Short Communication

Relevance of Histone H1 Kinase Activity to the G2/M Transition during the Cell Cycle of Dictyostelium discoideum

Tohru Arakane¹ and Yasuo Maeda*¹

Biological Institute, Graduate School of Science, Tohoku University, Aoba, Sendai, 980-77 Japan

The implication of histone H1 kinase activity for the G2/M transition during the cell cycle was investigated using Dictyostelium discoideum Ax-2. Histone H1 kinase with its activity was purified from cell extracts by the use of p13-succ subunit (Cdc2 protein kinase) (Dunphy et al. 1988, Gautier et al. 1988) and a regulatory subunit (cyclin B) (see Labbe et al. 1989). During the early development of Xenopus, cyclin B accumulates up to a maximal level at the G2/M boundary, allowing cells to enter the M phase (Evans et al. 1983, Standart et al. 1987), and is then destroyed to allow cells to leave mitosis (Murray et al. 1989). For other developmental cell lines, however, cyclin B is not necessarily the major regulatory factor in the G2/M transition (Edgar et al. 1994). Thus the details of the regulatory mechanism seem to vary from species to species and there may be various control mechanisms operating differently depending on developmental stages of a single organism. In Dictyostelium, it has been demonstrated that cyclin B mRNA and protein levels fluctuate during the vegetative cell cycle (Luo et al. 1994). However, Luo et al. (1995) have reported that cyclin B protein levels change only slightly during differentiation and that there is no increase in the activity of Cdc2-histone H1 kinase during the late developmental mitosis. Based on these results, they have raised the possibility that the control mechanism of mitosis during Dictyostelium differentiation may differ from that for vegetative growth. As presented here, however, histone H1 kinase activity was found to reach a

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The regulatory mechanism of the G2 to M phase transition in the cell cycle has been shown to be highly conserved in euakratory cells. Dictyostelium discoideum is a simple eukaryote which provides an excellent model system to analyze the cell cycle regulation, growth/differentiation transition, cell differentiation and pattern formation. D. discoideum Ax-2 cells grow and multiply by binary fission with a doubling time of about 7.5 hr, as long as nutrients are supplied. Upon starvation, however, the starving cells initiate developing to form a multicellular aggregate and eventually differentiate into either spores or stalk cells.

The cell cycle of vegetative Ax-2 cells is characterized by short M and S phases, little or no G1, and a long G2 phase (Weijer et al. 1984a, Maeda 1986), thus suggesting the G2/M transition may be the most weighty step of cell cycle regulation in this organism. In addition, the cell-cycle position at the onset of starvation has been shown to be a intrinsic variable which is important for the cell sorting, differentiation and pattern formation (McDonald and Durston 1984, Weijer et al. 1984b, Gomer and Firtel 1987, Ohmori and Maeda 1987, Maeda et al. 1989, Maeda 1993, Araki et al. 1994, Amagai and Maeda 1996). After the analysis using Ax-2 cells synchronized by the temperature shift method (Maeda 1986), starving Ax-2 cells are able to advance through the cell cycle to a particular point (PS-point) and enter the differentiation phase from this point (Maeda et al. 1989). Although the growth and differentiation phases are generally believed to be mutually exclusive, there are some indications that the cell cycle may progress during the differentiation phase (Zada-Hames and Ashworth 1978, Durston and Volk 1978, Zimmermann and Weijer 1993, Araki and Maeda 1995), possibly in response to prespore differentiation during the mound-tipped aggregate stage.

In general, initiation of mitosis (M phase) requires a protein kinase complex (MPF) consisting of a catalytic subunit (Cdc2 protein kinase) (Dunphy et al. 1988, Gautier et al. 1988) and a regulatory subunit (cyclin B) (see Labbe et al. 1989). During the early development of Xenopus, cyclin B accumulates up to a maximal level at the G2/M boundary, allowing cells to enter the M phase (Evans et al. 1983, Standart et al. 1987), and is then destroyed to allow cells to leave mitosis (Murray et al. 1989). For other developmental cell lines, however, cyclin B is not necessarily the major regulatory factor in the G2/M transition (Edgar et al. 1994). Thus the details of the regulatory mechanism seem to vary from species to species and there may be various control mechanisms operating differently depending on developmental stages of a single organism. In Dictyostelium, it has been demonstrated that cyclin B mRNA and protein levels fluctuate during the vegetative cell cycle (Luo et al. 1994). However, Luo et al. (1995) have reported that cyclin B protein levels change only slightly during differentiation and that there is no increase in the activity of Cdc2-histone H1 kinase during the late developmental mitosis. Based on these results, they have raised the possibility that the control mechanism of mitosis during Dictyostelium differentiation may differ from that for vegetative growth. As presented here, however, histone H1 kinase activity was found to reach a

¹ Present address: Division of Medical Pharmacology, Torei Co. Ltd., Muromachi 2-2-1, Nihonbashi, Chuo-ku, Tokyo, 103 Japan.

¹ Correspondence and reprints: Biological Institute, Graduate School of Science, Tohoku University, Aoba, Sendai, 980-77 Japan.

* Correspondence and reprints: Biological Institute, Graduate School of Science, Tohoku University, Aoba, Sendai, 980-77 Japan.
maximum just before the M phase during the late development (possibly prespore differentiation) as well as during the vegetative cell cycle, thus indicating use of basically the same machinery for the G2/M transition.

**Materials and Methods**

**Cells and culture conditions**

Vegetative cells of *Dictyostelium discoideum*, axenic strain Ax-2 (clone 8A), were grown in 10 ml of HL-5 medium supplemented with 1.5% glucose (Watts and Ashworth 1970) in a 200 ml–Erlenmeyer flask, with a doubling time of about 7.5 hr. To allow cells to develop, Ax-2 cells were harvested at the exponential growth phase, washed twice by centrifugations in 20 mM Na/K-phosphate buffer (pH 6.4), and suspended in the buffer. The washed cells were plated on 1.5% agar (Bacteriological Agar; Oxoid) at a density of about 7.0 x 10^7 cells/cm^2 and then incubated at 22.0 C.

**Synchronization of the cell–cycle phase**

To induce good cell synchrony, a slight modification of the temperature shift method (Maeda 1986) was used. Ax-2 cells growing exponentially at 22.0 C were shifted to 9.4 C, shaken for 14.5 hr, and then re-shifted to 22.0 C. Under this condition, cell doubling occurred over about a 2-hr period after a lag phase of about 1 hr.

**Preparation of cell extracts**

Cells were collected and washed once with extraction buffer (10 mM Tris–HCl, 15 mM MgCl2; 0.01% Brij 35, 80 mM Naβ-glycerophosphate, 1 mM Na3VO4, 15 mM glycoletherdiaminetetraacetic acid (EGTA), 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml pepstatin A, 20 μg/ml antipain, 20 μg/ml leupeptin, pH 7.5) in a 1.5 ml–Eppendorf microtube. The cell pellet (packed cell volume) was immediately resuspended in an equal volume of ice-cold extraction buffer and stood for 5 min in an ice bath, followed by centrifugation for 2 min at 2,000 x g. The resulting pellet was again suspended in an equal volume of the buffer and homogenized mildly by an electronic homogenizer (BM-KIKI), monitoring the degree of cell lysis under a phase-contrast microscope. The homogenate was centrifuged for 20 min at 13,000 x g to remove the membrane fraction and then ultracentrifuged for 1 hr at 100,000 x g. The supernatant thus obtained was stored at −80 C before use.

**Absorption to p13\textsuperscript{sucl} affinity gel**

p13\textsuperscript{sucl}-binding proteins from cell extracts was collected by the use of p13\textsuperscript{sucl}-Sepharose CL-4B (Pharmacia) gel (p13\textsuperscript{sucl}, 5 mg/ml) provided kindly from Dr. T. Kishimoto of Tokyo Institute of Technology. The cell extracts (50 μl) were absorbed with the gel (2 μl) overnight at 4 C and then centrifuged for 1 min at 13,000 x g. The pellet containing the gel was washed twice with the extraction buffer containing 0.4 M NaCl. The washed gel was used for the assay of histone H1 kinase activity.

**Measurement of histone H1 kinase activity**

The p13\textsuperscript{sucl} gel prepared as described in the preceding section was resuspended in 25 μl of kinase buffer containing 0.5 mg/ml histone H1 (Boheringer Mannheim), 100 mM ATP, 1 μCi [\textsuperscript{32}P] ATP (4,500 Ci/mmol, ICN), 3 mM MgCl2, and 25 mM Tris–HCl, pH 7.5. Reactions were carried out for 20 min at 25 C and stopped by addition of an equal volume of 2X sample buffer (4% sodium dodecyl sulfate (SDS), 200 mM dithiothreitol (DTT), 20% glycerol, 0.005% bromophenol blue (BPB), Tris–HCl, pH 6.8). Samples were boiled for 5 min, cooled in an ice bath, and analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Ten μl of the sample was applied onto each lane of 10% gel and electrophoresed. After staining of gels with Coomassie brilliant blue (CBB), bands containing histone H1 were cut off and their radioactivities were determined by a liquid scintillation counter.

**Results and Discussion**

In *Dictyostelium*, it has been believed that the kinds of Cdks and cyclins involved in the cell–cycle progression are a quite few as compared with those in animal cells (Michaelis and Weeks, 1993), thus providing a simpler system for the analysis of cell–cycle regulation. For the purification of Cdc2 homologue and Cdk cyclin complex from cell extracts, we used p13\textsuperscript{sucl}–affinity gel. Formerly Hinze et al. (1992) have purified Cdc2 kinase homologue from *Dictyostelium discoideum* Ax-2 cells using p13\textsuperscript{sucl}-agarose beads, but failed to detect its histone H1 kinase activity. Although the precise reason for this failure is presently unknown, histone H1 kinase activity was found to be retained both by cell homogenization under a milder condition and by a prolonged time (overnight) of cell extract absorption with p13\textsuperscript{sucl}–gel. The specificity of p13\textsuperscript{sucl} affinity purification for Cdc2 kinase was examined by using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and subsequent immunoblotting using the Cdc2–specific antibody kindly provided by Dr. G. Weeks of UBC. As a result, Cdc2 protein (p32) was found to be preferentially absorbed by the p13\textsuperscript{sucl}–gel used (data not shown). The p32 could be the product of either the *Dictyostelium* Cdc2 or Cdk2 genes or could be the sum of the two gene products (Hinze et al. 1992). Incidentally, when cell extracts were absorbed with Sepharose CL-4B without p13\textsuperscript{sucl}, the p32 was scarcely detected by immunoblotting using the Cdc2–specific antibody, and no histone H1 kinase activity was detectable (data not shown).

Hereupon, a temporal change in histone H1 kinase activity during the progression of cell cycle was examined using Ax-2 cells synchronized by the temperature shift method (Maeda 1986). As shown in Fig.1, it is evident that histone H1 kinase activity is highest just prior to mitosis (immediately after the temperature shift from 9.4 C to 22.0 C) and then decrease gradually, thus coming to almost zero at 5 hr after the temperature shift. The activity seemed to begin increasing prior to the next M