The p53-related p73 proteins regulate developmental processes, cell growth, and DNA damage response. p73 function is regulated by post-translational modifications and protein-protein interactions. At the G1/M transition, p73 is phosphorylated at Thr-86 by the p34cdc2/cyclin B complex; this is associated with its exclusion from condensed chromosomes and loss of DNA binding and transcriptional activation ability. Here we showed that p73 hypo-phosphorylated species reappear during mitotic exit, concomitant with p73 relocalization to telophase nuclei and recovered ability to activate transcription. Functional knock-out of p73 gene expression by small interfering RNAs (siRNAs) alters mitotic progression, yielding an increase of ana-telophase cells, the accumulation of aberrant late mitotic figures, and the appearance of abnormalities in the subsequent interphase. This p73 activity at the M-to-G1 transition is mediated by its transactivating function because expression of the transcription dominant negative mutant p73DD induces the same mitotic exit phenotype. We also found that the cyclin-dependent kinase inhibitor Kip2/p57 gene is a specific target of p73 regulation during mitotic exit and re-entry into G1. Both knock-out of p73 gene expression by siRNAs and abrogation of p73-dependent transcription by the p73DD mutant abrogate Kip2/p57 increase at the M-to-G1 transition. Moreover, similar abnormalities (e.g. delay in late mitotic stages with the accumulation of aberrant ana-telophase figures, and abnormalities in the following interphase) are observed in cultures in which the expression of Kip2/p57 is abrogated by siRNAs. These results identify a novel p75-Kip2/p57 pathway that coordinates mitotic exit and transition to G1.

The p53 paralog p73 encodes several pro-apoptotic (transactivation-competent, TA) and anti-apoptotic (dominant negative, DN) isoforms (1, 2). TAp73 transcripts mediate cell cycle arrest and/or apoptosis in response to DNA damage (3–8) and regulate developmental processes in the central nervous system and the immune system (9–13). The DN-p73 proteins are expressed from a second promoter, the P2 p73 promoter; these isoforms act as trans-repressors of p53- and p73-dependent transcription and possess both anti-apoptotic and pro-proliferative potential (1, 14, 15). These features allow a regulatory loop to take place following non-apoptotic DNA damage, whereby p53-dependent activation of the P2 p73 promoter induces DN-p73 proteins to terminate p53/p73-driven cell cycle arrest in those cells that do not undergo apoptosis (15). TAp73 proteins have also been implicated in positive regulation of cell growth (16–18). Indeed, TAp73 expression is itself modulated during the cell cycle (18). TAp73 proteins accumulate in S-phase cells (18), and the S-phase gene adenine deaminase gene (ADA) has been found to be a specific p73 target gene (19). The function of p73 proteins is also regulated by post-translational modifications and protein-protein interactions in different cellular and pathophysiological contexts (4, 16, 17, 20, 21). Recently, we and others have shown that p73 is a novel target of mitotic phosphorylation by the p34cdc2/cyclin B complex (16, 17). Both p73a and p73b are phosphorylated on threonine 86 in normal mitotic cells and during mitotic arrest induced by microtubule-targeting drugs, resulting in a sharp reduction of its DNA binding and transcriptional activation ability (16). p73 mitotic phosphorylation is also associated with its exclusion from condensed chromosomes in meta-anaphase cells (16). Altogether, these results indicate that M phase-specific phosphorylation of p73 by the p34cdc2/cyclin B complex negatively regulates its transcriptional activating function. Here we have investigated the recovery of p73 function during mitotic exit. We showed that Thr-86 p73 phosphorylation is lost during mitotic exit and is accompanied by p73 relocalization to telophase nuclei and recovery of transactivating ability. Abrogation of p73 expression by specific small interfering RNAs (siRNAs) led to delayed mitotic progression with an increase in ana-telophase cells, accumulation of aberrant late mitotic figures, and generation of abnormal (bimucleated and micronucleated) cells in the subsequent interphase. We also found that loss of p73 transcriptional activity recapitulates the same mi-

1 The abbreviations used are: TA, transactivation-competent; DN, dominant negative; siRNA, small interfering RNA; ADA, adenine deaminase; GFP, green fluorescent protein; cdki, cyclin-dependent kinase inhibitor; DAPI, 4',6-diamidino-2-phenylindole; FACs, fluorescence-activated cell sorter; MEF, mouse embryo fibroblasts; FBS, fetal bovine serum; HA, hemagglutinin; TRITC, tetramethylrhodamine isothiocyanate.
washing twice in phosphate-buffered saline plus 0.5% Tween 20, and to grow in 10% FBS-containing medium. Samples for flow cytometry were analyzed by immunoblotting with anti-p73 phosphoT86 and anti-p73 antibody. Lane A, asynchronous cells; lane N, nocodazole blocked cells. B, extracts of H1299-derived p73α-inducible cells treated as above were analyzed by immunoblotting with anti-p73 phosphateT86 and anti-p73 antibody. Lane N, nocodazole-blocked cells. C, localization of p73 in H1299-derived p73α-inducible cells, treated as in A. Immunofluorescence analysis using anti-p73 (1:100) (Texas Red secondary antibody; pseudocolored in red) and anti-α-tubulin (a-tub) (fluorescein isothiocyanate-conjugated secondary antibody, pseudocolored in green) was performed. Cells were counterstained with DAPI to detect DNA. p73 is excluded from chromosomes in prometaphase (top row) but reassociates with chromatin in telophase (middle row) and is again totally nuclear when cells re-enter G1 (bottom row).

**RESULTS**

**Fig. 1. Hypophosphorylated p73 proteins relocate with chromatin in telophase.** A, H1299-derived p73α-inducible cells were treated with ponasterone A (Pon A, 6 h) and synchronized in prophase in the presence of nocodazole (Noc) (lane N). Prophase-arrested cells were collected by mitotic shake off, washed, and replated in fresh culture dishes. Cell extracts were prepared at the indicated times after replating and analyzed for electrophoretic mobility of p73α by immunoblotting with anti-p73 antibody. The percentage of (G1, M) and G2 cells were determined by FACS analysis. Lane A, asynchronous cells; lane N, nocodazole blocked cells. B, extracts of H1299-derived p73α-inducible cells treated as above were analyzed by immunoblotting with anti-p73 phosphateT86 and anti-p73 antibody. Lane N, nocodazole-blocked cells. C, localization of p73 in H1299-derived p73α-inducible cells, treated as in A. Immunofluorescence analysis using anti-p73 (1:100) (Texas Red secondary antibody; pseudocolored in red) and anti-α-tubulin (a-tub) (fluorescein isothiocyanate-conjugated secondary antibody, pseudocolored in green) was performed. Cells were counterstained with DAPI to detect DNA. p73 is excluded from chromosomes in prometaphase (top row) but reassociates with chromatin in telophase (middle row) and is again totally nuclear when cells re-enter G1 (bottom row).

**MATERIALS AND METHODS**

**Cell Culture, Synchronization, and FACS Analysis—**T98G (glioma) cells were cultured in RPMI plus antibiotics, nonessential amino acids, glycine, sodium pyruvate, and 10% heat-inactivated fetal bovine serum (FBS). The H1299 ponasterone-inducible p73α cell line (24) was maintained in RPMI medium supplemented with 10% FBS. Where indicated, cells were synchronized in prophase by nocodazole treatment (50 ng/ml) for 18 h. p73α/−/− and p73β/−/−-reconstituted p73α/−/− MEFs (kindly provided by J. Y. J. Wang, University of California, San Diego (University of California San Diego, La Jolla) were cultured in Dulbecco’s modified Eagle’s medium plus antibiotics supplemented with 10% FBS. MEF cultures were synchronized by serum deprivation (48 h in Dulbecco’s modified Eagle’s medium, 0.5% FBS) and then restimulated to grow in 10% FBS-containing medium. Samples for flow cytometry analysis were prepared by fixing the cells in 70% ethanol (30 min, 4 °C), washing twice in phosphate-buffered saline plus 0.5% Tween 20, and staining with 50 μg/ml propidium iodide. Samples were analyzed in a FACStar Plus (BD Biosciences) flow-cytometer (10,000 events/sample) using the WinMDI software.

**Antibodies, Plasmids, Recombinant Proteins, and Reagents—**Antibodies directed against human cyclin B1 (catalog number G5115), p21 (catalog number H164), Kip2/p57 (catalog number C20), actin (catalog number 8G10), tubulin (catalog number C-2), and the HA tag (catalog number Y11) (Santa Cruz Biotechnology, Inc.) were used at a 1:200 dilution. The mouse monoclonal anti-p73 antibody (clone 1288) (Imgenex, Inc.) recognizes all known splicing variants of the human and mouse p73 (Ref. 4 and data not shown). The anti-GFP rabbit polyclonal antibody (1:1000) was from Clontech. The anti-p73 phosphateT86 was described previously (17). The mouse monoclonal fluorescein isothiocyanate-conjugated anti-α-tubulin antibody (catalog number F2168, 1:100) and the mouse monoclonal anti-α-tubulin antibody (catalog number B512, 1:2000) were from Sigma. Secondary anti-mouse fluorescein isothiocyanate (1:200) and anti-mouse Texas Red (1:800) antibodies were from Jackson Immunoresearch, Inc. and Vector, Inc., respectively. The pCDNA-HA-p73DD plasmid expresses a p73 miniprotein that acts as a selective dominant negative of p73-dependent, but not p53-dependent, transcription (18). pGFPN1 expression vector was from Clontech Inc. Double-stranded fluorescent siRNAs specific for GFP, p73, and Kip2/p57 (Dharmacon, Inc.) were transfected using the Lipofectamine Plus reagent (Invitrogen). The sequences of the siRNAs are available upon request. Nocodazole and propidium iodide were purchased from Sigma; ponasterone A was purchased from Alexis Biochemical, Inc.

**Semiquantitative Reverse Transcription-PCR—**RNA was isolated by the single step guanidinium isothiocyanate-phenol method. 5 μg of total RNA were reverse-transcribed using the First Strand cDNA synthesis kit (Amersham Biosciences), and 1/10 of reverse-transcribed material was used in PCR reactions. Semiquantitative PCR conditions were optimized to obtain reproducible and reliable amplification within the logarithmic phase of the reaction. Kip2/p57 transcript was amplified using primers F1 5′-ACATCCAGATGGAGCTCTTG-3′ and R1 5′-TGCTCTGTGGAGCTGAATCC-3′. β-Actin transcript was amplified using primers F1 5′-ATCGGCCACCATCTTCAATGGCTG-G-3′ and R1 5′-GCATGCTGCTCTGGTTGATCCACATCTGC-3′. The amount of amplified products relative to actin expression was quantified by ImageQuant 2.0 software (Amersham Biosciences).

**Cytological Preparations and Microscopy—**Cells were fixed in 3.7% paraformaldehyde and processed for indirect immunofluorescence before counterstaining in 0.1 μg/ml DAPI. Images were analyzed using an Olympus AX70 microscope configured for epifluorescence and equipped with a cooled camera device (Photometrics).

**RESULTS**

**Hypophosphorylated p73 Proteins Relocalize to Reforming Nuclei in Telophase—**Most substrates phosphorylated by the yeast p34cdc2/cyclin B complex homologous to mammalian p34cdc2/cyclin B become dephosphorylated at mitotic exit (22, 23). To establish whether p73 also undergoes dephosphorylation during exit from mitosis, H1299-derived p73α-ponasterone A-inducible cells (24) were subjected to nocodazole arrest and then released from the block and analyzed for both p73 Thr-86 phosphorylation and subcellular localization during mitotic completion. As shown in Fig. 1A, p73α fully recovers its faster mobility 1 h after release from nocodazole arrest. The use of specific antibodies raised against the Thr-86 phosphorylated forms (17) revealed that this was concomitant with a sharp reduction of phosphorylation at this site (Fig. 1B). Cells from the same cultures were analyzed by immunofluorescence to follow up mitotic progression after nocodazole removal. In prometaphase-arrested cells, p73 was found to be excluded from...
condensed chromosomes and distributed throughout the cytoplasm, as detected by anti-p73 antibody staining (Fig. 1C, in red). After nocodazole washout, p73 dispersal persists throughout metaphase, anaphase, and early telophase (Ref. 16 and data not shown). p73 then relocates to daughter nuclei in late telophase, as early as 1 h after nocodazole release (Fig. 1C), i.e. the time at which phosphorylation is lost. As cells progress through G1 re-entry, 3 h after nocodazole release, and chromatin decondensation is complete, the p73 signal regains its homogeneous pattern (Fig. 1C).

Abrogation of p73 Function Perturbs Mitotic Progression and Exit—The observation that p73 is rapidly dephosphorylated at Thr-86 and reassociates with nuclei already in late telophase prompted us to determine whether p73 function affects mitotic completion and the M-to-G1 transition under physiological conditions, i.e. in cells that were not exposed to spindle poisons. We first analyzed progression through an entire cell division cycle in MEFs derived from p73−/− mice after growth arrest and serum restimulation. FACS analysis of cultures progressing synchronously through the cell cycle revealed that lack of p73 causes an evident accumulation of cells in the G2 and M stages followed by a delay in the M-to-G1 transition, as compared with p73-null MEFs reconstituted with p73β (Fig. 2A, left panel). Moreover, fluorescence microscopy analysis revealed the frequent occurrence of lagging chromatids and chromatin bridges between separating nuclei in the p73−/− MEFs (Fig. 2A, right panel).

Next, we analyzed mitotic progression in human T98G p53-null glioma cells in which p73 expression was abrogated by p73-specific siRNAs (Fig. 2B). p73-siRNAs, although not affecting the organization of the mitotic apparatus proper (Fig. 2B, right panel, rows a–c), did perturb progression through the final stages of mitosis. The proportion of mitotic cells in anaphase stages increased in a statistically significant manner as compared with control cultures treated with siRNAs against GFP (Fig. 2B, lower left panel), indicating that loss of p73 delays the completion of mitosis. In addition, isolated lagging chromatids and persisting chromatid bridges between nuclei were also evident in late mitotic stages in p73-siRNA T98G cultures, as observed previously in p73−/− MEFs, indicating inaccurate or incomplete segregation to daughter cells (Fig. 2B, right panel, rows d and e). To substantiate these findings, we also analyzed interphase cells in cultures that were subjected to the RNA interference protocol for 24 h and then harvested by trypsinization and split in two fresh culture dishes for another 24 h, and thus likely to have undergone mitosis in the absence of p73 function. A statistically significant induction of abnormalities was recorded in the subsequent interphase in the absence of p73 (Fig. 2C, including binucleated cells and cells with micronuclei, possibly derived from lagging chromatids or broken chromatin bridges. These results confirmed that p73 function is required for accurate mitotic division.

p73 Function during Exit from Mitosis Is Linked to Its Transcriptional Activity—We previously showed that cdc2-dependent phosphorylation of p73 at Thr-86 occurs at the G2/M transition and results in a reduced capability to bind its DNA target sequences and to activate transcription (16). The observation that p73 is rapidly dephosphorylated at Thr-86 and relocalizes with chromatin already in late telophase (Fig. 1, A–C) prompted us to verify whether transcriptional activity of p73 is actually required during the M-to-G1 progression to regulate exit from mitosis or, alternatively, whether the loss of other p73 functions is responsible for mitotic exit abnormalities. Indeed, although mitosis is accompanied by a global repression of nuclear RNA synthesis in higher eukaryotes, some genes, including the human cyclin B1 gene (25), are transcribed even in the context of the highly
condensed mitotic chromatin. Interestingly, in *Saccharomyces cerevisiae*, a specific subset of genes is transcriptionally activated in telophase (26) by the Swi5p chromatin remodeling enzyme and the Gcn5p histone acetyltransferase (27, 28) to favor mitotic exit. Thus, we decided to analyze mitotic progression in T98G cells in which p73 transcriptional function was abrogated by overexpression of the dominant negative mutant p73DD (18). As shown in Fig. 3A, this caused a mitotic exit phenotype characterized by an increased proportion of anaphase figures, similar to that observed in cells in which p73

**FIG. 3.** *p73* transcriptional activity is required for mitotic exit. **A,** abrogation of *p73* transcriptional function using the p73DD dominant negative mutant delays late mitotic stages. Mitotic substages were recorded in T98G cultures transfected with pcDNA-HA or pcDNA-HA-p73DD by immunofluorescence to anti-α-tubulin (to visualize the spindle) and counterstained with DAPI (to visualize chromosomes). Pooled results from two independent experiments are shown (at least 250 mitotic cells were analyzed). Pro/Prometa, prophase/prometaphase; meta, metaphase; Ana/Telo, ana-telophase. **B,** aberrant late mitotic phenotypes in cultures expressing the p73DD mutant. Row a, an exemplifying field from p73DD-transfected cultures with two neighboring anaphase/early telophases, both of which show precociously decondensed chromatin in one of the separating nuclei (left column, DAPI staining) and a well shaped midbody (visualized by tubulin immunofluorescence, middle column). Row b, telophase from p73DD-transfected cultures with lagging chromatids trapped in the elongating midbody (bar, 10 μm). α-tub, α-tubulin.

**FIG. 4.** The Kip2/p57 cdki is a *p73* target during exit from mitosis. **A,** p21 and Kip2/p57 accumulate with different kinetics in T98G glioma cells released from nocodazole (Noc) arrest. Extracts from cells treated as for Fig. 1A were collected at the indicated times and analyzed by immunoblotting for p21 (row c) and Kip2/p57 (row b) protein levels and *p73β* electrophoretic mobility (row a). **Lower panel,** quantification of Kip2/p57 protein levels by densitometric analysis. Data are expressed as fold increase (mean ± S.D.) from two independent experiments. Lane N, nocodazole-blocked cells. **B,** abrogation of *p73* transcriptional activity prevents Kip2/p57 induction during exit from mitosis. T98G cells were transfected with either HA-p73DD or pcDNA-HA vectors. 8 h after transfection, cells were synchronized with nocodazole and released from arrest as described in Fig. 1A, collected at the indicated times, and analyzed by either immunoblotting using the indicated antibodies (upper panel, rows a to c) or semiquantitative reverse transcription-PCR using specific primers for Kip2/p57 (lower panel). Middle and lower panels, quantification of Kip2/p57 protein and mRNA levels, respectively, by densitometric analysis. Data are expressed as fold increase (mean ± S.D.) from two independent experiments. **C,** selective degradation of *p73* transcripts by specific siRNAs prevents Kip2/p57 induction during exit from mitosis. T98G cells were transfected with either GFP or *p73*-specific siRNAs. Cell extracts prepared as in B were assayed by immunoblotting for endogenous Kip2/p57 and p73. **Lower panel,** quantification of Kip2/p57 protein levels by densitometric analysis. Data are expressed as fold increase (mean ± S.D.) from two independent experiments.
expression was abolished by siRNAs (Fig. 2B). Again, the mitotic apparatus appeared to assemble normally, yet mitotic cells expressing the p73DD mutant exhibited a variety of abnormalities in late anaphase/early telophase stages (Fig. 3B). For example, chromatin decondensed precociously and often asymmetrically in one of two segregating nuclei and/or nuclei remained frequently connected through persisting chromatin bridges. Lagging chromatids were also often observed between nuclei. The abnormal late mitotic figures observed in p73 siRNA- or p73DD-transfected cultures were virtually indistinguishable. These observations strongly suggested that the transcriptional activating function of p73 is required for proper completion of mitosis.

The Kip2/p57 cdki Is a Transcriptional Target of p73 during Exit from Mitosis—Although p73 shares with p53 the ability to bind the same conserved elements (p53-responsive elements, p53RE) in the regulatory regions of their target genes (1, 2), increasing evidence indicates that specific subsets of genes are selectively activated in vivo by p73 or p53 (29). Since p73 and p53 are differentially regulated during mitotic progression (16, 30), we reasoned that any gene potentially involved in control of mitotic exit by p73 should be specifically targeted by p73 but not p53. Obvious target genes known to be regulated along the cell cycle, such as p21, GADD45, and 14-3-3, are activated by both p53 and p73 (1, 2, 29). Among genes that have been shown to be selectively regulated by p73, only the ADA and the Kip2/p57 genes are implicated in control of cell cycle progression (19, 29, 31). The ADA gene encodes an enzyme that controls nucleotide metabolism, the deficiency of which leads to defective DNA synthesis and repair. Of interest to the present study, the cyclin-dependent kinase inhibitor Kip2/p57 has been reported to cooperate with p21 to ensure coordination of M and S cycles so as to prevent endoreduplication (32). We therefore asked whether Kip2/p57 is also modulated at the M-to-G1 transition. We found that Kip2/p57 is up-regulated during nocodazole release in T98G cells (Fig. 4A, row b), with a kinetics that differs from that of p21 (row c). Kip2/p57 expression peaks 1 h after release of nocodazole-induced arrest, when most cells are in telophase and p73 phosphorylation is lost (Fig. 1B), and begins to decrease after 4 h, when cells have re-entered G1, whereas p21 increase is highest at this same time (Fig. 4A, compare row b and row c). Most importantly, selective abrogation of p73 transcriptional activity, by either overexpression of the dominant negative mutant p73DD (Fig. 4B, compare the left and right panels in row a) or targeted degradation of p73 mRNA using p73-specific siRNAs (Fig. 4C, compare the left and right panels in row a), prevented Kip2/p57 induction in cells released from nocodazole arrest but fail to affect Kip2/p57 expression in nocodazole-treated cells, a condition in which p73 is transcriptionally inactive due to p34cdc2-dependent phosphorylation. These data indicated that Kip2/p57 is specifically regulated by p73 during mitotic exit, and this regulation can account, at least in part, for the requirement of active p73 in coordination of mitotic progression and correct mitotic exit.

Abrogation of Kip2/p57 Induces a Mitotic Exit Phenotype—Next, we examined the effect of abrogating Kip2/p57 expression on mitotic progression and M-to-G1 transition. We reasoned that if Kip2/p57 is a p73 transcriptional target during exit from mitosis and plays an important role in the process, then knocking-out of the Kip2/p57 gene expression by siRNAs...
should result in a mitotic phenotype resembling that found when p73 function is impaired. Indeed, cells exposed to Kip2/p57 siRNAs showed an increased proportion of ana-telophase figures in the mitotic cell population (Fig. 5A) and accumulate both aberrant late mitotic figures (Fig. 5B, rows c and d) and nuclear abnormalities in the subsequent interphase (Fig. 5C). Again, recorded mitotic abnormalities mostly included lagging chromatids between the separating chromatid sets in ana-telophase figures and the persistence of trapped chromatin in the elongating midbody in telophase (Fig. 5B, rows c and d). Taken together these results highlighted the requirement of an intact p73-Kip2/p57 pathway for the cell to coordinate mitotic progression and correctly achieve mitotic exit.

**DISCUSSION**

We report here for the first time a role of p73 in mitotic exit and progression to interphase. The subcellular localization and function of p73 are regulated by phosphorylation and dephosphorylation events during mitosis. We previously showed that, at nuclear envelope breakdown, the endogenous p73 is phosphorylated, is excluded from condensed chromosomes, and disperses in the mitotic cytoplasm, unlike p53 that associates with mitotic microtubules and centrosomes. This differential localization first suggested that these proteins play specific, non-overlapping roles during mitosis (16). Hyperphosphorylated p73 persists during meta- and anaphase. We have now shown that in telophase, p73 is again hypophosphorylated and relocates to daughter nuclei. Consistent with the hypothesis that p73 plays a physiological role in mitosis, we have found that abrogation of p73 expression affects mitotic completion and the M-to-G1 transition, as evidenced by delayed progression through late mitotic stages (ana- and telophase), accompanied by an increased frequency of abnormalities in late mitotic stages and in the subsequent interphase. These results highlighted the specificity of the role played by p73 in mitosis.

Most agents that perturb the mitotic apparatus (i.e. microtubule-directed chemicals; genetic mutants; laser-directed ablation of specific mitotic or chromosome components) evoke the mitotic spindle checkpoint, which arrests mitosis before anaphase, thus preventing chromatid separation and segregation (33, 34). When cells "slip" through this checkpoint and begin chromatid segregation despite the presence of defects in the mitotic apparatus, p53 function is induced and arrests further proliferation of mitotic products generated under faulty conditions (30) through a regulatory mechanism involving ataxia telangiectasia-mutated (35). In the present experiments, a distinct, non-overlapping role for p73 clearly emerged. Cells similarly progressed through pro-, prometa-, and metaphase whether p73 is functional or not, consistent with the notion that, in these stages, phosphorylated p73 loses its trans-activating activity, as reported in previous work (16). Thus, the assembly of the mitotic apparatus and the spindle checkpoint function were insensitive to the presence or absence of p73. Only in the conclusive stages of the mitosis (i.e. late anaphase/telophase) was the trans-activating function of p73 required again; when this function was perturbed, mitotic completion was hindered and showed increased inaccuracy. These results highlighted the specificity of the role played by p73 in mitosis and suggested that p73 is part of the cellular machinery that regulates normal mitotic completion and transition to the following G0.

Our knowledge of factors that regulate mitotic exit in mammalian cells is still fragmentary and is far from the definition of integrated pathways such as those revealed by genetics in yeast. In an effort to identify specific transcriptional targets of p73 in mitotic exit, we have found that the cdki Kip2/p57 is activated by p73, but not p53, in late telophase, concomitant with p73 loss of phosphorylation, relocation in nuclei, and recovered ability to activate transcription. Indeed, abrogation of p73 function, using either the p73DD dominant negative mutant or p73-specific siRNAs, prevented Kip2/p57 accumulation. Furthermore, the abrogation of Kip2/p57 expression by specific siRNAs reproduced the mitotic exit phenotypes observed in cells lacking p73. Based on these data, Kip2/p57 can be viewed as one specific target of p73 important for coordination of mitotic progression and the M-to-G1 transition. The type of abnormalities detected in late mitosis (connecting chromatid bridges between separating nuclei, isolated chromatids that fail to segregate) and the absence of gross defects at the level of the mitotic apparatus suggested that p73, through p57, contributes to regulate the re-establishment of chromatin organization as cells accomplish segregation of the duplicated genomes and re-enter G1.

The identification of a physiological role for p73 at mitotic exit suggested that, besides its established role in mediating cell growth arrest and/or apoptosis in response to DNA damage and spindle poisons, p73 may play an important role in cell growth control. Our results, together with the observation that p73 levels increase in S-phase cells, may evoke a possible explanation for the lack of p73 mutations in most human tumors. Major "hot spots" of p53 mutations fall in the highly conserved DNA-binding core domain; mutant p53 protein cannot bind DNA or activate p53/p73 target genes. Similar mutations in the DNA-binding core domain of p73 would abrogate its ability to activate transcription at the M-to-G1 transition and would favor aberrant mitotic exit, thus causing a detrimental effect on cell growth and viability.

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