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# Construction of a cDNA Microarray Derived from the Ascidian *Ciona intestinalis*

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**ABSTRACT**—A cDNA microarray was constructed from a basal chordate, the ascidian *Ciona intestinalis*. The draft genome of *Ciona* has been read and inferred to contain ~16,000 protein-coding genes, and cDNAs for transcripts of 13,464 genes have been characterized and compiled as the "*Ciona intestinalis* Gene Collection Release I". In the present study, we constructed a cDNA microarray of these 13,464 *Ciona* genes. A preliminary experiment with Cy3- and Cy5-labeled probes showed extensive differential gene expression between fertilized eggs and larvae. In addition, there was a good correlation between results obtained by the present microarray analysis and those from previous EST analyses. This first microarray of a large collection of *Ciona intestinalis* cDNA clones should facilitate the analysis of global gene expression and gene networks during the embryogenesis of basal chordates.

**Key words:** ascidian, *Ciona intestinalis*, cDNA microarray, gene expression, development

## INTRODUCTION

Ascidians or tunicates (the subphylum Urochordata) are basal chordates, and they share a common ancestor(s) with vertebrates. Ascidians provide an appealingly simple experimental system for investigating the molecular mechanisms that underlie cell-fate specification during development (Satoh, 1994; Corbo *et al*., 2001; Satoh, 2001; Satoh *et al*., 2003). First, the ascidian tadpole is composed of only ~2600 cells, which constitute a small number of organs including epidermis, central nervous system (CNS), endoderm and mesenchyme in the trunk, and notochord and muscle in the tail. Extensive information on the cell lineages of most major tissues and organs of ascidians is available (Conklin, 1905; Nishida, 1987). Second, the blastomeres of early ascidian embryos are large and easy to manipulate (Nishida, 2002), and permit detailed visualization of changes in gene expression during development (Satoh, 2001). Third, *Ciona* embryogenesis is rapid (about 18 hr elapses from fertilization to a free-swimming tadpole at 18°C) and the entire life cycle takes less than 3 months, facilitating genetic analyses (Nakatani *et al*., 1999; Sordino *et al*., 2000). Fourth, novel functions of developmental genes can be determined by misexpressing or overexpressing a variety of regulatory genes that encode transcription factors (Takahashi *et al*., 1999) or signaling molecules (Imai *et al*., 2002) and by functional suppression of genes with morpholino oligonucleotides (Satou *et al*., 2001a). Finally, transgenic DNAs can be introduced into developing embryos using simple electroporation methods (Corbo *et al*., 1997) that permit simultaneous transformation of hundreds of synchronously developing embryos, facilitating studies of transcriptional regulation.

*Ciona intestinalis* is a cosmopolitan species that spawns all year round and is used by researchers worldwide. Very recently, the draft genome of *Ciona intestinalis* has been determined, and its ~160 Mbp genome is estimated to contain ~16,000 protein-coding genes (Dehal *et al*., 2002), similar to the number in other invertebrates, but

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only half that found in vertebrates. It has been proposed that large-scale gene duplications occurred in the vertebrate lineage after the divergence from cephalochordates and urochordates (Holland *et al.*, 1994). The ascidian, however, has a basic, non-duplicated set of the chordate-type genome (Dehal *et al.*, 2002).

In addition, we are now performing an in-depth cDNA analysis of *Ciona intestinalis* that includes the generation of expressed sequence tags (ESTs), the collection and sequencing of a non-redundant set of cDNAs, and the clarification of their expression profiles (Satoh *et al.*, 2003; Satou *et al.*, 2002a). Six cDNA libraries were made from fertilized eggs (Nishikata *et al.*, 2001), cleaving embryos (Fujiwara *et al.*, 2002), tailbud embryos (Satou *et al.*, 2001b), larvae (Kusakabe *et al.*, 2002), young adults (Ogasawara *et al.*, 2002), and the testes of mature adults (Inaba *et al.*, 2002). We then generated ESTs from both the 5' and 3' ends from ~90,000 cDNA clones. The total number of 3' ESTs was 81,637, and they were grouped into 13,464 independent cDNA clusters. The 13,464 clones were re-arrayed in 36 384-well plastic dishes for release as "*Ciona intestinalis* Gene Collection Release I (CiGCR1)" (Satou *et al.*, 2002b). This number of cDNAs corresponds to ~80% of all protein-coding genes of this ascidian. The 5' ESTs were searched against the DDBJ/Genbank/EMBL database to obtain information about the proteins the genes encode. In addition, ~10,000 cDNAs have been sequenced to determine their full-length insert. All of the *Ciona intestinalis* cDNA information can be seen at our website http://ghost.zool.kyoto-u.ac.jp/indexrl.html.

Given the present research circumstances described above, application of DNA microarray strategies may facilitate the identification of developmental genes with novel functions and characterization of gene networks in *Ciona intestinalis*. DNA microarrays have been used to analyze gene expression profiles during developmental stages in *Caenorhabditis elegans* (Hill *et al.*, 2000), *Drosophila melanogaster* (Furlong *et al.*, 2001; Arbeitman *et al.*, 2002), the zebrafish (Ton *et al.*, 2002), and the mouse (Miki *et al.*, 2001). To date, however, no DNA microarray containing an array of cDNAs or oligo DNAs corresponding to all the protein-coding genes of a vertebrate species is available. Our present study was therefore an attempt to construct a microarray of ascidian cDNAs containing a large number of cDNAs corresponding to ~80% of all protein-coding genes of this ascidian.

## MATERIALS AND METHODS

### Biological materials

*Ciona intestinalis* were cultivated at the Maizuru Fisheries Research Station of Kyoto University, Maizuru, Kyoto, Japan. Adults were maintained under constant light to induce oocyte maturation. Eggs and sperm were obtained surgically from the gonoduct. After insemination, eggs were washed and maintained in seawater at room temperature (18–20°C). Larvae hatched at about 17 hr of development.

### Construction of cDNA microarray

CiGCR1, which contains a set of 13,464 unique *Ciona* genes, was used. Clones were obtained from the 384-well plates and cDNA inserts were amplified by PCR using Accu Taq LA DNA polymerase (Sigma-Aldrich,USA) in a 20-μl reaction mixture. PCR products were purified using Corning FilterEXTM 384-well filter plates (Corning, USA). The quality and quantity of purified PCR products were confirmed using agarose gel electrophoresis. More than 90% of the clones gave a single band. Purified PCR products were dried and resuspended in 10 μl of distilled water, and then mixed with 10 μl of spotting solution (Microarray Crosslinking Reagent D, Amersham Biosciences, USA). cDNA solutions were spotted onto silianized microarray slides (type 7 Star, Amersham Biosciences) using a capillary pen type arrayer (Gene III , Amersham Biosciences). cDNA spotted slides were then exposed to 50mJ of 254nm light to crosslink DNA on slides. Each slide was spotted with duplicate sets of about 4,500 unique cDNAs on the left- and right-hand sides of the array; in total 13,396 cDNAs were spotted on four slides in duplicate. Lambda phage DNA (Takara, Japan) was used as an external control gene and was spotted onto each array block (32×12) with more than one spot. Spotting solution without DNA was spotted as a negative control on each block with at least 14 spots. This cDNA microarray was termed "*Ciona intestinalis* cDNA chip version 1 (Ci cDNA chip ver. 1)".

### Preparation of probes and hybridization to cDNA microarray

Total RNAs were isolated from fertilized eggs and larvae by ultracentrifugation in a cesium trifluoroacetate gradient, and mRNA was extracted using an mRNA purification kit (Amersham Biosciences). The RNA quality was verified by electrophoresis using an Agilent 2100 bioanalyzer (Agilent Technologies, USA). Labeling and hybridization were performed as follows: first-strand cDNA probes were generated by incorporation of 50 μM of either Cy3-dCTP or Cy5-dCTP (Amersham Biosciences) during reverse transcription with 400 units of SuperScript II (Gibco-BRL, USA), each 1 μg of mRNA, 200 pg of lambda polyA$^+$ RNA-A (Takara) as external control, 4 μg of random hexemer (Invitrogen, USA), and 1 μg of oligo-dT in a total volume of 20 μl reaction mixture at 42°C. After the reaction, residential RNAs were degraded and neutralized, and the resulting cDNA probes purified using a Cyscribe GFX purification kit (Amersham Biosciences), according to the manufacturers protocol, and then concentrated using a vacuum centrifuge dryer. The labeling reaction efficiency of each cDNA probe was confirmed by spectrophotometry, measuring the absorbance at 260nm, 550nm and 650nm. One-fourth of the labeled probe mixture was used for hybridization of one arrayed slide, and the four slides of one set were hybridized with the same probes and under the same conditions. Hybridization reactions were performed using a manufacturer's hybridization buffer (Amersham Biosciences) containing formamide for 16 hr at 55°C in an air phase incubator. After hybridization, the slides were washed in 2X SSC/0.1% SDS for 10 min, followed by washing in 1X SSC/0.1% SDS for 5 min each at 55°C, then in 0.1XSSC at room temperature to remove SDS. Spinning at low speed in a centrifuge for 1 min dried the slides.

### Scanning and data processing

The slides were scanned using a GenePix4000B DNA microarray scanner (Axon Instruments, USA) at ~635 nm (Cy5) and then at ~532 nm (Cy3). The voltage of both channels of the photo multiplier tubes were adjusted to obtain a ratio value of 1.0 for the external control spots. The resulting fluorescent intensities for each spot were quantified by an algorithm for adaptive circle segmentation of an image, and signal intensities calculated from the value of the median of the background intensities subtracted from the median of the intensities of each pixel ([F532 Median] - [B532] or [F635 Median] - [B635]) using GenePix Pro 4.0 microarray analysis software (Axon Instruments). Two independent hybridization exper-

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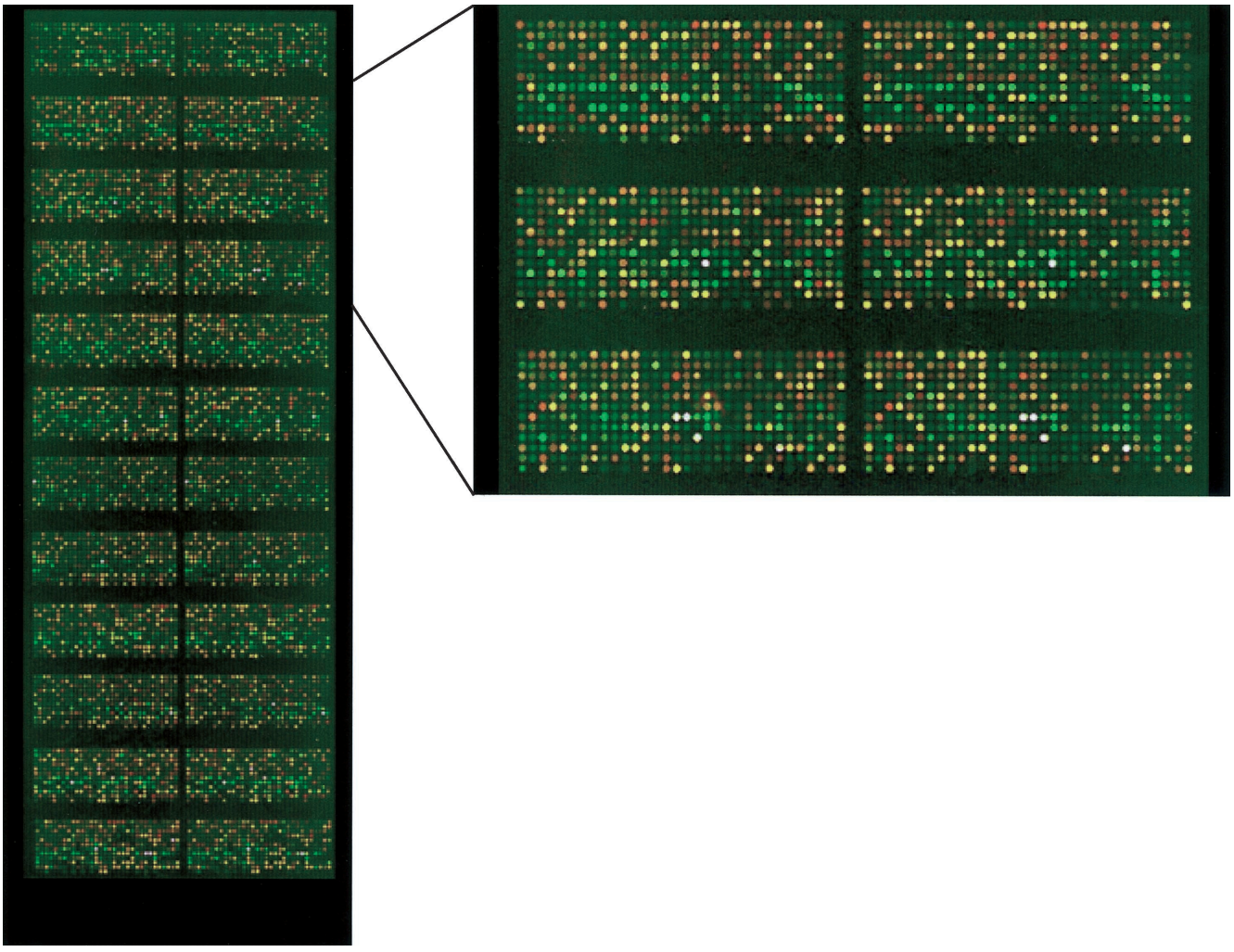


**Fig. 1.** The "*Ciona intestinalis* cDNA chip version 1 (Ci cDNA chip ver. 1)": a first-generation cDNA microarray with 13,394 PCR products derived from "*Ciona intestinalis* Gene Collection Release I". In total 13,396 kinds of cDNA were divided and spotted on four slides. A color-image of a slide of the four Ci cDNA chip ver. 1 are shown in Fig. 1, one slide was spotted with duplicate sets of about 4,500 unique cDNAs on the left- and right-hand sides of the array. Colors indicate the hybridization to each probe: green spots indicate up-regulated in larvae, red up-regulated in fertilized eggs, and yellow almost equally expressed at both stages.

iments, each using one set of slides, were performed. An intensity-dependent normalization (non-linear of LOWESS normalization) method (Clevel and Devlin, 1988) was applied to normalize the data from replicate experiments using Genespring software version 5.0 (Silicon Genetics, USA). The fluorescence units of each duplicate spot on each slide were averaged and the ratio value then calculated; so that the signal intensity of each tested sample (larvae sample) was divided by that of the control sample (fertilized egg sample). We labeled the same RNA samples with different dyes to evaluate the variation of the results depending on the labeled dye bias; and also to determine cutoff values for up- or down-regulated genes. Under our experimental conditions, we set the significance values at >2.0 for up-regulated genes and <0.5 for down-regulated genes, respectively. The processed overlay image of a hybridized slide is shown in Fig. 1.

## RESULTS AND DISCUSSION

DNA microarray analysis is a powerful technology for global gene expression profiling. The recent accumulation of huge amounts of data on *Ciona intestinalis*, in the form of EST analyses and cDNA clone collections, led us to successfully construct an ascidian microarray containing 13,396 cDNAs. To assess the qualities of the *Ciona* cDNA microarray, we used it to investigate transcriptional differences between fertilized eggs and larvae. The mRNAs obtained from larvae and fertilized eggs were used to prepare cDNA probes differentially labeled with Cy3 and Cy5, and hybridized to the Ci cDNA chip ver. 1. In total 13,396 cDNAs were divided and spotted on four slides. Each slide of four of Ci cDNA chip ver. 1 was spotted with duplicate sets of about 4,500 unique cDNAs on the left- and right-hand sides of the array. As we have shown in a color-image of the slide of the four in Fig. 1, the signal profiling of both sides exhibited the same patterns, and the fluorescent intensities derived from duplicate spots were also almost the same. An average of ratio value of lambda phage DNA controls which were spotted elsewhere onto four slides was 1.01±0.1 (n=152). These

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data indicated that the *Ciona* cDNA microarray provides reproducible results.

We spotted cDNA onto mirror-type slides (type 7 Star, Amersham); this type of slide gave a stronger signal intensity than those of the usual clear glass because the fluorescence derived from each spot was reflected on the surface of the mirror and with high efficiency detected by the scanner. Also, this slide shows the lower background signals. We detected fluorescent signals derived from around 70% of the 13,396 spots of the *Ciona* arrays, even though we used probes prepared from 0.25 μg of each mRNA for hybridization to one slide. To determine the significant cutoffs for up-regulated genes in fertilized eggs and in larvae, we first labeled mRNAs from the fertilized eggs with both dyes and then hybridized them to the arrays. The results of hybridization yielded a linear scatter plot, with almost all of the spots lying within the range of a twofold difference (Fig. 2A). On the basis of this result, we chose >2.0 as the cutoff value for up-regulated genes in larvae and <0.5 for genes specifically expressed in fertilized eggs. As shown in Fig. 2B, experiments with fertilized egg samples and larvae samples showed variations. A total of 2,400 genes exhibited signal intensity >2.0 (up-regulated in larvae), and 2,100 genes exhibited intensity <0.5 (up-regulated in fertilized eggs).

Next, we compared the microarray data to data from EST analyses that have already been included in the *Ciona* cDNA database (http://ghost.zool.kyoto-u.ac.jp/indexr1.html). The cDNA libraries of *Ciona* fertilized eggs (Nishikata *et al.*, 2001) and larvae (Kusakabe *et al.*, 2002) are neither normalized nor amplified. Therefore, the number of ESTs (hereafter we call this the EST count) corresponds to the quantity of at least the majority group of the mRNAs in the library. In other words, we can determine whether the gene is expressed either in eggs or in larvae or in both. Table 1 summarizes the 15 cDNAs with highest expression ratios as determined by the microarray analysis. Table 1 includes, for each cDNA, information about the EST count of the corresponding gene at the larval stage and fertilized egg stage, and the gene product predicted by BLASTX search. As is evident in Table 1, all of the cDNAs had large EST counts at the larval stage, but no or very small EST counts at the fertilized egg stage. For example, the ID00617 gene, which encodes a protein with sequence similarity to a mouse alpha tectorin, scored the ratio (larvae/eggs) of 240 in the array data, and its EST count was 119 in larvae and 2 in fertilized eggs. Another example is the ID00244 gene, which encodes a protein with sequence similarity to a mouse polydomain protein, and had a ratio (larvae/eggs) of 223 in the array data, while its EST count was 118 in larvae and 0 in fertilized eggs. The other 13 cDNAs showed similar results. Therefore, the quantitative expression of genes assessed by the array analysis corresponds to that assessed by EST analysis. Strictly speaking, the microarray ratio does not always show one-to-one relationship with the ratio determined by EST analysis, but in general the array ratio shows fairly good accordance with EST counts.

On the other hand, Table 2 summarizes the 15 cDNAs with the lowest array ratios (larvae/eggs). The occurrence of large EST counts in eggs clearly indicates that all of the genes are expressed maternally. The data shown in Table 2 also show a distinct co-relationship between the array ratio and EST count as to the qualitative expression of genes. For

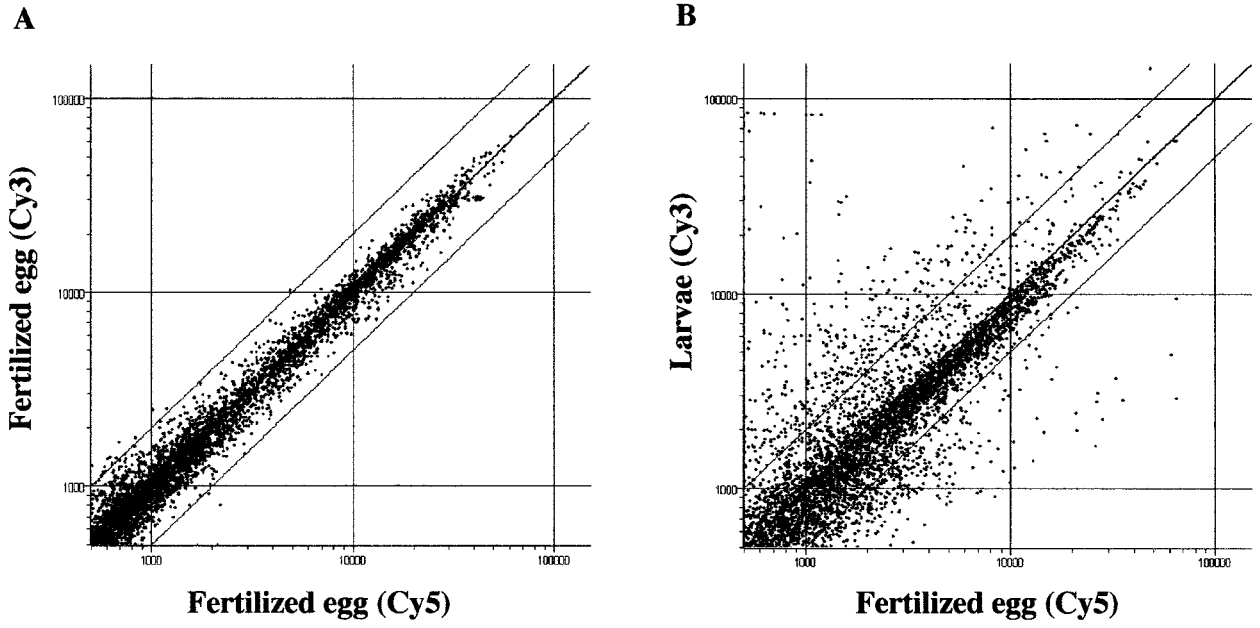



**Fig. 2.** (A) Scatter plot of fluorescent signal intensity from a hybridization of fertilized eggs in both the Cy3 and Cy5 channels to the Ci cDNA chip ver. 1. Lines show two-fold different expression in both channels. (B) Scatter plot of fluorescent signal intensity from a hybridization to the Ci cDNA chip ver. 1 of larvae and fertilized eggs with Cy3- and Cy5-labeled probes, respectively. Lines show twofold different expression in both channels. Spots with background-subtracted intensity lower than 500 in either the Cy3 or Cy5 channel were filtered out.

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**Table 1.** List of genes of which expression is up-regulated in larvae.

| Clutster ID | clone ID | Ratio LV/FE | Larvae(LV) /24532 EST clones | Fertilized eggs (FE) /29444 EST clones | Accession No | Data Base entry name | Organism | p |
|---|---|---|---|---|---|---|---|---|
| 00617 | cicl05f19 | 240 | 119 | 2 | CAA68138 | alpha tectorin | *Mus musculus* | 2e-16 |
| 00244 | cilv06e24 | 223 | 118 | 0 | BC008135 | polydomain protein | *Mus musculus* | 4e-13 |
| 00056 | cilv12n18 | 207 | 135 | 0 | AAA60229 | Pur protein | *Homo sapiens* | 1e-37 |
| 00171 | cilv30p03 | 194 | 75 | 0 | BAA23597 | CsEpi-1 | *Ciona savignyi* | 9e-74 |
| 00419 | cilv22b11 | 143 | 363 | 0 | BAA23647 | BAI 1 | *Homo sapiens* | 1e-18 |
| 00035 | cilv15p02 | 119 | 313 | 0 | AAA18911 | ribosomal protein L8 | *Xenopus laevis* | 1e-53 |
| 00093 | ciad01j05 | 81.7 | 44 | 0 | BAA36973 | alpha 1 type I collagen | *Cynops pyrrhogaster* | 1e-26 |
| 00065 | cilv10c11 | 75.4 | 1488 | 0 | BAA08112 | nonmuscle actin | *Halocynthia roretzi* | 2e-53 |
| 02124 | cilv14a06 | 60.5 | 51 | 1 | AAB33312 | Tamm-Horsfall protein (THP) | *Bos taurus* | 1e-18 |
| 00087 | cicl22b06 | 59.6 | 395 | 1 | AAA50025 | elongation factor 1-alpha | *Danio rerio* | 4e-34 |
| 00578 | cilv14e05 | 59.2 | 70 | 2 | AAA48675 | HMW/LMW collagen subunit precursor | *Gallus gallus* | 3e-22 |
| 00837 | cilv34n07 | 47.6 | 336 | 1 | CAB90289 | alpha 5 type IV collagen | *Homo sapiens* | 1e-51 |
| 01325 | cilv04i10 | 39.0 | 42 | 1 | – | | | |
| 00605 | cilv02p13 | 37.0 | 34 | 0 | CAB88872 | serine protease | *Anopheles gambiae* | 4e-16 |
| 00031 | cilv01j06 | 31.7 | 55 | 0 | AAB30787 | superfast myosin light chain-2 (MyLC2) | *Felis catus* | 2e-51 |

**Table 2.** List of genes of which expression is up-regulated in fertilized eggs.

| Clutster ID | clone ID | Ratio LV/FE | Larvae(LV) /24532 EST clones | Fertilized eggs (FE) /29444 EST clones | Accession No | Data Base entry name | Organism | p |
|---|---|---|---|---|---|---|---|---|
| 00585 | cieg07e03 | 0.08 | 3 | 112 | AAH02983 | similar to gene rich cluster, C9 gene | *Homo sapiens* | 1e-24 |
| 00032 | cicl10b06 | 0.11 | 16 | 136 | AAD22962 | claudin-1 | *Homo sapiens* | 1e-16 |
| 06444 | cieg28d21 | 0.11 | 0 | 19 | BAA91002 | unnamed protein product | *Homo sapiens* | 2e-16 |
| 02605 | ciad79i24 | 0.12 | 2 | 39 | AAF46189 | CG3184-PA | *Drosophila melanogaster* | 1e-63 |
| 02257 | citb10p18 | 0.12 | 0 | 20 | BAB33089 | hypothetical protein | *Macaca fascicularis* | 1e-24 |
| 01544 | cicl20p10 | 0.18 | 2 | 33 | BAA11926 | posterior end mark | *Ciona savignyi* | 4e-19 |
| 01137 | cieg01m07 | 0.19 | 0 | 78 | 1D8J | GENERAL TRANSCRIPTION FACTOR TFIIE-BETA | *Homo sapiens* | 1e-16 |
| 00734 | cicl01d09 | 0.19 | 0 | 77 | CAA45968 | cyclin B1 | *Mus musculus* | 1e-18 |
| 03719 | cieg22g14 | 0.20 | 0 | 21 | – | | | |
| 04422 | cicl08a24 | 0.23 | 0 | 25 | AAC18425 | NAD+-dependent isocitrate dehydrogenase | *Bos taurus* | 1e-40 |
| 06573 | cieg09n14 | 0.24 | 0 | 38 | BAB93517 | OK/SW-CL.48 | *Homo sapiens* | 1e-85 |
| 05917 | cicl15d13 | 0.27 | 2 | 29 | AAB84279 | OmpR | *Proteus vulgaris* | 1e-61 |
| 04248 | cicl52k02 | 0.30 | 0 | 20 | AAD09339 | NAD+-specific isocitrate dehydrogenase beta subunit isoform A | *Homo sapiens* | 2e-41 |
| 03061 | cieg08k24 | 0.32 | 1 | 37 | BAB28333 | unnamed protein product | *Mus musculus* | 1e-19 |
| 01271 | cieg0402 | 0.32 | 2 | 20 | CAA18425 | cell division control protein 18 | *Schizosaccharomyces pombe* | 1e-19 |

example, the ID00585 gene scored a ratio (larvae/eggs) of 0.08 in the array data, while the EST count was 112 in fertilized eggs and 3 in larvae. The array ratio of the ID00320 gene was 0.11, while the EST count was 136 in fertilized eggs and 16 in larvae. Therefore, as in the case of genes that are up-regulated at the larval stage, the quantitative expression of down-regulated genes assessed by the microarray analysis coincided with that assessed by EST analyses. Interestingly, five genes listed in Table 2 encode proteins with sequence similarity to vertebrate or *Drosophila* counterparts, but whose function is not known. Because these genes show dramatic changes in the quantity of their transcripts during embryogenesis, their roles should be elucidated in further studies.

Finally, Table 3 lists 15 cDNAs whose array ratio fell in the range of 2.0-0.5. Namely, these are cDNAs for genes whose expression was at the same level at both the fertilized egg stage and larval stage. As is evident in this Table, most of the genes showed similar EST counts at both the fertilized egg stage and larval stage.

All of the data described above clearly demonstrate that the quantitative expression of *Ciona* developmental genes can be assessed using cDNA microarrays and that the expression measured by the array analysis corresponds well with that deduced from EST analyses. Therefore, it can be concluded that the *Ciona intestinalis* cDNA microarray

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**Table 3.** List of genes which show non-differential expression.

| Clutster ID | clone ID | Ratio LV/FE | Larvae(LV) /24532 EST clones | Fertilized eggs (FE) /29444 EST clones | Accession No | Data Base entry name | Organism | p |
|---|---|---|---|---|---|---|---|---|
| 01501 | cicl20d13 | 1.35 | 80 | 86 | AAD24490 | phosphate transporter precursor | *Drosophila melanogaster* | 1e-63 |
| 00555 | ciad37a13 | 1.29 | 25 | 22 | AAC46585 | ribosomal protein DL11 | *Drosophila melanogaster* | 1e-72 |
| 00774 | cieg54d17 | 1.26 | 19 | 14 | AAA93261 | zinc finger protein C2H2-25 | *Homo sapiens* | 2e-17 |
| 00289 | cicl17o21 | 1.14 | 23 | 17 | CAB96129 | putative claudin | *Halocynthia roretzi* | 5e-20 |
| 01777 | ciad02b22 | 1.13 | 16 | 23 | AAB48626 | ribosomal protein L27 homolog | *Caenorhabditis elegans* | 1e-37 |
| 00051 | ciad06k02 | 1.08 | 18 | 14 | AAA42291 | tropomyosin TM4 | *Rattus norvegicus* | 1e-16 |
| 01786 | cieg01c10 | 1.05 | 28 | 24 | AAF50751 | CG10576-PA | *Drosophila melanogaster* | 1e-19 |
| 01131 | cilv10l18 | 1.01 | 119 | 94 | AAF52454 | CG10203-PA | *Drosophila melanogaster* | 1e-25 |
| 00086 | cieg57m19 | 0.88 | 84 | 81 | AAG15316 | beta tubulin | *Notothenia coriiceps* | 1e-71 |
| 00754 | cieg34k18 | 0.86 | 32 | 33 | BAB23963 | unnamed protein product | *Mus musculus* | 1e-41 |
| 00264 | cieg14o23 | 0.78 | 15 | 14 | BAA22933 | proteasome p45/SUG | *Rattus norvegicus* | 8e-93 |
| 03297 | cicl46l24 | 0.78 | 15 | 14 | P30153 | serine/threonine protein phosphatase 2A | *Homo sapiens* | 1e-70 |
| 01175 | citb07m06 | 0.73 | 22 | 21 | BAB02430 | nucleolar protein | *Arabidopsis thaliana* | 1e-30 |
| 02686 | ciad20i18 | 0.72 | 27 | 19 | CAB56042 | glycine rich RNA binding protein | *Ciona intestinalis* | 3e-40 |
| 03319 | cieg49o23 | 0.71 | 18 | 12 | AAA40884 | catalase (EC 1.11.1.6) | *Rattus norvegicus* | 1e-63 |

prepared in the present study is of a high enough quality so that it can be used for further analysis of the gene expression profiles.

## PERSPECTIVE

As described above, a cDNA microarray based on CiGCR1 was constructed. EST projects as well as cDNA projects of *Ciona intestinalis* are progressing. By adding new data for transcripts expressed in gastrulae/neurulae and those expressed in the gonad (ovary), endostyle (the primordium of vertebrate thyroid gland), neural complex, heart, and blood (coelomic) cells of the adult, the present data set consists of more than 480,000 ESTs (Satoh *et al*., 2003). The ESTs are categorized into 17,834 independent cDNA clusters. An additional 4,370 cDNA clones identified from the second series are ready for release as " the supplement of CiGCR1". Therefore, a total of 17,834 independent cDNA clones are now available as a *Ciona* gene resource. BLAST search suggests that this cDNA resource covers ~85% of the transcripts expressed in this ascidian. Along with these advances in *Ciona* cDNA projects, we are now making a "*Ciona intestinalis* cDNA chip version 2", which includes all cDNAs including "the supplement of CiGCR1", on a single slide. Furthermore, "*Ciona intestinalis* oligo chip version 1" can also now be prepared custom-made (Agilent Technologies). Despite their relatively high position in animal phylogeny, ascidians show several characteristic phenomena (Sawada *et al*., 2001). Some live as colonial ascidians, formed by budding. They synthesize cellulose-like carbohydrates in the tunic. Some ascidians extensively accumulate heavy metals such as vanadium from seawater. It is to be expected that complex gene networks are involved in each of the biological activities mentioned above. Therefore, it is intriguing to ask what kinds of gene networks are responsible for various biological phenomena in the basal chordates. Furthermore, reading of the *Ciona intestinalis* draft genome has been completed (Dehal *et al*., 2002). Thus, the results of analyses using the cDNA microarray can be interpreted using various sources of genetic information. The present construction of a cDNA microarray may therefore enormously facilitate research on the genes and gene networks of *Ciona intestinalis*.

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## REFERENCES

Arbeitman MN, Furlong EE, Imam F, Johnson E, Null BH, Baker BS, Krasnow MA, Scott MP, Davis RW, White KP (2002) Gene expression during the life cycle of *Drosophila melanogaster*. Science 297: 2270–2275

Clevel WS, Devlin SJ (1988) Locally-weighted regression: an approach to regression analysis by local fitting. J Amer Statis Assoc 83: 596–610

Corbo JC, Levine M, Zeller RW (1997) Characterization of a notochord-specific enhancer from the Brachyury promoter region of the ascidian, *Ciona intestinalis*. Development 124: 589–602

Corbo JC, Di Gregorio A, Levine M (2001) The ascidian as a model organism in developmental and evolutionary biology. Cell 106: 535–538

Conklin EG (1905) The organization and cell lineage of the ascidian egg. J Acad Nat Sci (Philadelphia) 13: 1–119

Dehal P *et al*. (2002) The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. Science 298: 2157–2167

Fujiwara S, Maeda Y, Shin-i T, Kohara Y, Takatori N, Satoh N (2002) Gene expression profiles in *Ciona intestinalis* cleavage-

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stage embryos. Mech Dev 112: 115–127
Furlong EEM, Andersen EC, Null B, White KP, Scott MP (2001) Patterns of gene expression during *Drosophila* mesoderm development. Science 293: 1629–1633
Hill AA, Hunter CP, Tsung BT, Tucker-Kellogg G, Brown EL (2000) Genomic analysis of gene expression in *C. elegans*. Science 290: 809–812
Holland PWH, Garcia-Fernodez J, Williams NA, Sidow A (1994) Gene duplications and the origins of vertebrate development. Development Suppl: 125–133
Imai KS, Satoh N, Satou Y (2002) Early embryonic expression of *FGF4/6/9* gene and its role in the induction of mesenchyme and notochord in *Ciona savignyi* embryos. Development 129: 1729–1738
Inaba K, Padma P, Satouh Y, Shin-i T, Kohara Y, Satoh N, Satou Y (2002) EST analysis of gene expression in testis of the ascidian *Ciona intestinalis*. Mol Reprod Dev 62: 431–445
Kusakabe T, Yoshida R, Kawakami I, Kusakabe R, Mochizuki Y, Yamada L, Shin-i T, Kohara Y, Satoh N, Tsuda M, Satou Y (2002) Gene expression profiles in tadpole larvae of *Ciona intestinalis*. Dev Biol 242: 188–203
Miki R *et al*. (2001) Delineating developmental and metabolic pathways *in vivo* by expression profiling using RIKEN set of 18,816 full-length enriched mouse cDNA arrays. Proc Natl Acad Sci USA 98: 2199–2204
Nakatani Y, Moody R, Smith WC (1999) Mutations affecting tail and notochord development in the ascidian *Ciona savignyi*. Development 126: 3293–3301
Nishida H (1987) Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. III. Up to the tissue restricted stage. Dev Biol 121: 526–541
Nishida H (2002) Patterning the marginal zone of early ascidian embryos: localized maternal mRNA and inductive interactions. BioEssays 24: 613–624
Nishikata T, Yamada L, Mochizuki Y, Satou Y, Shin-i T, Kohara Y, Satoh N (2001) Profiles of maternally expressed genes in fertilized eggs of *Ciona intestinalis*. Dev Biol 238: 315–331
Ogasawara M, Sasaki A, Metoki H, Shin-i T, Kohara Y, Satoh N, Satou Y (2002) Gene expression profiles in young adult *Ciona intestinalis*. Dev Genes Evol 212: 173–185
Satoh N (1994) Developmental Biology of Ascidians, Cambridge Univ Press, New York
Satoh N (2001) Ascidian embryos as a model system to analyze expression and function of developmental genes. Differentiation 68: 1–12
Satoh N, Satou Y, Davidson B, Levine M (2003) *Ciona intestenalis*: An emerging model for whole-genome analyses. Trends Genetics 19: 376–381
Satou Y, Imai KS, Satoh N (2001a) Action of morpholinos in *Ciona* embryos. Genesis 30: 103–106
Satou Y *et al*. (2001b) Gene expression profiles in *Ciona intestinalis* tailbud embryos. Development 128: 2893–2904
Satou Y, Takatori N, Fujiwara S, Nishikata T, Saiga H, Kusakabe T, Shin-i T, Kohara Y, Satoh N (2002a) *Ciona intestinalis* cDNA projects: expressed sequence tag analyses and gene expression profiles during embryogenesis. Gene 287: 83–96
Satou Y, Yamada L, Mochizuki Y, Takatori N, Kawashima T, Sasaki A, Hamaguchi M, Awazu S, Yagi K, Sasakura Y, Nakayama A, Ishikawa H, Inaba K, Satoh N (2002b) A cDNA resource from the basal chordate *Ciona intestinalis*. Genesis 33: 153–154
Sawada H, Yokosawa H, Lambert CC (Eds) (2001) The Biology of Ascidians. Springer-verlag, Tokyo
Sordino P, Heisenberg C-P, Cirino P, Toscano A, Giuliano P, Marino R, Pinto MR, De Santis R (2000) A mutational approach to the study of development of the protochordate *Ciona intestinalis* (Tunicata, Chordata). Sarsia 85: 173–176
Takahashi H, Hotta K, Erives A, Di Gregorio A, Zeller RW, Levine M, Satoh N (1999) *Brachyury* downstream notochord differentiation in the ascidian embryo. Genes Dev 13: 1519–1523
Ton C, Stamatiou D, Dzau VJ, Liew C-C (2002) Construction of zebrafish cDNA microarray: gene expression profiling of the zebrafish during development. Biochem Biophys Res Commun 296: 1134–1142

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