Cks1 was originally identified based on genetic interactions with CDC28, the gene that encodes Cdk1 in the budding yeast Saccharomyces cerevisiae. Subsequent work has shown that Cks1 binds Cdc28 and modulates its activity against certain substrates. However, the Cks1/Cdc28 complex also has a role in transcriptional chromatin remodeling not related to kinase activity. In order to elucidate protein networks associated with Cks1 transcriptional functions, proteomic analysis was performed on immunofinity-purified Cks1, identifying a physical interaction with the Paf1 complex. Specifically, we found that the Paf1 complex component Rtf1 interacts directly with Cks1 and that this interaction is essential for efficient recruitment of Cks1 to chromatin in the context of GAL1 gene induction. We further found that Cks1 in this capacity serves as an adaptor allowing Rtf1 to recruit 19S proteasome particles, shown to be required for efficient RNA production from some rapidly inducible genes such as GAL1.
Yeast growth. Regular growth medium for our yeast stains was yeast extract-peptone (YPE) with 2% dextrose (YPED). For GAL1 induction experiments, yeast cells were grown in YEP medium with 2% raffinose, galactose (Gal), or fructose (Fru) with a final concentration of 2% was added, and cells were incubated for 45 min. Mating pheromone arrest synchrony experiments were performed as described previously (33).

Antibodies. The following antibodies were obtained from commercial sources: anti-Flag (Sigma), anti-glutathione-S-transferase (anti-GST; Millipore), antithemagglutinin (anti-HA; Roche), anti-His (Millipore), anti-Myc (Sigma), and anti-Rpt1 (Abcam). Anti-Cdc28 antibodies were described previously (34).

Western blotting and coimmunoprecipitation. Western blotting and coimmunoprecipitations were carried out as previously described (4, 17). Purification of proteins for mass spectrometry analysis. Flag-Cks1 and untagged strains were grown to an optical density (OD) of 2.0, typically in 1 liter of YEPD medium. Cells were harvested and washed once with ice-cold water. The cell pellet was drop frozen in liquid nitrogen and ground to a fine powder using a Retsch grinder chilled with liquid nitrogen. The ground powder was collected in a 50-ml screw-cap tube and stored at −80°C. All binding assays were performed at 4°C for 4 h. Samples were resolved by 4 to 20% SDS-PAGE. Western blots were developed using antibodies as indicated below.

Reproducibility and significance. All experiments were carried out at least two times. Error bars, where shown, correspond to standard deviations (SD). P values were determined using Student’s t test.

RESULTS

Cks1 interacts with the Paf1 complex. To identify Cks1-interacting proteins, immunoprecipitates of Cks1 were subjected to high-resolution mass spectrometry analysis (multidimensional protein identification technology [MuDPIT]) (35, 36). Specifically, proteins bound to Flag-Cks1 immunoprecipitated using anti-Flag resin were compared to proteins bound to the resin when incubated with extract from a nontagged strain. Among Flag-Cks1-specific interactions detected (Table 2), components of the Paf1 transcriptional elongation complex were of particular interest from a transcriptional perspective. To confirm the interaction,
Cks1 was tagged with the Flag epitope and one component of the Pafl complex, Rtf1, was tagged with the HA (Fig. 1A) or Myc (Fig. 1B) epitope. To maintain endogenous expression levels, both proteins were tagged at their carboxyl termini by insertion of sequences into the respective chromosomal loci. Immunoprecipitation of Cks1 and Rtf1 was performed, and in each case, the heterologous protein was detected by Western blotting, revealing an interaction and thus confirming the proteomic analysis. In addition, Cdc28 (Cdk1), a binding partner of Cks1, was detected in all immune complexes. Note that whereas Cks1 immune complexes were highly enriched for Rtf1, a relatively smaller proportion of Rtf1 immune complexes contained Cks1 and Cdc28. This suggests that a significant fraction of the Cks1 pool in the cell is associated with Rtf1, whereas a much smaller fraction of the Rtf1 pool is bound to Cks1. It has been reported that Rtf1 binds non-specifically to anti-Flag resin (38, 39), possibly producing an artifactual result for the Flag-Cks1 pulldown component of the experiment. However, when extract containing HA-Rtf1 but not Flag-Cks1 was incubated with anti-Flag resin, HA-Rtf1 was not detected in the eluate, whereas it was detected in parallel anti-HA immunoprecipitates (see Fig. S1 in the supplemental material). Therefore, under the experimental conditions employed in the current study, Rtf1 does not bind to anti-Flag resin at a level that is detectable.

**GAL1 induction is defective in an rtf1 deletion mutant.** We have previously shown that Cks1 regulates transcription of GAL1 (25). Because of the physical interaction between Cks1 and Rtf1, we hypothesized that Cks1 is involved in GAL1 transcription through interaction with the Pafl complex. Thus, mutants with deletions of each component in the Pafl complex were generated and induction of GAL1 was measured 45 min after addition of galactose by quantitative real-time PCR analysis. Previously, it has been shown that deletion of some genes encoding Pafl complex subunits confers defective galactose-dependent transcription at some loci (40, 41). However, a complete analysis of all component genes has not been reported, and strain background differences appear to affect Pafl complex mutant phenotypes (our unpublished observations), justifying this partial repetition of previous work. As shown in Fig. 2A, the induction of GAL1 is attenuated in several of the mutants, but most severely in the rtf1 deletion mutant. To exclude the trivial possibility that the defect in GAL1 transcription in the Pafl complex mutants is due to altered expression of Cks1, endogenous Cks1 was Flag tagged in the various deletion mutant strains and Western blotting was carried out to compare the levels of Cks1 protein expression. Whereas the leo1 and cdc73 mutants indeed showed reduced expression of Cks1, there was no detectable alteration of Cks1 expression in the rtf1 mutant (Fig. 2B). Therefore, the defect in GAL1 induction in the rtf1 mutant is not due to the impairment of Cks1 expression but most likely is attributable to a direct contribution of Rtf1 to GAL1 transcription.

**Rtf1 is required for recruitment of Cks1 onto the GAL1 ORF.** Because failure to express Cks1 was ruled out as the mechanism accounting for the deficiency of GAL1 induction in the rtf1 mutant, we investigated whether recruitment of Cks1 to the promoter or the open reading frame (ORF) regions of the GAL1 gene was defective. Note that recruitment of Cks1 to the GAL1 gene has been shown to be essential for efficient transcriptional induction (25). Therefore, ChIP was carried out using anti-Flag antibodies to determine the occupancy of Flag-Cks1 on GAL1 chromatin during a 45-min time course. Four primer sets designed to amplify the upstream activating sequence (UAS)/promoter, 5′ ORF region, middle ORF region, or 3′ ORF region of the GAL1 gene were shown schematically in Fig. 2C (upper portion). In contrast to wild-type cells and the paf1 deletion mutant, the rtf1 mutant showed highly compromised Cks1 binding along the entire GAL1 ORF (Fig. 2C). Thus, a failure to efficiently recruit Cks1 to the GAL1 ORF could account for the defect in GAL1 induction observed in the rtf1 mutant.

The failure to accumulate GAL1 mRNA observed in the rtf1 mutant could be due to a defect in transcription initiation or in a downstream function, such as elongation, 3′ end processing, or mRNA transport. We therefore carried out an RNA polymerase II ChIP in parallel with the Flag-Cks1 ChIP described above (see Fig. S2 in the supplemental material). Clearly, there is no defect in RNA polymerase II occupancy as a function of GAL1 induction in the rtf1 mutant, ruling out a defect in transcription initiation.

To determine if Cks1 and Rtf1 are required mutually for load-
FIG 2 Rtf1 is required for effective recruitment of Cks1 to the GAL1 ORF. (A) Each component of the Paf1 complex was deleted separately. Wild-type (WT) and mutant cells were cultured in raffinose (−) for 2 h, and 2% galactose (+) was added for 45 min. GAL1 transcripts were quantified and normalized to actin (ACT1) mRNA. (B) Expression level of Flag-tagged Cks1 in WT and various Paf1 complex deletion mutant strains. Samples were analyzed by SDS-PAGE and Western blotting. Amido black staining (top) and Cdc28 (middle) served as loading controls. (C) Recruitment of Flag-tagged Cks1 to the GAL1 ORF in the absence of Paf1 or Rtf1. Upon galactose induction for 15 or 45 min, chromatin immunoprecipitation using anti-Flag antibodies was performed in WT, Flag-tagged Cks1, and paf1 and rtf1 deletion mutant strains. Cks1-associated chromatin fragments were isolated, amplified using the indicated primers, and normalized to the amount of input DNA prior to immunoprecipitation. All error bars represent SD. Statistical analysis was carried out comparing WT and ∆rtf1 in panels A and C. P values were determined using Student’s t test.

Cks1 Links the Paf1 Complex to the 19S Proteasome

...ing onto chromatin during transcription, ChIP analysis was conducted to compare the recruitment of Rtf1 to the GAL1 gene in the cks1 deletion mutant versus the wild-type strain. As previously shown (25), GAL1 expression is reduced in the absence of Cks1 (Fig. 3A). However, levels of Rtf1 (Fig. 3B) and binding of Rtf1 to the GAL1 gene are comparable in the cks1 mutant and wild-type strain (Fig. 3C). Taken together, these results indicate that whereas binding of Cks1 to the GAL1 ORF in the context of transcriptional induction is dependent on Rtf1, binding of Rtf1 is independent of Cks1.

Interaction between Cks1 and Rtf is direct. The binding detected between Cks1 and Rtf1 in Fig. 1 could be direct or mediated by other proteins. In order to distinguish between these mechanisms, Cks1 (GST tagged) and Rtf1 (Flag tagged) were expressed in E. coli. GST alone or GST-tagged Cks1 was immobilized on glutathione agarose beads and incubated with extracts containing Flag-Rtf1. The beads were washed, and eluted proteins were analyzed by SDS-PAGE and Western blotting. Rtf1 was captured on beads containing GST-Cks1 but not those containing GST (Fig. 4A), indicating a direct interaction. Through a series of domain mapping experiments analogous to the experiment described above, except that fragments of Rtf1 were tested for ability to bind Cks1 (see Fig. S3 in the supplemental material), the Cks1 binding domain of Rtf1 was identified as residing between residues 234 and 373 (Fig. 4B). In this case, the central fragment of Rtf1 was tagged with the 6×His epitope rather than the Flag epitope used to mark the full-length protein in Fig. 4A. This is a region shown genetically to be essential for a number of the transcriptional functions attributed to Rtf1 (42).

Cks1 is essential for recruiting the 19S proteasome particle to Rtf1. Since Rtf1 is required for efficient recruitment of Cks1 to GAL1 (Fig. 2C and 3C) and Cks1 is required for recruitment of the 19S proteasome particle (25), we speculated that Cks1 might serve as an adaptor to allow binding the 19S proteasome particle to Rtf1. To test this idea, we first carried out an immunoprecipitation experiment to determine whether Rtf1 and the 19S proteasome interact in vivo. Rtf1 was tagged using the HA epitope, and Rpt1, an ATPase component of the 19S proteasome base, was tagged using the Flag epitope. When lysates were immunoprecipitated using anti-Myc (negative control), anti-Flag, and anti-HA anti-
bodies (Fig. 4C) a strong band at the position of Rpt1 was detected in the lane corresponding to the anti-Flag immunoprecipitate, as expected (upper and lower portions). In the lane corresponding to the HA-Rtf1 pulldown, a weak band at the mobility corresponding to Rpt1 is detected over background. This minimal signal is not surprising, since only a very small fraction of 19S proteasome particles in the cell is likely to be involved in transcription rather than proteolysis, the primary function of proteasomes. It was not possible to determine whether Rtf1 was coimmunoprecipitated with Flag-Rpt1 because of the high background contributed by anti-Flag IgG at the relevant position on the blot. To examine whether Cks1 is essential for the interaction between Rtf1 and the 19S proteasome, we devised a modified pulldown experiment. The Cks1-interacting region of Rtf1, residues 234 to 373, containing a 6×His tag produced in E. coli, was immobilized on Ni-NTA beads, which were then incubated with wild-type yeast extract or extract from a cks1 deletion mutant strain. When proteins were eluted and subjected to SDS-PAGE and Western blotting, Rpt1 from wild-type but not cks1 deletion mutant strains was captured on the Rtf1 beads (Fig. 4D). Therefore, the 19S proteasome particle cannot bind directly to Rtf1 but can bind in the presence of Cks1. To confirm that Cks1 is necessary and sufficient to mediate this interaction, we performed an in vitro reconstitution experiment (Fig. 4E). Again, the Cks1-interacting region of Rtf1 was preloaded onto Ni-NTA beads, after which the beads were incubated with GST alone or GST-tagged Cks1. Lysates from a cks1 deletion strain were then incubated with the beads supplemented with GST-Cks1 or GST alone. Capture of 19S proteasome particles was again determined by Western blotting using anti-Rpt1 antibody. 19S particles were only captured by beads that had been preincubated with GST-Cks1 (Fig. 4E), confirming that Cks1 can serve as a mediator for recruiting 19S particles to Rtf1. If this relationship between Rtf1, Cks1, and the 19S proteasome particle exists in vivo, then Rtf1 should contribute to recruitment of the 19S proteasome to chromatin. We therefore used ChIP analysis to compare recruitment of the 19S particle to the GAL1 ORF in wild-type versus rtf1 deletion strains. There was a significant decrease in Rpt1 binding to the GAL1 ORF in the rtf1 mutant after 45 min of induction (Fig. 5B), similar to the reduction observed for Cks1 binding (Fig. 2C). Deletion of rtf1 did not affect the level of Rpt1 (Fig. 5A), thereby excluding changes in

FIG 3 Cks1 is not required for the recruitment of Rtf1 to the GAL1 ORF. (A) Wild-type or cks1 deletion mutant cells were cultured in raffinose for 2 h, and 2% galactose was added for 45 min. GAL1 transcripts were quantified and normalized to actin (ACT1) mRNA. (B) Expression level of Flag-tagged Rtf1 in wild-type and cks1 deletion mutant strains. Samples were analyzed by SDS-PAGE and Western blotting. Amido black staining (bottom) was used as a loading control. (C) Recruitment of Flag-tagged Rtf1 to the GAL1 ORF in the absence of Cks1. Before and after galactose induction for 45 min, chromatin immunoprecipitation using anti-Flag antibodies was performed on wild-type untagged (control) wild-type Rtf1-Flag and cks1 Rtf1-Flag strains. Rtf1-associated chromatin fragments were isolated, amplified using the indicated primers, and normalized to the amount of input DNA prior to immunoprecipitation. Error bars represent SD.
Cks1 Links the Paf1 Complex to the 19S Proteasome

Rpt1 expression as an explanation for reduced recruitment. These data are consistent with Cks1 serving as an adaptor that facilitates Rtf1-mediated recruitment of the 19S proteasome particle to actively transcribed chromatin in order to carry out transcriptional functions (Fig. 5C).

DISCUSSION

Transcriptional elongation is a highly regulated process involving both positive and negative factors (43). In this context, the Paf1 complex serves as a multifunctional platform for recruiting factors that alter chromatin dynamics (28). Nevertheless, the molecular mechanisms downstream of the Paf1 complex have not been completely elucidated. Our proteomic analysis of Cks1-interacting proteins identified 4 out of 5 components of the Paf1 complex (Table 2), implying a potential role for Cks1 in transcriptional functions of the Paf1 complex. In the current study, we demonstrated that Cks1 serves as an adaptor that allows Rtf1, a component of the Paf1 complex, to recruit the 19S proteasome particle to target genes, necessary for efficient transcriptional elongation and transcript processing (22, 23). Although we have not directly determined the specific role of Rtf1 within the GAL1 locus in our strain background, the fact that GAL1 message accumulation was impaired in the rtf1Δ mutant, but occupancy of RNA polymerase II on the GAL1 ORF was not, argues against a role in transcriptional activation or elongation and favors a role in mRNA processing. On the other hand, the facts that both Cks1 and the 19S proteasome particle have a role in nucleosome eviction (26) and that Rtf1 is required for efficient Cks1 and 19S proteasome particle recruitment are consistent with a role in transcriptional elongation. More direct experiments will be required to distinguish between these functions.

Rtf1-dependent recruitment of Cks1 and the 19S proteasome to the GAL1 gene during transcriptional induction. The Paf1 complex was identified based on its physical association with RNA polymerase II (44). Using the GAL1 gene as a model system, it was previously shown that deletion of genes encoding Ctr9 or Paf1, components of the Paf1 complex, led to reduced expression by impairing nucleosome eviction as well as decreasing association of
RNA Pol II (40). Yet the molecular mechanism whereby the Paf1 complex promotes nucleosome eviction has remained poorly understood. We have previously shown that Cks1 and the 19S proteasome particle are required for induction-dependent nucleosome eviction at the \textit{GAL1} gene (26). In the present study, we demonstrate that Cks1 promotes Rtf1-dependent recruitment of the 19S proteasome particle to the \textit{GAL1} gene, possibly providing an explanation for Paf1 complex-mediated nucleosome eviction. Interestingly, the recruitment of Cks1 was affected within the ORF region but not the UAS region of the \textit{GAL1} gene in the \textit{rtf1} deletion mutant (Fig. 2C). However, 19S proteasome particle loading was defective in both the UAS and ORF regions (Fig. 5B). This suggests that different mechanisms of proteasome recruitment might be operative with the UAS versus ORF regions. Indeed, it has been shown that recruitment of the 19S proteasome particle to the UAS region of the \textit{GAL1} gene for transcriptional activation functions is dependent on the transcription factor Gal4 and the chromatin-remodeling complex SAGA (16). Therefore, it appears that Cks1 is only important for recruitment of the 19S proteasome particle for elongation functions of Rtf1. This is also consistent with previous reports suggesting that the 19S proteasome particle has distinct functions in transactivation and elongation (22, 23).

**General applicability of the mechanisms proposed in this study.** Cks1 has been shown to regulate expression of two inducible genes, \textit{CDC20} (3) and \textit{PHO5} (26). Other inducible cell cycle regulated genes (45, 46) and \textit{PHO5} (47) have also been reported to be regulated by the Paf1 complex. These data suggest that Rtf1-Cks1-19S proteasome particle axis comes into play particularly when rapid gene induction requires energy for nucleosome eviction. Possibly the ATPases of the 19S particle are mobilized for this function. Interestingly, Rtf1 seemed to be the most critical member of the Paf1 complex in \textit{GAL1} expression in our genetic background (Fig. 2A). This is consistent with a recent study in which a fragment of Rtf1 could support H2B ubiquitylation even...
in the absence of other Paf1 complex members (48). In addition, Rtf1 is essential for the association of the Paf1 complex with chromatin and RNA Pol II in yeast (49). Therefore, Rtf1 appears to be central to the Paf1 complex and in some cases can carry out functions independently. Indeed, we observed no defect in GAL1 induction in the paf1 deletion mutant but a strong defect in the rtf1 deletion mutant. It should be pointed out, though, that in another study, the paf1 mutant was defective in GAL1 induction (40), most likely attributable to differences in genetic background.

Possible parallels between Cks protein transcriptional functions in yeast and mammalian cells and possible roles in cancer. We discovered novel physical and functional interactions between Cks1 and a component of the Paf1 complex in yeast. Several observations suggest that a similar functional relationship may exist in mammals. First, Cks1 (50) and the Paf1 complex (28) share significant structural similarity with their counterparts in mammals. Second, Cks paralogs, Cks1 and Cks2, are required for efficient expression of CDK1, CCNA2, and CCNB1 (encoding Cdk1, cyclin A2, and cyclin B1) (51, 52). A similar relationship exists between the Paf1 complex and expression of the same three genes (53). Moreover, in some ducal breast carcinomas, the expression of Cdk1, cyclin B1, and cyclin A2, as well as Cks proteins, is coordinately upregulated (54). However, although Cks protein overexpression is a characteristic of many forms of cancer (55–73) and Cks1 deletion has protective effects in some mouse cancer models (74–76), it is not clear that Cks-mediated oncogenicity is related to transcriptional functions. As Cks1 is a component of the SCFSkp2 ubiquitin ligase, it has been suggested that oncogenic functions of Cks1 might be mediated by affecting the stability of the Cdk inhibitor p27Kip1, an SCFSkp2 target (77). Yet disruption of Skp2 has only a modest effect on Myc-mediated lymphomagenesis in a mouse model, whereas deletion of Cks1 significantly attenuates the disease (78). On the other hand, it is more likely that oncogenic functions of Cks proteins are linked to override of cell cycle checkpoints and oncogene-induced stress barriers associated with Cks protein overexpression (76). A thorough understanding of the Cks1-proteasome-Paf1 interaction in humans, should it exist, will allow the determination of whether Cks protein-mediated transcription functions contribute to oncogenesis.

ACKNOWLEDGMENTS

We thank members of the Scripps Cell Cycle Group for discussion. This research was supported by NIH grants GM038328 and CA074224.

REFERENCES

1. Hadwiger JA, Wittenberg C, Mendenhall MD, Reed SI. 1989. The Saccharomyces cerevisiae CKS1 gene, a homolog of the Schizosaccharomyces pombe sucl + gene, encodes a subunit of the Cdc28 protein kinase complex. Mol. Cell. Biol. 9:2034–2041.
2. Hayles J, Beach D, Durack B, Nurse P. 1986. The fission yeast cell cycle control gene cdc2: isolation of a sequence suc1 that suppresses cdc2 mutant function. Mol. Gen. Genet. 202:291–293.
3. Morris MC, Kaiser P, Rudaky S, Baskerville C, Watson MH, Reed SI. 2003. Cks1-dependent proteasome recruitment and activation of CDC20 transcription in budding yeast. Nature 423:1009–1013.
4. Kaiser P, Moncollin V, Clarke DJ, Watson MH, Bertolaet BL, Reed SI, Bailly E. 1999. Cyclin-dependent kinase and Cks/Suc1 interact with the proteasome in yeast to control proteolysis of M-phase targets. Genes Dev. 13:1190–1202.
5. Sauer RT, Baker TA. 2011. AAA+ proteases: ATP-fueled machines of protein destruction. Annu. Rev. Biochem. 80:587–612.
scriptional elongation complex is required for ubiquitination of histone H2B. J. Biol. Chem. 278:33625–33628.
30. Wood A, Schneider J, Dover J, Johnston M, Shilatifard A. 2003. The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. J. Biol. Chem. 278:34739–34742.
31. Redd SL, Hagedorn A, Lorincz AT. 1985. Protein kinase activity associated with the product of the yeast cell division cycle gene CDC28. Proc. Natl. Acad. Sci. U. S. A. 82:4055–4059.
32. Longtine MS, McKenzie A, 3rd, Demarini DJ, Shah NG, Wach A, Brachat A, Philippens P, Pringle JR. 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast 14:953–961.
33. de Bruin RA, Kalashnikova TI, Chahwan C, McDonald WH, Westerhout C, Yates J, III, Russell P, Reed SI. 2003. Constraining G1-specific transcription to late G1 phase: the MBI-regulated corepressor Prof1 acts via negative feedback. Mol. Cell 23:483–496.
34. Mendenhall MD, Jones CA, Reed SI. 1987. Dual regulation of the yeast CDC28-p40 protein kinase complex: cell cycle, pheromone, and nutrient limitation effects. Cell 59:927–935.
35. Olson BL, Hock MB, Ekholm-Reed S, Wohlschlegel JA, Dev KK, Kralli AM, Mendenhall MD, Jones CA, Reed SI. 2006. X chromosomal abnormalities in basal-like human breast cancer. Cancer Cell 9:121–132.
36. Chow LS, Lam CW, Chan SY, Tsao SW, To KF, Tong SF, Hung WK, Dammann R, Huang DP, Lo KW. 2006. Identification of RASSF1A modulated genes in nasopharyngeal carcinoma. Oncogene 25:310–316.
37. de Vos S, Krug U, Hofmann WK, Pinkus GS, Swerdlow SH, Wachmann W, Grogan TM, Said JW, Koefler HP. 2003. Cell cycle alterations in the blastoid variant of mantle cell lymphoma (MCL-BV) as detected by gene expression profiling of mantle cell lymphoma (MCL) and MCL-BV. Diagn. Mol. Pathol. 12:35–43.
38. de Wit NJ, Rijntjes J, Diepstra JH, van Kuppevelt TH, Weidle UH, Ruitter DJ, van Muijen GN. 2005. Analysis of differential gene expression in human melanocytic tumour lesions by custom made oligonucleotide arrays. Br. J. Cancer 92:2249–2261.
39. Inui N, Kitagawa K, Miwa S,hattori T, Chida K, Nakamura H, Kitagawa M. 2003. High expression of Csk1 in human non-small cell lung carcinomas. Biochem. Biophys. Res. Commun. 303:978–984.
40. Kawakami K, Enokida H, Tachiwada T, Gotanda T, Tsuneyoshi K, Kubo H, Nishiyama K, Takiguchi M, Nakagawa M, Seki N. 2006. Identification of differentially expressed genes in human bladder cancer through genome-wide gene expression profiling. Oncol. Rep. 16:521–531.
41. Kitajima S, Kudo Y, Ogawa I, Bashir T, Kitagawa M, Miyahuchi M, Pagano M, Takata T. 2004. Role of Csk1 overexpression in oral squamous cell carcinomas: cooperation with Skp2 in promoting p27 degradation. Am. J. Pathol. 165:2147–2155.
42. Li WW, Lin YM, Hasegawa S, Shimokawa T, Murata K, Kameyama M, Ishikawa O, Katagiri T, Tsunoda T, Nakamura Y, Furukawa Y. 2004. Genes associated with liver metastasis of colon cancer, identified by genome-wide cDNA microarray. Int. J. Oncol. 24:305–312.
43. Masuda TA, Inoue H, Nishida K, Sonoda H, Yoshikawa Y, Kakeji Y, Utsunomiya T, Mori M. 2003. Cyclin-dependent kinase 1 gene expression is associated with poor prognosis in gastric carcinoma. Clin. Cancer Res. 9:5693–5698.
44. Ouellet V, Guyot MC, Le Page C, Filiali-Mouhim A, Lussier C, Tonin PN, Provencher DM, Mes-Masson AM. 2006. Tissue array analysis of expression microarray candidates identifies markers associated with tumor grade and outcome in serous epithelial ovarian cancer. Int. J. Cancer 119:599–607.
45. Ouellet V, Provencher DM, Maugaud CM, Le Page C, Ren F, Lussier C, Novak J, Ge B, Hudson TJ, Tonin PN, Mes-Masson AM. 2005. Discrimination between serous low malignant potential and invasive epithelial ovarian tumors using molecular profiling. Oncogene 24:4672–4687.
46. Shapira M, Ben-Izhak O, Bishara B, Futerman B, Minkov I, Krausz MM, Pagano M, Hershko DD. 2004. Alterations in the expression of the cell cycle regulatory protein cyclin kinase subunit 1 in colorectal carcinoma. Cancer 100:1615–1621.
47. Slotky M, Shapira M, Ben-Izhak O, Linn S, Futerman B, Talic M, Hershko DD. 2005. The expression of the ubiquitin ligase subunit Csk1 in human breast cancer. Breast Cancer Res. 7:R737–R744. doi:10.1186/bcr1278.
48. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, Febbo PG, Balk SP. 2006. Increased expression of genes converting ad-
renal androgens to testosterone in androgen-independent prostate cancer. Cancer Res. 66:2815–2825.
68. Urbanowicz-Kachnowicz I, Baghdassarian N, Nakache C, Gracia D, Mekki Y, Bryon PA, Ffrench M. 1999. ckshs expression is linked to cell proliferation in normal and malignant human lymphoid cells. Int. J. Cancer 82:98–104.
69. Wong YF, Cheung TH, Tsao GS, Lo KW, Yim SF, Wang VW, Heung MM, Chan SC, Chan IK, Ho TW, Wong KW, Li C, Guo Y, Chung TK, Smith DI. 2006. Genome-wide gene expression profiling of cervical cancer in Hong Kong women by oligonucleotide microarray. Int. J. Cancer 118:2461–2469.
70. van’t Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GI, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH. 2002. Gene expression profiling predicts clinical outcome of breast cancer. Nature 415:530–536.
71. Shapira M, Ben-Izhak O, Linn S, Futerman B, Minkov I, Hershcob DD. 2005. The prognostic impact of the ubiquitin ligase subunits Skp2 and Cks1 in colorectal carcinoma. Cancer 105:1336–1346.
72. Kawakami K, Enokida H, Tachiwada T, Nishiyama K, Seki N, Nakagawa M. 2007. Increased SKP2 and CKS1 gene expression contributes to the progression of human urothelial carcinoma. J. Urol. 178:301–307.
73. Lan Y, Zhang Y, Wang J, Lin C, Ittmann MM, Wang F. 2008. Aberrant expression of Cks1 and Cks2 contributes to prostate tumorigenesis by promoting proliferation and inhibiting programmed cell death. Int. J. Cancer 123:543–551.
74. Keller UB, Old JB, Dorsey FC, Nilsson JA, Nilsson L, MacLean KH, Chung I, Yang C, Spruck C, Boyd K, Reed SI, Cleveland JL. 2007. Myc targets Cks1 to provoke the suppression of p27Kip1, proliferation and lymphomagenesis. EMBO J. 26:2562–2574.
75. Lee EK, Kim DG, Kim JS, Yoon Y. 2011. Cell-cycle regulator Cks1 promotes hepatocellular carcinoma by supporting NF-kappaB-dependent expression of interleukin-8. Cancer Res. 71:6827–6835.
76. Liberal V, Martinsson-Ahlzen HS, Liberal J, Spruck CH, Widschwendter M, McGowan CH, Reed SI. 2012. Cyclin-dependent kinase subunit (Cks) 1 or Cks2 overexpression overrides the DNA damage response barrier triggered by activated oncoproteins. Proc. Natl. Acad. Sci. U. S. A. 109:2754–2759.
77. Harper JW. 2001. Protein destruction: adapting roles for Cks proteins. Curr. Biol. 11:R431–R435. doi:10.1016/S0960-9822(01)00253-6.
78. Old JB, Kratzat S, Hoellein A, Graf S, Nilsson JA, Nilsson L, Nakayama KI, Peschel C, Cleveland JL, Keller UB. 2010. Skp2 directs Myc-mediated suppression of p27Kip1 yet has modest effects on Myc-driven lymphomagenesis. Mol. Cancer Res. 8:353–362.