplicated later by Conklin (1905) who punctured (also within the chorion) the two cells resulting from one of the first two blastomeres (Fig. 4C, D). Cohen and Berrill (1936) achieved similar results after first dechorionating the embryos and actually removing the dead or inactive cells. Subsequently, Reverberi and Minganti (1946) began a series of blastomere isolation and recombination experiments which showed even more clearly that cell lineages in ascidians are effectively fate maps for larval parts.

Ascidian lineages themselves result from an invariant cleavage pattern which always segregates the same visibly distinct cytoplasmic regions of the zygote into lineages having restricted tissue and organ fates. This led to the conception that many aspects of ascidian larval development are conditioned by spatially localized and subsequently segregated egg cytoplasmic “organ-forming” substances, of presumably maternal origin. Davidson (1990) has characterized this as an autonomous specification process; he and others explain such spatially distributed tissue determinants as being maternally preformed gene regulatory factors or gene products of some kind. The experimental basis of this general conception has been reviewed extensively by Satoh (1994).

Conditional specification is dependent on cell-cell interactions in which some kind of inductive signal passes between cells that causes them to change or modify their fate. Terminal differentiation of the nervous system and the brain melanocytes of ascidian embryos are regulated by such conditional specification (Reverberi and Minganti, 1946). It is interesting, however, that the competence of only certain ectodermal tis-
sues of ascidians to respond to inductive signals may itself be controlled by autonomous specification (Satoh, 1994). Under experimental conditions a modest degree of neural expression may proceed even in the absence of inductive interactions (Whittaker, in preparation).

For purposes of illustrating developmental autonomy, that of larval endodermal tissues will be discussed briefly. During the late gastrulation of ascidian development, the mass of endodermal tissues sharing common lineages (founder cells) begins to produce a strong histochemical localization of alkaline phosphatase enzyme (Whittaker, 1977). The distribution of this enzyme staining is illustrated in Fig. 2, where staining can also be seen in the endodermal strand. Particular blastomeres of the 8- and 16-cell stages, when isolated and cultured, result in partial embryos that produce alkaline phosphatase but only if they are blastomeres from an endodermal lineage (Whittaker, 1990). At the 8-cell stage the endodermal lineages are already restricted to the vegetal quartet of cells and only cells from these make alkaline phosphatase. Cytoplasmic transfer experiments, involving the fusion of different lineage blastomeres with cytoplasmic fragments from various regions, give results which indicate that cytoplasm from an endodermal lineage causes non-lineage blastomeres to develop partial embryos with strong alkaline phosphatase activity (Nishida, 1993).

Similar kinds of experiments have now been done with muscle, ectoderm, and notochordal lineages; their results also point to an early autonomous specification (described by Satoh, 1994), with the odd exception of notochord. Specification of notochord cells appears to depend on blastomere interactions that occur before the 64-cell stage (Nakatani and Nishida, 1994). This notochordal difference highlights an important point about the determination of cell fate. Eventually in embryogenesis, all specification becomes autonomous in the sense that no further information, either intrinsic or acquired by cell interactions, is necessary to determine the ultimate organ fate of cells. At some point in most kinds of embryos there is a regionalization or modularity with respect to fates. Tunicates are unusual only with regard to how exceptionally early such specifications occur.

**Selection and the Larval/Adult Action-Systems**

In addition to his speculation about the paedomorphic origin of vertebrates from ascidian-like larvae, Garstang (1922) made an enduring contribution to our thinking about larval evolution in general. He was among the first to see that natural selection could act just as powerfully on the developmental or larval stages of an organism as upon the adult end-product, especially when larva and adult inhabit different zones of life. Grave (1935) independently recognized a similar dichotomy of parts in ascidians by describing the independent changes in their larval and adult action-systems during metamorphosis.

The various tissues and organs of the ascidian larva, and probably of other tunicate larvae as well, seem to have an extraordinary degree of developmental autonomy. In this sense, their development is uncoupled, and selection might be expected to act differentially and with considerable freedom on variants with mutations affecting single organs or systems. In fact, the whole of larval ontogeny, from the neurula stage onwards, can be dissociated from tissue changes leading to ascidiozooids. This is shown by two different kinds of observation: experimental preparations of half-embryos that result in normal ascidiozooids, and the occurrence of so-called anural species of ascidian in which major expressions of the larval stage structures have been reduced or eliminated presumably by environmentally mediated selection.

Half-embryos, and subsequently half-larvae, were produced by puncturing the egg chorion of *Styela plicata* embryos with a tungsten needle and destroying one of the blastomeres at the 2-cell stage (Nakauchi and Takeshita, 1983). The lateral half-larvae resulting from this operation were able to hatch, metamorphose, and ultimately develop into complete functional but smaller ascidiozooids. Not only does this illustrate that the adult action system has a largely conditional specification, but further indi-
cates its relative independence of interaction with larval tissues.

Some species of ascidian which find themselves living on sand and mud flats where wide dispersal and specific site selection are perhaps no longer likely to be an advantage, are discovered to be developing without a larval stage. That is, the larvae develop only about as far as the neural plate and neurula stages; most gene expressions for larval tissue-specific proteins will be reduced or absent (Whittaker, 1979; Jeffery and Swalla, 1990). Such structural larval features as differentiated brain tissues, including melanocytes, muscle myofilaments and notochordal cell extensions do not ordinarily occur. These tailless (anural) larvae are not in any sense primordial or more primitive. Larval loss occurs in the quite advanced families, Molgulidae and Styelidae; elegant 18S rDNA comparison methods show that anural species apparently originate independently and sometimes from sympatric tailed (urodele) species within the same geographic area (Hadfield et al., 1995). Anural development is polyphyletic in origin.

Perhaps the single most interesting experiment done with an anural species (Molgula occulta) was to fertilize their eggs with sperm from a closely related urodele species (Molgula oculata). Some of the hybrid embryos resulting from this cross developed a brain melanocyte and a short tail rudiment containing extended notochord cells (Swalla and Jeffery, 1990). The most important deduction from the results of such an interspecific hybridization experiment is that anural development may result from loss-of-function mutants. Perhaps some of the genes involved in suppression of the larval features are possible “master control genes” similar to the eyeless gene of Drosophila which appears to control eye formation (Halder et al., 1995). Genes encoding potential regulatory factors have already been identified with altered expression patterns in M. oculata and M. occulta (Swalla et al., 1993).

The results of experiments with expression of lacZ fusion constructs containing the 5’ upstream promotor regions of a larval muscle actin gene from each species suggest that loss of muscle cell differentiation is also accompanied by changes in the structure of muscle actin genes themselves rather than just in trans-acting regulatory factor genes involved in their expression (Kusakabe et al., 1996). An accumulation of mutations leading to malfunction in the structural genes for actin and other muscle proteins may, however, have been preceded by prior inactivation of regulatory genes which initiate notochord differentiation and tail organization in general.

There is evidence in some cases that individual larval organs and even features within the organs are differentially suppressed during anural transformations. In Molgula arenata (Whittaker, 1979) and Molgula occulta (Swalla and Jeffery, 1990), there is still a residuum of histochemically detectable muscle acetylcholinesterase even when there is obviously no structural muscle differentiation; in certain other anural species enzyme activity appears to be absent. In the anural molgulid species Bostrichobranchus digonas, expression of tyrosinase enzyme and melanin pigment occurs in 1–2 melanocytes that would ordinarily form parts of brain sensory structures in urodele species. So far, no other anural species enzyme activity appears to be absent. In the anural molgulid species Bostrichobranchus digonas, expression of tyrosinase enzyme and melanin pigment occurs in 1–2 melanocytes that would ordinarily form parts of brain sensory structures in urodele species. In B. digonas there are no expressions of such muscle-specific markers as acetylcholinesterase, α-actin, and myosin heavy chain (Swalla and Jeffery, 1992).

Berrill (1945, 1955) and others have compared larval structures in many ascidian species and have assumed adaptationist explanations of the differences observed. Changes seem to have occurred frequently involving reductions, deletions or modifications of the two brain sensory structures, the ocellus and statocyte. These alterations occur without obvious correlated changes in other organs and are deduced to be adaptations associated with shifts in sensory input needs related to altered habitat preference for a newly evolving species. In other species there are variations in tail muscle cell number which reflect increased larval size. The numbers range from 36 muscle cells in larvae of solitary ascidians to as many as 1,134 in Ecteinascidia turbinata.
an exceptionally large larva of a colonial species. There are no attendant changes in the number (about 40) of the adjacent notochordal cells. Possibly increased muscle cell numbers favor a greater swimming efficiency in the larger larvae, whereas changing the number of the incompressible notochordal cells would have little selective advantage. Larval organ systems clearly appear to be modified independently of each other.

As Gould (1992) reminds us, one of the first (1812) arguments against the possibility of evolution was rooted in Baron Cuvier's correlation of parts and their absolute dissociability. Animal parts were presumed to be so closely integrated that even minor changes would occasion a virtually prohibitive compensatory reorganization in order to maintain function.

In minor key, Gould and Lewontin (1979) have raised the same specter that single-feature adaptations of body parts, such as those postulated above in ascidians, do not occur easily or rapidly in evolution because of the highly integrated nature of developmental processes. Raff (1996), however, has revealed the somewhat illusory nature of this perception by emphasizing the compartmental and modular behavior of embryonic regions in many kinds of organisms. Such modularity can be interpreted as permitting natural selection to operate quite independently on individual body parts, essentially in a pattern of "mosaic evolution."

THE CAMBRIAN EXPLOSION AND CHORDATE RADIATION

One of the surprises of the last decade has been confirmation of the apparent speed at which the major animal phyla seem to have evolved during the Cambrian period, 510–545 Myr ago. Over an interval of about 15–20 Myr, virtually all the metazoan phyla, including the chordates, burst into being (Briggs et al., 1994). An amphioxus-like cephalochordate creature, Pikacea gracilens, has been recovered from the Burgess Shale formation of 520 Myr ago (Briggs et al., 1994). The larvacean specialist Lohmann (1922) identified a larvacean tunicate fossil, Oesia disjuncta, from the Burgess Shale, and another larvacean species was described from the Early Cambrian (Zhang, 1987), although both designations remain controversial. A fossil colonial ascidian, Palaeobotryllus, has been claimed from the Late Cambrian (Müller, 1977).

These fossil species, and others more problematical in their interpretation, indicate nonetheless that chordates have radiated explosively during Cambrian times. Identification of the conodont-bearing animal from fossils of the much later Ordovician and Carboniferous periods, and the discovery that their tooth-like conodont elements were functional teeth with a microstructure similar to the dermal bone and mesodentine of vertebrates, indicates these animals to be primitive vertebrate-like chordates (Janvier, 1995). Conodont fossils first occur in Upper Cambrian strata, as do fragments of an agnathan fish, Anatolepis (Smith et al., 1996).

On the basis of the fossil record as presently known, tunicates seemingly arose at some time in the Lower Cambrian. Paradoxically, it is the character of early autonomous specification in their development that may have favored their success both as a group and as a malleable precursor to more complex pre-vertebrate forms such as amphioxids. Given both their long history and their present day species numbers, estimated at approximately 3000 (Satoh, 1994), urochordates appear to be a well-adapted group. Although most of the species are ascidians living subtidally in varied niches, tunicates have radiated successfully into both pelagic and abyssal environments.

In contrast, living cephalochordates as represented by two main genera (Branchiostoma and Asymmetron = Epigonichthys) are undoubtedly collateral survivors of a once connecting group to the vertebrates, yet they are much less successful in their diversity. These modern forms are niche specialists limited to virtually a single kind of environment (coarse, current-swept sand) with only about 25 species worldwide. Not surprisingly, amphioxids are more conditionally specified (regulative) in their early stages and much less autonomously specified in their early embryogenesis than ascidians (Reverberi, 1971).
A thesis presented here is that the innate property of extreme autonomous specification in the early ascidian embryo is a basis for dissociation of the various development programs in larval organs, and their separation from those in the ascidiozooid. Because of this dissociability and a consequent rather strict modularity, natural selection might be expected to act more rapidly on the individual organ systems. Presumably independent organ changes would favor the success of ascidian species in making rapid adjustments to their preferred ecological niches, but equally favor rapid evolutionary change among the "dual animal" paedomorphs postulated by Garstang (1928), Berrill (1955) and Romer (1972).

Consideration of the continuous small improvements of design required to produce a structure as elaborate and precise as a vertebrate eye, beginning with a patch of light-sensitive cells, gives what is thought to be an over-estimate of about 1800 steps taking place in about 400,000 generations (Nilsson and Pelger, 1994). The generation time for small and medium-sized aquatic animals such as ascidians is usually one year. Even without a developmental regulatory mechanism that strongly favored selection on a very modular basis, important structural changes in organs could occur rapidly. During the Cambrian period rapid changes in the diverse structural features of chordates did unquestionably occur.

ACKNOWLEDGMENTS

Robert J. Crowther collaborated in much of the original investigative work discussed herein and contributed helpful discussions of the ideas. The work was supported in part by a grant from NSERC Canada, and by funds from the University of New Brunswick.

REFERENCES


Amsterdam: North-Holland.


Svan, I. 1982. Possible ascidian counterpart to the vertebrate saccus vasculosus with reference to Pyura tessellata (Forbes) and Boltenia echinita (L.). Acta Zool. (Stockh.) 63:85-89.


Wada, H. and N. Satoh. 1994. Details of the evolu-


