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Characterization of G_1 Transit Induced by the Mitogenic-Oncogenic Viral Ki-*ras* Gene Product

JON P. DURKIN* AND JAMES F. WHITFIELD

Cell Physiology Group, Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A OR6

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NRK rat kidney cells infected with a temperature-sensitive mutant of the Kirsten sarcoma virus (*ts*371) were transformed at 36°C but were phenotypically nontransformed at 41°C because of the abnormal thermolability of the oncogenic 21-kilodalton product of the viral Ki-*ras* gene. Thus *ts*K-NRK cells were rendered quiescent in a G_0-G_1 state by a 48-h incubation in serum-free medium at the nonpermissive, p21-inactivating temperature of 41°C. The serum-starved cells could then be stimulated to transit G_1 either (i) as nontransformed cells by adding serum at 41°C or (ii) as transformed cells by lowering the temperature to a p21-activating 36°C. The viral p21 protein was as effective as serum in stimulating *ts*K-NRK cells to transit G_1 and to start replicating DNA. While p21 effectively stimulated cells to transit G_1 even in unconditioned, serum-free medium, they still needed cell-derived conditioning factors to subsequently divide. The p21 protein also enabled the cells to transit G_1 in spite of an extracellular Ca^{2+} deficiency that inhibited the G_1 transit of serum-stimulated cells. p21 activity was needed to stimulate both early and late G_1 events. In contrast to serum, p21 did not stimulate total RNA or protein synthesis, but some RNA and protein synthesis must have been needed for the p21-driven G_1 transit because it could be stopped by actinomycin D or cycloheximide.

It is becoming increasingly evident that the polypeptide products of several oncogenes are variously linked to, and can activate, the proliferative machinery of the host cell. For example, the v-*sis* gene product is structurally similar to platelet-derived growth factor (12, 36), and the v-*erb*B product is homologous to part of the epidermal growth factor receptor (13). Moreover, the product of the v-*src* gene, $pp60^{v\text{-}src}$, is a complete mitogen, the activity of which is independent of external growth factors in serum (14, 15).

Oncogenic members of the *ras* family of oncogenes and their *ras* protein products have been shown to activate the proliferative machinery when microinjected into quiescent cells (1, 16, 30). The *ras* proteins are membrane-associated, GTP-binding GTPases, the oncogenic forms of which differ from the nononcogenic forms usually by having amino acid substitutions at position 12 as well as position 59 or 61 and reduced GTPase activity (18, 25, 31). These proteins are distantly related to the GTP-binding α subunits of the membrane-associated G proteins (21) that regulate, among other things, Ca^{2+} movements (20) and adenylate cyclase (19), both of which are strongly implicated in cell proliferation (for reviews, see references 5 and 37). Indeed, the *ras* proteins of yeast cells have been shown to stimulate adenylate cyclase activity and are required for G_1 transit (9, 23, 33, 34). By contrast, the mammalian Ha-*ras* protein does not stimulate adenylate cyclase (2, 9), although the viral Ki-*ras* protein may do so, as suggested by its ability to rapidly stimulate adenylate cyclase in NRK cells (17).

It is likely the ability of a v-*ras* gene to stimulate proliferation is related to its oncogenicity. If this is the case, it would be important to find out how a viral *ras* protein affects the proliferative machinery of the cell. While microinjection has been valuable in demonstrating that oncogenic *ras* gene products can trigger proliferation of quiescent cells, the usefulness of this technique for analyzing this phenomenon is limited because of the small number of cells that can be injected. Therefore, we used a NRK cell line, *ts*K-NRK, infected with a temperature-sensitive Kirsten murine sarcoma virus (Ki-MSV) mutant (*ts*371) to characterize the pattern of events that follow the intracellular activation of the mitogenic-oncogenic p21 protein of the virus and to compare this with the pattern of events that follows the stimulation of these cells by serum factors.

MATERIALS AND METHODS

Cell quiescence and stimulation. Uninfected NRK cells and *ts*K-NRK cells were the generous gifts of M. Scolnick (Merck Sharpe & Dhome, West Point, Pa.). The *ts*K-NRK cell line was produced by infecting NRK cells with the temperature-sensitive, transformation-defective Ki-MSV *ts*371 (29). Stock cultures actively growing at 36°C were replated in Dulbecco minimal essential medium containing 15% bovine calf serum and incubated for 48 h at 40°C. They were then rendered quiescent by incubation either in serum-free medium Dulbecco minimal essential medium-Ham F12 [1:1] containing 1 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.2]) or medium containing 0.2% calf serum for 48 h at 41°C. The cells were incubated for the first 48 h at 40°C rather than at 41°C because of a transiently reduced plating efficiency at the higher temperature that was caused by trypsinization. At 41°C the quiescent cells were stimulated to resume cycling either by adding serum to the medium to 10% or by lowering the incubation temperature from 41 to 36°C to activate the abnormally thermolabile p21 product of the virus.

Measurements of DNA synthetic activity or cell cycle distribution. The proportion of cells making DNA was determined autoradiographically by the procedure of Whitfield et al. (38) or cytofluorographically by the method of Boynton et al. (7).

[^{3}H]uridine and [^{35}S]methionine incorporation. At time zero, quiescent cells in serum-free medium were (i) left quiescent at 41°C or (ii) stimulated to transit G_1 by adding serum at 41°C or by dropping the temperature to 36°C. At

* Corresponding author.

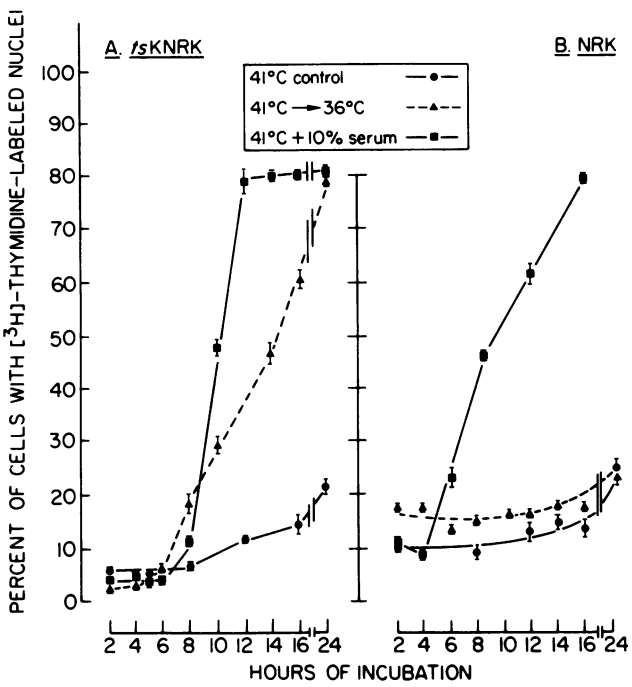

FIG. 1. The ability of quiescent, serum-deprived *ts*K-NRK cells (A) but not uninfected NRK cells (B) to transit G_1 and initiate DNA synthesis after a shift from 41 to 36°C. Cultures that were rendered quiescent by incubation in serum-free medium for the previous 48 h at 41°C were left at 41°C, stimulated to transit G_1 by adding serum to 10% at 41°C, or shifted to 36°C without the addition of serum. Cultures were labeled continously with [^{3}H]thymidine from time zero, and at the indicated times their DNA synthetic activities were determined autoradiographically, as described in the text. The points are means ± standard error of the means (SEM) of three cultures.

various times after stimulation, [^{35}S]methionine or [^{3}H]uridine was added to the culture medium to 15 or 2 μCi/ml, respectively. After a 1-h labeling period, the amount of ^{3}H incorporated into cellular RNA and the amount of ^{35}S incorporated into cellular protein were determined as described below. In other experiments, the conditioned medium from quiescent cultures at 41°C was pooled and put into tubes which were either empty or contained enough serum to give a final concentration of 10%. [^{35}S]methionine or [^{3}H]uridine was added to the pooled medium to 15 or 2 μCi/ml, respectively, and 2 ml of the appropriate serum-free or serum-containing medium was returned to the cultures at 41°C, which were then kept at 41°C or shifted to 36°C. At various times thereafter the amount of incorporated radiolabel was determined. To do this, the medium was discarded, the cultures were washed twice with 5 ml of ice-cold phosphate-buffered saline, and the cells were lysed on the dish in 2 ml of a buffer (at 4°C) containing 20 mM Tris hydrochloride (pH 7.0), 5 mM $MgCl_2$, 100 mM NaCl, 1% (vol/vol) Triton X-100, 0.5% sodium deoxycholate, and 3 mM phenylmethylsulfonylfluoride. The 2-ml lysates were put into tubes, and RNA or protein was precipitated by adding an equal volume of ice-cold 14% perchloric acid. The precipitates were pelleted by centrifugation, washed twice with 7% perchloric acid, and dissolved in 1 N NaOH, and their radioactivities were measured by liquid scintillation counting. To measure total protein contents, cells from identically treated, but unlabeled, cultures were removed from the culture dishes with a mixture of 0.25% trypsin–1 mM EDTA in phosphate-buffered saline, and their protein contents were determined exactly by the method of Durkin and Whitfield (14), using the colorimetric method of Bradford (8). Cell numbers were measured with a Coulter electronic cell counter (Coulter Electronics, Hialeah, Fla.). Identically treated cultures that had been exposed to [^{3}H]thymidine were used to determine DNA synthetic activity.

Protein degradation. Protein degradation rates were determined by the method of Poole and Wibo (27), as modified by Durkin and Whitfield (14).

Extracellular Ca^{2+} and G_1 transit. In some experiments, the effects of extracellular Ca^{2+} deprivation on serum and p21 actions were determined as described in the legend of Fig. 7. Ca^{2+}-free Dulbecco minimal essential medium and F12 were purchased from GIBCO Laboratories (Grand Island, N.Y.). The ionic (physiologically available) Ca^{2+} concentration in the serum was reduced to 0.02 mM with the highly specific Ca^{2+} chelator ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) rather than with the much less specific Chelex 100 ion-exchange resin (4, 6). The concentration of the nonchelated Ca^{2+} remaining in the EGTA-treated serum was measured fluorometrically by the method of Borle and Briggs (3).

RESULTS

*ts*K-NRK cells infected with the temperature-sensitive *ts*371 mutant of Ki-MSV produce an abnormally thermolabile oncogenic p21 *ras* protein (26) which, in these experiments, transformed the cells at 36°C but left them phenotypically untransformed at 41°C. Thus, *ts*K-NRK cells formed colonies in soft agar (a standard in vitro indicator of neoplastic transformation) at 36°C but not at the nonpermissive 41°C (data not shown). Cytofluorographic analysis revealed that removing serum from the medium of *ts*K-NRK cells that had been growing exponentially in complete medium at 41°C arrested ≥90% of the cells in a G_0-G_1 state. As expected, the addition of serum to the medium of these quiescent cells to a final concentration of 10% caused them to resume cycling at the nonpermissive temperature of 41°C as nontransformed cells and to start replicating DNA as early as 6 to 8 h later (Fig. 1A). However, these quiescent cells could also be induced to resume proliferation without the addition of any growth factors or nutrients by lowering the temperature to a level permissive for p21 activity and transformation. Thus, lowering the temperature from 41 to 36°C stimulated quiescent *ts*K-NRK cells to transit the G_1 phase and to initiate DNA replication as early as 6 to 8 h after p21 activation even in medium without serum growth factor (Fig. 1A). This striking proliferative response of *ts*K-NRK cells to the temperature shift did not result from temperature shock, because serum-starved, uninfected NRK cells did not transit G_1 and start replicating DNA when the temperature was reduced from 41 to 36°C (Fig. 1B). While the actual length of G_1 in p21-stimulated cultures varied between 6 and 10 h in this series of experiments, it was always the same as the length of G_1 in accompanying serum-stimulated cultures. Thus, activation of the Ki-*ras* gene product was a potent mitogenic signal which stimulated quiescent *ts*K-NRK cells to transit the G_1 phase and initiate DNA synthesis in medium free of serum growth factor. This ability of p21 to stimulate G_1 transit was aided by, but not dependent on, conditioning factors produced by the cells during their growth-arresting, 48-h preincubation in serum-free medium at the nonpermissive temperature of 41°C. Thus, replacement of the conditioned medium with fresh

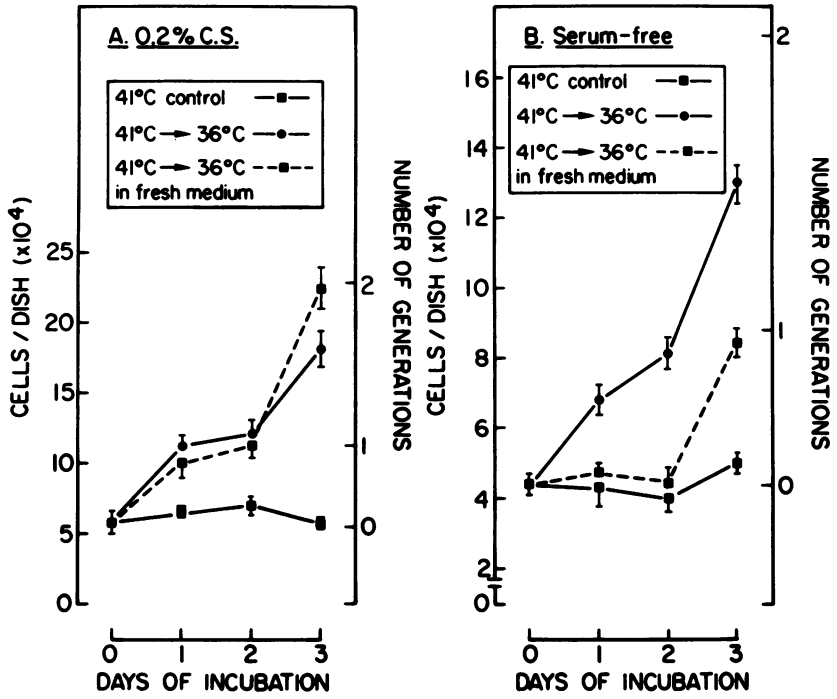

FIG. 2. p21 activity induces *ts*K-NRK cells in serum-free conditioned medium to divide. Cultures were rendered quiescent by incubation for 48 h at 41°C in medium containing 0.2% serum (A) or in medium without any serum (B). On day 0, some cultures were kept at 41°C, some were shifted to a p21-activating 36°C, and the rest were shifted to 36°C after their conditioned medium was replaced by fresh (i.e., unconditioned) medium containing 0.2% serum (A) or medium without any serum (B). At the indicated times the number of cells per dish was determined, as described in the text. The points are means ± SEM of three cultures.

medium immediately before the shift from 41 to 36°C reduced, by about 35%, the number of cells that entered S phase during the ensuing 24 h.

The *ts*K-NRK cells that had been stimulated by p21 activation to transit G_1 and replicate their DNA went on to divide. For example, cells arrested in G_0-G_1 by 48 h of incubation at 41°C in medium containing 0.2% serum divided within 24 h after the temperature was lowered to 36°C (Fig. 2A). Moreover, they did so even when this conditioned medium was replaced by fresh (i.e., unconditioned) 0.2% serum-containing medium at the time of the temperature shift (Fig. 2A). However, in serum-free medium the division of p21-stimulated cells was much more dependent on cell-derived conditioning factors than was the initiation of DNA synthesis, because the temperature-shifted cells were able to divide only if cellular conditioning factors were present after p21 activation (Fig. 2B). Thus, while as much as 65% of the stimulated cells initiated DNA replication at the normal time when the conditioned, serum-free medium was replaced by fresh, unconditioned medium immediately after the shift from 41 to 36°C, their subsequent division was delayed by 48 h (Fig. 2B), presumably until p21 had managed to recondition the medium.

The temperature-induced reactivation of p21 in quiescent *ts*K-NRK cells is a rapid event, as indicated by its ability to stimulate cellular adenylate cyclase activity within 30 min of a shift from 41 to 36°C (17). This rapid responsiveness of the abnormally thermolabile p21 to a temperature shift enabled us to trigger bursts of p21 activity of relatively short durations by transiently reducing the temperature from 41 to 36°C. Even an 8-h exposure to active p21 at 36°C was not enough to commit quiescent *ts*K-NRK cells to DNA synthesis (Fig. 3), despite the fact that the G_1 phase in these experiments lasted for only 9 to 10 h. This suggests that p21 is needed throughout the G_1 phase and is responsible for both starting and promoting G_1 transit in serum-free medium.

One of the most striking effects of mitogens in serum is to rapidly increase the rates of RNA and protein synthesis in quiescent cells (10, 28, 35). For example, the addition of serum to the conditioned, serum-free medium of quiescent *ts*K-NRK cultures at 41°C caused two- to threefold increases in the rates of [^{3}H]uridine incorporation into total cellular RNA (Fig. 4A), [^{35}S]methionine incorporation into protein (Fig. 4A and 5A), and total protein accumulation (Fig. 5C). These increases in [^{35}S]methionine incorporation into protein and the total cellular protein content were due almost entirely to increased synthesis rather than an observed decrease in protein degradation, which was much too small (~1%/h) to account for the increase in the rate of [^{35}S]methionine incorporation (data not shown). Despite the fact that p21 was as potent a mitogen as 10% serum (Fig. 5B), stimulation of the *ts*K-NRK cells by dropping the temperature from 41 to 36°C did not raise the rates of [^{3}H]uridine incorporation into RNA (Fig. 4A), [^{35}S]methionine incorporation into protein (Fig. 4B and 5B), or total protein accumulation (Fig. 5C). This apparent failure of protein synthesis to increase after the shift from 41 to 36°C was not due to the fact that an increase in protein synthesis was counterbalanced by an increase in protein degradation. The rate of protein degradation did not rise after the p21-activating temperature shift (data not shown).

Although p21-stimulated *ts*K-NRK cells did not increase their overall rates of RNA and protein synthesis, they still had to make new RNA and protein species to transit G_1 and start replicating DNA. Thus, the RNA synthesis (i.e., tran-

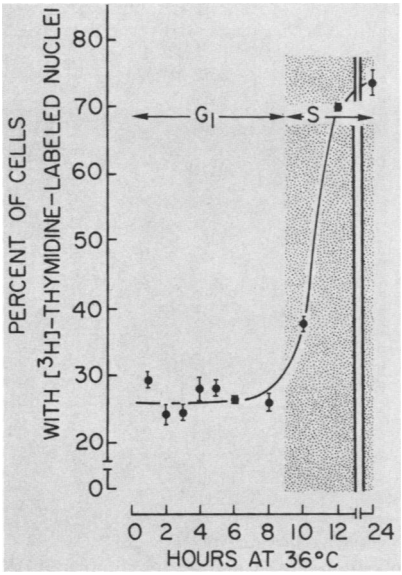

FIG. 3. p21 activity is required throughout G_1 for serum-deprived *ts*K-NRK cells to initiate DNA synthesis. Quiescent cultures in serum-free, conditioned medium at 41°C were shifted to the permissive temperature of 36°C at time zero for the indicated periods of time before being returned to the nonpermissive temperature of 41°C. The cultures were continuously exposed to [^{3}H]thymidine from 0 to 24 h, after which the total proportion of cells which had entered S phase was determined autoradiographically, as described in the text. The shaded areas represent the mean time at which cells, shifted to 36°C at time zero, would normally have entered S phase. The points are means ± SEM of three cultures.

scription) inhibitor actinomycin D and protein synthesis (i.e., translation) inhibitor cycloheximide prevented both serum-stimulated and p21-stimulated cells from transiting G_1 and replicating DNA when added to the culture medium in early G_1 (Fig. 6). However, serum-stimulated cells at 41°C rapidly became insensitive to actinomycin D in the second half of G_1 (Fig. 6A), which suggests that they had accumulated enough essential RNA transcripts by then to support the initiation of DNA replication. By contrast, the p21-stimulated *ts*K-NRK cells seemed unable to accumulate essential RNA transcripts at the same rate as serum-stimulated cells, because they remained vulnerable to the effects of actinomycin D throughout the G_1 phase (Fig. 6A). Serum-stimulated *ts*K-NRK cells became insensitive to the effects of cycloheximide 2.5 to 3 h after they became insensitive to actinomycin D (Fig. 6B), which indicates that these cells, with their heightened protein synthetic capacity, were able to build up a pool of essential proteins by late G_1 that was large enough to sustain S phase transit without further input. By contrast, p21-stimulated *ts*K-NRK cells remained sensitive to cycloheximide much longer (Fig. 6B), reflecting, perhaps, the combined effects of an unstimulated protein synthetic machinery and the slow production of essential RNA transcripts.

Another characteristic feature of tranformed cells is their ability to proliferate in the face of an extracellular Ca^{2+} deficiency that stops the proliferation of the corresponding untransformed cells (for a review, see reference 32). To determine the effect of p21 on the extracellular Ca^{2+} requirement of its host, we subjected quiescent cells to either a short or prolonged Ca^{2+} deprivation before stimulation by serum or p21. Thus, *ts*K-NRK cells were first rendered quiescent in a G_0 state by incubation in serum-free medium for 48 h at 41°C. During the last 2 or 16 h of this incubation the extracellular Ca^{2+} concentration was lowered drastically by replacing the original serum-free medium containing 1.8 mM Ca^{2+} with fresh serum- and Ca^{2+}-free medium. Even the short 2-h preliminary Ca^{2+} deprivation was enough to greatly inhibit the ability of factors in serum to stimulate G_1 transit (Fig. 7). By contrast, even the prolonged 16-h Ca^{2+} deprivation was unable to seriously impede the G_1 transit of p21-stimulated cells (Fig. 7). Thus, the endogenous reactivation of p21 initiated and then drove G_1 transit without the external Ca^{2+} and growth factors essential for the G_1 transit of nontransformed cells.

DISCUSSION

We have shown that the oncogenic, membrane-associated p21 *ras* protein of Ki-MSV can stimulate Ca^{2+}- and serum-

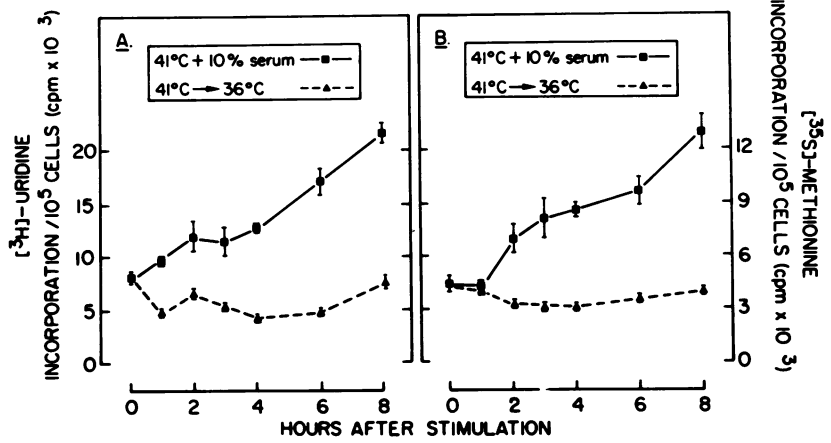

FIG. 4. p21 activation induces G_1 transit in serum-deprived *ts*K-NRK cells without the increase in total cellular RNA synthesis (A) or total cellular protein synthesis (B) that occurred in serum-stimulated cells. Quiescent *ts*K-NRK cells in serum-free, conditioned medium at 41°C were left at 41°C or stimulated at time zero to transit G_1 by adding serum at 41°C or by lowering the temperature to a p21-activating 36°C. One hour before the indicated times, the culture medium was supplemented with either [^{3}H]uridine (2 μCi/ml) (A) or [^{35}S]methionine (15 μCi/ml) (B). The amount of radioactivity incorporated into cellular RNA or protein that was precipitable by 7% perchloric acid was determined, as described in the text. The points are means ± SEM of four cultures.

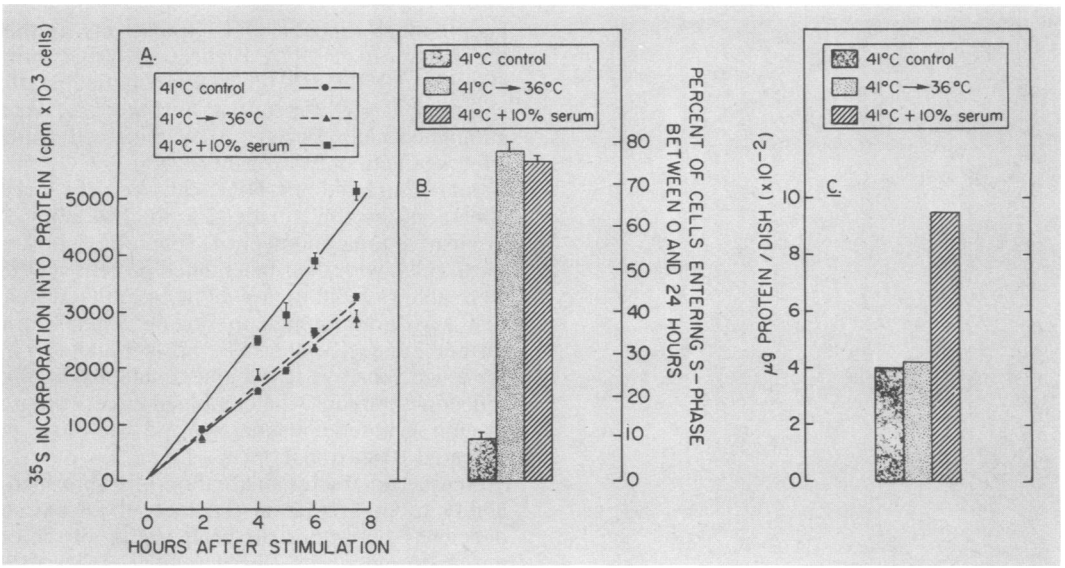

FIG. 5. The relation between protein synthesis, total protein content, and DNA replication in p21- and serum-stimulated *ts*K-NRK cells. Cultures of quiescent *ts*K-NRK cells in serum-free, conditioned medium at 41°C were kept at 41°C or were stimulated at zero hours to transit G_1 either by adding serum at 41°C or by lowering the temperature to 36°C without adding serum. (A) At time zero the culture medium was supplemented with [^{35}S]methionine (15 μCi/ml), and at the indicated times the amount of radioactivity incorporated into total cellular protein was determined, as described in the text. (B) Other cultures were continuously exposed to [^{3}H]thymidine from 0 to 24 h, after which the proportion of cells that had entered S phase was determined autoradiographically, as described in the text. (C) The total protein content of the cells in other cultures was determined by the method of Bradford (8) 6 h after stimulation.

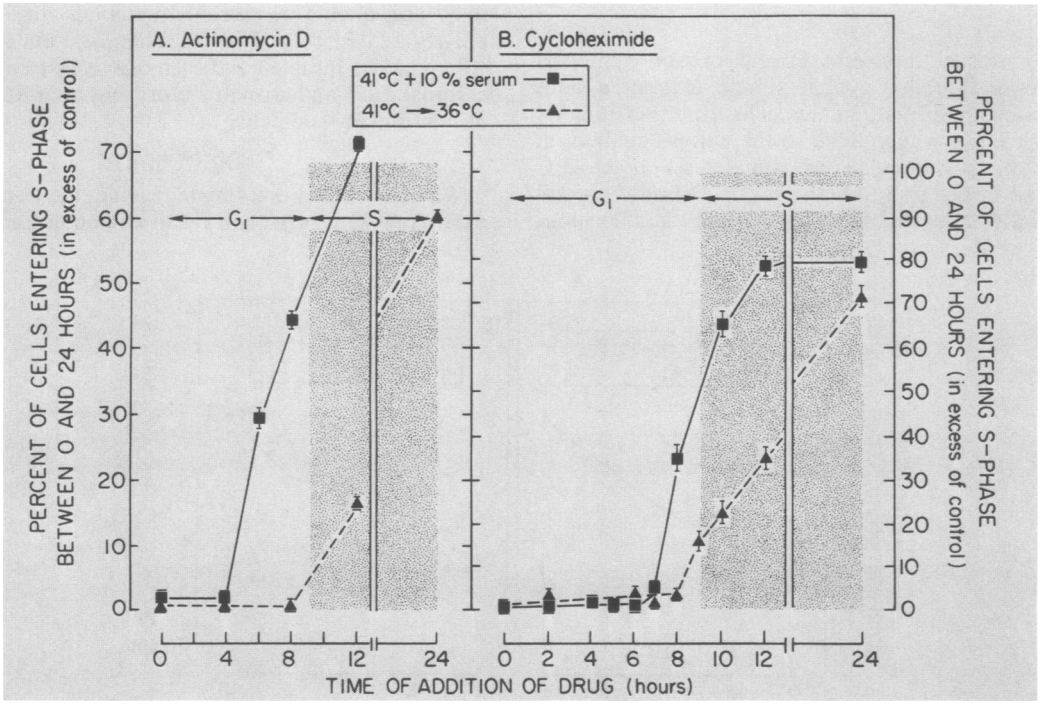

FIG. 6. Transcription-inhibiting levels of actinomycin D (A) and translation-inhibiting levels of cycloheximide (B) blocked the transit of both serum- and p21-stimulated cells through G_1. Cultures of quiescent *ts*K-NRK cells in serum-free, conditioned medium were stimulated at time zero to transit G_1 by dropping the temperature from 41 to 36°C or by adding serum to 10% at 41°C. At the indicated times after stimulation, actinomycin D or cycloheximide was added to a concentration of 0.5 or 1 μg/ml, respectively; and the effect on G_1 transit was determined by continuously exposing the cells to [^{3}H]thymidine from 0 to 24 h and determining the total proportion of cells that had initiated DNA synthesis during that time, as described in the text. The shaded area represents the mean time at which cells, stimulated at 0 h by p21 activation, would normally have entered S phase. The points are means ± SEM of three cultures.

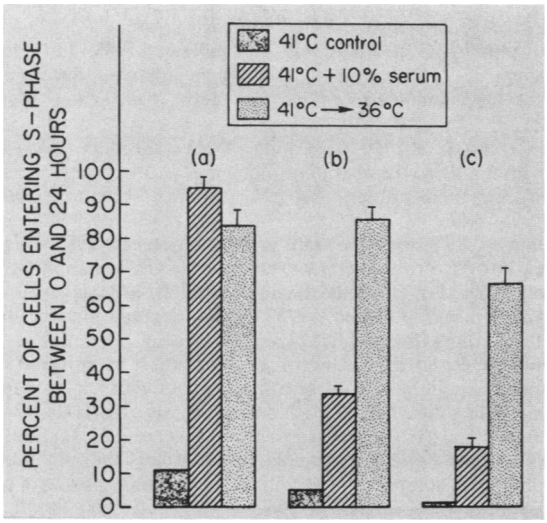

FIG. 7. The G_1 transit induced by p21 activity, but not by serum, is independent of extracellular Ca^{2+}. Cultures of *ts*K-NRK cells were rendered quiescent by (a) incubation in serum-free medium (containing the normal 1.8 mM Ca^{2+}) for 48 hours at 41°C; (b) same as for part a, except that for the last 2 h of the 48-h incubation the culture medium was replaced with fresh serum- and Ca^{2+}-free medium; (c) same as for part a, except that for the last 16 h of the 48-h incubation the culture medium was replaced with serum- and Ca^{2+}-free medium. After the 48-h preincubation, some cultures were left unstimulated at 41°C, while others were stimulated to transit G_1 either by adding Ca^{2+}-deficient serum (the Ca^{2+} concentration of which had been lowered to 0.02 mM, as described in the text) to a final concentration of 10% at 41°C or by lowering the temperature to a p21-activating 36°C without adding Ca^{2+}-deficient serum. The total proportion of cells which entered the S phase during the first 24 h after stimulation was determined autoradiographically, as described in the text. The points are means ± SEM of six cultures.

deprived *ts*K-NRK cells to transit G_1, replicate DNA, and ultimately divide, despite severe Ca^{2+} and serum deficiencies and without help from exogenous growth factors. In fact, as was first shown by Kaplan et al. (22) and confirmed by the results of this study, the viral protein is a complete mitogen than can drive cells through their whole cycle without any exogenously supplied growth factors. The viral protein does all of this without the increases in total RNA and protein syntheses that occur in serum-stimulated *ts*K-NRK cells. In this regard, p21 is like another viral mitogen, $pp60^{v\text{-}src}$, which also induces G_1 transit without increasing the rate of protein synthesis (14). Parenthetically, no other protein mitogen, to our knowledge, acts alone or in concert with other mitogens to reduce G_1 transit without increasing the rates of RNA and protein syntheses. However, p21-stimulated cells do need to make some RNA and proteins to transit G_1 because they can be prevented from initiating DNA replication by RNA and protein synthesis inhibitors such as actinomycin D and cycloheximide. This means that the p21-stimulated cells must make these critical RNA transcripts and proteins with the limited synthetic capabilities of quiescent cells, a restriction that makes them vulnerable to the inhibitors far longer than serum-stimulated cells with their much greater ability to rapidly accumulate and maintain pools of essential components.

p21 does not need any external growth factors to drive G_1 transit, and its prereplicative action is enhanced only slightly by cell-derived conditioning factors. However, while most of the p21-stimulated cells did not require conditioning factors to transit G_1, they did need these endogenous factors to divide. This suggests that conditioning factors produced by the cells as a result of p21 activity may play a role in later stages of the cell cycle.

In many of these respects, the viral p21 *ras* protein is like the $pp60^{v\text{-}src}$ *tyr* protein kinase of avian sarcoma virus (14). However, the two viral mitogens differ in one important respect. $pp60^{v\text{-}src}$ activity is needed only in early G_1 to commit cells to a round of DNA replication (14), while p21 activity is needed for most, if not all, of the G_1 phase. This difference might be due to the fact that $pp60^{v\text{-}src}$ is able to trigger a subsequently self-sustaining cascade of prereplicative events, while p21 cannot do this and must persist long enough to stimulate both early and late G_1 events.

It has recently been shown that normal cellular c-*ras* gene activity increases specifically in late G_1 (11) and that the c-*ras* product(s) is required at that late time to stimulate cellular events that are needed to initiate DNA replication in serum-stimulated mouse fibroblasts (26). Thus, the late G_1 function of the viral Ki-*ras* protein in the present study may be the same as the one carried out by the normal c-*ras* protein(s). However, the uncontrolled activity of the viral p21, on its activation by a shift from 41 to 36°C, is able to stimulate both early and late events that lead to DNA replication. The viral Ki-*ras* protein, therefore, behaves as a complete mitogen, and this abnormal multifunctional activity may be related to its oncogenicity.

The mitogenic action of the viral Ki-*ras* protein, and consequently at least part of its oncogenicity, might be mediated by Ca^{2+} and cyclic AMP. A link between the viral Ki-*ras* protein and Ca^{2+} is indicated by the ability of the protein to greatly reduce the amount of external Ca^{2+} needed by *ts*K-NRK cells to transit G_1. Thus, the Ki-*ras* protein may drive G_1 in part by stimulating extracellular Ca^{2+}-dependent events known to be needed for both the G_0 to G_1 and G_1 to S transitions in other cells (for a review, see reference 37). A link between the viral Ki-*ras* protein and cyclic AMP is suggested by the observation of Franks et al. (17) that this protein, directly or indirectly, stimulates cellular adenylate cyclase in the early G_1 phase of *ts*K-NRK cells.

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