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An Integrated Database of the Ascidian, *Ciona intestinalis*: Towards Functional Genomics

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ABSTRACT—An integrated genome database is essential for future studies of functional genomics. In this study, we update cDNA and genomic resources of the ascidian, *Ciona intestinalis*, and provide an integrated database of the genomic and cDNA data by extending a database published previously. The updated resources include over 190,000 ESTs (672,396 in total together with the previous ESTs) and over 1,000 full-insert sequences (6,773 in total). In addition, results of mapping information of the determined scaffolds onto chromosomes, ESTs from a full-length enriched cDNA library for indication of precise 5'-ends of genes, and comparisons of SNPs and indels among different individuals are integrated into this database, all of these results being reported recently. These advances continue to increase the utility of *Ciona intestinalis* as a model organism whilst the integrated database will be useful for researchers in comparative and evolutionary genomics.

Keywords: *Ciona intestinalis*, ascidian, genome, expressed sequence tag (EST), integrated database

INTRODUCTION

Recent decoding of genomic sequences of a variety of animals demonstrates their usefulness in a wide range of biological studies. Because the genome sequences themselves do not have any biological meaning, they should be properly annotated to show how many and what kind of genes are encoded. Additionally, especially for functional analyses of the genome, we should more precisely determine gene structures including transcription start sites, exons and introns of each gene, spatial and temporal expression profiles, and phylogenetic annotations of the genes to examine how many paralogs and related genes are encoded in the genome. Much work on genome-wide descriptions has been done for a variety of model organisms. *Drosophila melanogaster* and *Caenorhabditis elegans* are leading this field, and a variety of useful annotations continue to be added to their integrated databases, named flybase (Chen *et al*., 2005) and wormbase (Drysdale *et al*., 2005), respectively. Within the phylum Chordata, similar work has also begun recently. For example, the H-invitational database was published recently as a human integrated database (Imanishi *et al*., 2004).

The ascidian, *Ciona intestinalis*, is a basal chordate and the seventh animal whose genome sequence has been determined (Dehal *et al*., 2002). Together with 480,753 ESTs, the genome sequence is an important resource for ascidian biology (Satoh *et al*., 2003). The genome sequence and basic annotations are browsable in the web site of the Joint Genome Institute (http://genome.jgi-psf.org/ciona4/ciona4.home.html), and have provided new insights into the origin and evolution of chordates. The genome sequence of *Ciona* has also revealed that this animal contains less paralogs than those of vertebrates (*e.g*., Satou *et al*., 2002b). Because *Ciona* shares the basic body plan with vertebrates in spite of the simple genome, *Ciona* provides an efficient experimental system for studying development of the basic body plan common to chordates on a genome-wide scale (Satoh, 2003; Satoh *et al*., 2003).

For functional analyses of the *Ciona* genome which aim to explore molecular mechanisms of ascidian development and the origin of the chordates, abundant digitally available cDNA sequences and the availability of their source clones are essential. We have previously made a database based on EST sequences that is accessible via the internet (ghost.zool.kyoto-u.ac.jp) (Satou *et al*., 2002a). In this data-

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base, all the EST clones are grouped by similarity between their 3'-ESTs into cDNA clusters and therefore each cDNA cluster roughly corresponds to one gene. Various experimental results based on the cDNA clusters have been obtained. For example, we described the spatial expression patterns of 1,000 cDNA clusters each at five different developmental stages and showed the usefulness of the spatial or temporal expression profiles based on EST counts from 12 cDNA libraries for a given gene (Nishikata *et al*., 2001; Satou *et al*., 2001; Fujiwara *et al*., 2002; Kusakabe *et al*., 2002; Ogasawara *et al*., 2002; Satou *et al*., 2002c, 2003a). Full-insert sequences (FISs) of about 5,000 cDNA clones were determined and stored in this database. The source clones have been distributed to researchers all over the world via this database and utilized for their experiments (Satoh, 2004).

Although these two major *Ciona* web sites share some data with each other, they are not well linked, which is frustrating researchers in functional genomics. After publication of the draft genome of *Ciona intestinalis*, we have continued to make efforts to enrich the resources for functional genomics, and to update the Ghost web database. Namely, we have mapped the scaffolds to the chromosomes by fluorescent in situ hybridization (FISH) of BAC clones (Shoguchi *et al*., 2005, submitted). Determination of precise 5'-ends of genes by ESTs from a full-length enriched library has been performed (Satou *et al*., submitted). A global analysis of polymorphisms has been performed (Kawashima *et al*., in preparation). Additionally, we have systematically identified almost all transcription factor genes and performed phylogenetic analyses of them as well as genes involved in major signaling pathways such as wnt and TGF-β (Chiba *et al*., 2003; Hino *et al*., 2003; Kawashima *et al*., 2003; Sasakura *et al*., 2003a, 2003b; Satou *et al*., 2002b, 2003b, 2003c; Wada *et al*., 2003; Yagi *et al*., 2003; Yamada *et al*., 2003). Almost all of their developmental expression profiles have also been described (Imai *et al*., 2004). For the convenience of researchers, these data should be publicly available via the internet hopefully with the genomic sequence, and therefore we extended the Ghost database to the integrated *Ciona intestinalis* database, from which all of these data can be retrieved. Together with the ESTs and full insert sequences that we determined in the present study, this integrated database provides a powerful tool for studying functional, comparative and evolutionary genomics.

MATERIALS AND METHODS

Construction of cDNA libraries and EST sequencing

We made six cDNA libraries of *Ciona intestinalis*. Two cDNA libraries from two different stages of juveniles and one cDNA library from the whole body of a mature adult were constructed in pBluescript vector using a commercially available kit (Stratagene). Three libraries from embryos (mixture of eggs to tailbud embryos), the whole body of a mature adult, and the digestive gland were made into pDONR222 using another commercially available kit (Invitrogen). The bacteria containing the libraries were picked into 384-well plates. The cDNA inserts were amplified by polymerase chain reaction and the amplified cDNA inserts were end-sequenced.

Genome browser interface and computer software used in the present study

The genome browser interface was constructed based on the generic genome browser package (Stein *et al*., 2002). The alignments of ESTs and FISs were performed by BLAT (Kent, 2002) and the alignment of whole-genome shotgun reads were performed by BLAST (Altschul *et al*., 1990).

RESULTS AND DISCUSSION

Update of the web site

The web interface providing the cDNA infomation was not changed apart from the addition of a link to the genome browser on each page of cDNA information. The genome browser interface was constructed using the generic genome browser package (Stein *et al*., 2002). Together with ESTs and FISs of cDNA clones determined since a previous publication (Dehal *et al*., 2002), all of the available data based on cDNAs are also presented in this genome browser, and each of the cDNA information tracks is tightly linked to the cDNA web resources.

Update of the ESTs and the cDNA full insert sequences

In previous studies, we made 12 cDNA libraries and obtained 241,519 5'ESTs and 239,234 3'ESTs (480,753 in total) (Dehal *et al*., 2002). In this study, we newly made cDNA libraries from the digestive gland of the adult, whole adult body except tunic, embryos from the egg to the late tailbud stage and juveniles, and obtained 94,624 5'ESTs and 97,145 3'ESTs (191,769 in total; Table 1; DDBJ/Genbank/EMBL accession numbers, BW318210-BW509978). The combined ESTs cover about 85% of the predicted gene models (previously covering about 77%; Dehal *et al*., 2002). In addition, we collected 11,884 ESTs from the public database from other groups. In addition to the ESTs, we had previously deposited 4,983 FISs of *Ciona* cDNA clones, to which we added further 1,766 FISs determined in the present study (6,749 in total; DDBJ/Genbank/EMBL accession numbers, AK173344-AK175109). We also collected 349 full insert sequences from the public database. All of these sequences are aligned against the genome sequence using the BLAT program (Kent, 2002) and each EST or FIS appearing in the genome browser provides a web link to the corresponding cDNA cluster information, which includes the spatial and temporal expression profiles of the gene as well as its sequence. All of the cDNA clones we used for obtaining the ESTs are freely available for academic purposes.

As previously, we performed gene prediction in the draft genome using the Grail-Exp program (Uberbacher *et al*., 1996) and this updated cDNA information, which resulted in 19,682 gene models. Accuracy seems to be improved in most cases, which is useful for the ascidian studies. Determining as many cDNA sequences as possible is the only way to obtain accurate gene structures. As described

Table 1. Summary of ESTs obtained in the previous and present studies.

Library	5' EST	3' EST	Total
ESTs determined in the previous study			
Egg	29,810	29,444	59,254
Cleaving embryo	31,156	26,796	57,952
Gastrula/Neurula	23,066	23,475	46,541
Tailbud embryo	30,282	31,209	61,491
Larva	24,282	24,680	48,962
Young adult	28,547	29,138	57,685
Gonad	16,048	16,239	32,287
Testis	4,655	4,717	9,372
Endostyle	2,241	2,497	4,738
Neural complex	10,116	10,029	20,145
Heart	12,904	12,414	25,318
Blood cells	28,412	28,596	57,008
subtotal	241,519	239,234	480,753
ESTs determined in the present study			
Embryo mix	17,784	17,734	35,518
Juvenile 1	23,914	23,897	47,811
Juvenile 2	3,441	3,336	6,651
Digestive Gland	17,582	17,808	35,390
Mature adult	31,903	34,370	66,273
subtotal	94,624	97,145	191,643
Total	336,143	336,379	672,396

above, we have determined 6,773 cDNA sequences, although some of them are different cDNA clones for the same genes. Accurate gene structure and number will be determined by on-going efforts to determine the cDNA sequences in the future.

Additional useful cDNA and genomic resources

ESTs from a full-length cDNA library

To determine the 5'-end location of genes precisely, we made a full-length enriched cDNA library from embryos and have started to obtain 5'ESTs from this library (Satou *et al*., submitted). About 50% of the ascidian mRNAs contain the spliced-leader, which is added by *trans*-splicing, and this analysis has revealed gene species making mRNA with the spliced-leader. In addition, because this *trans*-splicing is used for resolution of polycistronic pre-mRNAs, this analysis has also been able to reveal polycistronic transcription units. At present, 2,079 5'ESTs corresponding to 662 different gene species are available (Fig. 1) and sequencing is on-going.

Chromosome mapping

We obtained 17,322 end sequences from 8,661 BAC clones in a previous study (Dehal *et al*., 2002). These sequences were also aligned against the genome. Using these sequences, the scaffolds have been mapped to chromosomes by FISH of BAC clones (Shoguchi *et al*., 2005). At present, the mapped scaffolds cover almost 60% of the whole genome (Shoguchi *et al*., submitted). In the web site we made in the present study, the results of the mapping of BACs and scaffolds are also shown (Fig. 2).

Polymorphisms revealed by whole-genome shotgun reads from different individuals

Ciona is known to have many polymorphisms, with 15 times as many as those in human (Dehal *et al*., 2002). The genome project of *Ciona intestinalis* has been carried out mainly by Japan and the United States. In Japan, two libraries for the whole-genome shotgun sequencing were made from three Japanese individuals, while in the United States several libraries were made from one American individual. Because of the abundance of polymorphisms, the current assembly is basically based on the American individual. We have mapped high-quality regions of the whole-genome shotgun reads in Japan to the current assembly, as well as those from the American one (Fig. 3).

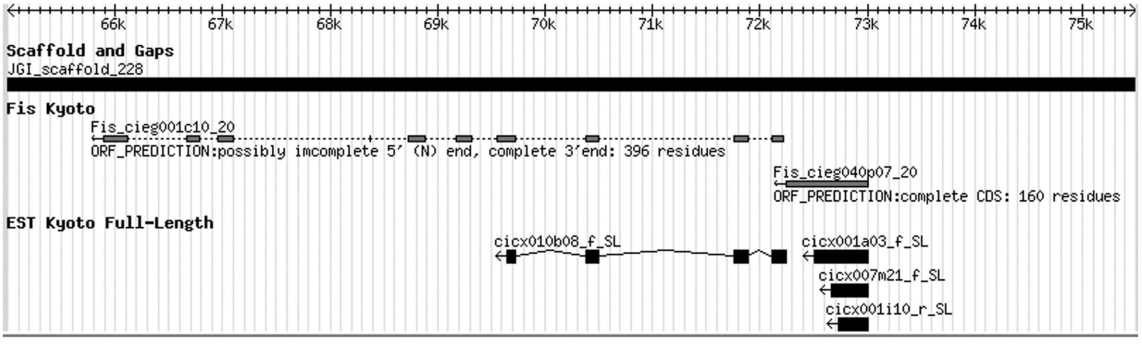

Fig. 1. A screen shot showing 5'-ESTs from a full-length enriched library. In this genomic region, two genes are encoded, which is proven by two full-insert sequences. For each of these two genes, 5'-ESTs from a full-length enriched library were found, indicating the precise 5'-ends of these two genes. The last two letters of the names of 5'-ESTs with the spliced-leader are 'SL'. In this example, these two genes make mRNA with the spliced-leader.

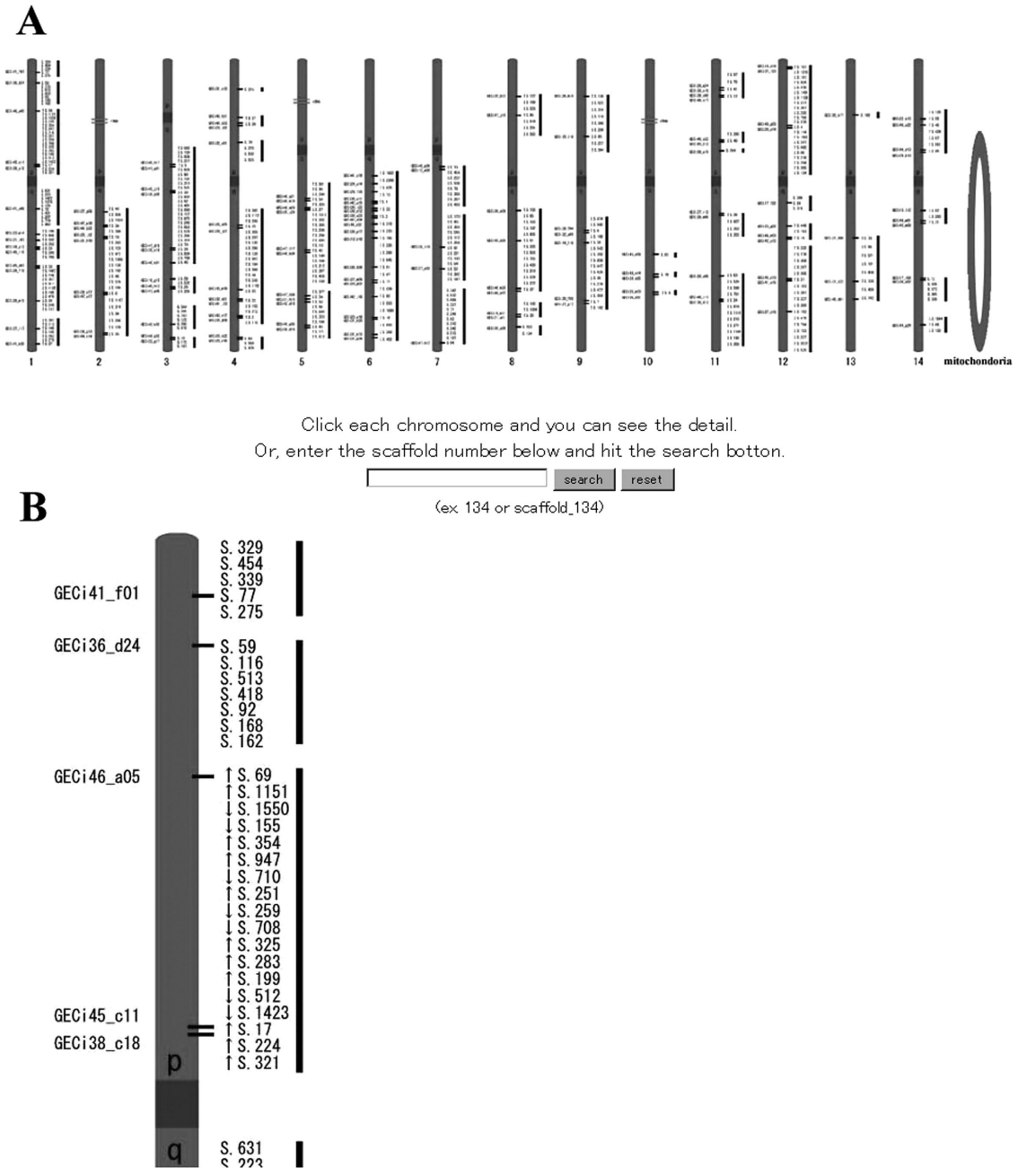

Fig. 2. Screen shots showing the results of mapping of scaffolds onto the chromosomes. (A) A screen shot showing the overview including the search field. (B) A screen shot showing the detailed view of each chromosome.

By this mapping, polymorphisms such as short insertions or deletions (indels) and single nucleotide substitutions (SNPs) can be browsed in the web site. For example, the in silico prediction of the biologically important regions in gene promoters using the polymorphism rate within a species is useful (Boffelli *et al*., 2004). It has been shown that the polymorphism rate is low in exons and high in introns and in intergenic regions (Kawashima *et al*., in preparation). Therefore, this alignment shown in the browser will give a powerful tool for predicting biologically important regions of promoters and exons/introns.

Gene expression profiles

As shown in the previous study, EST counts from each cDNA library for a given gene represent the abundance of the corresponding mRNA at each developmental stage or in each tissue, from which the cDNA libraries were made (Satou *et al*., 2003a). In the web site constructed based on the cDNA information, we have presented the expression profile of each cDNA cluster (nearly identical with 'gene', which is constructed by comparisons of 3' ESTs with one another). In the present study, the expression profile of each gene model predicted by Grail-Exp with the new dataset,

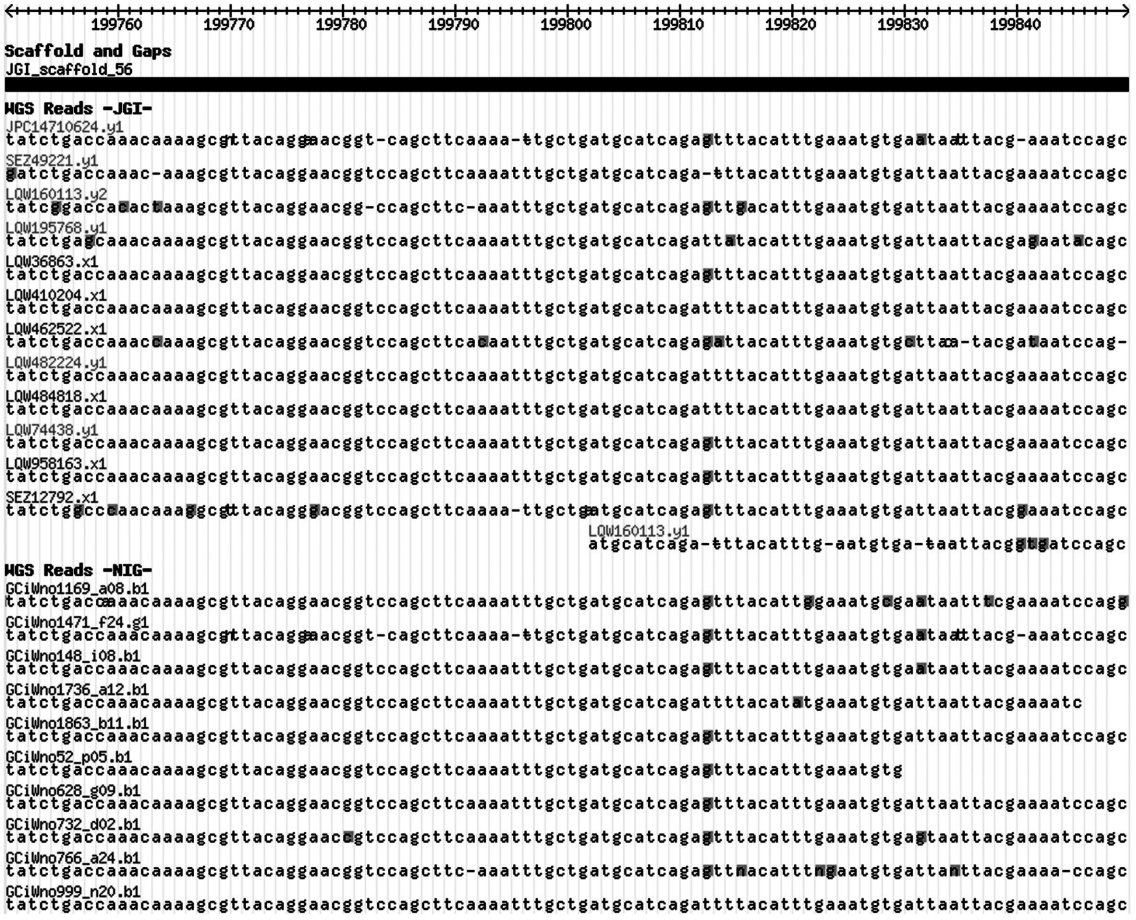

Fig. 3. A screen shot showing an alignment of whole genome shotgun reads from an American individual and three Japanese individuals. Grey boxes indicate nucleotides mismatched with the assembled draft genome sequence.

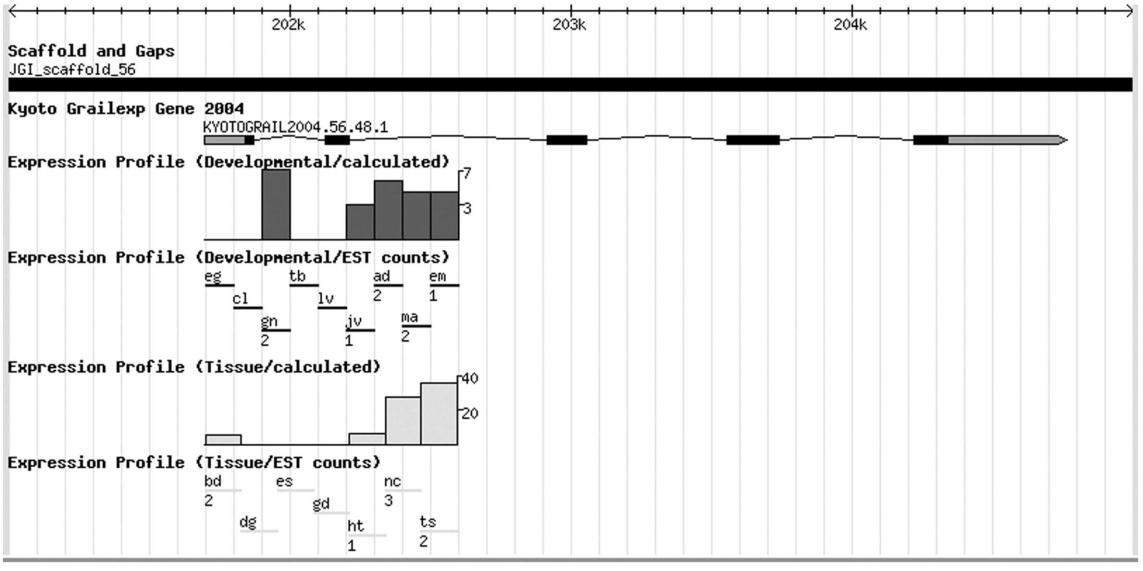

Fig. 4. A screen shot showing a developmental expression profile and a spatial expression profile in an adult. In this example, a gene designated as KYOTOGRAIL2004.56.48.1 is expressed between the gastrula and neurula stages (gn) and then again in juveniles (jv) and young adults (ad), and in a mature adult, this gene is expressed in blood cells (bd), heart (ht), neural complex (nc) and testis (ts).

which is described above, is shown in the genome browser (Fig. 4).

In addition, we have performed more than 6,000 whole-mount in situ hybridization experiments to disclose the spatial expression patterns of developmentally regulated genes (Nishikata *et al*., 2001; Satou *et al*., 2001; Fujiwara *et al*., 2002; Kusakabe *et al*., 2002; Ogasawara *et al*., 2002; Imai *et al*., 2004). These data have also been presented mainly by each cDNA cluster. The web links to these data are added as a track in the genome browser. Therefore, users can easily find the information about when and where the gene is expressed as long as it has been examined.

Microarrays

We have developed two oligonucleotide-based microarrays (oligomicroarrays) containing about 22,000 probes and 44,000 probes (Agilent technology). These arrays are useful for genome-wide studies of gene expression. Because the probes on the arrays were mapped onto the genome sequence, users can see directly where in the genome the probes of interest are mapped or what kind of genes the probes of interest are. Therefore, users can easily find the expression profile and the relevant information of the gene that the probe of interest indicates. Oligomicroarrays are known to be often severely affected by single-nucleotide substitutions (Hughes *et al*., 2001). Using mapped whole genome shotgun reads, possible effects of polymorphisms on results of oligomicroarrays can be browsed.

CONCLUSION

In *Ciona intestinalis*, lots of effort has been made to improve the genomic information of this basal chordate. Whole genome shotgun reads from a mixture of three individuals other than the one from whom the draft genome was determined correspond to 5 times as many as the genome size, which is useful for analysis of polymorphisms. The determined scaffolds have been mapped to each chromosome by FISH. About 480,000 ESTs and 5,605 FISs have been obtained, and spatial expression profiles of about 1,000 genes in each of 5 developmental stages (5,000 genes in total) have been described. Developmental expression profiles of almost comprehensively annotated transcription factor genes and signaling molecule genes in several major pathways have been described. 2,079 5'-ESTs from a full-length enriched library have been obtained. In this report, we determined an additional 191,643 ESTs and 1,766 FISs, and collected ESTs and FISs from the public database. We extended our web-based database, called Ghost, to show all of this information together. In addition to the conventional user interface based on cDNA clustering, we introduced a new user interface based on the generic genome browser. Both interfaces are tightly interlinked with each other. We believe that the resources presented in this integrated database are useful not only for researchers working on ascidians but also for researchers in comparative and evolutionary genomics.

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