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Source: Zoological Science, 20(7) : 809-818
Published By: Zoological Society of Japan
URL: https://doi.org/10.2108/zsj.20.809
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ABSTRACT—Retinoic acid (RA) regulates many of the chordate-specific and vertebrate-specific characters. These include the anteroposterior pattern of the dorsally located central nervous system, pharynx with gill slits, neural crest cells, limb morphogenesis and anteroposteriorly organized vertebrae. The necessity of endogenous RA and the RA receptor (RAR) has been demonstrated by mutant analyses, vitamin A-deficient animals and various other methods. Since RAR has been identified only in chordates, the acquisition of the RAR-mediated RA signaling pathway is thought to be an important event for the innovation of the chordate body plan. RA-synthesizing aldehyde dehydrogenases and RA-degrading enzymes also seem to be chordate-specific. The expression pattern of these genes in ascidian embryos is similar to that in vertebrate embryos. These results suggest that the RA signaling cascade, with various regulators and modifiers, had been already well established in the common chordate ancestor. RA also regulates morphogenesis during the asexual reproduction of ascidians, suggesting that RA may also have played a part in producing diversity within the chordate groups.

Key words: chordate, body plan, retinoic acid receptor, retinoic acid metabolism, evolution

INTRODUCTION

The origin of chordates, as well as that of vertebrates, has long attracted many scientists in various fields of biology (for review see Gee, 1996; Hall, 1998). Therefore, we would like to avoid repeating well-conceived theories and debates here. We would like to concentrate our discussion on the possibility that the acquisition of the metabolic and signaling pathways of retinoic acid (RA) have played important roles in the evolution of the chordate and vertebrate body plans and divergence within the phylum Chordata.

It is widely accepted that the innovation of a new body plan can be achieved by gene duplication (Ohno, 1970). Gene duplication allows one of the derivatives to change to an extent that may otherwise be fatal (Holland et al., 1994). This may finally result in the elaboration of a gene with novel function and novel expression pattern (Holland et al., 1994). This seems quite applicable to vertebrate evolution, where two rounds of genome-wide gene duplication are believed to have occurred (Sidow, 1996). Attention has been concentrated on the duplication of the Hox gene cluster (Ruddle et al., 1994; Holland and Garcia-Fernández, 1996). Indeed, the duplication of Hox genes produced a complicated vertebrate body plan. However, the vertebrate Hox genes are functionally interchangeable with Drosophila counterparts (Malicki et al., 1990; Lutz et al., 1996). The genes upstream and/or downstream to the Hox function, as well as Hox-independent developmental programs, should also be considered for seeking “difference” between vertebrates and invertebrates, as carefully pointed out by Holland and Garcia-Fernández (1996). In addition, cephalochordate amphioxus and urochordate ascidians belong to the phylum Chordata, together with vertebrates (Kowalevsky, 1866, 1867; Garstang, 1928; Katz, 1983). They share many characters with vertebrates, even though their genomes possess a single Hox gene cluster (Garcia-Fernández and Holland, 1994; Dehal et al., 2002). The genes important for the innovation of the basic chordate-specific body plan may therefore have to be sought among those commonly found in all chordate species but not in non-chordate species. The draft genome sequence of the ascidian Ciona intestinalis revealed that about one-sixth (ca. 2570) of the Ciona genes possess homologs only within chordates, suggesting that these genes arose in the common ancestor of chordates (Dehal et al., 2002). A gene encoding the RA receptor (RAR) belongs
to this category (Dehal et al., 2002). RA regulates proliferation, differentiation, morphogenesis and pattern formation in a wide variety of tissues, organs and cell lines in vertebrates (De Luca, 1991; Conlon, 1995; Ross et al., 2000). Similar effects have been observed exclusively in chordates as discussed below, suggesting that the acquisition of the RAR function was a driving force for innovation of the chordate body plan. In the following sections we show how RA and RAR, and RA-metabolizing enzymes, are involved in the cell differentiation and morphogenesis of key chordate-specific cell types. Then, we discuss the possibility that a urochordate-specific life, with asexual reproduction, was elaborated by the acquisition of the RA signaling pathway.

RAR-mediated RA signaling pathway is acquired in the chordate ancestor

RA signaling is mediated not only by RAR but also mediated by retinoid X receptor (RXR) (Mangelsdorf et al., 1990). RXR is a heterodimeric partner that can bind various nuclear receptors including RAR (Mangelsdorf and Evans, 1995). RAR, but not RXR, can bind all-trans RA and 9-cis RA when it forms a heterodimer with RXR (Kurokawa et al., 1994). By contrast, RXR can bind 9-cis RA (Heyman et al., 1992), when it dimerizes with RXR or a few other nuclear receptors (NURR1 and LXR) (for review see Leblanc and Stunnenberg, 1995). The RXR-encoding genes have been cloned from a wide variety of animals. In Drosophila an RXR homolog, Ultraspiracle (USP; Oro et al., 1990), does not bind RA but forms a heterodimer with the ecdysone receptor (Oro et al., 1990; Yao et al., 1992). Although there is no evidence suggesting endogenous RA function in Drosophila, the possibility cannot be excluded that 9-cis RA regulates some biological functions in other invertebrates.

In contrast, RAR-encoding genes have been identified exclusively in chordates. Vertebrates possess three RAR-encoding genes (Fig. 1; for review see Mangelsdorf and Evans, 1992). The RAR/RXR heterodimer binds to the specific DNA sequences, called RA response element (RARE), and mediates RA signaling (Umesono et al., 1991). The unliganded form of the RAR/RXR can still bind to RARE and functions as a transcriptional repressor through protein-protein interaction with nuclear receptor co-repressors SMRT and N-CoR (Chen and Evans, 1995; Hörlein et al., 1995). Ascidians (Hisata et al., 1998; Devine et al., 2002; Nagatomo et al., 2003) and amphioxus (Escriva et al., 2002) possess a single RAR-encoding gene (Fig. 1). Sequence-specific DNA-binding, heterodimerization with RXR, and RARE-dependent transcriptional activation of reporter genes were experimentally demonstrated for these protochordate RARs (Kamimura et al., 2000; Escriva et al., 2002). No RAR has been reported in any non-chordate species. The well-characterized genomes of Caenorhabditis elegans and Drosophila melanogaster do not contain any gene similar to RAR (The C. elegans Sequencing Consortium 1998; Adams et al., 2000).

RA-mediated malformations have been reported in a

number of non-chordates, including sponges (Imsiecke et al., 1994), cnidarians (Müller, 1984), molluscs (Crέton et al., 1993), crustaceans (Hopkins and Durica, 1995), and echinoderms (Sciarrino and Matranga, 1995). However, RA-induced phenotypes can hardly be interpreted by analogy from those of chordates, suggesting that the RA-mediated developmental programs, if any, are not homologous to those in chordates. Phenotypes induced by exogenous RA are similar among the chordate species as described in the following sections. The protochordate RARs are expressed during embryogenesis in a stage-specific manner (Escriva et al., 2002; Nagatomo et al., 2003), suggesting specific roles in the embryo. An RAR-mediated RA signaling pathway seems to have been established in the chordate ancestor.
RA signaling is important for the expression of chordate-specific characteristics

The central nervous system (CNS) in chordates is formed from the folding of the dorsally located neural plate and is subdivided along the anteroposterior axis (Gee, 1996). The anteroposteriorly organized, but ventrally located CNS is observed in the annelids and arthropods, and was once regarded to be homologous to the chordate CNS (Dohrn, 1875; see also Nübler-Jung and Arendt, 1994). However, the homology between chordate dorsal CNS and protostome ventral CNS is rather unsolid (Lacalli, 1995; Peterson, 1995). The homology between hemichordate dorsal “neurocord” and the chordate neural tube is not widely accepted either (Brusca and Brusca, 1990). It seems therefore likely that the organization of the dorsal, hollow CNS has been acquired during the evolution of the common “chordate” ancestor (Garstang, 1928).

RA causes the homeotic transformation of the identity of rhombomeres in the hindbrain by affecting the expression pattern of Hox genes along the anteroposterior axis of the neural tube in vertebrates (Morriss-Kay et al., 1991; Papalopulu et al., 1991). A dominant negative form of RARβ interferes with the normal rhombomere patterning and reduces RA-induced teratogenesis (van der Wees et al., 1998). Similarly, another type of the dominant negative RAR eliminates HoxD1 expression and affects the pattern formation in the hindbrain (Kolm et al., 1997). Since the dominant negative RAR inhibits RAR-mediated but not RXR-mediated signaling, the RAR/RXR heterodimer was revealed to play a key role in the normal hindbrain patterning (Kolm et al., 1997; van der Wees et al., 1998). The necessity of endogenous RA was also demonstrated by the defect of the hindbrain patterning in vitamin A-deficient quails (van der Wees et al., 1999). Koide et al. (2001) showed that the repressor activity of unliganded RAR is required for correct differentiation of the forebrain. This also suggests complicated but important endogenous roles of RAR for the chordate body plan.

In amphioxus and ascidians, the region-specific expression pattern of many developmental regulatory genes along the anteroposterior axis of the central nervous system is similar to that in vertebrates (Wada and Satoh, 2001). Although there is no obvious indication of the metamic regionalization in the putative hindbrain region, the expression pattern of Hox genes is also similar to that in vertebrates (Katsuyama et al., 1995; Gionti et al., 1998; Locascio et al., 1999; Wada et al., 1999; Nagatomo and Fujinawa, 2003). RA affects the Hox gene expression pattern in amphioxus (Holland and Holland, 1996) and ascidians (Katsuyama et al., 1995; Nagatomo and Fujinawa, 2003), although the malformation of the anterior neural tissues in ascidians can hardly be regarded as homeotic transformation (Fig. 2; Denucé 1991; Nagatomo et al., 2003).

The pharynx, with gill slits and the endoderm, is another chordate-specific characteristic (Young, 1981). The protochordate endoderm is thought to be homologous to the vertebrate thyroid gland (Gee, 1996; Ogasawara et al., 1999). The hemichordate pharynx possesses gill slits but no endoderm (Gee, 1996). RA causes the loss of pharynx in RA-treated lamprey (Kuratani et al., 1998) and mammalian (White et al., 1998) embryos. RA is involved in the transcriptional regulation of Pax1 and Pax9 genes in the pharyngeal endoderm in mice (Wendling et al., 2000). Targeted mutagenesis of RARs resulted in the loss of the second and third pharyngeal arches (Lohnes et al., 1994). Abnormalities are also obvious in the thymus and thyroid gland (Mendelsohn et al., 1994). The reduction of the posterior pharyngeal endoderm in vitamin A-deficient quails provides additional support for endogenous RA requirement for pharyngeal patterning (Quinlan et al., 2002).

In ascidians the development of pharyngeal basket starts after metamorphosis. RA treatment during postlarval development leads to reduced expression of otx gene and eventual loss of the pharyngeal basket (Hinman and Degnan, 1998, 2000). In amphioxus, the size of the pharynx is reduced and gill slits do not form in RA-treated embryos (Holland and Holland, 1996). In contrast, the pharynx is expanded in embryos treated with an RA antagonist, BMS009 (Escriva et al., 2002). In this case, RA affects the expression of a Pax1/9 homolog (Holland and Holland, 1996; Escriva et al., 2002). The pharynx of ascidians and hemichordates also expresses Pax1/9 homolog (Ogasawara et al., 1999). These results suggest that RA regulates the pharyngeal morphogenesis through a similar genetic cascade in all the chordate groups.

![Fig. 2. RA-induced phenotype in the central nervous system of ascidians. The photographs were obtained by Tomoko Ishibashi of our laboratory. (A) Head of a normal Ciona intestinalis larva, the anterior is to the left, and the dorsal is up. The adhesive papillae (pa) and the brain vesicle with two sensory pigment cells (arrows) are differentiated. (B) Head of an RA-treated larva, dorsal view. The anterior neural tube failed to close and was exposed to the dorsal surface (arrow), and the presumptive brain cells form a cell mass outside the body (arrowhead).](https://bioone.org/journals/Zoological-Science/article-pdf/16/4/811/6451936/0165-0092-16-4-811.pdf)
RA signaling is also important for the expression of vertebrate-specific characteristics

Many important vertebrate-specific characteristics are found in the complicated structure of the head, and thus vertebrates (and hagfish) are called, “craniates” (Gee, 1996). The neural crest cells give rise to the cranial nerves, cephalic skeletal components and many other tissues comprising the head structure (Gans and Northcutt, 1983). Since most of these and other vertebrate-specific cell types derive from the neural crest cells, their evolution is thought to be an extremely important event in vertebrate evolution (Hall, 2000). The neural crest cells come only from the posterior neural plate (the hindbrain and spinal cord regions), suggesting that posteriorization of the neural plate, according to “the activation/transformation model” (Nieuwkoop and Albers, 1990), is involved in the neural crest development (Villanueva et al., 2002). The neural crest differentiation depends, at least partly, on the induction from the paraxial mesoderm (Bonstein et al., 1998). This and the fact that the posterior paraxial mesoderm is the site of retinoic acid synthesis (discussed below, see Fig. 4) suggest that RA could be one of the candidate posteriorization factors. In fact, RA treatment and a constitutively active form of RAR can induce the neural crest in the presumptive forebrain region (Villanueva et al., 2002). The presumptive neural crest cells die by apoptosis in vitamin A-deficient quails (Maden et al., 1998). Wada (2001) proposed that the dorsal midline epidermis in ascidians and amphioxus is the origin of the neural crest and thus the neural crest cell population itself is not an innovation of vertebrates. An enhancer element of the amphioxus Hox-1 gene (AmphiHox-1) can be activated in the vertebrate neural crest, although AmphiHox-1 expression is restricted to the neural tube in amphioxus embryos (Manzanares et al., 2000). Since Hox-1 expression in the neural crest depends on RA (Manzanares et al., 2000), the acquisition of the RA signaling pathway could have conferred the neural crest cell properties on the dorsal midline epidermis of the vertebrate ancestor. Wada (2001) is skeptical to this idea because an AmphiHox-3 enhancer containing an RARE was not expressed in the neural crest cells (Manzanares et al., 2000). However, requirement of a few additional changes does not necessarily deny the importance of the acquisition of RA-responsiveness for the neural crest evolution. A large part of the amphioxus nerve cord is thought to be homologous to the vertebrate brain (Holland et al., 1992). The Hox-3 and Hox-5 homologs of ascidians are expressed only at the late stages of development (Giontì et al., 1998; Locascio et al., 1999). These observations suggest that the expression and function of the posterior (5’) Hox genes were largely modified after the divergence of protochordate and vertebrate groups.

The limbs are also vertebrate-specific, and missing in protochordates (Gee, 1996). RA affects the proximodistal axis in the regenerating amphibian limbs (Niazi and Saxena, 1978; Maden, 1982; Thoms and Stocum, 1984). The effect of RA on the anteroposterior pattern formation of developing chick wing bud was remarkable (Tickle et al., 1982). The organizing role of endogenous RA in the limb has long been expected, but also doubted. Although RA-induced ectopic digit formation follows the upregulation of RARβ expression, the endogenous organizing region (called ZPA) does not express RARβ (Noji et al., 1991). RA thus came to be regarded as a notorious example of the chemical that only mimics the action of endogenous factors. ZPA expresses Sonic hedgehog (Shh) gene that organizes the anteroposterior pattern in the limb bud (Riddle et al., 1993). The question then moved to how the Shh expression was activated in the posterior region of the limb bud (Johnson and Tabin, 1997). It seems that the anteroposterior organization of the limb bud is a sophisticated modification of the pre-existing pattern in the lateral plate mesoderm (Cohn et al., 1997) Lu et al. (1997) demonstrated that RAR antagonists block the formation of ZPA. In addition, the expression domain of Hoxb-8, a direct RA target gene, correlates with the domain of polarizing activity in the lateral plate mesoderm (Lu et al., 1997). Inhibition of the RA synthesis (Stratford et al., 1996) and the RAR/RXR function (Helms et al., 1996) in the lateral plate mesoderm disturbed limb formation. These results suggest that RA truly acts as an endogenous factor for ZPA induction and limb formation.

The name “vertebrates” comes from the vertebral column. RA induces homeotic transformations of the vertebræ along the anteroposterior axis (Kessel and Gruss, 1991). This phenotype is derived from altered pattern of Hox expression (Kessel and Gruss, 1991; Kessel, 1992).

It should be noted that all these vertebrate-specific characters are affected by targeted mutagenesis of at least two of three RAR subtypes (Lohnes et al., 1994). The phenotypes of RAR double mutants are similar to the fetal vitamin A-deficient syndrome (Wilson et al., 1953). This suggests that endogenous RAR plays important roles in the expression of these characteristics.

RA-synthesizing enzyme and RA-degrading enzyme are also chordate-specific

Is RA synthesized only in the chordates? In vertebrates, a major RA-synthesizing enzyme in the embryo is the aldehyde dehydrogenase encoded by Raldh2 gene (Zhao et al., 1996). Raldh2 is expressed in the posterior mesoderm in the pre-somite stage vertebrate embryos (Niederreither et al., 1997; Swindell et al., 1999). Targeted disruption of Raldh2 gene causes early embryonic lethality (Mic et al., 2002). Conditional rescue of the Raldh2 null mutant mice by providing RA within maternal food revealed that the forelimb formation requires normal RA synthesis (Niederreither et al., 2002b). These results suggest an essential role of RA in vertebrate embryogenesis. Although many experiments have been carried out in which exogenous RA was applied to various invertebrate species as described above, little attention has been paid to the RA-synthesizing activity in invertebrates.

Retinal, of various isomers, is stored in the unfertilized...
egg of the ascidian *Halocynthia roretzi* (Irie et al., 2003). Recently, we identified a candidate Raldh2 homolog (*Ci-Raldh2*) from the ascidian *Ciona intestinalis* (Fig. 3A; Fujiwara et al., 2002; Nagatomo and Fujiwara, 2003). The amino acid sequence deduced from the *Ci-Raldh2* cDNA shows the highest similarity to human RALDH2. However, low bootstrap values suggest that the branching pattern of a few related retinaldehyde dehydrogenases (RALDH3 (Grün et al., 2000; Niederreither et al., 2003), mitochondrial ALDH2 (Chen et al., 1994) and RALDH2) is uncertain (Fig. 3A). The expression of *Ci-Raldh2* was restricted in the anterior paraxial mesoderm (a few muscle cells on both sides of the notochord) (Fig. 4A, C; Nagatomo and Fujiwara, 2003). Although it is still under dispute, the ascidian muscle cells are thought to correspond to the segmented somites in vertebrates (Crowther and Whittaker, 1994). If so, the expression pattern of *Ci-Raldh2* is similar to that of vertebrate Raldh2 in the somites (Fig. 4A-D; Niederreither et al., 1997). The role of 11-cis-retinal in vision is thought to be evolutionarily ancient and retinal-metabolizing enzymes were isolated from invertebrates (for review see Duester, 2000). There is no evidence suggesting endogenous roles for all-trans RA and 9-cis RA in non-chordate invertebrates. Furthermore, no Raldh2 homolog has been reported in any non-chordate species. However, it is difficult to draw conclusions from sequence comparison. The sequence of aldehyde dehydrogenases is extremely highly conserved from bacteria, fungi and plants to animals, and we can hardly predict the substrate from the sequence.

Vertebrates possess cytochrome P450 enzymes (CYP26) that catalyze the reaction from RA to 4-oxo- and 4-hydroxy-RA (White et al., 1997, 2000). Cyp26 is expressed in the presumptive forebrain/midbrain region of the gastrulating embryo, while Raldh2 is expressed in the presomitic and lateral plate mesoderm at the same stage (Fig. 4B; Swindell et al., 1999). This complementary expression pattern suggests that CYP26 is limiting the range of RA action (Fig. 4A–D). The region where RA is deduced to be active coincides with the spatial expression pattern of an RARE-containing reporter gene (Fig. 4E; Rossant et al., 1991). Cyp26 null mutant mice exhibited the posterior transformations of cervical vertebrae and abnormal rhombomere pat-

![Fig. 3.](#)
gene is expressed in the anterior neural plate of the gastrula and neurula, and in the brain of the tailbud embryos (Fig. 4A, C; Nagatomo and Fujiwara, 2003). This expression domain does not overlap that of Raldh2 and is similar to that of vertebrate Cyp26 (Fig. 4A-D; Nagatomo and Fujiwara, 2003). The expression of Cyp26 in ascidian embryos is dramatically upregulated by exogenous RA (Nagatomo and Fujiwara, 2003), as is the case in vertebrates (White et al., 1997). The enzymatic activity of these ascidian homologs remains to be demonstrated biochemically.

Variation of the repertoire of RA-target genes in some chordate species

If the acquisition of the RAR gene is a key event during the chordate evolution, the history of the RAR’s recruiting target genes depicts at least a part of chordate morphological evolution. RAR regulates developmental programs mainly through transcriptional regulation of Hox genes (Simone et al., 1990; Gould et al., 1994; Manzanares et al., 2000). A few developmental regulatory genes are also known as RA target genes. These include RARß (Mendelsohn et al., 1991) and Meis2 (Oulad-Abdelghani et al., 1997). The systematic analyses of RA target genes, based on cDNA arrays, were performed using embryonic stem cells (Kelly and Rizzino, 2000) and embryonal carcinoma cells (Freemantle et al., 2001) but not in embryos of any animal species.

Recently we identified a number of RA target genes in the ascidian embryos by means of a cDNA microarray-based screening (Ishibashi et al., 2003). The strong activation of Hox-1 and Cyp26 was similar to that in vertebrates (Nagatomo and Fujiwara, 2003; Ishibashi et al., 2003). As previously shown by in situ hybridization analysis (Nagatomo et al., 2003), RAR expression was not activated by RA (Ishibashi et al., 2003). This is in marked contrast to the RA-inducibility of the RAR genes in amphioxus (Escriva et al., 2002) and vertebrates (Mendelsohn et al., 1991). Many candidate target genes seemed to be involved in the neuronal functions, suggesting that not only morphogenesis but also cellular differentiation of the nervous system are regulated by RA. However, RA-treated larvae can respond to natural metamorphosis inducers even though they lack the adhesive papilla that is responsible for the chemosensory response (Hinman and Degnan, 1998). This suggests that RA does not cause homeotic transformation of the anterior cells into completely different cell types in ascidians.

RA-mediated transdifferentiation in the budding ascidians

RA also plays an important role in the asexual reproduction of the ascidians, which is a characteristic acquired only in the urochordate lineage of chordate evolution. In the ascidian Polyandrocarpa misakiensis, the gut, pharynx, endostyle, neural gland and many other tissues are formed through the pluripotent transdifferentiation of the atrial epithelium (Fujiwara and Kawamura, 1992; Kawamura and...
Fujiiwara, 1994). Exogenous RA induces ectopic transdifferentiation, resulting in a completely duplicated body axis (Hara et al., 1992). Localized activation of aldehyde dehydrogenase suggests that endogenous RA acts as a regulator of transdifferentiation (Kawamura et al., 1993). Genes encoding RAR and RXR are expressed in the developing bud (Hisata et al., 1998; Kamimura et al., 2000). Functional analysis revealed that they bind specific DNA sequence as a heterodimer and activate reporter gene expression depending on RA (Kamimura et al., 2000). A differential display screening identified a few target genes, including a serine protease with a complex modular structure (Ohashi et al., 1999). This serine protease, named TRAMP, is a candidate transdifferentiation factor, since recombinant TRAMP protein can stimulate proliferation and probably dedifferentiation of the cell line derived from the atrial epithelium (Ohashi et al., 1999).

About a half of the ascidian species can proliferate asexually by budding. Various modes of budding have been described (for review see Nakauchi, 1982). The origin of the newly formed tissues is the atrial epithelium in Styelidae and Botryllidae, the stolonial septum in Perophoridae, and the epidermal epithelium in Polyclinidae, Polyclinidae and some other families (Nakauchi, 1982). Since the common chordate ancestor was thought to be a free-swimming tadpole-like animal (Wada, 1998), the ability of asexual reproduction seems to have been independently acquired several times during the ascidian evolution (Wada et al., 1992). This suggests that small genetic changes can cause the conversion of solitary to colonial lifestyle. Despite the extreme diversity in the type of multipotent formative tissues, the early buds of most species look similar to one another. In the developing bud, the gut and pharynx are usually the first organs to be formed (Nakauchi, 1982 and references therein). The molecular mechanisms underlying the bud development may be roughly common to all the budding ascidians. RA affects morphogenesis of the pharyngeal endoderm in all chordate groups, as described above. And Hox genes determine the anteroposterior pattern of the digestive tract in vertebrate embryos (Sakiyama et al., 2001). Considering these facts, the ability of asexual reproduction may have been acquired by re-activation of RA-driven developmental programs in some adult cell type. Since the asexual reproduction includes reconstruction of the whole body from a piece of tissues, the process can be thought as regeneration (Kawamura and Fujiwara, 2000). RA affects amphibian limb regeneration as described above. Inhibition of RA synthesis disturbs regeneration, suggesting that endogenous RA plays an important role in the amphibian limb regeneration (Maden, 1998). Although RA is also involved in the regeneration of a few other tissues (for review see Maden and Hind, 2003), we have little evidence to connect, in general, the ability of regeneration to the ability of re-activation of RA signaling pathway.

Conclusions and perspectives
The RAR-mediated RA signaling pathway is involved in the formation of most chordate-specific and vertebrate-specific characteristics. The necessity of endogenous RA functions has been demonstrated for each case, using dominant negative RARs, RAR mutants and vitamin A-deficient animals. RA-synthesizing and RA-degrading enzymes also seem to be chordate-specific. Considering this, the basic framework of the RA signaling cascade, target genes and metabolizing enzymes that modify RA functions has already been established in the common chordate ancestor. How were the complicated network and cascade established? How were RAR expression and function modified during the evolution of vertebrates? Thinking about the origin, it is important to obtain the information about the sister groups, hemichordates and echinoderms. In addition to the functional analysis of the genes involved in the RA signaling, screening and comparative analysis of homologous genes in hemichordates and echinoderms will be of great interest.

ACKNOWLEDGEMENTS
We are grateful to Kan-ichiro Nagatomo and Tomoko Ishibashi for photographs and other data shown in figures. We also thank Tom Suzuki for the construction of phylogenetic trees. Work from the author’s lab was supported by Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan. S.F. was also supported by the Asahi Glass Foundation.

REFERENCES


Dohrn A (1875) Der Ursprung der Wiebelthiere und das Princip des Functionswechsels. Verlag von Wilhelm Engelmann, Leipzig


Kowalevsky AO (1866) Entwickelungsgeschichte der einfachen Ascidien. Mém L'Acad St Petersbourg Ser VII 10: 1–19
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Kowalevsky AO (1867) Entwickelungsgeschichte des Amphioxus lanceolatus. Mém L'Acad St Petersbourg Ser VII 11: 1–17
Niazi IA, Saxena S (1978) Abnormal hindlimb regeneration in tadpoles of the toad, Bufo andersoni, exposed to excess vitamin A. Folia Biol (Krakow) 26: 3–11
in embryonal development. Physiol Rev 80: 1021–1054
Wada H, Makabe KW, Nakauchi M, Satoh N (1992) Phylogenetic relationships between solitary and colonial ascidians, as inferred from the sequence of the central region of their respective 18S rDNAs. Biol Bull 183: 448–455
(Accepted April 11, 2003 / Invited Review)