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[REVIEW]

Genes of the Ascidian: An Annotated List as of 1997

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ABSTRACT—The ascidians occupy a crucial phylogenetic position between invertebrates and vertebrates. Advances in molecular biological techniques and molecular cloning have enabled studies of molecular mechanisms to be more and more accessible in ascidians. Recently, an increasing number of ascidian genes have been cloned. In this review, we summarize the molecular features and expression profiles of all ascidian genes published to date and describe how and why they were cloned. Such information not only is valuable for understanding the recent advancements in ascidian molecular biology but also to bring out the importance of ascidians as an experimental system for a variety of fields in biology.

INTRODUCTION

Ascidians (subphylum Urochordata or Tunicata, class Ascidiacea), are sessile marine animals ubiquitous throughout the world. They have evolved rich patterns and modes of development. Some live as individuals (solitary or simple ascidians), whereas others form colonies (colonial or compound ascidians). Ascidians provide a unique and fine experimental system for studies of embryology (reviewed by Satoh, 1994). In addition to the embryology, ascidians have been used in various fields of biology. Several ascidians accumulate vanadium from sea water (reviewed by Michibata, 1996). The ascidian tunic contains tunicin, a type of cellulose (Ranby, 1952). Ascidians are thought to have a prototype of vertebrate immune system (e.g., Beck *et al*., 1993). In particular, their phylogenetic position as a primitive chordate is important in any fields of biology for understanding the vertebrates.

The first report of a cloned ascidian cDNA, an adult body wall muscle actin gene, was published by Tomlinson *et al*. in 1987. In this decade, numerous ascidian genes have been isolated. Previously, Satoh (1994) reviewed the inclusive data about the ascidians. In 1996, Satoh *et al*. reviewed the genes in the early development of ascidians. Recently, in addition to conventional screening to search for ascidian homologues of known genes, systematic large scale screening which aims to find all or most of the genes related to a certain intriguing biological mechanism has become available. This method should facilitate identification of a huge number of novel genes and provide extensive information about the genetic circuitry of biological mechanisms.

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Since our knowledge about the ascidian genes will increase massively in the forthcoming few years, it is worthwhile reviewing all the ascidian genes published to date at this point. Moreover, by reviewing the molecular biological data about the ascidians, we tried to bring out the importance of the ascidians as an excellent experimental system for the comprehensive biological studies including total genome sequencing.

GENE LIST

EMBRYONIC GENES

Muscle

Muscle actins Actins are highly conserved proteins found in all eukaryotes from yeast to higher vertebrates. Most organisms have genes that encode several actin isoforms. Vertebrate actins are classified into three isoforms (α, β and γ) according to their isoelectric points in two-dimensional gel electrophoretic analysis. Ascidian actins also exhibit considerable heterogeneity. *Styela plicata* embryos and adults contain three major and two minor isoforms of actin (Tomlinson *et al*., 1987a). Two of the major isoforms are likely to be β- and γ-actins, and the third may be an α-actin (Jeffery *et al*., 1990). Genomic southern blot, using the coding region of cytoplasmic actin cDNA as a probe, showed that the *S. plicata* genome has 10 to 15 actin genes (Beach and Jeffery, 1990).

HrMA2, *4a*, *4b*, *5*, *6* (*HrMA2/4* cluster) and *HrMA1a*, *HrMA1b* (*HrMA1* pair): All *HrMA* genes are muscle type α-actin genes isolated from *Halocynthia roretzi*. *HrMA2*, *4a*, *4b*, *5* and *6* were clustered in a 30 kb region of the genome and aligned in the same direction (*HrMA2/4* cluster) (Kusakabe *et al*., 1992). Nucleotide sequences of 5' flanking regions, as well as the coding regions, are well conserved among this

cluster. *HrMA1* pair consists of two genes, designated as *HrMA1a* and *1b*. These two genes are linked in a head-to-head arrangement on opposite strands and share a 340-bp 5' flanking sequence containing two symmetrically located TATA boxes (Kusakabe *et al*., 1995).

Expression of *HrMA2/4* is restricted to larval muscle cells (Kusakabe, 1995). Careful examination of timing of the gene expression by whole-mount *in situ* hybridization and RT-PCR analysis revealed that zygotic transcripts of *HrMA4a* are first evident at the 32-cell stage (Satou *et al*., 1995). Analysis of deleted and mutated constructs of the 5' upstream region of *HrMA4a* fused with *lacZ* showed that the two short sequences within the proximal region (–103 to –66) of the *HrMA4a* gene were essential for muscle specific expression (Satou and Satoh, 1996). The 5' upstream region of *HrMA4a* up to –103 bp was also proved to sufficient for appropriate spatial expression in larval muscle cells in *Ciona savignyi*, even when the proximal E-box sequence was mutated (Hikosaka *et al*., 1992, 1993, 1994).

The *HrMA1a* showed basically the same expression pattern as *HrMA4a* (Kusakabe *et al*., 1995). *HrMA1a* transcripts are first detected at the 64-cell stage (Satoh *et al*., 1996b). Microinjection of deletion constructs with 5' upstream region of *HrMA1a* and *HrMA1b* revealed that rather short sequences including the CArG box-like sequence are essential for the muscle-specific expression of these genes (Kusakabe *et al*., 1995; Satoh *et al*., 1996a).

ScTb1, *24*, *30*, *12/34*: Five cDNA clones (*ScTb1*, *ScTb24*, *ScTb30*, *ScTb12/34*), which encoded identical muscle type α-actin isoform, were isolated from an *S. clava* tailbud-stage library (Beach and Jeffery, 1992). *ScTb1* was detected in eggs, embryos, and adults, *ScTb24* and *ScTb12/34* were detected in embryos and adults, and *ScTb30* was detected only in embryos. The *ScTb24* gene was detected only in tail muscle cells, whereas the *ScTb30* gene was detected in embryonic tail muscle, mesenchyme, epidermal and neural cells.

MocuMA1 and *MoccMA1*: *Molgula oculata* and *M. occulta* are closely related species but different in their mode of development. *M. oculata* is a urodele developer, while the *M. occulta* is an anural developer which fails to differentiate an otolith, notochord and tail muscle cells (Swalla and Jeffery, 1990). *MocuMA1*, a single copy, larval-type muscle actin gene was isolated from a *M. oculata* genomic library (Kusakabe *et al*., 1996). The orthologus larval muscle actin genes *MoccMA1a* and *MoccMA1b* were also isolated from a *M. occulta* genomic library. *MocuMA1* is intron-less but is functional. Deletions, insertions, codon substitutions and frame shifts occurred in *MoccMA1a* and *1b* coding regions suggesting these genes are psudogenes (Kusakabe *et al*., 1996). In addition, microinjections of various promoter-*lacZ* fusion constructs in *Ciona intestinalis*, *M. oculata* and *M. occulta* eggs revealed that the 5' upstream regions of the *MoccMA1a* and *1b* retained muscle-specific promoter activity and *M. occulta* egg retained *trans*-acting factors responsible for expression of the *MocuMA* gene (Kusakabe *et al*., 1996). These results suggest that the regression of muscle cell differentiation in anural species is mediated by changes in the structure of muscle actin genes rather than in the *trans*-acting regulatory factors required for their expression (Kusakabe *et al*., 1996).

Muscle actin genes were isolated from various ascidians. Sequence analysis of these muscle-type actin genes provides new information on the evolution of chordate muscle actins. Invertebrate muscle actins are more closely related to vertebrate cytoplasmic than muscle actins (e.g., Macias and Sastre, 1990). Comparison of diagnostic amino acids, which distinguish vertebrate muscle actin from vertebrate cytoskeletal actin (Vandekerckhove and Weber, 1984), revealed ascidian muscle-type actin isoforms, such as *ScTb1*, *HrMA4* and *HrMA1*, to be more similar to vertebrate muscle-type actins than vertebrate cytoplasmic actins (Kovilur *et al*., 1993; Kusakabe *et al*., 1995). Furthermore, two distinct lineages of muscle actin isoforms, larval muscle type and adult body wall muscle type, could be distinguished in ascidians (Kusakabe *et al*., 1997). Although, chordate actin gene whose expression is known to be restricted to the embryonic stage is very rare, *ScTb30* and *HrMA4* cluster genes are expressed only in embryogenesis. In addition, arrangements of the introns in these ascidian muscle actins are conserved among vertebrate and echinoderm actins (Kusakabe *et al*., 1992, 1995, 1997). These results suggest that ascidians and vertebrates have a common ancestor, which developed vertebrate-type muscle actins after divergence from the invertebrates (Kusakabe *et al*., 1992; Kovilur *et al*., 1993).

As shown in the *HrMA4* cluster, *HrMA1* pair and *ScTb* gene family, unusually large numbers of genes encoding identical or similar proteins of the same muscle actin isoform are present in the ascidian genome. It is likely that the ascidian multiple actin genes arose by gene duplication. This may be advantageous for maximizing the synthesis of contractile proteins during rapid differentiation of ascidian larval muscle cells (Kusakabe *et al*., 1991; Beach and Jeffery, 1992). Clustering of the vertebrate-type muscle actin genes, such as the *HrMA4* cluster, has not been reported, yet.

Myosin heavy chain *HrMHC1*: Mu-2 is a monoclonal antibody specific to differentiating muscle cells of *H. roretzi* (Nishikata *et al*., 1987a). *HrMHC1* has been isolated for a gene that encodes the Mu-2 antigenic protein (Makabe and Satoh, 1989). *HrMHC1* resembles myosin heavy chain of vertebrate skeletal and cardiac muscles (Araki and Satoh, 1996). *HrMHC1* transcripts are expressed in the nuclei of primary muscle lineage blastomeres in gastrula and in the cytoplasm of muscle cells in neurula through tailbud embryo (Makabe and Satoh, 1989; Makabe *et al*., 1990). The 5' flanking regions of *HrMHC1* and *HrMA4* share several common sequence motifs, and two proximal motifs called box T1 and box T2 play a crucial role in the muscle-specific transcriptional activity of *HrMHC1* (Araki and Satoh, 1996).

Troponins *aTnT2/aTnT19* and *eTnT11/14*: Ascidian troponin T cDNA clones were isolated from *H. roretzi* by Endo *et al*. (1996). *aTnT2* and *aTnT19* mRNAs encode an identical protein and seem to be generated from a single gene by the alternative 3' end processing of a single pre-mRNA. *aTnT2/*

aTnT19 and *eTnT11* (identical to *eTnT14*) are derived from different genes. *aTnT2* transcripts are specifically expressed in adult body wall muscle, while *eTnT11* transcripts are specifically expressed in the embryonic/larval tail muscle cells (Endo *et al*., 1997).

AdTnC and *LaTnC*: Two distinct cDNAs encoding troponin C isoforms (*AdTnC* and *LaTnC*) were isolated from *H. roretzi*. *AdTnC* is expressed in adult body wall muscle and cardiac muscle, and *LaTnC* is expressed in larval tail muscle. Full genomic sequence of the single *TnC* gene revealed that these two isoforms were generated by the alternative splicing of the third exon. The 5' upstream region (about 1 kb) of the *TnC* gene contains four E-box, and one M-CAT box sequences (Yuasa *et al*., 1997a).

TnI: *C. intestinalis* expresses a homologous set of shorter and longer TnI isoforms in body wall muscle and heart, respectively. These two TnI isoforms are identical except for a 47-residue near N-terminal insertion in the heart TnI. The sequence analysis of *Ciona* genomic DNA showed that the two isoforms were generated by alternative splicing from a single gene (MacLean *et al*., 1997). Although the vertebrate cardiac muscle TnI isoforms also have insertion sequences (25-55 aa) in their N-termini, the vertebrate skeletal muscle TnI isoform and cardiac muscle TnI isoform are encoded by distinct genes. Molecular phylogenical analysis of the vertebrates and ascidian *TnI* genes suggests that the gene duplications that established the TnI family occurred after the ascidian/vertebrate divergence (Hastings, 1997).

AdTnI, *LaTnIα* and *LaTnIβ*: Three distinct cDNAs (*AdTnI*, *LaTnIα* and *LaTnIβ*) of TnI isoforms from the ascidian *H. roretzi* were isolated. These three isoforms are encoded by different genes. *AdTnI* encodes a protein of 173 aa, and expressed in adult body wall muscle and adult heart muscle. *LaTnIα* and *LaTnIβ* encode 142 aa highly conserved proteins (96.5% identity) and those are the shortest of all known TnIs. Both *LaTnIα* and *LaTnIβ* are expressed in larval tail muscle. The ascidian adult and larval TnIs show 77-79% identity to each other, but show lower identity to vertebrate TnIs (52-59%) (Yuasa *et al*., 1997b).

Myogenic factors *AMD1*: *AMD1*, ascidian MyoD-related factor 1, was cloned (Araki *et al*.,1994). Amino acid identity within bHLH domain between the AMD1 protein and each of the other myogenic bHLH factors ranges from 76% for mammalian *MyoD1* to 63% for the protein encoded by *hlh-1* from *C. elegans*. *AMD1* transcripts were first detected at the 64-cell stage by RT-PCR (Araki *et al*., 1994) and 32-cell stage by *in situ* hybridization analysis (Satoh *et al*., 1996a). Because the *AMD1* is expressed zygotically, and zygotic transcripts of a muscle actin gene, *HrMA4*, are first detected at 32-cell stage, *AMD1* is not a muscle determinant itself and may be involved in maintaining the differentiation state of muscle cells (Araki *et al*., 1994).

CiMDFa and *b*: Another MyoD family gene *CiMDF* is isolated from *C. intestinalis*. *CiMDF* is a single-copy gene and gives rise to two differentially regulated transcripts. A 1.8 kb transcript (*CiMDFa*) appeared first and was gradually replaced by a 2.7kb transcript (*CiMDFb*) during early development. Furthermore, northern blots revealed both *CiMDFa* and *b* transcripts in adult body wall muscle (Meedel *et al*., 1997). Deduced proteins encoded by *CiMDFa* and *CiMDFb* shared identical Cys-rich/bHLH domain, but CiMDFa lacked the domain III which has been implicated in the effector function and is conserved among vertebrate MyoD (Schwarz *et al*., 1992). Cys-rich/bHLH domain of CiMDF is 71% identical with mouse MyoD and 90% identical with AMD1 (Meedel *et al*., 1997).

Mesenchyme

HrCA1: Mesenchyme cell-specific gene was first isolated as a cytoskeletal actin gene (see also cytoplasmic actin) from the *H. roretzi* embryo (Araki *et al*.,1996). The *HrCA1* coding region shares 15 of 20 diagnostic amino acid positions with human cytoplasmic β-actins. *HrCA1* is a single copy gene. *In situ* hybridization analysis revealed that transcripts of this gene are expressed predominantly in mesenchyme cells. In addition, at tailbud stage, faint signals were evident in notochord cells and some neuronal cells. *HrCA1* transcripts were found in every tissue of the adult examined, including the gill (branchial basket), body wall muscle, gonad, digestive gland and intestine. When *HrCA1* expression was examined in cleavage-arrested embryos, it was found only in mesenchyme-lineage blastomeres suggesting that *HrCA1* can be used as a marker of mesenchymal differentiation (Araki *et al*., 1996).

Epidermis

HrEpiA, *B*, *C*, *D*=*HrSEC61*, *E*, *F*, *G* and *H*: When *H. roretzi* embryos were continuously treated with cytochalasin B from the 1-cell stage, they developed into 1-cell arrested embryos which expressed epidermal antigens (Nishikata *et al*., 1987c). Epidermis specific genes, *HrEpiA* through *HrEpiH*, were isolated from a cDNA library derived from this 1-cell arrested embryo (Ueki *et al*., 1991). *HrEpiD* is an ascidian homologue of the mammalian and yeast *SEC61* genes, and was designated *HrSEC61* (Ueki and Satoh, 1994). *In situ* hybridization studies revealed that only *HrEpiE* was expressed transiently in presumptive neural cells whereas the others exhibited lineage-associated expression in presumptive epidermal cells (Ishida *et al*., 1996). Ueki *et al*. (1994) clearly showed the conspicuous autonomy of expression of ascidian epidermis-specific genes and suggested a self-sustainable developmental system of ascidian epidermal lineage cells.

Comparison of the 5' flanking regions of these epidermis specific genes gave insights into the tissue specific transcriptional control mechanism. They shared 19 motifs which contained conserved 6-bases sequences. Lac-Z analysis of *HrEpiB* and *HrEpiD* revealed that the 5' upstream sequence up to –345 and from –166 to +108, respectively, was sufficient for the epidermis-specific expression (Ueki and Satoh, 1995).

Hmserp1: Taking advantage of the differential display to compare expression patterns in unfertilized eggs, gastrula, neurula, tailbud and larval stages of *Herdmania momus*, *Hmserp1* was isolated (Arnold *et al*., 1997b). A 1.3 kb *Hmserp1* transcript was present in gastrula, neurula and tailbud stages and was most abundant in the neurula stage. This gene en-

codes a novel serine protease containing a single kringle motif and a catalytic domain. *Hmserp1* was expressed in cells of the differentiating epidermis but not in progenitors of the central nervous system (CNS).

HmEGFL-1: With the same strategy as for the *Hmserp1* (Arnold *et al*., 1997b), a single transcript of about 1.2 kb (*HmEGFL-1*) which expressed specifically during the late tailbud and larval stage was cloned (Arnold *et al*., 1997a). *HmEGFL-1* encodes 337 aa polypeptides with four EGF-like domains. The temporal expression pattern of *HmEGFL-1* was confirmed by the Northern and RT-PCR analyses. Strong expression of *HmEGFL-1* was localized throughout the papillae and anteriormost trunk and weaker expression in the epidermis of the remainder of the embryo. As the anterior portion of the *H. momus* larvae associated with the papillae is thought to be important as a signaling center for metamorphosis (Degnan *et al*., 1997), *HmEGFL-1* may have a role in signaling the initiation of metamorphosis (Arnold *et al*., 1997a).

Notochord

T-box family *As-T*: T-box genes encode proteins containing T-domain which is a DNA binding domain of *Brachyury* (*T*) gene products. Mouse *Brachyury* (*T*) is expressed in mesoderm and notochord cells, and implicated in mesoderm formation and notochord differentiation in mouse embryos. *As-T* was isolated as an ascidian homologue of the mouse *Brachyury* (*T*) gene from *H. roretzi* (Yasuo and Satoh, 1993). The expression of *As-T* is restricted to notochord-lineage cells, and the transcripts appear immediately after the restriction of their developmental fate to notochord (Yasuo and Satoh, 1993, 1994). The expression patterns of *As-T* suggested that the primary function of *Brachyury* is to specify embryonic cells to differentiate into notochord (Yasuo and Satoh, 1993).

Ci-Bra: *Ci-Bra* was isolated from a gastrula-stage *C. intestinalis* cDNA library. Sequence analysis indicates that the *CiBra* DNA binding domain shares about 70% amino acid identity with vertebrate *Brachyury* genes as well as *As-T*. *Ci-Bra* transcripts are first detected at the 64-cell stage and are expressed only in the notochord cells. A 434 bp enhancer from the *Ci-Bra* promoter region mediates the notochord-restricted expression of both GFP and *lacZ* reporter genes (Corbo *et al*., 1997b).

As-T2: *As-T2* is isolated as another T-box gene which encodes a divergent T-box protein (Yasuo *et al*., 1996). *As-T2* transcripts are detected in the muscle-lineage blastomeres and the caudal tip of the embryo (Yasuo *et al*., 1996). Although *As-T2* is not a notochord-specific gene, we dare to list it in this category, because it is closely related to *As-T*. To date, several T-box genes have been isolated from various vertebrates (e.g., Ryan *et al*., 1996). *As-T2* was the first example that more than one T-related gene participated in the mesoderm formation.

These studies about ascidian T-box genes demonstrated that the function of the T-box genes is conserved among chordates. The genes upstream and downstream of the T-box genes might also be conserved among chordates (see, Body Patterning). Furthermore, cellular interactions are proved to be required for the fate specification of notochord in ascidian embryos with a fine microsurgery technique (Nakatani and Nishida, 1994). Ascidian embryos offer an ideal experimental system for studying the cellular mechanisms of body patterning of chordates.

Neural tissues

Na^+-channel protein *TuNaI*: A neural tissue-specific gene was isolated as the Na^+-channel protein gene, *TuNaI* from *H. roretzi* (Okamura *et al*., 1991, 1994). *TuNaI* transcripts were detected in the cells of the peripheral and central nervous systems in tailbud and young tadpole embryo. *TuNaI* protein is 52% identical to rat brain type II Na^+ channel. The classical (e.g., Rose, 1939), histochemical (e.g., Nishida, 1991) and electrophisiological (e.g., Okado and Takahashi, 1988) studies about the inductive signals for the neural cell differentiation from endodermal lineage cells were confirmed with using *TuNaI* transcript as a marker for the neural cell differentiation (Okamura *et al*., 1994). Using *TuNaI* as a neuronal marker of the *Halocynthia* larva, Okada *et al*. (1997) identified two distinct lineages of neurons. One is the a-line-derived neurons which situated in the brain and in the trunk epidermis, and the other is the A-line-derived neurons which consist of motor neurons in the neck neural tube. The a-line cells are controlled by a mechanism similar to vertebrate neural induction, while the A-line cells develop without close association with the epidermal lineage cells.

β-tubulin *HrTBB1* and *HrTBB2*: Two β-tubulin cDNAs (*HrTBB1* and *HrTBB2*) were cloned with degenerate PCR from the *H. roretzi* early-tailbud embryo cDNA library (Miya and Satoh, 1997). These ascidian β-tubulin genes are highly conserved and their amino acid sequences are 91-98% identical to other invertebrate and vertebrate β-tubulins. The expression of *HrTBB1* is maternal, while *HrTBB2* is expressed both maternally and zygotically. The zygotic expression of *HrTBB2* starts at the neural plate stage and is restricted to the differentiating and established CNS and cells in papilla and the peripheral nervous system. Thus, *HrTBB2* will be a useful early molecular marker for neural cell differentiation in the ascidian embryo.

Pigment cells

Tadpole larvae of ascidians have two sensory pigment cells in the brain. One is the otolith cell that functions as a gravity receptor, while the other is part of the primitive photosensory structure and termed ocellus (Dilly, 1962, 1964). These sensory cells, like vertebrate pigment cells, contain membrane-bounded melanin granules.

Tyrosinase *tyrosinase*: Tyrosinase is a key enzyme in melanin biosynthesis and is also involved in the formation of the melanosome, a specialized membrane-bounded organelle required for melanin synthesis in vertebrate melanocytes. The amino acid sequence of *H. roretzi* tyrosinase gene is 36-39% identical to vertebrate tyrosinases (Sato *et al*., 1997). Ascidian tyrosinase gene is a single gene in the *H. roretzi* genome, the transcripts of which are first detected at the early neurula stage in pigment precursor cells and then in pigment cells of larvae.

CiTyr: A *C. intestinalis* cDNA clone that encodes a tyrosinase was also cloned (Caracciolo *et al*., 1997). *Ciona* tyrosinase appears to be about 100 amino acid residues longer in the C terminal region compared to the vertebrate and *H. roretzi* tyrosinases. The spatial and temporal expression patterns of *CiTyr* were almost the same as those of *H. roretzi* tyrosinases (Sato *et al*., 1997).

Tail region

HrPost-1: By isolating the tail region and the trunk region of the *H. roretzi* tailbud embryo, Takahashi *et al*. (1997a) constructed tail and trunk cDNA library. The screening with the subtracted tail-specific cDNA probe yielded various cDNA clones which expressed in a tissue-restricted manners. One of them, *HrPost-1*, encodes a novel, possible secreted protein. The *HrPost-1* transcript was first detected in the posterior vegetal portion of the gastrula-stage embryo. At the early-to-mid tailbud stage, *HrPost-1* was expressed in the epidermal cells of the tail region. In the same paper, they also described other 13 cDNA clones which represented intriguing expression pattern. Six of them expressed specifically in the tail muscle-cells, two expressed specifically in the trunk-lateral cells, two expressed in the tip of the tail epidermis, one expressed in visceral ganglion and epidermis, one expressed in muscle and CNS and one expressed in notochord and CNS.

ADULT GENES

Muscle

actins *SpMA1* (= *SpMA*), *McMA1* (= *McMA*) and *MocuMA2*: *SpMA1* and *McMA1* cDNAs were isolated from *S. plicata* and *M. citrina* adult mantle (Tomlinson *et al*., 1987b; Jeffery *et al*., 1990; Swalla *et al*., 1994). These transcripts were found in adult tissues (mantle, branchial sac, myocytes and mesenchyme cells) and in mesenchyme cells of late tailbud embryo. Although they do not express in larval muscle cells, using the coding regions of these cDNAs as probes, relationship between the mode of development and α-actin gene expression was investigated (Jeffery *et al*., 1990).

MocuMA2 gene encodes an adult muscle-type actin (Kusakabe *et al*., 1997). Sequence and molecular phylogenetic analyses of these adult muscle actin genes revealed that two different types of muscle actin, larval muscle and adult body wall muscle actins, were present (Kusakabe *et al*., 1997).

HR-29 *HR-29*: HR-29 protein was first described by Takagi *et al*. (1993), as an *H. roretzi* body wall muscle-specific 29 kDa band on SDS-PAGE. The HR-29 protein is found only in body wall muscles of the class Ascidiacea. Temporal and spatial expressions of *HR-29* have yet to be analyzed. The C-terminal region had significant homology with small heat-shock proteins (33% identical) and lens protein α-crystallines (37% identical). The N-terminal region showed no significant homology with other proteins. The genomic DNA sequence of HR-29 has been partially determined (Takagi *et al*., 1993).

entactin/nidogen *AsEnt1 antigen*: AsEnt1 antigen was identified as an antigen recognized by an antibody AsEnt1 (Nakae *et al*., 1993). The antigen localized on the basement membrane of ascidian body-wall muscle. On screening of the adult body wall muscle cDNA library with this antibody, cDNA clones encoding AsEnt1 antigen were isolated and their deduced amino acid sequence exhibited high similarity to mouse entactin and human nidogen. Entactin/nidogen is a component of the basement membrane and interacts with other basement membrane components, laminin and collagen IV (Fox *et al*., 1991).

tropomyosin *CTm1*: *CTm1* encoded *C. intestinalis* tropomyosin (Tm) which resembles the vertebrate-striated muscle Tm isoform (Meedel and Hastings, 1993). An identical isoform, derived from *CTm1*, is expressed at high levels in both body wall muscle and heart muscle. Southern blot analysis showed no evidence for additional *Tm* genes in *Ciona*, while vertebrates and *Drosophila* contain multiple genes of *Tm* family. The *CTm1* gene was suggested to be diverged from the ancestor of the vertebrate *Tm* genes before the duplication/divergence of the latter to establish the present day vertebrate *Tm* multigene family.

Ascidian muscle cells are classified into three types; non-striated, multinucleated adult body wall muscle cells, striated cardiac muscle cells, and striated, uni-nucleated larval tail muscle cells (Kalk, 1970; Terakado and Obinata, 1987). In vertebrate, the troponin/tropomyosin regulatory system is highly characteristic of striated muscle (Endo and Obinata, 1981). Ascidian body wall muscle contained troponin/tropomyosin while lacking the sarcomeric organization, and therefore may provide evolutionary insights into the relationship among the vertebrate striated and smooth muscles.

Some actins, MHC and MyoD genes expressed in the embryonic muscle cells are also expressed in the adult body wall muscle cells. *aTnT* is expressed specifically in the adult body wall (see, Embryonic Genes).

Pharyngeal-gill

HrPhG1 and *2*: Differential screenings of an *H. roretzi* pharyngeal-gill cDNA library with total endostyle cDNA probes and total pharyngeal-gill cDNA probes yielded cDNA clones for two pharyngeal-gill-specific genes, *HrPhG1* and *2* (Tanaka *et al*., 1996) . The transcripts of both genes were detected in the adult pharyngeal-gill specifically. Mean hydropathy profiles of HrPhG1 and 2 suggested both genes contained a predicted signal peptide sequence. This implied that these genes are secreted proteins.

Endostyle

HrEnds1 and *2*: At the time at which pharyngeal-gill-specific genes were isolated, endostyle-specific gene, *HrEnds1* and *2* cDNA were also isolated from *H. roretzi* (Ogasawara *et al*., 1996). The cells of ascidian endostyle differentiate into eight or nine strips or zones that run parallel to one another in a longitudinal orientation. The transcripts of both genes were detected in zone 6, in which cells have numerous secretory granules. *HrEnds1* and *2* also contained a predicted signal peptide sequence suggesting that they are secreted proteins.

BODY PATTERNING

Homeobox Genes *HrHlx* (= *AHox1*): Using a genomic

DNA probe of the *Antennapedia*-type homeobox of silk worm or sea urchin, *HrHlx* cDNA, originally named *AHox1*, was isolated from *H. roretzi* genome (Saiga *et al*., 1991; Satoh *et al*., 1996b). Comparison of the amino acid sequence of the HrHlx homeodomain with those of several representative *Drosophila* homeobox genes revealed that HrHlx homeodomain shows 70% similarity with *Drosophila* H2.0. Northern blot analysis detected the *HrHlx* transcripts in larvae, juveniles, and adult digestive tract, digestive gland, coelomic cell, endostyle, pharyngeal epithelium, gonad and body wall muscle. *In situ* hybridization with the 7-day-old juveniles showed that *HrHlx* transcripts were present in endodermal cells that were differentiating to form the epithelium of the digestive system (Saiga *et al*., 1991).

Hrlim: *Hrlim* was isolated from an *H. roretzi* fertilized egg cDNA library by screening with degenerate oligonucleotides complementary to the third helix of the *Antp* homeobox (Wada S *et al*., 1995). Sequence analysis revealed that *Hrlim* belongs to the LIM class homeobox gene containing two cystein/histidine-rich motifs, known as the LIM domain, in addition to a homeodomain. Maternal *Hrlim* transcripts exhibit weak localization in the anterior animal blastomere pair of the 8-cell embryo. Zygotic expression of *Hrlim* is first detected at the 32-cell stage and can be divided into two phases. In the first phase expression is detected in endoderm and notochord precursors. After gastrulation, *Hrlim* is expressed in specific subset of cells in the CNS.

HrHox-1, *-2*, *-4/7A*, *-4/7B* and *-10*: To date, five *Hox* genes have been isolated from *H. roretzi* (Katsuyama *et al*., 1995). *HrHox-4/7A* and *HrHox-4/7B* exhibited the same degree of similarity to members of paralogous subgroups 4 through 7. *HrHox-1* shows a high degree of similarity to the *labial* group *Hox* genes and is expressed on ectodermal tissues, epidermis and CNS of swimming tadpole larvae.

Hroth: *Hroth* gene was isolated as an ascidian homologue of *orthodenticle* (Katsuyama *et al*., 1996; Wada S *et al*., 1996). *Hroth* expression is first detected at the 32-cell stage in specific cells of both ectoderm and mesoderm lineages (Wada S *et al*., 1996). At the neurula and tailbud stages, *Hroth* is expressed in the anterior region of the neural fold, closing to form a neural tube. At the larval stage, expression of *Hroth* is observed in the sensory vesicle, surrounding two types of pigment cells, the otolith and the ocellus. *HrHox-1* and *Hroth* expression domains do not overlap. The space between these expression is maintained throughout the embryogenesis (Katsuyama *et al*., 1996). *Hroth* and *Hrlim* are co-expressed in endoderm precursors in the initial but not in the late gastrula (Wada S *et al*., 1996).

HrPax-37: The expression patterns of *HrPax-37* have been well analyzed by *in situ* hybridization (Wada H *et al*., 1996). Expression of *HrPax-37* is first detected at the early gastrula stage. At the early embryonic stage, *HrPax-37* transcripts are expressed in the neural and epidermal precursor cells. Although *HrPax-37* is also expressed in the dorsal epidermis, the expression patterns up to the neurula stage are similar to those of vertebrate *Pax-3* and *Pax-7* in the differentiating dorsal neural tube. So, *HrPax-37* is regarded as a descendant of the precursor which gives rise to *Pax-3* and *Pax-7* in vertebrates. At the tadpole larval stage, *HrPax-37* transcripts were expressed in neural tube, sensory vesicle and visceral ganglion. Injection of *HrPax37* RNA into fertilized eggs causes ectopic expression of the dorsal neural marker, tyrosinase gene, confirming a regulatory role in dorsal patterning of the neural tube comparable to its vertebrate homologues (Wada H *et al*., 1997).

Ciona msh: One *Ciona msh*-like homeobox sequence was amplified by PCR (Holland, 1991). Yet, the temporal and spatial expression patterns of this gene have yet to be analyzed.

CiHbox1 to *9*, *Ci-Dll-A* and *B*, *Ci-hlx-A*, *B* and *Ci-NK5*: Fourteen *Ciona* homeobox containing genes were identified by PCR amplification (Gregorio *et al*., 1995). Recently, using a polyclonal antibody against *Dll/Dlx* homeodomain (Panganiban *et al*., 1995), Dll protein was proved to expressed in the distal tip of the ampulla of *Molgula occidentalis* (Panganiban *et al*., 1997).

AHox2 and *AHox3*: Homeobox containing genomic fragments were cloned from *S. clava* (*AHox2*) and *S. plicata* (*AHox3*) (Ge *et al*., 1994). The homeodomain sequences deduced from these fragments were identical and *Antennapedia*-like.

MocuMsx-a and *McMsx*: The *MocuMsx-a* and *McMsx* genes were isolated from *M. oculata* and *M. citrina*, respectively (Ma *et al*., 1996). Based on similarities in and around their homeodomains, *Msx-a* genes were classified into members of the *msh*-like subclass of *Msx* genes. Southern blot analysis suggests that there are one or two copies of the *Msx-a* gene in the *Molgula* genome. *Msx-a* gene expression is restricted to the developmental stages. The cells expressing *Msx-a* gene are mesoderm and ectoderm cells undergoing morphogenetic movements during embryogenesis or mesodermal cells interacting with endodermal or epidermal epithelia during organogenesis.

PPax-6: The amino acid sequence identity within the paired domain (87%) and the homeodomain (95%) to *Aniridia* (human) and *Small eye* (mouse), and the conserved genomic organization throughout the known *Pax-6* genes suggest that *PPax-6* is orthologous to the known vertebrate and invertebrate *Pax-6* genes (Glardon *et al*., 1997). Expression of *PPax-6* is first detected at late gastrula stages in distinct regions of the developing neural plate. At the tailbud stage, it is expressed in the nerve cord and the brain vesicle, where the sensory organs form. Ectopic expression of the ascidian *Pax-6* gene in *Drosophila* leads to the induction of supernumerary eyes (Glardon *et al*., 1997) supporting the idea that the morphogenesis of the different type of eyes is controlled by a *Pax-6* dependent genetic pathway (Halder *et al*., 1995).

It has been suggested that a number of the homeobox genes are involved in spatial patterning along the body axis in metazoan embryos. Studies of the ascidian homeobox genes are very important for understanding the molecular mechanisms of regional specification in the development of ascidian embryo. Furthermore, they provide insights into the evolutional

history of the body plan of vertebrates.

The presence of a single set of clustered homeobox genes, the *Hox* cluster, in insects and four *Hox* clusters in mammals suggests that a primordial cluster must have undergone successive duplications. To date, there is no evidence for more than one homeobox gene clusters exist in the ascidian genome (Katsuyama *et al*., 1995; Gregorio *et al*., 1995). Studies on the ascidian homeobox gene cluster will help to establish whether such a duplication preceded the appearance of chordates.

forkhead/HNF-3β The notochord and dorsal ectoderm induce dorsoventral compartmentalization of the vertebrate neural tube through the differential regulation of genes such as *HNF-3β* (e.g., Sasaki and Hogan, 1994), *Pax3*, *Pax6* (e.g., Goulding *et al*., 1993) and *snail* (e.g., Mansouri *et al*., 1996).

Ci-fkh: *Ci-fkh* gene was cloned from *C. intestinalis* by Corbo *et al*. (1997a). Ci-fkh proteins include winged-helix domain which shows strong conservation with other members of the forkhead/HNF-3β family (about 90% identity with mouse HNF-3β). *Ci-fkh* is initially expressed in the presumptive CNS, notochord and gut. Expression becomes progressively restricted to notochord precursor cells during gastrulation. It is subsequently reactivated in the ventral layer of nerve cord underlying the notochord during neurulation.

MocuFH1: *MocuFH1*, a member of the *forkhead/HNF-3* gene family in *M. oculata*, is a single copy gene but there is at least one other related forkhead gene in the *M. oculata* genome (Olsen and Jeffery, 1997). *MocuFH1* first expressed in the presumptive endoderm, mesenchyme and notochord cells in late cleavage stage embryo. *MocuFH1* expression continues in the same lineages during gastrulation and neurulation. When the *MocuFH1* transcripts level was reduced during gastrulation by using antisense oligo nucleotides, some endoderm and notochord cells failed to enter the embryo, while the muscle precursor cells undergo involution.

HrHNF3-1: Ascidian homologue of class I *forkhead/HNF-3* gene was also cloned from *H. roretzi* (Shimauchi *et al*., 1997). *HrHNF3-1* expressed as early as the 16-cell stage in blastomeres of the endoderm, notochord and mesenchyme lineage. This early *HrHNF3-1* expression dose not require cell-cell interaction. *HrHNF3-1* expressed in the cells of ventral layer of the nerve cord, which is reminiscent of the floor plate of vertebrate embryo.

The Ci-fkh, MocuFH1 and HrHNF3-1 proteins show high conservation in their forkhead domains (96%; Ci-fkh/MocuFH1, 91%; Ci-fkh/HrHNF3-1) but show little conservation outside this region. Although, the expression patterns of *Ci-fkh*, *MocuFH1* and *HrHNF3-1* are slightly different, these expression patterns are similar to those of vertebrate counterparts, suggesting that *forkhead/HNF-3* genes have a fundamental role in organizing the body plan in chordates.

snail *Ci-sna*: Ci-sna proteins include five putative zinc fingers. *Ci-sna* is specifically expressed in muscle and trunk mesenchyme precursors, as well as cells of the lateral neural plate border (Corbo *et al*., 1997a). *Ci-sna* expression is lost from the descendants of these cells by the onset of neurulation. Promoter fusion genes of *Ci-fkh* and *Ci-sna* revealed that *Ci-fkh* and *Ci-sna* are expressed in the ventral and lateral ependymal cells, respectively. These expression patterns of *Ci-fkh* and *Ci-sna* in the neural tube are quite similar to those seen in vertebrates.

BMP *HrBMPa*: *HrBMPa* is an ascidian homologue of vertebrate *BMPs-5–8* which belong to the 60A subclass of BMPs (Miya *et al*., 1996). *HrBMPa* transcripts were first detected at the gastrula and not in unfertilized egg or early embryo. *HrBMPa* transcripts were evident in the anterior part of the CNS and the midline of both ventral and dorsal ectoderm in neurula and early tailbud embryos. The expression profile of *HrBMPa* suggested that it plays a major role in neuroectoderm cell differentiation and resembled that of *Xenopus* BMP-7.

HrBMPb: *HrBMPb* is an ascidian homologue of vertebrate *BMP-2/BMP-4* and *Drosophila decapentaplegic* (*dpp*) (Miya *et al*., 1997). The zygotic expression of *HrBMPb* was observed in some cells at the lateral edge of the nural plate through gastrula to neurula, but not in the presumptive epidermis. Overexpression of *HrBMPb* functions as a neural inhibitor and as an epidermal inducer but not as a ventralizing agent in ascidian development.

Notch *HrNotch*: HrNotch contains 33 EGF repeats, 3 Notch/Lin-12 specific repeats, a RAM domain, 6 ankyrin repeats and a PEST sequence (Hori *et al*., 1997). Molecular phylogenic analysis revealed that the *HrNotch* branched off before the vertebrate *Notch* genes diverged. Zygotic expression of *HrNotch* is predominant in the epidermal and neural cells in the neurula and tailbud embryo including the sensory pigment cell precursors which constitute an equivalence group. In the down stream of notch signaling, RBP-Jκ (Su (H)) is thought to be important as a transcription factor. Ascidian homologue of *RBP-Jκ* has already cloned (see Table 1).

MATERNAL GENES

The ascidian egg is regarded as a typical 'mosaic' egg: blastomeres isolated from early embryos differentiate into tissues according to their normal fates. Moreover, a specific region of the egg cytoplasm which segregated into a certain tissue was visible in the living embryo. Various descriptive and experimental studies have provided conclusive evidence for the presence of prelocalized cytoplasmic information or determinants responsible for the tissue differentiation. A well-known example of a cytoplasmic determinant is that responsible for muscle cell differentiation (eg., Nishida, 1992). The muscle determinants are sequestered into the so called myoplasm which forms a crescent in the posterior region after ooplasmic segregation (reviewed by Jeffery 1985; Uzman and Jeffery, 1986; Satoh *et al*., 1990; Swalla 1992; Nishida 1992; Satoh 1994).

ScYC1, *3*, *4*, *5* and *10*: When the ascidian eggs were homogenized in high salt conditions, they fractionated into cytoplasmic mass of the myoplasmic region and soluble fraction of the other region (Jeffery, 1984, 1985). RNAs were extracted from both fractions of *S. clava* fertilized egg. Differen-

Table 1. Genes isolated from ascidians

Gene categories and Gene names	Species	Gene Source	Reference
EMBRYONIC GENES			
Muscle			
muscle actin			
HrMA2	*Hr*	c/g	Kusakabe *et al*., 1992
HrMA4a	*Hr*	c/g	Kusakabe *et al*., 1991
HrMA4b	*Hr*	g (partial)	Kusakabe *et al*., 1992
HrMA5	*Hr*	g (partial)	Kusakabe *et al*., 1992
HrMA6	*Hr*	g (partial)	Kusakabe *et al*., 1992
HrMA1a	*Hr*	c/g	Kusakabe *et al*., 1995
HrMA1b	*Hr*	c/g	Kusakabe *et al*., 1995
ScTb1	*Sc*	c	Beach and Jeffery, 1992
ScTb12/34	*Sc*	c	Beach and Jeffery, 1992
ScTb24	*Sc*	c (partial)	Beach and Jeffery, 1992
ScTb30	*Sc*	c (partial)	Beach and Jeffery, 1992
MocuMA1	*Mocu*	g	Kusakabe *et al*., 1996
MoccMA1a, *b*	*Mocc*	g	Kusakabe *et al*., 1996
myosin heavy chain			
HrMHC1	*Hr*	c/g	Araki and Satoh, 1996
troponin			
aTnT2/aTnT19	*Hr*	c	Endo *et al*., 1996
eTnT11/14	*Hr*	c	Endo *et al*., 1996
AdTnC and *LaTnC*	*Hr*	c/g	Yuasa *et al*., 1997a
TnI	*Ci*	c/g (partial)	MacLean *et al*., 1997
AdTnI, *LaTnIα* and *LaTnIβ*	*Hr*	c	Yuasa *et al*., 1997b
myogenic factors			
AMD1	*Hr*	g	Araki *et al*.,1994
CiMDFa, *b*	*Ci*	c/g	Meedel *et al*., 1997
As-MEF2	*Hr*	c	Araki, 1995**
Mesenchyme			
HrCA1	*Hr*	c	Araki *et al*., 1996
Epidermis			
HrEpiA, *C*, *E*, *F*, *G*, *H*	*Hr*	c (partial)	Ueki *et al*., 1991
HrEpiB, *D*	*Hr*	c/g	Ueki *et al*., 1991
Hmserp1	*Hm*	c	Arnold *et al*., 1997a
HmEGFL-1	*Hm*	c	Arnold *et al*., 1997b
Notochord			
T-box family			
As-T	*Hr*	c	Yasuo and Satoh, 1993
As-T2	*Hr*	c	Yasuo *et al*., 1996
Ci-Bra	*Ci*	c	Corbo *et al*., 1997b
Neural Tissue			
Na^+-channel protein			
TuNaI	*Hr*	c	Okamura *et al*., 1994
β-tubulin			
HrTBB1 and *HrTBB2*	*Hr*	c	Miya and Satoh, 1997
Pigment Cell			
tyrosinase	*Hr*	c/g (partial)	Sato *et al*., 1997
CiTyr	*Ci*	c (partial)	Caracciolo *et al*., 1997
Tail region			
HrPost-1	*Hr*	c	Takahashi *et al*., 1997a
ADULT GENES			
Muscle			
actin			
SpMA1	*Sp*	c	Tomlinson *et al*., 1987a
McMA1	*Mc*	c (partial)	Swalla *et al*., 1994
MocuMA2	*Mocu*	g	Kusakabe *et al*., 1997
HR-29			
HR-29	*Hr*	c/g	Takagi *et al*., 1993
entactin/nidogen			
AsEnt1 antigen	*Hr*	c	Nakae *et al*., 1993
tropomyosin			
CTm1	*Ci*	c	Meedel and Hastings, 1993

Table 1. (continued)

Gene categories and Gene names	Species	Gene Source	Reference
Pharyngeal Gill			
HrPhG1, 2	*Hr*	c	Tanaka *et al.*, 1996
Endostyle			
HrEnds1, 2	*Hr*	c	Ogasawara *et al.*, 1996
BODY PATTERNING			
Homeobox Genes			
Halocynthia roretzi			
HrHlx (*AHox1*)	*Hr*	c	Saiga *et al.*, 1991
Hrlim	*Hr*	c	Wada S. *et al.*, 1995
HrHox-1	*Hr*	c	Katsuyama *et al.*, 1995
HrHox-2, -4/7A, -4/7B, -10	*Hr*	c (partial)	Katsuyama *et al.*, 1995
Hroth	*Hr*	c	Katsuyama *et al.*, 1996; Wada S. *et al.*, 1996
HrPax-37	*Hr*	c (partial)	Wada H. *et al.*, 1996
Ciona intestinalis			
Ciona msh	*Ci*	g (partial)	Holland, 1991
CiHbox1 to 9	*Ci*	g (partial)	Gregorio *et al.*, 1995
Ci-hlx-A, B	*Ci*	g (partial)	Gregorio *et al.*, 1995
Ci-Dll-A, B	*Ci*	g (partial)	Gregorio *et al.*, 1995
Ci-NK5	*Ci*	g (partial)	Gregorio *et al.*, 1995
hox5	*Ci*	c	Gionti, 1997**
other ascidians			
AHox2	*Sc*	c/g (partial)	Ge *et al.*, 1994
AHox3	*Sp*	g (partial)	Ge *et al.*, 1994
MocuMsx-a	*Mocu*	g (partial)	Ma *et al.*, 1996
McMsx	*Mc*	g (partial)	Ma *et al.*, 1996
PPax-6	*Pmam*	c/g	Glardon *et al.*, 1997
HcPou1	*Hc*	c	Kennett and Lavin, 1997**
APou1, Ahox2, 3, 4	*Hm*	c (partial)	Kennett, 1994**
Asox1, 2, 3	*Hm*	c (partial)	Degnan, 1994**
PmHox1, 5	*Pm*	c (partial)	Fujiwara, 1996**
PmHbox1, 2, 3, 4,	*Pm*	g (partial)	Fujiwara, 1996**
Pm-CAD, PmNkx	*Pm*	g (partial)	Fujiwara, 1996**
fork head/HNF-3β			
Ci-fkh	*Ci*	c/g	Corbo *et al.*, 1997a
MocuFH1	*Mocu*	c	Olsen and Jeffery, 1997
HrHNF3-1	*Hr*	c	Shimauchi *et al.*, 1997
snail			
Ci-sna	*Ci*	c/g	Corbo *et al.*, 1997a
BMP			
HrBMPa	*Hr*	c	Miya *et al.*, 1996
HrBMPb	*Hr*	c	Miya *et al.*, 1997
Notch			
HrNotch	*Hr*	c	Hori *et al.*, 1997
RBP-Jκ			
RBP-Jκ	*Hr*	c	Kawaichi, 1997**
MATERNAL GENES			
ScYC1, 3, 4, 5, 10	*Sc*	c (partial)	Swalla and Jeffery, 1995
myoplasmin-C1	*Ci*	c	Nishikata and Wada, 1996
pem (*posterior end mark*)	*Cs*	c	Yoshida *et al.*, 1996
pem-2, -4, -5 and *-6*	*Cs*	c	Satou and Satoh, 1997
Cymric (*Uro-1*)	*Mocu*	c	Makabe *et al.*, unpublished
lynx (*Uro-2*)	*Mocu*	c	Swalla *et al.*, 1993
Manx (*Uro-11*)	*Mocu*	c	Swalla *et al.*, 1993
PCNA			
ScYC26b	*Sc*	c	Swalla and Jeffery, 1996b
ribosomal protein L5			
ScYC26a	*Sc*	c	Swalla and Jeffery, 1996c

Table 1. (continued)

Gene categories and Gene names	Species	Gene Source	Reference
IMMUNITY			
C-type lectin			
TC14-1 and *-2*	*Pm*	c (partial)	Shimada *et al*., 1995
BSCLT	*Bs*	c	Pancer *et al*., 1997
complement-control protein			
Bs.1 and *Bs.2*	*Bs*	c (partial)	Pancer *et al*., 1995
mannan binding protein-associated serine protease			
AsMASPa and *b*	*Hr*	c	Ji *et al*., 1997
immunoreceptor			
A74 antigen protein	*Hr*	c	Takahashi *et al*., 1997b
PAR_BOTSC	*Bs*	c	Pancer *et al*., 1996a
antimicrobial peptides			
clavanin A, C, D and E	*Sc*	c	Zhao *et al*., 1997a
clavaspirin	*Sc*	c	Zhao *et al*., 1997a
styelin C, D and E	*Sc*	c	Zhao *et al*., 1997b
FKBP			
Bs.6	*Bs*	c	Pancer *et al*., 1993
serine protease			
CTRL-BOSCH	*Bs*	c (partial)	Müller *et al*., 1994
GLUCAGON			
pituitary adenylate cyclase-activating polypeptide			
pacap1, *2*	*Cp*	c/g (partial)	McRory and Sherwood, 1997
gastrointestinal hormon			
Cionin	*Ci*	c	Monstein *et al*., 1993
MOLECULAR PHYLOGENY			
rDNA and rRNA			
5S rRNA	*Hr*		Kumazaki *et al*., 1983 Komiya *et al*., 1983
18S rDNA	*As*, *Cs*, *Pj*, *Hr*, *Pmir*, *Pm*, *Sc*, *Sr*		Wada H *et al*., 1992
	Ac, *Bd*, *Cf*, *Dg*, *Ea*, *Mb*, *Mc*, *Mcom*, *Me*, *Mm*, *Mp*, *Mocd*, *Mocc*, *Mocu*, *Ms*, *Mt*, *Pc*, *Pp*, *Sm*, *Sp*,		Hadfield *et al*., 1995
18S rRNA	*Sc*		Field *et al*., 1988
28S rDNA	*Hm*		Degnan, 1990
	Bd, *Ea*, *Mb*, *Mc*, *Mcom*, *Me*, *Mm*, *Mp*, *Mocc*, *Mocd*, *Mocu*, *Ms*, *Mt*		Hadfield *et al*., 1995
satellite DNA			
147-bp repeat sequence	*Ps*	g	Kumar *et al*., 1988
Cla I repeat sequence	*Hm*	g	Degnan and Levin, 1995
Bs321, 531, 711, 721, 811	*Bs*	g	Pancer *et al*., 1994
PB29, 41, 49, C1, Bs811	*Bs*	g	Stoner *et al*., 1997
GENERAL			
aldehyde dehydrogenase			
Pm-aldh9	*Pm*	g (partial)	Harafuji *et al*., 1996
transglutaminase			
CiTGase	*Ci*	c	Cariello *et al*., 1997
ADP/ATP translocase			
HrcATL1	*Hr*	c	Miya *et al*., 1994
protein phosphatase			
StyPTP	*Sp*	c (partial)	Matthews *et al*., 1991
HcPP1	*Hc*	c	Kennett and Lavin, 1997**
trypsinogen			
TRY1_BS and *TRY2_BS*	*Bs*	c	Pancer *et al*., 1996c
TRYP1	*Bv*	c	Roach *et al*., 1997
proteasome β-subunit			
PRCE_BOTSC	*Bs*	c	Pancer *et al*., 1996d
Ci-zeta	*Ci*	c	Marino, 1997**

Table 1. (continued)

Gene categories and Gene names	Species	Gene Source	Reference
nuclear components			
Hgv2	*Hr*	c	Fujiwara *et al*., 1993
H3 and H4 histone gene	*Sp*	g	Ishaq *et al*., 1993
transcription factors			
ETS	*Sm*	g (partial)	Degnan *et al*., 1993
EB1_BOTSC	*Bs*	c	Pancer *et al*., 1996b
Phallusia FTZ-F1	*Pmam*	g (partial)	Escriva *et al*., 1997
Phallusia COUP-TF	*Pmam*	g (partial)	Escriva *et al*., 1997
Phallusia ERR1	*Pmam*	c (partial)	Escriva *et al*., 1997
RARs	*Pm*	c (partial)	Fujiwara, 1996**
intermediate filament			
SpIF	*Sp*	c (partial)	Jeffery *et al*., 1990
ScIF	*Sc*	c (partial)	Jeffery *et al*., 1990
cytoplasmic actin			
SpCA8	*Sp*	c	Kovilur *et al*., 1993
ScCA15	*Sc*	c	Beach and Jeffery, 1990
*HrCA1**	*Hr*	g	Kusakabe *et al*., 1997
cadherin			
BS-cadherin	*Bs*	c/g	Levi *et al*., 1997
HSP70			
HSP70.1 and *HSP70.2*	*Bs*	g	Fagan and Weissman, 1996
hsp70	*Ci*	c	Marino, 1997**
calmodulin			
calmodulin	*Hr*	c	Yuasa, 1997**
CaM	*Ci*	c	Branno, 1997**
ribosomal protein			
L21	*Ps*	g	Ricketts, 1992**
MITOCHONDRION			
cytochrome oxidase subunit I			
CO I	*Hr*	c	Yokobori *et al*., 1993
CO I	*Am*	g (partial)	Hart, 1995**
CO III	*Ps*	g	Durrheim *et al*., 1993
rDNA			
16S rDNA	*Ps*	g	Crafford, 1993**
transfer RNA gene			
tRNA-His	*Ps*	g	Crafford, 1993**
COSMID CLONE			
COS1, 2, 41 and 46	*Ci*	g	Bird *et al*., 1996, 1997**

**HrCA1* is the same gene as the mesenchyme specific *HrCA1*.

**These genes were directly submitted to GenBank.

Species: *Ac*, *Ascidia ceratodes* (S/U); *Am*, *Ascidia mentula* (S/U); *As*, *Ascidia sydneiensis samea* (S/U); *Bd*, *Bostrichobranchus digonas* (S/A); *Bs*, *Botryllus schlosseri* (C/U); *Bv*, *Boltenia villosa* (S/U); *Cp*, *Chelyosoma productum* (S/U); *Ci*, *Ciona intestinalis* (S/U); *Cs*, *Ciona savignyi* (S/U); *Cf*, *Cnemidocarpa finmarkiensis* (S/U); *Dg*, *Dendrodoa grossularia* (S/U); *Ea*, *Eugyra arenosa* (S/A); *Hr*, *Halocynthia roretzi* (S/U); *Hc*, *Herdmania curvata* (S/U); *Hm*, *Herdmania momus* (S/U); *Mb*, *Molgula bleizi* (S/A); *Mc*, *Molgula citrina* (S/U); *Mcom*, *Molgula complanata* (S/U); *Me*, *Molgula echinosiphonica* (S/U); *Mm*, *Molgula manhattensis* (S/U); *Mo, Molgula occidentalis* (S/U); *Mocc*, *Molgula occulta* (S/A); *Mocu*, *Molgula oculata* (S/U); *Mp*, *Molgula provisionalis* (S/A); *Ms, Molgula socialis* (S/U); *Mt*, *Molgula tectiformis* (S/A); *Pc*, *Pelonaia corrugata* (S/A); *Pj, Perophora japonica* (C/U); *Pmam*, *Phallusia mammillata* (S/U); *Pm*, *Polyandrocarpa misakiensis* (C/U); *Pp*, *Polycarpa pomaria* (S/U); *Pmir*, *Pyura mirabilis* (S/U); *Ps*, *Pyura stolonifera* (S/U); *Sc*, *Styela clava* (S/U); *Sm*, *Styela montereyensis* (S/U); *Sp*, *Styela plicata* (S/U); *Sr*, *Symplegma reptans* (C/U). S, solitary ascidian; C, colonial ascidian; U, urodele species; A, anural species.

Gene Source: c, cDNA; g, genomic DNA.

tial screen yielded five overlapping clones (*S. clava* YC clones; *ScYC1*, *3*, *4*, *5* and 10) encoding a 1.2-kb polyadenylated RNA. The nucleotide sequence of the longest clone, *ScYC10* contains a short open reading frame, and high AT contents, but similarity to other cloned ascidian mitochondrial genes suggest that it may be a 16S rRNA of the mitochondria (B. Swalla, personal comm.). YC RNA is localized in the cortex of postvitellogenic oocyte, in the myoplasm during ooplasmic segregation, in the muscle lineage cells during cleavage, and in differentiating primary and secondary muscle cells (Swalla and Jeffery, 1995).

myoplasmin-C1: Nishikata *et al*. (1987b) produced monoclonal antibodies which specifically recognize components of the myoplasm of *C. intestinalis* egg. One of the antigens, designated myoplasmin-C1, is a single 40-kDa polypeptide (Nishikata, 1991). When the myoplasmin-C1 antibody was injected into the egg, the differentiation of the tail muscle cell was specifically inhibited (Nishikata *et al*., 1987b). cDNA clones for *myoplasmin-C1* were obtained by screenings of the *Ciona* ovary cDNA library with this antibody (Nishikata and Wada, 1996). Sequence analysis of *myoplasmin-C1* cDNA revealed that it encoded a novel protein with long heptad repeats in both N- and C-termini. Moreover, extraction with Triton X-100 indicated that myoplasmin-C1 was bound to the egg cytoskeleton. These results suggest that myoplasmin-C1 interacts with other protein molecules and plays an important role in the anchorage and the precise distribution of the muscle determinants.

posterior end mark (*pem*): Centrifugation of unfertilized eggs of the ascidian *C. savignyi* gave rise to four types of fragments: a large nucleated red fragment, and small enucleated black, clear, and brown fragments (Marikawa *et al*., 1994). Several experiments suggested that the black fragment contained mRNAs responsible for the muscle and endoderm determinants and factors for embryonic axis formation (Marikawa *et al*., 1994, 1995). A cDNA clone, designated as *pem* (*posterior end mark*) was obtained by differential screening of black fragment and red fragment cDNA libraries (Yoshida *et al*., 1996). The amino acid sequence of the *pem* gene product showed no significant homology to known proteins. Its maternal transcript initially concentrates to the posterior-vegetal cytoplasm of the fertilized egg and later is restricted to the very narrow posterior-most region of the embryo. However, overexpression of this gene by microinjection of synthesized *pem* mRNA into fertilized eggs resulted in development of tadpole larvae with deficiency of the anteiormost adhesive organ, dorsal brain and sensory pigment-cells. This result suggests that *pem* plays a role in the patterning of the anterior and dorsal structures of the larva.

pem-2, *pem-4*, *pem-5* and *pem-6*: Novel maternal genes were cloned from a cDNA library of *C. savignyi* fertilized egg mRNAs subtracted with gastrula mRNAs (Satou and Satoh, 1997). All these mRNAs are localized in the posterior-vegetal cytoplasm of the egg, and they later marked the posterior end of early embryo. These localization patterns resemble that of *pem* (Yoshida *et al*., 1996). The *pem-2* transcript is about 3.3 kb length, and encodes a new member of CDC24 family (Satou and Satoh, 1997). The *pem-4* transcript is about 2.3 kb and is suggested to encodes transcription factor with C2H2-type zinc finger motifs. The *pem-5* and *pem-6* transcripts are 2.7 and 1.9 kb, respectively. The PEM-5 and PEM-6 have no significant homology to any known proteins. All transcript showing *pem*-like localization pattern share a six-bases motif "UUAUUU" in their 3' UTRs, though its importance is not clear.

Cymric (*Uro-1*), *lynx* (*Uro-2*) and *Manx* (*Uro-11*): *Cymric*, *lynx* and *Manx* genes were isolated in a subtractive screen of cDNA libraries designed to identify maternal genes expressed differentially in *M. oculata*, tailed (urodele) species and *M. occulta*, tailless (anural) species (Swalla *et al*., 1993; Swalla, 1996). Southern blots show that the *lynx* and *Manx* genes are present in both species, but the corresponding mRNAs are expressed preferentially in the urodele species gonad (Swalla *et al*., 1993).

The *Manx* gene is a single-copy gene encoding 2.0- and 2.3-kb mRNAs that are expressed both maternally and zygotically (Swalla *et al*., 1993). Zygotic *Manx* transcripts are present only between the late cleavage stage and neurula stage and accumulate in prospective notochord, neural tube, tail muscle and posterior ectoderm cells. The predicted Manx protein contains a nuclear localization signal and a zinc finger motif. Antisense oligodeoxynucleotide treatment inhibited *Manx* expression and urodele features in hybrid embryos, which suggests that *Manx* is required for development of the chordate larval phenotype in ascidians (Swalla and Jeffery, 1996a).

The *Cymric* and *lynx* genes appear to produce only maternal transcripts (Swalla *et al*., 1993). The predicted lynx protein contains a leucine zipper motif. *Cymric* gene encodes a putative tyrosine kinase with two SH2 domains (Swalla, 1996; K. Makabe, personal comm.). The Cymric and lynx proteins are predicted to function in a signal transduction cascade (Swalla, 1996; Jeffery, 1997).

PCNA *ScYC26b*: Swalla and Jeffery (1996b) screened an *S. clava* gonad cDNA library with the YC probe to identify maternal YC-related RNAs in ascidian eggs. They reported a cDNA clone, *ScYC26b,* encoding the ascidian proliferating cell nuclear antigen (PCNA). The maternal transcript of *ScYC26b* was localized in ectoplasm and depleted in the myoplasm. Zygotic *ScYC26b* was confined to the developing nervous system and was abundant even after the neural cells had ceased to proliferate. However, the report that the antisense of YC RNA was located in 3' end of ScYC26b clone is likely to be a cloning artifact (B. Swalla, personal comm.).

ribosomal protein L5 mRNA *ScYC26a*: Swalla and Jeffery (1996c) also reported a *ScYC26a* cDNA clone which had a long 5' non-coding sequence complementary to YC RNA and encoded the ribosomal protein L5. Northern blot hybridization showed that *S. clava* eggs and embryos contained maternal *ScYC26a* transcript and that zygotic *ScYC26a* transcript did not accumulate until after metamorphosis. *In situ* hybridization showed that maternal *ScYC26a* transcript was localized in the myoplasm and was segregated primarily to

the muscle cell lineages during embryogenesis.

IMMUNITY

C-type lectin *TC14-1* and *-2*: TC14 is a calcium-dependent, galactose-binding lectin from colonial species *Polyandrocarpa misakiensis*. (Suzuki *et al*., 1990). Its relative molecular mass is 14×10^3. TC-14 protein is expressed in the atrial epithelium and mesenchymal cells of the bud (Kawamura *et al*., 1991). Two closely related cDNA clones, termed *pTC14-1* and *-2*, were cloned (Shimada *et al*., 1995). Northern blot analysis revealed that the amount of *TC14-1* mRNA increases during bud development, and peaks at 36 hr after separation of the bud from the parental body wall. Changes in the intensity of the *TC14-2* signals were not obvious during bud development. The preliminary results of *in situ* hybridization suggested that *TC14-1* mRNA is expressed in the ampullae of bud primordia just after the initiation of bud outgrowth. For these lectins have considerable homology to the variable region of the Ig κ-chain (Suzuki *et al*., 1990), they intriguing not only for the developmental system but also for the evolution of the vertebrate immunity.

BSCLT: Another cDNA clone encoded a C-type lectin was cloned during the screening with the microsatellite sequence probe ($(GACA)_{13}(GTG)_{13}$) in the cDNA library from *Botryllus schlosseri* during alloimmune response (Pancer *et al*., 1997). BSCLT has both a C-type lectin domain and an Ig domain. This was the first report of a soluble lectin that features a complete Ig domain.

Compliment control protein superfamily *Bs.1* and *Bs.2*: Using degenerate primers of mammalian TNF-α and an allogenic rejection-cDNA library of colonial ascidian, *B. schlosseri*, PCR based cloning yielded two partial cDNA clones, *Bs.1* and *Bs.2* (Pancer *et al*., 1995). Deduced amino acid sequences of *Bs.1* and *Bs.2* have substantial similarity to mammalian complement proteins, selectins and apolipoprotein H, but not to TNF-α. They also have a somatomedin B-like domain in the C terminus.

Mannan binding protein-associated serine protease *AsMASPa* and *AsMASPb*: Using degenerate primers of serine protease domain of Bf/C2, Ji *et al*. (1997) tried to identify the C3-activating enzyme in ascidians. Contrary to their expectation, *AsMASPa* and *AsMASPb*, which encode mannan binding protein-associated serin protease (MASP) were cloned. MASPs have structural similarity to C1q and C1r/C1s and can activate the complement system in an antibody-independent fashion (Sato *et al*., 1994). Ji *et al*. (1997) suggested that no Bf/C2 amplification argue against the presence of Bf or C2 in ascidians. Transcripts of *AsMASPa* and *AsMASPb* are 3.2 kb and 3.6 kb, respectively, and predominantly expressed in the hepatopancreas.

Immunoreceptor A74 antigen protein gene: The A74 monoclonal antibody inhibits phagocytosis and aggregation of hemocyte of *H. roretzi* and the A74 antigen is a membrane glycoprotein with a molecular mass of 160 kDa (Takahashi *et al*., 1995). Using the degenerate primers against the N-terminal amino acid sequence, the cDNA coding for the A74 antigen was cloned. The A74 antigen has two immunoreceptor tyrosine-based activation motifs (ITAMs) and SH2/SH3 binding motifs in its intracellular domain. Taking advantage of the GST fusion proteins carrying the ITAM motifes of A74 antigen, each of the two ITAM motifs was proved to be tyrosine-phosphorylated by human c-Src kinase *in vitro*. Thus, the A74 antigen might be involved in the initial stage of signal transduction (Takahashi *et al*., 1997b).

PAR_BOTSC: During the screening of the peptide transporter genes (*TAP1/TAP2*), *PAR_BOTSC* (possible antigen receptor-like molecule of *B. schlosseri*) was cloned. *PAR_BOTSC* encodes 267 aa single polypeptide which have sequence similarity to the vertebrate soluble antigen receptors (Pancer *et al*., 1996a). This gene provide an insight into the evolution of the vertebrate antigen receptors.

Antimicrobial peptides clavanin and styelin: Clavanin A, B, C, D (Lee *et al*., 1997a) and E (unpublished) and styelin A and B (Lee *et al*., 1997b) are antimicrobial peptides purified from *S. clava*. cDNA which encode clavanin A, C, D and E and clavaspirin (newly identified distantly related clavanin isoform) (Zhao *et al*., 1997a) and Styelin C, D and E (Zhao *et al*., 1997b) were cloned. Northern blot analyses with clavanin A and styelin C probes revealed that these mRNAs were expressed in both hemocytes and pharyngeal tissue. Styelins are cecropin-like molecules, while clavanins are 23-aa histidine-rich α-helical peptide. These findings are important for the understanding the evolution of innate immunity.

FKBP *Bs.6*: cDNA clone (*Bs.6*) encoded a FK506 and rapamycin-binding protein (FKBP) was cloned during the screening with the microsatellite sequence probe ($(GA)_{21}$) in the cDNA library from *B. schlosseri* during alloimmune response (Pancer *et al*., 1993). *Bs.6* encodes 134 aa polypeptide which shares substantial amino acid-sequence identity with all the known single-domain FKBPs (e.g. human FKBP-13: 62%, human FKBP-12: 48%). As the human FKBP-12 and -13 have been proposed as a mediator of immunosuppressive reactions, Bs.6 may be an important link in the evolution of the immunosuppressive functions of the immunophilins.

Serine protease *CTRL-BOSCH*: A cDNA, *CTRL-BOSCH*, encoding a putative serine protease was isolated from the *B. schlosseri* entire colony cDNA library (Müller *et al*., 1994). It encoded 248 aa polypeptide which have considerable sequence identity to mammalian chymotrypsinogens (e.g. 46%: rat chymotripsinogen B precursor, 45%: dog chymotripsinogen). Although the *CTRL-BOSCH* mRNA was detected in the zooids not in the test material, protease activity in the test material (including blood vessels and test cells) was 3.7-fold higher than that in the zooid. Authors proposed that the biological role of this protease was in the defense mechanisms or in the allogenic and xenogeneic interactions.

Studies on the ascidian immune systems are important for understanding the origin of vertebrate complex immune system. In addition to the above mentioned molecular clonings, Pestarino *et al*. (1997) revealed that the *Styela plicata* IL-1 β is expressed in neural cells probed with the human IL-1 β

sequence.

GLUCAGON

Pituitary adenylate cyclase-activating polypeptide (PACAP) *pacap1* and *pacap2*: Two cDNAs and two partial genes which encode glucagon superfamily peptides were identified from *Chelyosoma productum* (McRory and Sherwood, 1997). One of them, *pacap1*, encodes a signal peptides, a growth hormone-releasing factor (GRF)-like peptide$_{1-27}$, and PACAP$_{1-27}$ which is 96% identical to human PACAP. The other, *pacap2*, encodes a signal peptides, cryptic peptide, another GRF-like peptide$_{1-27}$, and PACAP-like peptide$_{1-27}$. By an RT-PCR and an *in situ* hybridization, *pacap1* mRNA was detected specifically in the cells of neural ganglion but not in the neural gland. While, *pacap2* mRNA was detected in the neural ganglion, dorsal strand and intestine. These data provide insights in the glucagon superfamily evolution and the evolution of the nervous system and the endocrine system of vertebrates.

Gastrointestinal hormone Cionin: Cionin was first identified as an unique octapeptide from the neural ganglion of *C. intestinalis* (Johnsen and Rehfeld, 1990). It has the same C-terminal active site as those of mammalian chorcystokinin (CCK) and gastrin. 3' and 5' RACE with using synthetic oligonucleotides based on the cionin peptide sequence revealed the pre-procionin cDNA sequence. The deduced 128 amino acid pre-procionin have some similarities to preproCCK. Northern blot analysis detected the cionin mRNA from the gastrointestinal tract corresponding to the cionin expression in both the neural ganglion and the intestinal tract. Immunological and biochemical studies revealed that the gastrointestinal cionin was less processed than the neuronal cionin (Monstein *et al*., 1993).

MOLECULAR PHYLOGENY

rDNA: An entire rDNA tandem repeat that includes the coding region of 18S, 5.8S and 26S rRNA was isolated from *H. momus* (Degnan *et al*., 1990). Comparison of *rDNA* primary sequence and *rRNA* secondary structures from *H. momus* with those from other organisms, demonstrated that the ascidians are more closely related to other chordates than invertebrates.

5S and 18S rRNA: Direct sequencing of rRNA was applied to the pioneer works of molecular phylogeny. *H. roretzi* 5S rRNA was used for the comprehensive analysis of the evolutionary relationships among distantly related organisms (Kumazaki *et al*., 1983; Komiya *et al*., 1983; Hori and Osawa, 1987). *S. clava* 18S rRNA was also used for molecular phylogenetic analysis of metazoans (Field *et al*., 1988).

18S and 28S rDNA: Owing to the PCR method, elucidation of the genomic sequence of rRNA gene is more accessible. Almost the entire sequence of 18S rDNA was applied to the phylogenetic comparison from advanced invertebrates through primitive chordates. The deuterostome group closest to vertebrates was the cephalochordates. Ascidians, larvaceans, and salps seem to form a discrete group (Wada and Satoh, 1994). The phylogenetic tree deduced from the central region of about 1000 bp of the 18s rDNAs suggests that the three species of Enterogona and the five spices of Pleurogona examined form discrete and separate groups irrespective of their potential to form colonies (Wada H *et al*., 1992). Phylogenetic analyses of the central region of the 18S rDNA and hypervariable D2 loop of 28S rDNA from 21 species of the families Ascididae, Styelidae, and Molgulidae suggest that anural development evolved independently in styelid and molgulid ascidians and is also polyphyletic in the Molgulidae (Hadfield *et al*., 1995).

147-bp repeat sequence: When *Pyura stolonifera* total DNA was digested with *Hin*dIII, approximately 150bp satellite band was appeared (Kumar *et al*., 1988). Cloning and sequencing of the fragments revealed a highly conserved 147-bp AT-rich (more than 80%) sequence. This sequence is tandemly repeat up to 20 kb in size and represents more than 5% of the total genomic DNA. The transcripts hybridizing to this sequence is present in unfertilized egg but not in the adult organism. The slot blot analysis with variety of ascidian DNAs represents that this repeat sequence was widely distributed in Ascidiacea.

*Cla*I satellite sequence: When the *H. momus* forma *curvata* genome was digested with *Cla*I, at least 10 distinct satellite bands were generated. One of them was non-rDNA, a 663 bp unique satellite sequence, while the other were ribosomal. The 663 bp *Cla*I satellite sequence is 54.2% AT-rich and contains many small repeats. This sequence was present only in the genome of *H. momus* forma *curvata* not in *H. momus* forma *grandis*. This molecular evidence, in addition to the reproductive and developmental differences, indicated the presence of strong barriers to gene flow between these two forms (Degnan and Lavin 1995).

microsatellite DNA: In order to study the allorecognition mechanism of colonial ascidians, population genetic molecular markers are necessary. Two independent groups cloned several loci of polymorphic microsatellite sequences from *B. schlosseri* by using specific primer sets. For example, Bs811 locus has 17 alleles with their size ranging from 238 bp to 332 bp and its core repeat sequence is uninterrupted $(AG)_{40}$ (Pancer *et al*., 1994). Another locus, PB29, has three alleles of 167 bp, 168 bp and 171 bp and its core repeat sequence is $(GTT)_5$ $(GTG)_5$ $(GTT)_1$ $(CTT)_1$ (Stoner *et al*., 1997). Some of these polymorphic loci, including PB29, follow a strict Mendelian pattern of segregation. PB29 and some other Mendelian loci are suitable for the population genetics and phylogenetic analysis (Stoner *et al*., 1997).

GENERAL

Aldehyde dehydrogenase *Pm-aldh9*: Retinoic acid can induce a secondary axis in the asexual developing bud of the *P. misakiensis* (Hara *et al*., 1992). The activity of aldehyde dehydrogenase (ALDH) was detected in the bud and the ALDH protein purified from this species could convert retinal to retinoic acid (Kawamura *et al*., 1993). Two genomic fragments encoding ALDHs, designated as *Pm-aldh9* and *Pm-aldh24*, were cloned by degenerate PCR (Harafuji *et al*., 1996). Amino acid

sequences deduced from *Pm-aldh9* showed high similarity to mouse retinaldehyde dehydrogenase (AHD2) and that from *Pm-aldh24* showed similarity to ALDHs of wide variety of organisms from vertebrate through bacteria. Northern blot analysis revealed that the expression of these genes was not changed throughout bud development.

Transglutaminase *CiTGase*: Transglutaminase (TGase) catalyzes the post-translational modification of proteins. *Ciona* TGase (*CiTGase*) gene was cloned by the screening with the degenerate oligonucleotide coding for the conserved amino acid sequence of the TGase active site (Cariello *et al*., 1997). The amino acid sequences of CiTGase was about 36% identical to other TGase sequences and the putative catalytic center was conserved. The *CiTGase* mRNA appeared in mesenchyme cells at gastrula stage and in primordial muscle cells from neurula to late tailbud stage.

ADP/ATP translocase *HrcATL1*: The ADP/ATP translocase is the most abundant integral protein of the inner mitochondrial membrane and is encoded by nuclear DNA. *HrcATL1* cDNA encodes an ADP/ATP translocase of *H. roretzi* (Miya *et al*., 1994). A large amount of *HrcATL1* mRNA was present in the unfertilized eggs, and was expressed not only in the embryo but also in all adult tissues. Although, in the ascidian embryo, mitochondria are predominantly segregated into muscle lineage precursor cells, the difference in the amount of mRNA for *HrATL1* between blastomeres of animal and vegetal hemisphere of the 8-cell embryos was less obvious.

Protein phosphatase *StyPTP*: Using PCR technique, 27 distinct cDNA sequences which contained PTPase domain were amplified (Matthews *et al*., 1991).

Trypsinogen *TRY1_BS* and *TRY2_BS*: Using a degenerate primer for the serine active site amino acid sequence which is fully conserved in all trypsins (Müller *et al*., 1993), two cDNAs encoding putative different trypsinogens were cloned from *B. schlosseri* rejection cDNA library (Pancer *et al*., 1996c). Both TRY1_BS and TRY2_BS are 243 amino acid long polypeptides. According to the comparison of the amino acid patterns in activation peptides and the numbers of the disulfide bridges in trypsinogens, the TRY1_BS sequence was suggested more closely related to the invertebrate sequence than those of vertebrates.

TRYP1: Ascidian trypsinogen cDNA (*TRYP1*) was isolated from *Boltenia villosa* intestine cDNA library with degenerate serine protease primers (Roach *et al*., 1997). It contains all of the important sequence features of a trypsinogen, such as the six cysteine residues absolutely conserved in all vertebrate trypsins, and possesses overwhelming similarity to the known trypsinogens. Authors made phylogenetic analysis to the vertebrate trypsinogens.

Proteasome β-subunit *PRCE_BOTSC* and *Ci-zeta*: The proteolytic core complex of proteasome, so called 20S proteasome, is a cylindrical particle consisting of four rings, each of which is organized from seven homologous, but not identical, α and β subunits (e.g., Lupas *et al*., 1993). The 20S proteasome has been implicated in processing for MHC class I-restricted antigen presentation (Rock *et al*., 1994). Upon stimulation with interferon-gamma, β-subunits of 20S proteasome are replaced by homologous β-type subunits X (also designated as epsilon, PRCC, PRCE and LMP7), Y (also designated as delta, PRCD and LMP2), and Z (also designated as MECL1). Pancer *et al*. (1996d) and Marino (see, Table 1) cloned the PRCE_BOTSC (cDNA encoded X subunit of *B. schlosseri*) and *Ci-zeta* (cDNA encoded Z subunit of *C. intestinalis*), respectively. As the X and Y subunits are encoded within the MHC class II region and Z subunit is encoded outside the MHC in the vertebrate genome, these protochordate homologues may offer some insights into the origin of the MHC.

Nuclear components *Hgv2*: A monoclonal antibody, Hgv-2, was raised against germinal vesicles of the *H. roretzi*, and its 83-kDa antigen, Hgv2, was found in the interphase nuclei of embryonic cells but not in those of juveniles (Fujiwara and Satoh, 1990). Fujiwara *et al*. (1993) isolated *Hgv2* cDNA clone and revealed that Hgv2 was closely related to the amphibian karyophilic histone-binding protein N1.

H3 and H4 histone genes: Ascidian H3 and H4 histone genes were isolated from *S. plicata* sperm genome (Ishaq *et al*., 1993). Sequence analysis of entire region containing H3 and H4 histone genes indicates that the two genes are transcribed in opposite directions and have structural similarities to cell-cycle dependent histones. H3 and H4 histone genes are represented approximately five times per haploid genome. The G+C content of the third base codon position for the *S. plicata* H3 and H4 genes is 60%, and the A content is 21%. This pattern is more similar to the pattern found in invertebrates (Ishaq *et al*., 1993).

Transcription factors *ETS*: Ets-family members of transcription factors are defined by the presence of the highly conserved ETS DNA-binding domain corresponding to approximately 80 amino acid residues. Using degenerate PCR primers for ETS domain, a 159 bp cDNA fragment of ETS domain was cloned from *Styela montereyensis* (Degnan *et al*., 1993).

EB1_BOTSC: During the screening of the peptide transporter genes (TAP1/TAP2), *EB1_BOTSC* (*B. schlosseri* EB1 homologue) was cloned by degenerate PCR. EB1_BOTSC is 209 aa and identical with 48% of the amino acid residues in human EB1 (Pancer *et al*., 1996b). In mammals it appears that impairment of the interaction between APC (adenomatous polyposis coli) and EB1 could result in tumorgenesis (Su *et al*., 1995). Recently, APC is suggested to play an important role in Wnt signaling (reviewed by, Cadigan and Nusse, 1997). A high degree of conservation between human and ascidian EB1 homologues is indicative of an essential regulatory mechanism within chordates.

Phallusia FTZ-F1, *COUP-TF* and *ERR1*: Fragments of nuclear receptor genes, including FTZ-F1 (fushi-tarazu factor 1), COUP-TF (chicken ovalbumin upstream-promoter transcription factor), and ERR (estrogen-related receptor) were amplified from *Phallusia mammillata* genomic DNA by using PCR techniques (Escriva *et al*., 1997).

Intermediate filament *SpIF* and *ScIF*: *SpIF* and *ScIF*

were isolated as homologues to the vertebrate Type III intermediate filament (IF) from *S. plicata* and *S. clava*, respectively (Jeffery *et al*., 1990). Southern blot analysis suggested that *SpIF* was a single-copy gene. Northern blot analysis revealed that *ScIF* mRNA was absent in eggs and cleaving embryos and accumulated after gastrula stage. *In situ* hybridization analysis revealed that *ScIF* transcripts were expressed in epidermis, neural tube, mesenchyme and muscle, but not in endoderm and notochord in larval development. And *ScIF* transcripts were expressed in test, mesenchyme and muscle cells in adult ascidians.

Cytoplasmic actin *SpCA8*: *SpCA8* was isolated as *Styela plicata* mRNA for cytoplasmic actin. The complete sequence of *SpCA8* was determined (Kovilur *et al*., 1993).

ScCA15: The derived amino acid sequence of *ScCA15* most closely resembled vertebrate β-actin. Maternal *ScCA15* mRNA is distributed uniformly in the cytoplasm of the oocyte and unfertilized egg. After ooplasmic segregation, it appears translocated into the ectoplasm, and then segregates into epidermal and neural precursor cells. Zygotic transcripts start to accumulate from the neurula stage in the epidermal and neural cells. In the young adults, *in situ* hybridization revealed that *ScCA15* transcripts were expressed strongly in the alimentary tract, the ovaries, the testes, and the endostyle (Beach and Jeffery, 1990 ; Jeffery *et al*., 1990).

HrCA1: Genomic sequence of *HrCA1* gene revealed by Kusakabe *et al*. (1997: See mesenchyme-specific genes).

Cadherin *BS-cadherin*: Using a differential display of mRNA expressed in *B. schlosseri* under allogeneic noncompatible conditions compared with mRNA expressed in naive parts of the same genotype, BS-cadherin was cloned as the mRNA which specifically expressed in a colony undergoing allogenic rejection processes (Levi *et al*., 1997). Low percentage of identity of BS-cadherin to known cadherins, intron-less genomic structure of BS-cadherin and molecular phylogenic study suggest that BS-cadherin is ancestral to classical cadherins type I and type II.

HSP70 *HSP70.1* and *HSP70.2*: A genomic library of *B. schlosseri* was screened with a probe specific for the *Xenopus HSP70* N-terminal portion (Fagan and Weissman, 1996). Two intronless genes (*HSP70.1* and *HSP70.2*) were cloned. These two genes are highly conserved both in the coding region (94%) and in the 5' and 3' flanking region (83% and 82%, respectively). These genes were heat inducible. *HSP70.1* and *HSP70.2* are good candidates for protochordate homologues of the MHC-linked HSP70 genes of vertebrates.

MITOCHONDRIA

Cytochrome oxidase subunit I *CO I*: Cytochrome oxidase subunit I (CO I) gene of mitochondria was isolated from an ascidian, *H. roretzi* (Yokobori *et al*., 1993). The amino acid sequence comparison of CO Is revealed that codons AGA and AGG are read as glycine in ascidian mitochondria.

CO III: Cytochrome oxidase subunit III (CO III) gene of mitochondria was isolated from *Pyura stolonifera*. Codons AGA and AGG are read as glycine also in this species (Durrheim *et al*., 1993).

COSMID CLONES

Sequences of *C. intestinalis* cosmid clones were directly submitted to GenBank by an Italian group. Several coding sequences, including homeobox gene and forkhead-like gene, are found in these sequences (see, Table 1).

ASCIDIAN GENES ON WWW

Today, with the popularization of the internet, databases of genetic resources are more accessible than ever. For example, the entire nucleotide sequence of any known gene can be obtained by accessing the GenBank (http://ncbi.nlm.nih.gov/Entrez/medline.html). For searching ascidian genes, "As-Genes (http://devl.bio.konan-u.ac.jp/as-genestitle.html)" is more informative. As-Genes contains nucleotide sequence, temporal and spatial pattern of expression, and summarized feature of each gene. The abstract and summarized result of related references are also available in "As-Genes". The complete reference list of this paper is also available on "As-Genes".

CONCLUSION AND PERSPECTIVE

In this review, we listed all the genes that have been cloned and reported at the end of 1997. We omitted some molecular biological studies on ascidian genes, whose sequence data were not available or available only with small PCR fragment. For example, purified acetylcholinesterase mRNA was tested for its translational capacity by microinjection into *Xenopus laevis* oocytes (Meedel and Whittaker, 1983). This paper was one of the pioneer works of ascidian molecular biology and revealed the temporal expression pattern of a single gene.

Ascidians have long been offering excellent model systems for experimental biology. Furthermore, advancements in molecular biological techniques and the accumulation of basic information about ascidian genes increase the importance of the ascidians as a good experimental system for molecular biological study. For example, as mentioned previously in this review, *As-T* and *As-T2* genes will shed light on the molecular mechanisms underlined the mesoderm formation in vertebrate and on the evolutionary process of chordates.

In addition, the haploid genome in ascidians is estimated to include about 1.8×10^8 nucleotide pairs and may contain 10,000-20,000 different genes (Mirsky and Ris, 1951; Atkin and Ohno, 1967; Lambert and Laird, 1971). This is only 5-6% of the size of the human haploid genome. The relatively small sized genome, in addition to the importance for the experimental system, makes the ascidians one of the most suitable animals in which to study the complete genome.

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