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Poly(ADP-ribosylation) of Atypical CS Histone Variants Is Required for the Progression of S Phase in Early Embryos of Sea Urchins

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Abstract The patterns of poly(ADP-ribosylation) in vivo of CS (cleavage stage) histone variants were compared in sea urchin zygotes at the entrance and the exit of S1 and S2 in the initial developmental cell cycles. This post-translational modification was detected by Western immunoblots with rabbit sera anti-poly(ADP-ribose) that was principally reactive against ADP-ribose polymers and slightly against ADP-ribose oligomers. The effect of 3 aminobenzamide (3-ABA), an inhibitor of the poly(ADP-ribose) synthetase, on S phase progression was determined in vivo by measuring the incorporation of ^{3}H thymidine into DNA. The results obtained indicate that the CS histone variants are poly(ADP-ribosylated) in a cell cycle dependent manner. A significantly positive reaction of several CS variants with sera anti-poly(ADP-ribose) was found at the entrance into S phase, which decreases after its completion. The incubation of zygotes in 3-ABA inhibited the poly(ADP-ribosylation) of CS variants and prevented both the progression of the first S phase and the first cleavage division. These observations suggest that the poly(ADP-ribosylation) of atypical CS histone variants is relevant for initiation of sea urchin development and is required for embryonic DNA replication.

Key words: ADP-ribosylation, CS histone variants, cell cycle, sea urchin zygotes, 3 aminobenzamide

The involvement of poly(ADP-ribosylation) of nuclear proteins in the regulation of processes such as differentiation, gene expression, DNA repair, DNA recombination, and DNA replication has been previously described. Such modification requires specific enzymes for their addition to or removal from acceptor nuclear proteins. The addition is catalyzed by the poly-(ADP-ribose) synthetase, which transfers the ADP moiety of NAD to acceptor proteins to form either oligo- or poly-(ADP-ribose) linear or branched homopolymers covalently attached to chromosomal proteins [1,2]. The removal is catalyzed by the enzymes poly(ADP-ribose) glycohydrolase and poly(ADP-ribose) protein lyase, yielding ADP-ribose, which is further hydrolyzed to AMP and ribose phosphate [3,4]. The overall pattern of poly(ADP-ribosylation) of nuclear proteins that can be determined in vivo is a consequence of the activity of these enzymes, of the intranuclear DNA concentration and of the steric availability of acceptor chromosomal proteins.

Several acidic nuclear proteins are known to be acceptors for this post-translational modification, such as RNA polymerase II [5], ligase II [6], topoisomerase I and II [7,8], Ca II, Mg II-dependent endonuclease [9], DNA polymerase α and β [10], the terminal nucleotidyltransferase [11], and the poly(ADP-ribose) synthetase itself [12]. Additionally, other nuclear proteins, including histones [13–15] and the high mobility group proteins (HMG) [16] are the most important acceptors for poly(ADP-ribosylation) in the nucleus. Poly(ADP-ribose) synthetase contains two zinc-binding domains, it participates in the recognition of DNA strand breaks, and its activity is strictly dependent on DNA single or double stranded interruptions [17]. This enzyme has been found in a wide variety of eukaryotic cells but several terminally differentiated cells lack its activity [reviewed in 1,2,18]. No clear information is available at present concerning poly(ADP-ribosylation) of nuclear proteins in the early stages of development, with the exception of a previous report by Koide et al. that the activity of poly(ADP-ribose) synthetase in sea urchin embryos rises during cleavage stages, peaks at the blastula stage, and then falls with the onset of gastrulation [19].

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These observations strongly suggest that ADP-ribosylation of chromosomal proteins is involved in DNA repair, recombination, and replication. Thus, a significant role for this post-translational modification during the cell cycle may be postulated. However, conflicting results have been published for several biological systems such as HeLa cells, the slime mold *Physarum polycephalum,* mitogen-activated lymphoid cells, and murine thymic cells [20–26]. The differences found within the same experimental system [16,20,21] may be attributable to variability in the experimental protocols used to assay the poly(ADP-ribosylation) of nuclear proteins.

Cleavage stages of sea urchin development are characterized by very short cell cycles with a minimal G1 phase, except for the first postfertilization cell cycle, which exhibits a short period of pronuclear remodeling preceding the first S phase. The S phases are extremely rapid with very short G2/M intervals [reviewed in 27]. In contrast to other eukaryotes, the chromatin of sea urchin eggs and early cleavage embryos is organized in atypical nucleoparticles formed by seven CS variants that are bound to 126 b.p of DNA [28,29]. According to their amino acid compositions, the seven CS variants differ from typical histones although their electrophoretic migration is consistent with histones in high-resolution polyacrylamide gels [28,30]. Except for the phosphorylation of one of the CS variants [31], other post-translational modifications affecting these proteins are unknown at present but each class is microheterogeneous, as judged by electrophoretic analysis in high resolution two-dimensional gels [32,33].

To avoid experimental variations in the in vitro determinations of ADP-ribosylation of nuclear proteins during the embryonic cell cycle, we have examined the patterns of poly(ADP-ribosylation) of CS histone variants in zygotes during the two initial S phases and in the period of the first G2/M, respectively. The poly(ADP-ribosylation) of CS variants was determined with polyclonal antibodies raised in rabbits against purified poly(ADP-ribose) polymers. The potential role of the poly(ADP-ribosylation) of CS variants during cell cycle progression was investigated by determining the effect in vivo of 3-ABA, a very specific inhibitor of the poly(ADP-ribose) synthetase [21,34]. Our results indicate that the CS variants are poly(ADP-ribosylated) in sea urchin zygotes. The patterns of poly(ADP-ribosylation) in vivo of CS histone variants at the onset of DNA replication are different from those found at the G2/M transition and suggest that this post-translational modification is absolutely necessary for DNA replication and cell cycle progression of early sea urchin embryos.

MATERIALS AND METHODS

Gametes and Zygotes

Sea urchins *Tetrapygus niger* were collected from the Bay of Concepcion and maintained at room temperature in an aquarium containing natural sea water under constant aeration. Unfertilized eggs, sperm, and embryo cultures were obtained as described previously [28].

Isolation of Chromosomal Proteins and Gel Electrophoresis

CS chromosomal proteins from embryos harvested at different times after insemination were isolated as described previously [28,32,33]. Sample preparation and one- and two-dimensional gel electrophoresis were performed essentially as described by Alfageme et al. [35] and Laemmli [36].

Preparation and Purification of ADP-Ribose Polymers and Oligomers

Polymers and oligomers were obtained from a cell free system, to obtain labeled ADP-ribose polymers and oligomers; ^{3}H-NAD^{+} was used as substrate (NEN, Boston, MA, sp. act 33.4 Ci/mmol). The purification of the synthesized polymers and oligomers was performed essentially as described by Burzio et al. [37] with the following minor modifications. The 0.1 M NaOH suspension of the nuclear extracts containing the ADP-ribose polymers and oligomers was neutralized with HCl. SDS was added to a concentration of 1% (w/v) and Proteinase K (Boehringer, Mannheim) to 100 μg/ml. This mixture was incubated for 4 h at 37°C. Subsequently, the same amount of enzyme was added and the 4 h incubation was repeated. To remove insoluble material, the suspension was centrifuged at 15,000g for 15 min and the supernatant was deproteinized three times with a mixture of chloroform/isoamyl-alcohol 24:1 (v/v). Then the aqueous layer was made 0.2 M in sodium acetate (pH 5.0) and 2.5 vol of absolute ethanol was added to precipitate the ADP-ribose polymers

and oligomers. The suspension was maintained at −20°C overnight; the precipitate was collected by centrifugation, washed with 70% cold ethanol and vacuum dried. The nucleic acids present in this preparation were eliminated by treatments with 100 μg/ml of DNAse I and 100 μg/ml of pancreatic ribonuclease (Sigma Chemical, St. Louis, MO) and finally with a 5 h incubation at 37°C with 300 units/ml of micrococcal nuclease (Worthington, Freehold, NJ). Then the poly(ADP-ribose) solution was made 0.5% (w/v) in SDS, incubated at 37°C with 50 μg/ml of Proteinase K (Boehringer, Mannheim) for 10 h, and deproteinized as described above. The ADP-ribose polymers and oligomers were precipitated with absolute ethanol as described above, centrifuged at 15,000g for 15 min, and dried under vacuum. The relative amount of polymers to oligomers, as defined previously by Burzio et al. [37], was determined with radiolabeled ^{3}H ADP-ribose by precipitating the whole ADP-ribose fraction obtained by the procedure outlined above, with 10% (w/v) tricholoroacetic acid (TCA). This treatment renders the oligomers containing chain lengths under 18–20 units of poly(ADP-ribose) soluble and precipitates the polymers containing over 18–20 residues of ADP-ribose [37]. In a typical experiment, the whole ADP-ribose preparation contained 40% polymers and 60% oligomers.

Preparation of Antisera and Western Immunoblot Analysis

The preparation of the anti-ADP-ribose serum was performed as described by Kanai et al. [38]. Whole ADP-ribose polymers and oligomers coupled to methylated serum albumin were utilized as antigens. Briefly, an initial dose of 700 μg of poly ADP-ribose in 1 ml of buffer (0.1 M sodium phosphate, pH 7.4) were emulsified with 1 ml of complete Freund's adjuvant and used to immunize rabbits. The same dose of antigen, but dissolved in incomplete Freund's adjuvant, was injected subcutaneously 20 days later. The serum against poly(ADP-ribose) was obtained 30 days after the first inoculation of the antigen. Subsequently this serum, diluted 1/50 (v/v) in a Tris-buffered saline solution (TBS) containing 0.02 M Tris, pH 7.5, 0.5 M NaCl, and 1% (w/v) gelatin, was used to incubate the nitrocellulose membranes for the Western immunoblot analysis. The binding capacity of the antiserum obtained to ADP-ribose polymers and to ADP-oligomers was determined by the millipore-filter technique as described by Kanai et al. [39]; 1 ml of serum diluted 1/50 retained a level of radioactivity on the millipore filters corresponding to 1.72 μg of ^{3}H-poly(ADP-ribose) and of 1.2 μg of ^{3}H-oligo(ADP-ribose), respectively. The procedures followed for the electrophoretic transfer, blotting, and immunodetection were essentially those described by Towbin et al. [40], modified as described previously [32,33].

DNA Synthesis

Zygotes were incubated continuously from 3 to 120 min after insemination in ^{3}H thymidine (NEN, Boston, MA, Sp. act. 108.2 Ci/mmol) at a concentration of 1 μCi/ml. At different times after insemination, 1 ml samples of the zygote suspension were collected and washed in cold sea water acidified with HCl to pH 4.0 in order to stop their metabolic activity [32,33]. The chromatin was isolated as previously described [28]. The radioactivity incorporated into DNA was measured by the paper filter disc method as described by Bollum [41].

RESULTS

Reactivity of the Anti-ADP-Ribose Sera Against Polymers and Oligomers

The binding capacity to ADP-ribose oligomers and polymers of the rabbit serum anti-whole ADP-ribose fraction was measured by the millipore filter technique described by Kanai et al. [39]. The ^{3}H-labeled whole ADP-ribose fraction was purified and the polymers were separated from the oligomers by precipitating the polymers with 10% (w/v) TCA and recovering the oligomers after their precipitation with absolute ethanol as described previously by Burzio et al. [37]. The ADP-ribose binding assay was performed for the anti-serum diluted 1/50 in phosphate-buffered saline containing 1% bovine serum albumin (PBS-BSA) analogous to the experimental condition used for the Western blot analysis. The amount of radioactivity retained by ADP-Ribose oligomers was measured relative to ADP-ribose polymers and expressed as 100% of the antiserum binding capacity (Fig. 1). As shown in Figure 1, the anti-serum dilution used in the Western blots analysis reported in this paper reacts principally with polymers of ADP-ribose and at a lower level with oligomers. These results are in agreement with previous reports for serum anti-ADP-ribose obtained in mice [38].

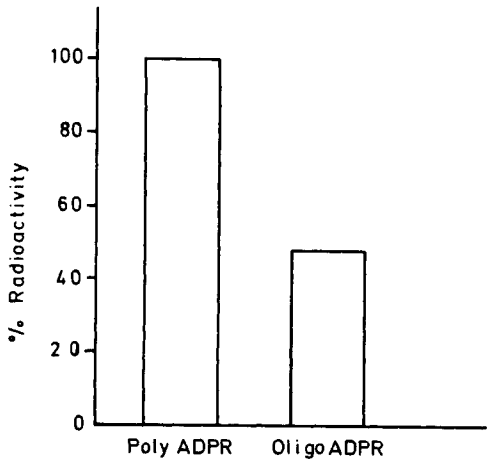

Fig. 1. Reactivity of the antisera obtained in rabbits by injection of MBSA-(ADP-ribose) complexes against polymers and oligomers of ^{3}H-(ADP-ribose). The polymers of (ADP-ribose) were insoluble in 10% (w/v) TCA and contained more than 18–20 residues of (ADP-ribose); the oligomers corresponded to the fraction soluble in 10% (w/v) TCA which contained molecules with less than 18–20 residues of (ADP-ribose), as defined previously by Burzio et al. [37]. The binding capacity of the tritium-labeled (ADP-ribose) polymers and oligomers to the rabbit antisera was determined by the millipore filter method described previously by Kanai et al. [39]. The binding of (ADP-ribose) oligomers is referenced to the retention of ADP-ribose polymers on millipore filters, which is considered as 100%.

Poly(ADP-Ribosylation) of CS Histone Variants at the Initial Cell Cycles of Sea Urchin Embryos and the Effect of 3-ABA on Cell Cycle Progression

The time course of the initial cleavage divisions was followed by light microscopy. The results (Fig. 2) show that the first cleavage occurs 90 min post insemination (p.i.) and the second is observed at 120 min p.i. To determine cell cycle parameters, the incorporation of ^{3}H thymidine into DNA was monitored in vivo. As shown in Figure 2, the first S phase occurs between 20 min p.i. and 40 min p.i. and the second S phase was observed between 80 min p.i. and 120 min p.i. The timing of DNA replication determined for these sea urchin embryos is consistent with previous results obtained for different species of sea urchins by others [27,42].

To examine the patterns of ADP-ribosylation of CS histone variants in vivo during the first cleavage cycle, total acid soluble proteins were isolated from zygotes harvested 30 min p.i. (first S phase), 60 min p.i. (first G2/M interval), and 90 min p.i. (second S phase). These proteins were separated by two-dimensional electrophoresis in gels containing Triton-DF 16/urea/acetic acid (TAU/PAGE) in the first dimension and

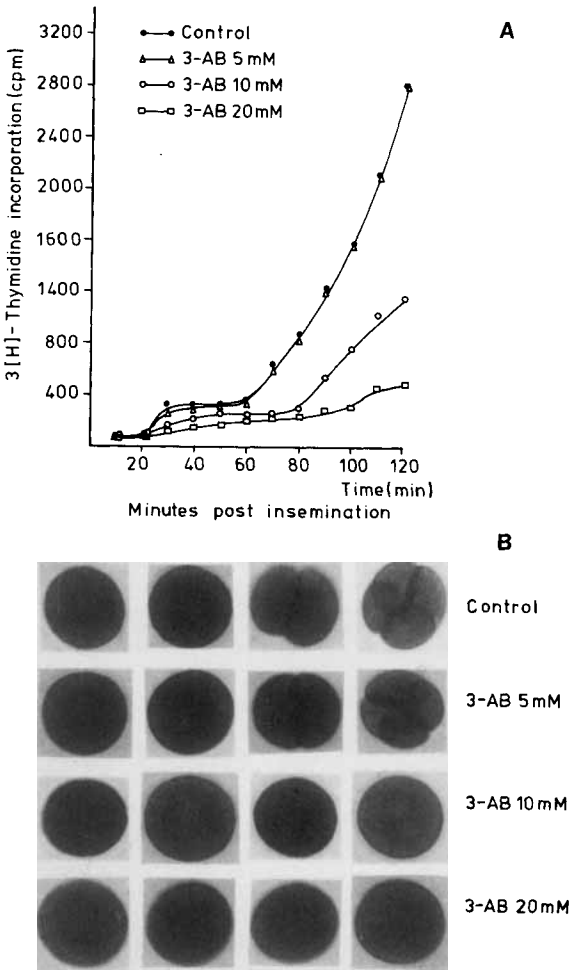

Fig. 2. **A:** Effect of 3-aminobenzamide (3-ABA) on the incorporation of ^{3}H thymidine into DNA. **B:** Effect of 3-ABA on the first cleavage cycle observed by light microscopy. The effects of 3-ABA on the incorporation of ^{3}H thymidine into DNA and on the first cleavage cycle were obtained by incubating the zygotes from 3 min post-insemination until 120 min p.i. into 5 mM, 10 mM, and 20 mM of 3-ABA, respectively.

sodium dodecylsulfate (SDS/PAGE) in the second dimension, transferred to nitrocellulose membranes, and analyzed by Western blots for ADP-ribose polymers and oligomers with antiserum. The results of the stained gels and the corresponding Western blots (Fig. 3) indicate that the CS variants from zygotes are poly(ADP-ribosylated) throughout the first embryonic cleavage cycle. Similar patterns of heterogeneous ADP-ribosylation were observed for the CS variants obtained from zygotes harvested at the first and second S phases. Strong positive signals were observed for the components that migrate in the one-dimensional gels in the regions corresponding to P1, CS A, and CS G. Weak signals were obtained for components mi-

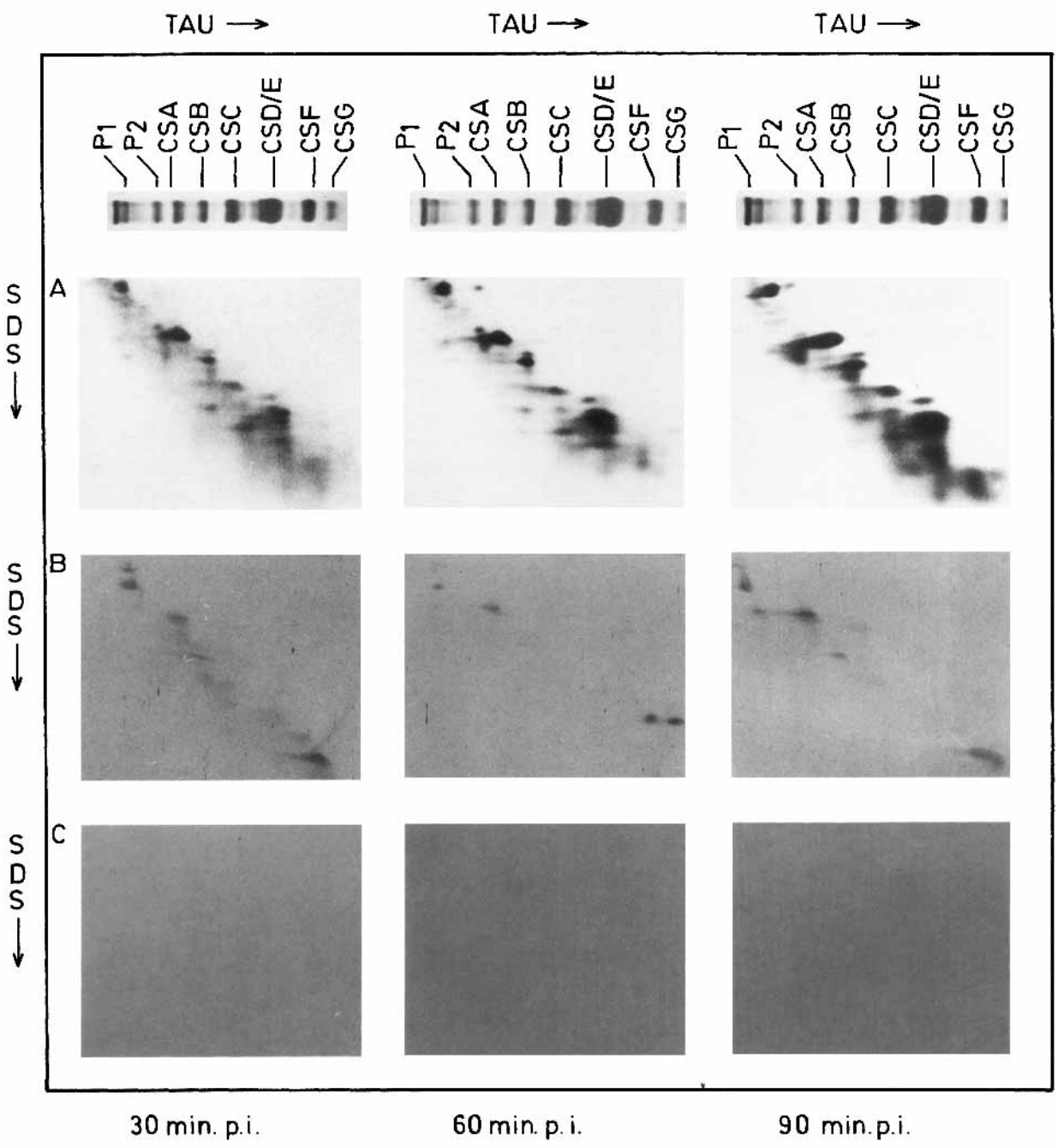

Fig. 3. Electrophoretic and Western blot analysis of CS variants from sea urchin zygotes isolated at different times p.i. using rabbit antisera against polymers and oligomers of ADP-ribose. Total acid soluble chromosomal proteins were isolated from zygotes harvested at the first S phase (30 min p.i.); the first G2/M transition (60 min p.i.) and at the second S phase (90 min p.i.), electrophoresed in two-dimensional polyacrylamide gels, transferred to nitrocellulose membranes and revealed using antisera against ADP-ribose. **A:** Total CS variants separated in two-dimensional gels. The first dimension was performed in 15% acrylamide/TAU gels and the second dimension in 18% acrylamide/SDS gels, and the gels were stained with Coomassie blue. **B:** Western blots of total CS variants from zygotes obtained 30 min p.i. (S1), 60 min p.i. (G2/M1), and 90 min p.i. (S2), respectively. **C:** Western blots of total CS variants isolated from zygotes at 30 min p.i. (S1), 60 min p.i. (G2/M1), and 90 min p.i. (S2) that were incubated contiguously with 3-ABA beginning at 3 min p.i. The Coomassie blue stained slots corresponding to the first-dimensional gel containing the CS variants from zygotes are included at the top of the two-dimensional gels and Western blots as a reference of electrophoretic migration. The direction of migration is indicated by arrows.

grating in the position of CS B – CS C and a very diffuse signal was found for CS D-E and CS F. The proteins P1 and P2 have HMG-like solubility properties, as reported previously [28]. Unlike the heterogenous ADP-ribosylation signals found during the S phase, at the G2/M interval, single spots of a positive reaction were found in the positions of P1, CS A, and two spots were detached in the position of CS F/CS G. Nothing was detected in the regions of the gel corresponding to CS B, CS C, and CS D-E.

To explore the potential role of poly(ADP-ribosylation) of CS histone variants in the replication of the embryonic genome and in the initial cleavage divisions, the zygotes were incubated continuously, from fertilization until 120 min p.i., in different concentrations of 3-ABA, an inhibitor of the poly(ADP-ribose) synthetase activity. The cleavage divisions were monitored by light microscopy and the replication of DNA was determined by ^{3}H-thymidine incorporation into DNA (Fig. 2). As shown in Figure 2, the inhibition of poly(ADP-ribose) synthetase activity by 3-ABA inhibits both cleavage divisions and DNA replication in a dose-dependent manner; 5 mM ABA has almost no effect, 10 mM ABA slows the first cleavage division and decreases DNA replication, and 20 mM ABA completely blocks cleavage and inhibits very strongly the replication of the embryonic genome. The pattern of poly(ADP-ribosylation) of CS histone variants isolated from zygotes incubated in 20 mM of 3-ABA was determined by Western immunoblots (Fig. 3) to assess more directly the relationship between poly(ADP-ribosylation) of CS histone variants, replication of the embryonic genome, and the initial cleavage divisions. As shown, a concentration of 20 mM 3-ABA inhibits the poly(ADP-ribosylation) of CS histone variants to nondetectable levels when assayed with the anti-serum used for the present study.

DISCUSSION

The finding reported here, that atypical CS histone variants that form the chromatin of early sea urchin embryos are acceptors of poly-(ADP-ribose), is consistent with previous reports indicating that histones are the main chromosomal proteins poly(ADP-ribosylated) in most eukaryotes [1,2]. This strengthens the idea that the CS chromosomal proteins function as histones in the chromatin of early sea urchin embryos. We could identify CS A, B, C, D-E, F, G, and the HMG-like protein P1 as acceptors of ADP-ribose polymers during S phase of the first embryonic cleavage cycle, while P1, CS A, and CS G were the major acceptors in the G2/M interval. These results demonstrate that the microheterogeneity reported previously [32,33] for CS A, B, C, D-E, and G is due to the poly(ADP-ribosylation) of these proteins. Obviously, other post-translational modifications, such as acetylation, ubiquitinylation, methylation, and phosphorylation, may also contribute to the observed microheterogeneity as has been documented for histones from other cells. Thus far it has been demonstrated that a phosphorylation of the CS variant migrating similarly to H2A occurs coincidentally with the first S phase in sea urchin embryos [31].

The reactivity of the antibodies used for this study decreases according to the length of ADP-ribose polymers [38] so the patterns of poly(ADP-ribosylation) of CS variants reported here are restricted to the limits of detection of the antibodies used for this study and therefore do not represent the total ADP-ribosylation of CS variants found in vivo for these embryos. Despite these methodological limitations, it was demonstrated that the extent of poly(ADP-ribosylation) of CS histone variants differs between S phase and the G2/M interval and that the CS histone variants are poly(ADP-ribosylated) throughout the first cleavage cycle. This study was not extended to chromosomal proteins obtained before the first S phase because sperm specific histones are still present in zygotes at that time, making the interpretations of the results less definitive [32,33]. Our results concerning a differential pattern of poly-ADPR found in vivo during the cell cycle of sea urchin embryos are consistent with the increased poly-(ADP-ribose) polymerase mRNA levels determined in vivo in regenerating rat liver. These findings suggest a role for this enzyme in cell proliferation [43] and are consistent with previous reports indicating a biphasic rise of the activity of poly(ADP-ribose) synthetase in S phase and at the S/G2 transition in HeLa cells [20] and in *Physarum polycephalum* [23,44]. The results obtained for sea urchin embryos are also in agreement with the increase of histone poly(ADP-ribosylation) found for mitogen-activated lymphoid cells [26] and in proliferating mouse and human cells in culture [45]. In contrast to these reports, Tanuma et al. have observed a maximal poly(ADP-ribosylation) in metaphase chromosomes of HeLa cells [16].

These discrepancies are probably due to differences in experimental approaches, as the measurement in vitro of this post-translational modification is not strictly analogous to the situation found in vivo.

The functional correlation between DNA replication and the cell cycle dependent poly(ADP-ribosylation) of CS histone variants was demonstrated by the inhibition of embryonic S phase progression by 3-ABA, a specific inhibitor of poly(ADP-ribose) synthetase which does not significantly inhibit poly(ADP-ribose) glycohydrolase and is nontoxic to most cells [reviewed in 18]. The inhibition of embryonic S phase reported here is dose dependent, as has been reported in other biological systems [46]. However, the effective 3-ABA inhibition doses are very difficult to assess in vivo. In contrast to permeabilized cells, little is known about the intracellular concentrations and compartmentalization of inhibitors of poly(ADP-ribose) synthetase in intact cells, as indicated by Cleaver et al. [47], but among all known inhibitors, the benzamide derivatives are the most specific and reliable for studies in intact cells [48]. To avoid the problem of the effective 3-ABA concentration in vivo, which can not be measured experimentally in sea urchin zygotes, we have chosen a dose of 3-ABA that clearly inhibits DNA replication in intact zygotes to establish a relationship between the poly(ADP-ribosylation) of CS variants and DNA replication. The inhibitory doses in vivo of 3-ABA reported here, similar to the nicotinamide doses used previously by Koide et al. [19] for sea urchin embryos, are much higher and should not be compared directly to the doses of inhibitors used by others to block poly(ADP-ribose) synthetase activity in intact cultured cells, in permeabilized cells, or in isolated nuclei.

The results linking the poly(ADP-ribosylation) of CS histone variants and DNA replication contribute support to the suggestion previously made by Poirier et al. [49] concerning the potential role of poly(ADP-ribosylated) histones in chromatin relaxation that may facilitate events such as DNA repair or DNA replication [45,49,50]. Additionally it was previously reported that the incubation of cells in 3-ABA promotes the accumulation of 10 kb DNA replication intermediates [51]. This finding can be related to the activity of ligase II, the enzyme presumed to be responsible for DNA ligation, the activity of which is dependent on poly(ADP-ribose) synthesis [6]. Recently it has been proposed that poly(ADP-ribosylated) histones are deposited on DNA during replication. These modified histones arise from the activation of poly-(ADP-ribose) synthetase at the replication fork by the Okazaki fragments [50]. The process by which poly(ADP-ribosylation) of CS variants may participate in embryonic DNA replication or repair remains to be determined. However, it is reasonable to anticipate that the mechanisms may be similar to those of histones from other cells.

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REFERENCES

1. Althaus FR, Hilz H, Shall S: In: "ADP-Ribosylation of Proteins." Berlin: Springer-Verlag, 1985, pp 1–578.
2. Althaus FR, Richter C: In: "ADP-Ribosylation of Proteins." Berlin: Springer-Verlag, 1985, pp 1–237.
3. Miwa M, Tanaka M, Matsushima T, Sugimura T: J Biol Chem 249:3475–3482, 1974.
4. Okayama H, Honda M, Hayaishi O: Proc Natl Acad Sci USA 75:2254–2257, 1978.
5. Taniguchi T, Susuki S, Shizuta Y: Biochem Biophys Res Commun 127:526–532, 1985.
6. Creissen D, Shall S: Nature 296:271–272, 1982.
7. Darby M, Schmitt B, Jongstra-Bilen J, Vosberg HP: EMBO J 4:2129–2134, 1985.
8. Ferro AM, Olivera B: J Biol Chem 259:547–554, 1984.
9. Tanaka Y, Yoshihara K, Itaya A, Kamiya T, Koide S: J Biol Chem 259:6579–6585, 1984.
10. Yoshihara K, Itaya A, Tanaka Y, Ohashi Y, Ito K, Teraoka H, Tsukada K, Matsukage A, Kamiya T: Biochem Biophys Res Commun 128:61–67, 1985.11.
11. Tanaka Y, Ito K, Yoshihara K, Kamiya T: Eur J. Biochem 155:20–25, 1986.
12. Kawaichi M, Ueda, Hayaishi O: J Biol Chem 256:9483–9489, 1981.
13. Riquelme PT, Burzio LO, Koide SS: J Biol Chem 254: 3018–3028, 1979.
14. Burzio LO, Riquelme PT, Koide SS: J Biol Chem 254: 3029–3037, 1979.
15. Huletsky A, Niedergang C, Fréchette A, Aubin R, Gaudreau A, Poirier G: Eur J Biochem 146:277–285, 1985.

16. Tanuma S, Yagi T, Johnson GS: Arch Biochem Biophys 237:38–42, 1985.
17. Menissier-de-Murcia J, Molinete M, Gradwohl G, Simonin F, de Murcia, G: J Mol Biol 210:229–233, 1989.
18. Shall S: Biochem Soc Trans 17:317–322, 1989.
19. Koide SS, Ellison RP, Burzio LO, Koide SL: Biol Bull (Woods Hole MA) 151:416–417, 1976.
20. Kidwell WR, Mage MG: Biochem 15:1213–1217, 1976.
21. Adolph KW: Arch Biochem Biophys 243:427–438, 1985.
22. Tanuma S, Kanai Y: J Biol Chem 257:6565–6570, 1982.
23. Golderer G, Schneider R, Auer B, Loidl P, Gröbner P: Biochem J 253:859–867, 1988.
24. Sóoki-Tóth A, Bánfalvi G, Szöllösi J, Kristen E, Staub M, Antoni F, Kun E: Exp Cell Res 184:44–52, 1989.
25. Marini M, Zunica G, Monti D, Cossarizza A, Ortolani C, Franceschi C: FEBS Lett 253:146–150, 1989.
26. Boulikas T, Bastin B, Boulikas P, Dupuis G: Exp Cell Res 187:77–84, 1990.
27. Poccia D: In Bourne C, Jeon K, Friedlander M (eds): "International Review of Cytology," Vol 105. New York: Academic Press, pp 1–65, 1986.
28. Imschenetzky M, Puchi M, Massone R: Comp Biochem Physiol 84B:23–27, 1986.
29. Imschenetzky M, Puchi M, Gutierrez S, Massone R: Exp Cell Res 182:436–444, 1989.
30. Newrock RM, Alfageme ECR, Nardi RV, Cohen LH: Cold Spring Harb Symp Quant Biol 42:421–431, 1978.
31. Green GR, Poccia DL: Dev Biol 134:413–420, 1989.
32. Imschenetzky M, Puchi M, Massone R, Oyarce AM, Rocco M: Arch Biol Med Exp 21:409–416, 1988.
33. Imschenetzky M, Puchi M, Massone R, Roco M, Gamboa S: Arch Biol Med Exp 21:241–245, 1988.
34. Purnell M, Whish J: Biochem J 185:8755–8777, 1980.
35. Alfageme CR, Sweidler A, Mahowald A, Cohen LH: J Biol Chem 249:3729–3736, 1974.
36. Laemmli UK: Nature 227:680–685, 1970.
37. Burzio L, Riquelme P, Koide S: Analytical Biochem 66:434–445, 1975.
38. Kanai Y, Miwa T, Matsushima T, Sugimura T: Immunology 34:501–508, 1978.
39. Kanai Y, Kawaminami Y, Miwa T, Matsushima T, Sugimura T: Nature 265:175–177, 1977.
40. Towbin H, Staehelin T, Gordon J: Proc Natl Acad Sci USA 76:4350–4354, 1979.
41. Bollum F: In Grossman, Moldave, K (eds): "Methods in Enzymology." New York: Academic Press, 1969, pp 169–173.
42. Wagenar E: Exp Cell Res 144:393–403, 1983.
43. Menegazzi M, Carcereri de Prati A, Ledda-Columbano G, Columbano A, Uchido K, Miwa M, Susuki H: Arch Biochem Biophys 279:232–236, 1990.
44. Grobner P, Loidl P: Biochem J 232:21–24, 1985.
45. Farzaneh F, Panayotou FN, Bowler LD, Hardas BD, Broom T, Walther C, Shall S: Nucleic Acids Res 16: 11320–11328, 1988.
46. Cesarone CF, Scarabelli L, Giannoni P, Gallo G, Orunescu M: Biochem Biophys Res Commun 171:1037–1043, 1990.
47. Cleaver J, Borek C, Milam K, Morgan W: Pharmacol Ther 313:269–294, 1987.
48. Rankin P, Jacobson E, Benjamin R, Moss J, Jacobson M: J Biol Chem 264:4312–4317, 1989.
49. Poirier G, de Murcia G, Jongstra-Bilen J, Niedergang C, Mandel P: Proc Natl Acad Sci USA 79:3423–3427, 1982.
50. Boulikas T: J Biol Chem 265:14638–14647, 1990.
51. Lönn U, Lönn S: Proc Natl Acad Sci USA 82:104–108, 1985.