Translational Control of p27^{Kip1} Accumulation During the Cell Cycle

Ludger Hengst and Steven I. Reed*

Cell cycle phase transitions in eukaryotic cells are driven by regulation of the activity of protein kinases known as cyclin-dependent kinases (Cdks). A broad spectrum Cdk-inhibitory activity associated with a 28-kilodalton protein (p28^{Ick1}) was induced in cells treated with the drug lovastatin or upon density-mediated growth arrest and was periodic in the cell cycle, with peak activity in G_1. The p28^{Ick1} protein was shown to be identical to p27^{Kip1}, and the periodic or induced inhibitory activity resulted from a periodic accumulation of the protein. Variations in the amount of p27 protein occurred, whereas the abundance of the p27 messenger RNA remained unchanged. In every instance investigated, the posttranscriptional alteration of p27 protein levels was achieved in part by a mechanism of translational control, although in density-arrested fibroblasts and thymidine-arrested HeLa cells the half-life of the protein was also changed.

Cell cycle progression is regulated at several irreversible transition points, passage through which is controlled by the activity of Cdks (*1–3*). Progression from G_1 to S phase in mammalian cells is regulated by the accumulation of cyclins D, E, and A, which bind to and activate different Cdk catalytic subunits (*2*). However, cyclin accumulation and Cdk binding do not constitute the only levels of regulation of Cdk activity. Cdk activity is also regulated by both positive and negative phosphorylation events (*3*), as well as by association with inhibitory proteins (*4*). Two major classes of Cdk inhibitors have recently been identified in mammalian cells. Whereas p15 [p15^{INK4B,MTS2} (*5*)], p16 [p16^{INK4,MTS1} (*6*)], and p18 (*7*) specifically inhibit Cdk4 and Cdk6 by binding to the Cdk subunit alone, p21 [p21^{Cip1,Waf1,Sdi1} (*8, 9*)], p27 [p27^{Kip1}, p28^{Ick1} (*10–13*)], and p57 [p57^{Kip2} (*14*)] can bind to and inhibit a broad range of Cdk-cyclin complexes.

We identified a heat-stable Cdk-inhibitory activity that was cell cycle–regulated and accumulated in lovastatin-arrested HeLa cells (*13*). This activity could be attributed to a 28-kD protein, p28^{Ick1}. We purified the protein from lovastatin-arrested HeLa cells and isolated the corresponding complementary DNA (cDNA) on the basis of peptide sequences obtained (*15*). Both peptide and cDNA sequences were identical to those of the Cdk inhibitor p27^{Kip1} (*11, 12, 15*). The p27^{Kip1} protein is implicated in the negative regulation of G_1 progression in response to a number of antiproliferative signals (*11*). For example, studies in macrophages have linked cyclic AMP–induced growth arrest to an increase in the amount of p27^{Kip1} protein, whereas the drug rapamycin abrogates a small reduction in p27 abundance observed after colony-stimulating factor–1 stimulation (*16*). Likewise, interleukin-2–induced proliferation of T cells results in a decrease in the amount of p27^{Kip1} protein, an effect that can be prevented by addition of the antiproliferative drug rapamycin (*17*).

On the basis of our previous observations, we investigated whether p27 protein or mRNA levels were induced in response to lovastatin treatment. HeLa cells treated with lovastatin accumulated increased amounts of p27 protein over the course of treatment compared with asynchronous cells (Fig. 1). In contrast, lovastatin caused no increase in p27 mRNA relative to asynchronous cells (Fig. 1), suggesting a posttranscriptional mode of regulation.

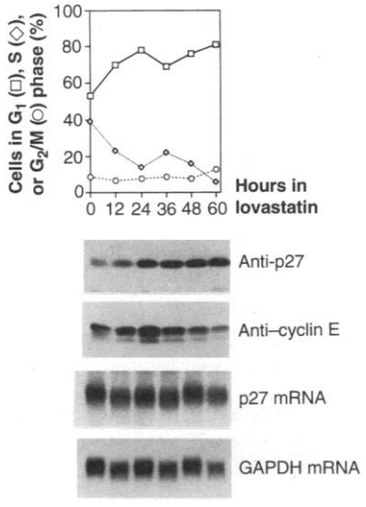

Fig. 1. Posttranscriptional regulation of p27 abundance in lovastatin-arrested cells. HeLa cells were treated with lovastatin, and samples were taken over the next 60 hours. The cell cycle distribution in G_1, S, or G_2/M phase was determined by FACS analysis of BrdU pulse–labeled cells. Protein extracts from cells at each time point were immunoblotted with antibodies to p27 (anti-p27) or cyclin E (anti–cyclin E) (*24*). RNA was isolated from the same samples and hybridized with probes specific for p27 mRNA or glyceraldehyde phosphate dehydrogenase mRNA (GAPDH mRNA) (*24*).

To determine whether posttranscriptional control represented a general pattern of p27 regulation, we analyzed various cell lines for the regulation of p27 protein levels. Human diploid fibroblasts accumulate p27 when they exit the cell cycle and enter a quiescent state as they grow to high density (*10, 13, 17*). Therefore, asynchronous cells were analyzed for p27 mRNA and protein as they underwent density-mediated arrest. Whereas the level of p27 mRNA remained constant during the exit from the cell cycle and maintenance in a quiescent state, p27 protein accumulated to large amounts (Fig. 2), demonstrating that this accumulation of p27 is also regulated posttranscriptionally.

HL60 promyelocytic leukemia cells can be induced to exit the cell cycle and differentiate into monocytes by treatment with 1α,25-dihydroxy vitamin D3 and indomethacin (*18*). Because p27 inhibitory activity exists in this cell line (*13*), we examined whether p27 accumulation was correlated with cell cycle exit associated with differentiation. HL60 cells were monitored for several days as they differentiated and ceased proliferation in response to treatment with vitamin D3. Under these conditions, p27 protein accumulated in the absence of growth to high density (Fig. 3). Thus, p27 may be involved in exit from the cell cycle in conjunction with differentiation. As in the

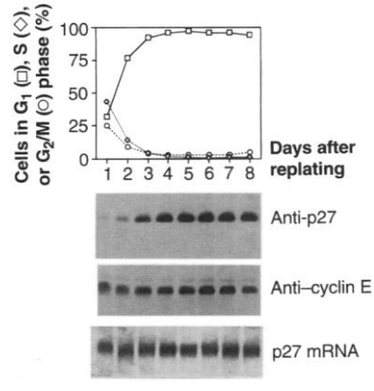

Fig. 2. Posttranscriptional regulation of p27 abundance in growth-arrested fibroblasts. Human diploid fibroblasts were analyzed as they grew into density-mediated growth arrest. Samples were taken at daily intervals after replating. Cells at each time point were analyzed for their distribution in the cell cycle, amounts of p27 and cyclin E proteins, and p27 mRNAs as described (Fig. 1). Equal amounts of total protein or mRNA were examined (*24, 25*).

Department of Molecular Biology, MB-7, Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA.

*To whom correspondence should be addressed.

previous instances, the regulation of p27 accumulation was not attributable to transcriptional control, because p27 mRNA levels remained constant during cell differentiation (Fig. 3). Therefore, p27 accumulation appears to be regulated by a posttranscriptional mechanism in cells that undergo cell cycle arrest or exit from the cell cycle.

Inhibitory activity of p27 is periodic in the cell cycle, with peak activity in G_1. To investigate the cell cycle regulation of inhibitory activity, we synchronized HeLa cells and observed their passage from mitosis through G_1 into S phase. When lysates were analyzed for the abundance of p27 protein, a periodicity was observed similar to that of the heat-stable Cdk-inhibitory activity (Fig. 4A). Cells released from nocodazole had no detectable amounts of p27; accumulation of p27 began as cells progressed into G_1 and decreased as cells entered S phase. To determine the periodicity of the p27 levels over the entire cell cycle, we synchronized HeLa cells with a double thymidine block and release protocol. When these cells were released from the final thymidine block and monitored through S, G_2/M phase, and then through G_1 to the next S phase, the amount of p27 decreased from the release point in S phase and then increased during the subsequent G_1 interval, indicating a single peak of accumulation during the G_1 phase of each cell cycle (*19*). Because the amount of protein paralleled the inhibitory activity in all instances investigated, protein abundance rather than protein modification seems to be the primary mode of regulation of p27 activity. Similarly, analysis of elutriated HL60 promyelocytic leukemia cells revealed a strong correlation between a G_1-specific inhibitory activity (*13*) and cell cycle–regulated levels of p27 that peak in G_1 (*19*).

Analysis of mRNA from the nocodazole-synchronized HeLa cells demonstrated no fluctuation in p27 mRNA levels (Fig. 4A). This result demonstrates that the cell cycle function of p27 is regulated at the level of protein accumulation by posttranscriptional mechanisms.

To investigate cell cycle–dependent periodicity of p27 in a nontransformed cell line, we synchronized HS68 human diploid fibroblasts by density-mediated growth arrest. The released cells were monitored through the cell cycle; as in HeLa cells, p27 accumulated in G_1 (Fig. 4B). Because of imperfect synchronization (only about 60% of the cells entered G_1 synchronously), the variation in the amount of p27 observed should be taken as an underestimate. The persistent basal amount of p27 is most likely due to cells that still remain in G_0 during the course of the experiment.

To determine whether p27 protein levels are regulated at the level of turnover or by translational control, we measured the rate of ^{35}S-methionine and ^{35}S-cysteine incorporation into p27 and the half-life of the labeled protein. Compared with asynchronous HeLa cells, lovastatin-arrested cells synthesized p27 at an increased rate. The different rates of translation (three times more p27 was synthesized during a 1-hour pulse in lovastatin-arrested cells) appear to account for the different steady-state amounts of protein observed under these conditions, because the half-life of the p27 protein (2.5 hours) was identical for arrested and asynchronous HeLa cells (Fig. 5A and Table 1). Small differences in the stability of p27 apparently do not influence the amount of radioactivity incorporated into p27, because even when the time of incorporation was much shorter than the

Table 1. Half-life of p27 in HeLa cells and human diploid fibroblasts. All data were quantitated on the basis of PhosphorImager scans.

Cell line	Growth condition	Half-life (hours)
HeLa	Asynchronous	2.5
HeLa	Lovastatin arrest	2.5
HeLa	Thymidine block	0.4
HS68	Asynchronous	2
HS68	Contact inhibited	>5

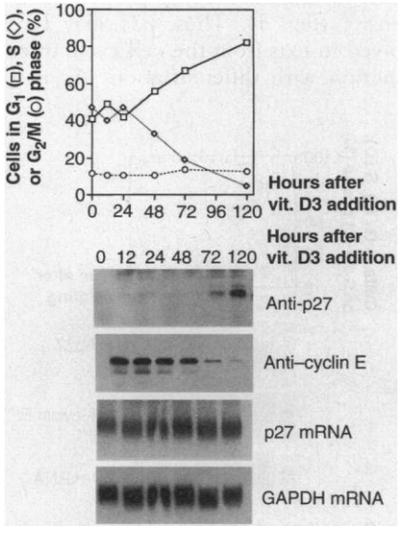

Fig. 3. Induction of p27 in HL60 cells treated with vitamin D3. Differentiation to monocytes was induced by treatment with 1α,25 dihydroxy vitamin D3 and indomethacin. Samples were taken at 12-hour intervals after addition of the differentiation agent. The distribution of cells in the cell cycle, the amounts of p27 and cyclin E protein, and the amounts of p27 and GAPDH mRNA were determined as described (Fig. 1) (*24, 26*).

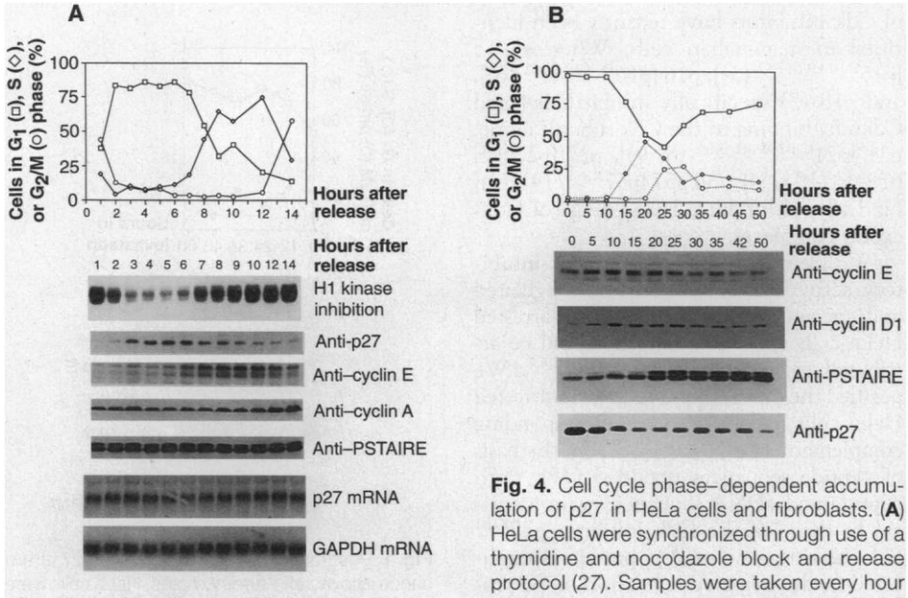

Fig. 4. Cell cycle phase–dependent accumulation of p27 in HeLa cells and fibroblasts. (**A**) HeLa cells were synchronized through use of a thymidine and nocodazole block and release protocol (*27*). Samples were taken every hour after release from the nocodazole block. The extent of cell cycle synchrony was determined by FACS analysis. Heat-stable Cdk inhibitory activity was measured with cyclin A–associated kinase as a substrate as described (*13*). Protein extracts from each time point were analyzed for the amount of p27 (anti-p27), cyclins (anti–cyclin E and anti–cyclin A), or Cdks (anti-PSTAIRE) by immunoblotting. RNA was isolated from the same samples and hybridized with probes specific for p27 mRNA or GAPDH mRNA (*24, 27*). (**B**) Accumulation of p27 during G_1 in human diploid fibroblasts. HS68 cells were synchronized in G_0 by density-mediated growth arrest. Arrested cells were released by plating at low density and monitored through the cell cycle (*28*). The fraction of cells in G_1, S, or G_2/M phase was determined by FACS analysis of cells exposed briefly to BrdU. Abundance of various proteins was analyzed as in (A).

half-life of p27, the same difference in the amount of labeled p27 was observed. Thus, a difference in the rate of synthesis is responsible for the observed different accumulation of p27 protein.

Compared with asynchronous cells, thymidine-arrested (S phase) cells showed reduced accumulation of labeled p27 (Fig. 5, A and C). However, the half-life of the protein was reduced compared with that of asynchronous or lovastatin-arrested cells (Fig. 5, A and B, and Table 1). Using two shorter times of incorporation, 15 and 30 min, we compared the results with the experiment shown in Fig. 5A. We observed similar differences (over threefold in every case) in rates of synthesis for thymidine-arrested cells and asynchronous cells (Fig. 5C). Therefore, translational control of p27 synthesis also contributes to the decrease in p27 levels observed during thymidine arrest.

To examine whether translational control is a general mechanism of regulation of p27 abundance, we also analyzed asynchronously growing and contact-inhibited human diploid fibroblasts (Fig. 6). An increase in the rate of p27 translation was detected in the arrested cells (Fig. 6A), indicating that an increase in p27 translation is at least in part responsible for the increase in the steady-state amount of p27 observed in density-arrested cells (Fig. 2). In addition, the half-life of the p27 protein was shown to be increased in the arrested cells (Fig. 6, A and B, and Table 1). The importance of translational control for the induction of p27 in density-arrested cells was further confirmed by an experiment (Fig. 6C) in which radioactive amino acids were present for a time (30 min) much shorter than the half-life of the protein in asynchronous cells (2 hours) (Fig. 6B and Table 1). Thus, in density-arrested fibroblasts, the abundance of p27 is regulated both at the level of translation and turnover.

Ubiquitin-regulated degradation of p27 contributes to regulation of the amount of p27 in serum-starved fibroblasts (*20*). Our results indicate that translational control is in part or predominantly responsible for the regulation of p27 abundance under various conditions. A rapid increase in translation of p27 may be essential for negative regulation of G_1 progression in response to antiproliferative signals. Alternatively, regulation of the half-life of the protein could be important for maintaining the arrested state.

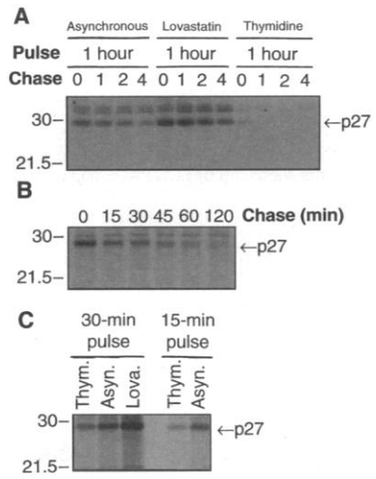

Fig. 5. Translational control of p27 levels in HeLa cells. (**A**) Thymidine- or lovastatin-synchronized cells or asynchronous cells were incubated for 1 hour with ^{35}S-methionine and ^{35}S-cysteine (pulse) and subsequently incubated in the presence of an excess of nonradioactive methionine and cysteine for the additional times indicated (chase). Cells were lysed, extracts were adjusted for equal amounts of protein, and p27 was precipitated from nondenatured protein extracts with polyclonal antibodies (*23*). The immunoprecipitates were separated by SDS-PAGE, and the radioactive p27 was detected by autofluorography. (**B**) Thymidine-arrested HeLa cells were labeled for 30 min and chased for the times indicated. p27 was immunoprecipitated from denatured protein extracts with a polyclonal anti-peptide antibody, the precipitates were separated, and radioactive p27 was detected by autoradiography (*29*). (**C**) Thymidine (Thym.)- or lovastatin (Lova.)-synchronized or asynchronous (Asyn.) HeLa cells were labeled for 15 or 30 min with ^{35}S-methionine and ^{35}S-cysteine. p27 was immunoprecipitated from protein extracts of these cells, separated by SDS-PAGE, and detected by autoradiography (*23*). Molecular size standards are indicated on the left (in kilodaltons).

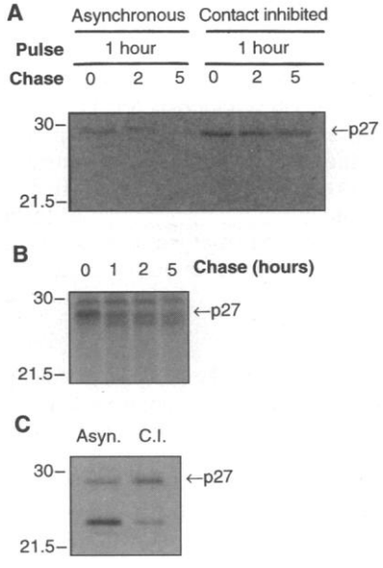

Fig. 6. Translational control of p27 levels in human diploid fibroblasts. (**A**) A pulse-chase experiment was performed with human diploid fibroblasts (HS68). Contact-inhibited cells or asynchronous cells were pulse-labeled for 1 hour with ^{35}S-methionine and ^{35}S-cysteine (pulse) and subsequently incubated in the presence of an excess of nonradioactive methionine and cysteine for the additional times indicated (chase). Cells were lysed, extracts were adjusted for equal cpm, and p27 was immunoprecipitated with a polyclonal anti-peptide antibody. The immunoprecipitates were separated by SDS-PAGE, and p27 was detected by autofluorography (*30*). (**B**) Asynchronously growing HS68 cells were labeled for 90 min and incubated in the presence of nonradioactive methionine and cysteine for 0, 1, 2, or 5 hours. p27 was precipitated from protein extracts of these cells and analyzed by SDS-PAGE and autoradiography (*31*). (**C**) Contact-inhibited or asynchronous cells were labeled with ^{35}S-methionine and ^{35}S-cysteine for 30 min, protein extracts of the cells were denatured by boiling, and p27 and p21 were immunoprecipitated with antibodies generated against a conserved peptide sequence of these inhibitor proteins. The precipitates were separated, and p27 and p21 (lower band) were detected by autofluorography (*32*). Molecular size standards are indicated on the left (in kilodaltons).

REFERENCES AND NOTES

1. R. W. King, P. K. Jackson, M. W. Kirschner, *Cell* **79**, 563 (1994); J. Pines, *Trends Biochem. Sci.* **18**, 195 (1993).
2. C. J. Sherr, *Cell* **79**, 551 (1994); G. F. Draetta, *Curr. Biol.* **6**, 842 (1994).
3. T. R. Coleman and W. G. Dumphy, *Curr. Opin. Cell Biol.* **6**, 877 (1994).
4. C. J. Sherr and J. M. Roberts, *Genes Dev.* **9**, 1149 (1995); G. Peters, *Nature* **371**, 204 (1994).
5. G. J. Hannon and D. Beach, *Nature* **371**, 257 (1994).
6. M. Serrano, G. J. Hannon, D. Beach, *ibid.* **366**, 704 (1993).
7. K.-L. Guan *et al.*, *Genes Dev.* **8**, 2939 (1994).
8. W. S. El-Deiry *et al.*, *Cell* **75**, 817 (1993); J. M. Slingerland, L. Hengst, D. Alexander, M. R. Stampfer, S. I. Reed, *Mol. Cell. Biol.* **14**, 3683 (1994).
9. J. W. Harper, G. R. Adami, N. Wei, K. Keyomarsi, S. J. Elledge, *ibid.*, p. 805; Y. Xiong *et al.*, *Nature* **366**, 701 (1993); A. Noda, Y. Ning, S. F. Venable, O. M. Pereira-Smiths, J. R. Smith, *Exp. Cell Res.* **211**, 90 (1994).
10. K. Polyak *et al.*, *Genes Dev.* **8**, 9 (1994).
11. K. Polyak, M. H. Lee, H. Erdjument-Bromage, P. Tempst, J. Massague, *Cell* **78**, 59 (1994).
12. H. Toyoshima and T. Hunter, *ibid.*, p. 67.
13. L. Hengst, V. Dulic, J. Slingerland, E. Lees, S. I. Reed, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 5291 (1994).
14. M. H. Lee, I. Reynisdóttir, J. Massague, *Genes Dev.* **9**, 639 (1995); S. Matrsuoka *et al.*, *ibid.*, p. 650.
15. To purify the inhibitor protein from lovastatin-arrested HeLa cells, we separated extracts obtained from 25 liters of arrested culture by size chromatography on a Superdex 200 column (Pharmacia). Fractions containing inhibitory activity (150 to 250 kD) were collected, and the proteins of these fractions were denatured at 100°C for 5 min. The heat-stable proteins were separated by Superdex 200 chromatography, and the proteins of fractions containing inhibitory activity were separated by SDS–polyacrylamide gel electrophoresis (PAGE) and transferred to a polyvinylidene difluoride membrane. The resulting band at 28 kD was digested by trypsin and peptides were microsequenced. The following peptide sequences were obtained: NLFGPDHEEL, NDFQNHKP, YEWQEVEK, LPEFYYRP, and RPQFR (*21*). The first two peptides exhibit more than 50% sequence identity with the sequence of the inhibitor p21 and were therefore used to design degenerate oligonucleotides to perform a PCR reaction that resulted in the cloning of an incomplete p27 cDNA. Using this cDNA fragment as probe, we cloned the complete p27cDNA sequence.
16. J. Kato, M. Matsuoka, K. Polyak, J. Massague, C. J. Sherr, *Cell* **79**, 487 (1994).
17. J. Nourse *et al.*, *Nature* **372**, 570 (1994).
18. C. M. Bunce *et al.*, *Leukemia* **8**, 595 (1994).
19. L. Hengst and S. I. Reed, unpublished data.

20. M. Pagano *et al.*, *Science* **269**, 682 (1995).
21. Abbreviations for the amino acid residues are as follows: C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; K, Lys; L, Leu; N, Asn; P, Pro; Q, Gln; R, Arg; T, Thr; V, Val; W, Trp; and Y, Tyr.
22. V. Dulić, E. Lees, S. I. Reed, *Science* **257**, 1958 (1992).
23. HeLa cells were grown to 3×10^5 cells/ml and arrested with thymidine (2 mM) or lovastatin (40 μM) for 24 hours. Cells (5×10^7) were collected by centrifugation, washed in phosphate-buffered saline (PBS), and resuspended in 50 ml of Dulbecco's modified Eagle's medium (DMEM) without methionine and cysteine (ICN) and with thymidine, lovastatin, or no drug. Tran^{35}S label (1 mCi; ICN) was added, and the cells were pulse-labeled for 60 min. After the pulse, methionine and cysteine were added to final concentrations of 100 or 150 mg/liter. At the time points shown, samples of cells were collected by centrifugation, washed in PBS, and lysed in RIPA buffer [in PBS: 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, phenylmethylsulfonyl fluoride (PMSF; 0.1 mg/ml), aprotinin (30 μl/ml; Sigma), and 0.1 mM sodium orthovanadate]. Protein concentrations were determined by absorbance at 280 nm, and equal amounts of protein extracts were used for p27 immunoprecipitation with an antibody to p27, generated against amino acids 181 to 198 of p27 [p27-C19 (Santa Cruz Biotechnology, Santa Cruz, CA), suitable for immunodepletion of the nondenatured protein]. Immune complexes were collected on protein A–Sepharose beads and washed five times in RIPA buffer and once in RIPA buffer containing methionine and cysteine (each at 1 mg/ml). The immunoprecipitates were separated by SDS-PAGE, treated with Amplify (Amersham), and used for autofluorography. For pulse-labeling experiments, increased amounts of cells (3.5×10^7 cells/10 ml) and radioactivity (1.5 mCi/10 ml) were used, and cells were starved for 60 min in medium lacking methionine and cysteine before labeling.
24. HeLa cells were grown in suspension culture at densities of 2×10^5 to 6×10^5 cells/ml in DMEM supplemented with newborn calf serum (10%). Asynchronous growing cells were arrested in G_1 by treatment with 66 μM lovastatin for 33 hours as described [K. Keyomarsi, L. Sandoval, V. Band, A. B. Pardee, *Cancer Res.* **51**, 3602 (1991)]. For fluorescence-activated cell sorting (FACS) analyses, 5-ml samples were labeled for 15 min with bromodeoxyuridine (BrdU; 30 μg/ml). The distribution of the cells in the cell cycle was determined with monoclonal antibodies to BrdU (anti-BrdU) and staining with propidium iodide (Becton Dickinson) according to the manufacturer's directions. Proteins and RNA were isolated with the Tri Reagent system (Molecular Research Center). Immunoblots of cyclins, Cdks, and p27 were performed with polyclonal (anti-p27 and anti–cyclin A) or monoclonal antibodies (anti–cyclin E and anti-PSTAIRE) as described (*22*). Antibodies to p27 were generated against a peptide sequence (CRNLFGPVDHEELTRDLE) (*21*) from the p27 protein, and antibodies affinity-purified against the immunogenic peptide were used. Polyclonal antibodies to p21 were generated with the full-length protein as antigen. RNA was separated through 1% agarose gels, transferred to Nytran plus membrane (Schleicher & Schuell), and hybridized at 65°C in 1 M NaCl, 10% dextran sulfate, 1% SDS, and salmon testis DNA (100 μg/ml).
25. Asynchronously growing human newborn foreskin fibroblasts (HS68) cells were diluted 1:3, seeded in DMEM supplemented with fetal bovine serum (FBS; 10%) at 20% confluency, and grown into contact inhibition. For FACS analyses, cells on one plate were labeled for 30 min with BrdU (30 μg/ml).
26. HL60 human promyelocytic leukemia cells were grown in RPMI 1640 medium supplemented with 20% heat-inactivated FBS. Differentiation to monocytes was induced by adding 1α, 25-dihydroxy vitamin D3 (Biomol; 10^{-7} M final concentration) and indomethacin (Biomol; 18.75 μg/ml final concentration).
27. HeLa cells were grown in suspension culture at densities of 2×10^5 to 6×10^5 cells/ml in DMEM supplemented with newborn calf serum (10%). Cells were synchronized by a thymidine and nocodazole block release protocol with 2 mM thymidine for 19 hours, released for 3 hours, and treated with nocodazole (75 ng/ml) for 12 hours. Samples of the synchronized cells were harvested every hour after release from the nocodazole block, and the percentage of cells in different phases of the cell cycle was determined by FACS analysis as described (*13*).
28. HS68 human diploid fibroblasts were grown to confluency and incubated for three additional days in DMEM growth medium, supplemented with 10% heat-inactivated FBS. Cells were trypsinized and seeded in a dilution of 1:5. Samples were taken at 3-hour intervals after release from the contact inhibition. For FACS analyses, samples were labeled for 15 min with BrdU (30 μg/ml).
29. HeLa cells were incubated in 2 mM thymidine for 22 hours. For each time point, a 10-ml cell suspension was incubated in DMEM without methionine and cysteine (ICN) at 6×10^6 cells/ml for 30 min. After addition of 1.8 mCi Tran^{35}S label (ICN) per sample (10 ml), cells were labeled for 30 min and incubated for 0, 15, 30, 60, or 120 min in the presence of methionine (100 mg/liter) and cysteine (150 mg/liter). Cells were collected by centrifugation, washed in PBS, and lysed in RIPA buffer (in PBS: 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, PMSF (0.1 mg/ml), aprotinin (30 μl/ml; Sigma), and 0.1 mM sodium orthovanadate). Protein concentrations were determined by absorbance at 280 nm, and samples were adjusted for equal protein amounts. Proteins were denatured by boiling at 100°C for 5 min. p27 was precipitated with a polyclonal antibody to p27 (C19) (*23*). Immune complexes were collected on protein A–Sepharose beads and washed seven times in RIPA buffer and once in RIPA buffer containing methionine and cysteine (each at 1 mg/ml). The immunoprecipitates were separated by SDS-PAGE and used for autofluorography.
30. HS68 human diploid fibroblasts were grown to contact inhibition and used after 6 days of density-mediated growth arrest. Asynchronous cells were grown to <30% confluency on plates. Labeling was done as described (*23*). Labeled cells were washed with PBS, scraped in RIPA buffer, extracts were normalized for equal counts per minute (cpm), and p27 immunoprecipitated as described (*23*).
31. HS68 human diploid fibroblasts were labeled 4 days after density-mediated growth arrest. Asynchronous cells were grown to <30% confluency on plates. Pulse-chase labeling was done as described (*23*). The labeled cells were washed with PBS, trypsinized, and washed several times with ice-cold PBS. Cells were lysed in RIPA buffer, the extracts were normalized for equal cpm, and p27 immunoprecipitated as described (*23*).
32. Contact-inhibited HS68 human diploid fibroblasts were labeled 3 days after density-mediated growth arrest cells. Asynchronous cells were grown to 45% confluency on plates. Cells were labeled for 30 min with 350 μCi of Tran^{35}S label in 10 ml of DMEM without methionine and cysteine (ICN) (per plate). Cells were washed in ice-cold PBS and scraped in PBS containing 1% NP-40 and PMSF (0.1 mg/ml), aprotinin (30 μl/ml; Sigma), and 0.1 mM sodium orthovanadate. The extracts were normalized for equal cpm and boiled for 10 min. The heat-stable supernatant containing almost all p27 protein was adjusted to 0.5% sodium deoxycholate and 0.1% SDS. The p27 protein was precipitated with affinity-purified antibody generated against a p27 peptide sequence (*24*). The second antibody cross-reacts with the p21 protein. Immunoprecipitates were washed once in RIPA and three times in PBS containing 1% NP-40, separated by SDS-PAGE, and analyzed by autofluorography.
33. We thank A. W. Alberts for lovastatin; T. Hunter, R. Poon, and H. Toyoshima for communicating results before publication; and S. Haase, F. Melchior, B. Niculescu, and K. Sato for critical reading of the manuscript. Supported by fellowships from the Leukemia Society of America (to L.H.) and by U.S. Public Health Service grant GM46006 and U.S. Army grant DAMD17-94-J4208 (to S.I.R.).

21 August 1995; accepted 30 January 1996

Altered Sensory Processing in the Somatosensory Cortex of the Mouse Mutant Barrelless

E. Welker,* M. Armstrong-James, G. Bronchti, W. Ourednik, F. Gheorghita-Baechler, R. Dubois, D. L. Guernsey, H. Van der Loos, P. E. Neumann

Mice homozygous for the *barrelless* (*brl*) mutation, mapped here to chromosome 11, lack barrel-shaped arrays of cell clusters termed "barrels" in the primary somatosensory cortex. Deoxyglucose uptake demonstrated that the topology of the cortical whisker representation is nevertheless preserved. Anterograde tracers revealed a lack of spatial segregation of thalamic afferents into individual barrel territories, and single-cell recordings demonstrated a lack of temporal discrimination of center from surround information. Thus, structural segregation of thalamic inputs is not essential to generate topological order in the somatosensory cortex, but it is required for discrete spatiotemporal relay of sensory information to the cortex.

Segregation in the processing of peripheral information is a common principle in the organization of sensory cortical areas. This principle was first demonstrated in the primary visual cortex of cats and monkeys, in which segregation of thalamocortical axons forms the anatomic basis for ocular dominance columns (*1*). In rodents, the pattern of mystacial whisker follicles is replicated in layer IV of the primary somatosensory cortex (SI) by an array of cell clusters named "barrels" (*2*). Each barrel is activated by an individual whisker, the "center" whisker, through fast thalamocortical relay, but it also integrates information from neighboring whiskers (*3*) through slower intracortical circuitry (*4*). The one-to-one correspondence between whisker follicles and their