Redundant cyclin overexpression and gene amplification in breast cancer cells

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ABSTRACT Cyclins are prime cell cycle regulators and are central to the control of major check points in eukaryotic cells. The aberrant expressions of two cyclins (i.e., cyclins A and D1) have been observed in some cancers, suggesting they may be involved in loss of growth control. However, in spite of these occasional changes involving only two cyclins, there are no clear connections between general derangements of other cyclins or their dependent kinases in a single tumor type. We detected general cyclin overexpression in 3 of 3 breast tumor tissue samples. In addition, using proliferating normal vs. human tumor breast cells as a model system, we observed a number of alterations in cyclin expression: (i) an 8-fold amplification of cyclin E gene in one tumor line, a 64-fold overexpression of its mRNA, and altered expression of its protein; (ii) deranged expression of cyclin E protein in all (10 of 10) tumor cell lines studied; (iii) increased cyclin mRNA stability, resulting in (iv) general overexpression of RNAs and proteins for cyclins A and B and CDC2 in 9 of 10 tumor lines and (v) deranged order of appearance of cyclins in synchronized tumor vs. normal cells, with mitotic cyclins appearing prior to G1 cyclins. These multiple general derangements in cyclin expression in human breast cancer cells provide evidence linking aberrant cyclin expression to tumorigenesis.

Cyclins were first identified in marine invertebrates on the basis of their dramatic cell cycle periodicity during meiotic and early mitotic divisions (1–3). More than 30 cyclin sequences are now available for comparison. They fall into three categories; A-type, B-type, and G1 cyclins (cyclins C, D1–D3, and E). These can be distinguished on the basis of conserved sequence motifs, patterns of appearance, and apparent functional roles during specific phases and check points of the cell cycle in a variety of species (for review, see refs. 4 and 5).

Cyclins function by forming a complex with and activating a family of cyclin-dependent protein kinases (CDKs), at various stages in the cell cycle. The activated kinase starts a complex kinase cascade that directs the cell into DNA synthesis and/or mitosis (6–8). Since the major regulatory events leading to proliferation in animal cells occur in the G1 phase of the cell cycle (9), the deranged expression of cyclins and CDKs active in G1 may be the key to oncogenesis. The link between oncogenesis and cyclins has been made with the discovery of inappropriate expression of two cyclins in tumors (10). (i) The cyclin A gene is the site of integration of a fragment of hepatitis B virus genome in a hepatocellular carcinoma (11). Cyclin A is also associated with the adenovirus transforming protein E1A in adenovirus-transformed cells (12). (ii) In some parathyroid tumors, the Pradl (cyclin D1) locus is overexpressed due to a chromosomal rearrangement translocating it to the enhancer of the parathyroid hormone gene (13). Recently, translocation/amplification of cyclin D1 has been associated with a small percentage of other cancers including centrocyclic lymphomas and squamous cell, esophageal, and breast carcinomas (14–16).

Although these observations emphasize the importance of cyclins in cancer, the question remains of how the altered expression of only two cyclins (i.e., cyclins A and D1), which are only very occasionally deranged, can be responsible for transformation. As yet, there have been no clear connections between cyclin derangements and cancer involving the aberrant expression of more than one cyclin, or any of the CDKs, in a single type of cancer. A survey of all cyclins and CDKs in the same system of normal vs. tumor cells is essential to show whether cyclins can function collectively or redundantly in cancer by bypassing crucial check points in the cell cycle. We have chosen breast cancer as a model system for these studies because of its clinical significance and it is only in the mammary system that the normal and tumor derived-epithelial cells can be grown in long-term culture and in the same medium, allowing their comparative analysis (17). By using six cyclins (cyclins A, B, C, D1, D3, and E) and two CDK (CDC2 and CDK2) DNA sequences and antibodies as molecular probes, we describe abnormal expression of various cyclins and CDKs in human breast cancer cell lines when compared to normal mammary epithelial cells. We show gene amplification and overexpression of cyclin E in 1 of 10 human breast tumor cell lines and altered expression of cyclin E protein in all tumor lines studied. We also show the untimely appearances of mitotic cyclins prior to G1 cyclins in synchronized tumor vs. normal cells. We conclude from these findings that aberrant expression of cyclins may be intimately associated with the neoplastic process.

MATERIALS AND METHODS

Materials. [methyl-3H]Thymidine (81 Ci/mmol; 1 Ci = 37 GBq) and [α-32P]dCTP (3000 Ci/mmol) were purchased from New England Nuclear. Lovastatin was kindly provided by A. W. Alberts (Merck Sharp & Dohme). Mevalonic acid lactone and serum were purchased from Sigma and cell culture medium was from GIBCO. All other chemicals used were of reagent grade.

Cells and Culture Conditions. Table 1 lists all the cell lines used in this study. Normal human mammary epithelial cell strains 70N, 81N, and 76N were obtained from reduction mammaplasties of three individuals (17). The ZR75T cell line was derived from a human tumor produced in a nude mouse from a procloned derivative of ZR75-1, ZR-75-1; MDA-MB-157, MDA-MB-231, MDA-MB-436, T47D, BT-20, HBL-100, HS578T, and SKBR3 cells were all obtained from the American Type Culture Collection. MCF-7 cells were obtained from Michigan Cancer Foundation (Detroit). All cells were cultured in DFCI-1 medium as described (17) and were

*Abbreviations: CDK, cyclin dependent kinase; DRB, 5,6-dichloro-benzimidazole.

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Table 1. Characterization of normal and tumor-derived breast epithelial cells

<table>
<thead>
<tr>
<th>No.</th>
<th>Cell line strain</th>
<th>Cell type</th>
<th>ER status</th>
<th>Tumorigenicity</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>70N</td>
<td>N</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>81N</td>
<td>N</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>76N</td>
<td>N</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>MCF-7</td>
<td>A (pe)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>MDA-MB-157</td>
<td>C (pe)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>MDA-MB-231</td>
<td>A (pe)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>MDA-MB-436</td>
<td>A</td>
<td>-</td>
<td>-</td>
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<td>8</td>
<td>T-47D</td>
<td>DC (pe)</td>
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<td>+</td>
</tr>
<tr>
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<td>BT-20</td>
<td>C</td>
<td>+</td>
<td>-</td>
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<tr>
<td>10</td>
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<tr>
<td>11</td>
<td>HS-578T</td>
<td>DC</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>SKBR-3</td>
<td>A (pe)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>ZR75T</td>
<td>IDC</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

N, normal breast cells from reduction mammoplasty; A, adenocarcinoma; C, carcinoma; DC, ductal carcinoma; T(bm), tumor (breast milk); IDC, infiltrating DC. Estrogen receptor (ER) status of the cell lines were determined by Northern blot analysis using a cDNA to the full-length estrogen receptor as a probe. +, Positive; −, negative.

maintained free of Mycoplasma as determined by the MycoTect test kit (GIBCO).

Cell Synchronization. Synchronization by lovastatin treatment or growth-factor deprivation were performed as described (18). Briefly, medium was removed 36–48 h after the initial plating of ZR75T cells and replaced with fresh medium plus 20 μM lovastatin for 33 h. At time 0 h, cells were stimulated with fresh medium containing 2 mM mevalonic acid. Cells were harvested at indicated time points, and DNA synthesis and cell density were measured.

Synchronization of normal mammary epithelial 76N cells by growth-factor deprivation was as follows: At 48 h after plating subconfluent 76N cells, cells were washed three times and incubated in DFCI-3 medium for 48 h. DFCI-3 medium is DFCI-1 medium without essential growth factors (17). At time 0 h, cells were stimulated by the addition of DFCI-1 medium and harvested at the indicated times thereafter.

Southern and Northern Blot Analyses. DNA isolation and Southern blot analysis were performed as described (19). Total RNA was isolated from frozen tissue samples, obtained from the National Disease Research Interchange, Eastern Division (Philadelphia), by placing the tissue in 3 ml of guanidinium isothiocyanate and homogenizing with 10–20 strokes using a glass tube and Teflon pestle. Homogenates of tissues and cell lines were subjected to Northern blot analysis as described (18). The DNA probes were prepared by random-primer labeling (20) (Boehringer Mannheim) with [α-32P]dCTP to a specific activity of 1 × 10⁶ dpm/μg of DNA.

Total Cell Extract Preparation and Western Blot Analysis. Cell lysates were prepared by addition of 1 vol of sonication buffer (50 mM Tris-HCl, pH 7.4/0.25 M NaCl/1 mM dithiothreitol) to a cell pellet and sonication at 4°C by using the cup-horn mode for five 2-min bursts. Homogenates were centrifuged at 100,000 × g for 45 min at 4°C. Aliquots of the supernatants were subjected to Western blot analysis as described (21). The following hybridization conditions were used for each antibody: affinity-purified anti-p34cdc2 kinase peptide antibody (GIBCO) was incubated for 3 h at 1 μg/ml; monoclonal antibodies C160 to human cyclin A or GNSII to human cyclin B were incubated overnight at 4°C at a dilution of 1:3; affinity-purified rabbit anti-human cyclin E serum (specific for cyclin E) as described (22, 23) was incubated for 3 h at a dilution of 1:2500 in blocking buffer [20 mM Tris-HCl, pH 7.5/150 mM NaCl/5% (wt/vol) bovine serum albumin/5% (wt/vol) dried milk/0.05% Tween 20]. Blots were then washed extensively and developed with detection reagents as directed by the manufacturer (cyclin E, ECL-Amersham; cyclins A and B and p34cdc2, Promega).

RESULTS

Overexpression of Cyclins in Breast Tissues. To directly examine the relevance of cyclin derangement to in vivo conditions, we measured the expression of cyclin mRNAs in breast tumor samples vs. normal adjacent breast tissue. Total RNA was isolated from three human infiltrating ductal and metastatic breast carcinomas (Fig. 1, lanes 3–5) and two adjacent nontumorous tissues (lanes 1 and 2) and subjected to Northern blot analysis using probes for cyclins A and B and CDC2. These analyses revealed that mRNAs for cyclins A and B and CDC2 were significantly overexpressed in tumor samples when compared to the normal adjacent tissue, suggesting that they may be involved in transformation. However, the question remains whether cyclin overexpression is dependent on cell proliferation or represents a true difference between normal and tumor cells. To answer this question we switched to an in vitro system, examining cultured cells.

Differential Cyclin Expression in Proliferating Normal vs. Breast Cancer Cells. Ten breast cancer cell lines were compared against three proliferating normal mammary epithelial cell strains obtained from reduction mammoplasties and used early—i.e., at passages 9–11 (17) (Fig. 2). The tumor cell types, their tumorigenicity potentials, and their estrogen receptor status are listed in Table 1.

We found a common pattern of overexpression of the mitotic cyclins A and B and their dependent kinase, CDC2, by up to 12-fold, in most (9/10) of the tumor cell lines studied (Fig. 2). The pattern and extent of overexpression of the cyclin A, cyclin B, and protein CDC2 were very similar to their mRNAs in most of the tumor lines (Fig. 2B). These in vitro observations are consistent with the breast tissue results (Fig. 1), suggesting that cyclin overexpression in tumor cells is not dependent on proliferation, since the normal cell strains used are rapidly proliferating. Hence, the difference in cyclin expression is due to a normal vs. tumor difference, one that is general among most tumor lines studied.

Whereas the mitotic cyclins and CDC2 are overexpressed in 90% of the tumor lines studied, the G1 cyclins and their dependent kinase, CDK2, are overexpressed in only 50% of the tumor lines. Furthermore, cyclins D1 and D3 (25) are only moderately overexpressed (15) or even underexpressed in two tumor lines (Fig. 2A, lanes 7 and 10, cyclin D1) as compared to normal cell strains. Cyclin C mRNA is expressed equally in normal and tumor cells and c-myc mRNA levels in the tumor lines examined here are generally equal to or lower than normal, as observed (19).

The most striking abnormality is that of cyclin E expression. We found a remarkable overexpression of cyclin E mRNA in only one tumor line (MDA-MB-157) (Fig. 2A, lane

![Fig. 1. Overexpression of cyclins in primary breast cancer tissues.](image-url)

Overexpression of cyclins in primary breast cancer tissues. Total RNA was extracted from two adjacent normal human breast tissue samples (lanes 1 and 2) and three human breast cancer tissues (lanes 3–5), corresponding to infiltrating ductal carcinoma, metastatic breast carcinoma, and infiltrating ductal carcinoma, respectively, and analyzed on Northern blots (20 μg of RNA per lane). Blots were hybridized with the indicated probes or 36B4 (24), used for equal loading.)
Fig. 2. Cyclins are overexpressed in human tumor breast epithelial cell lines. Northern blot (A) and Western blot (B) analyses of cyclin expression in normal vs. tumor breast epithelial cells. RNA was analyzed on Northern blots (20 µg of RNA per lane). The list of normal cell strains (lanes 1–3) and tumor cell lines (lanes 4–13) is presented in Table 1 (using identical numbers). (B) Western blot analysis for cyclin A, cyclin B, cyclin E, and CDC2 from cell extracts obtained from the same cell lines used in A. The large arrowheads point to the respective proteins for which each blot was probed at the following molecular masses: cyclin E, 50 kDa; cyclin A, 60 kDa; cyclin B, 62 kDa; CDC2, 34 kDa. The small arrowheads point to the two extra cyclin E-like proteins observed in the tumor cells at 42 and 35 kDa. Molecular mass standards were used on each gel to estimate the position of each band.

5) and moderate overexpression in five other tumor lines (Fig. 2A, lanes 4, 6, 7, 11, and 12). Western blot analysis however, revealed derangements of cyclin E protein in all these tumor lines (Fig. 2B, lanes 4–13), including those with no apparent overexpression of cyclin E mRNA (Fig. 2A, lanes 8–10 and 13). Cyclin E antibody (22), recognized one major protein migrating at ~50 kDa in the normal mammary epithelial cell lysates, corresponding to the size of the cyclin E protein (22, 26). In the tumor lines, however, cyclin E antibody recognized three major proteins migrating at 50, 42, and 35 kDa. Each tumor line overexpressed one, two, or all three cyclin E-related proteins, revealing a deranged pattern of cyclin E protein expression that in all cases was different from that of the normal cell strains (Fig. 2B).

Amplification of Cyclin E Gene. We examined cyclin E overexpression in the MDA-MB-157 cell line at the levels of DNA, RNA, and protein, as compared to the normal cell strain 76N. Southern blot analysis revealed an 8-fold amplification of the cyclin E gene (Fig. 3A), and no gross genomic rearrangement. No coamplification of the other cyclin or CDK genes were observed in this tumor line (data not shown), even though there was a general overexpression (up to 10-fold) of their mRNAs.

Northern blot analysis indicated that though cyclin E mRNA is overexpressed by 64-fold in the tumor cell line, it is present in only one size (2.1 kb) in both normal and tumor cells (Fig. 3B). Furthermore, the one size cyclin E mRNA in the tumor line is translated into at least three overexpressed proteins (Fig. 3C). Whereas, in normal cells, cyclin E antibody reacted strongly with only one protein of 50 kDa (22, 26). The cyclin E proteins in MDA-MB-157 cells have a much higher H1 kinase activity associated with them compared to that of the normal cell strain (K.K., G. Molnar, and A.B.P., unpublished observations). Collectively these observations provide evidence of a genetic alteration of cyclin E in cancer, implying an oncogenic role for this cyclin.

Cell Cycle Expression of Cyclin and CDK mRNAs in Normal vs. Tumor Cells. To gain insight into the possible functions of cyclins A and B and CDC2, which were overexpressed not only in the three breast tumor tissues examined but also in 90% of all tumor cell lines, we compared the behavior of their RNAs through the cell cycles of normal vs. tumor cells (Fig. 4). The normal cell strain we examined is 76N. We chose the ZR75T tumor cell line for this analysis since it is the only one among the 10 we studied that overexpresses only cyclins A and B and CDC2 and none of the G1 cyclin mRNAs. ZR75T cells were synchronized in early G1 phase by lovastatin (18). 76N cells were synchronized by growth-factor deprivation and with lovastatin, which gave very similar patterns of cyclin expression. Therefore, only the results after growth-factor deprivation are shown. Synchrony of both cell types was monitored by [3H]thymidine incorporation (Fig. 4B).

Northern blot analysis revealed similarities and differences in the expression patterns of cyclins in these synchronized cells. Importantly, the patterns and timing of expression of G1 cyclins were very similar in both cell types. Cyclins D1, D3, C, and E mRNA levels were low to undetectable at the early hours in G1 phase and peaked at mid (cyclins D1, D3, and C) to late (cyclin E) phase, and most of them dropped rapidly as cells entered S phase (Fig. 4A). Hence, the predominant accumulation of these cyclins in the G1 phase of normal and tumor cells, suggests that aberration in G1 cyclins is not responsible for deranged growth of the ZR75T cells.

However, the overexpressed mRNAs for cyclins A and B and CDC2 in tumor cells show a perturbed appearance in the G1 phase of the cell cycle (Fig. 4). In normal cells these mRNA levels were very tightly regulated, demonstrated by their dramatic fluctuations during the cell cycle, peaking by ~50-fold at S phase (15–21 h after release) for cyclin A andcdc2 and late S/G2 phase (21–24 h after release) for cyclin B. Subsequently, all rapidly disappeared at G2/M phase. On the other hand, in tumor cells, the basal levels of cyclin A and B mRNAs were high during the G1 phase and the maximal levels reached were >10-fold higher than in the normal cells. In addition, cyclin mRNA levels did not disappear as rapidly in G2/M phase in tumor cells as in normal cells, resulting in their prolonged overexpression and, possibly, in higher protein levels (Fig. 2B).
Fig. 4. Expression of cyclins in synchronized normal 76N and tumor ZR75T breast cells. (A) Normal cells were synchronized by growth factor deprivation and tumor cells by lovastatin (20 μM for 33 h). At indicated times after growth-factor stimulation (normal cells) or addition of 2 mM mevalonate (tumor cells), total RNA was extracted from cells and analyzed on Northern blots (20 μg of RNA per lane). Blots were hybridized with the indicated probes or 36B4 (used for equal loading). (B) DNA synthesis rates in 76N (open symbols) and ZR75T cells (solid symbols) as measured by [3H]thymidine incorporation. ND, not detectable.

These differences in the tumor cells of cyclin mRNA levels and patterns of expression during the cell cycle results in a deranged order of appearance. In normal 76N cells the order of expression of cyclins was: D1(C) → D3 → E → A → B, as expected (27). In tumor cells, on the other hand, the mitotic cyclins appeared earlier than the G1 cyclins: B → A → D3(D1) → E. This untimely appearance in the cell cycle is due to their high basal levels in G1 phase.

**Mechanism of Cyclin mRNA Overexpression in Tumor Cells.** The mechanism by which cyclins are deregulated in ZR75T appears to depend mainly upon greater stability of cyclin mRNAs in tumor cells. (There were no gross genetic alterations of any cyclins in this tumor line compared to the normal cell DNA, data not shown.) To determine mRNA stabilities, we used the transcriptional inhibitor 5,6-dichlorobenzimidazole (DRB), which inhibits polymerase II activity responsible for mRNA synthesis. DRB was used at 100 μM, which in normal cells is sufficient to completely inhibit c-myc transcription (within >0.5 h after addition of DRB). mRNAs levels were detectable in ZR75T cells.) At the indicated times after the addition of DRB, total RNA was extracted from cells and subjected to Northern blot analysis (Fig. 5). In all cases, cyclin and CDK mRNAs were more stable in tumor than in normal cells. The relative mRNA half-life values of cyclins and CDKs ranged from 0.3 to 1.5 h in normal cells, whereas those in tumor cells ranged from 3 to 8 h. The increase in stability of cyclins A, B, and CDC2 in tumor cells of both normal cells (Fig. 5) quantitatively accounts for the higher steady-state mRNA levels observed previously for this tumor line (Fig. 2, lane 13).

mRNAs of G1 cyclins were also significantly more stable in the ZR75T cells. This is surprising since G1 cyclins were not overexpressed in this cell line. Furthermore, mRNA of another gene, histone H4, was also more stable in tumor cells than in normal cells (Fig. 5). Hence, the mechanism by which cyclin mRNAs are stabilized in this cell line does not depend on genetic alterations of cyclin genes.

**DISCUSSION**

The purpose of this study is to establish evidence correlating the deranged expression of cyclins to loss of growth control. To emphasize the role that cyclins can play in the process of tumor formation, it is crucial to provide evidence for their general derangement, not just the derangement of one or two cyclins in rare isolated cell lines and/or tumors. In this report we have strengthened the link between cause and effect by describing several changes that are seen in all or most breast tumor cell lines. These alterations are (i) deranged expression of cyclin E protein in 10 of 10 tumor lines, (ii) general overexpression of mRNAs and proteins for cyclins A and B and CDC2 in 9 of 10 tumor lines and 3 of 3 tumor tissue samples, and (iii) in one tumor line, increased mRNA stability of all cyclins and CDKs, which may cause untimely appearance of the mitotic cyclins at the G1 phase of the tumor cell cycle. Because of this generality, we suggest that these changes in cyclin expression are not merely consequences, but contribute to the loss of growth control.

**Is Cyclin E an Oncogene?** Two lines of evidence suggest that cyclin E may be the prime candidate for oncogene action. (i) The kinetics of appearance of cyclin E in late G1 phase (Fig. 4; refs. 22, 26, and 28) coincides with timing of the restriction point protein that is required for moving cells toward S phase (ref. 9, and Q. P. Dou and A.B.P., unpublished results). The deregulation of this process can lead to abnormal proliferation. (ii) The deranged production of cyclin E protein in all cancer cell lines examined suggests an oncogenic role for cyclin E. Irrespective of their tumorigenicity potentials or estrogen-receptor status, we observed overexpression of one, two, or all three of the cyclin E-like proteins ranging in size from 35 to 50 kDa, whereas in the normal cell strains (70N, 76N, and 81N), we observed only one major protein of ~50 kDa. Although the mechanisms responsible for this deranged appearance of cyclin E (like) protein(s) remain to be elucidated, the fact that only cyclin E protein is altered in all tumor lines suggests that the deregulation of this protein may be involved in transformation and also that it can be used as a diagnostic tool for breast cancer.

**Can Overexpressed G1 Cyclins Function Redundantly in G1 Phase?** Redundancy of cyclins has been observed in yeast (29) and Xenopus oocytes (2, 30). Remarkably G2 cyclins were generally overexpressed not only in most of tumor lines but also in tumor tissue samples. Consequently, high levels of G2 cyclins appeared prior to G1 cyclins (in a tumor line that overexpresses G2 but not G1 cyclins; Fig. 4). The regulatory mechanisms that are deranged leading to abnormal prolifer-
overcome a G1 block. Hinds et al. (32) report that a human osteogenic sarcoma cell line that lacks full-length nuclear retinoblastoma protein (pRb) product but has received a wild-type pRB-encoding expression construct arrests in G0 or G1 phase in a metabolically active state. Cotransfection of cyclin A or E overrides this pRB-induced block, causing cell cycle progression such that 60–70% of the pRB-positive cells were found in the S, G2, and M phases of the cell cycle. Hence, cyclin A and/or E is able to direct the cell into S phase. Similarly, we propose that when these cyclins are overexpressed, as in the breast cancer cell lines, the cells will overcome checkpoint controls in the cell cycle.

We thank R. Sager for providing three normal cell strains, 70N, 76N, and 81N; L.-H. Tsai, S. Schiff, and E. Harlow for monoclonal antibodies to cyclins A (C160) and B (GNS11) and for cDNA to CDC2; V. Dulic and S. Reed for cDNAs to cyclins C and E as well as polyclonal antibody to cyclin E; T. Motokura and A. Arnold for cDNAs to cyclins D1 and D3; J. Pines and T. Hunter for cDNA clones to cyclins A and B; H. Piwnicka-Worms for cDNA to CDC2; L. Averboukh and G. Molnar for technical assistance; A. Crozat, M. Corbley, K. Swisswolme, and Q. P. Dou for helpful discussions; and P. Liang, N. Jahroudi, and D. Anderson for critical analysis of this manuscript. This work was supported in part by Grant CA 22427 (to A.B.P.), Basic Research Support Grant 507RR05526-29 (to K.K.), and National Research Service Award CA08949-01 (to K.K.), all from the National Institutes of Health.


**Fig. 5.** Stability of cyclin RNAs in normal vs. tumor breast cells after DRB treatment. Exponentially growing 76N and ZR75T cells were treated with 100 μM DRB. At indicated times after addition of DRB, RNA was extracted from cells and analyzed on Northern blots with the indicated probes. Hybridization signals were normalized to the amount loaded by using signal intensity of 36B4 as loading control and expressed as a percentage of the values at time 0 h. Half-life values were then estimated as the time needed to get 50% signal intensity. ND, not detectable.

**Why Are Cyclins More Stable in Tumor Cells?** An initial posttranscriptional derangement, such as increased RNA stability of cyclins, may lead to a cascade of changes resulting in increased net cyclin protein production. This preliminary step in the early stages of transformation may be compensated so as to keep the mRNA levels of cyclin genes from rising. For example, cyclins D1, D3, and E mRNAs are more stable in ZR75T tumor cells than in 76N normal cells, though at the RNA level they are not overexpressed (Figs. 2 and 5). Upon loss of this feedback control, cyclin levels increase, as seen with overexpression of RNAs and proteins for cyclins A and B and CDC2 in 90% of tumors examined (Fig. 2); the general stability of cyclin mRNAs may lead to their increased abundances that could persist during all phases of the cell cycle (Fig. 4). Cell cycle regulation of the overexpressed cyclins is also perturbed, as seen in the untimely appearances of cyclins A and B at the G1 phase of the cell cycle (Fig. 4).

Our observations that cyclins E and/or A may be intimately involved in the process of tumor formation is consistent with recent evidence on the ability of these cyclins to