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Evolutionary Conservation of Repetitive Sequence Expression in Sea Urchin Egg RNA's

Abstract. *Cloned repetitive DNA sequences were used to determine the number of homologous RNA transcripts in the eggs of two sea urchin species,* Strongylocentrotus purpuratus *and* S. franciscanus. *The eggs of these species contain different amounts of RNA, and their genomes contain different numbers of copies of the cloned repeats. The specific pattern of repetitive sequence representation in the two egg RNA's is nonetheless quantitatively similar. The evolutionary conservation of this pattern suggests the functional importance of repeat sequence expression.*

The sea urchin genome contains 10^5 to 10^6 repetitive sequence elements belonging to several thousand nonhomologous families. These repeat families are represented in nuclear RNA's and in egg RNA in a manner specific to the state of differentiation (*1, 2*). The individual repetitive sequence fragments were isolated by S1 nuclease digestion of partially renatured DNA from *Strongylocentrotus purpuratus*, and cloned by the addition of chemically synthesized restriction enzyme recognition sequences (*3*). The cloned fragments were labeled and strand-separated, and were used as probes to detect RNA's homologous to individual repeat families. The results can be summarized as follows: (i) All of nine cloned repeat sequences studied are represented in nuclear RNA's and egg RNA, and at least 80 percent of the various repeat families in the genome are represented in egg RNA. This is in marked contrast to single copy sequences, of which only a minor fraction are found in nuclear or egg RNA's. (ii) Each repeat sequence family is represented to a particular extent in each RNA. The sequence concentrations of transcripts complementary to particular cloned repeats may differ more than 100-fold in a given RNA, and different families are highly represented in each RNA investigated. Thus the levels of representation are a function of the state of differentiation. (iii) Both strands of each cloned repeat are represented in all the RNA's studied. The complementary repeat transcripts in general reside on different RNA molecules, and probably derive from asymmetric transcription of separate multiple genomic copies oriented oppositely (*4*). A number of repeat elements of each sequence family are probably utilized in transcription. Recent studies (*5*) have shown that many of the single copy maternal messenger RNA transcripts stored in the egg are linked covalently with short repetitive sequence elements, and most of the repeat transcripts are associated with this interspersed RNA fraction.

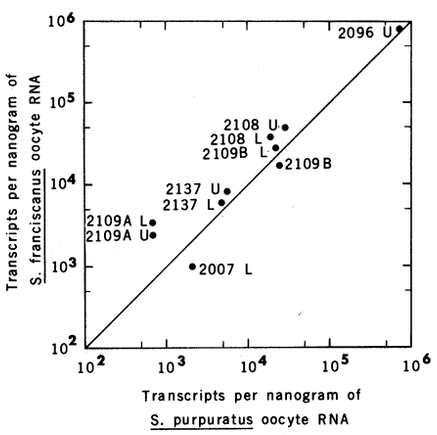

Fig. 1. Comparison of repeat sequence concentrations in the RNA of *Sf* and *Sp* eggs. Data are from *S. purpuratus* (abscissa) and *S. franciscanus* (ordinate) of Table 1. The solid line with slope 1 is the equivalence curve that would represent equal transcript concentrations in the eggs of the two species.

We now report experiments demonstrating that the specific pattern of repeat sequence representation in egg RNA has been quantitatively conserved since the divergence of two sea urchin species. The lineages leading to these species, *S. purpuratus* (*Sp*) and *S. franciscanus* (*Sf*), apparently separated 15 to 20 million years ago (*6*). They are of particular interest since their genomes contain clearly different numbers of copies of many repeat sequences (*7*). An issue addressed in the following experiments is whether the pattern of repeat sequence expression reflects these large evolutionary changes in repetitive sequence family size, or is independent of them.

In Table 1 are listed the results of measurements made with six cloned repeat fragments. The two strands of each cloned repeat were separated and reacted individually with *Sp* and *Sf* egg RNA's. The fraction of the total egg RNA complementary to each probe sequence was calculated from either kinetic or titration measurements (legend to Table 1) [see (*1*), the source of much of the *Sp* data listed]. In order to estimate the number of specific RNA transcripts, the mass of RNA per egg was multiplied by the specific fraction of the RNA hybridizing with the cloned probe. This calculation is of interest because the diameter of the *Sf* egg is about 120 μm compared to 80 μm for *Sp*, and thus the *Sf* egg is three times larger in volume. Measurements by the phloroglucinol method (*8*) show that the mass of RNA per average *Sf* egg is 8.3 ng compared to about 3 ng for the *Sp* egg (*9*). Thus the concentration of total RNA (most of which is ribosomal RNA) in the eggs of the two species is almost the same. In Table 1 the number of transcripts complementary to each cloned probe per egg is given for *Sp* and *Sf*, respectively; these values have been normalized for the egg RNA content of each species to give the number of specific transcripts per nanogram of total RNA. Measurement of transcript prevalence by kinetic or titration analysis is accurate only to within a factor of about 2.

The conclusions (*1*) for *Sp* egg RNA hold as well for *Sf* egg RNA (Table 1). Apart from clone CSp2096, which in several ways is atypical (*10*), the specific representation of the individual clones differs in *Sf* egg RNA by factors as large as 50. Both strands of all but one cloned repeat are also represented in *Sf* egg

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RNA, just as in *Sp* egg RNA. The exception is CSp2096, the representation of which is highly asymmetric in the eggs of both species (*10*).

The major result is that the pattern of representation of the six cloned probes in egg RNA in essentially similar in the two species (Table 1). The data on transcripts per oocyte show that the sequences highly represented in *Sp* egg RNA are also highly represented in *Sf* egg RNA. The number of specific transcripts per unit of RNA mass were normalized for the egg RNA content of the two species. With one exception (CSp2109A) the number of transcripts per unit of RNA mass is the same within a factor of 2 in *Sp* and *Sf* eggs, while the size of the genomic repeat sequence families represented by this set of clones differs as much as ninefold. In the case of the CSp2109A sequence, the *Sf* egg has three to four times more homologous transcripts per nanogram of egg RNA, but the *Sp* genome has 20 times the number of homologous repeats as the *Sf* genome. When one DNA strand is more highly represented in RNA, this transcriptional asymmetry is retained in the eggs of both species. This is clear in the case of CSp2096, and is probably also evident for the CSp2108 sequence. The degree of proportionality in the numbers of specific transcripts per egg for the two

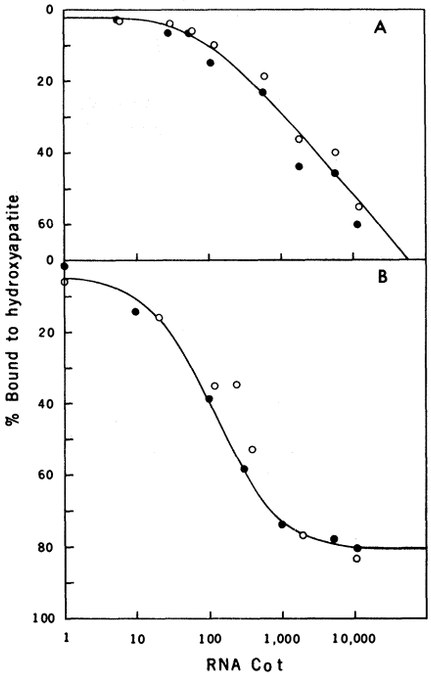

Fig. 2. Hybridization of *S. purpuratus* repetitive ^{3}H-labeled DNA with excess *S. franciscanus* and *S. purpuratus* egg RNA. (A) Hybridization of total *S. purpuratus* repetitive ^{3}H-labeled DNA tracer with *Sp* and *Sf* egg RNA. The *S. purpuratus* DNA was labeled in vivo to a specific activity of 1.1×10^6 cpm/μg. The ^{3}H-labeled DNA was denatured, reassociated to a C_0t of 40, treated with S1 nuclease, and chromatographed on Sepharose CL-2B. Included material of mode size 300 nucleotides was hybridized with a 10^6-fold mass excess of *S. purpuratus* egg RNA (●) and *S. franciscanus* egg RNA (○) at 55°C in 0.4*M* phosphate (Na^+) buffer. The fraction of ^{3}H-labeled DNA in RNA-DNA hybrids was assayed by binding to hydroxyapatite at 50°C in 0.12*M* phosphate (Na^+) buffer and elution at 99°C. The tracer was that described by Costantini *et al.* (*1*). The data were pooled and fit with the assumption of two kinetic components (solid line). The line shown is a least squares solution based on two kinetic components. This analysis suggests that 38.6 percent of the ^{3}H-labeled DNA repeat tracer hybridizes with a second-order rate constant of $1.79 \times 10^{-3} M^{-1}$ sec^{-1} and 38.5 percent hybridizes with a second-order rate constant of $5.64 \times 10^{-5} M^{-1}$ sec^{-1}. (B) Hybridization of highly expressed *S. purpuratus* repetitive ^{3}H-labeled DNA with *Sp* and *Sf* egg RNA. The *Sp* repetitive ^{3}H-labeled DNA tracer used in (A) was fractionated to select for those repeat sequences whose transcripts are most abundant by preparative reassociation with *Sp* egg RNA. This tracer was twice reassociated to RNA C_0t 500 with a 3×10^4-fold excess of *Sp* egg RNA as described (*1*). The ^{3}H-labeled DNA which bound to hydroxyapatite after the two RNA excess hybridizations constituted 19 percent of the repetitive tracer. The average sequence concentration of the RNA's represented in this tracer was 10^5 copies per egg. This selected ^{3}H-labeled DNA fraction was hybridized with a 10^6-fold excess of *Sp* (●) and *Sf* (○) egg RNA, and the kinetics of the reaction was determined. Hybridizations and assay conditions were as in (A). On the basis of a single second-order kinetic component, the second-order rate constants were $7.9 \times 10^3 M^{-1}$ sec^{-1} and $3.5 \times 10^3 M^{-1}$ sec^{-1}, respectively. The solid line shown is the least squares solution of the *Sp* hybridization points.

Table 1. Abundance of transcripts complementary to six cloned repetitive sequences in oocyte RNA's of *S. purpuratus* and *S. franciscanus*.

Clone	Strand*	Length† (NT)	Genomic‡ frequency *S. purpuratus*	Transcripts per oocyte§		Transcripts per nanogram of RNA		Genomic frequency ratio *S. purpuratus*/ *S. franciscanus*††
				S. purpuratus‖	*S. franciscanus*¶	*S. purpuratus***	*S. franciscanus***	
CSp2007	U	1,100	400	7,000	N.D.	2,100	N.D.	9
	L			7,000	8,300	2,100	1,000	
CSp2108	U	204	20‡‡	97,000	420,000	29,000	50,000	0.7
	L			59,000	310,000	18,000	37,000	
CSp2109A	U	180	900	2,500	20,000	800	2,400	20
	L			2,800	29,000	800	3,500	
CSp2109B	U	111	200	83,000	140,000	25,000	17,000	4
	L			73,000	240,000	22,000	29,000	
CSp2137	U	226	530	19,000	68,000	5,800	8,200	3
	L			16,000	50,000	4,800	6,000	
CSp2096	U	203	80	2,400,000	6,000,000	720,000	720,000	2.5
	L			Low§§	Low§§	Low§§	Low§§	

*The two complementary strands of each cloned repeat were designated "upper" (U) and "lower" (L) according to their electrophoretic mobility on neutral polyacrylamide gels (*2*). †The length of each cloned repetitive element except CSp2007 is known from DNA sequence analysis (*11*). The length of CSp2007 was estimated from electrophoretic mobility of the duplex fragment (*12*). ‡Haploid genomic repetition frequency was estimated from the kinetics of reassociation of each cloned repeat with an excess of *S. purpuratus* DNA (*12*). §The limit of detection of RNA transcripts is dependent on the specific activity of the cloned repeat DNA probe. The ^{32}P-labeled DNA probes used in this study were routinely labeled to about 10^7 cpm/μg, which allows detection of about 100 transcripts per nanogram of egg RNA. ‖The number of RNA transcripts complementary to a cloned DNA sequence was determined by comparing the rate of hybridization of labeled, strand-separated cloned repeats to the rate of hybridization of single copy ^{3}H-labeled DNA with excess oocyte RNA (*13*). The kinetics of the RNA-driven hybridizations with the cloned repeats are approximately second order since both complementary strands are present in the RNA. Titration measurements were carried out by reacting increasing amounts of oocyte RNA with a constant quantity of labeled, strand-separated DNA tracer. Data analysis was as described (*1, 2*). The values presented under *S. purpuratus* are averages of previous determinations (*1*) and new determinations. The latter are one determination for the CSp2108 upper strand, one determination for the CSp2109B lower strand, and the determination shown for the CSp2096 upper strand. These three measurements were obtained by the kinetic method. The rate constants (M^{-1} sec^{-1}) were 2.5×10^{-3} for the CSp2108 upper strand, 4.5×10^{-3} for the CSp2109B lower strand, and 2.2×10^{-1} for the CSp2096 upper strand. ¶The values shown were determined by the kinetic method except in the case of CSp2109A upper strand and one determination of CSp2137 upper strand, which were obtained by the titration method. The second-order rate constants (M^{-1} sec^{-1}) were: CSp2108 U, 8.0×10^{-3}; CSp2108 L, 5.9×10^{-3}; CSp2109A U, 5.3×10^{-4}; CSp2109B U, 4.0×10^{-3}; CSp2109B L, 7.0×10^{-3}; CSp2137 U, 1.1×10^{-3}; CSp2137 L, 9.8×10^{-4}; CSp2007 L, 3.4×10^{-4}; CSp2096 U, 2.2×10^{-1}. In the titration measurements the fractions of the total RNA found to consist of homologous transcripts were for CSp2109A, U, 3.1×10^{-7}; and for CSp2137 U, 1.1×10^{-6}. **The number of RNA transcripts per nanogram of oocyte RNA comlementary to a cloned DNA sequence is the number of transcripts per egg divided by 3.3 (nanograms of RNA per *Sp* egg) or 8.3 (nanograms of RNA per *Sf* egg). ††Data from (*6, 14*). ‡‡Recent work (*15*) has shown that there are many sequences distantly related to CSp2108 in the genome of *Sp*. The value shown refers to the size of a discrete subset of closely related sequences, which also exist in *Sf*. §§The case of CSp2096 L is complicated by the asymmetry of the RNA transcripts. During hybridization, labeled DNA probe must compete for its scarce RNA complement with the more abundant RNA strand. We estimate that the maximum prevalence of the CSp2096 L transcript is 7 percent of the CSp2096 U prevalence, or about 50,000 transcripts per nanogram of egg RNA in both species.

species is shown in Fig. 1, where the quantitative similarity in the pattern of repeat sequence expression in the two egg RNA's extends over the full range of transcript sequence concentrations.

The results in Table 1 and Fig. 1 derive from only six cloned repeat sequences and could be nonrepresentative. We attempted to generalize these results by using repetitive DNA tracers prepared from whole sheared *Sp* DNA. As a control, in Fig. 2A the reactions of such a tracer with *Sp* and *Sf* egg RNA's are shown. The kinetics of these reactions are almost indistinguishable, and it follows that the overall distribution of repeat transcript sequence concentrations in the two egg RNA's is similar. The generalization that those repeat families highly represented in *Sp* egg RNA are also highly represented in *Sf* egg RNA is tested in Fig. 2B. A subfraction of the genomic repeat tracer enriched for the sequences prevalent in *Sp* egg RNA was reacted with both egg RNA's. The experiment shows that the repeat sequences prevalent in *Sp* egg RNA are represented in about the same concentrations in *Sf* egg RNA. We conclude that the quantitative similarity in the patterns of repeat sequence representation is general in *Sp* and *Sf* egg RNA's.

The distribution of repeat sequence concentrations in egg RNA is almost certainly physiologically significant. This distribution is highly sequence specific, and different distributions have been found in other RNA's that have been studied. Specific processes of repetitive sequence transcription and RNA accumulation must operate during oogenesis. Our results show that these processes have been adjusted during evolution so as to preserve the repeat transcript sequence concentrations despite significant changes in genomic repeat family sizes and in egg volume and total RNA content. Conceivably all the repeat sequences belonging to expressed families are transcribed in both genomes, but the turnover and transcription rates have altered so as to compensate for the changes in repeat family size. The following argument suggests an alternative view. The particular pattern of repeat sequence representation present in the eggs of both of these species probably existed in their common ancestor as well. Therefore only a subset of the repeats of some repetitive families may be transcribed during oogenesis, since in the cases where the *Sp* families are larger than the *Sf* families this is not reflected in higher *Sp* transcript sequence concentrations. Possibly only repetitive sequence elements of given families which are present in the genomes of both species are expressed. The general implication would be that although multiple homologous repeats are utilized in transcription, these may often represent a subset of the whole genomic sequence family.

Our results emphasize the functional importance of repetitive sequence transcription. The particular pattern of repeat sequence representation in mature eggs is quantitatively conserved between two sea urchin congeners, although the size of the various repeat families in their genomes differs significantly. The repeat sequences themselves have diverged less than single copy sequences during the evolution of this genus (*7*). Conservation of repeat sequence expression suggests that the mechanism restraining change in the primary sequences is evolutionary selection, based on important but unknown functions of the repetitive RNA transcripts.

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Simian Virus 40 Crystals

Abstract. *Small (10 to 150 micrometers) cubic crystals of simian virus 40 have been grown by ammonium sulfate precipitation. Electron micrographs of thin sections from these crystals reveal ordered arrays of virus particles.*

During the past few years a number of crystalline RNA plant viruses have been studied by x-ray diffraction analysis; high-resolution images of tomato bushy stunt virus (*1*) and of southern bean mosaic virus (*2*) have become available. Polyoma became the first animal virus subjected to serious crystallographic investigation (*3, 4*). I now report the crystallization of simian virus 40 (SV40).

In the wild, SV40 causes inapparent infections in certain primates. In the laboratory it can cause tumors in immature animals, and can transform cells in culture. It serves as an important probe in studies of transformation, gene regulation and expression, and DNA replication (*5*). This virus is also of considerable structural interest. It is a nonenveloped particle whose capsid is built on a $T = 7d$ icosahedral surface lattice (*6, 7*). Its 420 subunits are clustered into 12 pentamers and 60 hexamers, giving a total of 72 morphological units or capsomeres in the coat (*7*). The particle diameter has been estimated, from electron microscopy (*8*), as 41 nm and, from x-ray solution scattering (*9*), as 48 nm. The molecular size is 17.6 million daltons (*8*). SV40 is known to code for three coat proteins (VP1, VP2, and VP3). Hexamers contain almost exclusively VP1,

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