Reovirus-Induced σ 1s-Dependent G₂/M Phase Cell Cycle Arrest Is Associated with Inhibition of p34^{cdc2}

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Serotype 3 reoviruses inhibit cellular proliferation by inducing a G₂/M phase cell cycle arrest. Reovirus-induced G₂/M phase arrest requires the viral S1 gene-encoded σ 1s nonstructural protein. The G₂-to-M transition represents a cell cycle checkpoint that is regulated by the kinase p34^{cdc2}. We now report that infection with serotype 3 reovirus strain Abney, but not serotype 1 reovirus strain Lang, is associated with inhibition and hyperphosphorylation of p34^{cdc2}. The σ 1s protein is necessary and sufficient for inhibitory phosphorylation of p34^{cdc2}, since a viral mutant lacking σ 1s fails to hyperphosphorylate p34^{cdc2} and inducible expression of σ 1s is sufficient for p34^{cdc2} hyperphosphorylation. These studies establish a mechanism by which reovirus can perturb cell cycle regulation.

Mammalian reoviruses are nonenveloped, double-stranded RNA viruses having a broad host range in nature (68). Mechanisms underlying reovirus-induced cytopathicity are not completely understood. Although reovirus replication is thought to be entirely cytoplasmic (reviewed in reference 47), reovirus infection is associated with dramatic alterations of nuclear function that likely contribute to cell death. Reovirus induces apoptosis (57, 70) and inhibits cellular proliferation (55, 69).

Reovirus inhibits cellular proliferation by inducing a G₂/M phase cell cycle arrest. Serotype 3 prototype strains type 3 Abney (T3A) and type 3 Dearing (T3D) induce G₂/M cell cycle arrest to a greater extent than the prototype serotype 1 strain Lang (T1L) (55). Strain-specific differences in the capacity of reovirus to induce G₂/M cell cycle arrest are determined by the viral S1 gene (55, 69). The S1 gene is bicistronic, encoding the viral attachment protein σ 1 and the nonstructural protein σ 1s in overlapping but out-of-sequence reading frames (15, 30, 62). The σ 1s protein is necessary and sufficient for reovirus-induced G₂/M cell cycle arrest since a σ 1s-deficient reovirus mutant fails to induce G₂/M cell cycle arrest and ectopic expression of σ 1s results in accumulation of cells in the G₂/M phase of the cell cycle (55).

The transition from G₂ to M is under the control of the cyclin-dependent kinase p34^{cdc2}/cdk1 (cdc2) (29, 31, 49, 50). Activation of cdc2 requires its association with cyclin B (13, 22, 35, 42, 44, 46). Cyclin B expression varies with the phase of the cell cycle, with levels peaking during G₂ and early M phase (27, 28, 40, 54). Regulated expression of cyclin B ensures that the cdc2-cyclin B heterodimeric complex forms at the appropriate time during cell cycle progression (54).

The cdc2-cyclin B complex is maintained in an inactive state as a result of hyperphosphorylation of cdc2 (5, 12, 14, 54, 63).

Cdc2 migrates in acrylamide gels as three distinct bands corresponding to the level of inhibitory phosphorylation. Cdc2-PP is phosphorylated on both Thr14 and Tyr15, and cdc2-P is phosphorylated on Thr14 or Tyr15 (48). The protein kinases wee1 and myt1 are capable of phosphorylating cdc2 (4, 18, 45, 51, 52, 60). Entry into mitosis requires removal of the inhibitory phosphorylation on cdc2 by the dual-specificity phosphatase cdc25C (23, 34, 37, 43, 59, 65). Dephosphorylation results in cdc2 kinase activation, which initiates events necessary for mitosis (reviewed in reference 29).

Having shown that reovirus-induced inhibition of cellular proliferation (69) results from G₂/M phase cell cycle arrest (55), we conducted experiments to determine how reovirus infection perturbs G₂-to-M checkpoint regulatory mechanisms. We found that the capacity of cdc2 kinase to phosphorylate a cdc2-specific histone H1 peptide substrate is dramatically inhibited in cell lysates following infection with serotype 3 reovirus. Reovirus-induced inhibition of cdc2 kinase activity is associated with an increase in hyperphosphorylated forms of cdc2. Expression of σ 1s results in hyperphosphorylation of cdc2, and a σ 1s-deficient mutant reovirus fails to inactivate cdc2. These results indicate that serotype 3 reovirus-induced G₂/M phase cell cycle arrest is associated with cdc2 kinase inhibition and provide biochemical evidence linking the σ 1s protein to this process.

MATERIALS AND METHODS

Cells and viruses. Spinner-adapted mouse L929 cells (ATCC CCL1) were grown in Joklik's modified Eagles's minimal essential medium, which was supplemented to contain 5% heat-inactivated fetal bovine serum (Gibco BRL, Gaithersburg, Md.) and 2 mM L-glutamine (Gibco). Human embryonic kidney (HEK293) cells (ATCC CRL1573), Madin-Darby canine kidney (MDCK) cells (ATCC CCL34), C127 cells (ATCC CRL1616), and HeLa cells (ATCC CCL2) were grown in Dulbecco's modified Eagle's medium, which was supplemented to contain 10% heat-inactivated fetal bovine serum (HEK293, MDCK, and C127) or 10% non-heat-inactivated fetal bovine serum (HeLa), 2 mM L-glutamine, 1 mM sodium pyruvate (Gibco), 100 U of penicillin per ml, and 100 μ g of streptomycin per ml (Gibco).

Reovirus strains T1L and T3A are laboratory stocks. The reovirus field isolate

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strain type 3 clone 84 was isolated from a human host (11). T3C84-MA was isolated as previously described (9). Type 3 clone 84 and T3C84-MA are abbreviated herein as T3/ σ 1s+ and T3/ σ 1s−, respectively. Viral strains were plaque purified and passaged two to three times in L929 cells to generate working stocks as previously described (67).

Reovirus infection. Cells were seeded in six-well plates (Costar, Cambridge, Mass.) at 5×10^5 cells per well in a volume of 2 ml of the appropriate medium. After 24 h of incubation, when cells were 50 to 60% confluent, the medium was removed, and cells were infected with reovirus at a multiplicity of infection (MOI) of either 100 PFU per cell (T1L and T3A) or 1,000 PFU per cell (T3/ σ 1s+ and T3/ σ 1s−) in a volume of 200 μ l at 37°C for 1 h. After viral infection, 2 ml of fresh medium was added to each well. At various times postinfection, cells were harvested and washed with 1 ml of phosphate-buffered saline.

Inducible expression of σ 1s. C127 stable transformants expressing T3D σ 1s (BPX6-2) from the mouse metallothionein promoter and vector control cells (BPV1-2) were gifts of Aaron Shatkin (16). BPX6 (σ 1s-expressing) and BPV1 (vector control) cells were seeded in six-well plates at 5×10^5 cells per well in a volume of 2 ml per well. After 24 h of incubation, cells were incubated with 1 μ M CdCl₂ to induce σ 1s expression (17) and harvested at various times postinduction for cdc2 kinase migration analysis.

Gel electrophoresis and immunoblot analysis. Cell pellets were lysed in 2 $\times$ Laemmli buffer (36) (approximately 10^6 cells per 50 μ l) containing 4% sodium dodecyl sulfate, 20% glycerol, 10% β -mercaptoethanol, 0.004% bromophenol blue, and 0.125 M Tris-HCl (pH 6.8) (Sigma, St. Louis, Mo.). Lysates were sonicated for 5 to 10 s on ice, boiled for 10 min, and electrophoresed in 10 or 15% polyacrylamide gels as described previously (3, 36) (Hoefer Pharmacia Biotech, San Francisco, Calif.) at constant voltage (60 V through the stacking gel and 150 V through the resolving gel).

Following electrophoresis, gels and Hybond-C nitrocellulose membranes (Amersham, Piscataway, N.J.) were separately equilibrated to Towbin's transfer buffer (0.025 M Tris base, 0.15 M glycine, 20% methanol [pH 8.0]) for 5 min (66). Proteins were electroblotted onto nitrocellulose membranes for 30 to 45 min at 100 V in a Hoefer Scientific blotting tank at 4°C. Membranes were rinsed in Tris-buffered saline (TBS; 20 mM Tris base, 137 mM NaCl [pH 7.6]), incubated with fresh 5 to 10% nonfat dry milk (NFD) in TBS at 22°C for 2 h, and rinsed with TBS.

Immunoblots were normalized for actin content as determined by the density of actin staining detected using an antiactin antibody (CP01; Calbiochem, San Diego, Calif.) (1:5,000 dilution in TBS containing 5% NFD) at 22°C for 1 h) and probed for either cyclin B1 (554177, PharMingen; San Diego, Calif.) at a 1:375 dilution in TBS at 22°C for 16 h, cdc2 (SC-54; Santa Cruz Biotechnology, Santa Cruz, Calif.) at a 1:500 dilution in TBS at 22°C for 3 to 4 h, or cdc25C (67211A; PharMingen) at a 1:300 dilution in TBS at 22°C for 3 h. Bound antibodies were probed with an anti-mouse immunoglobulin conjugated to horseradish peroxidase (NA931; Amersham) at a 1:2,000 dilution in TBS containing 2% NFD at 22°C for 2 to 3 h. Antibody binding was visualized using enhanced chemiluminescence (Amersham), and densitometric analysis was performed using a FluorS MultiImager system and Quantity One software (Bio-Rad, Hercules, Calif.).

The fold increases in cyclin B1 levels following reovirus infection were calculated by dividing the densitometric value for cyclin B1 following infection by the densitometric value for cyclin B1 following mock infection for each time point. The fraction of cdc2-PP was calculated by dividing the densitometric value for cdc2-PP by the densitometric value for the total amount of cdc2 kinase. The fold increase in cdc2-PP following reovirus infection was calculated by dividing the fraction of cdc2-PP following virus infection by the fraction of cdc2-PP following mock infection for each time point. Nocodazole treatment (5 μ g per ml for 20 h) resulted in the fastest-migrating, nonphosphorylated/active form of cdc2 (data not shown). The three phosphorylation states for cdc2 and cdc25C were confirmed by treating L929 cell lysates with 0, 2, 5, or 10 U of potato acid phosphatase (Roche Molecular Biochemicals, Indianapolis, Ind.) for 1.5 h and 15 min, respectively, in 50 mM PIPES [piperazine-*N,N'*-bis(2-ethanesulfonic acid)] containing 1 mM dithiothreitol at 30°C prior to cdc2 or cdc25C detection (data not shown).

Cdc2 protein kinase assay. Cdc2 kinase activity was determined by using the SignaTECT cdc2 protein kinase assay system (Promega, Madison, Wis.). Cells were lysed by sonication in cdc2 extraction buffer containing 50 mM Tris-HCl (pH 7.4), 250 mM NaCl, 1 mM EDTA, 50 mM NaF, 1 mM dithiothreitol, 0.1% Triton X-100, 10 μ M leupeptin, and 100 μ g of aprotinin per ml and normalized for actin content. Aliquots of cell lysate corresponding to equivalent amounts of actin content were incubated with 50 μ M [γ -³²P]ATP and 25 μ M histone H1 biotinylated peptide substrate. The radiolabeled phosphate substrate was recov-

FIG. 1. T3A infection induces increased cyclin B1 levels. L929 cells were either mock infected (M) or infected with T3A (T3) at an MOI of 100 PFU per cell. Cell lysates were collected, normalized for actin content, and probed for cyclin B1 (A). At each time point postinfection, the band intensity for cyclin B1 following T3A infection was compared to that following mock infection and represented as the fold increase in cyclin B1 level (B).

ered on a streptavidin matrix and analyzed by scintillation counting. Nocodazole treatment (5 μ g per ml for 20 h) was used as a positive control.

RESULTS

Cyclin B1 levels are increased following infection with reovirus T3A. Cyclin B1 levels oscillate with the cell cycle, peaking during G₂ and early M phase (27, 28, 40, 54). To determine whether reovirus infection alters levels of cyclin B1, we analyzed cyclin B1 levels in L929 cells following T3A infection (Fig. 1). At various intervals after infection, L929 cell lysates were collected, normalized for actin content, and probed for cyclin B1 protein (Fig. 1A). T3A infection resulted in increased cyclin B1 levels at 24 h postinfection (1.4-fold increase over mock-infected cells), and these levels were further increased at 31 h postinfection (1.75-fold increase over mock-infected cells) (Fig. 1B). These results indicate that reovirus-induced G₂/M phase cell cycle arrest does not result from loss of cyclin B1.

T3A reovirus infection inhibits cdc2 kinase activity. Cdc2 kinase activity plays an important regulatory role at the G₂-to-M checkpoint (29, 31, 49, 50). We therefore analyzed cdc2 kinase activity following T3 reovirus infection. L929 cells were infected with T3A, and cdc2 kinase activity in cell lysates was assessed at 48 h postinfection. Lysates were normalized for actin content to allow kinase activity in lysates from infected and mock-infected cells to be directly compared. T3A infection resulted in a 4.8-fold reduction in cdc2 kinase activity in comparison to mock-infected cells, $P = 0.028$ (Fig. 2). Thus, reovirus-induced G₂/M phase cell cycle arrest is associated with inhibition of the major G₂-to-M transition control kinase, cdc2.

FIG. 2. Cdc2 kinase activity is inhibited following T3A infection. L929 cells were mock infected, infected with T3A at an MOI of 100 PFU per cell, or treated with nocodazole. Cell lysates were collected at 48 h postinfection, normalized for actin content, and assayed for cdc2 kinase activity. Results are presented as the mean product formed in counts per minute minus a no-cell lysate control for three independent experiments. The error bars indicate the standard errors of the mean.

T3A induces inhibitory phosphorylation of cdc2 kinase. Reduced cdc2 kinase activity may result from either protein degradation or kinase inactivation by inhibitory hyperphosphorylation (5, 12, 14, 54, 63). We therefore analyzed the amounts of phosphorylated cdc2 by immunoblot assay at 6, 24, and 48 h postinfection (Fig. 3). In comparison to mock-infected control cells, T3A infection was associated with an increase in the slower-migrating, hyperphosphorylated form of cdc2 (cdc2-PP) at both 24 (1.5-fold) and 48 (2.5-fold) h postinfection (Fig. 3A). The increase in cdc2-PP following T3A reovirus infection was significantly greater than the increase in cdc2-PP following T1L infection at 24 and 48 h postinfection, $P = 0.006$ and $P = 0.003$, respectively (Fig. 3B). These results indicate that T3 reovirus infection is associated with hyperphosphorylation of cdc2. Furthermore, infection with T1 reovirus, which induces substantially less G_2/M cell cycle arrest than T3 reovirus (55), likewise induces less inhibitory phosphorylation of cdc2 (Fig. 3C).

Cdc2 is phosphorylated in cell lines that undergo G_2/M phase cell cycle arrest in response to reovirus infection. Given that the capacity of reovirus to induce G_2/M phase cell cycle arrest is not cell type specific (55), we considered whether hyperphosphorylation of cdc2 occurred following reovirus infection of other cell types. MDCK, C127, HEK293, and HeLa cells were either infected with T3A or mock infected as a control, and cdc2 phosphorylation status in cell lysates was determined at 48 h postinfection. Increases in the fraction of cdc2-PP following T3A infection was observed in all cell lines tested except HeLa cells (Fig. 4). Therefore, reovirus-induced hyperphosphorylation of cdc2, like G_2/M cell cycle arrest, is not limited to cell type.

The $\sigma 1s$ protein is necessary and sufficient to inhibit cdc2 kinase. We have previously shown that $\sigma 1s$ expression is required for G_2/M phase cell cycle arrest in response to serotype 3 reovirus infection (55). To determine whether $\sigma 1s$ is required for reovirus-induced cdc2 kinase inhibition, we compared levels of hyperphosphorylated cdc2 in L929 cells following infection with reovirus strains T3/ $\sigma 1s^+$ and T3/ $\sigma 1s^-$, which vary in the capacity to express $\sigma 1s$ (58). The accumulation of phosphorylated cdc2 following infection with T3/ $\sigma 1s^-$ was signifi-

cantly less than the accumulation of phosphorylated cdc2 following infection with its $\sigma 1s$ -expressing parent virus, T3/ $\sigma 1s^+$ (Fig. 5A). Therefore, functional $\sigma 1s$ is required for serotype 3 reovirus-induced hyperphosphorylation of cdc2.

We also have shown that ectopic expression of $\sigma 1s$ results in G_2/M phase cell cycle arrest (55). To determine whether $\sigma 1s$ expression alone is sufficient to induce cdc2 kinase inhibition, we analyzed the phosphorylation status of cdc2 in C127 cells engineered to express the T3D $\sigma 1s$ protein under the control of the mouse metallothionein promoter (16). The fraction of cdc2-PP following induction of $\sigma 1s$ by $1 \mu M$ CdCl₂ was 1.6- and 2.0-fold greater in cells expressing $\sigma 1s$ than in vector control cells at 45 and 55 h postinduction, respectively (Fig. 5B). These results indicate that $\sigma 1s$ is necessary and sufficient to induce inhibition of cdc2 kinase.

FIG. 3. Cdc2 kinase inhibition following T3A infection results from hyperphosphorylation. L929 cells were either mock infected (M) or infected with T3A (T3) (A) or T1L (T1) (B) at an MOI of 100 PFU per cell. Cell lysates were collected at the indicated times postinfection, normalized for actin content, and probed for cdc2 kinase. Phosphorylated forms of cdc2 are shown. Cdc2-P indicates either Thr14 or Tyr15 phosphorylation, and cdc2-PP indicates Thr14 and Tyr15 phosphorylation. (C) The fraction of cdc2-PP was determined for each condition, and at each time point postinfection, the fraction of cdc2-PP following T1L (triangles) or T3A (squares) infection was compared to that following mock infection and represented as the fold increase in cdc2-PP. Results are presented as the mean fold increase in cdc2-PP for four (T3A) or two (T1L) independent experiments. The error bars indicate the standard errors of the mean.

FIG. 4. T3A infection inhibits *cdc2* kinase in a variety of cell lines. MDCK (A), C127 (B), HEK293 (C), and HeLa (D) cells were either mock infected (M) or infected with T3A (T3) at an MOI of 100 PFU per cell. Cell lysates were collected at 48 h postinfection, normalized for actin content, and probed for *cdc2* kinase. Phosphorylated forms of *cdc2* are shown. *Cdc2*-P indicates either Thr14 or Tyr15 phosphorylation, and *cdc2*-PP indicates Thr14 and Tyr15 phosphorylation.

Cdc25C is inhibited following serotype 3 reovirus infection.

To determine whether increased inhibitory phosphorylation of *cdc2* following reovirus infection resulted from inactivation of the *cdc2*-specific phosphatase *cdc25C*, we analyzed the different phosphorylation states of *cdc25C* in polyacrylamide gels since *cdc25C* activity is regulated by phosphorylation. At 6, 24, 31, and 48 h postinfection, L929 cell lysates were run on polyacrylamide gels, transferred to nitrocellulose, and probed for *cdc25C* phosphatase (Fig. 6). T3A infection resulted in a decrease in the slower-migrating/hyperphosphorylated or active form of *cdc25C* (hyperphosphorylated *cdc25C*) at 24, 31, and 48 h postinfection compared to mock-infected controls. There was a corresponding increase in the inactive Ser216-phosphorylated and nonphosphorylated forms of *cdc25C* compared to mock-infected controls. These results suggest that one pathway by which reovirus inhibits *cdc2* is to prevent *cdc2* dephosphorylation by inhibiting the *cdc2*-dependent phosphatase *cdc25C*.

DISCUSSION

Serotype 3 reovirus strains induce G_2/M phase cell cycle arrest in a variety of target cells in vitro (55). The progression from G_2 to M is regulated by the association of cyclin B1 with the *cdc2* kinase (13, 22, 35, 42, 44, 46). We hypothesized that either decreased levels of cyclin B1 or decreased activity of *cdc2* kinase might mediate reovirus-induced cell cycle arrest at the G_2 -to-M checkpoint. Our findings demonstrate that reovirus infection does not result in decreased cyclin B1 levels.

FIG. 5. The $\sigma 1s$ protein is necessary and sufficient to inhibit *cdc2*. (A) L929 cells were mock infected (M), infected with T3A (T3) at an MOI of 100 PFU per cell, or infected with T3C84 (T3/ $\sigma 1s^+$) or $\sigma 1s$ -null mutant T3C84-MA (T3/ $\sigma 1s^-$) at an MOI of 1,000 PFU per cell. Cell lysates were collected at 48 h postinfection, normalized for actin content, and probed for *cdc2* kinase. Phosphorylated forms of *cdc2* are shown. *Cdc2*-P indicates either Thr14 or Tyr15 phosphorylation, and *cdc2*-PP indicates Thr14 and Tyr15 phosphorylation. (B) C127 cells stably transfected with T3D- $\sigma 1s$ under the control of the mouse metallothionein promoter ($\sigma 1s$) or vector control cells (V) were induced with CdCl₂, harvested at the indicated times postinfection, normalized for actin content, and probed for *cdc2* kinase. Phosphorylated forms of *cdc2* are shown.

Instead, we found a striking reduction in *cdc2* kinase activity and a concomitant increase in *cdc2* hyperphosphorylation.

Cdc2-cyclin B activity is inhibited by phosphorylation of *cdc2* (12, 54, 63). Following serotype 3 reovirus infection, there is a significant reduction in *cdc2* kinase activity and an increase in inhibitory phosphorylation of *cdc2*. Inhibition of *cdc2* kinase by

FIG. 6. *Cdc25C* is inhibited following reovirus infection. L929 cells were either mock infected (M) or infected with T3A (T3) at an MOI of 100 PFU per cell. Cell lysates were collected at the indicated times postinfection, normalized for actin content, and probed for *cdc25C*. Phosphorylated forms of *cdc25C* are shown. Reovirus infection results in reduction of the hyperphosphorylated/active form of *cdc25C*. Results are representative of three independent experiments.

reovirus is not cell type specific and occurs in all cell lines that were found to undergo significant amounts of G₂/M phase cell cycle arrest following serotype 3 reovirus infection except in HeLa cells, which show the least amount of reovirus-induced G₂/M arrest.

We have previously shown that σ 1s expression is required for reovirus-induced G₂/M phase cell cycle arrest (55). We now show that σ 1s expression is also required for inhibition of cdc2 kinase activity. Furthermore, σ 1s expression alone, which induces an increase in the percentage of cells in the G₂/M phase of the cell cycle (55), is capable of inhibiting cdc2. These results provide strong evidence that the viral S1 gene product σ 1s induces G₂/M phase cell cycle arrest at the G₂-to-M checkpoint by activating a pathway leading to hyperphosphorylation of cdc2 kinase; however, alternative mechanisms may contribute to reovirus-induced G₂/M phase cell cycle arrest. These results are consistent with observations that σ 1s is present in the nucleus of serotype 3-infected cells (8, 58), where it could interact with proteins responsible for G₂-to-M transition.

Several viruses are influenced by or alter cdc2 kinase activity. For example, the herpes simplex virus U_L13 and α 22/U_S1.5 (ICP22) gene products (1), *Autographa californica* nucleopolyhedrovirus ODV-EC27 (6, 7), and human papillomavirus E6 and E7 (64) can all increase cdc2 activity. In cases where viruses increase cdc2 kinase activity, it is possible that this activity is required to phosphorylate viral proteins. For example, varicella-zoster virus glycoprotein gI (72), Epstein-Barr virus EBNA-LP (33), hepatitis E virus ORF3 (73), and herpes simplex virus ICP0 (2) are phosphorylated by cdc2. Conversely, human immunodeficiency virus (HIV) Vpr (25, 56) and human papillomavirus E2 (19) inhibit or delay the activation of cdc2. These observations suggest that viruses have evolved mechanisms to alter cdc2 function. At least in the case of HIV Vpr, cdc2 inhibition and subsequent G₂/M cell cycle arrest result in increased viral replication (24, 71). The role of reovirus-induced G₂/M arrest in viral replication remains unknown. However, it is clear that reovirus-induced G₂/M cell cycle arrest does not require apoptotic DNA damage (55).

The mechanism by which reovirus inhibits cdc2 kinase activity remains to be established. Cdc2 kinase activity is negatively regulated by the kinase wee1 and positively regulated by the phosphatase cdc25C (reviewed in reference 29). Following reovirus infection, cdc25C is inhibited by dephosphorylation. It is suggested that HIV Vpr can inhibit cdc2 activity (25, 56) via activation of wee1 and inactivation of cdc25C (26, 41). Vpr may inactivate cdc25C through physical interaction with protein phosphatase 2A (26), which inhibits cdc25C activity by dephosphorylation (10, 32, 38). Cdc25C activity is also inhibited following phosphorylation by the kinases chk1 and chk2 (20, 21, 39, 53, 61). Based on these observations, it is possible that the reovirus σ 1s protein mediates inactivation of cdc2 kinase by increasing the levels of wee1 and/or chk1 or by increasing the translocation of protein phosphatase 2A to the nucleus. Wee1 directly phosphorylates and inactivates cdc2, whereas chk1 inactivates cdc25C so that cdc25C is no longer available to dephosphorylate and activate cdc2. Recent supportive investigation revealed that reovirus infection is associated with increased expression of wee1 and chk1 (G. J. Poggioli and R. L. DeBiasi, unpublished observation). Our ongoing work is directed at defining the biochemical mecha-

nisms underlying σ 1s-mediated inactivation of cdc2 kinase and establishing the pathological significance of this process.

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