scribed [L. M. Traub and R. Sagi-Eisenberg, *J. Biol. Chem.* **266**, 24642 (1991)] except that for the first dimension, 3.5% polyacrylamide rods containing 2% ampholytes (Pharmalyte 3–10 and 5–8, 1:4) were used and focusing was for 12,000 *V*·hours.

30. Antibodies used included affinity-purified AS/8 antibody to the α common peptide: CGAGESGK-STIVKQM (anti-G_{common}); AS/6 antibody to the $G\alpha_o$ peptide: CNLKEDGISAAKDVK (anti-G_o); AS119 antibody to the $G\alpha_{i1}$ peptide: LDRIAQPNYI (anti-G_{i1}); AS/64 antibody to the $G\alpha_{i2}$ peptide: CTGANKYDEAAS (anti-G_{i2}); AS/7 antibody to the $G\alpha_t$ peptide: KENLKDCGLF (known to recognize both $G\alpha_{i1}$ and $G\alpha_{i2}$) (anti-$G_{i1,2}$); EC antibody to the $G\alpha_{i3}$ peptide: KNNLKECGLY (anti-G_{i3}).
31. Permeabilization was done in the absence of divalent cations in a buffer (buffer A) containing 137 mM NaCl, 2.7 mM KCl, 5.6 mM glucose, bovine serum albumin (BSA) (1 mg/ml), and 20 mM Hepes (pH 7.5). The cells were permeabilized for 6 min at 30°C with 50 μM ATP in the presence of EGTA (15 μM). The cells were resealed by the addition of 3 mM $MgCl_2$ for 10 min, transferred and diluted in new tubes containing buffer A, and incubated for 10 min. The cells were then treated with 48/80 (5 μg/ml) for 20 min at 37°C. The reactions were quenched by the addition of ice-cold buffer without BSA. The cells were sedimented and the amount of secreted histamine was measured as described (*7*).
32. Purified mast cells suspended in buffer A (*31*) were allowed to adhere to glass cover slips for 90 min at 37°C under a humidified atmosphere of 93% air and 7% CO_2. The cells were washed twice with phosphate-buffered saline (PBS) and fixed for 30 min at room temperature in 3% paraformaldehyde. The cells were washed three times with PBS and transferred to ice. They were subsequently permeabilized on ice for 3 min with a buffer containing 100 mM NaCl, 300 mM sucrose, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM Pipes (pH 7.0), and 0.5% Triton X-100. After one wash with PBS and two washes with 0.05% Tween in PBS (Tween-PBS), the cells were incubated for 30 min at room temperature with affinity-purified AS/7 (33.5 μg/ml), affinity-purified EC (50 μg/ml), or antiserum to mannosidase II (1:200) diluted in Tween-PBS. After three washes with Tween-PBS buffer, the cells were incubated for 30 min at room temperature with lissamine rhodamine–labeled antibody [affinity-purified goat antibody to rabbit immunoglobulin G (Jackson Immunoresearch Laboratories, West Grove, PA), 1:40 dilution in Tween-PBS buffer]. Subsequently, the cells were washed once with Tween-PBS and twice with PBS. The cells were viewed on a Zeiss Axioskop microscope with a 100× Plan-Neofluar objective and photographed on Fujichrome 3200 film. For peptide inhibition, each antibody was incubated for 2 hours at room temperature with the respective peptide (100 μg/ml). For treatment with BFA, a similar labeling procedure was used. However, the adhered cells were washed and treated with BFA (10 μg/ml) for 10 min in PBS at 37°C before fixation.
33. We thank Y. Zick and J. R. Gillette for helpful discussions and a critical reading of this manuscript and A. M. Spiegel, G. Schultz, and J. G. Donaldson for their generous gift of antibodies. Supported by grants from the Israel Cancer Research Fund (R.S.E.), the Israel Academy of Sciences (R.S.E.), and the Israel Cancer Association (R.S.E.).

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A Link Between Cyclin A Expression and Adhesion-Dependent Cell Cycle Progression

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Cell adhesion has an essential role in regulating proliferation during the G_1 phase of the cell cycle, and loss of this adhesion requirement is a classic feature of oncogenic transformation. The appearance of cyclin A messenger RNA and protein in late G_1 was dependent on cell adhesion in both NRK and NIH 3T3 fibroblasts. In contrast, the expression of Cdc2, Cdk2, cyclin D1, and cyclin E was independent of adhesion in both cell lines. Transfection of NRK cells with a cyclin A complementary DNA resulted in adhesion-independent accumulation of cyclin A protein and cyclin A–associated kinase activity. These transfected cells also entered S phase and complete multiple rounds of cell division in the absence of cell adhesion. Thus, cyclin A is a target of the adhesion-dependent signals that control cell proliferation.

Adhesion to substratum is required for the proliferation of most mammalian cell types; nonadherent cells fail to proliferate despite the presence of growth factors and nutrients (*1*, *2*). In NRK and NIH 3T3 fibroblasts, this adhesion requirement can be explained in terms of a discrete cell cycle transition that is manifest in late G_1 and prior to the rise in histone H1 kinase activity characteristic of cells entering S phase (*2*–*6*). G_1/S histone H1 kinase activity likely results from the activation of the Cdc2 or Cdk2 cyclin-dependent kinase (Cdk) by cyclin A or cyclin E (*6*–*8*). Thus, we asked whether the synthesis of these Cdk's or cyclins was anchorage-dependent.

Adherent and nonadherent fibroblasts synchronized in G_0 were exposed to growth factors (fetal calf serum and epidermal growth factor) (*9*) and collected at times corresponding to transit through G_1 (*10*). RNA blot hybridizations (*11*) showed that the accumulation of Cdc2 and cyclin D1 mRNAs was independent of cell anchorage, whereas the accumulation of cyclin A mRNA was strictly anchorage-dependent in both NRK and NIH 3T3 cells (Fig. 1A). In adherent cells, cyclin A mRNA was first detected in late G_1 (Fig. 1A), suggesting a possible role for cyclin A in mediating anchorage-dependent cell cycle progression from late G_1 to S.

Adherent and nonadherent NRK and NIH 3T3 cells synchronized at early S phase by incubation with hydroxyurea (*2*) were analyzed for Cdk2, cyclin E, and cyclin A protein (Fig. 1B). Immunoblotting (*12*) showed that the overall amounts of Cdk2 and cyclin E proteins were anchorage-independent, although the distribution

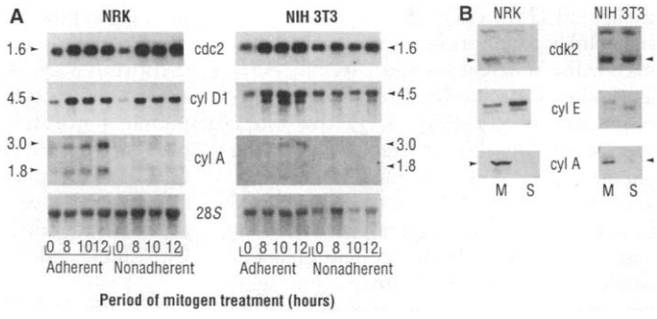

Fig. 1. Effect of cell adhesion on cell cycle–dependent expression of cyclin A. (**A**) Attachment-dependent expression of cyclin A mRNA. Adherent and nonadherent NRK and NIH 3T3 fibroblasts synchronized at G_0 were exposed to growth factors. Similar amounts of isolated total RNA (see 28*S*) were fractionated and hybridized to cDNA probes for $p34^{cdc2}$, cyclin D1, and cyclin A. Flow cytometry confirmed that both the adherent and nonadherent cells remained in G_1 throughout the time points tested. Molecular size markers are indicated at left (in kilobases). (**B**) Attachment-dependent expression of the cyclin A protein. Adherent (M, monolayer) and nonadherent (S, suspension) hydroxyurea-synchronized NRK and NIH 3T3 cells were prepared, collected, and extracted. Equal amounts of protein from each extract were subjected to immunoblot analysis. Cell viability was 90%, as determined by trypan blue exclusion, throughout the experiments.

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of cyclin E protein between its two isoforms (*13*) was anchorage-dependent in NIH 3T3 cells. Because this regulation of cyclin E isoforms was not observed in NRK cells, it is unlikely to be essential for maintaining the anchorage-dependent phenotype. In both cell types, however, accumulation of cyclin A was anchorage-dependent (Fig. 1B), consistent with the anchorage-dependent accumulation of its mRNA.

NRK and NIH 3T3 fibroblasts were infected with a cyclin A cDNA expressed from a retroviral promoter (*14*) in order to uncouple the expression of cyclin A from the adhesive state of cells. Cells were also infected with the vector alone and vectors encoding cyclin E and Cdk2 (*14*). G-418–resistant colonies were obtained for all infections, and both cell types overexpressed cyclin E and Cdk2 mRNAs. However, ectopically expressed cyclin A mRNA and protein was only detected in NRK cells. Infection of Rat-1 and primary human foreskin fibroblasts also failed to yield stable cyclin A expressors. Thus, cyclin A expression is likely controlled at multiple levels; our use of NRK cells was fortuitous in allowing us to examine the consequence of ectopic cyclin A expression.

RNA (Fig. 2A) and protein (Fig. 2B) blot analysis from asynchronous cultures of each NRK transfectant (NRK^{neo}, NRK^{cyA}, NRK^{cyE}, and NRK^{Cdk2}) (*15*) showed that cyclin A, cyclin E, and Cdk2 mRNAs and proteins were expressed by the corresponding infected cells in both the presence and absence of cell adhesion. The amounts of these proteins were three to five times those in cells infected with the vector alone (NRK^{neo}, Fig. 2B). All of these NRK transfectants cycled similarly in monolayer (*16*), indicating that overexpression did not alter cell cycle progression in general. However, only the cells expressing cyclin A were able to cycle in suspension (*17*) (Fig. 2C). Thus, cyclin A specifically restored cell cycling in nonadherent NRK fibroblasts. These results were observed in at least four separate experiments (with pools and isolated clones). NIH 3T3 transfectants overexpressing cyclin E or Cdk2 also failed to proliferate in suspension.

To demonstrate that ectopic expression of cyclin A rescues cell cycle progression at the G_1/S adhesion-dependent transition, we treated adherent and nonadherent NRK^{neo} and NRK^{cyA} cells synchronized in G_0 with growth factors and incubated them with [^{3}H]thymidine for consecutive 2-hour periods spanning S phase (*18*). Both NRK^{neo} and NRK^{cyA} cells entered S phase at 12 hours when cultured in monolayer, but only NRK^{cyA} cells entered S phase when cultured in suspension (Fig. 3A). Although the rate of entry into S phase was slower than that observed with the adherent counterparts, the expression of cyclin A at the G_1/S boundary was sufficient to override the adhesion requirement for G_1/S transit. Because the delayed entry into S phase was observed with two isolated NRK^{cyA} clones and a pool of NRK^{cyA} cells, heterogeneity in the cells expressing cyclin A cannot account for the altered kinetics of G_1/S transit. Thus, anchorage-dependent expression of cyclin A

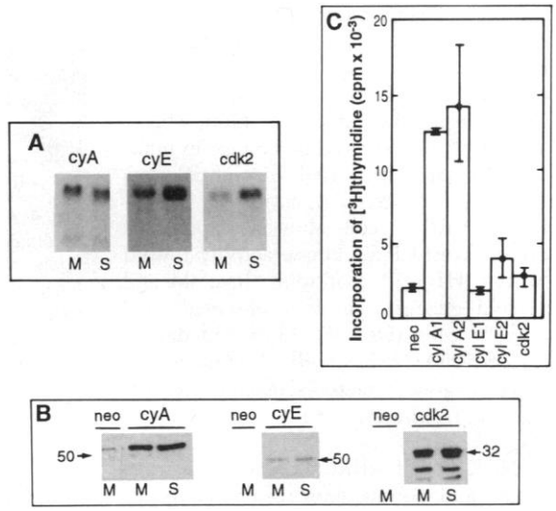

Fig. 2. Analysis of NRK transfectants. NRK transfectants were incubated for 2 days in growth factor–supplemented DMEM prior to collection and extraction. (**A**) Ectopic gene expression. RNA isolated from adherent (M, monolayer) and nonadherent (S, suspension) NRK^{cyA}, NRK^{cyE}, and NRK^{Cdk2} cells was fractionated and hybridized to the corresponding cDNA probe. Staining for ethidium bromide showed similar amounts of ribosomal RNA in each lane, and parallel hybridizations with RNA isolated from NRK^{neo} cells showed that the major bands represent specific hybridization to the ectopically expressed mRNA. (**B**) Ectopic protein expression. Extracts of adherent (M) and nonadherent (S) NRK^{cyA}, NRK^{cyE}, and NRK^{Cdk2} cells were analyzed by SDS–gel electrophoresis and immunoblotting with antibodies specific for the ectopically expressed protein. Results with NRK^{neo} cells show the corresponding endogenous expression for each ectopically expressed protein. (**C**) Cell cycling in nonadherent NRK transfectants. NRK^{neo}, NRK^{cyA}, NRK^{cyE}, and NRK^{Cdk2} cells were incubated in suspension for 2 days, and the extent of cell cycling was assessed by incorporation of [^{3}H]thymidine into DNA during the last 24 hours. Two distinct pools of stable transfectants were tested for cyclin A (cyl A1 and A2) and cyclin E (cyl E1 and E2). Cell viability was 90%.

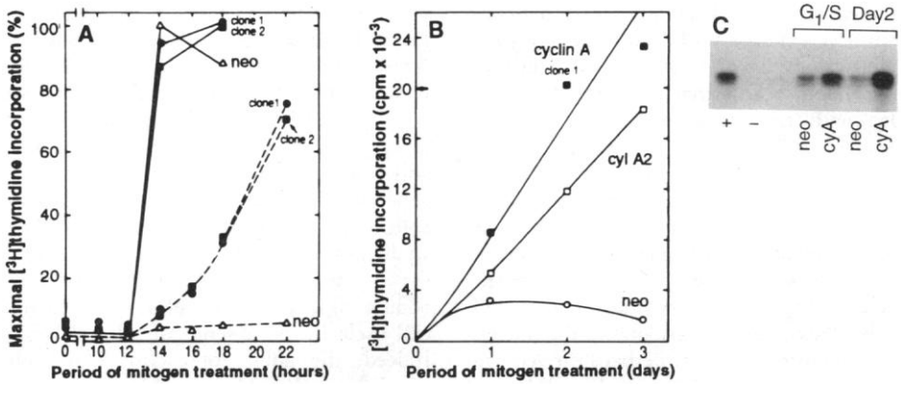

Fig. 3. G_1/S transit of NRK^{cyA} cells in the absence of cell attachment. (**A**) Entry into S phase. Adherent (solid lines) and nonadherent (dashed line) NRK^{neo} (△) and NRK^{cyA} [clone 1 (●) and clone 2 (□)] cells synchronized in G_0 were treated with growth factors, and the extent of DNA synthesis was determined from duplicate cultures by incorporation of [^{3}H]thymidine during consecutive 2-hour periods (ranges were within 10% of the means shown). NRK^{cyA} clone 1 and clone 2 were isolated from an NRK^{cyA} pool. (**B**) Sustained DNA synthesis in nonadherent NRK^{cyA} cells. Nonadherent NRK^{neo} (○) and NRK^{cyA} cells synchronized in G_0 [clone 1 (■) and the cylA2 pool (□)] were exposed to growth factors, and duplicate cultures were incubated with [^{3}H]thymidine for three consecutive 24-hour periods. Data are plotted as mean [^{3}H]thymidine incorporation minus background (ranges were within 7% of the means shown). Background [^{3}H]thymidine incorporation is defined as TCA-insoluble radioactivity in 1-day cultures lacking growth factors. The arrowhead shows the amount of [^{3}H]thymidine incorporation when NRK^{neo} and the NRK^{cyA} cells were incubated with growth factors for 1 day in monolayer. (**C**) Cyclin A–associated H1 kinase activity. Nonadherent NRK^{neo} and NRK^{cyA} cells synchronized in G_0 were exposed to growth factors, either for 16 hours in the presence of hydroxyurea (G_1/S) or for 2 days, before extraction and determination of histone H1 kinase activity. Symbols: (+) and (−) show the extent of histone H1 phosphorylation when extracts of adherent G_1/S-synchronized NRK^{neo} cells were incubated with antiserum to cyclin A and normal rabbit serum, respectively. NRK^{neo} and NRK^{cyA} remained 90% viable during 2 days in suspension.

underlies a large part, but not all, of the adhesion requirement for G_1/S transit.

The stimulation of G_1/S transit that we observed with the cyclin A transfectant was persistent: Growth factor–treated, nonadherent NRK^{cyA} fibroblasts continued to incorporate [^{3}H]thymidine into DNA (Fig. 3B) during three consecutive 24-hour incubations with radiolabel (*18*). The number of NRK^{cyA} cells also increased five- to sixfold during the 3-day incubation, whereas NRK^{neo} cells showed no change in number. Consistent with their differential rates of cell cycle progression, extracts of nonadherent NRK^{cyA} cells showed amounts of cyclin A–associated kinase activity toward histone H1 (*19*) that were threefold and fivefold greater than that observed with NRK^{neo} cells (Fig. 3C, G_1/S and day 2, respectively). NRK^{cyA} cells also formed colonies in growth factor–supplemented soft agar (*20*), whereas NRK^{neo} cells did not (Fig. 4).

NRK^{neo} and NRK^{cyA} cells have a well-spread and nonovergrowing phenotype in monolayer (Fig. 4). We never observed focus formation in NRK cells expressing the cyclin A gene despite the fact that these cells were anchorage-independent for growth. In fact, the phenotype of our cyclin A transfectants resembles that of NRK cell mutants that have lost their adhesion requirement, but not their growth factor requirement, for cell proliferation (*2*). In molecular terms, this might mean that constitutive expression of cyclin A is not sufficient to bypass the earlier growth factor–dependent events that now seem to correlate with the expression of cyclins D and E (*12, 21, 22*).

Most eukaryotic cells commit to cell division during the late G_1 phase of the cell cycle (*23, 24*). The notion of an irreversible commitment step during late G_1 in mammalian cells was first described in terms of the cellular response to growth factors (*23*), but cell adhesion also contributes to the process of commitment (*1, 2*). Perhaps the sequential synthesis and action of cyclins D, E, and A, together with that of their catalytic subunits, the Cdk's, may integrate the growth factor– and adhesion-induced signals that control the process of commitment to cell proliferation in high eukaryotes. Collectively, these events may comprise what has classically been called the restriction point in Balb/c 3T3 cells (*23*). They may also have roles analogous to the CLN gene products that mediate transit through START in *Saccharomyces cerevisiae* (*24*).

Anchorage-independent cell proliferation is a hallmark of oncogenic transformation, and perturbations in the expression or activity of cyclin A are associated with abnormal growth control (*3, 7, 25*). The results described here connect these observations by showing that (i) cyclin A expression is a target of anchorage-dependent signals and (ii) enforced expression of cyclin A induces anchorage-independent growth. These data both support and extend previous studies demonstrating that cyclin A is necessary for entering S phase (*4, 26*).

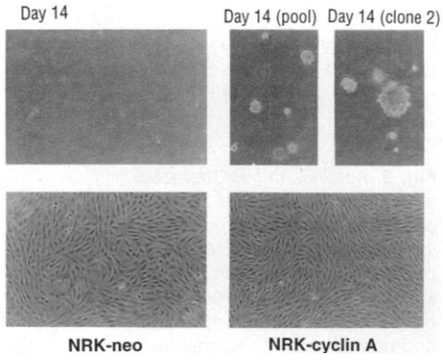

Fig. 4. Anchorage-independent growth of NRK fibroblasts ectopically expressing cyclin A. The figure shows NRK^{neo} (left panels) and NRK^{cyA} (right panels) cells that were cultured in growth factor–supplemented soft agar for 14 days (top panels) or brought to confluence in monolayer with DMEM containing 5% FCS (bottom panels). Each photograph was taken with a ×10 objective.

Cyclin A expression appears to be a general target of adhesion-dependent signaling since this link is observed in both NRK and NIH 3T3 cells. However, we succeeded in expressing cyclin A constitutively only in NRK cells. The similar phenotypes of cell cycle arrest in nonadherent NRK and NIH 3T3 cells (*2*) suggest that ectopic cyclin A expression could mediate adhesion-dependent signals in fibroblasts generally. But it is also possible that NRK cells represent a uniquely simple system for studying the role of cyclin A in adhesion-dependent cell proliferation. In other cells, additional levels of control may restrict the cell cycle in the absence of cell adhesion. Indeed, the failure of cyclin A to fully rescue normal proliferation kinetics in nonadherent NRK cells hints at the presence of other regulatory mechanisms.

Cell adhesion is mediated by the interaction of extracellular matrix proteins with cell surface integrin, but integrins also act as signaling receptors that stimulate tyrosine phosphorylation of certain cytosolic proteins (*27*). Some of the adhesion-dependent signals are likely the consequence of altered cell shape (*28*), but the existence of both growth factor– and integrin-stimulated kinase activities provides a direct biochemical basis for the existence of both growth factor– and adhesion-dependent cell cycle transitions. Regulation of cyclin A gene expression in NRK and NIH 3T3 cells provides an exquisite example of how these distinct signaling systems can cooperate at the molecular level: Adhesion induces cyclin A gene expression only after growth factors bring the cells to an adhesion-responsive portion of the cell cycle.

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8. Complexes between cyclin D1 and cdk's are also required for cell cycle progression through G_1, but histone H1 kinase activity has not been detected in association with cyclin D immunoprecipitates [(*22*); H. Matsushime *et al.*, *Cell* **71**, 323 (1992)].
9. Adherent and nonadherent NRK and NIH 3T3 fibroblasts (2.5×10^6 cells) synchronized in G_0 were prepared as described (*2*) except that the NIH 3T3 cell medium contained 0.2% calf serum. Cell cycle progression was stimulated by addition of growth factors (5% dialyzed fetal calf serum, 2 nM epidermal growth factor for NRK cells, and 5% calf serum for NIH 3T3 cells).
10. The G_0 to S phase interval is approximately 12 hours in both NRK and NIH 3T3 cells.
11. Total RNA was isolated from NRK and NIH 3T3 cells synchronized in G_0 and exposed to growth factors for the times indicated in Fig. 1A. We fractionated and hybridized equal amounts of RNA with nick-translated or random-primed DNA probes using the following final wash conditions: 1× saline-sodium phosphate-EDTA (SSPE), 0.2% SDS, 60°C (human Cdc2 and cyclin A probes); and 0.2× SSPE, 0.5% SDS, 42°C (human cyclin D1 probe). Exposure times were 12 to 24 hours (Cdc2 and cyclin D1) or 4 to 9 days (cyclin A). Poor cross-hybridization between the human Cdk2 and cyclin E probes and rodent mRNA precluded detection of these transcripts.
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13. The two cyclin E isoforms are thought to be encoded by alternatively spliced cyclin E mRNAs (K. Carlson, J. Schumacher, M. Ohtsubo, J. Roberts, unpublished observations).
14. Retroviruses encoding human cyclin A, cyclin E, and Cdk2 cDNAs were prepared with pLXSN and the PA317 or PE501 retrovirus packaging cell lines [A. D. Miller and G. J. Rosman, *BioTechniques* **7**, 980 (1989); R. A. Hock, A. D. Miller, W. R. A. Osborne, *Blood* **74**, 876 (1989); (*12*)]. Virus-containing medium was used to infect NRK and NIH 3T3 cell monolayers as follows: Day 1, 7.5×10^5 cells were plated in 10-cm dishes in 5% serum–Dulbecco's modified Eagle's medium (DMEM); Day 2, the medium was changed to 5% serum–DMEM containing polybrene, 4 μg/ml (Sigma) and 0.1 to 0.2 ml of virus-containing

medium; Day 3, the cells were split into 10-cm dishes at 1:3, 1:10, and 1:30 dilutions and cultured in 5% serum–DMEM containing geneticin. The medium was replaced every 3 to 4 days, and pools of G-418–resistant colonies (either 8 or >100 colonies for each infection) were prepared.
15. Exponentially growing monolayers of NRK^{neo}, NRK^{cyA}, NRK^{cyE}, and NRK^{cdk2} cells were trypsinized and seeded on uncoated or agar-coated 150-mm dishes (2×10^6 cells in DMEM containing growth factors). Duplicate cultures of each transfectant were incubated for 48 hours and collected. One set of cells was extracted for RNA blot hybridizations with equal amounts of ribosomal RNA (rRNA). The other set was extracted in 0.1 ml of nonreducing SDS sample buffer. Protein concentrations were estimated by SDS–gel electrophoresis and silver staining; normalized portions of each sample were subjected to the immunoblot analysis.
16. Adherent, subconfluent cultures of NRK^{neo}, NRK^{cyA}, NRK^{cyE}, and NRK^{cdk2} cells were prepared as in (*17*). After an overnight incubation, [^{3}H]thymidine (1 μCi/ml) was incubated with each culture for 24 hours. Cells were fixed with 5% trichloroacetic acid (TCA), and TCA-insoluble radioactivity was isolated. [^{3}H]thymidine incorporation varied by no more than 20% between the four cell lines. Standard mitogen assays showed that the interval from G_0 through S phase was also similar in NRK^{neo} and NRK^{cyA} cells.
17. Exponentially growing monolayers of NRK^{neo}, NRK^{cyA}, NRK^{cyE}, and NRK^{cdk2} cells (4×10^4 cells per 35-mm dish) were incubated in suspension with growth factors and [^{3}H]thymidine (*2*). Incorporation of radiolabel into DNA was determined during the last 24 hours of a 2-day incubation.
18. NRK^{neo} and NRK^{cyA} cells synchronized in G_0 were suspended (2×10^4 in 2 ml of DMEM containing growth factors) and added to 35-mm dishes coated with either 100 μg of type I collagen (adherent cultures) or agar (nonadherent cultures). S phase progression was monitored by incubation of the growth factor–supplemented cultures with [^{3}H]thymidine (1 μCi/ml) for the 2-hour periods shown in Fig. 3A. DNA synthesis over a 3-day period was determined similarly except that the incubation with [^{3}H]thymidine was for three consecutive 24-hour periods.
19. Cyclin A–associated kinase activity in extracts of adherent and nonadherent NRK cells (2.5×10^6) was determined similarly to the procedure described in (*2, 12*) except that (i) the extraction buffer consisted of 10 mM sodium phosphate buffer (pH 7.0), 0.25 M NaCl, 0.1% SDS, 1% NP-40, 1% sodium deoxycholate, 2 mM EDTA, 0.1 mM sodium orthovanadate, 50 mM sodium fluoride, aprotinin (10 μg/ml), leupeptin (10 μg/ml), and 1 mM phenylmethylsulfonyl fluoride; (ii) a 100-fold dilution of antiserum to cyclin A was used; and (iii) the immunoprecipitates were suspended in 30 μl of kinase buffer [J. Pines and T. Hunter, *Cell* **58**, 833 (1989)] containing 50 μM adenosine triphosphate (ATP), 5 μCi of [γ-^{32}P]ATP (3000 Ci/mmol), and 4 μg of histone H1.
20. The analysis in soft agar was done as described (*2*) except that 35-mm dishes were coated with 1 ml of 0.5% agar and top layers contained 2 ml of 0.3% agar with 10^4 cells. The cultures were incubated for 14 days, during which time the majority of cells in the NRK^{cyA} pool formed colonies in soft agar as did at least 80% of the cells from the isolated NRK^{cyA} clone.
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Characterization of a Pathway for Ciliary Neurotrophic Factor Signaling to the Nucleus

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Components of a signaling pathway that couples the ciliary neurotrophic factor (CNTF) receptor to induction of transcription were identified. CNTF stimulated the tyrosine phosphorylation of p91, a protein implicated in interferon signaling pathways, and of two proteins that are distinct but related to p91. Tyrosine-phosphorylated p91 translocated to the nucleus, where p91 and p91-related proteins bound to a DNA sequence found in promoters of genes responsive to CNTF. This DNA sequence, when inserted upstream of a reporter gene, conferred a transcriptional response to CNTF. A pathway that transduces interferon signals may therefore have a more general function in the propagation of responses to certain neurotrophic factors.

Ciliary neurotrophic factor enhances the survival and differentiation of distinct populations of neurons and glia (*1, 2*). It binds to the α component of its receptor and then sequentially associates with two structurally related β signal-transducing receptor components, gp130 and the leukemia inhibitory factor receptor β (LIFRβ), whose heterodimerization apparently transduces a signal across the membrane (*3, 4*). Activation of the receptors for CNTF and its related cytokines (*5*) results in phosphorylation of intracellular proteins on tyrosine and induction of transcription of immediate early genes (*6, 7*). However, the mechanisms by which signaling proceeds from the membrane to the nucleus remain almost completely unknown.

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A CNTF signaling pathway might share components of the signaling pathways for interferon alpha (IFN-α) and gamma (IFN-γ) (*8–10*). The CNTF receptor is related in structure to the IFN receptors (*11*). Protein tyrosine phosphorylation is required for signaling by CNTF and IFNs (*6, 9, 10*). An important DNA promoter site in genes activated by interleukin-6 (IL-6) (*12*) bears similarity to the IFN-γ–activated site (GAS) (*13*), which mediates transcriptional induction of IFN-γ–responsive genes. These observations and the finding that the CNTF and IL-6 receptors share the signal-transducing subunit gp130 (*5, 6*) suggest that the pathways that propagate the CNTF, IL-6, and IFN signals to the nucleus might also be similar.

A 91-kD protein (p91) becomes tyrosine-phosphorylated in cells exposed to IFN-α or IFN-γ (*9, 10*). Phosphorylated p91 then translocates to the nucleus where it participates in the transcriptional activation of interferon-responsive genes. It is believed that IFN-γ induces the binding of a p91 dimer to the GAS (*10*), and IFN-α causes the association of p91 with two newly tyrosine-phosphorylated 84- and 113-kD pro-