In sea urchin embryos the translation of cleavage stage histone variants is confined to S phase and is partially uncoupled from DNA replication

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We have analyzed the intracellular distribution of the basic proteins synthesized during the first cell cycle of sea urchin development to obtain information on the temporal relationship between DNA replication and the ***de novo*** *synthesis of cleavage stages (CS) histone variants. The newly synthesized CS histone variants were labeled* ***in vivo*** *with a mixture of ^{3}H-aminoacids and the CS histone variants were isolated from chromatin, analyzed by electrophoresis on two-dimensional polyacrylamide gels and the labeled polypeptides were detected by fluorography. The coupling between DNA replication and the translation of CS histone genes was investigated by measuring the synthesis of CS histone variants in zygotes treated with aphidicolin, an inhibitor of α-DNA polymerase. The results obtained indicate that during the first cleavage cell cycle of sea urchin development, the* ***de novo*** *synthesis of CS histone variants is coincident with DNA replication as in most eukaryotic cells, but differently from somatic cells, the translation of the CS histone gene products is partially uncoupled from DNA replication.*

Key words*: cell cycle, DNA replication, histone synthesis, sea urchins, zygotes.*

INTRODUCTION

During early cleavage stages (CS) of sea urchin development the events leading to cell DNA replication and division and those involved in cell growth are uncoupled, as opposed to other eukaryotic cells in which these events are coupled. This peculiarity makes sea urchin zygotes an attractive model to investigate the mechanisms involved in cell proliferation. Unlike most eukaryotic cells, sea urchin zygotes contain a set of histone like proteins, the CS histone variants, that are distinct from the well conserved histones found in most cells. The electrophoretic migration of these variants is similar to the typical histones isolated from other sources, but their aminoacid composition differs significantly from somatic histones, as well as from histones obtained from sperm and larvas of the same species (Imschenetzky *et al*, 1984, 1986, 1989a, 1990). The aminoacid compositional difference between CS histone variants and somatic type histones is consistent with the immunological unrelatedness between these variants and the histones present in larval stages of development (Imschenetzky *et al*, 1993). Despite their distinct composition, the CS variants should be regarded as histones in terms of their function, since these proteins are responsible for the primary folding of DNA into beaded nucleoparticles during early cleavage stages of sea urchin development. (Imschenetzky *et al*, 1989b, 1993).

A tight relationship between DNA replication and histone neosynthesis seems to be an obvious need for each replication

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round in all eukaryotes forming chromatosomes, which are the basic structural arrangement of newly synthesized chromatin. It has been previously shown that almost 90 percent of the total histones synthesized during each cell cycle is confined to the synthesis (S) phase and that only one tenth of the total histones is synthesized *de novo* throughout the cell cycle (Wu and Bonner, 1981). Furthermore, it has been clearly demonstrated that histone gene expression is coupled to DNA replication at the transcriptional as well as at the translational level in most cells (Old and Woodland, 1984; Stein and Stein, 1984; Stein *et al*, 1994; Doenecke *et al*, 1994). Related to the coupling of DNA replication and histone gene transcription it is well known that the cell cycle-dependent histone gene expression is under the control of regulatory elements (AAACACA and the CCAAT box) that are located in proximal and distal positions as referred to the histone coding regions. Such regulatory DNA elements interact with transcription factors, namely HiNF-B or H1TF, thus promoting the coupling of histone gene expression and DNA replication at the transcriptional level (Stein *et al*, 1994; Doenecke *et al*, 1994).

The coupling of DNA replication to the translation of histone gene products is less well understood than the transcriptional control. Previous results published by Hereford and Osley (1981, 1982) indicate that DNA replication is linked to the stability of histone gene mRNA, since the inhibition of DNA replication induces an arrest of histone gene translation, as well as the degradation of polysomal histone mRNA (Baumbach *et al*, 1987). The regulation of histone translation also involves a quantitative control of the amount of histones synthesized during each S phase since an increased amount of histone genes does not affect the normal level of histone gene products, indicating that histone gene expression exhibits a dose compensation at the translational level (Osley and Hereford, 1981; Osley, 1991). The precise mechanisms involved in the several levels of translational control are not fully understood so far. Related to the translational control of histone gene expression, the early developmental stages of the sea urchin is a unique model that permits one to focus on this particular problem, without interference from events related to the transcriptional control of histone gene expression. In contrast to most cells, during early cleavage stages of sea urchin development the coupling between DNA replication and histone gene transcription is not observed, as has been shown previously. Transcription inhibition does not prevent DNA replication throughout the cleavage divisions. These results strongly suggest that the mRNA coding for histones are already present in unfertilized eggs, presumably as one of the maternal masked mRNA that are synthesized during oogenesis and activated after fertilization (Arceci and Gross, 1977; Poccia *et al*, 1981, 1986; Deleon *et al*, 1983; Angerer *et al*, 1984). The identity of this masked mRNA and the mechanisms involved in its unmasking for translation are still far from being understood. Differently from somatic cells that exhibit a temporal coupling between histone synthesis and DNA replication, in sea urchin zygotes an inhibition of the histones synthesized after fertilization does not affect the first replication round of zygotes, but prevents the following S phases and the first mitotic division (Tsanev and Russev, 1974; Wagenaar and Mazia, 1978). These results suggest that in zygotes there is a lag period between histone translation and DNA replication. Alternatively, a pool of preformed CS histone variants may be present in unfertilized eggs (Poccia *et al*, 1981). Due to the peculiarities outlined above, we have decided to use the early cleavage cells of the sea urchin *Tetrapygus niger* to determine whether the translation of CS histone gene RNA messengers is confined to S phase in the initial embryonic cell cycle, as it is in all eukaryotes known so far. We have also explored whether the inhibition of embryonic DNA replication affects the translation of CS histone gene products.

MATERIALS AND METHODS

Gametes and zygotes

Sea urchins *Tetrapygus niger*, were collected from the bay of Concepción and maintained

at room temperature in an aquarium containing natural sea water under constant aeration. Unfertilized eggs, sperm and embryos cultures were obtained as described (Imschenetzky *et al*, 1980, 1986, 1988, 1991b, 1993). As it was previously determined, the first S phase occurs between 30 min and 60 min post insemination (p-i) when embryonic development takes place at room temperature (18-21° C). Based on this information, we consider as pre-replicative phase that occurring from 3 min to 30 min p-i. As informed previously under standard conditions the replicative phase proceeds from 30 min to 60 min p-i (phase S1) and the second replication round occurs from 70 min to 110 min p-i (phase S2) (Imschenetzky *et al*, 1980, 1991a, 1992, 1993).

Histones isolation and electrophoretic analysis

Histones were isolated from the chromatin purified from sea urchin zygotes as previously described (Imschenetzky *et al*, 1986, 1993) and analyzed by electrophoresis in two dimensional gels. The first dimension was performed in 12% polyacrylamide gels containing urea/ acetic acid/Triton DF-16 as described by Alfageme *et al* (1974) and the second dimension was performed on 18% (w/v) polyacrylamide/SDS gels (Laemmli, 1970).

Intracellular distribution of the proteins synthesized ***de novo*** *during the first cell cycle of sea urchin zygotes*

To obtain the proteins synthesized *de novo* during the first embryonic cell cycle, the zygotes were incubated for different periods after fertilization in sea water containing a mixture of ^{3}H-aminoacids at a concentration of 10 mCi/ml of culture (algal protein hydrolyzate, NEN, Boston, Massachusetts). To analyze the proteins synthesized in the pre-replicative phase, the zygotes were harvested at the onset of the first S phase (30 min p-i). To analyze the proteins synthesized during the first S phase, the zygotes were incubated in sea water containing a mixture of ^{3}H-aminoacids from 30 min to 60 min p-i and harvested at 60 min p-i. Whole acid soluble proteins were isolated from the cytoplasm or alternatively from the chromatin, with 0.25 N HCl (Imschenetzky *et al*, 1991b). The incorporation of ^{3}H-aminoacids *in vivo* into the basic proteins located in the cytoplasm or to those isolated from the chromatin of zygotes was measured as described by Bollum (1969). Alternatively, the whole acid soluble proteins isolated either from the cytoplasm or from the chromatin of sea urchin zygotes were obtained and analyzed by two dimensional electrophoresis as described by Imschenetzky *et al* (1991b). The identification of each of the CS histone variants was performed according to their electrophoretic migration in two dimensional PAGE as informed previously (Imschenetzky *et al*, 1986, 1991b, 1993). The radioactivity associated to individual CS variant was determined by fluorography (Laskey, 1975; Chamberlain, 1979; Imschenetzky *et al*, 1991b).

Effect of aphidicolin on DNA replication and on the synthesis of CS histone variants

The timing of the S phase was followed by measuring ^{3}H-thymidine incorporation into DNA during the two first cleavage divisions. For this purpose the zygotes were incubated continuously from 3 min to 120 min p-i in sea water containing ^{3}H-thymidine at a concentration of 1 μCi/ml of zygote suspension (NEN, Boston, MA.). The ^{3}H-thymidine incorporated into DNA was determined as described by Imschenetzky *et al* (1991a). To prove that aphidicolin abolishes DNA replication in sea urchin zygotes, the incorporation of ^{3}H-thymidine into DNA from control cultures was compared with those treated with 30 μM of aphidicolin. The zygotes were incubated in sea water containing 30 μM of aphidicolin from 15 min until 120 min p-i and at each 5 min intervals the ^{3}H-thymidine incorporated into 10% (w/v) TCA insoluble material was measured. To improve the solubility of aphidicolin in sea water, this compound was first dissolved in 25 mM dimethylsulfoxide (DMSO) and then added to the zygote suspension. Therefore, in parallel to each inhibition experiment the effect of DMSO on

thymidine incorporation into DNA and on the morphology of the embryos was compared with that of zygotes cultured under standard conditions.

To determine the effect of the inhibition of DNA replication on the level of CS histone variants synthesized *de novo* during the first S phase, the fluorographic pattern of whole CS variants synthesized in normal zygotes was compared with that obtained from zygotes treated with 30 μM aphidicolin to inhibit DNA replication.

Under standard conditions the S phase occurs in zygotes between 30 min and 60 min p-i. To label the CS histone variants synthesized *de novo* during the first S phase, the zygotes were incubated in ^{3}H-aminoacids (10 μCi/ml) from 30 min to 60 min p-i. The zygotes were then harvested at 60 min p-i and processed to isolate the labeled CS histone variants from the chromatin of normal zygotes, or alternatively from those treated with 30 μM aphidicolin. The isolated CS histone variants were analyzed by two dimensional electrophoresis and the label associated with each electrophoretic spot was evidenced by fluorography. The identification of CS histone variants was performed according to their migration in two dimensional PAGE as compared with standard CS variants stained with Coomasie blue (Imschenetzky *et al*, 1986, 1991b, 1993).

RESULTS

Intracellular distribution of the basic proteins synthesized ***de novo*** *during the initial cell cycle of sea urchin embryos*

The intracellular distribution of the total basic proteins synthesized *in vivo* during the first cell cycle of sea urchin development was determined by labeling the proteins synthesized *de novo* between 3 min and 60 min p-i. At 60 min the zygotes were harvested and the radioactivity associated with the whole basic proteins isolated from the chromatin and from the cytoplasm was determined. The results, shown in Figure 1, indicate that around 80 percent of the total proteins synthesized during the first cell cycle of sea urchins zygotes are chromosomal basic proteins as these proteins are associated to the chromatin during the first cell cycle of zygotes.

To determine whether these newly synthesized basic chromosomal proteins are confined mainly to the S phase or are constitutively expressed during the first cell cycle, the proteins synthesized *de novo* were labeled with ^{3}H-aminoacids during the pre-replicative phase (from 3 min until 30 min p-i) or alternatively during the first S phase (from 30 min until 60 min p-i). The acid soluble proteins were then isolated from chromatin and from the cytoplasmic fraction (S30) and the radioactivity associated to these proteins was determined. The results indicate that the basic chromosomal proteins are synthesized *de novo* only during the first S phase, as shown in Table I. The basic proteins isolated from chromatin during the pre-replicative phase were not labeled.

The electrophoretic migration of the whole basic chromosomal proteins synthesized *in vivo* during S phase was analyzed in two dimensional polyacrylamide gels. The labeled polypeptides were detected by fluorography. The results shown in Figure 2 indicate that the basic chromosomal proteins labeled during S phase comigrate with CS

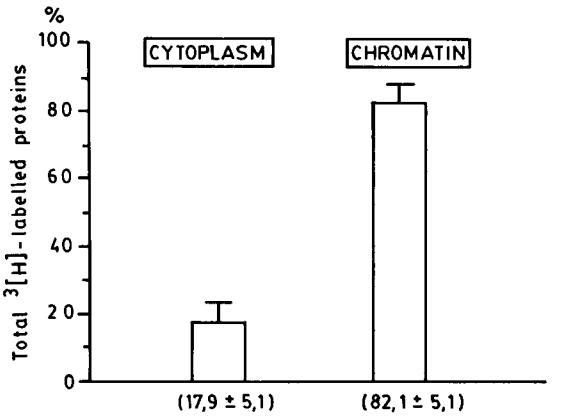

Fig 1. Incorporation of ^{3}H-aminoacids into basic proteins synthesized during the first cleavage cycle of sea urchins development. Zygotes were incubated continuously in sea water containing 10 μCi/ml of a mixture of ^{3}H-aminoacids from 3 min until 60 min p-i. Whole basic proteins were isolated from the cytoplasm and from the chromatin. The radioactivity associated to cytoplasmic proteins (S30 fraction) was measured as compared to chromosomal proteins. Means ± SD's from four independent experiments.

TABLE I

Basic proteins synthesized during the first cell cycle

Prereplicative phase (3-30 min p.i.)	cpm
Cytoplasm	30,545 ± 1,430
Chromatin	non-detectable
Replicative phase (30-60 min p.i.)	**cpm**
Cytoplasm	31,965 ± 1,320
Chromatin	355,610 ± 70,300

Basic proteins synthesized *de novo* during the first cell cycle of sea urchin development. Zygotes were incubated from 3 min until 60 min p-i in sea water containing a mixture of ^{3}H-aminoacids (10 μCi/ml of culture). Whole basic proteins synthesized *de novo* in the interval between fertilization and the onset of S phase (3 min-30 min p-i) and during S1 (30 min-60 min p-i) were isolated from the cytoplasm and from the chromatin and their radioactivity was determined. Average data of four independent experiments.

histone variants in the gel, whereas the cytoplasmic basic proteins labeled during S phase differ from the CS histone variants as judged by their electrophoretic migration. As shown in Figure 2A, the labeled polypeptides found in the cytoplasm of S phase zygotes do not migrate in the positions of the polypeptides identified as CS histone variants by comparison with the electrophoretic migration of standard CS histone variants. These results also indicate that CS histone variants are synthesized *de novo* and targeted to the nucleus, coincidently with the first S phase.

Coupling between DNA replication and the translation of CS histone variants

To investigate if DNA replication is coupled to the translation of CS histone mRNA, the synthesis *in vivo* of CS histone variants were compared in zygotes cultured in sea water with those cultured in the presence of 30 μM aphidicolin.

To confirm that the treatment of zygotes with 30 μM aphidicolin inhibits DNA replication, the incorporation of ^{3}H-thymidine into DNA was compared in normal zygotes with that of zygotes treated with 30 μM aphidicolin or with those incubated in sea water containing DMSO. The results shown in Figure 3 demonstrate that in zygotes treated with aphidicolin the incorporation of thymidine into DNA is strongly inhibited and that the initial cleavage divisions were not observed. It was also shown that the presence of DMSO does not alter the normal incorporation of ^{3}H-thymidine into DNA, the initial cleavage divisions or the early developmental stages of sea urchins.

The effect of the treatment of zygotes with 30 μM aphidicolin on the synthesis *in vivo* of whole chromosomal proteins was measured for total acidic proteins (Fig 4A) and for total basic chromosomal proteins (Fig 4B). As it has been previously demonstrated, the total basic chromosomal proteins are mostly represented by the CS histone variants (Imschenetzky *et al*, 1986, 1989b, 1993). To

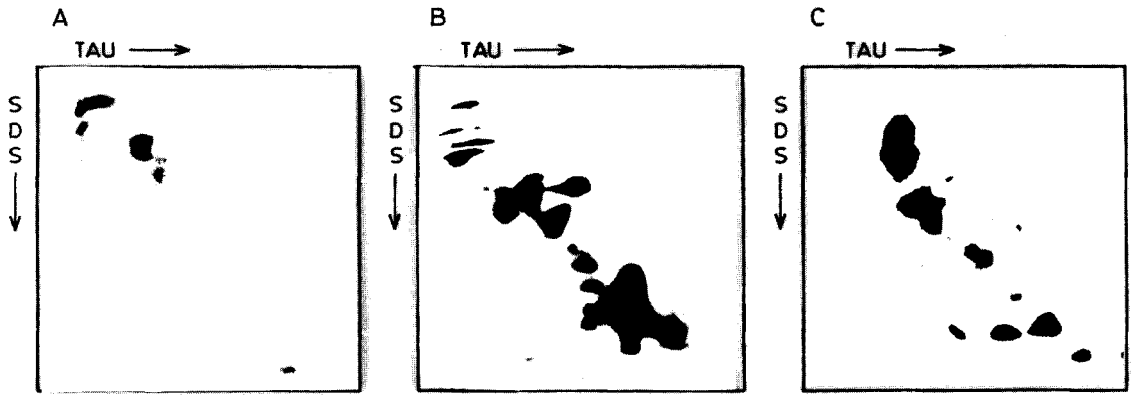

Fig 2. Analysis of the basic proteins synthesized during phase S1. Zygotes were labeled with a mixture of ^{3}H-aminoacids (algal protein hydrolyzate) from 30 min until 60 min p-i. The acid soluble proteins were obtained from the cytoplasm and from the chromatin, electrophoresed in two dimensional gels and subjected to fluorography. The first dimension gels were 15% acrylamide/TAU and the second dimension were 18% acrylamide/SDS. The direction of electrophoretic migration is indicated by arrows. Coomasie blue stained gels containing CS histone variants isolated from zygotes harvested 60 min p-i are included as a standard of electrophoretic migration (2B), fluorography of the labeled basic chromosomal proteins (2C) and of the basic cytoplasmic proteins (2A).

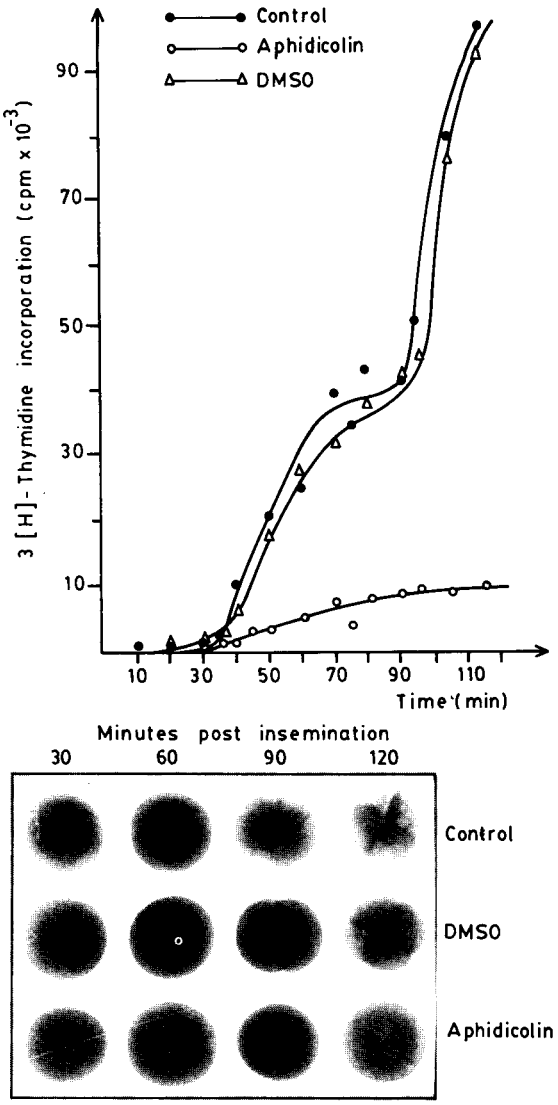

Fig 3. S phase inhibition by aphidicolin. Zygotes were incubated in sea water containing 30 µM aphidicolin from 3 min until 120 min p-i. The incorporation of ^{3}H-thymidine into DNA was measured for normal zygotes, for zygotes treated with aphidicolin and for zygotes cultured in sea water containing DMSO. The effect of aphidicolin or of DMSO on the morphology of zygotes was followed by light microscopy and compared with normal conditions.

determine the amount of neosynthesized chromosomal proteins bound to the chromatin during phases S1 and S2, the incorporation of ^{3}H-aminoacids into basic or into acidic chromosomal proteins was determined in normal zygotes and compared with those obtained from zygotes treated with 30 uM aphidicolin. As shown in Figure 4A, the amount of acidic chromosomal protein synthesized during phases S1 and S2 is very similar in normal embryos and in those treated with aphidicolin. On the other hand, the amount of basic chromosomal proteins decreases when the zygotes are treated with 30 µM aphidicolin, as shown in Figure 4B. These results indicate that the inhibition of DNA replication diminishes specifically the neosynthesis of basic chromosomal proteins. It is also obvious from the results shown in Figure 4B, that in the zygotes treated with aphidicolin the incorporation of ^{3}H-aminoacids into whole basic chromosomal proteins is diminished but not abolished.

To analyze the effect of the treatment of zygotes with aphidicolin on the synthesis

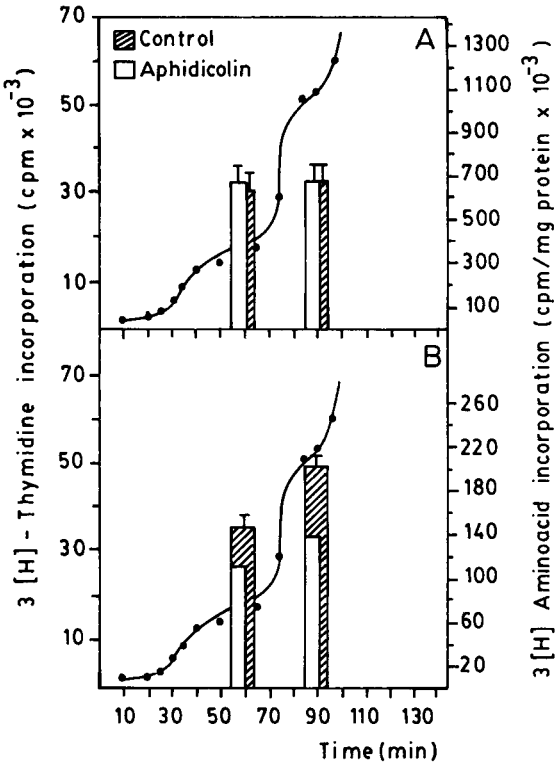

Fig 4. Effect of aphidicolin on the synthesis of basic chromosomal proteins. Zygotes were cultured in sea water containing 10 µCi/ml of a mixture of ^{3}H-aminoacids (algal protein hydrolyzate) from 3 min until 120 min p-i. At 120 min p-i, whole chromosomal proteins were isolated and the radioactivity was determined. Aphidicolin was added at 3 min p-i and the radioactivity of chromosomal proteins obtained from this cultures was determined and compared with those obtained from zygotes cultured in normal sea water. Total chromosomal proteins were isolated from both cultures, and the radioactivity associated to acidic proteins (4A) was determined as compared with that of basic proteins (4B). Means ± SD's from four independent experiments.

of individual CS histone variants, total CS histone variants were purified from chromatin as described by Imschenetzky *et al* (1986). Total proteins synthesized *in vivo* during phase S1 were labeled continuously with ^{3}H-aminoacids from 30 min until 60 min p-i. At 60 min the zygotes were harvested and the CS variants were isolated from normal zygotes, or alternatively from zygotes treated with aphidicolin and analyzed by two dimensional electrophoresis. The radioactivity associated with each protein was determined by fluorography. The results are shown in Figure 5. The CS histone variants synthesized in normal zygotes (Fig 5B) were compared with those treated with aphidicolin (Fig 5C). As illustrated, only very minor differences are evident, indicating that the major CS variants are synthesized when DNA replication is inhibited. The minor differences found between the fluorographic patterns shown in Figures 5B and 5C, are defined by a small group of spots migrating in the upper position of the gel and to one spot that exhibits the highest mobility of the proteins analyzed. These differences are signaled by arrows in Figures 5B and 5C. As is obvious from this comparison there are no clear differences in the spots located in the positions of the gel corresponding to the CS histone variants.

DISCUSSION

Similarly to the translation of histone gene products in most cells, it has now been shown that the synthesis of the CS histone variants coincides with DNA replication during the initial cleavage cell cycles of sea urchin development. This conclusion is based on the following three lines of experimental evidence. The first of these, is the demonstration that basic chromosomal proteins are very actively synthesized during the first cell cycle of sea urchin development, as shown in this report. The major fraction of basic proteins synthesized *de novo* during the first cell cycle are bound to chromatin during the first cell cycle. The second concerns the identification of whole basic chromosomal proteins as the CS histone variants (Imschenetzky *et al*, 1986). Other basic proteins that are co-extracted with CS histone variants from the chromatin of zygotes with dilute mineral acid, are four HMG like proteins, but their amount is insignificant as compared to the CS histone variants (Oñate *et al*, 1992). The third line of evidence is based on the demonstration that the synthesis *de novo* of CS histone variants occurs exclusively during S phase. The binding to the chromatin of labeled basic proteins was not detected before the onset of the first DNA replication round in zygotes.

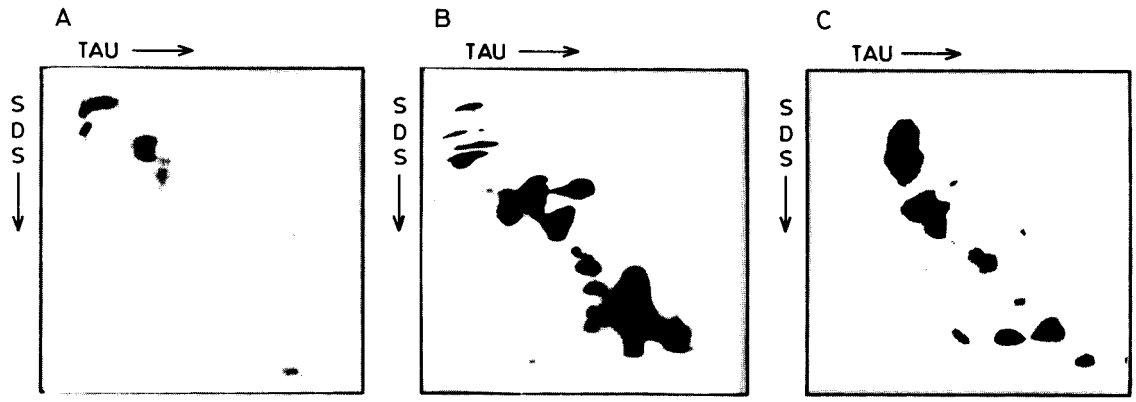

Fig 5. Effect of aphidicolin on the synthesis of basic chromosomal proteins synthesized during S phase. Zygotes were cultured in normal sea water or in sea water containing 30 μM aphidicolin. The newly synthesized proteins were labeled in both cultures with a mixture of ^{3}H-aminoacids from 30 min until 60 min p-i. Whole basic chromosomal proteins were isolated, electrophoresed in two dimensional gels and subjected to fluorography. The first dimension gels were 15% acrylamide/TAU and the second dimension were 18% acrylamide/SDS. Coomasie blue stained gels containing CS histone variants isolated from zygotes harvested 60 min p-i are included as standards of electrophoretic migration (5A). Fluorography of basic chromosomal proteins synthesized in normal zygotes (5B) and of basic proteins synthesized in the presence of aphidicolin (5C). The direction of electrophoretic migration is indicated by arrows

Furthermore, the two dimensional electrophoretic pattern of the labeled basic proteins is consistent with the identification of CS histone variants as the unique proteins synthesized during S phase, as opposed to the labeled cytoplasmic proteins that are clearly different from the CS variants in terms of their electrophoretic migration. As documented previously, the basic proteins synthesized at the time between fertilization and the onset of the first S phase are clearly different from CS histone variants (Imschenetzky *et al*, 1991b). An alternative explanation may also be a S phase confined targeting into nuclei of CS histone variants located in the cytoplasm as part of the maternal preexisting pool in the zygotes. Such control of nuclear input has been previously demonstrated to operate in other systems, but yet there is no clear evidence linking this control step to DNA replication or to the S phase of the cell cycle (Silver, 1991). The information presented in this report is insufficient to resolve the question of the nuclear targeting of the CS variants that are forming the maternal pool (Poccia *et al*, 1981, 1986), so this issue remains to be faced in the future.

Previous evidence had demonstrated clearly that transcription is not a prerequisite for DNA replication during the initial cleavage divisions (Gross and Cousineau, 1964). Therefore it may be expected that the CS histone variants translated during the initial cell cycles of zygotes are mainly encoded in mRNA of maternal origin. Since the translation of the CS is activated at the onset of the first DNA replication round and not before, based on the results discussed above, we suggest that one or more mechanisms of selective translational control of the mRNA of maternal origin are operating after fertilization in sea urchin zygotes. One of these control mechanisms could be responsible for the signal(s) linking a contemporaneous replication and translation of CS histone variants. The precise nature of such control mechanisms remains to be determined. The existence of specific control mechanisms that prevent the translation of histone mRNA beyond the actual needs of a given cell is also suggested by the results of Rousseau *et al* (1991, 1992) for the translation of H1°, the H1 variant that is expressed in non proliferative cells, indicating that the increased level of mRNA exceeds the resulting level of newly synthesized protein. Taking into consideration all these data, it appears that in early cleavage cells the control mechanisms linking histone gene expression to DNA replication are mainly located at the translational level, in contrast to most cells in which the coordinated control of S phase gene expression operates principally at the transcriptional level (Stein *et al*, 1994).

In sea urchin cleavage cells, the translational regulation of histone genes expression can be further dissected into several levels of control according to the data reported herein. The first is related to the partial coupling of CS histone translation to DNA replication. As shown in Figure 4, the arrest of embryonic DNA replication decreases approximately one third of the whole amount of newly synthesized CS histone variants incorporated to chromatin during the initial cell cycles of embryonic development. The observed decrease is histone specific, since it was shown that the binding to chromatin of newly synthesized acidic chromosomal proteins, remains unaltered when DNA replication was inhibited. As in other systems, in sea urchin cleavage cells, the coupling between DNA replication and the translation of CS histone variants might be related to the stability of CS histone mRNA (Stein and Stein, 1984; Marzluff and Pandey, 1988; Heintz, 1991; Morris *et al*, 1991; Wolffe, 1991). However, an additional level of control might be expected for CS translation, since their mRNA are pre-existing in the unfertilized eggs and are not totally degraded after each cell cycle.

The second level of CS translational control may be related to the stoichiometry of CS histones to DNA replication needed in order to organize a successful newly synthesized chromatin. In this respect, a pool of pre-synthesized CS histone variants in the unfertilized eggs of sea urchins has been previously suggested by Poccia *et al* (1981). The presence of a maternally derived cytoplasmic CS pool argues against the lack of labeled histones found in the cytoplasm of zygotes pulsed during phase S1 with ^{3}H-

aminoacids. Such a result, reported herein, clearly indicates that the newly synthesized CS histone variants are targeted into nuclei during S1 phase and do not remain in the cytoplasm of the zygote, as opposed to the cytoplasmic CS histone pool suggested previously by Poccia *et al* (1981). One of the possible explanations for this controversy, is the existence in zygotes of S phase dependent nuclear recruitment mechanisms that are responsible for the chromatin assembly of newly synthesized as well as of those CS variants that are pre-existent in the eggs. Alternatively, the CS variants from the pre-formed CS histone maternal pool might be designed for the replacement of spermatic histones that are degraded during male pronuclei remodeling, as shown previously (Imschenetzky *et al*, 1991b). Obviously it is not possible to derive a definitive conclusion thus far regarding the relationship between the pre-formed histones pool and the newly made CS variants. It seems clear however that this relationship has to be regulated in order to maintain the correct stoichiometry between the replicated DNA and the CS variants present in the cell during S phase. On the other hand, the S phase constrained binding of synthesized CS histone variants to the newly made chromatin reported here, adds further support to the idea that CS histone variants are genuine histones in terms of their function of organizing chromatin during early developmental stages in sea urchins.

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