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# Transcripts Containing the Sea Urchin Retroposon Family 1 (SURF1) in Embryos of the Sea Urchin *Anthocardis crassispina*

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**ABSTRACT**—We isolated two cDNAs, termed D7 and C2 in the present study, from a cDNA library of the 16-cell embryo of the sea urchin *Anthocardis crassispina*. The nucleotide sequence was determined completely for D7, and partially for C2. D7 does not have any significant open reading frames. Both D7 and C2 contain a common sequence that is 62% homologous to the sea urchin retroposon family 1 (SURF1). The SURF1 is a short interspersed repetitive element identified from the sea urchin *Strongylocentrotus purpuratus*, and is reported to be transcribed by RNA polymerase III. The structural feature of D7 and C2, however, suggests that they may be transcribed by RNA polymerase II. RT-PCR analyses revealed that (1) both D7 and C2 transcripts exist as a maternal RNA in the egg, (2) they appear evenly distributed in the 16-cell embryo, and (3) C2 transcripts are present throughout the development up to the gastrula, while D7 transcripts decrease in amount after the early cleavage stage.

## INTRODUCTION

Short interspersed repetitive elements (SINEs) are present in most, probably all, eukaryotic genomes. SINEs appear to have been amplified and dispersed in the genome via RNA intermediates, and are referred to as retroposons (Rogers, 1985; Weiner *et al.*, 1986). Among SINEs, the human *Alu* family and the mouse B1 family are homologous to 7SL RNA of the signal recognition particle, and are therefore considered to be of 7SL RNA origin (Rogers, 1985). However, all other SINEs appear to have been derived from tRNAs (Lawrence *et al.*, 1985; Daniels and Deininger, 1985; Sakamoto and Okada, 1985; Endoh and Okada, 1986; Matsumoto *et al.*, 1986). Although functions of SINEs are still uncertain, it is clear that amplification and dispersion of SINEs have greatly contributed to genomic evolution in many species as a mechanism to maintain the fluidity of genomes (for reviews, see Okada, 1991a, b).

In studies of the sea urchin genome, some short repetitive sequence families have been reported (Posakony *et al.*, 1981; Carpenter *et al.*, 1982; Johnson *et al.*, 1984; Cohen *et al.*, 1985; Nisson *et al.*, 1988; Calzone *et al.*, 1988). The best characterized is the sea urchin retroposon family1 (SURF1). The SURF1 was first identified as an element located upstream of the muscle actin gene of the sea urchin *Strongylocentrotus*

*purpuratus* (Hickey *et al.*, 1987). Approximately 800 copies of the SURF1 are estimated to be dispersed in the genome (Nisson *et al.*, 1988). The SURF1-1, a member of the SURF1, has the following features characteristic of SINEs: (1) it consists of three distinct regions, the tRNA-related, the tRNA-unrelated, and the AT-rich regions, (2) it is flanked by direct repeats, and (3) it is transcribed by RNA polymerase III *in vitro* (Nisson *et al.*, 1988).

In the present study, we isolated two cDNAs that contain the SURF1-like sequence from a cDNA library of the 16-cell embryo of the sea urchin *Anthocardis crassispina*. We present the structures and the expression patterns during the development. The structural features suggest that they may be transcribed not by RNA polymerase III but by RNA polymerase II. We also discuss possible functions as well as the evolutionary origin of the SURF1-like sequence.

## MATERIALS AND METHODS

### Sea urchin embryo

*A. crassispina* were collected near the Noto Marine Laboratory, Kanazawa University. Gametes were obtained by intracoelomic injection of 0.5 M KCl. Eggs were fertilized in filtered sea water, and embryos were cultured at a concentration of  $3 \times 10^3$  embryos/ml in filtered sea water at 20°C with gentle stirring.

### Blastomere separation

Eggs were inseminated in filtered sea water containing 1 mM 3-amino-1,2,4-triazole to prevent the fertilization membrane from hardening (Showmann and Foerder, 1979). The fertilization membrane

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was removed by the hatching enzyme as described previously (Yamaguchi *et al.*, 1994). Denuded embryos were cultured up to the 16-cell stage in sea water at 20°C at a concentration of  $5 \times 10^4$  embryos/ml. The 16-cell embryos were washed twice with ice-cold calcium-magnesium-free sea water containing 1 mM ethylene glycol-bis ( $\beta$ -aminoethyl ether) tetraacetic acid (EGTA). Approximately  $2 \times 10^6$  embryos were applied to the Hitachi SRR6Y elutriation system, and three types of blastomeres, the micromere, the mesomere and the macromere, were elutriated and fractionated depending on the size by an increase of flow rates (Yamaguchi *et al.*, 1994).

#### A. *crassispina* 16-cell embryo cDNA library

Total RNA was isolated from the 16-cell embryo by homogenization in guanidium thiocyanate followed by LiCl precipitation (Cathala *et al.*, 1983). Polyadenylated RNA was prepared from total RNA by oligo(dT)-cellulose chromatography. Complementary DNA copies of polyadenylated RNAs were synthesized by cDNA synthesis system (Amersham) using oligo(dT) as a primer. Complementary DNA library was constructed in  $\lambda$ gt11 (Amersham) according to the manufacturer's instructions. It included  $1.8 \times 10^6$  independent clones, and their insert size was 1.9 kb in average. The cDNA library was amplified once in our experiment.

#### Differential screening

Poly(A)<sup>+</sup> RNA was isolated from each blastomere of the 16-cell embryo as described above. <sup>32</sup>P-labeled cDNA was synthesized from 2  $\mu$ g poly(A)<sup>+</sup> RNA with 100 mCi [ $\alpha$ -<sup>32</sup>P]dCTP (600 Ci/mmol) and 100 units MMLV reverse transcriptase (BRL) using oligo(dT) as a primer. Approximately  $2 \times 10^4$  recombinants were differentially screened for the 16-cell embryo library using labeled cDNAs as probes. Filters (Hybond-N, Amersham) were hybridized in 5  $\times$  SSC, 5  $\times$  Denhardt's solution, 0.3% SDS, 60% formamide containing denatured salmon sperm DNA (100  $\mu$ g/ml) and labeled cDNA ( $5 \times 10^6$  cpm/ml) at 37°C. The filter was washed at 55°C in 0.1  $\times$  SSC, 0.1% SDS, and then exposed to a film (Kodak X-omat AR) for 7 days at -70°C using an intensifying screen. Isolated recombinants were subcloned into Bluescript II (Stratagene), and their sequences were determined by the dideoxy-chain termination method (Sanger *et al.*, 1977).

#### Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from *A. crassispina* embryos by GTC/LiCl method as described above. Complementary DNA was synthesized from total RNA with SuperScript reverse transcriptase (BRL) using oligo(dT) as a primer. RT-PCR was carried out using Taq DNA polymerase (Toyobo) on a thermal cycler, Quick Thermo Personal QTP-1 (Nippon Genetics). Two sets of oligonucleotides, P1S (5'-TGGATGCATTCCTTTTGTCTCGT-3') and P1A (5'-CAGCAGTAAATGGTATTGTGTCC-3'), and P2S (5'-CACTAGCGTCCTTTGGCA-3') and P2A (5'-GCATCCATACAGCAGCTG-3'), were used as a primer set for D7, which corresponded to nucleotide 642-665 and 1243-1266, and 2-19 and 261-278, respectively. For C2, P3S (5'-TTACTCACGTTACATTTGGAAAGG-3') and P3A (5'-GTGTGCATTGTTGTAACATAAACT-3') were used as a primer set, which were directed to nucleotide 14-37 and 389-412, respectively. Primers for the elongation factor were synthesized according to the sequence of the *A. crassispina* EF-1 $\alpha$  (M. Saito and K. Yamasu, personal communication). A reaction mixture containing cDNA synthesized from 5 ng total RNA as a template in a final volume of 25  $\mu$ l was subjected to PCR for 27-33 cycles of 92°C, 1 min for denaturing, 60°C, 1 min for annealing, and 72°C, 2 min for extension for primer sets of P1S/P1A and P3S/P3A, and for 27-33 cycles of 92°C, 1 min, 55°C, 1 min, and 72°C, 1 min for a set of P2S/P2A. PCR conditions for EF-1 $\alpha$  was 24-30 cycles of 92.5°C, 30 sec, 50°C, 1 min, and 72°C, 1 min. After PCR, 5  $\mu$ l of the reaction mixture was applied to a 1.5% agarose or a 3% NuSieve 3:1 agarose gel, and electrophoresed in 1  $\times$  TAE buffer. Gels were stained with ethidium bromide (0.5  $\mu$ g/ml) to detect PCR products.

## RESULTS

### Structural analyses

As a result of differential screening, we isolated 4 cDNAs from  $2 \times 10^4$  recombinants, named B5, C2, D7, and K4 in the present study, which appeared to be specific to the micromere of the 16-cell embryo. Restriction analysis revealed that B5 and D7 are identical with C2 and K4, respectively. We determined the complete nucleotide sequence of D7, and the partial sequence of C2.

D7 consists of 1392 bp, but no significant open reading frame was identified in either strand. One end of D7 terminates with oligo(A), and also the polyadenylation consensus sequence (AATAAA) locates 7 nucleotides upstream from the oligo(A) (Fig. 1). This structural feature is characteristic of the 3' end of RNA polymerase II transcripts (Proudfoot and Brownlee, 1976). We, therefore, refer to the end as the putative 3' end in the present study. Tandemly repeated sequences

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1  GCACTAGCGTCCTTTGGCAAGGCTAATCTACATTGCCACTCTCCACCCAGGTGTAA  60
61  ATGGGTACCGGCAGGAATGAATTCCTGAATGCTGTGAGCGCCGAAATCGGCAGCCTAGC  120
121 TAGAGCCGGGTAATATTGGAGCGCTTGAGCACCATTGCTGGATATGAGCGCTATA  180
181 TAAATATCCAATATTATTATTATTAGTCCCAATTCAAGCTCTTTGGATTGGACT  240
241 TCAAAACCCAGGTTTCTTAACCGCTGCTGATGGATGACCCAATACTTTCTTCATGGTC  300
301 TGGTAAGATCAACTTGAAATCCATAGTAAAGCAACAACATCCACCTATCCTTCAATT  360
361 GCAGGGCTGATGACACATGAACCTAAATGTTTGGTGATCTGTTGTCCTTTTAAGGTG  420
421 ATCTTTTCAAGCACCATTATTTCTATTAGTTCTGTTTGAACGCACTATTACAGGC  480
481 AGACTCTACTACAACCTTAAACACAAATCTTGTGAAAAATCGATCAATAATTCATAA  540
541 TTGCCATGTACTTCCATTTTGGATGTTTATCCCAAACTTACGATAATCTTTTAATC  600
601 TCCAATGGTTTTAGTTGTACAGGATGCTGATTTTTTTTGGATGATTCCTTTTGT  660
661 CTCGTAATCAAGATACCTTTTCAAGAAATGATTATAATGAATTGAAATAATTCATCTCT  720
721 CTTTTTAACTATTTTCTACATACCATCTGCTGATTTTACGAACCGACATACAAATCTAG  780
781 CAGGGATTAACCAACAATACTGCTGATTTTACGAACCCGATATGAATCTAGCAGGAT  840
841 TAAACCAAAAGTACTGCGTTTCTTTTGAATATCCAATGATATGAAAGAAATATTC  900
901 CACTCTCATCACCTGTTTAACTTCTATGATGATTGAATGAAAGTTAAGATATTCAAATTAG  960
961 CATTTTAAACAGAAAAGTCTTATACACAAATATCTCAGAAAAAATTTAGTCAGTG  1020
1021 TTCATTTTGTGTTTGTGTTTGTGTTATCTTCCCCCCCCAAATATTTTGTGCTGTTT  1080
1081 AGCATCATGCTATTTAAAAAAATTAAGAATAAATCATGATACCTCAACGATATGGG  1140
1141 AACAAAAAGGAAGTTAGATTTCAAAATTCAGTCGCTCTTATAATGCTCTACCTTGA  1200
1201 TCACAAAGCTCAAAATTCATTGAATACTACTTCTTTGTAGGAACACAATACCATTTA  1260
1261 CTGCTGTGAAGCTGCTGATGAGTACATGTGATCCAACTTTCTCCATATGTGCAATGT  1320
1321 TCAGTATTTTGTAACTATTGCCTACATATTCAAACTACTATAAGTAATGATAATGAATA  1380
1381 AATGATTAAAA

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**Fig. 1.** Nucleotide sequence of D7. Boxes indicate tandemly repeated sequences of 52 nucleotides. Arrows indicate sequences to which oligonucleotide primers are directed in RT-PCR. Internal EcoRI sites are thick-underlined. The Spec repeat-related region of the SURF1-like sequence is in italics, whereas the AT-rich region is in underlined italics. Polyadenylation consensus sequence (AATAAA) is dotted. This sequence has been deposited in the DDBJ database under accession No. D87213.

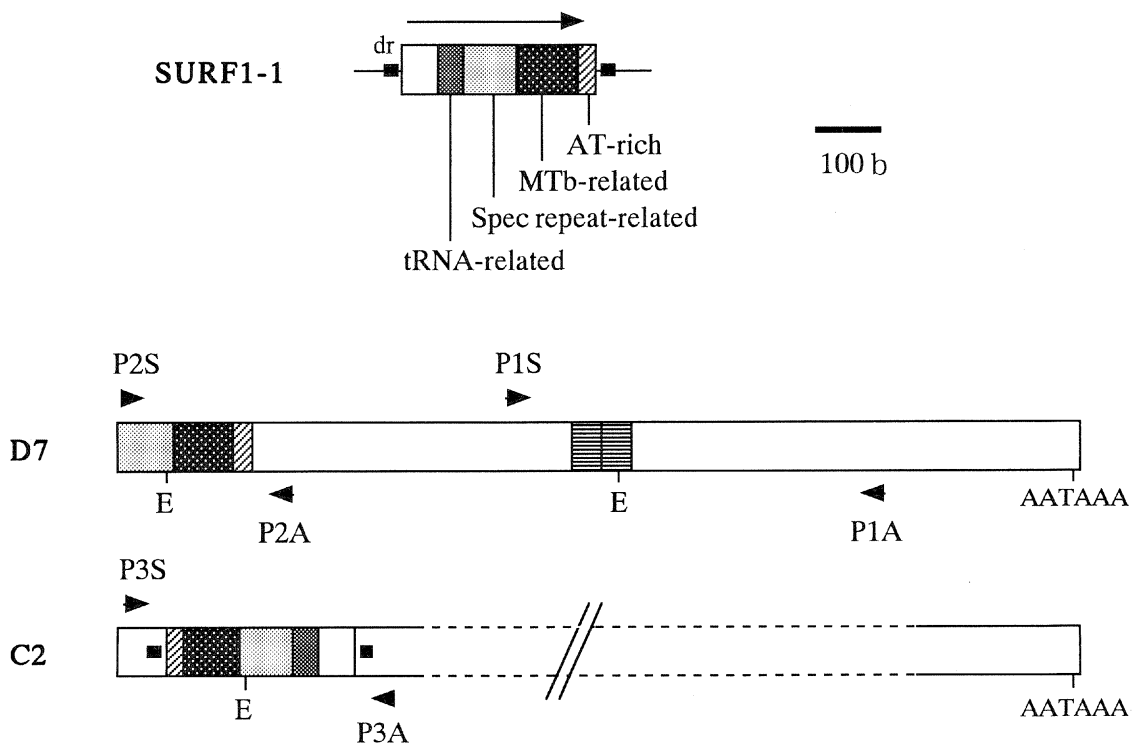
of 52 nucleotides exist around nucleotide 800, whose homology is 88%.

Homology search for D7 sequence showed that the 5' 210 bp has 62% homology to the sea urchin retroposon family 1 (SURF1), whereas the remaining 1.2 kb does not have any significant similarity to the sequences available through the DDBJ database. As other SINES, the SURF1-1 consists of three distinct regions: the tRNA-related region, the tRNA-unrelated region, and the simple AT-rich region (Fig. 2). The 5' portion of the tRNA-unrelated region is similar to the Spec repeat, and is referred to as the Spec repeat-related region (Nisson *et al.*, 1988). The Spec repeat was first identified as a common sequence between Spec1 and Spec2, the ectoderm-specific mRNAs of *S. purpuratus* embryos (Carpenter *et al.*, 1982). In the present study, the 3' portion of the tRNA-unrelated region was found to be 57% homologous to the 3' non-coding region of metallothionein B mRNA of *S. purpuratus* (Wilkinson and Nemer, 1987). Thus, we refer to this portion as the MTb-related region. The 5' end of D7 corresponds to the boundary between the tRNA-related and the Spec repeat-related regions. That is, D7 lacks the tRNA-related region of the SURF1-1.

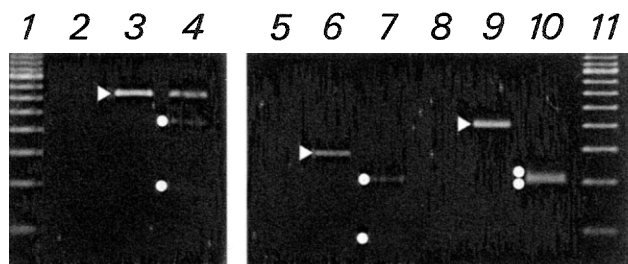
Transcription of the SURF1-1 by RNA polymerase III starts at the 5' portion and terminates at the AT-rich region (Nisson *et al.*, 1988). In order to confirm that the SURF1-like sequence constitutes a structural part of D7, we carried out RT-PCR

using D7-specific primers, P2S and P2A. P2S and P2A are directed to the sequences in and out of the SURF1-like sequence, respectively (Fig. 2). The PCR amplified a DNA fragment of expected size and also with an expected internal *Eco* R I site (Fig. 3, lanes 5-7). This indicates that the SURF1-like sequence is a part of a longer transcript. The original sequence of D7 may include the tRNA-related region, which serves as a promoter for RNA polymerase III. The SURF1-like sequence in D7, however, is followed by the AT-rich region and also 16 TTTTs and 8 longer oligo(T)s (Fig. 1). These sequences are considered to be a strong termination signal for RNA polymerase III (Endoh *et al.*, 1990). These sequences together with the presence of polyadenylation signal near the 3' end suggest that D7 may be transcribed by RNA polymerase II.

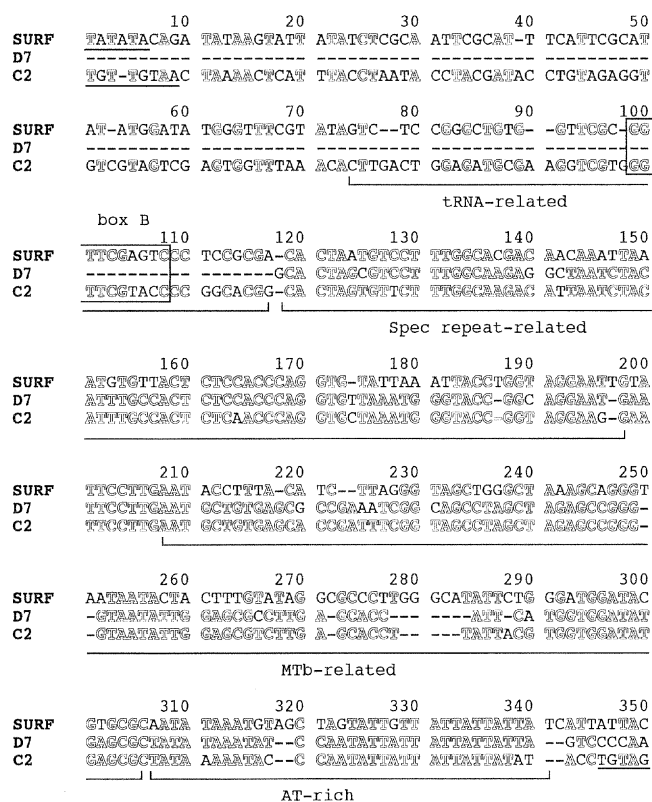
Turning to C2 cDNA, it is about 2.3 kb in size. We determined the sequence of about 0.3 kb from one end and about 0.5 kb from another (Fig. 2). One end of C2 terminates the polyadenylation consensus sequence followed by poly(A). We, therefore, refer to the end as the putative 3' end. The 5' region of C2 also contains the SURF1-like sequence, which is flanked by short direct repeats. The SURF1-like sequence in C2 is 62% and 89% similar to the SURF1-1 and the SURF1-like sequence in D7, respectively. However, the orientation is inverted. As in the structural analysis of D7, we performed RT-PCR for C2 using C2-specific primers, P3S and P3A. P3S and P3A correspond to the sequences in the 5' and the 3'



**Fig. 2.** Structures of the SURF1-1, D7, and C2. The SURF1-1 consists of the tRNA-related (dark shadow), the Spec repeat-related (light shadow), the MTb-related (white dots), and the AT-rich (diagonal stripes) regions, which are flanked by direct repeats (dr). A transcriptional unit and the direction by RNA polymerase III are indicated by an arrow. D7 and C2 include the SURF1-like structure in their 5' regions. Horizontally striped boxes in D7 indicate tandemly repeated sequences of 52 nucleotides. E and AATAAA show the position of internal *Eco* R I sites and polyadenylation consensus sequences, respectively. Arrowheads indicate positions of oligonucleotide primers used in RT-PCR.



**Fig. 3.** RT-PCR analyses using total RNA from the 16-cell embryo as a template. P1S/P1A (lanes 2-4) and P2S/P2A (lanes 5-7) were used as primer sets specific for D7, whereas P3S/P3A (lanes 8-10) was used as a C2-specific primer set (see Figs. 1 and 2). Lanes 2, 5, and 8 indicate PCR without reverse transcription, showing no genomic amplification under the condition used in the present study. Lanes 3, 6, and 9 show RT-PCR products of expected sizes (arrowheads). Lanes 4, 7, and 10 indicate *Eco* R I-digested RT-PCR products of expected size (white dots). Lanes 1 and 11 represent size markers (100 bp ladder, BRL).



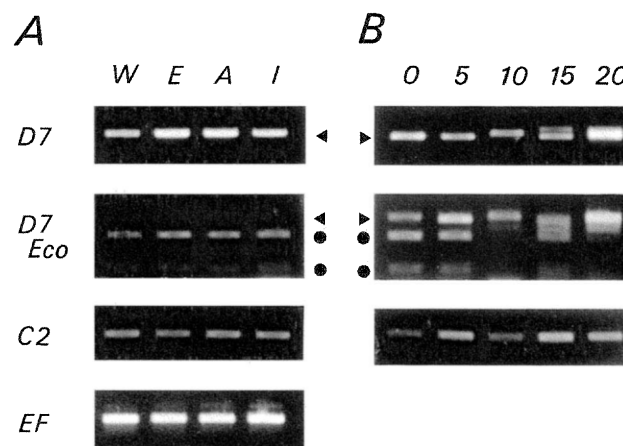
**Fig. 4.** Alignment of the nucleotide sequences between the SURF1-1 and the SURF1-like sequences in D7 and C2. Outlined letters represent the conserved nucleotides. Direct repeats flanking the SURF1-1 and the SURF1-like sequence in C2 were underlined. Note that the tRNA-related region is less conserved than the Spec repeat-related and the MTb-related regions.

flanking regions of the SURF1-like sequence, respectively (Fig. 2). The PCR amplified a DNA fragment of expected size and also with an expected internal *Eco* R I site (Fig. 3, lanes 8-10). This result, together with the presence of direct repeats flanking the SURF1-like sequence, and also the polyadenylation signal, suggest that the SURF1-like sequence in C2 may be also embedded within a RNA polymerase II transcription unit.

Figure 4 shows an alignment between the SURF1-1 and the SURF1-like sequences in D7 and C2. Most SINES include box A and box B, consensus sequences for the promoter of RNA polymerase III, in the tRNA-related regions (Galli *et al.*, 1981). Both the SURF1-1 and the SURF1-like sequence in C2 contain box B (GGTTCGANNCC), but no apparent box A was detected. It should be noted that the Spec repeat-related and the MTb-related regions (tRNA-unrelated region) are more conserved than the tRNA-related region between the SURF1-1 and the SURF1-like sequences (see discussion).

#### Distribution of D7 and C2 transcripts in the 16-cell embryo

To examine the spatial expression patterns of D7 and C2 within the *A. crassispina* embryo, we isolated total RNAs from the micromere, the mesomere, and the macromere of the 16-



**Fig. 5.** Expressions of D7 and C2 in the *A. crassispina* embryo analyzed by RT-PCR. D7 and C2 transcripts were amplified using P1S/P1A and P3S/P3A as a primer set, respectively. Arrowheads indicate RT-PCR products with expected size (D7), whereas dots show expected *Eco* R I-digested fragments of the product (D7 Eco). (A) Spatial expressions of D7 and C2 in the 16-cell embryo. Total RNAs from the whole embryo (W), the mesomere (E), the macromere (A), and the micromere (I) were used as a template. Both D7 and C2 transcripts appear to be uniformly distributed in the embryo, judged from an amount of the PCR product of EF-1 $\alpha$  (EF). (B) Temporal expressions of D7 and C2 during development. Total RNAs extracted from the unfertilized egg (0 hr), the early blastula (5 hr), the hatching blastula (10 hr), the mesenchyme blastula (15 hr), and the gastrula (20 hr) were used as a template. Judged from the presence of *Eco* R I-digested fragments (dots in D7 Eco), D7 is a maternal RNA. The transcript, however, decreases in amount after hatching. C2 is also stored in the egg as a maternal RNA, and the transcript exists in the embryo throughout the development.

cell embryo. We then carried out RT-PCR using oligonucleotides, P1S/P1A and P3S/P3A, as primer sets for D7 and C2, respectively. When analyzed by agarose gel electrophoresis, a DNA fragment of expected size for D7 (623 bp) and C2 (398 bp) was detected. This fragment was almost equal in amount among the three types of blastomeres (Fig. 5A). RT-PCR using another D7-specific primer set, P2S/P2A, also brought the same result (data not shown). Therefore, transcripts of D7 and C2 appear to be present evenly in the 16-cell embryo.

### Expressions of D7 and C2 during development

To examine the temporal expression of D7 and C2 during the development, we carried out RT-PCR using P1S/P1A and P3S/P3A as primer sets for D7 and C2, respectively (Fig. 5B). D7-specific primers generated about 0.7 kb DNA fragment(s) of expected size throughout the development from the unfertilized egg to the gastrula. Some of the fragments, however, did not contain an expected internal *Eco* R I site, suggesting that PCR might co-amplify a non-specific DNA fragment with a similar size. *Eco* R I-digested fragments were detected in the unfertilized egg and the early blastula, but decreased in amount after hatching. Contrary to this, C2-specific primers amplified a DNA fragment of expected size (Fig. 5B) and also with an expected internal *Eco* R I site (data not shown) throughout the development. These results show that both D7 and C2 are maternal RNAs, but their temporal expression appear to be regulated separately during development.

## DISCUSSION

We isolated two cDNAs, D7 and C2, which contain the SURF1-like sequence, from a cDNA library of the 16-cell embryo of the sea urchin *A. crassispina*. RT-PCR analyses using primers directed to sequences in and out of the SURF1-like sequences indicated that the SURF1-like sequence constitutes a structural part of D7 and C2. Nisson *et al.* (1988) reported that there is little expression of the SURF1 family by RNA polymerase II in early embryos. Structural features of D7 and C2, however, suggest that they may be transcribed by RNA polymerase II because of the following three reasons. First, both D7 and C2 have the polyadenylation signal followed by oligo(A), which is a common feature of the 3' end of RNA polymerase II transcripts (Proudfoot and Brownlee, 1976). Second, the SURF1-like sequence is followed by many AT-rich sequences, which serve as a strong stop signal for RNA polymerase III (Endoh *et al.*, 1990). Third, the SURF1-like sequence in C2 is embedded in a longer transcript in an inverted orientation. D7 and C2, therefore, are the first examples suggesting that the SURF1 may be also present as RNA polymerase II transcripts. It is probable that the SURF1-like sequences would be inserted into some maternal RNA genes by retroposition.

Concerning the number of the Spec repeat, two estimates have been reported. Carpenter *et al.* (1982) demonstrate that 2-3,000 copies are present in the genome of *S. purpuratus*,

and that four cloned versions of the Spec repeat do not have any features of retroposons. On the other hand, Nisson *et al.* (1988) estimate about 800 copies of the SURF1 in the genome. Judging from difference in the copy number between the Spec repeat and the SURF1, Nisson *et al.* (1988) suggest the following scenario for the formation of the SURF1 in the sea urchin genome. Initially, the Spec sequence became amplified and dispersed throughout the genome by a DNA mediated transposition, and one copy of this sequence was inserted just downstream of a tRNA gene. Subsequently, a transcript of this tRNA gene, which extended into the Spec sequence, was copied by the reverse transcriptase. Multiple copies of these retroposons were then dispersed in the genome. It is important to note that the tRNA-related region is less conserved than the Spec repeat-related region in the SURF1-1, D7, and C2 (Fig. 4). If a rate of the sequence divergence is uniform within the SURF1 sequence, the scenario suggested by Nisson *et al.* (1988) cannot explain the above fact. Instead, it seems more likely that the Spec sequence was amplified, and some of them were inserted just downstream of tRNA genes that had already diverged in the genome. Subsequently, transcripts of these tRNA genes, which acquired the Spec sequence in the 3' region, were reverse transcribed and dispersed in the genome. Deininger and Daniels have proposed that the tRNA-related regions of SINES have been generated from tDNAs which have accumulated mutations that did not hinder the intrinsic functions of tRNAs (Deininger and Daniels, 1986).

Alternatively, it is possible to consider that the Spec repeat may have a function to be conserved. Inverted repeat sequences often serve as target sites for transcription factors, which interact facing each other as homotypic or heterotypic pairs (Johnson and Smith, 1992; Ellis *et al.*, 1990; Cao *et al.*, 1991). Recently, Anderson *et al.* (1994) reported that conserved inverted repeat sequence serves as a target site for a sea urchin maternal DNA-binding factor. They estimate about 460 copies of the inverted repeat target sites or single sites in the *S. purpuratus* genome. The Spec repeat may also serve as a target site for a DNA-binding factor. It is also probable that the Spec repeat sequence in transcripts may regulate stability or translation of other mRNAs containing Spec repeat sequence. Spec1 and Spec2 mRNAs, for example, include the Spec repeat sequence in the 3' non-coding region, which is complementary to the Spec repeat-related region in D7 or the SURF1-1. This implies that D7 or the SURF1-1 transcript could hybridize with Spec1 and Spec2 mRNAs to destabilize them in the cytoplasm. Bruskin *et al.* (1981) reported that Spec1 and Spec2 mRNAs are rare or undetectable during the cleavage stage, but increase in amount from the blastula stage. In contrast, D7 transcript is present in the cleavage stage, but then decreases in amount (Fig. 5). Expression of the SURF1-1 is also similar to that of D7, that is, the highest around the 128-cell stage and drops as development proceeds (Nisson *et al.*, 1988). These results suggest that D7 and the SURF1 transcripts may work as a negative regulator against the Spec repeat-containing mRNAs during the early

stage of development. It may also explain the observation that C2 transcript is present throughout development unlike D7 transcript (Fig. 5).

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## REFERENCES

- Anderson R, Britten RJ, Davidson EH (1994) Repeated sequence target sites for maternal DNA-binding proteins in genes activated in early sea urchin development. *Dev Biol* 163: 11–18
- Bruskin AM, Tyner AL, Wells DE, Showman RM, Klein WH (1981) Accumulation in embryogenesis of five mRNAs enriched in the ectoderm of the sea urchin pluteus. *Dev Biol* 87: 308–318
- Calzone FJ, Lee JJ, Le N, Britten RJ, Davidson EH (1988) A long, nontranslatable poly(A) RNA stored in the egg of the sea urchin *Strongylocentrotus purpuratus*. *Genes Dev* 2: 305–318
- Cao Z, Umek RM, McKnight SL (1991) Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells. *Genes Dev* 5: 1538–1552
- Carpenter CD, Bruskin AM, Spain LM, Eldon ED, Klein WH (1982) The 3' untranslated regions of the two related mRNAs contain an element highly repeated in the sea urchin genome. *Nucl Acids Res* 10: 7829–7840
- Cathala G, Savouret J-F, Mendez B, West BL, Karin M, Martial JA, Baxter JD (1983) A method for isolation of intact, translationally active ribonucleic acid. *DNA* 2: 329–335
- Cohen JB, Hoffman-Liebermann B, Kedes L (1985) Structure and unusual characteristics of a new family of transposable elements in the sea urchin *Strongylocentrotus purpuratus*. *Mol Cell Biol* 5: 2804–2813
- Daniels GR, Deininger PL (1985) Repeat sequence families derived from mammalian tRNA genes. *Nature* 317: 819–822
- Deininger PL, Daniels GR (1986) The recent evolution of mammalian repetitive DNA elements. *Trends Genet* 2: 76–80
- Ellis HM, Spann DR, Posakony JW (1990) *extramachrochaetae*, a negative regulator of sensory organ development in *Drosophila*, defines a new class of helix-loop-helix proteins. *Cell* 61: 27–38
- Endoh H, Okada N (1986) Total DNA transcription in vitro. A procedure to detect highly repetitive and transcribable sequences with tRNA-like structures. *Proc Natl Acad Sci USA* 83: 251–255
- Endoh H, Nagahashi S, Okada N (1990) A highly repetitive and transcribable sequence in the tortoise genome is probably a retroposon. *Eur J Biochem* 189: 25–31
- Galli G, Hofstetter H, Birnstiel ML (1981) Two conserved sequence blocks within eukaryotic tRNA genes are major promoter elements. *Nature* 294: 626–631
- Hickey RJ, Boshart MF, Crain WR (1987) Transcription of three actin genes and a repeated sequence in isolated nuclei of sea urchin embryos. *Dev Biol* 124: 215–227
- Johnson AD, Smith DL (1992) A molecular mechanism for combinatorial control in yeast: MCM1 protein sets the spacing and orientation of the homeodomains of a2 dimer. *Cell* 68: 133–142
- Johnson SA, Davidson EH, Britten RJ (1984) Insertion of a short repetitive sequence (D881) in a sea urchin gene: a typical interspersed repeat? *J Mol Evol* 20: 195–201
- Lawrence CB, McDonnell DP, Ramsey WJ (1985) Analysis of repetitive sequence elements containing tRNA-like sequences. *Nucl Acids Res* 13: 4239–4252
- Matsumoto K, Murakami K, Okada N (1986) Gene for lysine tRNA1 may be a progenitor of the highly repetitive and transcribable sequences present in the salmon genome. *Proc Natl Acad Sci USA* 83: 3156–3160
- Nisson PE, Hickey RJ, Boshart MF, Crain WR (1988) Identification of a repeated sequence in the genome of the sea urchin which is transcribed by RNA polymerase III and contains the features of a retroposon. *Nucl Acids Res* 16: 1431–1452
- Okada N (1991a) SINES: short interspersed repeated elements of the eukaryotic genome. *Trends Ecol Evol* 6: 358–361
- Okada N (1991b) SINES. *Curr Opin Genet Develop* 1: 498–504
- Posakony JW, Scheller RH, Anderson DM, Britten RJ, Davidson EH (1981) Repetitive sequence of the sea urchin genome. III. Nucleotide sequences of cloned repeat element. *J Mol Biol* 149: 41–67
- Proudfoot NJ, Brownlee GG (1976) 3' noncoding region sequences in eukaryotic messenger RNA. *Nature* 263: 211–214
- Rogers J (1985) The structure and evolution of retroposons. *Int Rev Cytol* 93: 231–279
- Sakamoto K, Okada N (1985) Rodent type 2 Alu family, rat identifier sequence, rabbit C family, and bovine or goat 73-bp repeat may have evolved from tRNA genes. *J Mol Evol* 22: 134–140
- Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74: 5463–5467
- Showmann RM, Foerder C (1979) Removal of the fertilization membrane of sea urchin embryos employing aminotriazole. *Exp Cell Res* 120: 253–255
- Weiner AM, Deininger PL, Efstratiadis A (1986) Nonviral retroposons: genes, pseudogenes and transposable elements generated by the reverse flow of genetic information. *Annu Rev Biochem* 55: 631–661
- Wilkinson DG, Nemer M (1987) Metallothionein genes MTa and MTb expressed during distinct quantitative and tissue-specific regulation in sea urchin embryos. *Mol Cell Biol* 7: 48–58
- Yamaguchi M, Kinoshita T, Ohba Y (1994) Fractionation of micromeres mesomeres, and macromeres of 16-cell stage sea urchin embryos by elutriation. *Develop Growth Differ* 36: 381–387

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