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# Discordant Expression of the Cyclin-Dependent Kinases and Cyclins in Rat Liver Following Acute Administration of the Hepatocarcinogen [4-Chloro-6-(2,3-Xylidino)-2-Pyrimidinylthio] Acetic Acid (WY14,643)

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**ABSTRACT.** Cellular proliferation is an essential aspect of chemical carcinogenesis. At the core of cell cycle regulation is a family of serine/threonine protein kinases termed cyclin-dependent kinases (cdk). Cdk activity, which directs progression through the cell cycle, is dependent upon cdk binding to the appropriate, phase-specific cyclin proteins. Alterations in hepatic cdk1, cdk2, cdk4, cdk5, and cyclin protein expression were determined in response to acute dosing of the prototypic peroxisome proliferator and hepatocarcinogen [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid (WY14,643). Intraperitoneal dosing of 45 mg WY14,643/kg daily for 4 days to young, male rats produced dramatic increases in hepatic protein expression of all cdk analyzed as well as cyclins B, D2, D3, and proliferating cell nuclear antigen (PCNA). The largest relative increases, 6.1-, 2.8-, 11-, 83-, and 7.9-fold, were seen with cdk1, cdk4, cyclin B, cyclin D3, and PCNA, respectively. Increases of only 1.8-, 2-, 1.6-, and 1.4-fold were noted, respectively, for cdk2, cdk5, cyclin D2, and cyclin E. Analysis of gel filtration fractions indicated that PCNA co-eluted with cdk1 from the WY14,643-treated rats as a 70–80 kDa molecular complex. In contrast, cdk4, cdk5 and D cyclins migrated as much larger complexes with an estimated MW of approximately 180–190 kDa. *Copyright © 1996 Elsevier Science Inc.* BIOCHEM PHARMACOL 52;11:1749–1755, 1996.

**KEY WORDS.** peroxisome proliferator; cell cycle; hepatocarcinogenesis; cyclin-dependent kinase

The mechanisms underlying chemical carcinogenesis by nongenotoxic compounds have been an area of intensive investigation, but are still poorly understood. Peroxisome proliferators represent a subclass of these compounds; they are structurally diverse, with the majority being either hypolipidemic drugs (clofibrate, nafenopin, WY14,643)§ or industrial chemicals used in plastic production such as DEHP. The hallmark biochemical response to these compounds *in vivo* is an increase in the number or volume of peroxisomes within hepatocytes [1]. These compounds bind to an intracellular receptor known as the PPAR, which, once activated by ligand binding, alters gene transcription directly [2–6]. Activation of the PPAR also initiates signal transduction pathways as denoted by changes in protein tyrosine phosphorylation‖ similar to those induced by TCDD [7]. Moreover, peroxisome proliferators are highly hepatocarcinogenic in rodent species, with carcinogenicity correlating well with the capacity to induce and maintain cellular proliferation [8–11].

Recent investigations into the eukaryotic cell cycle have described the process as being governed by two families of proteins: cdk and cyclins. Cdk are a family of closely related serine/threonine kinases that mediate cell cycle progression from quiescence ($G_0$) through mitosis [12–19]. Their kinase activity is regulated in actively dividing cells by cycles of phosphorylation and dephosphorylation of critical threonine and tyrosine residues, and by complexing with cyclin proteins that target the substrate for phosphorylation [20, 21]. *In vivo*, transcriptional regulation has also been described as a critical control mechanism [22–25].

$p34^{cdc2}$kinase (cdk1) was the first cdk described and is currently the best characterized of the family. Moreover,

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§ *Abbreviations:* BRDU, bromodeoxyuridine; cdk, cyclin dependent kinase; CYP4A1, cytochrome P-450 4A1; DEHP, di(2-ethylhexyl)-phthalate; PCNA, proliferating cell nuclear antigen; Rb protein, retinoblastoma protein; TBS, Tris-buffered saline; TBST, Tris-buffered saline plus Tween 20; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; and WY14-463, [4-chloro-6(2,3-xylidino)-2-pyrimidinylthio] acetic acid.

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‖ Unpublished observations.

cdk1 is required for cellular division in all eukaryotic cells and serves as a marker of cellular proliferation [12, 14, 26]. Previous work in our laboratory has shown that both WY14,643 and TCDD, an unrelated rodent hepatocarcinogen, significantly induce the expression of hepatic cdk1 in exposed animals [27, 28]. In the present study, WY14,643 effects on the hepatic expression of cdk1 and other critical cell cycle control proteins are described.

## MATERIALS AND METHODS

### *Chemicals*

WY14,643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid; CAS 50892-23-4) was purchased from Chem-Syn Science Labs (Lenexa, KS). Bicinchoninic acid was obtained from Pierce (Rockford, IL); molecular size standards were supplied through BioRad (Melville, NY). Bicinchoninic acid was used for the spectrophotometric determination of protein concentration following manufacturer's instructions (Pierce, Rockford, IL). Anti-cdk1 (C-terminus polyclonal), anti-PCNA (PC10 monoclonal), anti-rabbit and anti-mouse antibodies were obtained from Pederson Biotech Resources (Ithaca, NY). Antibodies to cdk2, cdk4 and cdk5 as well as antibodies to cyclins A, D1, D2, D3, E and Rb protein were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody to cyclin B was purchased from Oncogene Sciences (Uniondale, NY). Alkaline phosphatase conjugated anti-mouse and anti-rabbit IgG were purchased from Jackson Immunoresearch (West Grove, PA). Antisera to CYP4A1 was purchased from Gentest (Woburn, MA). All other chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO) and were of the highest purity available.

### *Animals*

Young, adult male Sprague-Dawley rats (~200 g) were purchased from Charles River Laboratory (Charles River, MA) and acclimated for a minimum of 2 weeks before assignment to treatment groups. Rats were fed Prolab RMH 1000 (Agway, Cortland, NY) and received tap water *ad lib.* All rats were housed two or three per cage and maintained at constant temperature (22 ± 2°), humidity (50 ± 5%) and lighting (12/12 hr).

Rats were administered daily i.p. injection of either corn oil, as a control, or WY14,643 in corn oil at 45 mg/kg/day for 4 days. The volume of the injections ranged from 0.4 to 0.5 mL per rat. Preparation and −80° storage of the liver homogenates (S9) for SDS-PAGE, immunoprecipitation, immunoblotting, as well as cdk1 and PCNA quantification was performed as previously described [27–30].

### *Gel Filtration*

A disposable polystyrene column (Pierce, Rockford, IL) was packed with Sephacryl S-200 HR (Pharmacia, Piscataway, NJ) yielding a bed volume of 2 mL and equilibrated with TBS at a flow rate of 0.5 mL/min. Eluted fractions were monitored at 280 nm. The column was calibrated with alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), and cytochrome c (12.4 kDa). Fifty microliters of pooled (N = 5; 4-day post-treatment) rat hepatic S9 from WY14,643-treated rats or corn oil-treated rats were injected onto the column and fifteen 30-sec fractions collected beginning immediately post-injection. This procedure was performed three times for each treated and control sample. Eluted fractions were then pooled, flash frozen with liquid nitrogen, and stored at −80°.

### *Chromatography and ELISA Analysis*

Quantification of cdk1 and PCNA was performed using ELISA kits following the manufacturer's instructions (Paracelsian, Ithaca, NY). For analysis of cdk1, cdk2, cdk4, cdk5, Rb protein, PCNA, and cyclins A, B, D1–D3 and E, gel

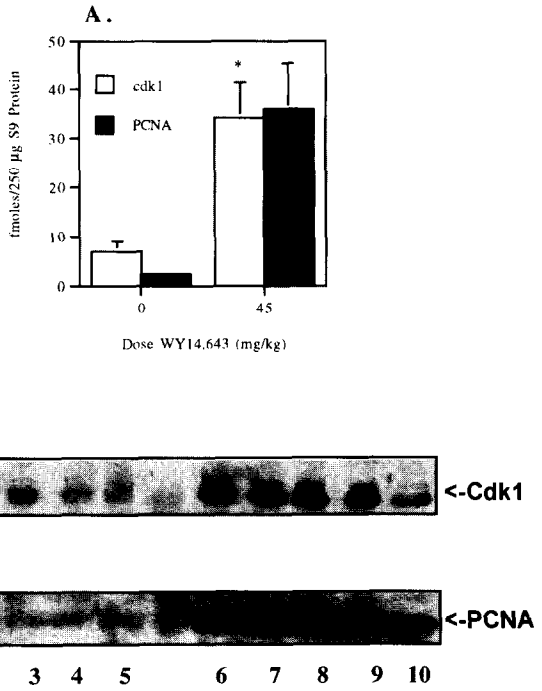


**FIG. 1. Effect of WY14,643 to induce expression of cell cycle regulatory proteins cdk1 and PCNA. Panel A: ELISA quantification of cdk1 and PCNA of hepatic S9 from control and WY14,643-treated animals. Samples were run in duplicate and represent the mean ± SD of five rats per treatment. Mean cdk1 fmol value ± SD: control, 6.65 ± 2.3, WY14,643-treated, 34.15 ± 7.4. Mean PCNA fmol value ± SD: control, 2.14 ± 0.92, WY14,643-treated, 36.06 ± 9.37. Panel B: western blot demonstrating hepatic induction of cdk1 expression following WY14,643 dosing performed as described in Materials and Methods. Lanes 1–5 are control samples, and lanes 6–10 are WY14,643-treated samples; each lane represents a single rat. Panel C: western blot demonstrating hepatic induction of PCNA expression following WY14,643 dosing performed as described in Materials and Methods. Lanes 1–5 are control samples, and lanes 6–10 are WY14,643-treated samples; each lane represents a single rat.**

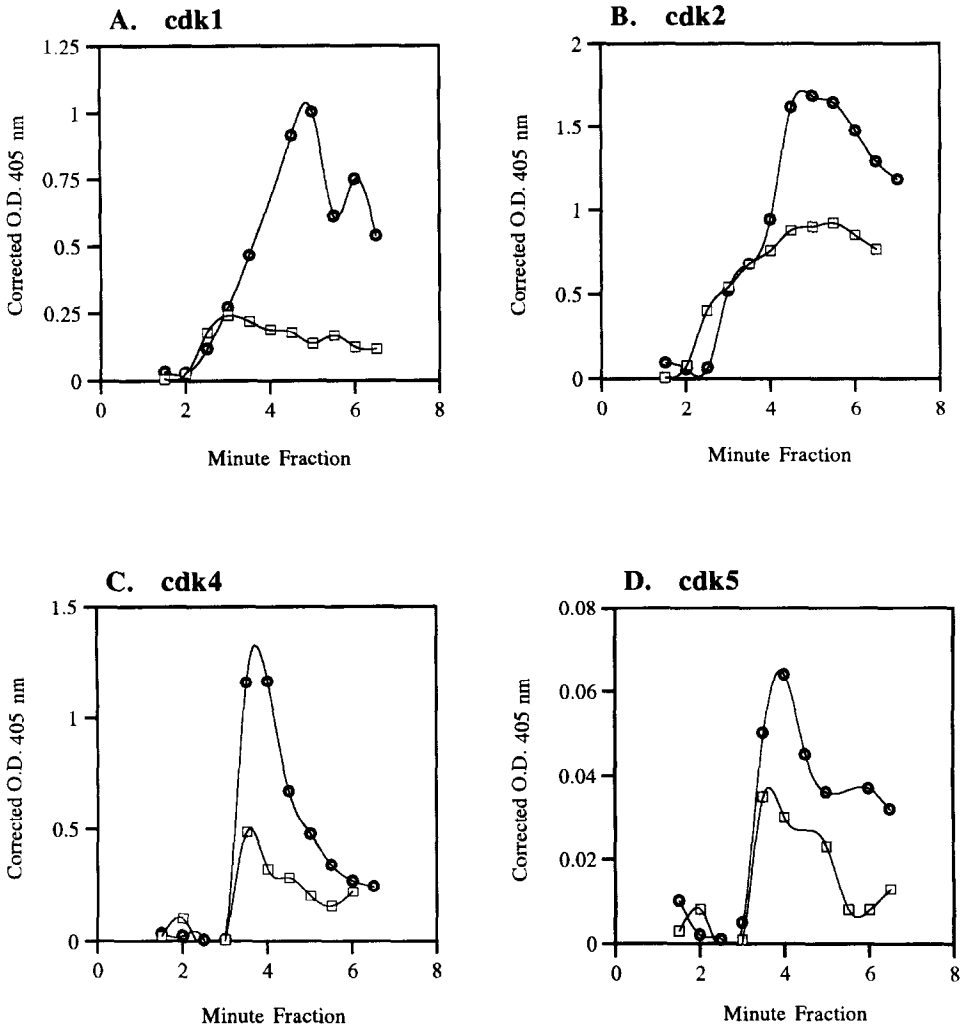


**FIG. 2. Elution profiles of cyclin-dependent kinases from control (open squares) and WY14,643-treated (open circles) hepatic S9. Fractions 3–14 eluting from a 2 mL Sephacryl S-200 HR column (0.5 mL/min flow rate) were diluted 40-fold into an ELISA plate and probed with antibodies specific for each cdk as described in Materials and Methods. Data in the figure are background subtracted optical densities read at 405 nm. Panel A: cdk1 profile. Panel B: cdk2 profile. Panel C: cdk4 profile. Panel D: cdk 5 profile.**

filtration fractions 3 through 14 were diluted 40-fold into 0.2 M borate buffer pH 10.5 and coated onto Immulon 3™ DividaStrips (Dynatech Laboratories, Chantilly, VA) at 37° for 1 hr. Plates were then washed three times with TBST, and working primary antibody solutions (1 μg/mL in TBST plus 0.1% BSA, 200 μL per well) for the respective proteins were added to the wells and allowed to incubate at room temperature for 1 hr. The plates were then washed and 200 μL of working secondary antibody (alkaline phosphatase conjugated anti-rabbit IgG, anti-mouse IgG; 1 μg/mL) added and allowed to incubate at room temperature for 1 hr. The plates were then again washed and color was developed using *p*-nitrophenylphosphate tablets dissolved (1.5 mg/mL) in diethanolamine substrate buffer (45 mM diethanolamine, 20 mM $MgCl_2 \cdot 6H_2O$, 0.02% $NaN_3$, pH 9.8). Optical density readings at 405 nm were taken 30, 60, and 120 min post-substrate addition using a Bio-Tek Instruments (Winooski, VT) Model EL312 ELISA plate reader.

### *Statistical Analysis*

Analysis of variance and regression analysis were performed using JMP v2 statistical software (SAS Institute, Inc., Cary, NC). Statistical significance between treatment groups and control was assessed with Student's *t*-test, using a Bonferroni correction factor for multiple comparisons. Data are reported as means ± 95% population confidence intervals or SD for the stated number of animals.

## RESULTS

Dosing of WY14,643 to young-adult male rats resulted in characteristic increases in liver/body weight ratios as well as CYP4A1 induction (data not shown). Induction of cellular proliferation was determined by quantification of cdk1 and PCNA protein levels by ELISA as shown in Fig. 1A. Hepatic cdk1 expression in WY14,643 treated rats was elevated approximately 5.1-fold relative to controls. Enhanced expression seen with ELISA results was corroborated by western blotting for cdk1 (Fig. 1B) as well as PCNA (Fig. 1C).

Size exclusion chromatography of hepatic S9 from treated and control rats followed by ELISA detection of cell cycle proteins demonstrated marked increases in cdk1 and cdk4 (Fig. 2); cyclin D3 (Fig. 3); and cyclin B and PCNA (Fig. 4) in WY14,643-treated samples relative to controls.

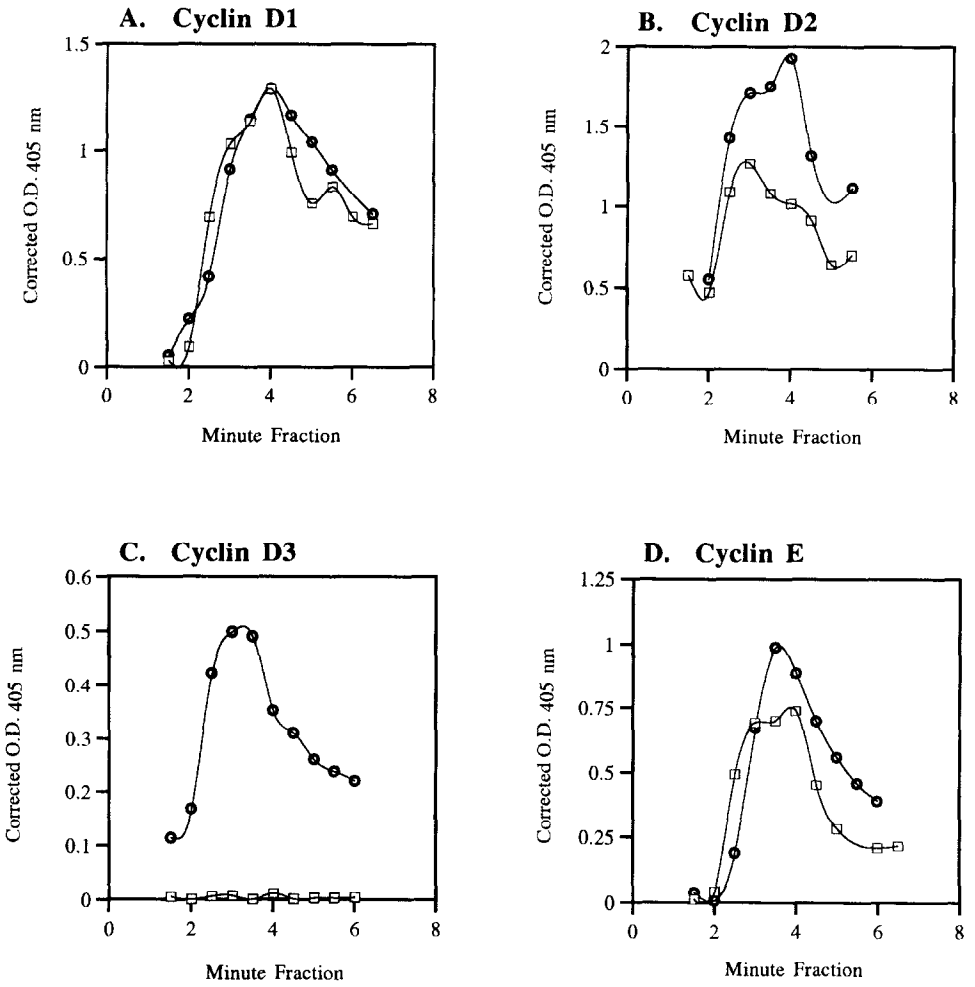


**FIG. 3. Elution profiles of $G_1$/S phase cyclins from control (open squares) and WY14,643-treated (open circles) hepatic S9. Fractions 3–14 eluting from a 2-mL Sephacryl S-200 HR column (0.5 mL/min flow rate) were diluted 40-fold into an ELISA plate and probed with antibodies specific for each cyclin as described in Materials and Methods. Data in the figure are background-subtracted optical densities (O.D.) read at 405 nm. Panel A: Cyclin D1 profile. Panel B: Cyclin D2 profile. Panel C: Cyclin D3 Profile. Panel D: Cyclin E profile.**

All cdk and cyclins with the exception of cyclin D3 were found to be expressed in control tissue, and statistical analysis of the three peak fraction optical density readings at 405 nm indicated that expression of all cdk were elevated ($P < 0.05$) as a result of WY14,643 dosing. With regard to cyclin proteins, however, only cyclins B, D2, and D3 were found to be increased ($P < 0.05$) relative to controls.

Estimation of the apparent molecular masses of the protein complexes with protein standards indicated that cdk1 and cdk2 complexes migrated with a molecular mass of approximately 70–80 kDa. In addition, PCNA co-eluted with cdk1 enriched fractions from the WY14,643-treated rats as determined by the chromatography profile as well as anti-cdk1 immunoprecipitation followed by immunoblotting (Fig. 5). Cdk4, cdk5 and D cyclins migrated as much larger complexes with a calculated molecular mass of approximately 180–190 kDa.

Superimposing the size exclusion chromatography ELISA elution profiles demonstrated good correlation for specific cdk/cyclin complexes as they occur *in vivo* compared with complexes identified from *in vitro* experiments. Cdk1/cyclin B, cdk4/D cyclins, and cdk2/cyclins A and E have repeatedly been demonstrated to associate *in vitro* and *in vivo* [12, 14, 21]. The higher molecular mass complexes might be from association with Rb protein, a known substrate of cdk4/5 and D cyclins [31, 32], which also eluted in the same fractions (Figs. 1 and 4).

## DISCUSSION

In this series of experiments, we have described the expression of the primary cdk (cdk1, cdk2, cdk4, cdk5) and cyclins (A, B, D1, D2, D3, and E) in WY14,643-treated rat liver relative to corn oil-treated controls. Results of this study can be compared to characterizations of cell cycle protein expression during normal regenerative proliferation following partial hepatectomy [22, 23, 33] for the purpose of identifying qualitative or quantitative differences between these two processes. Such a comparison may identify dysregulation of cell cycle signaling pathways involved in the early stages of chemical carcinogenesis.

Our analysis of cdk and cyclin expression in response to carcinogen-induced proliferation corroborates several findings described by Loyer *et al.* [22], with regard to normal liver regeneration. First, cdk2 and cyclins D1 and E are expressed in quiescent as well as proliferating hepatocytes. This study expands those observations by reporting that cdk4 and cyclins A and D2 were expressed in both quies-

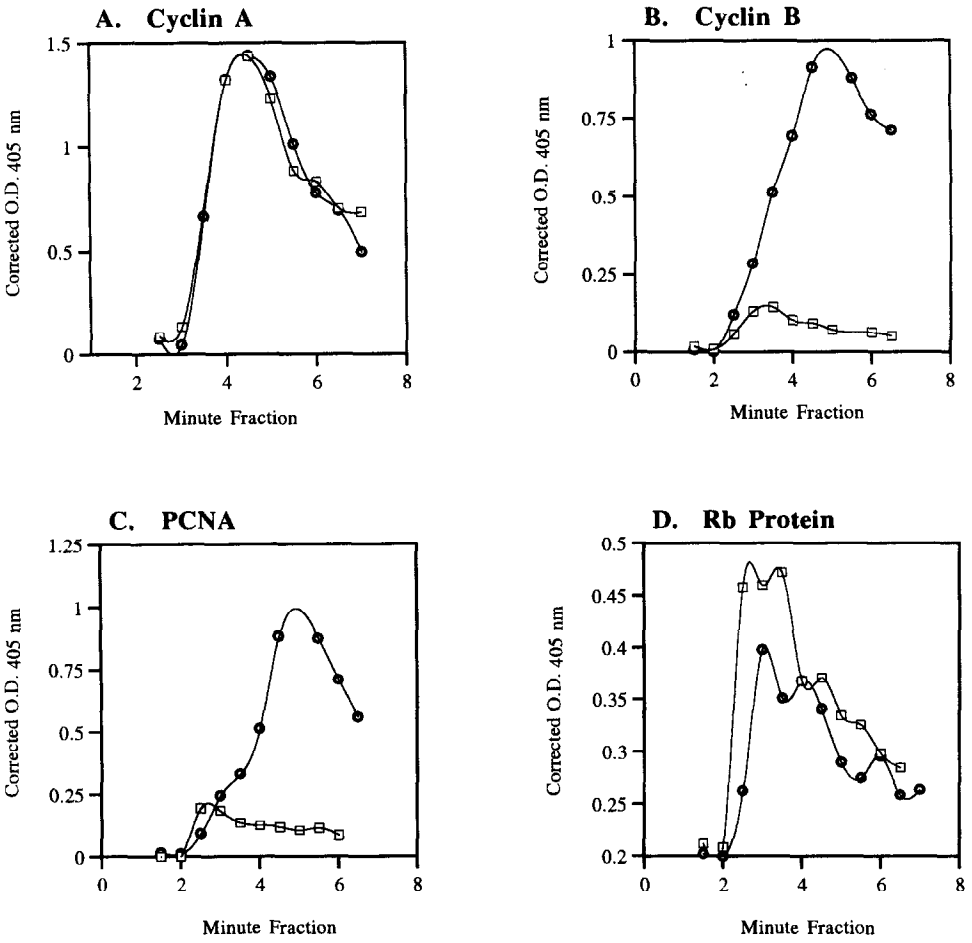


**FIG. 4. Elution profiles of $G_2$/M phase cyclins, PCNA, and Rb protein from control (open squares) and WY14,643-treated (open circles) hepatic S9. Fractions 3–14 eluting from a 2 mL Sephacryl S-200 HR column (0.5 mL/min flow rate) were diluted 40-fold into an ELISA plate and probed with antibodies specific for each cyclin as described in Materials and Methods. Data in the figure are background-subtracted optical densities read at 405 nm. Panel A: Cyclin A Profile. Panel B: Cyclin B profile. Panel C: PCNA profile. Panel D: Rb protein profile.**

cent and stimulated hepatic tissue. Second, we confirmed the finding that cdk1, PCNA, and cyclin B are upregulated transcriptionally in response to mitogenic stimuli, and further demonstrated that cdk2, cdk4, cdk5 and cyclin D3 protein concentrations are also greatly increased through hepatic mitogenic stimulation.

However, our results from this and a 5-day time course WY14,643 dosing experiment demonstrate that continuous WY14,643 exposure maintains elevated or dysregulated expression of cdk1, a phenomenon often seen in tumor cells [34, 35]. In contrast, cdk1 expression in normal liver regeneration begins to decrease 48 hr after partial hepatectomy [22–24].

Size exclusion chromatography and fraction analysis also served to aid in elucidating cdk/cyclin complexes *in vivo*. Work by others [36, 37] has suggested that all cdk and cyclins exist in quaternary complexes with PCNA and a 21-kDa protein ($p21^{Waf1/Cip1}$). If this were the case, these complexes would have a molecular mass >110 kDa. Our results indicate that in proliferating hepatocytes *in vivo*, cdk1 and cdk2/cyclin complexes migrate at approximately 70–80 kDa—more indicative of binary complexes. However, cdk4 and cdk5 and their presumed partners, the D cyclins, eluted as 180–190-kDa protein complexes, indicating association with other cellular proteins such as Rb protein [31, 32], which demonstrated an identical elution profile (Fig. 4D). However, in nonproliferative tissue, cdk1 and PCNA clute as a high molecular mass peak similar to that of cdk4/cyclins D.

Probable associations from the elution profile suggest complexes of cdk1 with cyclins A and B, cdk4 with D cyclins, and cdk2 with cyclins A and E. PCNA eluted primarily in fractions with cdk1 showing a possible association between cdk1 and PCNA; however, PCNA may also exist in the form of a homodimer [38]. Immunoprecipitation experiments from these samples have confirmed that a fraction of PCNA does coprecipitate with cdk1 (Fig. 5). This association has also been demonstrated in normal lymphocytes but not in transformed Jurkat cells [39].

This work does not elucidate a mechanism of action for the hepatocarcinogen WY14,643. However, WY14,643 binding to PPAR may initiate proliferative signals similar to results reported for the dioxin receptor [40]. Aberrant cell proliferation is a fundamental characteristic of cancer, and tumor promoters are hypothesized to act by increasing cellular division [41, 42]. Therefore, the described effects of WY14,643 on recently discovered signalling pathways involved in cell cycle control may provide a basis for a new hypothesis concerning the mechanisms by which nongenotoxic carcinogens may function.

It is the belief of the authors that the carcinogenicity of WY14,643 and other nongenotoxic carcinogens is in part a

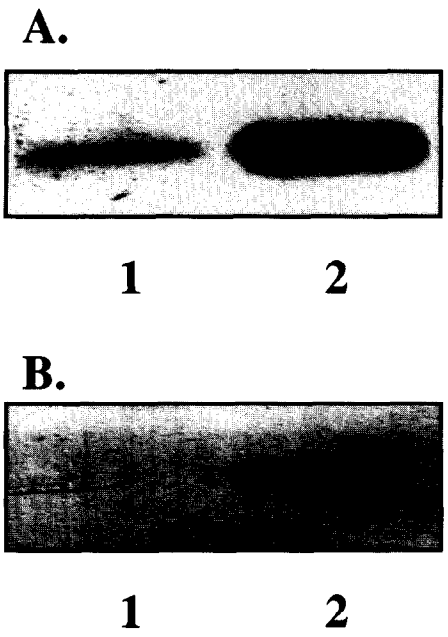


**FIG. 5. Detection of PCNA complexed with cdk1. Hepatic S9 and gel filtration column fractions containing 70–80 kDa proteins and protein complexes were subjected to immunoprecipitation with anti-cdk1 antibody followed by SDS–PAGE and PCNA immunoblot analysis as described in Materials and Methods. Panel A: lanes 1 and 2: control hepatic S9 and WY14,643 hepatic S9, respectively; Panel B: lanes 1 and 2: control and WY14,643 hepatic S9 gel filtration column fractions, respectively.**

function of their ability to stimulate signalling for cellular proliferation. In an effort to stimulate research into the role of cell cycle control proteins in nongenotoxic carcinogenesis, we propose the following model: The mitogenic stimulus that WY14,643 produces is manifest in the increased and discordant expression of the cell cycle control proteins, generating cellular proliferation within target cells. *In vivo*, WY14,643 produces a rapid increase in the rate of DNA synthesis as measured by [$^3$H]thymidine or BRDU incorporation into hepatocytes [8, 10, 11]. However, the magnitude of this response drops dramatically after a few days of dosing relative to initial time points [8, 10].

Maintenance of the signaling for proliferation, produced by continuous exposure to the chemical, results in the expression of cell cycle inhibitory proteins* in an effort to arrest cell division, thereby decreasing the rate of incorporation of S-phase labels. Exposure to stimulatory and inhibitory signaling simultaneously produces a signaling conflict in the cell. Signaling conflicts may be resolved in several ways: (i) removal of the chemical inducing the proliferative stimulus, (ii) apoptosis, or (iii) transformation of the cell to a phenotype that circumvents inhibitory pathways.

With continuous exposure to the chemical agent maintaining proliferative stimuli, the only options available to the cell are (ii) or (iii). It is becoming increasingly apparent that proliferation and apoptosis are closely related alternative cellular responses that share many of the same molecular mechanisms (cdk1, p53, cyclin D1, cyclin B, etc). Therefore, a signaling conflict that blocks cellular division may also serve to block programmed cell death. Genomic instability created by the signaling conflict facilitates transformation of the cell to a phenotype that is capable of circumventing the tissue-specific inhibitory and apoptotic pathways.

Discordant expression or dysregulation of both cdk and cyclins has been described for nearly all tumor types [34, 35]. Such dysregulation allows G2-specific signals to be generated in G1 or vice versa. The extent to which such dysregulation in normal cells may function to facilitate transformation is unknown, and a description of the capacity of WY14,643 to produce such changes with acute exposure was one of the objectives of this study. Our laboratory is currently exploring this hypothesis by examining cell cycle and apoptotic protein expression following chronic and subchronic periods of xenobiotic exposure.

* Manuscript in preparation.

## References

1. Green S, Tugwood JD and Issemann I, The molecular mechanism of peroxisome proliferator action: A model for species differences and mechanistic risk assessment. *Toxicol Lett* **64/65:** 131–139, 1992.
2. Sher T, Yi H-F, McBride OW and Gonzalez FJ, cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor. *Biochemistry* **32:** 5598–5604, 1993.
3. Bartles JR, Khuon S, Lin X, Zhang L, Reddy JK, Rao MS, Osoye ST, Nehme CL and Fayos BE, Peroxisome proliferator-induced alterations in the expression and modification of rat hepatocyte plasma membrane proteins. *Cancer Res* **50:** 669–676, 1990.
4. Muerhoff AS, Griffin KJ and Johnson EF, The peroxisome proliferator-activated receptor mediates the induction of CYP4A6, a cytochrome P450 fatty acid omega-hydroxylase, by clofibric acid. *J Biol Chem* **267:** 19051–19053, 1992.
5. Kliewer SA, Umesono K, Noonan DJ, Heyman RA and Evans RM, Convergence of 9-cis retinoic acid and peroxisome proliferative signalling pathways through heterodimer formation of their receptors. *Nature* **358:** 771–774, 1992.
6. Chen N and Crane DI, Induction of the major integral membrane protein of mouse liver peroxisomes by peroxisome proliferators. *Biochem J* **283:** 605–610, 1992.
7. Ma X, Mufti NA and Babish JG, Protein tyrosine phosphorylation as an indicator of 2,3,7,8-tetrachloro-*p*-dioxin exposure *in vivo* and *in vitro*. *Biochem Biophys Res Commun* **189:** 59–65, 1992.
8. Wada N, Marsman DS and Popp JA, Dose-related effects of the hepatocarcinogen, WY-14,643 on peroxisomes and cell replication. *Fundam Appl Toxicol* **18:** 149–154, 1992.
9. Busser M-T and Lutz WK, Stimulation of DNA synthesis in rat and mouse liver by various tumor promoters. *Carcinogenesis* **8:** 1433–1437, 1987.
10. Marsman DS, Cattley RC, Conway JG and Popp JA, Relationship of hepatic peroxisome proliferation and replicative DNA synthesis to the heaptocarcinogenicity of the peroxisome proliferators di-(2-ethylhexyl)phthalate and [4-chloro-

6-(2,3-xylidino)-2]-pyrimidinylthio]acetic acid (WY-14,643) in rats. *Cancer Res* **48,** 6739–6744, 1988.
11. Eacho PI, Lanier TL and Brodhecker CA, Hepatocellular DNA synthesis in rats given peroxisome proliferating agents: Comparison of WY14-14,643 to clofibric acid, nafenopin, and LY171883. *Carcinogenesis* **12:** 1557–1561, 1991.
12. Kamb A, Cell cycle regulators and cancer. *Trends Genet* **11:** 136–140, 1995.
13. Hartwell LH and Kastan MB, Cell cycle control and cancer. *Science* **266:** 1821–1828, 1994.
14. Draetta G, Mammalian G1 cyclins. *Curr Opin Cell Biol* **6:** 842–846, 1994.
15. King RW, Jackson PK and Kirschner MW, Mitosis in transition. *Cell* **79:** 563–571, 1994.
16. Nurse P, Ordering S phase and M phase in the cell cycle. *Cell* **79:** 547–550, 1994.
17. Sherr CJ, G1 phase progression; cycling on cue. *Cell* **79:** 551–555, 1994.
18. Heichman KA and Roberts JM, Rules to replicate by. *Cell* **79:** 557–562, 1994.
19. Hunter T and Pines J, Cyclins and cancer II: cyclin D and CDK inhibitors come of age. *Cell* **79:** 573–582, 1994.
20. Nigg EA, Cellular substrates of p34cdc2 and its companion cyclin dependent kinases. *Trends Cell Biol* **3:** 296–301, 1993.
21. Pines J, The cell cycle kinases. *Semin Cancer Biol* **5:** 305–313, 1994.
22. Loyer P, Glaise D, Cariou S, Baffet G, Meijer L and Guguen-Guillouzo C, Expression and activation of CDKs (1 and 2) and cyclins in the cell cycle progression during liver regeneration. *J Biol Chem* **269:** 2491–2500, 1994.
23. Albrecht JH, Hoffman JS, Kren BS and Steer CJ, Cyclin and cyclin-dependent kinase 1 mRNA expression in models of regenerating liver and human diseases. *Am J Physiol* **265:** G857–G864, 1993.
24. Lu XP, Koch KS, Lew DJ, Dulic V, Pines J, Reed SI, Hunter T and Leffert HL, Induction of cyclin mRNA and cyclin-associated histone H1 kinase during liver regeneration. *J Biol Chem* **267:** 2841–2844, 1992.
25. Furukawa Y, Piwnica-Worms H, Ernst TJ, Kanakura Y and Griffin JD, cdc2 gene expression at the G1 to S transition in human T lymphocytes. *Science* **250:** 805–808, 1990.
26. Wolowiec D, Deviller P, Simonin D, Souchier C, Rimokh R, Benchaib M, Bryon P-A and Ffrench M, CDK1 is a marker of proliferation in human lymphoid cells. *Int J Cancer* **61:** 381–388, 1995.
27. Ma X and Babish JG, Acute 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure results in enhanced tyrosylphosphorylation and expression of murine hepatic cyclin dependent kinases. *Biochem Biophys Res Commun* **197:** 1070–1077, 1993.
28. Ma X, Stoffregen D, Wheelock GD, Rininger J and Babish JG, Acute dosing of the hepatocarcinogen [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid (WY14,643) to rats results in enhanced hepatic expression of the cell division control enzyme p34cdc2 kinase. *Mol Pharmacol*, in press, 1996.
29. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685, 1970.
30. Towbin H, Staehelin T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* **76:** 4350–4354, 1979.
31. Dowdy SF, Hinds PW, Louie K, Reed SI, Arnold A and Weinberg RA, Physical interaction of the retinoblastoma protein with human D cyclins. *Cell* **73:** 499–511, 1993.
32. Ewen ME, Sluss HK, Sherr CJ, Matsushime H, Kato J-Y and Livingston DM, Functional interactions of the retinoblastoma protein with mammalian D-type cyclins. *Cell* **73:** 487–497, 1993.
33. Albrecht JH, Hu MY and Cerra FB, Distinct patterns of cyclin D1 regulation in models of liver regeneration and human liver. *Biochem Biophys Res Commun* **209:** 648–655, 1995.
34. Yasui W, Ayhan A, Kitadai Y, Nishimura K, Yokozaki H, Ito H and Tahara E, Increased expression of p34cdc2 and its kinase activity in human gastric and colonic carcinomas. *Int J Cancer* **53:** 36–41, 1993.
35. Keyomarsi K and Pardee AB, Redundent cyclin overexpression and gene amplification in breast cancer cells. *Proc Natl Acad Sci USA* **90:** 1112–1116, 1993.
36. Xiong Y, Zhang H and Beach D, D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. *Cell* **71:** 505–514, 1992.
37. Zhang H, Xiong Y and Beach D, Proliferating cell nuclear antigen and p21 are components of multiple cell cycle kinase complexes. *Mol Biol Cell* **4:** 897–906, 1993.
38. Dietrich DR, Toxicological and pathological applications of proliferating cell nuclear antigen (PCNA), a novel endogenous marker for cell proliferation. *Crit Rev Toxicol* **23:** 77–109, 1993.
39. Szepesi A, Gelfand EW and Lucas JJ, Association of proliferating cell nuclear antigen with cyclin-dependent kinases and cyclins in normal and transformed human T lymphocytes. *Blood* **84:** 3413–3421, 1994.
40. Enan E and Matsumura F, Evidence for a second pathway in the action mechanism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Biochem Pharmacol* **49:** 249–261, 1994.
41. Cayama E, Tsuda H, Sarma DSR and Farber E, Initiation of chemical carcinogenesis requires cell proliferation. *Nature* **275:** 60–62, 1978.
42. Schulte-Hermann R, Tumor promotion in the liver. *Arch Toxicol* **57:** 147–158, 1985.