Transcriptional control of tektin A mRNA correlates with cilia development and length determination during sea urchin embryogenesis

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SUMMARY

Previous studies have shown that tektin A, one of three integral filamentous protein components of outer doublet microtubules, is synthesized in sea urchins in an amount correlating to the length of embryonic cilia initially assembled or experimentally regenerated. To investigate further the molecular mechanism for the regulation of tektin synthesis, tektin cDNA clones were used to assess mRNA levels during ciliogenesis, zinc-induced animalization, deciliation-induced regeneration and theophylline-induced elongation. Possibly involved in centriole replication, low, near-constant levels of mRNA for all three tektins are present in the unfertilized egg and during cleavage stages. Preceded by new synthesis of tektin B and C mRNAs, tektin A mRNA is up-regulated during ciliogenesis, but only tektin A mRNA levels correlate directly with ciliary length in animalized embryos; the others augment larger, non-limiting pools of tektins B and C. Tektin mRNAs decrease to steady-state levels after ciliogenesis, but are up-regulated again when the embryos are deciliated, correlating with the length of cilia to be deployed. In a species where a 3-fold ciliary length increase can be induced by theophylline treatment of zinc-arrested embryos, the mRNAs accumulate to proportionately higher levels during arrest but are not translated until induction, whereupon they decrease inversely with ciliary elongation. This suggests transcriptional control with respect to mRNA amounts but post-transcriptional control with respect to the expression of this phenotype. These data are consistent with a model in which (1) tektin filaments serve as linear determinants of microtubule doublet structure, and (2) the fixed amount of tektin A mRNA and protein synthesis consequently limit the length of doublets that can be co-assembled from larger pools of tektins B and C, tubulin, and other components.

Key words: tektin, cilia, ciliogenesis, length regulation, sea urchin embryo, messenger RNA

INTRODUCTION

Ciliogenesis and flagellar development are cellular events marked by coordinated gene expression, culminating in the assembly of over 150 polypeptides to form the 9+2 microtubular axoneme (Lefebvre and Rosenbaum, 1986). While ciliogenesis and regeneration in the sea urchin embryo involve synthesis of the major ciliary proteins including tubulin and dynein (Merlino et al., 1978; Alexandraki and Ruderman, 1981; Pratt, 1989). However, a few prominent structural polypeptides, most notably the tektins, are synthesized de novo specifically at the onset of ciliogenesis and again upon regeneration (Stephens, 1977, 1989). The tektins are a class of proteins that were originally characterized from sperm flagellar microtubules (Linck et al., 1982; Linck and Stephens, 1987; Steffen and Linck, 1989) and which share some properties with intermediate filaments proteins and nuclear lamins (Norrander et al., 1992; Chen et al., 1993). Assembled in equimolar amounts, tektins A, B, and C are organized as extended polymers associated with a particularly stable set of protofilaments in ciliary and flagellar doublet microtubules (Linck, 1976; Linck and Langevin, 1982; Linck et al., 1985; Steffen and Linck, 1988; Stephens et al., 1989). Tektin filaments have periodicities matching the tubulin lattice (Pirner and Linck, 1994) and can serve as the primary constituent of at least one of the protofilaments of the microtubule doublet (Nojima et al., 1995).
embryos experimentally induced to grow longer cilia, the total amount of tektin A synthesized is directly proportional to ciliary length and the synthesis itself is coincident with ciliary elongation (Stephens, 1989). Together, these findings support the basic hypothesis that ciliary length in sea urchin embryos is determined by the quantal synthesis of tektin A, with this amount specifying the extent to which outer doublet microtubules can grow by co-assembly from pools of the other two tektins, tubulins and associated proteins. However, the mechanism for the quantitative regulation of tektin A synthesis is unknown.

In this study, we use cDNA probes for tektin A, B and C mRNAs to explore the molecular mechanism for the quantal synthesis of tektin A. We find that tektin A mRNA is present at near-constant low levels during early development; it then increases dramatically and coincidently with ciliogenesis; up-regulates again in response to deciliation; correlates with ciliary length in animalized embryos; and accumulates to proportionately elevated levels in zinc-arrested embryos, undergoing translation and degradation coincident with induced ciliary elongation.

**MATERIALS AND METHODS**

**Culturing of sea urchin embryos**

*Strongylocentrotus purpuratus* and *Lytechinus pictus* were obtained from Marinus, Inc., Long Beach, CA; *Strongylocentrotus droebachiensis* were obtained from the Marine Biological Laboratory, Woods Hole, MA; *Tripneustes gratilla* were collected locally on Oahu, HI. Eggs and sperm were collected, handled and fertilized at their natural growth temperature, as previously described (Stephens, 1972a, 1986). Routinely, eggs were washed by settling several times, resuspended in at least 10 volumes of seawater and fertilized by the addition of dilute sperm. Egg batches from individual females were handled separately and only those having >95% fertilization were used. In cases where large quantities of embryos were needed, fertilized eggs were pooled from 2-6 females. Fertilized eggs were washed twice by settling to remove excess sperm, resuspended in sterile seawater at 4-8°C for 10 minutes at 4°C to pellet nuclei and cellular debris. An equal volume of 0.5 mM Tris-HCl, pH 8, 0.35 M NaCl, 1 mM EDTA containing 0.25% diethyl pyrocarbonate were added to fresh embryos, or to frozen embryo samples which were then thawed on ice. RNA was isolated by previously described methods (Maniatis et al., 1982; Alexandraki and Ruderman, 1985). In brief, the embryos were resuspended, homogenized and centrifuged at 15,000 g for 20 minutes at 4°C to pellet nuclei and cellular debris. An equal volume of a 1:1 mixture of phenol and CHCl3, followed by extraction with two volumes of CHCl3. The cytoplasmic RNA was concentrated by ethanol precipitation. Polyadenylated RNA was isolated by oligo(dT)-cellulose chromatography, as described by Maniatis et al. (1982).

**Gel electrophoresis and blotting of RNA samples**

RNA samples were resolved on 1.5% agarose gels containing 2.2 M formaldehyde as described by Maniatis et al. (1982). Lambda DNA, digested with EcoRI and HindIII and denatured by boiling, was resolved in an adjacent lane to provide molecular weight standards. The RNA and denatured DNA samples were transferred to nitrocellulose, as described by Thomas (1980). After baking, the DNA section of the nitrocellulose was removed and stained using the method described for RNA bound to nitrocellulose by Maniatis et al. (1982).

**cdDNA probes**

Tektin A mRNA was detected by hybridization with *S. purpuratus* clones teka10-2 or teka5-8, characterized by Norrander et al. (1992); tekt B mRNA with clone tekb1 of Chen et al. (1993); and tektin C mRNA with clone tekC9-3 of Norrander et al. (unpublished data). Ubiquitin mRNA was detected with the *L. pictus* clone bUbT7, a gift of Dr Bruce Brandhorst. The cDNA probes were 32P-radiolabeled by nick translation using the BRL Nick Translation Reagent Kit (Bethesda Research Laboratories, Gaithersburg, MD).

**Hybridization of RNA blots**

Following electrophoresis, blotting and baking, the nitrocellulose filters were rehydrated in 2× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate) and prehybridized at 42°C for 2 hours in a solution containing 50% deionized formamide, 5× SSC, 40 mM sodium phosphate, pH 6.5, 5× Denhardt’s (1× = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA), 0.1% SDS and 200 μg/ml sheared, denatured salmon sperm DNA. 10 ml of prehybridization solution were used per 100 cm² of nitrocellulose. Hybridizations were carried out at 42°C for 16 hours in a solution containing 50% deionized formamide, 5× SSC, 1× Denhardt’s, 20 mM sodium phosphate, pH 6.5, 0.1% SDS, 50 μg/ml sheared, denatured salmon sperm DNA, and 25-40 ng/ml denatured, radiolabeled probe (specific activity = 1-5×10⁷ cts/min/μg). 4 ml of hybridization solution were used per 100 cm² of nitrocellulose. After the hybridization, filters were washed 4 times for 10 minutes in 2× SSC, 0.1% SDS at room temperature, followed by two 15 minute washes in 0.1× SSC, 0.1% SDS at 50°C. Filters were air dried and exposed to Kodak XAR X-ray film at ~80°C, using intensifying screens. After analysis, blots were stripped of the initial probe (Maniatis et al., 1982), hybridized with a different probe and then reprocessed as above.

**Densitometry**

 Autoradiograms were quantitated and expressed in terms of integrated optical density by computer-based video analysis (Stephens, 1992).
using the JAVA software/hardware package (Jandel Scientific, San Rafael, CA) and the general approaches outlined by Haselgrove et al. (1985). Optical density was obtained either by calibrating the program with a stepped-density film standard or by using the mathematical transform, \( OD = \log(\text{background intensity}/\text{band intensity}) \). These methods were in full agreement up to an optical density of 1.3, above which the camera response increasingly limited the accuracy of both. Maximum band optical densities generally fell below 1.0 for any given exposure, assuring linearity of both film and camera response. Scans were typically taken perpendicular to the migration axis, across the lanes and inclusive of the bands to be compared, using the ‘vertical average’ feature of the software. This produced the equivalent of a bar graph of relative band densities. To correct for any lane width differences, the scans were printed out and the peaks were manually integrated with a digitizing tablet, using SigmaScan (Jandel Scientific).

**RESULTS**

**Tektin mRNA levels increase markedly during ciliogenesis**

To determine the relative levels of tektin mRNAs during normal sea urchin development, *S. purpuratus* eggs were fertilized and grown in mass culture. Equal volume samples were taken during development for later processing and analysis. Equal amounts of mRNA, determined spectrophotometrically, were analyzed by northern blotting. The blots were probed consecutively for mRNAs corresponding to the three tektins and also for ubiquitin, a non-ciliary protein whose mRNA remains relatively constant throughout early development, transiently increasing by less than a factor of two at the mid-blastula stage (Nemer et al., 1991). The resulting autoradiograms from a representative experiment are illustrated in Fig. 1 and the corresponding quantitations, normalized to the ubiquitin signal to correct for any isolation or loading differences, are illustrated in Fig. 2.

The tektin cDNA probes each hybridized to different-sized mRNA species: 2750 bases for tektin A; 2900 bases for tektin B; and a tight doublet of 2700 and 2800 bases for tektin C. None of the tektin probes cross-hybridized (Fig. 1). The ubiquitin probe hybridized to a single mRNA species of 3200 bases (cf. Nemer et al., 1991). Low levels of all three tektin mRNAs were detected in the unfertilized egg, and there were no changes in message size observed throughout development. Ciliogenesis in this species begins at about 8 hours (cf. Harlow and Nemer, 1987b), after which point there was a marked rise in the level of each tektin mRNA (lane 4 of Fig. 1). With respect to peak levels, tektin A mRNA increased >20-fold over post-fertilization levels, while tektin B and C mRNAs each increased about 7-fold (Fig. 2). Before ciliogenesis, the level of tektin A mRNA was low and relatively constant, but the levels of tektin B and C mRNAs began to increase well before ciliogenesis, at the 8 hour time point. The messages for all three tektins fell rapidly after hatching, to values between 1/5 and 1/3 of their peak levels. However, these post-ciliogenesis levels were nevertheless somewhat higher than those seen in the early cleavage stages, a point that will be dealt with again under additional circumstances below.

Similar but interestingly different results were obtained in *L. pictus*. In contrast to *S. purpuratus*, multiple maternal \( \alpha \)- and \( \beta \)-tubulin mRNAs are present in *L. pictus* and these are differentially utilized or re-expressed at ciliogenesis (Alexandraki and Ruderman, 1985); we found similar behavior for the tektin messages. In the case of tektin A (Fig. 3), transient mRNAs of 3075, 2700, 2300 and 1675 bases were detected in eggs and very early in development but they declined to almost undetectable levels by the 16-cell stage. At later time points, the largest message disappeared entirely while the remaining ones increased more than 20-fold coincident with ciliogenesis, the data being normalized with respect to the ubiquitin signal. Very weak hybridization was found with the similarly behaving maternal and late-expression tektin B mRNAs, while hybridization to tektin C mRNAs was too weak to analyze (not shown).

**Tektin A mRNA levels are proportionately augmented during ciliogenesis in animalized embryos**

To determine if tektin mRNA levels were increased in embryos deploying cilia of increased ciliary length, normal embryos were compared with zinc-animalized embryos. Embryos grown in the presence of zinc ions reach the ciliated blastula stage and

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**Fig. 1.** Expression of ciliary tektin mRNAs during the early development of *S. purpuratus* embryos. A single northern blot was probed successively with cDNAs for tektins A (clone tekA5-8), B (clone tek-B1) and C (clone tekC9-3) and ubiquitin (clone bUbT7). Equal amounts of cytoplasmic RNA (35 \( \mu \)g) were loaded in each lane. Lane 1: unfertilized eggs; lane 2: 3 hours; lane 3: 8 hours; lane 4: 12.5 hours, blastula; lane 5: 16 hours 50 mins, rotating blastula; lane 6: 18.75 hours, hatched, swimming blastula; lane 7: 19 hours; lane 8: 24 hours 55 mins, mid-gastrula. In this species, ciliogenesis begins by 12 hours (lane 4) and is complete by 19 hours (lane 7), coinciding with the peak of tektin mRNAs; the ubiquitin message changed little during this period.
then differentiation is suspended (Nemer, 1986). Such zinc-treated embryos, referred to as ‘animalized’ because they have exaggerated features of the animal hemisphere, develop and regenerate cilia that are unusually long, resembling those of the apical tuft (see Stephens, 1989 or 1994b, for examples). In *S. purpuratus*, the cilia are initially about 1.3-1.4 times longer than normal, but show a diminishing population average as development proceeds (Harlow and Nemer, 1987b).

We examined the period of ciliogenesis in *S. purpuratus* during which the tektin mRNAs reached a peak, measuring the relative levels of tektin A, tektin B, tektin C and ubiquitin messages in normal and animalized embryos at 6 intervals from 14-16.5 hours. During this period, ciliary elongation was essentially linear and the levels of these mRNAs remained relatively constant. The averaged results, including the separate assessment of ubiquitin, are compared graphically in Fig. 4. In the case of zinc-treated embryos, ubiquitin mRNA was up-regulated 2.3-fold, in basic agreement with the report of Nemer et al. (1991) for this same species. Earlier reports also showed 2-fold or greater increases for various tubulin messages as a consequence of animalization (Harlow and Nemer, 1987b), consistent with measurements of up-regulated, overall ciliary protein pools seen after animalization (Stephens, 1989). Tektin B and tektin C mRNAs were similarly up-regulated by factors of 2.2 and 2.4, respectively, but tektin A mRNA increased only by about 1/3. This average increase in tektin A message alone correlates with the 1/3 ciliary length increase seen after the minimal animalization that is characteristic of this species.

**Tektin A mRNA is up-regulated in response to both animalization and deciliation**

In comparison with *S. purpuratus*, the cilia of *S. droebachiensis* animalized embryos are initially nearly twice normal length

![Graph of relative mRNA levels](image)

**Fig. 2.** Quantitation of tektin mRNA expression during the early development of *S. purpuratus* embryos. The autoradiograms in Fig. 1 were quantitated densitometrically, normalizing each tektin mRNA band with its corresponding ubiquitin signal to correct for any minor variations in loss, loading, or total mRNA modulation. Each of the three tektin data sets was then expressed in terms of the maximum mRNA level within that set, taken as 1.0. Although the absolute amounts of mRNA were not determined, relative to their peak values, tektin A lagged tektins B and C in expression, yet all peaked coincident with ciliogenesis.

**Fig. 3.** Expression of tektin A during the early development of *L. pictus* embryos. Equal amounts of cytoplasmic RNA were loaded in each lane; the resulting northern blot was probed with *S. purpuratus* cDNA clone tekA10-2. Lane 1: eggs; lane 2: 1 hour 8 mins, 2-cell stage; lane 3: 2 hour, 4-cell; lane 4: 2 hour 40 mins, 8-cell; lane 5: 3.25 hours, 16-cell; lane 6: 4.5 hours, 64-cell; lane 7: 5.5 hours; lane 8: 6.5 hours; lane 9: 7.5 hours; lane 10: 9 hours, rotation detected; lane 11: 10 hours, rotating blastula. Ciliogenesis in this species begins at about 8 hours. Note that considerable maternal mRNA of several size-classes is present in the unfertilized egg but that these messages are rapidly degraded during the cleavages. The major 2.7 kb and two smaller mRNAs are resynthesized upon ciliogenesis.

![Graph of relative mRNA levels](image)

**Fig. 4.** Peak levels of mRNAs for ubiquitin and tektins B, C and A during ciliogenesis in control and zinc-animalized *S. purpuratus* embryos. Data sets were normalized with respect to control levels, taken as 1.0. During the period from 14 to 16.5 hours, all mRNA levels, measured at six 15-30 minute intervals, remained relatively constant, as shown by the small standard deviations. Ubiquitin and tektin B and C mRNA levels were more than doubled by zinc treatment, whereas the level of tektin A message was increased only 1.34 times. Differences within each set were significant at $P<0.05$ by $t$-test, as was the difference between tektin A mRNA enhancement and the enhancement of the other sets.
(Stephens, 1989), the population average does not diminish with time, and the long cilia phenotype is stable to regeneration. Consequently, we turned to this species to study the effects of deciliation on normal and animalized embryos. Embryos were grown to the gastrula stage, with or without the addition of zinc at fertilization. Control and animalized embryos were deciliated by brief hypertonic salt treatment and allowed to regenerate cilia. Samples were taken during the linear phase of regrowth (cf. Stephens, 1989). Polyadenylated RNA was isolated from an equivalent number of embryos of the following kind: (1) control gastrula-stage embryos; (2) contemporaneous zinc-arrested animalized embryos; (3) deciliated control gastrula embryos in the process of regenerating cilia; and (4) deciliated animalized embryos in the process of regenerating cilia. In this species, the tektin A probe hybridized to a major 1450 base band and minor 1725 and 2900 base bands. The cDNAs for tektins B and C hybridized too weakly to obtain reliable quantitation, but their general behavior paralleled that of tektin A. In the case of ubiquitin, a major 1650 and a minor 3200 base message were seen; hybridization was also weak but quantifiable. Fig. 5 shows northern blots of the major ubiquitin band and also the tektin A mRNAs for the above conditions. The relative levels of the major ubiquitin and tektin A mRNAs in this representative experiment are compared quantitatively in Fig. 6.

Zinc-animalization caused only slight elevation of ubiquitin mRNA levels in *S. droebachiensis* (2nd versus 1st bar), in contrast to the marked effect of zinc in *S. purpuratus* embryos (Fig. 4). The level of ubiquitin mRNA in *S. droebachiensis* was barely stimulated by deciliation of control embryos (3rd versus 1st bar) but deciliation more than doubled the level of ubiquitin mRNA in zinc-animalized embryos (4th versus 1st bar).

From Fig. 6, it can be seen that the level of tektin A mRNA in fully ciliated zinc-animalized embryos was 2.8 times greater than that of control embryos (2nd versus 1st bar), even though neither embryo was undergoing active ciliogenesis. These constitutive levels may reflect the >2-fold longer cilia characteristically maintained by late-stage zinc-animalized embryos in this species (Stephens, 1989). Although the ubiquitin levels were minimally affected after deciliation of control embryos, the level of tektin A mRNA increased 2.5-fold in response to control embryo deciliation (3rd versus 1st bar). The tektin A mRNA increased to a similar degree when zinc-animalized embryos were deciliated (2nd versus 4th bar), as would be expected in both cases for the necessary deployment of new cilia.

**Proportionately accumulated mRNAs are utilized during the induction of long cilia**

Embryos of the Indo-Pacific sea urchin *T. gratilla* are arrested at the hatched blastula stage by zinc ions present since fertilization but, unlike *S. droebachiensis*, they do not normally express a long-cilia phenotype. Theophylline will arrest *T. gratilla* embryos when applied at the blastula stage, but it produces a minimally animalized embryo with only moderately elongated cilia. However, pulse treatment of zinc-arrested...
embryos with theophylline, at any stage from blastula through late gastrula, will immediately induce ciliary elongation, producing cilia nearly 3 times normal length (Stephens, 1994b). Just as in fully expressed animalization, tektin A is synthesized quantally during the induction, and the remaining ciliary proteins constitute proportionately larger pools in the induced embryos (Stephens, 1994c). Therefore, it should be informative to examine the behavior of tektin A and other mRNAs that gave rise to these proteins, before, during and after this unique induction process.

A large culture of zinc-arrested blastulae was induced to form long cilia with 5 mM theophylline and samples at successive time points were taken for both RNA isolation and ciliary length determination. Single northern blots were probed successively for tektin A, tektin B and ubiquitin: the cDNA probe for tektin C message hybridized too weakly for accurate quantitation. Representative mRNA blots for tektin A and ubiquitin are illustrated in Fig. 7, while all three messages are quantitated in Fig. 8.

Single mRNAs of 2650 bases were detected for both tektins A and B. These were both rapidly depleted to about 12% of the initial level during the induction process. The time course of mRNA translation/degradation approximated, inversely, the elongation of the cilia. The single 2600 base ubiquitin mRNA fell less rapidly, to less than 30% of its initial level. The kinetics were near-linear, discounting the apparently under-loaded sample at 90 minutes.

Animalization can result in the accumulation of certain mRNAs, for example β-tubulin message accumulates in the minimally animalized S. purpuratus (Harlow and Nemer, 1987b). To determine if a relative accumulation of tektin A or ubiquitin mRNAs took place during zinc treatment of T. gratilla, and also to determine the response of these mRNAs to theophylline, we prepared four parallel cultures from a single batch of fertilized eggs: (1) untreated control embryos; (2) control embryos treated with 5 mM theophylline; (3) zinc-arrested embryos; and (4) zinc-arrested embryos treated with 5 mM theophylline. At the beginning of this treatment, control embryos were just beginning to gastrulate. The cultures were all processed together, 3 hours after the introduction of theophylline, i.e. when ciliary elongation was complete. Single northern blot were successively probed for tektin A and ubiquitin mRNAs; relative mRNA levels under these four conditions, for one such blot, are illustrated Fig. 9.

In control embryos treated with theophylline, the level of ubiquitin mRNA was essentially the same as that of untreated embryos. In contrast, the amount of tektin A mRNA was reduced substantially after these embryos underwent their characteristic moderate ciliary elongation (1st versus 2nd bar). This demonstrates that theophylline treatment, which alone can minimally animalize embryos (Riederer-Henderson, 1988; Stephens, 1994b), leads to the degradation of tektin A mRNA, but further demonstrates, by lack of effect on the ubiquitin mRNA, that this is not accompanied by more general mRNA degradation.

In zinc-arrested embryos, the level of ubiquitin mRNA was >2.5-fold higher than in untreated controls (3rd versus 1st bar), i.e. it was up-regulated by zinc-treatment in T. gratilla embryos just as in S. purpuratus. Treatment of these zinc-arrested embryos with theophylline reduced this elevated ubiquitin mRNA back to near-control levels (3rd versus 4th bar). Similarly, the level of tektin A mRNA in zinc-arrested embryos was about 2.5-fold higher than that of untreated control embryos (1st versus 3rd bar) and, in this case, theo-
levels in fertilized embryos up to the blastula stage, at which mRNA is detectable in unfertilized eggs and it remains at low when embryos are animalized by zinc treatment. Tektin A regeneration and that it increases in proportion to ciliary length mRNA is up-regulated during both ciliogenesis and ciliary Our data show that the level of tektin A mRNA relative to total DISCUSSION

Fig. 9. The effect of theophylline on ubiquitin and tektin A mRNA levels in control and zinc-arrested T. gratilla embryos. 3 hours after induction, cytoplasmic RNA (35 μg) from control embryos, control embryos treated with theophylline, zinc-arrested embryos, and zinc-arrested embryos treated with theophylline was separated, blotted and analyzed with probes for tektin A (clone tekA5-8) and ubiquitin. The densitometric data were normalized with respect to the untreated control, taken as 1.0. Zinc-arrest resulted in a 2.5-fold increase in ubiquitin mRNA; theophylline reduced this to control levels, with no effect on the control itself. Zinc-arrest resulted in a comparable increase in tektin A mRNA levels; theophylline reduced this below untreated control levels and similarly reduced the tektin mRNA level of control embryos.

Theophylline treatment reduced this elevated level about 3.5-fold (3rd versus 4th bar).

The information in Figs 8 and 9 together demonstrates that zinc-arrested embryos of T. gratilla have elevated mRNA levels and, when ciliary elongation is induced by theophylline, the accumulated mRNAs fall back to (or below) control levels as the cilia elongate. That this mRNA is first translated is implicit from the fact that protein labeling during induction is proportionate with length (Stephens, 1994c). The >3-fold relative excess of tektin A mRNA that non-induced, zinc-arrested embryos accumulate (versus which remained after induction) correlates with the approximate 3-fold ciliary length increase that takes place after induction. However, a substantial steady-state level of tektin A mRNA remains after induction, as is also true after ciliogenesis or regeneration in the Strongylocentrotus species noted above. The final tektin A mRNA levels in control and zinc-arrested embryos treated with theophylline (2nd versus 4th bar) may reflect the >2-fold ciliary length difference that must be maintained after these two kinds of animalization (cf. Stephens, 1994b).

Our data show that the level of tektin A mRNA relative to total mRNA is up-regulated during both ciliogenesis and ciliary regeneration and that it increases in proportion to ciliary length when embryos are animalized by zinc treatment. Tektin A mRNA is detectable in unfertilized eggs and it remains at low levels in fertilized embryos up to the blastula stage, at which point the relative level rises about 20-fold. Messages for the other two tektins accumulate somewhat earlier and rise about 7-fold, probably reflecting their larger pool sizes, estimated by label-depletion to be about 3 times that needed to construct the one cilium existing on each cell (Stephens, 1994c). Therefore, the expression of all three tektins is likely to be required for the initial ciliogenesis. Tektin mRNAs fall off to steady-state levels after the blastula stage but rise again in response to deciliation. Like tubulin mRNAs, the tektin messages thus fall into the postcleavage-stage-specific (PCS) class of maternal mRNAs as defined by Kelso-Winemiller and co-workers (1993). Animalized embryos that form longer than normal cilia express proportionately higher tektin A mRNA levels, and these levels are further up-regulated upon deciliation. Finally, when cilia are induced to elongate on embryos where animalization is not fully expressed, proportionately accumulated tektin A and other mRNAs are evidently translated and degraded coincident with ciliary growth. This apparent inhibition of translation in zinc-arrested embryos contrasts with normal ciliogenesis or regeneration where the ciliary mRNAs are transcribed and immediately utilized.

These mRNA results, correlating message production with both ciliogenesis and the length of cilia to be deployed, are consistent with previous studies showing the correlation of quantal tektin A protein synthesis with ciliogenesis, regeneration and ciliary length (Stephens, 1977, 1989). The synthesis of all three tektin messages at ciliogenesis, with one of them being potentially limiting, is consistent with the structural model wherein the three tektins together form extended polymers within the wall of the microtubule doublet (cf. Linck, 1976; Linck and Langevin, 1982; Linck et al., 1982; Pirner and Linck, 1994; Nojima et al., 1995) and the consequent developmental hypothesis that the quantal amount of tektin A limits the final length of tektin filament, thus specifying the length of the co-assembling ciliary (or flagellar) axonemal microtubules (Stephens, 1977, 1989).

Our work has been limited by the relatively low levels of tektin mRNAs, even at ciliogenesis, and hence we have not been able to assess the number of copies of tektin A mRNA per cell under various conditions. However, some rough approximations may be useful. To detect a reasonable tektin A signal, we have had to use 25-50 times the number of embryos as other workers have used to quantitate tubulin mRNAs under similar experimental conditions. Tektin A represents 1-2% of the mass of the cilium (Stephens, 1977; Linck, unpublished), which itself is about 2/3 tubulin, thus giving an average mass ratio between tektin A and tubulin of 1:44. This suggests that the amounts of tektin A and tubulin mRNAs may be stoichiometric with respect to the amounts of each protein that must be synthesized for one round of ciliogenesis or for one round of replacement. Thus, it is likely that it is the messages that are proportionately or quantally synthesized as a result of coordinate regulation.

The mechanism for the observed increase in tektin A mRNA levels at ciliogenesis is not presently known. This up-regulation could be due to an increase in the transcription rates of tektin A genes or processing of existing nuclear message, an increase in tektin A mRNA stability, or, in the case of a multiple gene family, an increase in the number of genes being transcribed. In terms of message stability, the high level of tektin A mRNA seen in non-induced, zinc-animalized embryos...
of *T. gratilla* and its degradation coincident with ciliary elongation demonstrates that stable mRNA can accumulate and that post-transcriptional regulation is operative in animalized embryos, as has been clearly shown for β-tubulin mRNA (Harlow and Nemer, 1987b). Our only evidence for possible multiple gene regulation comes from embryos where several mRNA species can be detected (Fig. 1, tektin C; Fig. 3).

After deciliation, transcription may be somehow metered to produce sufficient tektin A mRNA to translate enough protein for one length of cilium and, coordinately, sufficient mRNAs for tubulin and the other non-limiting ciliary proteins to replenish their proportionately depleted pools. In partially expressed animalization, as in *T. gratilla*, zinc treatment leads to the production and accumulation of ciliary mRNAs (Fig. 9, 1st versus 3rd tektin A bar) without a concomitant increase in cilary length. At the same time, zinc may secondarily prevent translation. Theophylline treatment, by some unknown mechanism that does not involve global modulation of cAMP (cf. Riederer-Henderson, 1988; Stephens, 1994b), may release this translational block, allowing the full reading of the accumulated mRNAs and the consequent elongation of cilia. The moderate animalizing effect of theophylline on control embryos, accompanied by a marked decrease in tektin A mRNA but not ubiquitin mRNA (Fig. 9, 1st versus 2nd bars) may demonstrate just such an increased utilization of existing, steady-state ciliary mRNAs to produce the characteristic >50% augmentation in cilary length (Stephens, 1994b).

The fact that cilia in all of these embryos turn over at rates approaching those of regeneration (Stephens, 1994a) would suggest that the substantial steady-state levels of tektin A and other mRNAs seen after ciliogenesis, regeneration, or elongation reflect ciliary maintenance. It follows then that embryos that must maintain longer cilia would have proportionately higher tektin A mRNA levels if cilary length regulation takes place primarily at the transcriptional level. This >2-fold proportionality, in fact, was found consistently between tektin A levels in control versus animalized embryos at steady-state (Fig. 6, 1st versus 2nd or Fig. 9, 1st versus 3rd bars) and also after theophylline-induced length changes (Fig. 9, 2nd versus 4th bars).

Turnover aside, we can say with certainty that regeneration involves the coordinate and proper re-expression of essentially all of the ciliary proteins, while animalization involves the further up-regulation of these same proteins (cf. Stephens, 1977, 1989). Furthermore, for at least one of these proteins, tektin A, the mRNA and hence the final amount of protein that is synthesized for a single round of ciliogenesis appears to be strictly limiting. Since regeneration mimics ciliogenesis, and since regeneration can be repeated many times without affecting the timing of development, this precise, coordinate re-expression of ciliary genes can be considered as a ‘subroutine in the program of development’, a process whose function is likely to be ciliary replacement during an extended larval life (Stephens, 1994c).

The presence of low levels of tektin mRNAs at stages of development prior to ciliogenesis may indicate that the tektins are also involved in other cellular processes such as centriole replication, mitosis and cell division. This possibility is supported by the fact that antibodies raised against flagellar tektin cross-react with centrioles, centrosomes, mitotic spindles and/or midbodies in a variety of organisms (Steffen and Linck, 1988, 1989, 1992; Steffen et al., 1994). In regard to centriole formation, it may be significant that the ratio of tektin A mRNA during ciliogenesis compared with cleavage stages (i.e. about 20:1) approximates the ratio between the lengths of the single ciliary axoneme and the centriole that must be constructed in each blastomere. The availability of tektin probes, both antibodies and cDNAs, should now make it possible to address the important questions of centriole and basal body structure and genesis.

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### REFERENCES


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