THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 51, Issue of December 19, pp. 51786–51795, 2003
Printed in U.S.A.

Aurora-A Kinase Maintains the Fidelity of Early and Late Mitotic Events in HeLa Cells*[S]

Received for publication, June 13, 2003, and in revised form, September 30, 2003
Published, JBC Papers in Press, October 1, 2003, DOI 10.1074/jbc.M306275200

Tomotoshi Marumoto‡§, Shinobu Honda‡, Toshihiro Hara‡, Masayuki Nitta‡, Toru Hirota‡, Eiji Kohmura§, and Hideyuki Saya‡¶

From the ‡Department of Tumor Genetics and Biology, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan, and the §Department of Neurosurgery, Kobe University Graduate School of Medicine, 7-5-1, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

Aurora-A, a member of the Aurora/Ipl1-related kinase family, is overexpressed in various types of cancer and considered to play critical roles in tumorigenesis. To better understand the pathological effect of Aurora-A activation, it is first necessary to elucidate the physiological functions of Aurora-A. Here, we have investigated the roles of Aurora-A in mitotic progression with the small interfering RNA, antibody microinjection, and time lapse microscopy using human cells. We demonstrated that suppression of Aurora-A by small interfering RNA caused multiple events to fail in mitosis, such as incorrect separation of centriole pairs, misalignment of chromosomes on the metaphase plate, and incomplete cytokinesis. Antibody microinjection of Aurora-A into late G_2 cells induced dose-dependent failure in separation of centriole pairs at prophase, indicating that Aurora-A is essential for proper separation of centriole pairs. When we injected anti-Aurora-A antibodies into prometaphase cells that had separated their centriole pairs, chromosomes were severely misaligned on the metaphase plate, indicating that Aurora-A is required for proper movement of chromosomes on the metaphase plate. Furthermore, inhibition of Aurora-A at metaphase by microinjected antibodies prevented cells from completing cytokinesis, suggesting that Aurora-A also has important functions in late mitosis. These results strongly suggest that Aurora-A is essential for many crucial events during mitosis and that the phosphorylation of a series of substrates by Aurora-A at different stages of mitosis may promote diverse critical events in mitosis to maintain chromosome integrity in human cells.

To accurately divide genetic material between two daughter cells during mitosis, cells undergo various crucial events such as chromosome condensation, nuclear envelope breakdown, centrosome separation, bipolar spindle assembly, chromosome segregation, and cytokinesis. Studies of diverse lower organisms have found that these events are controlled by reversible phosphorylation conducted by several serine/threonine kinases, known as mitotic kinases (1). Mitotic kinases include cyclin-dependent kinase 1 ($p34^{cdc2}$), Polo-like, NimA-related, Warts, and Aurora/Ipl1-related kinases; their structures have been well conserved through evolution (1, 2).

Prominent among the mitotic kinases implicated in the regulation of mitotic progression is Aurora-A, one of the Aurora/Ipl1-related kinases. Studies in several species agree that Aurora-A localizes to centrosomes and the spindle and that the expression and activity peak at the G_2/M transition (3–5). In *Drosophila,* dysfunction of Aurora-A causes centrosome disorganization and a reduction in the length of astral microtubules (6, 7). These phenotypes may relate to the ability of Aurora-A to interact with and phosphorylate *Drosophila*-transforming acidic coiled-coil, a centrosome-associated protein implicated in the regulation of microtubule dynamics (7). In *Caenorhabditis elegans* embryos, the elimination of Aurora-A by RNA-mediated interference leads to failures in spindle assembly and centrosome maturation (8, 9). In *Xenopus* egg extracts, inhibition of Eg2, a *Xenopus* Aurora-A kinase, provokes a failure in the mitotic spindle assembly (10). These investigations indicate that Aurora-A has similar but slightly different roles between these organisms. On the other hand, less is known about human Aurora-A. Aurora-A might regulate TPX2, a prominent component of the spindle apparatus in human cells (11). Overexpression of human Aurora-A was found to interfere with mitotic exit, causing tetraploidization and concomitant amplification of centrosomes (12). Interestingly, the human *aurora-A* gene is mapped to chromosome 20q13, a region frequently amplified in various human malignant tumors (13–27). Moreover, overexpression of human Aurora-A overrides the cell cycle checkpoint (5, 28) and induces transformation in rodent fibroblasts (29, 30). These findings suggest that dysregulation of Aurora-A activation may lead to genomic instability, which results in development and progression of malignant tumors. Thus, to better understand the pathological effect of Aurora-A activation, it is first necessary to elucidate the physiological functions of Aurora-A in human cells.

In this study, we interfered with the function of Aurora-A by antibody microinjection in addition to silencing Aurora-A by RNA interference (small interfering RNA; siRNA)[1] in HeLa cells to precisely examine the effect of Aurora-A inhibition on each mitotic event. Our data demonstrate that human Aurora-A is required for multiple crucial events during mitosis, such as mitotic entry, separation of centriole pairs, and accurate

* This work was supported by a grant for cancer research from the Ministry of Education, Science, and Culture of Japan (to H. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[S] The on-line version of this article (available at http://www.jbc.org) contains four additional figures and two movies.

¶ To whom correspondence should be addressed. Tel.: 81-96-373-5116; Fax: 81-96-373-5120; E-mail: hsaya@gpo.kumamoto-u.ac.jp.

[1] The abbreviations used are: siRNA, small interfering RNA; MG132, carbobenzoxyl-leucinyl-leucinyl-leucinal; MTOC, microtubule-organizing center; TPX2, targeting protein for *Xenopus* kinesin-like protein 2; GST, glutathione *S*-transferase; PIPES, 1,4-piperazinediethanesulfonic acid; GFP, green fluorescent protein; DIC, differential interference contrast.

bipolar spindle assembly. Aurora-A plays a crucial role in events that occur at middle and late mitosis, including alignment of chromosomes on the metaphase plate and completion of cytokinesis. Thus, Aurora-A is a critical component for orchestrating mitotic progression in human cells.

EXPERIMENTAL PROCEDURES

Synchronization and Transfections—HeLa cells were synchronized at the beginning of the S phase by a double thymidine block and release protocol (2). Proteasome inhibitor carbobenzoxyl-leucinyl-leucinyl-leucinal (MG132; Sigma) was used in culture medium at 10 μM to block the cell cycle at metaphase (31, 32). For transient transfection, COS7 cells in 6-well plates were transfected using a FuGene6 transfection reagent following the manufacturer's instructions (Roche Applied Science).

Plasmids and Constructs—The open reading frames of Aurora-A, centrin3, and Mad2 were amplified by PCR from the HeLa cDNA library, and PCR products were sequenced and subcloned into a pGEM-T Easy vector (Promega). The fragment of Aurora-A was then cloned into the pcDNA3 vector (Invitrogen). Full-length cDNA fragments of Aurora-B and Aurora-C, kindly provided by Dr. Masashi Kimura (Gifu University), were subcloned into the pcDNA3 vector (Invitrogen), respectively. To generate GST and histidine-tagged Aurora-A proteins, the sequence corresponding to residues 1–387 of Aurora-A was amplified by PCR using the pGEM-T Easy-Aurora-A plasmid as a template and subcloned into pGEX4T-1 (Amersham Biosciences) or pRSET (Invitrogen) vectors. To generate GST-centrin3 protein or GST-Mad2 protein, full-length centrin3 or full-length Mad2 was subcloned into the pGEX4T-1 vector.

Antibody Preparation and Microinjection—Anti-centrin3 antibody was generated in rats by injecting GST-centrin3 (Supplemental Fig. S2). Anti-Mad2 antibody was generated in rabbits by injecting GST-Mad2 (Fig. 1*G*). Anti-Aurora-A antibody was generated in rabbits by injecting them with the GST-N-terminal domain of Aurora-A (amino acids 1–129) (Fig. S1) (5). The bleeds were affinity-purified using an Affi-Gel 10 gel coupled with the histidine-tagged N-terminal-domain of Aurora-A or GST-Mad2 following the manufacturer's instructions (Bio-Rad). For antibody microinjection, rabbit IgG from preimmune serum was affinity-purified using ImmunoPure (A) (Pierce). Anti-Aurora-A antibodies and control IgG were concentrated in 0.5× Dulbecco's phosphate-buffered saline (Sigma) in Vivaspin M_r 5,000 cut-off microconcentrators (Vivascience). Equal concentrations of anti-Aurora-A antibodies or control IgG, with or without a rhodamine-labeled tubulin (Cytoskeleton), were injected into the cytoplasm of synchronized HeLa cells at the specific phase (G_2 phase, prometaphase, or metaphase) of the cell cycle using an Eppendorf semiautomatic microinjector (Eppendorf) attached to an Olympus IX 70 microscope (Olympus). We identified the cell cycle stages of the cells by careful observation using DIC and/or fluorescence images. G_2 phase was defined as the time 8 h after release from a double thymidine block, when chromosomes have not yet been condensed. Prometaphase cells were selected based on the findings that nuclear envelope breakdown were completed but chromosomes had not been aligned on the metaphase plate. Metaphase cells were selected by the finding that chromosomes were completely aligned on the equator of the cell.

Time Lapse Imaging and Analysis—HeLa cells were plated and synchronized in ΔT 0.15-mm dishes (Biopteches). Before microinjection and observation, the culture medium was replaced with dye-free L-15 medium, pH 7.2 (Sigma), supplemented with 10% fetal calf serum and overlaid with mineral oil. Dishes were maintained at 37 °C using the ΔT Culture Dish System (Biopteches) and imaged on an Olympus IX 70 microscope equipped with a sensitive SenSys-1401E CCD camera (Roper Scientific). After antibody microinjection, images were obtained using a ×10 or ×40 UPlan Apo objective (Olympus). The camera, shutters, and the filter wheel were controlled by MetaMorph imaging software (Universal Imaging), and the images were collected at every 3 min with exposure times of 50 ms. Through-focus *z*-series stacks consisting of three frames were acquired at each time point. For quantitation of mitotic intervals, the duration of early mitosis (prophase, prometaphase, and metaphase) was defined as the time elapsed from the first sign of chromosome condensation (12:27 ± 2:53 (min:s) before completion of nuclear envelope breakdown in control HeLa cells, $n = 10$) to partial separation of chromosomes into more than two masses. The duration of late mitosis (anaphase and telophase) was defined as the time from chromosome separation to spreading of divided daughter cells.

Immunoblotting—For immunoblotting, equal amounts of cell lysates were denatured at 95 °C for 5 min in SDS sample buffer, electrophoresed on 12% SDS-PAGE Laemmli gels (33), and transferred to a nitrocellulose membrane (Hybond; Amersham Biosciences). The membranes were probed with the primary antibody. The following antibodies were used: anti-α-tubulin antibody (B-5-1-2; Sigma), anti-FLAG antibody (M5; Sigma), anti-Aurora-A antibody (5), and rat polyclonal anti-centrin3 antibody (Fig. S2). The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies and visualized by a chemiluminescence detection system from PerkinElmer Life Sciences.

siRNA—We used the following target sequences for Aurora-A siRNA: ^{725}AUG CCC UGU CUU ACU GUC A^{743} and ^{155}AUU CUU CCC AGC GCG UUC C^{173}. We obtained the similar results using above two sequences of oligonucleotides. The results obtained using the former sequence are represented here. Oligonucleotides were transfected into HeLa cells using oligofectamine (Invitrogen) as described (34). As a control, we used the GL-2 duplex to target the luciferase gene (34).

Immunofluorescence—For immunostaining, HeLa cells were rinsed for 1 min with PHEM buffer (25 mM HEPES, 60 mM PIPES, pH 6.9, 10 mM EGTA, and 2 mM $MgCl_2$) at 37 °C and then fixed with −20 °C methanol for Aurora-A or centrin3 staining. For Mad2 staining, the cells were fixed with 4% paraformaldehyde/PHEM. After washing with PHEM buffer containing 0.05% Triton-X, cells were incubated with blocking solution (3% bovine serum albumin in PHEM). Sequential incubation of primary and secondary antibodies was done in PHEM containing 0.2% bovine serum albumin. The stained cells were mounted with 1,4-diazabicyclo-(2,2,2)-octane/glycerol and observed with confocal laser-scanning microscopy (Fluoview; Olympus). Images were obtained separately by independent excitation at 488/568/633 nm to minimize overlapping signals. Antibodies used were anti-Aurora-A antibody (Fig. S1) (5), anti-centrin3 antibody (Fig. S2), anti-Mad2 antibody (Fig. 1*G*), anti-α-tubulin antibody (B-5-1-2; Sigma), anti-γ-tubulin antibody (GTU-88; Sigma), fluorescein isothiocyanate-conjugated anti-rabbit IgG (BIOSOURCE), fluorescein isothiocyanate-conjugated anti-mouse IgG (BIOSOURCE), fluorescein isothiocyanate-conjugated anti-rat IgG (BIOSOURCE), and Cy3-conjugated anti-mouse IgG (Amersham Biosciences). DNA was stained with propidium iodide (Sigma) or TOTO-3 dye (Molecular Probes, Inc., Eugene, OR).

RESULTS

Reduction of Aurora-A Expression by siRNA Leads to Failure of Various Mitotic Events—As a first approach to understand the function of Aurora-A in human cells, we used siRNA duplexes in HeLa cells (34). A 48-h treatment with siRNA targeted to a region located 725 bp downstream of the start codon in Aurora-A mRNA resulted in a considerable, but incomplete, reduction of the protein expression (Fig. 1, *A*, *E*, and *H*).

We first examined the effect of Aurora-A reduction on cell cycle progression. Asynchronized HeLa cells were transfected with Aurora-A siRNA. At 48 h after the transfection, we fixed the attached cells, stained them with anti-α-tubulin antibodies (microtubules) and TOTO-3 dye (DNA), and scored the number of cells in different cell cycle stages. We found that 91.9 ± 0.1% (mean ± S.D.) of the cells transfected with Aurora-A siRNA and 96.2 ± 0.2% of the control cells were in interphase (Fig. 1*B*). The proportion of the Aurora-A-depleted cells in earlier stages of mitosis (prophase, prometaphase, and metaphase) was more than double that of the control cells (4.9 ± 0.4% in the Aurora-A siRNA transfected cells *versus* 2.3 ± 0.1% in the controls) (Fig. 1*B*). However, cells at later stages of mitosis (anaphase and telophase) were less detected when Aurora-A siRNA was transfected (0.9 ± 0.5%), whereas a normal number of late mitotic figures was observed in control cells (1.6 ± 0.2%). These results indicated that reduction of Aurora-A expression causes early mitotic delay in HeLa cells. Moreover, although only 0.1 ± 0.1% of control cells showed an apoptosis-like appearance, 2.2 ± 1.0% of Aurora-A siRNA-transfected cells did. These phenotypes were observed consistently in all experiments using Aurora-A siRNA in HeLa cells.

As we have recently reported, complete depletion of Aurora-A expression blocked mitotic entry in mammalian cells (35). However, a fraction of cells showing significantly reduced but

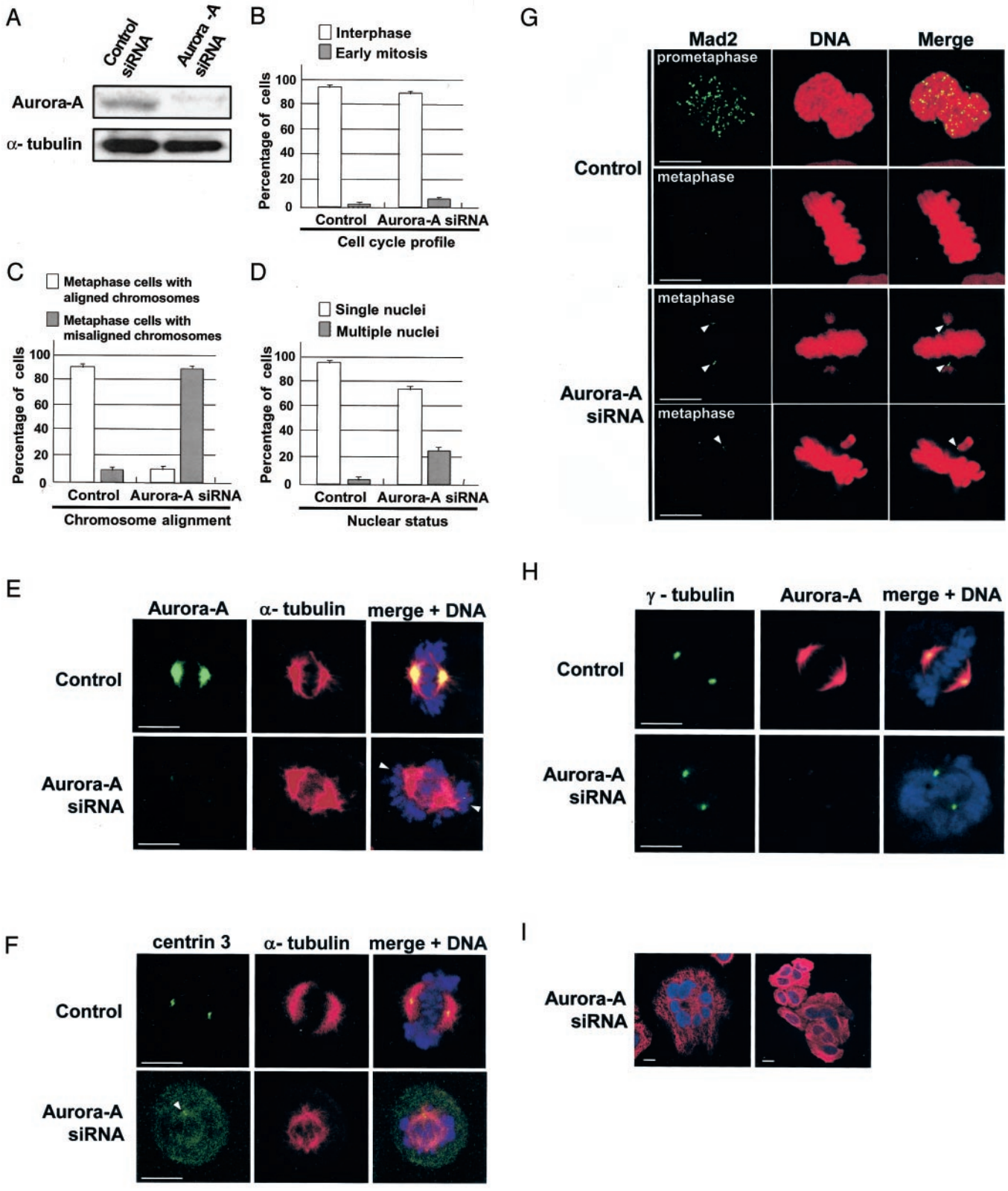


FIG. 1. **Suppression of Aurora-A expression by siRNA impairs various mitotic events in HeLa cells.** *A*, Western blot of HeLa cell lysates, prepared 48 h after transfection with either a siRNA duplex-targeting Aurora-A or a control duplex (GL2), probed with anti-Aurora-A or anti-α-tubulin (loading control) antibodies. *B*, graph showing the increased fraction of cells in early mitosis after treatment with Aurora-A siRNA for 48 h. Values shown are the means from three independent experiments in which over 300 cells were counted; the *error bars* (*B–D*) indicate S.D. *C*, graph showing a dramatic increase in the percentage of cells with misaligned metaphase chromosomes after treatment with Aurora-A siRNA (~9-fold greater than control). Values shown are the means from three independent experiments in which over 50 cells were counted. *D*, graph showing the increased percentage of multinucleate cells after treatment with Aurora-A siRNA for 48 h (~8-fold greater than control). Values shown are the means from three independent experiments in which over 300 cells were counted. *E–I*, cells were transfected with Aurora-A siRNA or control GL2 siRNA for 48 h and fixed with cold methanol (*E*, *F*, *H*, and *I*) or 4% paraformaldehyde (*G*) and processed for immunofluorescence with the indicated antibodies. DNA was visualized with TOTO-3 dye (*E*, *F*, *H*, and *I*) or propidium iodide (*G*). *Scale bars*, 10 μm. *E*, chromosomes are misaligned in cells transfected with Aurora-A siRNA. Aurora-A, α-tubulin, and DNA are stained in *green*, *red*, and *blue*, respectively. Misaligned

still detectable levels of Aurora-A were observed to enter mitosis after Aurora-A RNAi. These cells with attenuated Aurora-A expression revealed mitotic phenotypes described above.

We employed immunocytochemistry to examine further the phenotype of cells arrested in early mitosis by Aurora-A siRNA. In most of these Aurora-A-reduced mitotic cells (89.6 ± 2.0%, Fig. 1*C*), although chromosomes were condensed and bipolar spindles were formed, several chromosomes were not aligned on the metaphase plate and positioned in the vicinity of two spindle poles (Fig. 1*E*, *lower panel*). In contrast, fewer than 10% of control mitotic cells showed misaligned chromosomes (9.9 ± 1.6%) (Fig. 1, *C* and *E*, *upper panel*). The kinetochores of the misaligned chromosomes found in Aurora-A-reduced mitotic cells were positive for Mad2, which localizes selectively to the kinetochores that are not bound to the spindle microtubules (Fig. 1*G*) (36). It is thus possible that misalignment of chromosome in Aurora-A-reduced cells is caused by loose connections between microtubules and kinetochores during early mitosis.

The centrosome consists of a pair of centrioles and the surrounding pericentriolar materials, both of which play a central role in organizing mitotic spindles (37). In lower organisms, Aurora-A is thought to function in the centrosome separation and the recruitment of γ-tubulin to centrosomes (centrosome maturation) during mitosis (6, 9, 38). Thus, we examined mitotic centrosomes in Aurora-A-reduced cells by immunocytochemistry using an antibody against γ-tubulin, a component of pericentriolar material. We found that γ-tubulin staining was normal at each spindle pole in both control and Aurora-A-down-regulated cells during mitosis (Fig. 1*H*). We have recently shown that centrosomes remain immature in cells arrested at late G_2 phase by complete depletion of Aurora-A (35). Based on these observations, we speculate that low levels of activated Aurora-A, which allow the cells to enter mitosis, are sufficient for recruitment of γ-tubulin to centrosomes during mitosis in HeLa cells.

To analyze the centrosome separation in Aurora-A down-regulated cells, we have generated highly specific antibody against centrin3 (Fig. S2), a component of centriole (39). Two distinct dots of centrin3-positive staining that represent a pair of centrioles were clearly visible on each spindle pole in control cells (100%, 39 of 39; Fig. 1*F*, *upper panel*), but in Aurora-A-reduced cells, relatively low centrin3 staining was seen only on one spindle pole, and no staining was recognized on the other (Fig. 1*F*, *lower panel*). This abnormality was observed in 74% (28 of 38) of Aurora-A-reduced mitotic cells. These results suggest that Aurora-A is involved in the separation of centriole pairs during early mitosis.

We also found that a significant population (24.4 ± 5.0%) of the multinucleated cells was generated when Aurora-A was reduced, whereas control siRNA induced only 3.3 ± 1.0% of the multinucleated cells (Fig. 1, *D* and *I*). These data suggest that Aurora-A is directly or indirectly required for cytokinesis.

The findings described above suggest the possibility that Aurora-A is involved in various critical events related to the centrosome, such as separation of centriole pairs, chromosome alignment on the metaphase plate, and cytokinesis. However, given that the centrosome plays lead roles in the progression of various mitotic events, there is a possibility that dysfunction of the centrosome might be responsible for several mitotic failures found in Aurora-A-depleted cells. Thus, the possibility cannot be ruled out that reduction of Aurora-A levels by siRNA interference affects interphase events and that mitotic failures are caused as a secondary consequence.

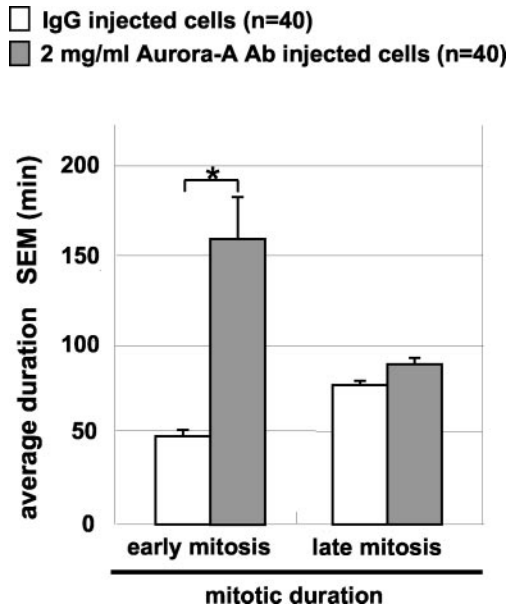


FIG. 2. **Injection of anti-Aurora-A antibodies into late G_2 cells delays mitotic progression.** HeLa cells were synchronized at the beginning of the S phase by a double thymidine block. At 8 h after release from the block, when the cells entered the late G_2 phase, equal concentrations (2 mg/ml) of anti-Aurora-A antibodies or control IgG were injected into the cells ($n = 40$ in each experiment). Cells were followed by DIC time lapse microscopy to determine durations of early mitosis (prophase, prometaphase, and metaphase) and late mitosis (anaphase and telophase) as described under "Experimental Procedures." For statistical analysis, all data were evaluated by two-tailed Student's *t* test. *, $p < 0.01$.

Anti-Aurora-A Antibody Injection Interferes with the Progression of Early Mitosis—We were very keen to find whether Aurora-A is directly involved in the mitotic events described above. For this purpose, at specific phases of cell cycle, we microinjected affinity-purified anti-Aurora-A antibodies into HeLa cells at a concentration of 2 mg/ml ($\sim 3.0 \times 10^{-3}$ pmol of the anti-Aurora-A antibodies per cell). This antibody was highly specific for Aurora-A kinase and did not recognize Aurora-B and Aurora-C (Fig. S1). We microinjected anti-Aurora-A antibodies into the cytoplasm at the late G_2 phase when the replication of centrosomes was completed (~8 h after release from a double thymidine block) and observed the cells by time lapse DIC microscopy. In each experiment, control cells microinjected with protein A-purified IgG from preimmune serum were simultaneously visualized in the same field as the experimental cells. As we have previously shown, injection of anti-Aurora-A antibodies into late G_2 cells results in significant delay of entry into mitosis, suggesting that Aurora-A is involved in the mechanisms of entry into mitosis in HeLa cells (5, 35).

We next quantitatively analyzed time lapse images to determine the duration of each mitotic event in the anti-Aurora-A antibody-injected cells. The duration of early mitosis

chromosomes are indicated by *white arrowheads*. *F*, impairment of centriole separation by reduction of Aurora-A expression. Weak staining of centrin3 was seen only on one spindle pole (*white arrowhead*) but not on the other in HeLa cells treated with Aurora-A siRNA. Centrin3, α-tubulin, and DNA are stained in *green*, *red*, and *blue*, respectively. *G*, Mad2 was positive on the misaligned chromosomes in cells transfected with Aurora-A siRNA. Mad2 and DNA are stained in *green* and *red*, respectively. The *white arrowheads* indicate Mad2 signals at misaligned chromosomes. *H*, γ-tubulin staining was unchanged in cells transfected with Aurora-A siRNA. γ-Tubulin is shown in *green*, and Aurora-A and DNA are shown in *red* and *blue*, respectively. *I*, multinucleate cells were found at 48 h after transfection with Aurora-A siRNA. Microtubules and DNA are stained in *red* and *blue*, respectively.

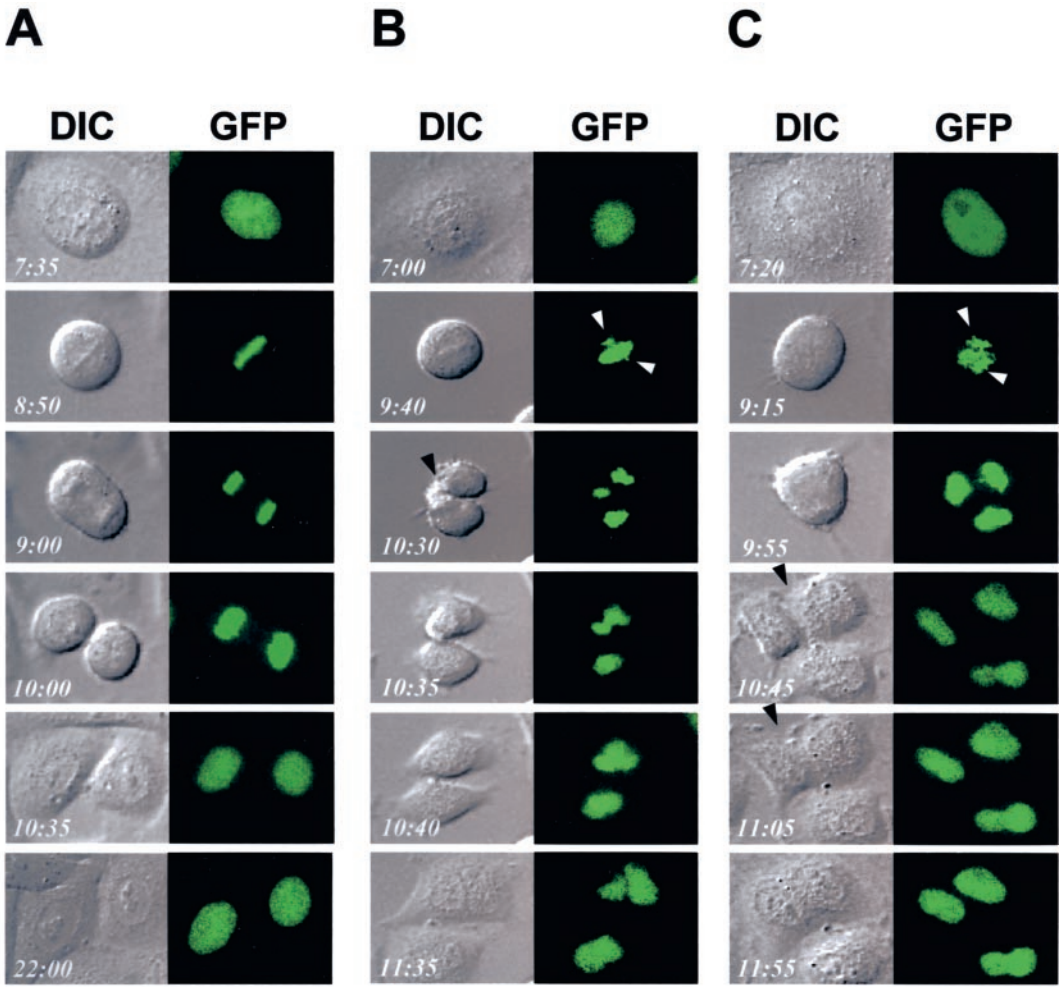


FIG. 3. **Abnormal mitosis was found in cells injected with anti-Aurora-A antibodies.** HeLa cells stably expressing histone H2B-GFP fusion protein were injected with anti-Aurora-A antibodies (2 mg/ml) or control IgG (2 mg/ml) at the late G_2 phase (~8 h after release from a double thymidine block) and followed by DIC and fluorescence time lapse microscopy. *A*, cells injected with control IgG underwent normal mitosis. *B* and *C*, cells injected with anti-Aurora-A antibodies underwent abnormal cell division. Misaligned chromosomes that appeared in the anti-Aurora-A antibody-injected cells are indicated by *white arrowheads*. Cells injected with anti-Aurora-A antibodies attempted to divide into three daughter cells. However, as for the cells indicated by *black arrowheads*, cytokinesis was reversed to yield multinucleate cells.

(prophase, prometaphase, and metaphase) in the anti-Aurora-A antibody-injected cells was significantly prolonged (160 ± 25 min in anti-Aurora-A antibody-injected cells (n = 40) *versus* 49 ± 3.8 min in control cells (n = 40) (Fig. 2). The duration of late mitosis (anaphase and telophase) was slightly prolonged in the anti-Aurora-A antibody-injected cells (89 ± 3.8 min in anti-Aurora-A antibody-injected cells (n = 40) *versus* 78 ± 2.5 min in control cells (n = 40) (Fig. 2). We speculated that inhibition of Aurora-A induces abnormalities in mitotic machinery during early mitosis and, as a consequence, activates a mitotic checkpoint that delays mitotic progression.

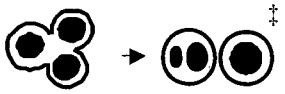
Anti-Aurora-A Antibody Injection Leads to Failure of Accurate Mitotic Spindle Assembly, Chromosome Segregation, and Cytokinesis—We next attempted to investigate more precisely about the mitotic failures in cells injected with anti-Aurora-A antibodies. For this purpose, we microinjected anti-Aurora-A antibodies (2 mg/ml) into HeLa cells stably expressing a histone H2B-GFP fusion protein at the late G_2 phase and analyzed the nuclear dynamics of cells by time lapse DIC/fluorescence microscopy and immunocytochemistry. Of the cells injected with control IgG, 95% (38 of 40) showed normal cell division (Fig. 3*A* and Table I), and in these cells the bipolar spindles formed normally, and chromosomes were aligned in normal fashion on the metaphase plate (Fig. 4, *A* and *B*). Interestingly, in 65% (26 of 40) of the cells injected with anti-Aurora-A antibodies (2 mg/ml), chromosomes were unequally segregated, and then the cells attempted to divide into three daughter cells in late anaphase, and two of the three fused in telophase, resulting in the formation of multinucleate cells (Fig. 3, *B* and *C*, Table I, and Supplemental Movie 1). We found that, although chromosome condensation and nuclear envelope breakdown occurred normally, multipolar spindles with misaligned chromosomes were formed in these cells (Fig. 4, *C–F*). These results indicated that Aurora-A is directly or indirectly involved in accurate mitotic spindle assembly, chromosome segregation, and cytokinesis.

Anti-Aurora-A Antibody Injection Causes Improper Separation of Centriole Pairs—We next used anti-centrin3 antibodies to examine whether each spindle pole contained a normal centriole pair. In all control cells (100%; 15 of 15), one pair of centrin3-positive dots was seen in each spindle pole (Fig. 5, *A–C*). However, in the majority of cells injected with the anti-Aurora-A antibodies (93%; 14 of 15), the centrin3-positive dots were unevenly distributed in each spindle pole; a pair of centrin3-positive dots was found in two of three or four spindle poles, and only weak and blurred staining of centrin3 was observed in the remaining pole (Fig. 5, *D–I*). These results raised the notion that Aurora-A is required for proper separation of centriole pairs and that inhibition of Aurora-A using antibody microinjection at a concentration of 2 mg/ml leads to

TABLE I

Various mitotic and postmitotic phenotypes induced by microinjection of anti-Aurora-A antibody

Synchronized HeLa cells at the late G_2 phase were microinjected with anti-Aurora-A antibody (2 or 12 mg/ml) or control IgG (2 mg/ml). Observation using time lapse DIC microscopy allowed the calculation of the percentage of injected cells with the phenotypes listed. *, typical phenotype of cells that divided into two daughter cells. ‡, typical phenotype of cells that attempted to divide into three in late anaphase and two of the three fused in telophase. §, typical phenotype of cells that attempted to divide into two daughter cells and subsequently fused. **, cells that underwent apoptosis or arrested before mitosis.

Phenotype	Contol IgG injected cells (n = 40)	2 mg/ml Anti-Aurora-A Antibody Injected Cells (n = 40)	12 mg/ml Anti-Aurora-A Antibody Injected Cells (n = 40)
	38 (95%)	14 (35%)	16 (40%)
	1 (2.5%)	26 (65%)	3 (7.5%)
	0 (0%)	0 (0%)	11 (28%)
Others **	1 (1.3%)	0 (0%)	10 (25%)

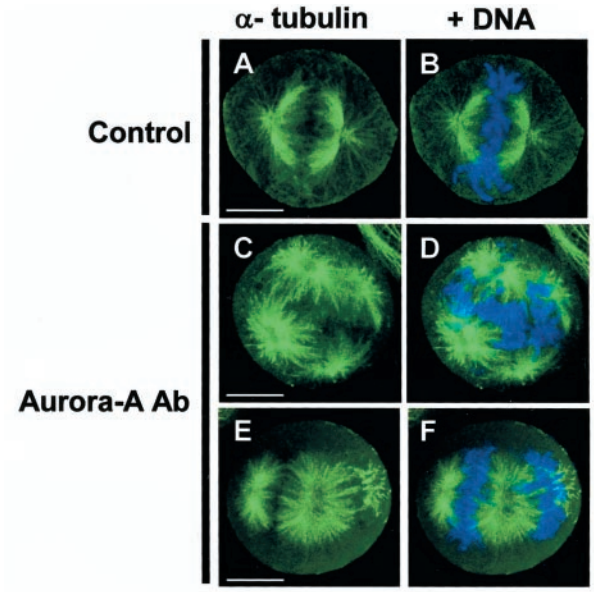


FIG. 4. **Inhibition of Aurora-A by antibody microinjection results in formation of multipolar spindles and improper chromosome alignment on the metaphase plate.** HeLa cells were synchronized at the beginning of S phase by a double thymidine block. At 8 h after release from the block, when cells entered the late G_2 phase, the cells were injected with 2 mg/ml of control IgG (*A* and *B*) or 2 mg/ml of anti-Aurora-A antibodies (*C–F*). The cells were then incubated for 2 h and fixed with −20 °C methanol and stained with anti-α-tubulin antibodies (*green*). DNA was stained with TOTO-3 (*blue*). *Scale bars*, 10 μm.

incomplete inhibition of Aurora-A function, resulting in incomplete separation of centriole pairs, followed by the formation of multipolar spindles. We next performed the antibody microinjection experiments using anti-Aurora-A antibodies at a higher concentration (12 mg/ml; $\sim 1.8 \times 10^{-2}$ pmol of the anti-Aurora-A antibodies per cell). Most of these cells were able to form bipolar spindles (85%; 28 of 33), and two spindle poles were stained with anti-γ-tubulin antibodies, suggesting that they acted as functional microtubule-organizing centers (MTOCs) (data not shown). However, it was frequently found (80%; 12 of 15) that two pairs of centrin3 dots were found in only one spindle pole, and faint and blurred staining of centrin3 was seen in the other (Fig. 5, *J–O*), implying that separation of centriole pairs was completely inhibited and that a small fraction of centrin3 was recruited to the acentriolar spindle pole. This is consistent with the previous result obtained by siRNA experiments (Fig. 1*F*). These results strongly suggest that Aurora-A is required for proper separation of centriole pairs that is essential for intact mitotic spindle assembly in HeLa cells.

Aurora-A Is Required for Proper Movement of Chromosomes to the Metaphase Plate—The cells injected with anti-Aurora-A antibodies at the higher concentration (12 mg/ml) formed bipolar spindles regardless of improper separation of centriole pair (Fig. 5, *J–O*). However, chromosome alignment on the metaphase plate was severely impaired (Fig. 5, *L* and *O*, *white arrowheads*). These results raised two possibilities. First, improper separation of centriole pairs could affect metaphase chromosome alignment. Second, Aurora-A is directly involved in chromosome alignment on the metaphase plate. To discriminate these possibilities, we synchronized HeLa cells at the beginning of the S phase by a double thymidine block protocol, and at 9 h after release from the block, we microinjected anti-Aurora-A antibodies (12 mg/ml) into prometaphase cells. Prometaphase cells were selected based on the following criteria; nuclear envelope breakdown was completed, centriole pairs were separated, and chromosomes had not been aligned on metaphase plate. Then the cells were incubated for 2 h in the presence of 10 μM MG132, an inhibitor of the 26 S proteasome, and observed for chromosome alignment at metaphase. MG132 is known to inhibit degradation of securin and other target proteins for anaphase-promoting complex, leading to cell cycle arrest at metaphase with alignment of chromosomes on the metaphase plate (40, 41). As previously reported (31, 32), we found that the treatment of HeLa cells with 10 μM MG132 induced metaphase arrest without impairment of chromosome movement or centrosome behavior in prometaphase (data not shown). In most control IgG-injected cells (92%; 23 of 25), all

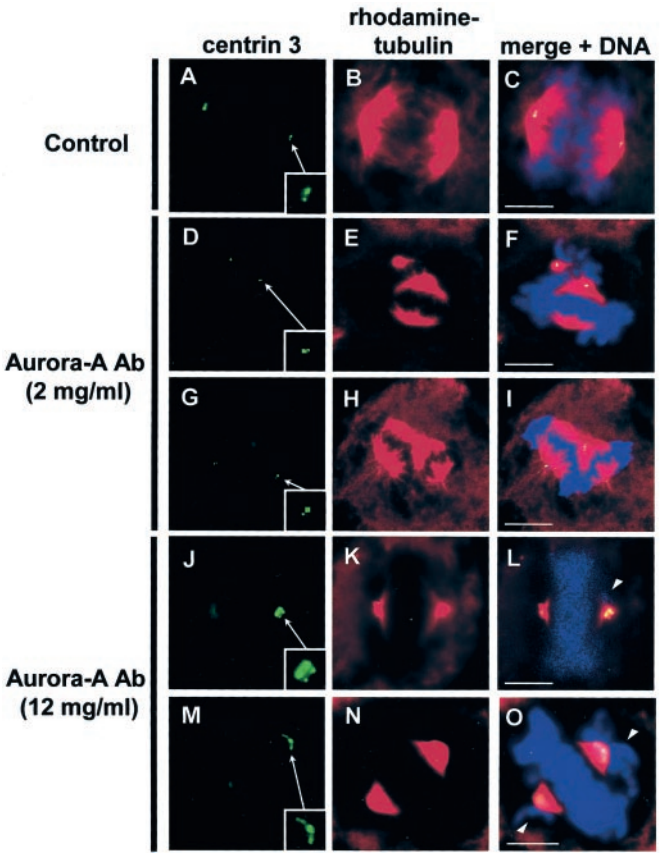


FIG. 5. **Separation of centriole pairs and chromosome alignment on the metaphase plate are impaired in anti-Aurora-A antibody-injected cells.** HeLa cells were synchronized at the beginning of the S phase by a double thymidine block. At 8 h after release from the block, the late G_2 cells were injected with control IgG (12 mg/ml; *A–C*) or anti-Aurora-A antibodies at concentrations of 2 mg/ml (*D–I*) or 12 mg/ml (*J–O*) supplemented with rhodamine-labeled tubulin (*red*; *B*, *E*, *H*, *K*, and *N*). Cells were then stained with anti-centrin3 antibodies (*green*; *A*, *D*, *G*, *J*, and *M*) and the DNA-specific dye TOTO-3 (*blue*; *C*, *F*, *I*, *L*, and *O*). Misaligned chromosomes are indicated by *white arrowheads*. In cells injected with the anti-Aurora-A antibodies at the low concentration (2 mg/ml), two of three spindle poles have paired centrin3-positive dots, and faint and blurred staining of centrin3 was observed in the remaining pole (*D–I*). In cells injected with anti-Aurora-A antibodies at the higher concentration (12 mg/ml), two paired centrin3-positive dots were found in one of the two spindle poles, and very faint and blurred staining of centrin3 was seen in the other (*J–O*). *Insets* show enlarged images of paired centrin3-positive dots. *Scale bars*, 10 μm.

chromosomes were properly aligned on the metaphase plate in the presence of MG132 (Fig. 6*A*). In contrast, 23 of 27 (85%) cells injected with anti-Aurora-A antibodies contained misaligned chromosomes (Fig. 6, *B* and *C*, *white arrowheads*). Thus, we concluded that the inhibition of human Aurora-A function interferes with the ability of chromosomes to align properly. In other words, these results indicate that Aurora-A is directly involved in proper alignment of chromosomes on the metaphase plate in HeLa cells.

Inhibiting Aurora-A Causes the Failure in Cytokinesis—Cells injected with anti-Aurora-A antibodies also frequently failed in cytokinesis (Fig. 3, *B* and *C*, Table I). Piel *et al.* (59) proposed that the completion of cell division is tightly connected with the behavior of the centriole. Therefore, this defect of cytokinesis could also be due to improper centriole behavior caused by the inhibition of Aurora-A function in the early mitotic phase. Another possibility is that Aurora-A has some direct function in cytokinesis. To examine these possibilities, we microinjected control IgG or anti-Aurora-A antibodies into metaphase cells

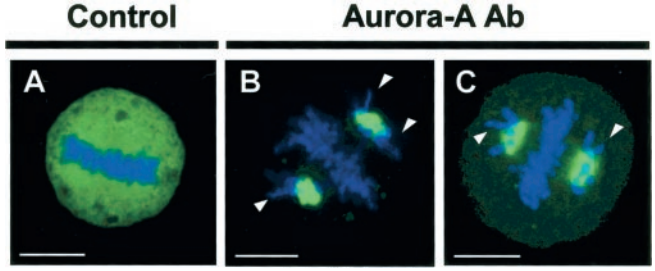


FIG. 6. **Microinjection of anti-Aurora-A antibodies into prometaphase cells causes improper alignment of chromosomes on the metaphase plate.** HeLa cells were synchronized at the beginning of the S phase by a double thymidine block. At 9 h after release from the block, cells at prometaphase, in which separation of the centriole pairs was completed, were injected with 12 mg/ml anti-Aurora-A antibodies (*B* and *C*) or control IgG (*A*). After further incubation for 2 h in the presence of MG132 (10 μM) to block the cell cycle at metaphase, the cells were fixed with −20 °C methanol and stained with fluorescein isothiocyanate-conjugated anti-rabbit antibodies (*green*) and TOTO-3 iodide (*blue*). In cells injected with anti-Aurora-A antibodies, misaligned chromosomes were frequently found near the spindle poles (*white arrowheads*). Note that the injected anti-Aurora-A antibodies (*B* and *C*) were concentrated on the spindle microtubules, where endogenous Aurora-A is localized (see Fig. 8, *D–F*). In contrast, control IgG (*A*) was diffusely distributed throughout the cytoplasm. *Scale bars*, 10 μm.

that had completed centrosome separation, bipolar spindle assembly, and chromosome alignment on the metaphase plate. All of the cells (100%; 20 of 20) injected with control IgG (12 mg/ml) underwent normal cytokinesis to produce two daughter cells (Fig. 7*A*). On the contrary, in 78% (14 of 18) of cells injected with anti-Aurora-A antibodies (12 mg/ml) at metaphase, chromosomes were segregated, but eventually the daughter cells fused to form binucleate cells (Fig. 7*B* and Movie 2). These results suggest the possibility that Aurora-A directly plays a role in completion of cytokinesis.

Aurora-A Is Localized on Centrosomes and the Spindle Midzone during Late Mitosis—The subcellular localization of Aurora-A has been examined previously, and it is well known that Aurora-A is localized on the centrosomes and mitotic spindles during early mitosis (prophase, prometaphase, and metaphase) (29, 30). However, less is known about the localization of Aurora-A during late mitosis (anaphase and telophase). To investigate it, we fixed asynchronized HeLa cells and stained them with anti-Aurora-A antibodies, anti-α-tubulin antibodies (microtubules), and TOTO-3 dye (DNA). We found that Aurora-A remained on the centrosomes and the spindles at the onset of anaphase (data not shown), and it started to localize to the spindle midzone in late anaphase (Fig. 8, *G–I*). In telophase, Aurora-A was found on the centrosomes and the spindle midzone (Fig. 8, *J–L*). These results support our finding described above that Aurora-A plays a role in late mitotic events, such as completion of cytokinesis.

DISCUSSION

We have demonstrated in the previous reports that Aurora-A is required for HeLa cells to properly enter mitosis (5, 35). In this study, with the use of antibody microinjection and siRNA, we have shown that Aurora-A is necessary for various mitotic events including separation of centriole pairs, chromosome alignment on the metaphase plate, and cytokinesis in addition to mitotic entry. These results lead to the idea that Aurora-A has multiple targets that are required for proper mitotic progression and maintenance of ploidy in human cells.

Role of Aurora-A in the Separation of Centriole Pairs during Mitosis—Our data show that down-regulation of Aurora-A by siRNA inhibits the separation of centriole pairs in HeLa cells (Fig. 1*F*). Impairment of centriole separation by Aurora-A siRNA was also observed in another cell line, HCT116 colorec-

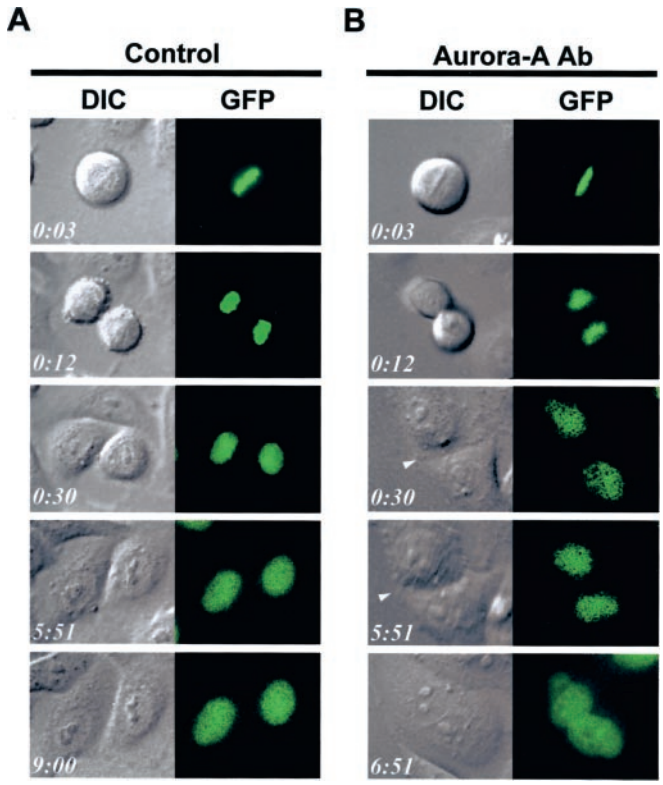


FIG. 7. **Microinjection of anti-Aurora-A antibodies into metaphase cells results in reversion of cytokinesis.** HeLa cells stably expressing histone H2B-GFP fusion protein were injected with control IgG (12 mg/ml) or anti-Aurora-A antibodies (12 mg/ml) at metaphase and observed by time lapse DIC and fluorescence microscopy. Representative images of control IgG-injected cell (*A*) and anti-Aurora-A antibody-injected cells (*B*) are shown. All of the cells (100%; 20 of 20) injected with control IgG underwent normal cytokinesis to produce two daughter cells. Contrarily, 78% (14 of 18) of cells injected with anti-Aurora-A antibodies failed to divide into two daughter cells, although chromosomes were segregated. Cytokinesis was reversed, as indicated by the *white arrowheads*.

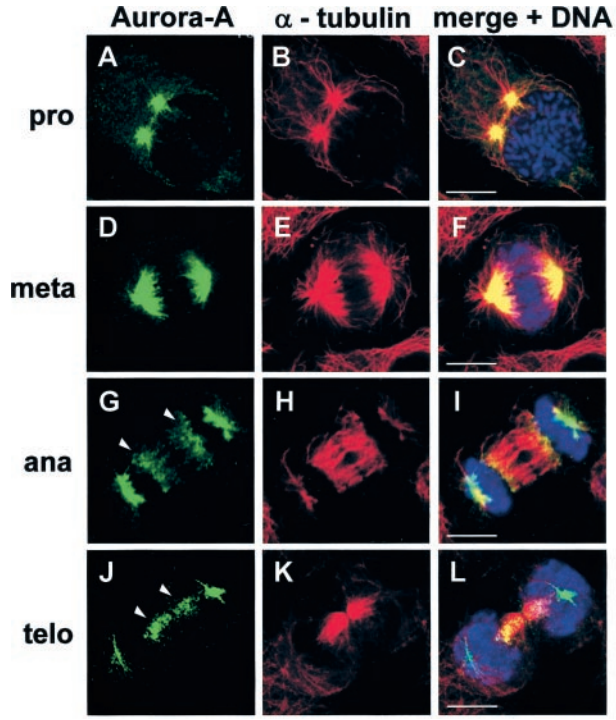


FIG. 8. **Subcellular localization of endogenous Aurora-A in HeLa cells during mitosis.** HeLa cells at various stages of mitosis (*A–C*, prophase; *D–F*, metaphase; *G–I*, anaphase; *J–L*, telophase) were stained with anti-Aurora-A antibodies (*green*) and anti-α-tubulin antibodies (*red*). DNA was stained with TOTO-3 dye (*blue*). The *merged images* are shown in *C*, *F*, *I*, and *L*. Note that Aurora-A is localized on both the spindle poles and mitotic spindles during early mitosis and on both the poles and the spindle midzone (*white arrowheads*) during late mitosis. *Scale bars*, 10 μm.

tal cancer cell (Fig. S4), which has a normal p53 and a relatively stable near diploid karyotype (42) and thus in principle should more closely resemble normal human cells. In mammalian cells, the separation of centriole pairs occurs in two steps (early and late) and is regulated by various phosphorylation reactions (1). At an early step, Nek2, a NIMA family member kinase, is thought to phosphorylate a centrosomal protein, C-Nap1, causing the dissolution of a dynamic structure that tethers duplicated centrosomes to each other (43, 44). At a later step, the movement of centriole pairs to both poles is promoted by several kinesin-related motor proteins and cytoplasmic dynein (1). We demonstrate that Aurora-A reduction by siRNA or microinjection of anti-Aurora-A antibodies (12 mg/ml) at the late G_2 phase in HeLa cells impaired the separation of centriole pairs (Figs. 1*F* and 5, *J–O*). However, when the antibodies were injected into the prometaphase cells in which the early step of centrosome separation had been completed, each centriole pair was normally localized to each pole of the bipolar mitotic spindle, although some chromosomes were not aligned on the metaphase plate (Fig. 6 and data not shown). Therefore, we speculate that Aurora-A is involved in the early step rather than the later step in the centrosome separation pathway. The possibility that Aurora-A and Nek2 cooperatively regulate C-Nap1 is currently under investigation.

HeLa cells injected with the anti-Aurora-A antibodies (12 mg/ml) were able to form bipolar spindles, although centriole pairs were not separated (Fig. 5, *J–O*). Therefore, in these cells, one of the poles functioned as MTOC in the absence of centrosome. It is known that the cells of higher plants, as well as the oocytes of some species, can assemble bipolar spindles without centrosomes (45, 46). Furthermore, recent elegant experiments in which centrosomes were specifically destroyed by laser microsurgery have confirmed that normal animal cells are able to utilize the centrosome-independent pathway to form bipolar spindles (47). Our findings, together with those previous observations, indicate that animal cells can assemble bipolar spindles through a centrosome-independent pathway.

The cells injected with anti-Aurora-A antibodies at a low concentration (2 mg/ml) showed incomplete centriole separation with formation of multipolar spindles (Fig. 5, *D–I*). In these cells, more than three MTOCs were often identified in a single cell (Fig. 4 and 5, *D–I*). Multipolar spindles are frequently formed in consequence of centrosome overduplication (48, 49). However, our immunocytochemical studies using anti-centrin3 antibody revealed that one of the three MTOCs did not contain centrioles (Fig. 5, *D–I*). These results suggest that multipolar spindles found in the anti-Aurora-A antibody-injected cells are formed in three steps: 1) incomplete separation of two pairs of centrioles; 2) formation of two MTOCs having a pair of centrioles and a third MTOC containing no centrioles; and 3) nucleation of microtubules from those three MTOCs. Our data are consistent with the previously described notion that centrosomes influence spindle assembly mostly by orienting the spindle in the cell but are not essential organizers of spindle bipolarity (50, 51).

It should be noted that monopolar spindle was more frequently formed in HCT116 cells than HeLa cells when Aurora-A was down-regulated (Fig. S4). This phenotype is very similar to that observed in the Aurora-deficient *Drosophila* (6). The phenotypic difference between HeLa and HCT116 cells

might be due to the difference in checkpoint activity that monitors separation of centriole pairs during early mitosis.

Inhibition of Aurora-A Delays Mitotic Progression—Reduction of Aurora-A by siRNA induced early mitotic delay in both HeLa (Fig. 1*B*) and HCT116 cells (Fig. S3*A*). Furthermore, the same results were yielded when an alternative Aurora-A siRNA was used (data not shown). We also performed antibody microinjection experiments and found that the duration of early mitosis was significantly prolonged in anti-Aurora-A antibody-injected cells (Fig. 2). One potential mechanism of this delay is that the spindle assembly checkpoint is activated by improper formation of mitotic spindle and/or unattached kinetochores of misaligned chromosomes in Aurora-A inhibited cells. However, these cells eventually accomplished the late mitotic events, such as chromosome segregation and nuclear envelope reconstitution. This might be due to the insufficiency of spindle assembly checkpoint function in HeLa cells or the requirement of Aurora-A for maintenance of the checkpoint.

Involvement of Aurora-A in Chromosome Alignment on the Metaphase Plate—Cells injected with anti-Aurora-A antibodies at prometaphase after the initial centrosome separation frequently formed misaligned chromosomes at metaphase (Fig. 6, *B* and *C*). Mad2 was positive on the kinetochores of misaligned chromosomes in Aurora-A-down-regulated metaphase cells (Fig. 1*G*). These results suggest that Aurora-A is required for kinetochore-microtubule interaction and is directly involved in chromosome alignment on the metaphase plate. Ipl1p, a yeast homologue of aurora kinase, is thought to play a key role in the regulation of chromosome segregation and the attachment of sister kinetochore to microtubules (52–55). In human cells, Aurora-B kinase is also found to be involved in chromosome alignment on the metaphase plate (32). Based on these data and our present findings, it might be reasonable to speculate that Aurora-A and Aurora-B cooperate to regulate kinetochore function to interact with microtubules, which is essential for faithful chromosome segregation in human cells.

Another potential mechanism for misalignment of chromosomes is that inhibition of Aurora-A may affect the assembly of functional mitotic spindles. It has recently been proposed that in *Xenopus* oocyte extract-based systems, Aurora-A kinase (Eg2) activated by RanGTP phosphorylates its substrates, including Eg5, TPX2, and protein phosphatase type 1, leading to spindle assembly (11, 56–58). Although there was no statistical difference in the size or microtubule density of mitotic spindles between Aurora-A-down-regulated cells and control cells in our experimental setting (data not shown), we can not completely exclude the possibility that microtubule dynamics and stability to form functional mitotic spindles are impaired by the down-regulation of Aurora-A, resulting in misalignment of chromosomes on the metaphase plate.

Requirement of Aurora-A for Completion of Cytokinesis—Inhibition of Aurora-A by siRNA and antibody microinjection led to failure to complete cytokinesis, resulting in the formation of multinucleate cells (Fig. 1, *D* and *I*, and 3). It has been described recently that the completion of cell division is tightly connected with the behavior of the mother centriole (59). Piel *et al.* (59) showed that the mother centriole visits the midbody at the late mitotic phase and induces the release of central microtubules from the midbody, leading to completion of cell division. In the Aurora-A-inhibited cells, because the centrioles were not evenly distributed to each MTOC as shown in Figs. 1*F* and 5, *J–O*, the behavior of mother centrioles might have been heavily impaired, resulting in a defect in cytokinesis. However, even when Aurora-A antibodies were injected into metaphase cells that had already completed centrosome separation and formed bipolar spindles normally, cytokinesis was reversed, and daughter cells eventually fused (Fig. 7*B*). This observation raises a notion that Aurora-A is directly involved in the signaling pathway for exit from mitosis. This possibility is supported by the fact that Aurora-A becomes localized on the spindle midzone toward the end of mitosis (Fig. 8).

In summary, we have demonstrated that Aurora-A is involved in various critical events throughout mitosis. We speculate that Aurora-A would function by phosphorylating substrates and/or binding important partners at each stage in mitosis. It will be important to determine how interfering with Aurora-A function leads to the failure of those multiple events in mitosis. In recent years, it has been thought that overexpression of Aurora-A would lead to centrosome amplification (30), which causes chromosome instability. However, our data suggest that not only overfunction, but also dysfunction, of Aurora-A might cause abnormal mitotic progression, leading to aneuploidy, which is a hallmark of malignant tumors.

Acknowledgments—We thank all of the members of the Tumor Genetics and Biology Department at Kumamoto University for helpful discussions; Drs. M. Kimura and Y. Okano for providing cDNA of Aurora-B and Aurora-C; Drs. G. M. Wahl and T. Kanda for providing the GFP-tagged histone H2B expression plasmid; Dr. N. Furuno for technical assistance; and Y. Fukushima for secretarial assistance. We are also grateful to members of the Gene Technology Center at Kumamoto University for important contributions to the experiments.

REFERENCES

1. Nigg, E. A. (2001) *Nat. Rev. Mol. Cell Biol.* **2,** 21–32
2. Hirota, T., Morisaki, T., Nishiyama, Y., Marumoto, T., Tada, K., Hara, T., Masuko, N., Inagaki, M., Hatakeyama, K., and Saya, H. (2000) *J. Cell Biol.* **149,** 1073–1086
3. Bischoff, J. R., and Plowman, G. D. (1999) *Trends Cell Biol.* **9,** 454–459
4. Giet, R., and Prigent, C. (1999) *J. Cell Sci.* **112,** 3591–3601
5. Marumoto, T., Hirota, T., Morisaki, T., Kunitoku, N., Zhang, D., Ichikawa, Y., Sasayama, T., Kuninaka, S., Mimori, T., Tamaki, N., Kimura, M., Okano, Y., and Saya, H. (2002) *Genes Cells* **7,** 1173–1182
6. Glover, D. M., Leibowitz, M. H., McLean, D. A., and Parry, H. (1995) *Cell* **81,** 95–105
7. Giet, R., McLean, D., Descamps, S., Lee, M. J., Raff, J. W., Prigent, C., and Glover, D. M. (2002) *J. Cell Biol.* **156,** 437–451
8. Schumacher, J. M., Ashcroft, N., Donovan, P. J., and Golden, A. (1998) *Development* **125,** 4391–4402
9. Hannak, E., Kirkham, M., Hyman, A. A., and Oegema, K. (2001) *J. Cell Biol.* **17,** 1109–1116
10. Roghi, C., Giet, R., Uzbekov, R., Morin, N., Chartrain, I., Le Guellec, R., Couturier, A., Doree, M., Philippe, M., and Prigent, C. (1998) *J. Cell Sci.* **111,** 557–572
11. Kufer, T. A., Sillje, H. H., Korner, R., Gruss, O. J., Meraldi, P., and Nigg, E. A. (2002) *J. Cell Biol.* **158,** 617–623
12. Meraldi, P., Honda, R., and Nigg, E. A. (2002) *EMBO J.* **21,** 483–492
13. Bigner, S. H., Mark, J., Friedman, H. S., Biegel, J. A., and Bigner, D. D. (1988) *Cancer Genet. Cytogenet.* **30,** 91–101
14. Bockmuhl, U., Petersen, I., Schwendel, A., and Dietel, M. (1996) *Laryngorhinootologie* **75,** 408–414
15. Courjal, F., Cuny, M., Rodriguez, C., Louason, G., Speiser, P., Katsaros, D., Tanner, M. M., Zeillinger, R., and Theillet, C. (1996) *Br. J. Cancer* **74,** 1984–1989
16. Iwabuchi, H., Sakamoto, M., Sakunaga, H., Ma, Y. Y., Carcangiu, M. L., Pinkel, D., Yang-Feng, T. L., and Gray, J. W. (1995) *Cancer Res.* **55,** 6172–6180
17. James, L. A., Mitchell, E. L., Menasce, L., and Varley, J. M. (1997) *Oncogene* **14,** 1059–1065
18. Kallioniemi, A., Kallioniemi, O. P., Piper, J., Tanner, M., Stokke, T., Chen, L., Smith, H. S., Pinkel, D., Gray, J. W., and Waldman, F. M. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 2156–2160
19. Larramendy, M. L., Tarkkanen, M., Blomqvist, C., Virolainen, M., Wiklund, T., Asko-Seljavaara, S., Elomaa, I., and Knuutila, S. (1997) *Am. J. Pathol.* **151,** 1153–1161
20. Muleris, M., Salmon, R. J., Dutrillaux, A. M., Vielh, P., Zafrani, B., Girodet, J., and Dutrillaux, B. (1987) *Cancer Genet. Cytogenet.* **29,** 289–301
21. Reznikoff, C. A., Belair, C. D., Yeager, T. R., Savelieva, E., Blelloch, R. H., Puthenveettil, J. A., and Cuthill, S. (1996) *Semin Oncol.* **23,** 571–584
22. Schlegel, J., Stumm, G., Scherthan, H., Bocker, T., Zirngibl, H., Ruschoff, J., and Hofstadter, F. (1995) *Cancer Res.* **55,** 6002–6005
23. Solinas-Toldo, S., Wallrapp, C., Muller-Pillasch, F., Bentz, M., Gress, T., and Lichter, P. (1996) *Cancer Res.* **56,** 3803–3807
24. Tanner, M. M., Tirkkonen, M., Kallioniemi, A., Collins, C., Stokke, T., Karhu, R., Kowbel, D., Shadravan, F., Hintz, M., and Kuo, W. L. (1994) *Cancer Res.* **54,** 4257–4260
25. Tanner, M. M., Tirkkonen, M., Kallioniemi, A., Isola, J., Kuukasjarvi, T., Collins, C., Kowbel, D., Guan, X. Y., Trent, J., Gray, J. W., Meltzer, P., and Kallioniemi, O. P. (1996) *Cancer Res.* **56,** 3441–3445
26. Tirkkonen, M., Johannsson, O., Agnarsson, B. A., Olsson, H., Ingvarsson, S., Karhu, R., Tanner, M., Isola, J., Barkardottir, R. B., Borg, A., and Kallion-

iemi, O. P. (1997) *Cancer Res.* **57,** 1222–1227
27. Yaseen, N. Y., Watmore, A. E., Potter, A. M., Potter, C. W., Jacob, G., and Rees, R. C. (1990) *Cancer Genet. Cytogenet.* **44,** 83–97
28. Anand, S., Penrhyn-Lowe, S., and Venkitaraman, A. R. (2003) *Cancer Cell* **3,** 51–62
29. Bischoff, J. R., Anderson, L., Zhu, Y., Mossie, K., Ng, L., Souza, B., Schryver, B., Flanagan, P., Clairvoyant, F., Ginther, C., Chan, C. S., Novotny, M., Slamon, D. J., and Plowman, G. D. (1998) *EMBO J.* **17,** 3052–3065
30. Zhou, H., Kuang, J., Zhong, L., Kuo, W. L., Gray, J. W., Sahin, A., Brinkley, B. R., and Sen, S. (1998) *Nat. Genet.* **20,** 189–193
31. Topper, L. M., Bastians, H., Ruderman, J. V., and Gorbsky, G. J. (2001) *J. Cell Biol.* **154,** 707–717
32. Kallio, M. J., McCleland, M. L., Stukenberg, P. T., and Gorbsky, G. J. (2002) *Curr. Biol.* **12,** 900–905
33. Laemmli, U. K. (1970) *Nature* **227,** 680–685
34. Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., and Tuschl, T. (2001) *Nature* **411,** 494–498
35. Hirota, T., Kunitoku, N., Sasayama, T., Marumoto, T., Zhang, D., Nitta, M., Hatakeyama, K., and Saya, H. (2003) *Cell* **114,** 585–598
36. Chen, R. H., Waters, J. C., Salmon, E. D., and Murray, A. W. (1996) *Science* **274,** 242–246
37. Zimmerman, W., Sparks, C. A., and Doxsey, S. J. (1999) *Curr. Opin. Cell Biol.* **11,** 122–128
38. Berdnik, D., and Knoblich, J. A. (2002) *Curr. Biol.* **12,** 640–647
39. Paoletti, A., Moudjou, M., Paintrand, M., Salisbury, J. L., and Bornens, M. (1996) *J. Cell Sci.* **109,** 3089–3102
40. Sherwood, S. W., Kung, A. L., Roitelman, J., Simoni, R. D., and Schimke, R. T. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 3353–3357
41. Wojcik, C., Schroeter, D., Stoehr, M., Wilk, S., and Paweletz, N. (1996) *Eur. J. Cell Biol.* **70,** 172–178
42. Lengauer, C., Kinzler, K. W., and Vogelstein, B. (1997) *Nature* **386,** 623–627
43. Fry, A. M., Mayor, T., Meraldi, P., Stierhof, Y. D., Tanaka, K., and Nigg, E. A. (1998) *J. Cell Biol.* **141,** 1563–1574
44. Mayor, T., Stierhof, Y. D., Tanaka, K., Fry, A. M., and Nigg, E. A. (2000) *J. Cell Biol.* **151,** 837–846
45. Karsenti, E., and Vernos, I. (2001) *Science* **294,** 543–547
46. Wittmann, T., Hyman, A., and Desai, A. (2001) *Nat. Cell Biol.* **3,** E28–E34
47. Khodjakov, A., Cole, R. W., Oakley, B. R., and Rieder, C. L. (2000) *Curr. Biol.* **10,** 59–67
48. Brinkley, B. R. (2001) *Trends Cell Biol.* **11,** 18–21
49. Doxsey, S. J. (2001) *Nat. Cell Biol.* **3,** E105–108
50. Karsenti, E., Newport, J., Hubble, R., and Kirschner, M. (1984) *J. Cell Biol.* **98,** 1730–1745
51. Steffen, W., Fuge, H., Dietz, R., Bastmeyer, M., and Muller, G. (1986) *J. Cell Biol.* **102,** 1679–1687
52. Chan, C. S., and Botstein, D. (1993) *Genetics* **135,** 677–691
53. Francisco, L., Wang, W., and Chan, C. S. (1994) *Mol. Cell. Biol.* **14,** 4731–4740
54. Biggins, S., Severin, F. F., Bhalla, N., Sassoon, I., Hyman, A. A., and Murray, A. W. (1999) *Genes Dev.* **13,** 532–544
55. Tanaka, T. U., Rachidi, N., Janke, C., Pereira, G., Galova, M., Schiebel, E., Stark, M. J., and Nasmyth, K. (2002) *Cell* **108,** 317–329
56. Tsai, M. Y., Wiese, C., Cao, K., Martin, O., Donovan, P., Ruderman, J., Prigent, C., and Zheng, Y. (2003) *Nat. Cell Biol.* **5,** 242–248
57. Giet, R., Uzbekov, R., Cubizolles, F., Le Guellec, K., and Prigent, C. (1999) *J. Biol. Chem.* **274,** 15005–15013
58. Katayama, H., Zhou, H., Li, Q., Tatsuka, M., and Sen, S. (2001) *J. Biol. Chem.* **276,** 46219–46224
59. Piel, M., Meyer, P., Khodjakov, A., Rieder, C. L., and Bornens, M. (2000) *J. Cell Biol.* **149,** 317–330

Aurora-A Kinase Maintains the Fidelity of Early and Late Mitotic Events in HeLa Cells

Tomotoshi Marumoto, Shinobu Honda, Toshihiro Hara, Masayuki Nitta, Toru Hirota, Eiji Kohmura and Hideyuki Saya

J. Biol. Chem. 2003, 278:51786-51795.
doi: 10.1074/jbc.M306275200 originally published online October 1, 2003