Effects of 5-aza-2’-deoxycytidine on the Gene Expression Profile During Embryogenesis of the Ascidian Ciona intestinalis: A Microarray Analysis

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DNA methylation is an important epigenetic factor that participates in silencing genes. Genomic approaches to studying DNA methylation promise to be particularly fruitful, since DNA methylation is involved in global control of gene expression in many organisms. With its draft genome completed and a large quantity of available cDNA data, Ciona intestinalis is newly emerging as an invaluable model organism for investigating genome-wide gene expression and function. Here we examine the effects of 5-aza-2’-deoxycytidine (5-aza-CdR), a chemical that blocks CpG methylation, on the gene expression profile of early C. intestinalis embryos, using oligonucleotide-based microarray analysis. Embryos treated with 5-aza-CdR show delayed gastrulation and are developmentally arrested at the neurula stage. They subsequently lose cellular adhesion and finally die. Apoptosis was not detected in these embryos by TUNEL staining at 12 h, indicating that the defects observed did not result from 5-aza-CdR-induced apoptosis. Gene expression profiles of 12-h-old 5-aza-CdR-treated embryos compared to wild-type revealed 91 upregulated genes and 168 downregulated genes. Although nearly half of these encoded proteins with unknown functions, several encoded cell-signaling molecules and transcription factors. In addition, genes associated with the stress response and cell defense were upregulated, whereas genes involved in cell adhesion were downregulated.

Key words: Ciona intestinalis, embryogenesis, gene expression profiles, DNA methylation, 5-aza-CdR, microarray

INTRODUCTION

With the recent progress in compiling genome project data, the ascidian Ciona intestinalis has become an attractive experimental organism for studying developmental genomics, particularly for genome-wide approaches to studying the molecular mechanisms underlying embryonic cell specification (Satoh et al., 2003). The draft C. intestinalis genome predicts 15,852 protein-coding genes (Dehal et al., 2002), with those encoding transcription factors and cell-signaling molecules benefitting from precise annotation (e.g., Satou et al., 2003a; Wada et al., 2003). Together with the availability of a large quantity of expressed sequence tags (ESTs) and cDNA data (Satou et al., 2005), this information has facilitated characterization of gene expression profiles during early C. intestinalis embryogenesis (Imai et al., 2004, 2006). Furthermore, an oligonucleotide-based microarray covering more than 85% of transcripts expressed during Ciona embryogenesis has been generated (Yamada et al., 2005) and has been used to examine zygotic gene expression profiles up to the 64-cell stage (Hamaguchi et al., 2007).

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The molecular mechanisms responsible for epigenetic control of gene expression in early ascidian embryos are still poorly understood. DNA methylation of dinucleotide 5'-CpG sequences and histone acetylation are two major mechanisms known to be involved in global regulation of gene expression (Bird, 2002). The genome of C. intestinalis is reportedly a stable mosaic of methylated and non-methylated domains (Simmen et al., 1999). For instance, multiple copies of an apparently active long terminal repeat retrotransposon, as well as a long interspersed element and a large fraction of abundant short interspersed elements, have been shown to be non-methylated. In contrast, genes are predominantly methylated, indicating that cytosine methylation in C. intestinalis may be preferentially directed to genes (Simmen et al., 1999).

The effects of DNA methylation on biological processes can be studied using 5-aza-2’-deoxycytidine (5-aza-CdR), a chemical that blocks methylation and thus causes demethylation of CpG sequences (Taylor and Jones, 1979). In C. intestinalis, 5-aza-CdR was observed to produce general, non-specific effects during embryogenesis (Puccia et al., 1986), but no studies examining its effects on ascidian embryogenesis have since been reported. Here we investigate the effects of 5-aza-CdR on gene expression profiles in Ciona embryos using microarray analysis.
MATERIALS AND METHODS

Maintenance of animals

Ciona intestinalis were cultivated at the Maizuru Fisheries Research Station of Kyoto University, Maizuru Bay, Kyoto, Japan. Eggs and sperm were surgically obtained from the gonoduct. After fertilization, they were dechorionated chemically with seawater containing 0.1% actinase E (Kaken Pharmaceutical Co., Tokyo) and 1.3% sodium thioglycolate (Wako Pure Chemical Industries, Osaka). Embryos were allowed to develop at about 18°C on 1% agar-coated plastic dishes filled with Millipore-filtered seawater containing 50 μg/ml streptomycin sulfate.

5-aza-2'-deoxycytidine (5-aza-CdR) treatment

5-aza-CdR (Sigma), a cytosine analog, was directly dissolved in seawater immediately before use. Fertilized eggs were incubated in seawater containing various concentrations of 5-aza-CdR.

TUNEL staining

TUNEL staining was basically performed as described previously (Chambon et al., 2002). Embryos treated with 5-aza-CdR were fixed 12 h after fertilization with 3.7% formaldehyde in Millipore-filtered seawater at room temperature for 20 min. As a control, normal embryos were collected and fixed at the same time. Embryos were washed for 5 min in phosphate-buffered saline (PBS) with 0.2% Triton-X100 (PBS-Tx) three times and twice with PBS. For positive controls, normal and experimental embryos were incubated with 0.5 μM DNase I in PBS for 10 min. After two washes in PBS-Tx and one wash in TdT buffer, embryos were incubated in 10 μM biotin-16-dUTP (Roche Molecular Biochemicals) and 0.6 μg/ml TdT (Promega) in TdT buffer for 1 h at 37°C. Embryos were subsequently washed for 10 min in PBS-Tx three times, then incubated for 45 min in 1:200 FITC-streptavidin at room temperature. Finally, embryos were washed in PBS-Tx, and mounted in 80% glycerol/N-propyl-gallate for visualization.

Oligonucleotide-based microarray

Ciona intestinalis oligonucleotide-based microarray version 2 was custom-made by Agilent Technologies. Oligonucleotide probes (60-mer) were synthesized on a base chip by the inkjet-based Sure Print technology. The 44,290 spots on the chip consisted of three types of features or probes: one negative control distributed evenly on the chip at 314 spots, 57 positive controls at 1,942 spots, and 44,205 experimental spots. For dye swapping, the same procedure was performed using reciprocal pairings of labeled CTP and total RNA.

Five μg of total RNA from each sample were amplified linearly and used as a template to generate labeled cRNA for a microarray probe. The amplified target probe was labeled with fluorescently tagged cyanine 3-CTP or cyanine 5-CTP (Perkin Elmer/NEN), using an Agilent Low RNA Input Fluorescent Linear Amplification Kit (Agilent Technologies). The quality and size of the probe were tested using an RNA 6000 Nano Lab-on-chip (Agilent Technologies) and an Agilent 2100 Bioanalyzer, and the quantity was determined using a NanoDrop microscale spectrophotometer (NanoDrop Technologies).

For dye swapping, the same procedure was performed using reciprocal pairings of labeled CTP and total RNA. A set of 1-μg targets from two samples was used for competitive hybridization on the C. intestinalis microarray version 2 with an In Situ Hybridization Kit Plus (Agilent Technologies). After hybridization, chips were washed and dried according to the manufacturer's protocol and scanned with a G2565BA Agilent DNA Microarray Scanner.

Microarray data analysis and annotation of gene function

The signal intensity of each fluorescent dye was extracted from scanned microarray images and normalized using Agilent G2567AA Feature Extraction Software (version 7.5). The local background method was used as a background subtraction algorithm, and other algorithms and parameters were used at default settings. Some spots that exhibited non-uniformity or saturated or low signal intensity were excluded from the following analysis. Genes that gave an average two-fold change in processed signal intensity were extracted, and those that showed this differential signal intensity in both sets of dye swap data were collected. Because low signal intensity often led to non-reproducible results, we excluded genes whose average processed signal was below 1,000.

Because the genes used to design our microarray were predicted from pre-existing sequence data, we were able to confirm which genes corresponded to each probe. A genomic sequence corresponding to each probe was found on the genome browser of the Ghost Database (http://ghost.zool.kyoto-u.ac.jp/index1.html). We preferentially used Kyoto Grail 2005 gene models (e.g., KYOTOGRAIL2005.1.1.1), which had high consistency with EST data because they were derived from RNA fragments with poly(A) sequences. When no appropriate Kyoto Grail 2005 gene model was found, appropriate CDNA (e.g., Fis_citb001a01), clusters of assembled ESTs (e.g., CLSTR00001r1), or JGI gene models predicted from Ciona intestinalis draft genome ver.1 (e.g., ci00000000001) were adopted. When assembled sequences from more than two clusters were used as gene models, cluster names were connected by an underscore (e.g., CLSTR00001r1_CLSTR00002r1). When no gene model exactly matched the probes, but the open reading frame of a predicted gene appeared to be correct, that gene model was adopted. More details of other sequence data are available from the corresponding author of this paper.

Sequence similarity searches of gene models were performed using the BLASTX algorithm (Altschul et al., 1997) against four protein databases to annotate for gene function as accurately as possible. We searched the Homo sapiens protein database with UniProt/SPTREMBL ID based on the NCBI36 genome assembly (http://www.ensembl.org/Homo_sapiens/index.html), the Drosophila melanogaster protein database with UniProt/SPTREMBL ID based on the BDGP4.3 genome assembly (http://www.ensembl.org/Drosophila_melanogaster/index.html), the NCBI refseq database (November 1, 2006 release, http://www.ncbi.nlm.nih.gov/RefSeq/), and the NCBI non-redundant protein database (November 1, 2006 release). Parameters were set at default values, and hits with expected values lower than 1e-15 were used to identify genes with similarities. When two or more hits were found, the best hit with a clear annotation of gene function was used. Hits with significant similarity to C. intestinalis proteins were picked up at the same time.

To exclude the possibility that gene models corresponded to non-coding RNA fragments, a search against the NCBI non-redundant RNA database was performed for those with no hits against four protein databases, but no significant similarities were found.

Following the similarity searches, gene models were classified based on Lee et al. (1999) into 17 categories. The basic classification criteria are described in Fig. 3C. Gene ontology (http://www.geneontology.org/index.shtml) was used for a reference. In cases in which function could be classified in more than two groups, we used the following criteria: transmembrane transporters of ions and small molecules were classified to Al, transmembrane complements to AVII, transmembrane signaling receptors to Bi, and other transmembrane proteins to AIV. Proteins involved in protein folding
were assigned to AV, those involved in wound recovery (e.g., blood coagulation) to AVII, and proteases other than those involved in intracellular signaling to AVIII. Ciona intestinalis transcription factors were assigned to CI, and zinc finger proteins to DI. When other hits with clear annotations were found for gene models, they were categorized to class DI. Molecules without definite assignment to DI and with no similarity were categorized as DII.

Relative EST number as a measure of temporal expression level

In previous studies, we performed large-scale EST analysis of transcripts expressed at several C. intestinalis developmental stages (e.g., Satou et al., 2002). Because these libraries were not normalized or amplified, the number of cDNA clones from each library accurately reflected the quantity of the transcripts of the corresponding genes (Satou et al., 2003b). Thus, the relative number of ESTs corresponding to any given gene model at different developmental stages can be used to trace the spatio-temporal expression level of the predicted gene.

RESULTS AND DISCUSSION

Effects of 5-aza-CdR on Ciona embryogenesis

When fertilized C. intestinalis eggs were dechorionated and allowed to develop in seawater containing 1 μM 5-aza-CdR, their early cleavage steps appeared to be normal, both in terms of timing and pattern of cleavage. However, development of 5-aza-CdR-treated embryos was delayed at the onset of gastrulation. Twelve hours after fertilization, when control embryos had reached the mid tailbud stage (Fig. 1A), experimental embryos appeared to be at the neurula stage (Fig. 1C). Sixteen hours after fertilization, when normal embryos had developed to the late tailbud stage (Fig. 1B), experimental embryos remained arrested at the neurula stage (Fig. 1D). At 19–21 hours post-fertilization, cells of the outermost epidermal layer began to detach from 5-aza-CdR-treated embryos (data not shown). Subsequently, most cells in the experimental embryos became dissociated, all cells were disconnected by 2 days of development, and the embryos finally died.

TUNEL staining of 5-aza-CdR-treated embryos

Treatment with 5-aza-CdR is known to induce nitric acid production, which causes death in pre-implantation mouse embryos (Athanassakis et al., 2000). Moreover, 5-aza-CdR treatment causes abnormal mitosis and apoptosis of neural progenitor cells in the fetal rat brain (Ueno et al., 2006). It is likely that similar damage occurred in Ciona embryos treated with 5-aza-CdR for more than 16 hours. However, the delayed gastrulation and neurulation arrest observed in these embryos suggests that 5-aza-CdR has different effects at earlier stages. To rule out the possibilities that induction of apoptosis by 5-aza-CdR accounts for these defects and that noise from the RNA of dead cells disturbs the expression profile, we performed TUNEL staining on 12-h-old 5-aza-CdR-treated embryos. As a control, apoptosis was induced in both 5-aza-CdR-treated and untreated embryos by DNase-I treatment. As shown in Fig. 2A, untreated tailbud-stage embryos showed no TUNEL staining, while control embryos treated with DNase I showed distinct TUNEL staining (Fig. 2B). Similarly, 12-h-old 5-aza-CdR-treated embryos showed no TUNEL staining (Fig. 2C), while those treated with DNase I did (Fig. 2D). These results indicate that apoptosis does not occur in 5-aza-CdR-treated embryos at 12 h of development.

**Fig. 1.** Effects of 5-aza-CdR on embryogenesis of Ciona intestinalis. Fertilized and dechorionated eggs were allowed to develop in seawater that contained 1 μM 5-aza-CdR. (A, B) Control embryos and (C, D) experimental embryos, at 12 hrs (A, C) and 16 hrs of development (B, D). Scale bar, 50 μm.
Gene Expression in Ciona Embryogenesis

Fig. 2. TUNEL staining of embryos at 12 hours of development. (A) Normal control embryo and (B) control embryo treated with DNase I. (C) Experimental embryo treated with 5-aza-CdR and (D) that treated with DNase I. Scale bar, 50 μm.

Fig. 3. Classification of genes which are (A) upregulated or (B) downregulated by treatment of embryos with 5-aza-CdR. Classification was carried out according to Lee et al. (1999), as shown in (C). The numbers with each pie show the rounded percentage of genes in each model.
Microarray analysis of changes in gene expression profiles in 5-aza-CdR-treated embryos

To examine the global effects of 5-aza-CdR on gene expression, total RNA was isolated from 5-aza-CdR-treated or control embryos, labeled with either Cy-3 or Cy-5, and hybridized to a 60-mer oligonucleotide-based microarray. Dye swapping was carried out to reduce false-positive data. Genes represented by probes or features were characterized carefully, as described in the Materials and Methods. Transcripts that were enriched in experimental embryos were selected by using a threshold value of 2.0 for the ratio of experimental to control expression. Ninety-one genes were identified as upregulated (Supplementary Table 1), while 168 genes appeared to be downregulated (Supplementary Table 2).

Because the EST library we previously generated was not normalized or amplified, the relative number of ESTs corresponding to any given gene can be used to trace spatiotemporal expression levels (see Materials and Methods). Supplementary Tables 1 and 2 show EST data for up- and downregulated genes, which are classified according to function. These data indicate that 54 of the 91 upregulated genes were expressed actively in normal embryos at the neurula and/or tailbud stage, whereas the other 37 genes were not expressed during these stages. Of the 168 downregulated genes, 106 were expressed in normal neurulae and/or tailbud embryos, while the other 62 genes were not expressed during these stages. It is likely that the upregulation of the 37 genes and the downregulation of the 62 genes which were not expressed in normal neurulae and/or tailbud embryos were indirect effects of 5-aza-CdR.

Genes upregulated in 5-aza-CdR-treated embryos

As shown in Fig. 3A, about 53% (48/89) of upregulated genes fell into Class D, whose protein functions are unknown. Twenty-seven genes were in Class A, 10 in Class B, and 4 in Class C. We could not identify appropriate gene sequences for two genes in the Ghost Database, so these were not included in this analysis. Two conspicuous groups among Class-A genes were subclass AII, which includes genes associated with cell replication and DNA modification, and AVII, which includes genes associated with stress response and cellular defense. It is possible that 5-aza-CdR somehow affects cell division (Ueno et al., 2006), leading to delayed development and arrest at the neurula stage, accompanied by a failure of tail elongation. Upregulation of stress-response and cellular-defense genes is likely due to general damage caused by 5-aza-CdR treatment.

In addition, 10 genes associated with cell signaling and four genes involved in transcription machinery were upregulated (Fig. 3A; Table 1). This suggests that 5-aza-CdR is not simply toxic, but also has specific effects on the transcription of certain genes. EST data suggest that all four genes encoding transcription factors, KYOTOGRAIL2005.258.1.1, KYOTOGRAIL2005.73.36.1, snail (Ci-sna), and Distal-less (Ci-Dll-B) (Table 1), are expressed in wild-type Ciona embryos. Although the functions of the first two are unknown, Ci-sna encodes a transcriptional repressor that inhibits Brachyury (Ci-Bra) expression in muscle cells by binding to a 434-bp enhancer in the promoter region of Ci-Bra, thus acting as a boundary repressor to restrict Ci-Bra expression to the neighboring notochord cells (Corbo et al., 1997, Fujiwara et al., 1998). In addition, the C. intestinalis genome contains three Distal-less genes, Ci-Dll-A, Ci-Dll-B and Ci-Dll-C, which are presumably derived from a single ancestral gene by duplication (Caracciolo et al., 2000) but which show different expression patterns. Future studies should focus on whether upregulation of these genes is accompanied by ectopic expression in cells of other lineages or whether upregulation affects expression of other genes.

Genes downregulated in 5-aza-CdR-treated embryos

As shown in Fig. 3B, 49% (81/167) of downregulated genes fell in Class D, which comprises proteins of unknown function (Supplementary Table 2). Of the remaining genes, 52 were in Class A, 27 in Class B, and 7 in Class C. Among Class A genes, a conspicuous group was subclass AVI, which includes genes associated with intermediate enzymes in synthetic and catabolic pathways, suggesting that 5-aza-CdR affected negatively the activity of these important intermediate enzymes. We could not find the appropriate gene model sequence for one gene in the Ghost Database, so it was not included in further analysis.

As shown in Table 1, Class B genes that were downregulated in experimental embryos included precursors of integrin β4, collagen α-1 (XIV) chain, spondin-1, P-selectin, and fibulin-2. The gene ontology (GO) suggests that these genes are involved in the interaction of cells with the extracellular matrix (ECM). Downregulation of these genes may contribute to the loss of cell-ECM adhesion and the gradual disintegration of 5-aza-CdR-treated embryos. In addition, the plakin family member plectin-1, which is a component of the desmosome (Huber, 2003; Litjens et al., 2006), and the actin-binding protein Shroom, which is essential for neural tube morphogenesis in vertebrate embryos (Hildebrand, 2005), were included in Class B. It is likely that 5-aza-CdR treatment suppresses the activity of these genes involved in maintenance of cell architecture and embryo shape.

Among seven downregulated transcription factors was Ci-Hox5, which is first expressed at the early tailbud stage in the anterior nerve cord in an anterior-to-posterior gradient, as well as in the lateral trunk (Ikuta et al., 2004). Future studies should examine the mechanisms of Ci-Hox5 downregulation and its effects on the expression of other genes.

One downregulated Class-D gene was Ci-META2, which was originally identified in a differential screen for genes expressed in metamorphosing juveniles, but not in swimming larvae (Nakayama et al., 2001). However, the function of Ci-META2 is unknown.

Conclusion

The spectrum of methylation levels and patterns is very broad among animals (Bird, 2002; Colot and Rossignol, 1999). For instance, 5-methylcytosine (m5C) has not been detected in the DNA of Caenorhabditis elegans, whose genome does not contain a conventional DNA methyltransferase. Drosophila melanogaster, whose DNA was long thought to be devoid of methylation, in fact does have a DNA methyltransferase-like gene (Tweedie et al., 1999) and has very low m5C levels (Lyko et al., 2000). In contrast, vertebrate genomes have the highest levels of m5C found in the animal kingdom, and methylation is dispersed over
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<th>Gene model</th>
<th>Accession No</th>
<th>Description</th>
<th>Organism</th>
<th>E value</th>
<th>Data base</th>
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<td></td>
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<td></td>
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<td><strong>Up-regulated genes in Class B</strong></td>
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<td>P97796</td>
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<td>Homo sapiens</td>
<td>8e-35</td>
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<td>Q04721</td>
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<td>1e-138</td>
<td>H</td>
<td>BIB</td>
<td>CLSTR42441/1</td>
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| **Up-regulated genes in Class C** |
| 26  | 2.76      | KYOTOGRAIL2005.258.1.1 | BAEG6603 | Transcription factor protein | Ciona intestinalis | 2e-172 | N | CL | CLSTR20175/1 | 0 | 2 |
| 59  | 2.34      | KYOTOGRAIL2005.2.32.1 | Q43623 | Zinc finger protein SLUG (Small homolog 2) | Homo sapiens | 4e-52 | H | CL | CLSTR20539/1 | 9 | 0 |
| 60  | 2.34      | KYOTOGRAIL2005.10.5.1 | NP_60107672 | Distal-less | Ciona intestinalis | 3e-91 | R | CL | CLSTR11516/1 | 5 | 1 |
| 83  | 2.16      | KYOTOGRAIL2005.73.38.1 | BAEG6608 | Transcription factor protein | Ciona intestinalis | 0.0 | N | CL | CLSTR30172/1 | 0 | 0 |

| **Down-regulated genes in Class B** |
| 6   | 6.26      | CLSTR4468/1/1 | P16144 | Intaglin beta-4 precursor | Homo sapiens | 4e-24 | H | BIB | CLSTR40461/1 | 0 | 5 |
| 6  | 5.30      | KYOTOGRAIL2005.61.8.1 | O60241 | Brain-specific angiogenisis inhibitor 2 precursor | Homo sapiens | 3e-21 | H | BIB | CLSTR30344/1 | 0 | 3 |
| 10  | 4.70      | KYOTOGRAIL2005.49.25.1 | Q2T376 | Rydoncine receptor 2 | Homo sapiens | 0.0 | H | BIB | CLSTR49351/1 | 2 | 1 |
| 17  | 4.16      | KYOTOGRAIL2005.159.22.1 | P46531 | Neurogenic locus notch homolog protein 1 precursor (Notch-1) | Homo sapiens | 5e-55 | H | BIB | CLSTR33147/1 | 0 | 2 |
| 38  | 3.87      | Fis. ciad017c21 | O60241 | Brain-specific angiogenisis inhibitor 2 precursor | Homo sapiens | 2e-23 | H | BIB | CLSTR30352/1 | 0 | 2 |
| 25  | 3.75      | KYOTOGRAIL2005.17.44.1 | Q50707 | Collagen alpha-1(IV) chain precursor | Homo sapiens | 2e-22 | H | BIB | CLSTR36794/1 | 0 | 0 |
| 35  | 3.29      | KYOTOGRAIL2005.46.32.1 | Q9HDB9 | Spordin-1 precursor (F-spordin) | Homo sapiens | 1e-141 | H | BIB | CLSTR56346/1 | 0 | 0 |
| 48  | 2.85      | KYOTOGRAIL2005.20.60.1 | P16109 | P-selectin precursor | Homo sapiens | 1e-56 | H | BIB | CLSTR30278/1 | 0 | 7 |
| 56  | 2.76      | KYOTOGRAIL2005.530.7.1 | Q14CC2 | NEXN protein | Homo sapiens | 3e-19 | H | BIB | CLSTR40041/1 | * | 0 |
| 57  | 2.76      | KYOTOGRAIL2005.26.81.1 | P36383 | Gap junction alpha-7 protein (Connexin-45) | Homo sapiens | 3e-49 | H | BIB | CLSTR10009/1 | 0 | 1 |
| 59  | 2.72      | KYOTOGRAIL2005.756.2.1 | P16066 | Atrial natriuretic peptide receptor A precursor | Homo sapiens | 0.0 | H | BIB | CLSTR40890/1 | 0 | 0 |
| 60  | 2.72      | KYOTOGRAIL2005.410.2.1 | NP_01027680 | Preproangiotensin-releasing hormone 2 | Ciona intestinalis | 5e-87 | R | BIB | CLSTR70695/1 | 0 | 0 |
| 66  | 2.65      | KYOTOGRAIL2005.52.32.1 | Q15149 | Plecrin-1 | Homo sapiens | 1e-105 | H | BIB | CLSTR60961/1 | * | 2 |
| 74  | 2.55      | KYOTOGRAIL2005.781.9.1 | Q9HPC2 | Insoluble hexaphosphate kinase 3 | Homo sapiens | 2e-46 | H | BIB | CLSTR13695/1 | 0 | 3 |
| 81  | 2.48      | KYOTOGRAIL2005.23.55.1 | P98695 | Fibulin-2 precursor | Homo sapiens | 1e-89 | H | BIB | CLSTR13475/1 | 0 | 1 |
| 83  | 2.48      | Fis. ciad009c03 | Q969Q1 | Ubiquitin ligase TRIM63 | Homo sapiens | 5e-39 | H | BIB | CLSTR10565/1 | 0 | 0 |
| 85  | 2.45      | CLSTR16591/1 | P16109 | P-selectin precursor | Homo sapiens | 7e-18 | H | BIB | CLSTR30077/1 | * | 0 |
| 88  | 2.42      | KYOTOGRAIL2005.143.32.1 | P16109 | P-selectin precursor | Homo sapiens | 1e-66 | H | BIB | CLSTR70725/1 | * | 0 |
| 89  | 2.41      | KYOTOGRAIL2005.257.11.1 | P98695 | Fibulin-2 precursor | Homo sapiens | 6e-60 | H | BIB | CLSTR11246/1 | 0 | 2 |

To be continued.
much of the genome. Most other invertebrate genomes have moderately high levels of methyl-CpG concentrated in large domains of methylated DNA that are separated by similarly large domains of unmethylated DNA (Bird et al., 1979). This mosaic methylation pattern has been confirmed at higher resolution in mosaic methylation pattern has been confirmed at higher resolution in mosaic methylation pattern has been confirmed at higher resolution in genetic contexts.

Our study is the first attempt to examine the relationship between DNA methylation and gene expression in Ciona embryos. It is unclear whether the changes in transcription we observed are directly associated with the state of DNA methylation, and we plan to address this in future studies.

The supplementary data for this article can be found online at “http://dx.doi.org/10.2108/zsj24.648.s1”.

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REFERENCES


Footnotes for Table 1.

** shows that a number of clusters containing only one clone are put together.


Taylor SM, Jones PA (1979) Multiple new phenotypes induced in 10T1/2 and 3T3 cells treated with 5-azacytidine. Cell 17: 771–779


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