We have identified a novel gene, *Clast3*, by subtraction of cDNAs derived from activated and naive B lymphocytes. *Clast3* expression is elevated in cycling cells and down-regulated in cells undergoing growth arrest, indicating that its expression is controlled in a cell cycle-dependent manner. The deduced amino acid sequence of *Clast3* cDNA exhibits no significant homology to the known proteins in mammalian and other species. Immunofluorescence staining revealed that *Clast3* localizes into discrete nuclear foci. Forced expression of *Clast3* results in growth retardation, polyploidy, and generation of multinucleated cells. Treatment of *Clast3* transfectants with nocodazole, a spindle-damaging agent, greatly enhances the incidence of the multinucleated cells, suggesting that *Clast3* overexpression impairs the same checkpoint activated by nocodazole. Down-regulation of *Clast3* expression by antisense oligonucleotides results in a decrease of cells at G2-M phase and a concomitant increase of apoptotic cells. These findings indicate that *Clast3* is a novel cell cycle-regulated protein and that its constitutive overexpression induces polyploidy and multinucleation by interfering with the mitotic spindle checkpoint.

Genetic stability is achieved by the coordinated regulation of DNA replication and repair, chromosomal segregation, and cell cycle checkpoints (1, 2). There are several checkpoints that act to ensure the orderly progression of critical events in the cell cycle. The mitotic spindle checkpoint functions to delay the metaphase to anaphase transition until all pairs of sister chromatids are attached to spindle microtubes, thereby ensuring the correct segregation of duplicated chromosomes into the two daughter cells (3, 4). A number of proteins have been identified that sense the kinetochore microtubule attachment and regulate the separation of sister chromatids. These include the kinetochore-associated CENPE, MAD, and BUB proteins, the anaphase-promoting complex/cyclosome and its associated cofactors CDC20, the separin-securin complex, and others (3, 4). Biochemical and genetic evidence suggests that kinetochores unattached to spindle microtubes generate a signal that leads to the activation of MAD2. The activated MAD2 inhibits the ubiquitylating activity of anaphase-promoting complex/cyclosome and thus the degradation of the securin Pds1, thereby preventing the progression into anaphase. Accumulating evidence indicates that dysregulated expression as well as functional inactivation of these checkpoint proteins can cause abnormal mitosis, leading to chromosomal mis-segregation, apoptosis, polyploidy, or multinucleated cells.

In addition to the proteins that directly monitor the microtube attachment and regulate chromosomal segregation, other proteins are also involved in the spindle checkpoint response. The tumor-suppressor P53 has been shown to prevent polyploidy by keeping cells with mitotic spindle damage from reinitiating DNA synthesis (5–8). Overexpression of the anti-apoptotic molecule Bcl-xL can cooperate with the loss of P53 to prevent postmitotic exit of cells (10–12). Overexpression of the Chk tyrosine kinase inhibitor p21<sup>WAF/CIP1</sup> appears to be involved in the spindle checkpoint by preventing endoreduplication after aberrant mitotic exit of cells (10–12). Overexpression of the Chk tyrosine kinase can induce polyploidy and multinucleation (13), whereas overexpression of putative serine/threonine kinase Sak-a or nuclear matrix protein HET/SAF-B or Rad6 can lead to multinucleation (14–16).

Viral gene products can also cause abnormal mitosis. Human T-cell leukemia virus type I Tax protein was shown to induce multinucleated cells by directly binding to human MAD1 and thus abrogating the mitotic spindle checkpoint (17). Simian virus 40 large T antigen (18), Epstein-Barr virus LMP-1 (19), and human immunodeficiency virus Vpr (20) induce polyploidy, multinucleation, or micronuclei formation when overexpressed in mammalian cells. Although the precise mechanisms of polyploidy and multinucleation are still not fully understood, it appears that these cellular and viral gene products induce abnormal mitosis by abrogating the mitotic spindle checkpoint.

During the course of searching for genes that are selectively expressed in activated B cells, we have identified a novel growth response gene, *Clast3*. We show that *Clast3* expression is controlled in a cell cycle-dependent manner and that its overexpression interferes with mitotic spindle checkpoint, leading to polyploidy and multinucleation.

**MATERIALS AND METHODS**

**Reagents—**Goat F(ab′)<sub>2</sub> antibodies to mouse IgM were purchased from Southern Biotechnology Associates (Birmingham, AL) and exten-
sively dialyzed in phosphate-buffered saline (PBS) before use. Culture supernatants of a myeloma cell line producing soluble CD40 ligand (CD40L) were used at a 3-fold dilution as described previously (21). Recombinant interleukin 4 was purchased from R&D Systems (Minneapolis, MN). The anti-FLAG antibodies (M5 and M2) and lipopolysaccharides were obtained from Sigma. The horseradish peroxidase-labeled antibodies to mouse IgG1 were obtained from Southern Biotechnology Associates.

Purification of Splenic B Cells and Cell Culture—Splenic B cells were isolated from 8–12-week-old C57BL/6 mice after T-cell depletion with anti-Thy-1 antibodies and complement treatment, followed by Ficoll-Hypaque density centrifugation as described previously (21). The splenic B cells and WEHI231 B lymphoma cells were cultured at 37°C with 5% CO2 in a humidified atmosphere in RPMI 1640 medium containing 5 × 10^{-5} M 2-mercaptoethanol and 50 units of penicillin-streptomycin supplemented with 10% fetal calf serum (FCS). NIH3T3 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% FCS and 50 units of penicillin-streptomycin.

Isolation of Clast3 Gene—Total RNA was extracted using TRIzol reagent (Invitrogen) from fresh splenic B cells and B cells activated for 2 days with soluble CD40L. Polyadenylated mRNA was then purified with an oligo(dT) cellulose column (Amersham Biosciences). cDNA synthesis and subtraction procedures were performed using the PCR-Select cDNA subtraction kit (Clontech) according to the manufacturer’s protocol. Candidate gene fragments were resolved in a 1% agarose gel, transferred simultaneously to two nylon membranes, and subjected to a reverse Northern blot with [32P]-labeled total cDNAs from either unstimulated or CD40L-activated B cells as probes. Gene fragments that selectively hybridized with cDNA probes of activated B cells were used to isolate the full-length cDNA. The 5’ end of Clast3 mRNA was determined with a RNA ligase-mediated rapid amplification of cDNA ends (SMART) protocol (Clontech, Austin, TX) following the manufacturer’s protocol. The outer and inner primers used to amplify the cDNAs derived from capped mRNA were 5’-CGATGCTGTCTGAGAGAA-A and 5’-CTCTCTTCTTGACCTG-3’, respectively.

Northern and Western Blot Analyses—For Northern blot analysis, 20 μg of total RNA was resolved in a formaldehyde-agarose gel, transferred onto a Hybond N+ nylon membrane (Amer sham Pharmacia Biotech), and hybridized with a [32P]-labeled Clast3 probe. For Western blot analysis, cells were lysed in a buffer containing 50 mM Tris-HCl (pH 6.8), 2% SDS, 1 M 2-mercaptoethanol, 10% glycerol, and a mixture of protease inhibitors (Sigma). The lysate proteins were sonicated for 2 min, heated at 100°C for 5 min, and resolved in a 10% SDS-polyacrylamide gel. The resolved proteins were transferred to a cellulose nitrate membrane with a semidy blotter, blocked with 5% nonfat milk, and reacted with the anti-FLAG antibody (M5) and then with horseradish peroxidase-labeled antibodies. The membrane was developed with the ECL system (Amer sham Pharmacia Biotech).

Construction of Expression Vectors and Establishment of Clast3 Stable Transfectants—A full-length mouse Clast3 cDNA was first subcloned into pFLAG-CMV-2 (Sigma). After confirming the Clast3 protein expression in COS7 cells, the FLAG-tagged Clast3 cDNA was excised from pFLAG-CMV-2 and subcloned into the pcXN2 expression vector that harbors a neomycin-resistant gene. NIH3T3 cells were transfected with 5 μg of pcXN2-Clast3 construct using Lipofectin reagent (Invitrogen) and selected 2 days later with 400 μg/ml Geneticin. Two weeks after the selection, the resulting colonies were picked up with cloning cylinders.

Induction of Cell Cycle Progression—NIH3T3 cells were washed once with serum-free DMEM and cultured for 48 h in the same medium containing 10% FCS to induce cell cycle arrest. The cells were then washed with Dulbecco’s modified Eagle’s medium containing 10% FCS to remove floating dead cells and cultured in the same medium to allow cell cycle progression. Cells were collected at the indicated time for RNA extraction and DNA content analysis.

Cell Cycle Synchronization and DNA Content Analysis—Exponentially growing NIH3T3 cells were treated for 24 h with 50 ng/ml nocodazole or 5 μM Taxol to induce G2-M arrest. For cell cycle analysis, cells were incubated in PBS containing 0.1% RNase A and 0.1% Triton at room temperature for 10 min and then incubated in PBS containing 100 μg/ml propidium iodide on ice for 30 min. DNA content was analyzed with a FACScan flow cytometer (BD Biosciences).

Immunofluorescence—NIH3T3 transfectants grown on coverslips were rinsed with PBS and fixed in PBS containing 1% paraformalde-
WEHI231 cells are known to undergo G\textsubscript{1} arrest by αIgM, and this G\textsubscript{1} arrest can be rescued by simultaneous stimulation with CD40L (22). Treatment of WEHI231 cells with αIgM for 18 h resulted in cell cycle arrest at G\textsubscript{1} phase (Fig. 1B, αIgM) and a dramatic decrease of the Clast3 transcript levels (Fig. 1C, αIgM, 18h). Co-stimulation with CD40L prevented the cell cycle arrest (Fig. 1B, CD40L+αIgM) and the Clast3 down-regulation (Fig. 1C, CD40L+αIgM, 18h) in WEHI231 cells. These results demonstrate that Clast3 mRNA levels are up-regulated in proliferating cells and dramatically down-regulated in cells undergoing growth arrest.

**Clast3 Expression Is Cell Cycle-regulated**—The induction of Clast3 expression by various mitogenic stimuli and its down-regulation in growth-arrested cells suggested that Clast3 expression may be controlled in a cell cycle-dependent manner. We therefore examined Clast3 transcript levels in different phases of the cell cycle. We synchronized NIH3T3 cells at G\textsubscript{1} phase by serum deprivation and then induced cell cycle progression by serum addition. Cell cycle analysis revealed that the majority of the cells were at G\textsubscript{1} phase before serum addition (Fig. 2A, 0h). Cells were at early and late S phases 12 and 16 h after serum addition, and by 20 h after serum addition, many cells entered into G\textsubscript{2}-M phase (Fig. 2A). Similar to G\textsubscript{1}-arrested WEHI231 cells, Clast3 expression was low in G\textsubscript{1}-arrested NIH3T3 cells (Fig. 2B, 0h), increased during S phase, and remained at G\textsubscript{2}-M phase (Fig. 2B). These results suggest that Clast3 expression is cell cycle-regulated.

**Clast3 Is a Novel Nuclear Protein**—The longest Clast3 cDNA obtained was 1110 bp (Fig. 3A), which was close to its mRNA size. To determine the 5' end of Clast3 mRNA, we generated cDNA from tobacco acid pyrophosphatase-treated, i.e. capped, DNA content (A) and Clast3 mRNA expression (B) in unsynchronized NIH3T3 cells after serum deprivation (0 h) or 12, 16, and 20 h after serum addition.
mRNA. We obtained a specific PCR product of ~400 bp only from tobacco acid pyrophosphatase-treated mRNA (Fig. 3B). Sequence analysis of this PCR product confirmed that the longest cDNA represents the full-length mRNA. The Clast3 cDNA predicts a single open reading frame, encoding a protein of 264 amino acids with a molecular mass of 30 kDa (Fig. 3A). Extensive homology searches against databases of known genes revealed a partial human Clast3 cDNA sequence lacking the 5’ portion (GenBank™ accession number AF068295). However, no other homologous genes were found in mammalian and other species. A search for the protein motifs identified two myristoylation sites, two protein kinase C phosphorylation sites, and one N-glycosylation site. A sequence with very weak homology to a motif found in the XPG protein and yeast RAD nucleic acid-joining protein family was also detected (data not shown).

Whereas we could not find any apparent nuclear localization signal in the predicted Clast3 amino acid sequence, immunofluorescence staining of NIH3T3 fibroblasts transfected with a Clast3 expression vector indicated that Clast3 localized to the nuclei, forming discrete dot-like foci (Fig. 3C).

**Forced Expression of Clast3 Causes Growth Retardation**—

The elevated Clast3 expression during S phase and metaphase implicated a potential role for Clast3 in cell division. To elucidate the function of Clast3, we stably transfected NIH3T3 cells with a Clast3 expression construct. We isolated eight Clast3 stable clones as well as three vector controls. Northern blot analysis of representative transfectants revealed high levels of the exogenous 1.2-kb Clast3 mRNA in all three Clast3 clones, whereas only endogenous 1.1-kb Clast3 mRNA was detected in the two vector controls (Fig. 4A). Western blot analysis confirmed the protein expression of the introduced Clast3 gene (Fig. 4B). Because Clast3 transcript levels increased upon cell growth, we anticipated an increased growth rate in Clast3 transfectants. Unexpectedly, cells overexpressing Clast3 exhibited a reduced growth rate compared with vector controls (Fig. 4C).

**Forced Expression of Clast3 Induces Polyploidy**—We then examined the cell cycle distributions of these transfectants. In the Clast3 transfectants, we observed a small population with 8n DNA content, as well as an increase of the cells with a 4n DNA (Fig. 5A, untreated). Because cells with a 4n DNA content at G1 phase would be indistinguishable from cells with a 2n DNA content undergoing mitosis, we then treated cells with the microtubule-depolymerizing agent nocodazole to induce cell cycle arrest at M phase. Cell cycle analyses of treated cells revealed an increased percentage of cells with an 8n DNA content in all three Clast3 stable clones as compared with the two vector controls (Fig. 5A, Nocodazole). A similar result was obtained when these cells were treated with the microtubule-stabilizing agent Taxol, which also arrests cells at M phase (Fig. 5A, Taxol). We further analyzed the DNA content of five additional Clast3 clones and one vector control, which confirmed the significant increases in the polyploid cells in the Clast3 transfectants before and after the nocodazole or Taxol treatment (Table I).

Clast3 stable clone 1 and vector control 1 were then treated with nocodazole or Taxol, and the DNA content was analyzed over the course of 48 h. A small population with an 8n DNA content could be recognized in Clast3 transfectants at 16 h and became more evident at 24 h (Fig. 5B). At 48 h, there was a significantly higher percentage of 8n cells in Clast3 stable than in Vector#1 in both nocodazole- and Taxol-treated cells. Moreover, an apparent accumulation of 16n cells was observed in Clast3 transfectants, but not in the vector control. A similar increase of polyploid cells was observed in Clast3 stable clones 2 and 18. These results demonstrate that cells overexpressing Clast3 rapidly accumulated polyploid cells.

We also examined the effect of Clast3 overexpression on polyploidy induction in 293T cells by co-transfecting an enhanced green fluorescent protein vector (pEGFP-C1) with either a Clast3 or control construct. Using enhanced green fluorescent protein as an indicator for transfected cells, we found a higher incidence of 8n cells in Clast3-transfected cells (15.5%) than in vector-transfected cells (3.8%), indicating that Clast3 overexpression can also induce polyploidy in 293T cells.

**Polyploidy Induction by Clast3 Is Accompanied by Multinucleated Cells**—

Immunofluorescence staining of Clast3 stable clones revealed elevated frequency of binucleated and multinucleated cells (Fig. 6A). The average percentages (± S.D.) of multinucleated cells in Clast3 clones 1 and 18 were 6.1 ± 0.5% and 4.1 ± 0.35%, respectively, which were significantly higher than those found in the two vector controls (0.79 ± 0.27% and 0.74 ± 0.66%) or in parental NIH3T3 cells (0.38 ± 0.33%) (Fig. 6B). Clast3 overexpression thus led to multinucleation as well as polyploidy.

**Spindle-damaging Agent Dramatically Enhances Clast3-mediated Multinucleation**—

To explore the potential mechanism of multinuclei formation induced by Clast3, we treated Clast3 clones and vector controls with the microtubule-depolymerizing agent nocodazole. We reasoned that if Clast3 overexpression interferes with the spindle damage checkpoint, then nocodazole treatment would be expected to greatly enhance Clast3-mediated multinucleation. On the other hand, if Clast3 is not related to the spindle checkpoint, then nocodazole treatment should not affect the multinuclei formation induced by Clast3 overexpression.

Treatment of Clast3 transfectants with nocodazole resulted in a dramatically high incidence of multinucleated cells (72.5 ± 3.5% and 61.3 ± 2.9%) (Fig. 6C). In contrast, treatment of the
control transfectants or parental NIH3T3 cells with nocodazole only increased the multinucleated cells to \(20-24\%\), perhaps reflecting an effect inherent to nocodazole. These results strongly suggest that Clast3 interferes with the same checkpoint activated by nocodazole.

**Down-regulation of Clast3 Expression Results in a Decrease of Cells at G2-M Phase and an Increase of Sub-G1 Cells**

The elevated Clast3 expression in cycling cells implicated a potential role for Clast3 in cell division, therefore we examined whether Clast3 is normally essential for cell cycle progression. For this purpose, we sought to transiently reduce the expression of Clast3 by the use of antisense oligonucleotides. Transfection of NIH3T3 cells with Clast3 antisense oligonucleotides resulted in slightly decreased levels of endogenous Clast3 transcripts as revealed by Northern blot analysis, which was in agreement with the approximately 20% transfection efficiency estimated by pEGFP-C1 vector (data not shown). DNA content analysis of cells transfected with Clast3 antisense oligonucleotides revealed a significant decrease of G2-M cells and a concomitant increase of the sub-G1 population in two independent experiments (Fig. 7, *Antisense*). These effects were not observed when Clast3 sense oligonucleotides were similarly transfected (Fig. 7, *Sense*). The sub-G1 population typically represents cells undergoing apoptosis. Clast3 thus appears to be essential for

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**Fig. 5. Clast3 overexpression induces polyploidy.** A, vector and Clast3 transfectants were either untreated or treated with 50 ng/ml nocodazole or 5 \(\mu\)M Taxol for 24 h. DNA content was determined as described under “Materials and Methods.” The experiments were done at least twice, and similar results were obtained. B, polyploidy induction as a function of time. Vector 1 and Clast3 clone 1 were treated as described in A, and their DNA histograms were determined over the course of 48 h.
TABLE I
Polyploid cells (DNA content >4N) in vector and Clast3 transfectants

| Treatment   | Vector (n = 3) | Clast3 (n = 8) |
|-------------|---------------|---------------|
| Untreated   | 0.7 ± 0.2     | 5.0 ± 1.6a    |
| Nocodazole  | 7.7 ± 1.0     | 17.3 ± 3.3b   |
| Taxol       | 5.1 ± 0.1     | 18.1 ± 3.1b   |

*p < 0.05 compared with vector controls.
*p < 0.01 compared with vector controls.

Abnormal Mitosis Induced by Clast3

Three vector and eight Clast3 transfectants were treated with 50 ng/ml nocodazole or 5 μM Taxol for 24 h. DNA content was analyzed as described under “Materials and Methods.” Data represent means ± S.D.

Several lines of evidence presented here indicate that Clast3 expression is associated with cell proliferation in normal B lymphocytes, B lymphoma cells, and fibroblasts, suggesting a potential role for Clast3 in cell cycle progression. Consistent with this possibility, treatment of NIH3T3 cells with Clast3 antisense oligonucleotides resulted in a decrease of cells at the G2-M phase and an increase of apoptotic sub-G1 cells. It remains to be elucidated how decreased Clast3 expression might affect normal mitosis.

Whereas Clast3 appears to play a role in cell division, its overexpression did not enhance cell growth but rather caused growth retardation. The vector we used to express Clast3 was pCXN2, which harbors the chicken β-actin promoter, the cytomegalovirus enhancer, and the SV40 origin. Our recent study revealed that pCXN2 drives high levels of gene expression in all phases of the cell cycle, even in cells deprived of serum for 48 h (23). The negative effects on cell growth by Clast3 overexpression suggest that Clast3 transcript levels must be precisely regulated for its normal function and that its constitutive overexpression can lead to normal cell division. The growth retardation observed in Clast3 transfectants is likely caused by the abnormal mitosis that generates polyploidy and multinucleated cells, rather than a direct growth-inhibitory effect of Clast3.

Although we could not detect any potential nuclear localization signal in the predicted Clast3 protein, immunofluorescence staining indicates that Clast3 is localized to the nuclear foci. In addition, preliminary analysis suggests that Clast3 is associated with condensed chromosomes during mitosis. Clast3 may be transported to and localized in the nuclei through interactions with other proteins. It is possible that Clast3 may bind to chromosomes including the centromere region and thus interfere with the functions of kinetochores that are known to play critical roles in spindle microtube binding and mitotic checkpoint response. The dramatic increase of the multinucleated cells in Clast3 transfectants treated with the spindle-damaging agent is consistent with a role for Clast3 in spindle checkpoint response. A detailed biochemical analysis is in progress to determine the precise intracellular localization of Clast3, including its potential co-localization with the kinetochore-associated proteins.

A wide variety of gene products have been shown to interfere with the mitotic spindle checkpoint. Thus, overexpression of the serine/theronine kinase Sak-a, the nuclear matrix protein HET-SAF-B, the Csk homologous kinase Chk, SV40 large T antigen, or human immunodeficiency virus Vpr can lead to multinucleated cells. Whereas multinucleation induced by these gene products is frequently accompanied by growth retardation and polyploidy, T antigen-induced multinucleation is not associated with growth inhibition, and Sak-a overexpression does not seem to induce polyploidy. These observations suggest that growth retardation, multinucleation, and polyploidy can be caused by different mechanisms. The induction of all these phenotypes by Clast3 overexpression suggests that Clast3 may interfere with multiple events during cell division.

Among the various cellular and viral gene products that are known to induce abnormal mitosis, Clast3 is most similar to Chk and Vpr in that they all induce multinucleation and polyploidy accompanied by growth retardation. Vpr, an accessory molecule of human immunodeficiency virus, is localized in the perinuclear region and appears to induce multinucleation by impairing the cytoskeletal proteins (20). On the other hand, Chk is localized in both nuclei and cytoplasm and has been

DISCUSSION

In the present study, we have identified a novel protein, Clast3, whose expression is elevated in cycling cells and down-regulated in growth-arrested cells. Clast3 overexpression induces growth retardation, polyploidy, and multinucleation, whereas its down-regulation by antisense oligonucleotides appears to cause a decrease in G2-M cells and an increase in apoptotic cells. These results collectively implicate a critical role for Clast3 in cell division.

Several lines of evidence presented here indicate that Clast3 transcript levels are controlled in a cell cycle-dependent manner. Clast3 expression is barely detectable in nonproliferating primary splenic B lymphocytes but is rapidly up-regulated by a variety of stimuli that induce B-cell proliferation. Conversely, Clast3 transcripts in WEHI231 B lymphoma cells diminish as they undergo G1 arrest. In addition, Clast3 expression is low in growth-arrested NIH3T3 cells and elevated upon cell cycle entry. Therefore, Clast3 expression is associated with cell proliferation in normal B lymphocytes, B lymphoma cells, and fibroblasts, suggesting a potential role for Clast3 in cell cycle progression.
suggested to induce multinucleation through phosphorylation and thus inhibition of the tyrosine kinase activity of Lyn, which appears to be associated with chromosomes (13). In contrast to Clast3, whose expression is up-regulated upon cell growth, Chk expression is not correlated with cell proliferation (24). Moreover, Chk-deficient cells grow normally (25), whereas Clast3 seems to be required for normal cell cycle progression. These observations suggest that although similar phenotypes are induced by the overexpression of these three divergent proteins, the underlying mechanisms are likely to be different. Clast3 may possess a unique function that is distinct from that of other proteins involved in mitosis and the mitotic spindle checkpoint.

Except for a truncated human Clast3 sequence in the data bases, we were unable to find homologous genes in other eukaryotes, including yeast. This is in contrast to many checkpoint proteins, which are conserved among species as distant as human and yeast. Clast3 may have evolved relatively recently and have a more specialized role in mammalian cell division.

Accumulating evidence suggests that defects in spindle checkpoint can cause chromosomal instability, which is a common feature of human cancers (1–3). Chromosomal instability is implicated in tumorogenesis through the loss of tumor suppressor gene function or the gain of oncogene function. Recently, MAD2 haploinsufficiency has been shown to abrogate the spindle checkpoint and cause chromosomal instability in human colon carcinoma cells (26). Consistently, Mad2+/− mice were found to develop papillary lung adenocarcinomas at high rates. In addition, mutations in the checkpoint gene BUB1 were identified in a fraction of colorectal cancers and have been shown to impair the spindle checkpoint response (27). Moreover, the gene encoding securin Pds1 was identified as the “pituitary tumor-transforming gene” and seems to be overexpressed in several tumor types (28–30). These observations indicate that defective spindle checkpoint is closely associated with tumorigenesis. It would be interesting to investigate whether Clast3 is overexpressed in human cancers.

In conclusion, we have identified a novel cell cycle-regulated protein, Clast3, which appears to play a critical role in cell division. Our results suggest that the precise regulation of Clast3 expression is important for its normal function and that its constitutive overexpression results in polyploidy and multinucleation accompanied by growth inhibition. Elucidation of the exact role of Clast3 in cell division should provide important insights into the regulatory mechanisms of mitosis and the spindle checkpoint, the defects of which are linked to chromosomal instability and cancer development.

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