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Rare maternal mRNAs code for regulatory proteins that control lineage-specific gene expression in the sea urchin embryo

(cleavage/mRNA prevalence/*Strongylocentrotus purpuratus*)

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ABSTRACT **The prevalence of mRNAs coding for the sea urchin embryo regulatory factors P3A1 and P3A2 was measured by single-strand probe excess solution hybridization. P3A1 and P3A2 are not homologous proteins, though they both bind specifically to a particular cis-regulatory sequence. Interaction at this target site is known to be required for lineage-specific expression of an aboral ectoderm-specific gene and probably for several other genes as well. Genome blot hybridizations show that both factors are encoded by single-copy genes. Maternal mRNAs for both factors are present at less than 10^3 molecules per egg, which places them in the rare mRNA class. During development to the mesenchyme blastula stage, the amount of P3A1 mRNA (per embryo) increases severalfold while that of P3A2 remains approximately constant. Specification of the aboral ectoderm founder cells and of their initial patterns of gene expression must occur during early to mid-cleavage stage. Therefore, the regulatory proteins needed for this process must be produced by this stage. We show that the quantities of the P3A proteins that can be synthesized from the numbers of mRNA molecules present in the large blastomeres of the early embryo are sufficient to be functional, because these proteins will be accumulated in the nuclei. Thus maternal P3A1 or P3A2 proteins are not required, nor were these detected in earlier studies. Furthermore, differential spatial (as well as temporal) distribution of both of these newly synthesized factor species could result from the unequal cleavage pattern utilized in the sea urchin egg.**

Two DNA-binding proteins, P3A1 and P3A2, that recognize the canonical target site $\overset{T}{C}N\overset{T}{C}GCGC\overset{A}{T}$ were recently cloned from embryonic materials of *Strongylocentrotus purpuratus*. P3A1 was cloned by direct screening of a cleavage-stage cDNA library by using a version of the oligomerized binding site as ligand (ref. 1; C.H., F.J.C., and E.H.D., unpublished data), and P3A2 was cloned by sequencing a protein purified from mesenchyme blastula-stage nuclear extracts by affinity chromatography over Sepharose bearing the same DNA target site (F.J.C., D. Teplow, C.H., and E.H.D., unpublished data). The P3A target site appears in the regulatory domains of three known genes active in the early embryo of this species, and in *in vitro* reactions with the regulatory DNA of these genes this site is bound tightly and specifically by proteins present in embryo nuclear extracts (2–4). These genes are the *CyIIIa* cytoskeletal actin gene (5) and the *Spec1* Ca^{2+}-binding-protein gene (6), which are expressed coordinately, exclusively in the aboral ectoderm lineages (7), and the *SM50* skeletal matrix protein gene, which is expressed in skeletogenic lineages (8, 9). *In vivo* competition experiments have shown that interference with the interactions mediated by the P3A site in the regulatory domain of the *CyIIIa* gene results in ectopic expression of a *CyIIIa*–chloramphenicol acetyltransferase gene (*CAT*) fusion reporter construct (10). Thus when excess copies of the P3A target site ligated to carrier DNA are coinjected into fertilized eggs together with the *CyIIIa–CAT* fusion construct, CAT mRNA is observed in mesenchyme, gut, and other cell types rather than exclusively in aboral ectoderm, while in controls injected with *CyIIIa–CAT* plus carrier DNA alone, such ectopic expression is almost never observed (10). The role of the P3A sites in the *SM50* gene is not yet known, though preliminary unpublished data (H. Sucov and E.H.D.) suggest that it may be involved in control of the wholly different spatial pattern of expression of this gene as well. Both factors are present in whole embryo nuclear extracts (M. Harrington and R. Zeller, personal communication), and the identity of the actual effectors of spatial control over *CyIIIa* and *SM50* expression *in vivo* will depend, in the different cell types, at least in part on their relative local concentrations at the time that specification of spatial expression occurs, i.e., in early to mid-cleavage stage (11). Though both factors react with the same target site, they are almost wholly distinct in primary amino acid sequence. P3A1 is a "finger protein," most similar to the *Drosophila* hunchback regulator (C.H., F.J.C., A.E.C., and E.H.D., unpublished data), whereas P3A2 is not and displays homology to no known type of DNA-binding protein.

In this paper we report measurements of the prevalence of the P3A1 and P3A2 mRNAs in whole embryos obtained by using the cloned probes. Both message species are present in unfertilized eggs as transcripts belonging to the rare class of maternal mRNAs, and neither increases greatly in concentration per embryo during embryogenesis. Quantitative considerations based on these and other measurements suggest the principle that such rare maternal mRNAs may nonetheless produce effective quantities of regulatory protein products if these are required in the early cleavage stage, since they will be concentrated in the nuclei, and since at the beginning of development each nucleus is serviced by a relatively large mass of cytoplasm.

MATERIALS AND METHODS

Isolation of DNA and RNA. High molecular weight genomic DNA was isolated from the sperm of individual *S. purpuratus* (12). Total RNA for the probe excess titrations was purified from unfertilized eggs or embryos (13), and poly(A)$^+$ RNA was prepared for RNA gel blot hybridizations from the total RNA according to Lee *et al.* (14).

RNA Blot Hybridizations. For RNA gel blots 3 μg of purified poly(A)$^+$ RNA were denatured and electrophoreses in a 2.2 M formaldehyde/1% agarose gel in 1× Mops buffer (20 mM buffer/5 mM NaOAc/1 mM EDTA, pH 7.0) at 100 V for 6 hr. The RNA was then transferred to a nitrocellulose

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filter. Filters were hybridized overnight at 42°C in 10 ml of hybridization solution (50% formamide/0.75 M NaCl/0.15 M Tris, pH 8/10 mM EDTA/0.1% Ficoll/0.1% polyvinylpyrrolidone/0.1% bovine serum albumin/50 mM sodium phosphate, pH 7.4/1% SDS containing sonicated salmon sperm DNA at 100 μg/ml) with the appropriate ^{32}P-labeled DNA probes (15). For P3A1 RNA blots the probe consisted of a fragment extending from the *Hin*dIII site in the polylinker to position 182 of the cDNA sequence (ATG is at position 25); for P3A2 RNA blots the probe consisted of the sequence from the polylinker *Hin*dIII site to a *Hin*dIII site at position 2883. The filters were washed twice with 0.3 M NaCl, 0.03 M sodium citrate, pH 7/0.2% SDS for 15 min at 60°C and twice with 15 mM NaCl/1.5 mM sodium citrate, pH 7/0.2% SDS for 30 min at 60°C and exposed at −70°C with an intensifying screen.

Single-Strand Probe Excess Titrations of mRNA. The number of P3A1 and P3A2 mRNA molecules transcribed in *S. purpuratus* eggs and early embryos was determined using single-stranded antisense RNA probe excess titration, as described by Lee *et al.* (14). Reactions were carried out in tracer excess of 10-fold or greater, at a solution hybridization criterion of 50°C, 0.4 M NaCl, and 50% formamide. The amount of RNA·RNA hybrid formed was assayed by trichloroacetic acid precipitation after digestion with RNase A and RNase T1 (14). P3A1 and P3A2 single-stranded antisense RNA probes were prepared from linearized plasmid DNA. A plasmid containing a fragment complementary to positions 500–740 of the P3A1 sequence (C.H., F.J.C., and E.H.D., unpublished data) was linearized with *Xho* I, and T3 polymerase was used to make the P3A1 antisense RNA probe. The P3A2 antisense RNA probe included the sequence from nucleotide 289 to 668. The probe was made from *Sal* I-linearized DNA, transcribed with T3 polymerase. The specific activity of the P3A1 probe was 6.4×10^2 dpm/pg and that of the P3A2 probe was 3.8×10^2 dpm/pg for the respective hybridization reactions, and 0.15 ng of P3A1 probe or 0.25 ng of P3A2 probe was used per reaction. The number of transcripts per embryo was determined from least-squares slopes of the titration measurements (i.e., probe protected as a function of total RNA per reaction; ref. 14).

RESULTS

P3A1 and P3A2 Are Encoded by Single-Copy Genes. Coding-region probes from the cloned cDNA were reacted with *Eco*RI-, *Hin*dIII-, or *Xba* I-digested genomic DNAs of individual sea urchins (data not shown). The genome blot patterns indicate unequivocally that both factors are encoded by single-copy genes (as noted above, the sequences of these genes are nonhomologous and the probes cannot crossreact). Restriction polymorphism is commonly observed in *S. purpuratus* genome blots because of the 4–5% single-copy DNA sequence polymorphism of this species (9, 12, 16, 17). The regions of the *S. purpuratus* genome encoding P3A1 and P3A2 are typically polymorphic for this species. Thus, for example, in four individual diploid DNAs digested with *Xba* I, two P3A2 fragment length alleles were found, *a* and *b*; these animals were *aa*, *bb*, *bb*, and *ab*, respectively.

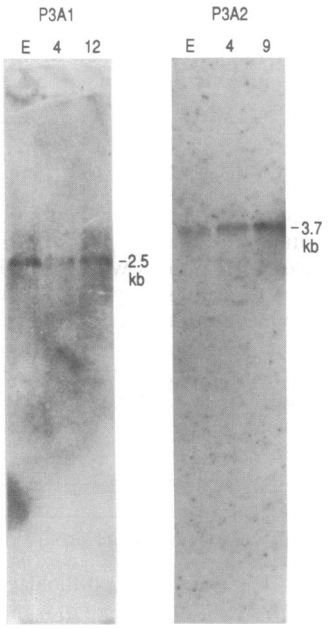

FIG. 1. RNA gel blots with P3A1 and P3A2 probes. A 194-nucleotide DNA probe was used to detect P3A1 transcripts, and a 2895-nucleotide probe to detect P3A2 transcripts (see *Materials and Methods*). Poly(A)$^+$ RNAs were prepared from egg (E), 4-hr early-cleavage embryos, and 12-hr late-cleavage embryos and reacted with these probes after electrophoresis through a denaturing formaldehyde gel. Size of the single-band observed in each series was estimated by comparison with BRL RNA size standards (not shown). As these are exceedingly rare transcripts, the relative intensities of the bands observed are not reliable indices of transcript concentration in the poly(A)$^+$ RNA. kb, Kilobases.

RNA Gel Blots. Fig. 1 displays RNA gel blots that clearly demonstrate only a single size of transcript for both the P3A1 and P3A2 probes, 2.5 and 3.7 kilobases, respectively. The most important conclusion from this experiment is that both messages are evidently maternal. Neither appears from these exposures to undergo a very striking increase in content per embryo during cleavage. However, this is at best a qualitative impression, first because of the difficulty of quantitative detection by gel blot methods of transcripts of such low prevalence as these (see below), and second because the level

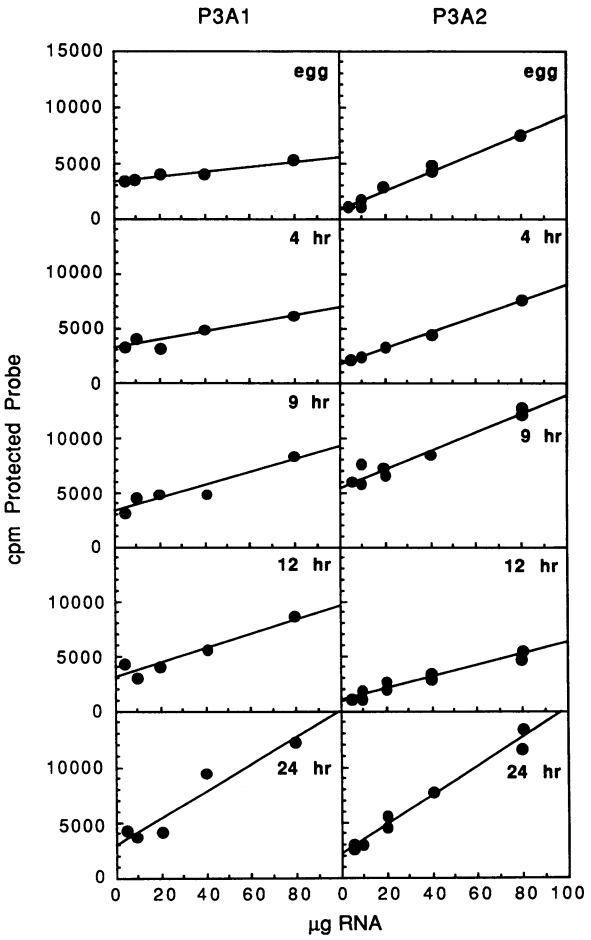

FIG. 2. Single-stranded probe excess titrations of P3A1 and P3A2 transcripts in egg and embryo RNA. Total RNAs extracted from the stages indicated were reacted in the amounts shown with labeled antisense probes representing regions of the P3A1 and P3A2 mRNAs. The P3A1 probe was 240 nucleotides long and the P3A2 probe was 379 nucleotides long. RNase-resistant hybridized cpm are shown on the ordinates. The lines represent the linear least-squares solutions. Quantitative data extracted from these measurements—i.e., slope ± SE, correlation coefficients (*r*), and calculated number of transcripts per embryo—are shown in Table 1. In each case the experiments illustrated here are listed as Exp. 2 in Table 1.

of polyadenylylation of specific sea urchin embryo mRNAs often changes during early development (7), and the experiments shown necessarily were carried out with poly(A)$^+$ rather than total RNA. The P3A proteins were not detected (at least by an assay requiring binding to target-site DNA) in unfertilized egg extracts (4). Therefore, the P3A factor(s) required for the specification process is probably synthesized on the mRNA forms revealed in Fig. 1.

Measurement of P3A1 and P3A2 Transcript Prevalence by Single-Strand Probe Excess Titration. To obtain quantitative estimates of the number of P3A1 and P3A2 transcripts per embryo, single-strand probe excess titrations were carried out on RNAs extracted from a developmental series of early embryonic stages. A set of reactions for each transcript species is shown in Fig. 2, and the data extracted from these measurements are listed in Table 1, where an additional series of results for each transcript is also shown. For rare transcripts, titration with antisense RNA probes is the obvious method of choice (18), due to (*i*) the great sensitivity afforded by the high-specific-activity synthetic probe transcripts; (*ii*) the inherent accuracy of the procedure, which is carried out in solution and is independent of reaction kinetics and of efficiency factors relevant to filter binding and hybridization; and (*iii*) the use of total rather than poly(A)$^+$ RNA.

Table 1 shows that there are only about 300 P3A1 transcripts and only about 1100 P3A2 transcripts per unfertilized egg. These values are slightly less than the average prevalence for the rare maternal mRNA transcript class typical of the sea urchin egg (20, 21). At the 24-hr mesenchyme blastula stage, the latest to which the data in Table 1 and Fig. 2 extend, the mass of the egg has been divided up into about 500 cells per embryo. Thus at fertilization there are only about one or two maternal transcripts (respectively) per blastula "cell equivalent" of egg cytoplasm. During development the quantity of P3A1 transcripts increases, though only about 6-fold, while P3A2 mRNA appears to remain approximately constant. Thus at least the gene encoding P3A1 mRNA is expressed in the embryo. Unless P3A2 is encoded by a most unusual mRNA, this gene is probably active during embryogenesis as well, so that the steady-state content observed is maintained by a continuous flow of newly synthesized but unstable mRNA. Earlier work has shown that in these embryos essentially all the rare maternal mRNAs have been replaced with homologous zygotic transcripts by the blastula stage, and these new transcripts typically turn over with a half-life of several hours (22). According to the values in Table 1, by the 24-hr blastula stage the P3A1 mRNA would be present at only about 4 molecules per cell, and the P3A2 mRNA at only about 1–2 molecules per cell, were they more or less evenly distributed throughout the embryo. This is probably a fair assumption, since (unpublished) *in situ* hybridization studies exclude any striking localization for either P3A1 or P3A2 RNAs. If some cells lack these mRNAs this might not have been noticed, and the exact disposition of either transcript or protein factor at these stages remains to be determined. Conceivably some fraction of the measured transcript content of either species represents nuclear RNA precursor, but this is unlikely to be large, as a precursor is not detectable in RNA gel blots (Fig. 1), and on the average the turnover rate of sea urchin embryo mRNAs is 5–10 times lower than that of their nRNA precursors (7).

Table 1 shows, in sum, that the unfertilized *S. purpuratus* egg contains P3A1 and P3A2 mRNAs, though at a very low level, and that on a per-embryo basis the abundance of these mRNAs remains low throughout early development. Yet it is in this period that the factor encoded by at least one of these mRNAs exercises an essential function in the spatial control of embryonic gene expression. Though there is apparently no maternal P3A1 or P3A2 protein in the egg, nuclear proteins binding the P3A site are clearly detectable by the fifth or sixth cleavage (4). The data in Table 1 permit us to construct a quantitative image of the mechanism by which the functional level of factor molecules can be synthesized on these low-abundance messages and supplied to the founder cell nuclei.

DISCUSSION

Our object is to understand the provenance of the P3A regulatory protein molecules, given these mRNA prevalence data. The relevant period of development is cleavage, since that is when specification of the aboral ectoderm lineages occurs (11), of which the *CyIIIa* gene provides a marker, and it is for the *CyIIIa* gene that there is direct evidence that

Table 1. Prevalence of P3A1 and P3A2 transcripts during development

mRNA	Stage	cpm hybridized/μg of RNA, mean ± SE (r)		No. of transcripts per egg or embryo		Average no. of transcripts	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2	Per egg or embryo	Per cell
P3A1	Egg	13 ± 4.9 (0.84)	22 ± 2.9 (0.98)	263 ± 99	365 ± 47	310	310
	4 hr	—	37 ± 9.1 (0.92)	—	613 ± 151	610	80
	9 hr	32 ± 4.6 (0.97)	61 ± 11.3 (0.95)	1077 ± 155	1011 ± 187	1040	8
	12 hr	56 ± 19.8 (0.85)	66 ± 11.2 (0.96)	1884 ± 666	1094 ± 186	1490	6
	24 hr	64 ± 9.4 (0.97)	122 ± 21.1 (0.96)	2160 ± 317	2021 ± 350	2090	4
P3A2	Egg	109 ± 15 (0.96)	85 ± 4.1 (0.99)	1026 ± 141	1202 ± 58	1110	1110
	4 hr	—	73 ± 1.6 (0.99)	—	1039 ± 23	1040	130
	9 hr	83 ± 19 (0.89)	83 ± 6.6 (0.98)	781 ± 179	698 ± 56	740	6
	12 hr	63 ± 12.3 (0.92)	53 ± 3.9 (0.98)	593 ± 116	754 ± 55	670	3
	24 hr	80 ± 24.7 (0.82)	131 ± 7.2 (0.99)	753 ± 232	1864 ± 102	1310	3

Measurements were made by single-stranded probe excess titration (14, 18) as described in *Materials and Methods*. Total RNA was extracted at the stages indicated (egg or hours postfertilization) from samples of a culture derived from pooled eggs of about 30 females. The number of transcripts per embryo (T) was calculated using the equation $T = N/\alpha\beta\gamma m\Sigma$ (18), where m is the experimentally determined slope of the titration curve in cpm/μg, α is the ^{32}P fractional counting efficiency (90%), β is the probe specific activity in dpm/ng, γ is the molar mass of the hybridizable portion of the probe in ng/mmol, N is Avogadro's constant in molecules per nmol, and Σ is the mass of RNA per embryo (2.8 ng at the stages investigated; ref. 19). Data are shown for two series of P3A1 reactions carried out at 37°C (Exp. 2) and 50°C (Exp. 1); Fig. 2 displays the 37°C reactions. There should not be, and is not, any significant difference between these two series of data. The averages shown were used to calculate transcripts per cell. The two P3A2 reaction series were both obtained at 50°C. The error shown on the individual transcript per embryo values is proportional to the standard error (SE) values calculated on the slope in each experiment (i.e., cpm/μg of RNA).

interaction at the P3A site is required for normal lineage-restricted spatial expression (10). Transcription of the *CyIIIa* gene begins in the aboral ectoderm precursor lineages perhaps as early as the eighth cleavage (5, 23). Similarly by late cleavage two other genes in which P3A interactions are likely to play a role have been activated as well: the *Spec1* gene, also expressed in aboral ectoderm (2, 6), and the *SM50* gene (2, 4, 11), expressed in the skeletogenic mesenchyme (9). In the following we focus on P3A2, since that is the protein for which our earlier measurements *in vitro* (4) provide an estimate of factor concentration in the embryo nuclei, and in nuclear extracts it is P3A2 that reacts with the *CyIIIa* target site. However, much the same conclusions would hold for P3A1.

Expected Accumulation of Newly Synthesized P3A2 Protein. In Fig. 3*a* the data for P3A2 mRNA in Table 1 are presented graphically against a real-time axis. As noted above, the amount of this mRNA per embryo changes but little over the period measured, and we take for the following calculations 800 mRNA molecules per embryo throughout. Calzone *et al.* (4) estimated that there are 1.9×10^5 molecules of P3A2 protein in the 7-hr (sixth cleavage) embryo and 2.4×10^5 molecules in the 24-hr mesenchyme blastula. The first question that arises is whether these amounts of P3A2 factor could be synthesized from a steady-state quantity of about 800 molecules of mRNA in the available time. The answer is shown in Fig. 3*b*. Here the dashed line describes the expected time course of accumulation of P3A2 protein, assuming that the synthesis rate per mRNA is 1 molecule of protein per min. This is an appropriate standard rate measured for translation of histone mRNAs in *S. purpuratus* [i.e., 0.8 codon per sec per polysome (27)]. The spacing of ribosomes is about 140 nucleotides. Other measurements gave a slightly higher rate, 1.5 codons per sec, so the estimate here is a conservative one (7, 27). It is assumed, in generating the accumulation profile in Fig. 3*b* (see legend), that the P3A2 protein is not infinitely stable, since there is evidently a decline in the overall rate of increase of this protein between 7 and 24 hr, compared to 0 and 7 hr (4), while the amount of mRNA remains the same (Fig. 3*a*). For simplicity, a constant P3A2 protein turnover rate is assumed, such that the steady-state content would be that measured in the 24-hr embryos. By this argument the half-life of P3A2 would be about 3.5 hr (where k_s is synthesis rate, k_d is decay rate, and S is steady-state content, $k_d = k_s/S$; here 800 molecules·min^{-1}/2.4×10^5 molecules = 3.33×10^{-3} min^{-1}; and $t_{1/2} = 0.693/k_d$, or 3.5 hr). Fig. 3*b* shows, in fact, that 800 molecules of P3A2 mRNA is just what would be required to generate by 7 hr approximately the measured amount of P3A2 protein starting from zero [including a 1-hr lag to permit mobilization of the maternal mRNA (18)]. The calculation also predicts that the steady state is achieved at about the point that cleavage terminates, 10–12 hr postfertilization. The excellent correspondence between predicted synthesis by 7 hr and measurement of P3A2 protein (Fig. 3*b*) indicates, incidentally, that it is unnecessary to postulate any cryptic P3A2 protein in the unfertilized egg—i.e., factor that would not have been measured by Calzone *et al.* (4) because it was at that stage inactive in DNA binding but that could later have contributed to the pool of active P3A2 protein by modification, rather than by *de novo* synthesis.

Effective Levels of P3A2 Protein Per Nucleus in Cleavage-Stage Blastomeres. The highest concentration of P3A2 protein per nucleus was measured at 7 hr (3000 molecules per nucleus) (4). This is consistent with the concentration requirements for occupancy of a large fraction of specific P3A sites, given the sequence preference constant (k_r) measured *in vitro* for this factor for its specific sites, relative to nonspecific DNA sites (4, 28). We assume, then, that the level of P3A2 protein required for function is in fact several thousand molecules per nucleus. That P3A2 is indeed a nuclear protein is shown not only by its presence in nuclear extracts (4) but also by immunocytological observations (R.

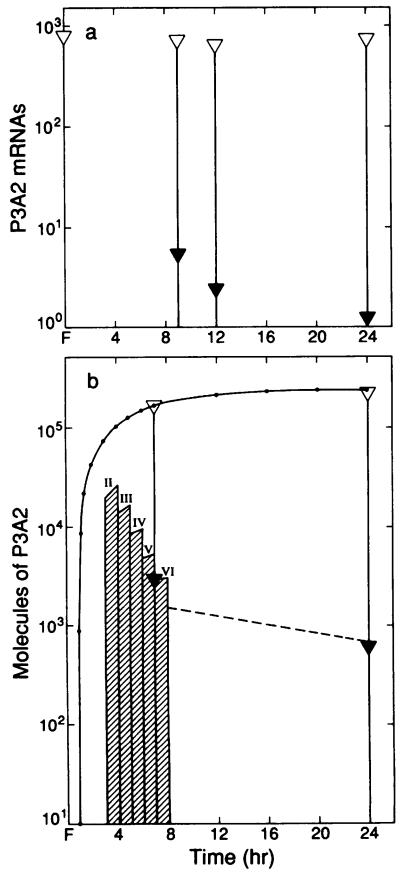

FIG. 3. Prevalence of P3A2 mRNA and calculated provenance of P3A2 protein. (*a*) P3A2 mRNA prevalence. Results from Table 1 for P3A2 transcripts are shown per embryo as vertical lines headed by triangles (open symbols) and per cell (solid symbols) on a real-time axis, on the assumption that all transcripts are mRNAs. The 9-hr embryo has about 130 cells, the 12-hr embryo about 250 cells, and the 25-hr embryo about 500 cells. (*b*) Provenance of P3A2 protein. The vertical lines headed by triangles show measurements of the P3A binding factor of Calzone *et al.* (4), i.e., the P3A2 protein (see text), obtained by quantitative probe excess titrations of nuclear extracts with the gel shift procedure. Open symbols give P3A2 protein per embryo and closed symbols the amount per cell. The 7-hr embryo has 60 cells. The generated solid curve shows the amount of P3A protein that would be accumulated on a constant average of 800 mRNA molecules, assuming the synthesis function $P(t) = k_s/k_d(1 - e^{-k_d(t-60)})$ (7, 22). Here k_d is taken as 2.22×10^{-3} min^{-1} and k_s as 800 molecules per min (see text). The lag of 1 hr was assumed because this amount of time is required for full mobilization of maternal mRNA on polysomes in sea urchin embryos (7). At 7 hr the calculated accumulation almost exactly matches the measured quantity of P3A2, 1.9×10^5 molecules per embryo. The calculated provenance of P3A2 per blastomere is shown for successive idealized cleavage cycles, second to sixth cleavage (Roman numerals). The calculation begins at the four-cell stage, when the oral quadrant is first separated from the aboral quadrants (11, 24). The quantity of P3A2 factor in each nucleus is taken as the amount synthesized from the generated curve at the beginning and end of each cleavage stage, as shown by the hatched bars. Two assumptions that are in detail inaccurate are made for simplicity: (*i*) that all the cells are the same size, which they are not (with interesting consequences; see text) and (*ii*) that the cleavages are wholly synchronous, while in fact after the first few cleavages the skeletogenic lineages are retarded relative to the others, and the animal cap lineages slightly precede the vegetal lineages (25, 26). Cell number at the sixth cleavage is taken as 60 rather than 64 over the time interval shown, in accord with observation. However, for the present purposes, which are heuristic, these digressions from reality are minor.

Zeller, A.E.C., and E.H.D., unpublished data). Fig. 3*b* also shows the amount of P3A2 protein that would be present per blastomere nucleus at each successive cleavage, from second through sixth cleavage, assuming 800 equally distributed P3A2 mRNA molecules, synchronous cleavage, the translation rate cited above, and a 40-min intercleavage cycle time. As noted in the legend some of these are not exactly realistic assumptions, though they are accurate for the first three cleavages. Thereafter they are acceptable except for the skeletogenic mesenchyme, which consists of smaller cells than the remainder and which divides on a slightly retarded schedule. However, irrespective of these simplifications the main point of this idealized calculation in Fig. 3*b* is relevant. This is that the amount of P3A2 mRNA per embryo, the fraction included in each successive blastomere as cleavage progresses, and the length of the cleavage cycles indeed suffice to provide effective levels of P3A2 protein per nucleus throughout early and mid-cleavage. It is interesting that at the earliest cleavages even more P3A2 protein would be present than at 7 hr. The second through the sixth cleavage is precisely when this factor is required for lineage-specific repression of *CyIIIa* function in founder cells of lineages other than the aboral ectoderm lineages, as it is in this interval that the aboral ectoderm lineages are definitively segregated out. The factor concentration per nucleus then falls, by the seventh cleavage, approaching that still present in 24-hr blastula nuclei (on the average). It is not known whether P3A2 function continues to be required at later stages or whether the average level of 700 molecules per nucleus is still functionally significant.

A Generality: The Function of Rare Maternal mRNAs Coding for Regulatory Proteins. The heuristic model shown in Fig. 3*b* implies an interesting principle that illuminates the role of maternal transcripts in the provenance of regulatory proteins in embryos that display holoblastic cleavage (i.e., most embryonic forms): *if the protein is one that localizes to the nucleus*, the large cytoplasmic domains of the early blastomeres may include a sufficient number of even very low-abundance mRNA molecules to provide effective nuclear concentrations of the protein. Thus the important variable is the number of mRNAs per blastomere, as cleavage proceeds, rather than the number per ribosome, per egg, or per mature cell equivalent of cytoplasm, as for cytoplasmic proteins. It is impressive that mRNAs present at the level of one or two molecules per blastula "cell equivalent" of cytoplasm can produce effective levels of nuclear protein, and this is of course a temporally limited mechanism that obtains only during early to mid-cleavage stages, when the cells contain >10 times the amount of cytoplasm that services each nucleus from the blastula stage onwards.

Spatial regulatory information will be developed by the same mechanism if, as is the case in the sea urchin embryo, the canonical cleavage pattern results in unequal-sized blastomeres that differ in lineage and fate. Thus even if the mRNA is evenly distributed in the egg, large blastomeres, such as those that constitute the veg_2 lineage (i.e., the gut–secondary mesenchyme lineage) in the sea urchin egg, will contain higher concentrations of the factor in their nuclei than will other blastomeres. Particularly small blastomeres, such as those that constitute the skeletogenic mesenchyme of the sea urchin embryo, could well contain subeffective concentrations of the factor. The mechanism discussed here depends in a sense of the constraints implicit in the low abundance of the message. In principle, this mechanism provides an elegant means by which the embryo can modulate quantitatively the concentration of gene-regulatory effector molecules in both time and space, beginning with a uniformly distributed pool of rare maternal transcripts.

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