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Mammalian p50^{Cdc37} is a protein kinase-targeting subunit of Hsp90 that binds and stabilizes Cdk4

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***CDC37*, an essential gene in *Saccharomyces cerevisiae*, interacts genetically with multiple protein kinases and is required for production of Cdc28p/cyclin complexes through an unknown mechanism. We have identified mammalian p50^{Cdc37} as a protein kinase-targeting subunit of the molecular chaperone Hsp90. Previously, p50 was observed in complexes with pp60$^{v\text{-}src}$ and Raf-1, but its identity and function have remained elusive. In mouse fibroblasts, a primary target of Cdc37 is Cdk4. This kinase is activated by D-type cyclins and functions in passage through G_1. In insect cells, Cdc37 is sufficient to target Hsp90 to Cdk4 and both in vitro and in vivo, Cdc37/Hsp90 associates preferentially with the fraction of Cdk4 not bound to D-type cyclins. Cdc37 is coexpressed with cyclin D1 in cells undergoing programmed proliferation in vivo, consistent with a positive role in cell cycle progression. Pharmacological inactivation of Cdc37/Hsp90 function decreases the half-life of newly synthesized Cdk4, indicating a role for Cdc37/Hsp90 in Cdk4 stabilization. This study suggests a general role for p50^{Cdc37} in signaling pathways dependent on intrinsically unstable protein kinases and reveals a previously unrecognized chaperone-dependent step in the production of Cdk4/cyclin D complexes.**

[*Key Words*: Cyclin-dependent kinase; D-type cyclin; molecular chaperone; Hsp90; p50^{Cdc37}]

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The decision of whether to enter the eukaryotic cell cycle is made in G_1, a period during which cells respond to both positive and negative growth signals (Pardee 1989). The ultimate recipients of these signals are cyclin-dependent protein kinases (Cdk) that regulate passage through sequential cell cycle transitions (Hunter and Pines 1994; Sherr 1994). D-type cyclins are induced by mitogens and activated Cdk4/cyclin D complexes are thought to phosphorylate the retinoblastoma susceptibility protein (Rb), thereby facilitating G_1 progression (for review, see Sherr 1994; Weinberg 1995). Cdk2/cyclin E is also required for entry into S-phase (Otsuba et al. 1995) and may also play a role in Rb inactivation. In fibroblasts, ectopic expression of D- and E-type cyclins can shorten the G_1 interval (for review, see Sherr 1994; Sherr and Roberts 1995) suggesting that these proteins are rate limiting for cell cycle entry. Various mechanisms have evolved to regulate the timing and extent of Cdk activation. These include regulation of cyclin abundance (Sherr 1994), activating phosphorylation on a conserved threonine by Cdk activating kinase (CAK) (Morgan 1995), inhibitory phosphorylation by Weel (for review, see Coleman and Dunphy 1994), and Cdk inhibitory proteins (for review, see Sherr and Roberts 1995; Harper and Elledge 1996). Together, these mechanisms allow temporal control of cell cycle transitions, facilitate checkpoint function, and contribute to cell cycle exit during development.

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Cdks are inactive in their monomeric form. Although a simple two-step mechanism involving cyclin binding and phosphorylation by CAK is sufficient to describe the assembly and activation of Cdk2/cyclin complexes (Desai et al. 1992; Connell-Crowley et al. 1993; for review, see Morgan 1995), production of some Cdk complexes is more complex and can involve additional steps. For example, p36^{MAT1} is an assembly factor for CAK and remains tightly associated with the Cdk7/cyclin H complex in vivo (Fisher et al. 1995; Tassan et al. 1995). Cdk4/cyclin D is also subject to addition layers of regulation. In quiescent fibroblasts stimulated to enter the cell cycle with growth factors, Cdk4/cyclin D complexes assemble in mid-G_1 as D-type cyclins accumulate (Matsushime et al. 1994; for review, see Sherr 1994). In quiescent fibroblasts expressing Cdk4 and cyclin D3 ectopically, both subunits are present but kinase complexes are not formed (Matsushime et al. 1994). Assembly of the Cdk4/cyclin D3 complex in this context occurs in mid-G_1 and requires an uncharacterized mitogen dependent step that is independent of CAK function. Additional steps in Cdk4 activation could involve an assembly factor anal-

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ogous to p36^{MAT1}, or alternatively, assembly could require factors that stabilize forms of Cdk4 or cyclin D compatible with activation, but that are not part of the holoenzyme.

Assembly of Cdc28p/cyclin complexes in *Saccharomyces cerevisiae* is also complex and requires Cdc37p function through an unknown mechanism. *CDC37* was identified as a START mutant (Reed 1980; Reed et al. 1985; Ferguson et al. 1986) but is required at other points in the cell cycle as well (Gerber et al. 1995). In *cdc37-1* mutant strains at the restrictive temperature, Cdc28p and cyclin proteins are present but not assembled (Gerber et al. 1995). We have cloned and characterized genes encoding mouse and human Cdc37. We find that Cdc37 encodes a 50-kD protein kinase targeting subunit of the chaperone heat shock protein 90 (Hsp90) and that Cdk4 is a major Cdc37-associated protein in fibroblasts. Previously, p50 was observed in association with Raf-1 and pp60$^{v\text{-}src}$/Hsp90 complexes (Hunter and Sefton 1980; Brugge et al. 1981; Opperman et al. 1981; Brugge 1986; Whitelaw et al. 1991; Stancato et al. 1993), but its molecular identity and function has remained unknown. Cdk4 is destabilized in the absence of Hsp90 function in fibroblasts, indicating a role for Cdc37/Hsp90 in kinase stabilization. This study suggests a general role for p50^{Cdc37} in kinase stabilization and reveals a previously unrecognized role for a molecular chaperone in Cdk regulation and G_1 control.

Results

Cloning of mammalian Cdc37 cDNAs

The requirement for Cdc37p function in the formation of Cdc28p/cyclin complexes in *S. cerevisiae* (Gerber et al. 1995) led us to examine the role of Cdc37 in mammalian Cdk function. Mouse and human Cdc37 cDNAs (see Materials and methods) contain open reading frames of 379 and 378 amino acids, respectively (Fig. 1A), and encode proteins that migrate at 50 kD in SDS-PAGE (see below). Mouse Cdc37 displays 90%, 44%, and 19% identity with human, *Drosophila melanogaster*, and *S. cerevisiae* Cdc37 proteins, respectively (Fig. 1A). Cdc37 sequences display no obvious sequence similarity with proteins of known function. The transcript size (~1.6 kb) (Fig. 1B) indicates that the mouse Cdc37 cDNA (1607 bp) is near full-length. Although the 5′ UTR lacks in-frame stop codons, anti-Cdc37 antibodies recognize a 50-kD protein in extracts from NIH-3T3 cells that comigrates with recombinant Cdc37 produced in insect cells (Fig. 1C) and with in vitro-translated Cdc37 (data not shown).

A subset of Cdks associate with Cdc37 in vitro

We tested whether Cdc37 could associate with mammalian Cdks or cyclins expressed in insect cells using in vitro-translated Cdc37 (Fig. 2A). Cdc37 was found to associate most efficiently with Cdk4, less efficiently with Cdk6 and Cdk7, but did not associate with Cdc2, Cdk2 (Fig. 2A), or with gluthionine *S*-transferase (GST)–cyclin

A

B

C

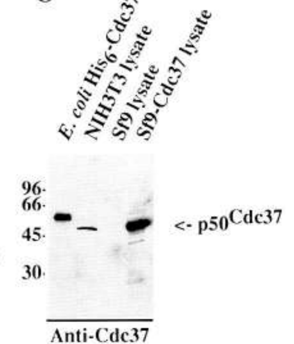

Figure 1. Mammalian Cdc37 encodes a widely expressed 50-kD protein. (*A*) Deduced amino acid sequences of mouse and human Cdc37 and alignment with *D. melanogaster* and *S. cerevisiae* Cdc37 homologs. (+) Similarities. GenBank accession nos. are U43076 for mouse and U43077 for human Cdc37 cDNAs. (*B*) Mouse tissue Northern blot (Clonetech) containing poly(A)$^+$ RNA was probed with [α-^{32}P]dCTP-labeled Cdc37 cDNA (exposure, 5 hr). (*C*) Anti-Cdc37 immunoblot of purified His$_6$-Cdc37 (20 ng) and the indicated cell lysates (30 μg) (exposure, 30 sec).

A, B, D1, or E (data not shown). Identical results were obtained in baculovirus coinfection experiments and in this analysis, Cdc37 also did not associate with Cdk3 or Cdk5 (data not shown). To test whether Cdc37/Cdk4 association is direct, binding reactions were performed using purified Cdc37 from *Escherichia coli* and purified GST–Cdk4 from insect cells (Fig. 2B). Both proteins were >90% homogeneous (Fig. 2B). At least 75% of the input Cdc37 was found to associate efficiently with GST–Cdk4, but was absent in control binding reactions. Thus, Cdc37 and Cdk4 can interact directly.

Cdk4 is a major target of Cdc37 in vivo

To assess the significance of these in vitro results, Cdc37/Cdk association was examined using extracts of mouse fibroblasts and human lymphoblasts. Anti-Cdc37 antibodies immunoprecipitate two prominent polypeptides from [^{35}S]methionine-labeled NIH-3T3 cells not found in control normal rabbit sera immunoprecipitates;

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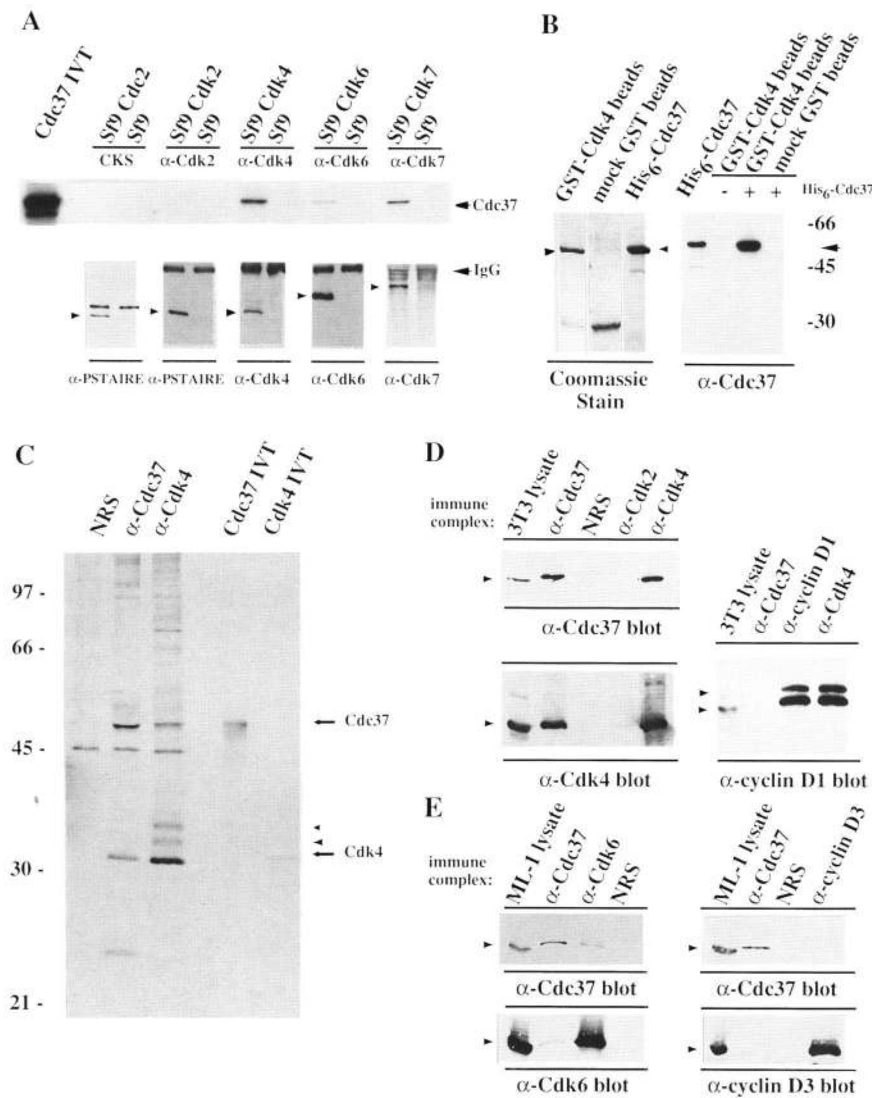

Figure 2. Cdc37 associates with a subset of Cdks in vitro and in vivo. (*A*) In vitro association of Cdc37 with Cdks. [^{35}S] methionine-labeled Cdc37 (at ~5 nM) was incubated with Sf9 cell lysates containing recombinant Cdks at 100–200 nM before immunoprecipitation. Cdc2 was purified using p9^{CKS}–agarose (Elledge et al. 1992) although identical results were obtained using anti-Cdc2 antibodies (data not shown). Complexes were analyzed by SDS-PAGE/autoradiography to visualize Cdc37 (*A, top*) and by immunoblotting to verify precipitation of the target kinase (*A, bottom*). Cdks are indicated by arrowheads. (*B*) Direct association of Cdk4 and Cdc37. (*Left*) SDS-PAGE of purified GST–Cdk4, His$_6$-Cdc37, and proteins from a mock GST–Cdk4 purification. The positions of His$_6$-Cdc37 and GST–Cdk4 are indicated by arrowheads. The 29-kD protein is insect cell glutathione *S*-transferase. (*Right*) GST–Cdk4 (1 μg) or mock Sepharose beads were incubated with or without 0.4 μg of His$_6$-Cdc37 (1 hr, 4°C) and washed complexes were then immunoblotted using anti-Cdc37 antibodies. His$_6$-Cdc37 (0.1 μg) was used as a blotting control. (*C*) [^{35}S]methionine-labeled NIH-3T3 extracts were immunoprecipitated using normal rabbit sera (NRS), anti-Cdc37, or anti-Cdk4 IgG and analyzed by SDS-PAGE and autoradiography using in vitro-translated Cdk4 and Cdc37 as controls (exposure, 24 hr). The positions of cyclin D1 and cyclin D2 are indicated by small and large arrowheads, respectively. (*D*) Cdc37 associates with Cdk4 but not cyclin D1 in vivo. NIH-3T3 lysates were immunoprecipitated using the indicated antibodies or NRS and complexes immunoblotted using lysate (30 μg of protein) as a control. Cdc37 was blotted using a monoclonal antibody recognizing p50^{Cdc37}. (*E*) Association of Cdc37 with Cdk6. ML-1 extracts were immunoprecipitated as indicated and immunoblots probed with the indicated antibodies.

a 50-kD protein that comigrates with in vitro translated Cdc37 and a 32-kD protein that comigrates with Cdk4 from anti-Cdk4 immune complexes (Fig. 2C). An additional protein at 24 kD was also detected in Cdc37 immune complexes (Fig. 2C). Cdk4 immune complexes also contain a protein at 50 kD that comigrates with Cdc37, as well as proteins at 36 and 34 kD that were identified as cyclins D1 and D2 by immunoblotting (data not shown). [NIH-3T3 cells express low levels of cyclin D3 (Matsushime et al. 1994)].

Immunoblot analysis confirmed the presence of Cdk4 in Cdc37 immune complexes and the presence of Cdc37 in Cdk4 immune complexes (Fig. 2D). Consistent with the in vitro binding assays, Cdc37 was not detected in Cdk2 immune complexes and vice versa (Fig. 2D; data not shown). We estimate that as much as 30% of Cdk4 in extracts from asynchronous NIH-3T3 cells is associated with Cdc37 (Fig. 2C; data not shown).

Although Cdc37 interacts weakly with Cdk6 in vitro (Fig. 2A), we did not detect this complex in NIH-3T3 lysates at the sensitivity level afforded by available Cdk6 antibodies (data not shown). This may reflect the low levels of Cdk6 in these cells. In ML-1 lymphoblasts, which have high levels of Cdk6 in complex with cyclin D3 but low to undetectable levels of Cdk4, cyclin D1, and cyclin D2, (Meyerson and Harlow 1994), we detected small quantities of Cdc37 in Cdk6 complexes and vice versa (Fig. 2E). Thus far, we have not detected associa-

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tion of Cdc37 with Cdk7 in NIH-3T3 or mouse embryonic fibroblasts (data not shown).

Although D-type cyclins are in Cdk4 immune complexes from [^{35}S]methionine-labeled NIH-3T3 cells, we did not detect proteins migrating at the expected position for D-type cyclins in Cdc37 immune complexes (Fig. 2C). Using a more sensitive immunoblotting method, we did not detect cyclin D1 in association with Cdc37 in NIH-3T3 cells (or vice versa) (Fig. 2D; data not shown). Similar results were obtained for cyclins D2 and D3 using mouse embryonic fibroblasts and ML-1 cells, respectively (Fig. 2E; data not shown).

These data indicate that Cdk4, and to a lesser extent Cdk6, are targets of Cdc37 in vivo and suggests that Cdc37 associates preferentially with forms of Cdks lacking a cyclin subunit. In addition, Cdk4 appears to be a major Cdc37-associated protein in NIH-3T3 cells as assessed by incorporation of [^{35}S]methionine (Fig. 2C).

Cdc37 encodes a 50-kD subunit of the molecular chaperone Hsp90

When coexpressed in insect cells, Cdc37 and GST–Cdk4 associate efficiently, forming an apparently stochiometric complex (Fig. 3A). In these complexes, we observed the recruitment of an additional 90-kD protein into the GST–Cdk4/Cdc37 complex that was not present in the absence of Cdc37. Amino-terminal sequence analysis revealed that this protein was a homolog of the ubiquitous and highly conserved heat shock protein Hsp90 (see Materials and methods). This identification was confirmed by immunoblotting of purified GST–Cdk4/Cdc37 complexes with anti-Hsp90 monoclonal antibodies (Fig. 3B).

Hsp90 is a chaperone that functions in signaling pathways involving steroid-receptor activation and protein kinases (for review, see Jakob and Buchner 1994; Bohen et al. 1995). Previous studies have shown that both Raf-1 and pp60$^{v\text{-}src}$ immune complexes contain Hsp90 and a 50-kD subunit of unknown identity and function (Brugge et al. 1981; Brugge 1986; Whitelaw et al. 1991; Stancato et al. 1993). The molecular size of p50^{Cdc37} and its association with Hsp90 in insect cells led us to examine whether Cdc37 encodes the p50 subunit of Hsp90. Monoclonal antibodies generated previously against p50 (Whitelaw et al. 1991) recognize recombinant Cdc37 (Fig. 3C), and react with a 50-kD protein in both NIH-3T3 extracts (Figs. 2D,E and 3C) and in anti-Cdc37 immune complexes (Fig. 2D,E). These data reveal that Cdc37 encodes a p50 subunit of two kinases that are known to associate with Hsp90. Moreover, our results demonstrate that Cdc37 is sufficient to mediate Hsp90/Cdk4 association when expressed in insect cells (Fig. 3A), suggesting that Cdc37 can function as a protein kinase targeting subunit of Hsp90. Consistent with our immunological identification of p50 as Cdc37, a partial Cdc37 cDNA has been isolated using peptide sequences derived from purified p50 (G. Perdew, pers. comm.).

Cdc37 is located in the cytoplasm

Previous studies have suggested that p50^{Cdc37} is associ-

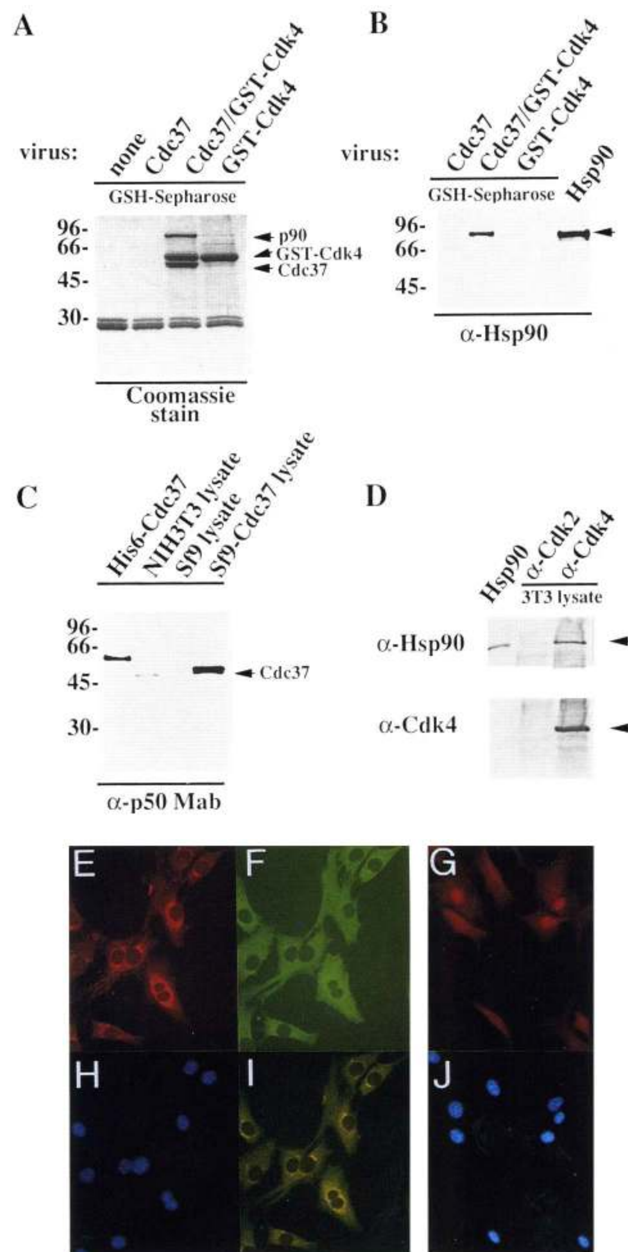

Figure 3. Cdc37 is the 50-kD subunit of Hsp90 and can target Cdk4 to Hsp90. (*A*) Cdc37 recruits Hsp90 into Cdk4 complexes. Sf9 cells were infected with the indicated viruses and after 40 hr, complexes were purified using GSH–Sepharose. Proteins were analyzed by SDS-PAGE and Coomassie blue staining. (*B*) Samples from *A* were immunoblotted using anti-Hsp90 antibodies. (*C*) The indicated protein extracts were immunoblotted using mAb α-p50 (Perdew and Whitelaw 1991). (*D*) Anti-Cdk4 and anti-Cdk2 immune complexes were immunoblotted using mAb anti-Hsp90. Purified bovine Hsp90 (~20 ng, Stress Gen, Inc.) was used as a control in *B* and *D*. (*E–J*) Cdc37 is localized in the cytoplasm. Indirect immunofluorescence of Cdc37 (*E*), Hsp90 (*F*), Cdk4 (*G*), and Hsp90/Cdc37 (*I*) in NIH-3T3 cells. Anti-Cdc37 IgG label, Texas Red; anti-Hsp90 label, fluorescein; anti-Cdk4 label, Texas Red. (*H,J*) Nuclei labeled with 4',6-diamidino-2-phenylindole for *E*, *F*, *I*, and *G*, respectively.

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ated quantitatively with Hsp90 (Perdew and Whitelaw 1991). Using indirect immunofluorescence with either polyclonal anti-Cdc37 (Fig. 3E) or anti-p50 monoclonal antibodies (data not shown), we find that Cdc37 is also largely cytoplasmic in NIH-3T3 cells and appears to colocalize with cytoplasmic Hsp90 (Fig. 3E,F,I). Importantly, Cdk4 is located in both the cytoplasm and the nucleus (Fig. 3G), whereas active Cdk4/cyclin D complexes are thought to be largely nuclear (Baldin et al. 1993). Thus association of Cdc37 with Cdk4 is predicted to take place primarily in the cytoplasm and therefore, may involve newly synthesized Cdk4.

Hsp90, Cdc37, and Cdk4 exist in a high molecular weight complex in fibroblasts

Although the association of Cdk4 with Cdc37 predicts that Hsp90 would be contained in Cdk4 complexes in mammalian cells, we did not observe strongly labeled proteins migrating at the position expected for Hsp90 in our Cdk4 or Cdc37 immune complexes (see Fig. 2C), although weakly labeled proteins were detected in this size range. The absence of labeled Hsp90 likely reflects the fact that Hsp90 is among the most abundant and stable proteins in the cell (1%–2% of the soluble protein) and therefore, would be expected to have a low specific activity under the labeling conditions used. Therefore, to examine association of Hsp90 and Cdk4, Cdk4 immune complexes were immunoblotted with anti-Hsp90 antibodies (in Fig. 3D). As expected, Hsp90 was observed in anti-Cdk4 immune complexes but not in anti-Cdk2 immune complexes.

Hsp90 is thought to function as a dimer and exists in high molecular weight complexes (Jakob and Buchner 1994). The finding that Cdk4 is associated with Hsp90 and Cdc37 predicts that a fraction of Cdk4 will be contained in high molecular weight complexes. In addition, the absence of D-type cyclins in Cdc37 complexes indicated that Cdk4/cyclin D complexes will be distinct from Cdk4/Cdc37/Hsp90 complexes. To examine Cdk4/Cdc37 complexes in greater detail, gel filtration analysis was performed on extracts from NIH-3T3 cells (Fig. 4). Hsp90 eluted from gel filtration columns as a single peak centered at ~450 kD, and comigrated with the bulk of Cdc37 (Fig. 4A). Detectable levels of Cdc37 were also found in the lower size ranges and could be detected as low as 50 kD upon long exposures of the blot. Consistent with the formation of high molecular weight complexes, a substantial fraction of Cdk4 comigrated with the peak of Cdc37 and Hsp90, although significant levels of Cdk4 were found throughout the column eluate. The existence of Cdk4/Cdc37/Hsp90 complexes was verified by the finding that anti-Cdk4 immune complexes from fractions containing ~450 kD complexes contain both Cdc37 and Hsp90 (Fig. 4B). Although Cdc37 was also found in Cdk4 immune complexes from lower molecular weight fractions, Hsp90 was absent from these complexes. Previous studies indicate that Hsp90 complexes are typically unstable (for review, see Jakob and Buchner 1994). Thus, the presence of Cdc37/Cdk4 complexes in the 80- to 200-kD size range may reflect dissociation from Hsp90 during chromatography. These data verify that the Cdk4/Cdc37/Hsp90 complex observed with recombinant proteins produced in insect cells is also formed in mammalian cells.

The results of gel filtration analysis are also consistent with our findings in crude cell extracts that Cdc37 and D-type cyclins are in mutually exclusive complexes with Cdk4. Although cyclin D1 and D2 eluted in a peak centered at ~150 kD and are associated with Cdk4, a fraction of D-type cyclin was found to comigrate with Hsp90 and Cdc37 (Fig. 4). However, Cdc37 immune complexes from these fractions did not contain D-type cyclins and vice versa (Fig. 4B; data not shown).

Cdc37 expression in vivo

If Cdc37 is a critical positive regulator of Cdk4 function, as expected of a chaperone, we reasoned that it would be

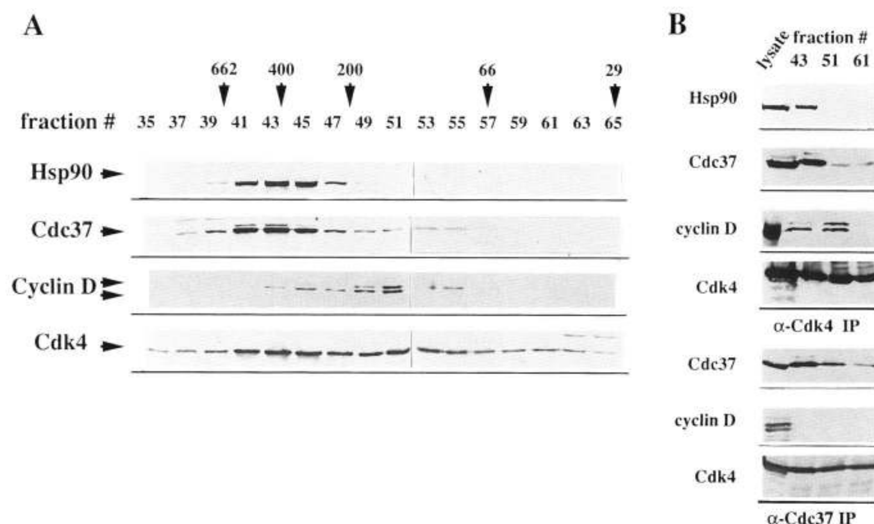

Figure 4. Cdk4, Cdc37, and Hsp90 exist in a high molecular weight complex in fibroblasts. (*A*) Cdk4, Cdc37, Hsp90, and D-type cyclins in nondetergent NIH-3T3 lysates were analyzed by gel filtration and immunoblotting. (*Top*) The positions of molecular weight standards. Anti-cyclin D antibodies used here react with both cyclin D1 and cyclin D2, allowing simultaneous visualization of the predominant D-type cyclins in these cells. (*B*) Cdk4 is associated with Hsp90 and Cdc37 in high molecular weight complexes. Fractions from ~450-, 150-, and 80-kD size ranges were precipitated with the indicated antibody and immune complexes subjected to immunoblotting.

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coexpressed in cells that require D-type cyclins for proliferation in vivo. In the mouse mammary epithelium, cyclin D1 is required for ductal epithelial cell proliferation during pregnancy (Sicinski et al. 1995). This requirement reflects the selectivity of cyclin expression in these cells. Cyclin D1, but not D2 or D3, mRNA is highly induced in these cells during pregnancy but is largely absent in the epithelial ducts from virgin or lactating mice (Fig. 5A–C; S.J. Elledge and S.B. Parker, unpubl.). Cdc37 exhibits a similarly striking pattern of expression during pregnancy. Cdc37 mRNA is absent from epithelial ducts of virgin mice but, like cyclin D1, is induced in the proliferating epithelium during pregnancy (Fig. 5D–F). In lactating breast, Cdc37 mRNA levels are reduced and more diffuse, but do not decline as much as does cyclin D1 mRNA.

Other adult tissue types that have discrete proliferative zones, such as small intestine and stomach, also display highly similar patterns of cyclin D1 and Cdc37 expression (Fig. 5G–J). In addition, highly proliferative zones in the embryo at day 11 postcoitum (p.c.) display high Cdc37 expression (Fig. 5L). For example, Cdc37 mRNA levels are high in the ventricle of the brain, a site of expression of cyclin D1 at this time in development (Sicinski et al. 1995). Although there is a strong correlation, Cdc37 expression is not limited to proliferative zones. For example, we can detect expression of Cdc37 mRNA in the hippocampus and forebrain sections of the adult brain, both of which are postmitotic (Fig. 5K). These results are consistent with a positive role for Cdc37 in the production of active cyclin D-dependent kinase complexes and cell cycle progression in vivo and suggest a selective role for Cdc37 in some postmitotic tissues.

Cdc37 and cyclin D/Cdk4 assembly

Having shown that Cdc37 can target Cdk4 to Hsp90, we wished to understand the biochemical consequences of this association. Initially, we examined whether Cdc37 could function directly to recruit cyclin D2 into Cdk4 complexes in an in vivo setting. Although insect cells expressing Cdk4 and cyclin D2 yield active kinase complexes, it is possible to identify infection conditions where only a fraction of the individual subunits are stably associated into active complexes. Therefore, excess subunits are available to serve as substrates for assembly by exogenous Cdc37. GST–Cdk4 and various combinations of cyclin D2 and Cdc37 viruses were used to infect insect cells and after 40 hr, GST–Cdk4 and associated proteins were isolated on GSH–Sepharose (Fig. 6). Under conditions where ~4% of the available cyclin D2 expressed in Sf9 cells remained stably associated with GST–Cdk4, an apparent stochiometry of 10:1 was ob-

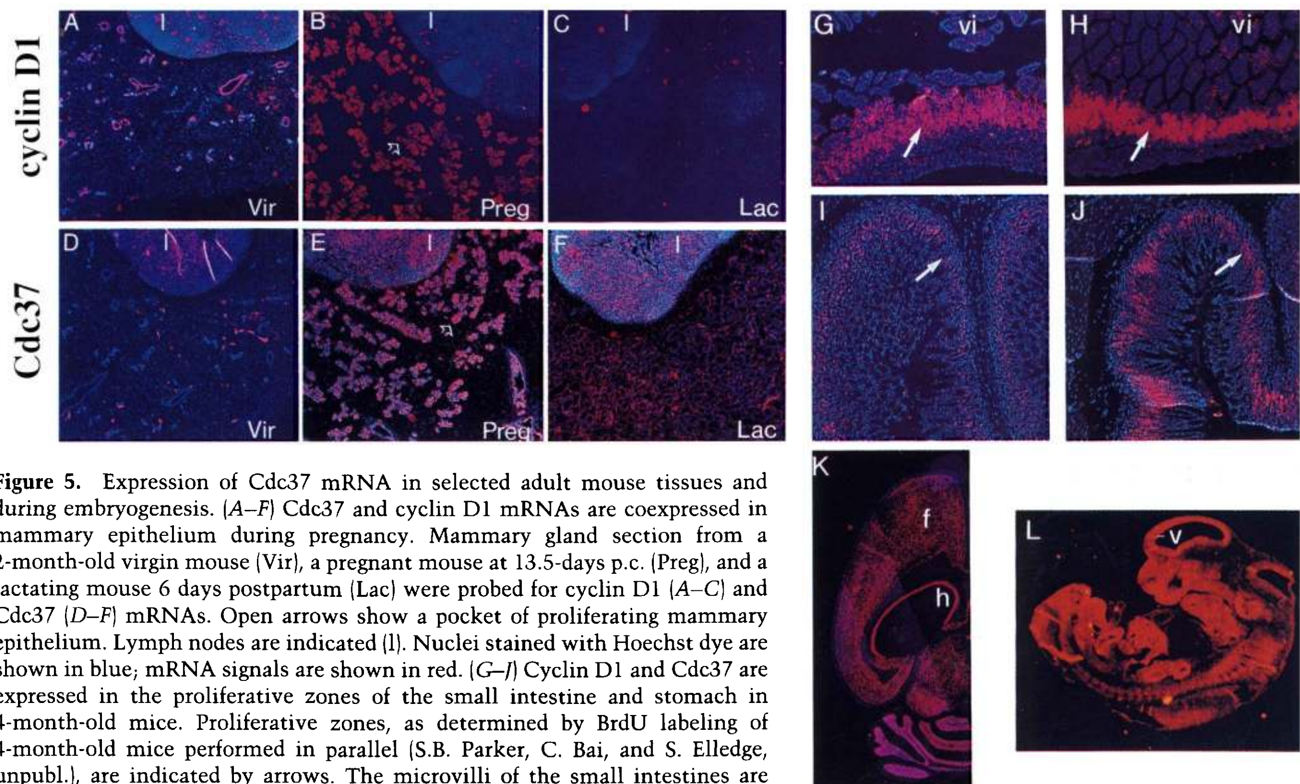

Figure 5. Expression of Cdc37 mRNA in selected adult mouse tissues and during embryogenesis. (*A–F*) Cdc37 and cyclin D1 mRNAs are coexpressed in mammary epithelium during pregnancy. Mammary gland section from a 2-month-old virgin mouse (Vir), a pregnant mouse at 13.5-days p.c. (Preg), and a lactating mouse 6 days postpartum (Lac) were probed for cyclin D1 (*A–C*) and Cdc37 (*D–F*) mRNAs. Open arrows show a pocket of proliferating mammary epithelium. Lymph nodes are indicated (l). Nuclei stained with Hoechst dye are shown in blue; mRNA signals are shown in red. (*G–J*) Cyclin D1 and Cdc37 are expressed in the proliferative zones of the small intestine and stomach in 4-month-old mice. Proliferative zones, as determined by BrdU labeling of 4-month-old mice performed in parallel (S.B. Parker, C. Bai, and S. Elledge, unpubl.), are indicated by arrows. The microvilli of the small intestines are indicated (vi). (*K*) Expression of Cdc37 in the adult mouse brain. Forebrain and hippocampus are indicated by f and h, respectively. (*L*) Expression of Cdc37 in an 11-days p.c. mouse embryo (sagittal section). The ventricle of the brain is indicated (v).

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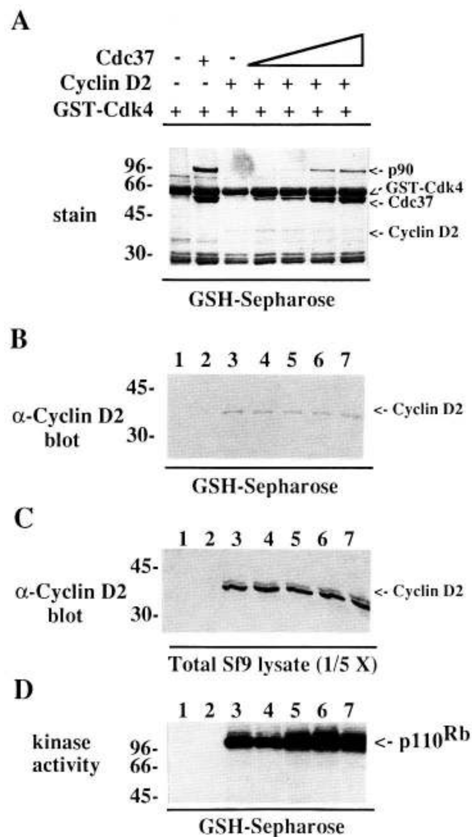

Figure 6. Cdc37 is not sufficient to recruit cyclin D into Cdk4 complexes in insect cells. (*A*) Sf9 cells infected with the indicated viruses for 40 hr were lysed in binding buffer and GSH–Sepharose-purified proteins subjected to SDS-PAGE and Coomassie blue staining. For Cdc37 virus, increasing amounts of virus stocks (25, 50, 75, and 100 μl) were added where indicated. (*B–C*) Samples of GST–Cdk4-associated proteins (*B*) or total cell lysate (*C*) were immunoblotted with anti-cyclin D2 antibodies. The quantity of GST–Cdk4-associated proteins used for blotting corresponds to five times more than that used for the total lysate blot. (*D*) p110^{Rb} kinase assays (Harper et al. 1995) were performed on 5% of the GST–Cdk4-associated proteins shown in *A*.

served for the purified GST–Cdk4/cyclin D2 complex (Fig. 6A–C). Coinfection with Cdc37 virus under these conditions led to accumulation of GST–Cdk4/Cdc37 complexes containing Hsp90, but additional cyclin D2 was not recruited into GST–Cdk4 complexes as assessed by both Coomassie staining and immunoblotting (Fig. 6A–C). Similar results were obtained in experiments using untagged Cdk4 isolated using anti-Cdk4 antibodies and with cyclin D1 (data not shown). Thus, Cdc37/Hsp90 is not sufficient to recruit cyclin D2 into Cdk4 complexes. We note, however, that the specific p110^{Rb} kinase activity of the existing cyclin D2/GST–Cdk4 complexes was increased approximately threefold in the presence of Cdc37 (Fig. 6D). In contrast, no increase in activity was observed when excess Cdc37 produced in *E. coli* was added to preassembled and purified GST–Cdk4/cyclin D2 complexes (data not shown), indicating that increased kinase activity was not attributable to direct activation by Cdc37. This modest increase in activity may reflect a function of Cdc37 that is independent of cyclin association.

Newly synthesized Cdk4 is unstable in the absence of functional Hsp90

Three previous findings suggested that Hsp90/Cdc37 might function in Cdk4/cyclin D activation through Cdk stabilization or folding, as opposed to recruiting cyclin D into Cdk4 complexes directly. First, in budding yeast expressing pp60$^{v\text{-}src}$, the levels and activity of this kinase are reduced with certain temperature sensitive alleles of *hsp82*, one of two Hsp90 homologs in *S. cerevisiae* (Nathan and Lindquist 1995) or in the absence of *CDC37* function (Boschelli 1993; Nathan and Lindquist 1995). Second, Cdc28p levels are reduced with loss of Cdc37p function (Gerber et al. 1995). Third, Hsp90/Cdc37 complexes are largely cytoplasmic (Fig. 3E) and Hsp90 is generally considered a cytoplasmic chaperone (Jakob and Buchner 1994). Because active cyclin D-dependent kinase complexes are largely nuclear, it seemed plausible that Cdc37/Hsp90 might interact with newly synthesized Cdk4 in the cytoplasm. Our finding that Cdk4 is partitioned into at least two distinct complexes—one containing cyclin D and the other containing Cdc37—is also consistent with Cdc37 being involved in a step distinct from, but perhaps required for, cyclin binding.

To examine whether Hsp90/Cdc37 may regulate Cdk4 stability, initially we determined the levels of several Cdks in fibroblasts treated with geldanamycin (GA). GA binds specifically to Hsp90 and disrupts its interaction with accessory proteins without affecting Hsp90 levels (Whitesel et al. 1994). Loss of Hsp90 function has been shown to affect pp60$^{v\text{-}src}$ and Raf-1 stability negatively (Whitesel et al. 1994; Schulte et al. 1995). Thus, the effects of GA appear to mimic, in part, the effects of *HSP82* and *CDC37* mutants in *S. cerevisiae*. Cdk4 levels in NIH-3T3 fibroblasts were reduced by about fivefold after treatment with GA for 16 hr. In contrast, the overall levels of Cdk6 and Cdk2, which are not detected in Cdc37 immune complexes in these cells, are either slightly increased (Cdk6) or not affected (Cdk2) (Fig. 7A). We note that in the case of Cdk2, there was an approximately twofold increase in the level of unphosphorylated Cdk2 with a concominant decrease in the level of more rapidly migrating T160-phosphorylated Cdk2. In addition, the levels of anti-PSTAIRE reactive Cdks and Cdk7 are unaltered upon GA treatment (data not shown).

To examine whether disruption of Hsp90 function by GA affects the stability of newly synthesized Cdk4, a pulse-chase experiment was performed. Asynchronous NIH-3T3 cells were [^{35}S]methionine labeled for 1.5 hr in the presence or absence of GA (0.5 μM), followed by a chase with unlabeled methionine for periods of 0, 5, or 20 hr. Anti-Cdc37 or anti-Cdk4 immune complexes were purified from whole cell lysates and analyzed (Fig. 7B,C).

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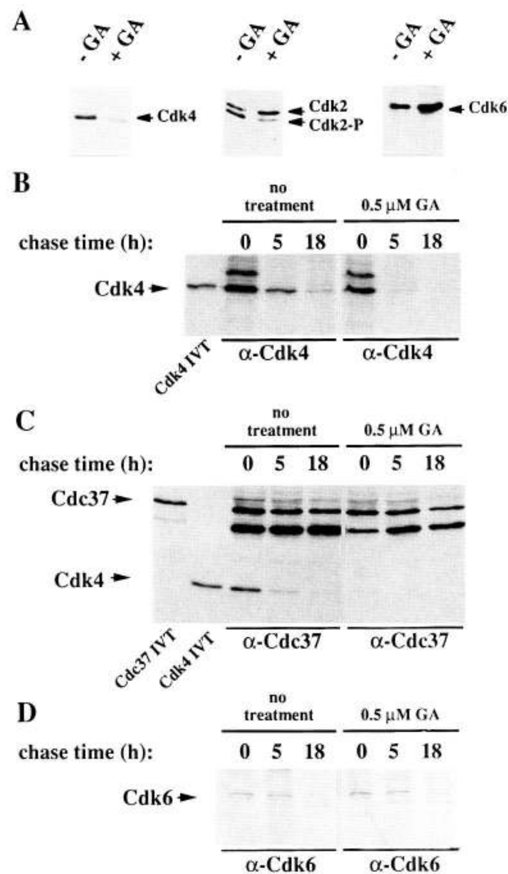

Figure 7. Loss of Hsp90 function destabilizes newly synthesized Cdk4. (*A*) Cdk4, but not Cdk2 or Cdk6, levels decrease in NIH-3T3 cells after treatment with geldanamycin (GA). Replica immunoblots of nondetergent extracts from NIH-3T3 cells in the presence or absence of GA (2 μM, 16 hr) were probed with the indicated antibodies. The positions of phosphorylated and unphosphorylated Cdk2 are indicated. (*B–D*) Effects of GA on the stability of newly synthesized Cdk4, Cdk6, and Cdk4/Cdc37 complexes. Cells were incubated alone or in the presence of GA (500 nM) during a 1.5-hr pulse with [^{35}S]methionine (0.2 mCi/ml). For chase, cells were washed with PBS and placed into fresh media (DMEM containing 5% FBS) with or without GA for 0, 5, or 20 hr. Equal portions of cell extracts for each time point were immunoprecipitated with anti-Cdk4 (*B*), anti-Cdc37 antibodies (*C*), or anti-Cdk6 antibodies (*D*) and immune complexes analyzed by SDS-PAGE and autoradiography (exposure, 30 hr). Cdk4 levels were determined using PhosphorImager analysis (Molecular Dynamics) after correction for background radioactivity. Proteins migrating at 34 and 36 kD in *B* are cyclin D2 and cyclin D1, respectively. The band migrating at 45 kD in *C* is the same nonspecific band found in NRS immune complexes (see Fig. 2C).

In the absence of GA, newly synthesized Cdk4 is readily detectable in anti-Cdk4 immune complexes at 5 and 20 hr after chase. In contrast, Cdk4 displayed markedly reduced stability in the presence of GA. Although comparable levels of Cdk4 were found in anti-Cdk4 immune complexes from treated and untreated cells at 0 hr, indicating the absence of strong translational effects, Cdk4 was essentially undetectable 5 and 20 hr after chase in the presence of GA. In comparison, the stability of newly synthesized Cdc37 or Cdk6 was essentially unaffected by GA (Fig. 7C,D). On the basis of two independent experiments, the half-life for Cdk4 is minimally fourfold lower in the presence of GA than in its absence. We also note that newly synthesized Cdk4 present at 0 hr in the presence of GA (Fig. 7B) does not stably associate with Cdc37 (Fig. 6C). Although these results indicate that association with Cdc37/Hsp90 is required for stabilization of Cdk4 in its newly synthesized form, we cannot rule out the possibility that other Hsp90 regulated proteins also contribute to Cdk4 stabilization independent of Cdc37. Although we and others have shown that treatment of lymphoblasts or fibroblasts with GA results in G_1 arrest (Yamaki et al. 1982; data not shown), it is highly unlikely that G_1 arrest alone contributes to the instability of Cdk4 observed in the pulse-chase experiment. This conclusion is based on the fact that the dramatic reduction in Cdk4 levels observed within 6.5 hr of treatment with GA (Fig. 7B) is substantially shorter than the cell cycle time for NIH-3T3 cells (~18 hr) and on the fact that 24–48 hr is required to begin to observe arrest in GA-treated cells (Yamaki et al. 1982).

Discussion

Protein kinases constitute one of the most highly regulated classes of signaling molecules known. The interaction of particular kinases with diverse regulators may require that they be able to adopt multiple conformational states that facilitate or inhibit interactions with regulatory partners. Any particular conformation may be simultaneously poised to receive signaling information and be intrinsically unstable (Bohen et al. 1995). As such, mechanisms are thought to exist that stabilize unstable forms of protein kinases, thereby facilitating signaling interactions. Although the molecular chaperone Hsp90 has been implicated in the stabilization of several kinases, including the oncoprotein kinases Raf-1 and pp60$^{v\text{-}src}$ (Brugge et al. 1981; Stancato et al. 1993; Nathan and Lindquist 1995), the mechanism by which selective recognition of misfolded or unstable forms of these kinases is achieved is unknown.

We have found that Cdc37 encodes a 50-kD subunit of Hsp90 previously observed in complexes with Raf-1 and pp60$^{v\text{-}src}$ and have shown that Cdc37 is sufficient to target Hsp90 to Cdk4 in insect cell (Fig. 3). These results indicate that p50 can function as a protein kinase targeting subunit of Hsp90. We have also found that Cdk4 is a primary target of Cdc37/Hsp90 in fibroblasts (Figs. 2–4) and loss of association of Cdc37 with Cdk4 by pharmacological disruption of Hsp90 function results in a reduction in the half-life of newly synthesized Cdk4 (Fig. 7). Thus, Cdc37 may regulate the abundance of forms of Cdk4 that can associate productively with D-type cyclins. In addition, Cdc37 displays cell type-specific expression patterns in adult tissues and is coexpressed with cyclin D1 in cells undergoing programmed cell division

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(Fig. 5), providing an in vivo correlate for a positive role in cell cycle progression.

Given the essential role of cyclin D-dependent kinases in cell cycle entry (Sherr 1994; Weinberg 1995), the identification of a chaperone for Cdk4 has two implications. First, factors like Cdc37/Hsp90 that may control Cdk4 stability could serve as points of regulation during exit from or entry into the cell cycle. One situation where Cdk4 levels are regulated is in the differentiation and cell cycle exit of murine erythroleukemia cells in vitro (Kiyokawa et al. 1994). These cells differentiate with high cyclin and Cdk2 levels, but have markedly reduced levels of Cdk4 as a result of decreased Cdk4 half-life. We note that p50^{Cdc37} is a phosphoprotein (Perdew and Whitelaw 1991) and therefore, its activity or specificity might be regulated post-translationally in response to external growth control signals. Second, cyclin D1 was identified as the *bcl1* oncogene and is overexpressed in particular tumor types, consistent with a role in transformation (Hunter and Pines 1994; Sherr et al. 1994; Weinberg 1995). Thus, proteins like Cdc37 that function in the generation of cyclin D-dependent kinases are likely to be required for the growth-promoting effects of cyclin D1, and therefore might be subject to altered regulation during transformation.

Cdc37/Hsp90 as a general protein kinase chaperone

Hsp90 is known to associate with steroid-receptors and protein kinases in vivo (Jakob and Buchner 1994; Bohen et al. 1995; Kimura et al. 1995). With steroid-receptors, Hsp90 accessory proteins including Hsp56, Hsp70, DnaJ, and p23 mediate selective interaction with receptors and catalyze turnover upon binding of ligand. The interaction of Hsp90 with protein kinases appears to depend on kinase recognition by p50^{Cdc37}. In vitro, Hsp90/p50 associates specifically with misfolded, largely inactive forms of the pp60v-src and the src-like tyrosine kinase *lck*, but does not associate with the folded and highly active form of the kinase (Brugge et al. 1981; Hartson and Matts 1994). Consistent with this, all three kinases demonstrated to associate physically with Cdc37/Hsp90 (Raf-1, pp60$^{v\text{-}src}$, and Cdk4) are destroyed rapidly in the absence of functional Hsp90 (Fig. 7; Whitesel et al. 1994; Nathan and Lindquist 1995; Schulte et al. 1995). Previous studies have shown that the levels of Cdc28p are reduced substantially in *cdc37-1* strains at the restrictive temperature (Gerber et al. 1995). This decrease in Cdc28p levels appears to be attributable at least in part to reduced half-life (D. Morgan and A. Farrell, pers. comm.), consistent with a role for Cdc37p in kinase stabilization. Decreased stability in the absence of chaperone function suggests that these kinases are intrinsically unstable in their newly synthesized form.

Genetic evidence indicates that the Cdc37/Hsp90 chaperone may have a broader substrate specificity than has been revealed biochemically. *CDC37* displays genetic interactions with several kinases in budding yeast, including *mps1* (M. Winey and A. Schutz, pers. comm.), casein kinase II (C. Glover, pers. comm.), *kin28* (Valey et al. 1995), pp60$^{v\text{-}src}$ (Boschelli 1993; Xu and Lindquist 1993), and *cdc28* (Reed et al. 1985). In addition, Hsp90 homologs interact genetically with *wee1*$^{+}$ in fission yeast (Aligue et al. 1994), with pp60$^{v\text{-}src}$ in budding yeast (Nathan and Lindquist 1995), and with *torso* and *sevenless* tyrosine kinases in *Drosophila* (Doyle and Bishop 1993; Cutforth and Rubin 1994). Interestingly, both *cdc37* and Hsp90 homologs were identified as *sevenless* enhancers in *Drosophila* (Cutforth and Rubin 1994), providing further genetic support for the physical interaction we have found. This network of interactions suggests that Cdc37 and Hsp90 are components of machinery normally involved in the production of a broad range of kinases. Alternatively, these interactions may reflect recognition of misfolded kinases that result from mutation. Chaperones, such as Cdc37/Hsp90, may also be important for the evolution of kinases that would be unstable in the absence of their regulatory partners.

Unlike the situation in mammalian cells, Cdc37p in budding yeast has not been detected in physical association with the critical target Cdc28p or with Hsp90 (Gerber et al. 1995). However, Hsp90 is observed in pp60$^{v\text{-}src}$ immune complexes from yeast (Dey et al. 1996), suggesting that the genetic interactions between Cdc37, Hsp90, and pp60$^{v\text{-}src}$ observed in yeast reflect the physical association observed in mammalian cells. It is possible that in budding yeast, these Cdc37p/Hsp90/Cdk complexes are less stable and consequently, more difficult to detect than in mammalian cells.

Implications of Cdc37/Cdk4 association for regulation of cell cycle entry

The finding that Cdk4 is associated with a chaperone in fibroblasts suggests that regulation of Cdk4 stability is an important step in the production of active cyclin D/Cdk4 complexes required for G_1 progression. Steps dependent on Cdc37 could contribute to the timing of Cdk4/cyclin D assembly and activation during G_1. Our results, coupled with data summarized above, are consistent with a hypothetical model shown in Figure 8. We propose that newly synthesized Cdk4 is relatively unstable in its monomeric form and requires association with

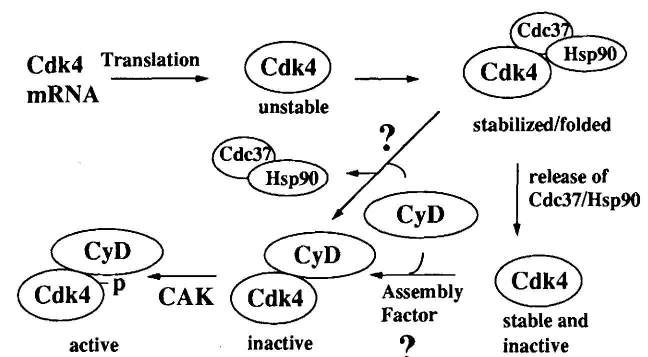

Figure 8. A hypothetical model depicting the role of Cdc37/Hsp90 in the Cdk4 activation process. (See text for details.)

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the Cdc37/Hsp90 chaperone for stabilization. The notion that Cdk4 is unstable is based on our finding that the half-life of newly synthesized Cdk4 is reduced substantially when Hsp90 function is blocked pharmacologically (Fig. 7). The finding that Cdk4 is not associated with Cdc37 in the absence of Hsp90 function is consistent with an active role for Cdc37 in Cdk4 stabilization. A direct test of the role of Cdc37 in kinase stabilization will likely require the development of dominant negative Cdc37 mutants.

Two pathways—one involving dissociation of Cdk4 from Cdc37/Hsp90 before cyclin association and the other involving association of cyclin D with a Cdc37/Cdk4/Hsp90 complex followed by release of Cdc37/Hsp90—can be envisioned for formation of Cdk4/cyclin complexes (Fig. 8). In the first pathway, the timing of Cdk4/cyclin D complex formation and hence, the timing of G_1 transit, could be controlled by the kinetics of release of Cdk4 from Cdc37, which in turn could be regulated by mitogenic signals. In the second pathway, Cdc37 could function directly in assembly through a mechanism similar to that envisioned for assembly of Cdk7/cyclin H by $p36^{MAT1}$, and in this case, the timing of Cdk4/cyclin D assembly would be dictated by cyclin accumulation. Our data indicate that Cdc37 and D-type cyclins form mutually exclusive complexes with Cdk4, suggesting that if tetrameric complexes containing cyclin D exists, they are extremely unstable. Thus, unlike the situation with $p36^{MAT1}$ (Fisher et al. 1995; Tassan et al. 1995), Cdc37 does not remain stably associated with the assembled kinase. In addition, Cdc37 does not function directly to recruit cyclin D2 into Cdk4 complexes when expressed in insect cells (Fig. 6). Although we cannot rule out the possibility that insect cells lack sufficient levels of a factor that collaborates with Cdc37 in Cdk4/cyclin D assembly, our results indicate that Cdc37/Hsp90 alone is not sufficient to recruit cyclin D2 into Cdk4 complexes. Further studies are required to determine whether release of Cdk4 from Cdc37 is the rate-limiting step in Cdk4/cyclin D assembly and, if so, whether accessory factors such as DnaJ/Hsp70 are required to catalyze kinase release, as suggested by genetic interactions in budding yeast (Kimura et al. 1995; Dey et al. 1996). In addition, it will be important to determine whether Cdc37/Hsp90 plays an active role in kinase folding or simply binds and stabilizes misfolded forms of kinases. Finally, it will be important to map out the signaling pathways in which Cdc37 participates.

Materials and methods

Cloning of mammalian cDNAs for Cdc37

Using a partial Cdc37-like chicken cDNA (GenBank accession no. U20281), two oligonucleotides [5′ primer; GAGCAGAA(A/G)CACAA(G/A)ACC; 3′ primer; GT(G/T)GA(C/T)CCCCG(G/C)CG(G/C)GC(T/G)TG] were designed and used to amplify a 270-bp fragment of *Cdc37* from a human liver cDNA library [30 cycles, denaturation: 94°C, 45 sec; annealing: 48°C, 45 sec; elongation: 72°C, 1 min]. This fragment was subcloned into pCRII (Invitrogen) and then used to screen mouse T-cell and human B-cell cDNA libraries in λ_{ACT} (Durfee et al. 1993). Candidate cDNAs were subcloned into pBluescript (Stratagene) and sequenced on both strands using Sequenase (U.S. Biochemicals).

Protein expression

The mouse Cdc37 cDNA, as a 1.6-kb *Xho*I fragment, was subcloned into RSET-C (Invitrogen) for expression in *E. coli* as a His_6 fusion or into a modified version of pGEX2TK for expression as a GST fusion protein. Fusion proteins contain 29 amino acids from the 5′ untranslated region of Cdc37 with the sequence AEHSLALVGPCSAPGRVEPREPVRASEAK in addition to the tag. Expression of GST–Cdc37 [in strain BL21(DE3)] was accomplished using standard procedures (Harper et al. 1995). His_6-Cdc37 was purified on a Ni–NTA column (Qiagen) as recommended by the supplier. For expression of Cdc37 in insect cells, the Cdc37 cDNA was cloned into pVL1392 and virus generated using Baculogold transfer DNA (Pharmingen). Sf9 cell lysates were prepared as described previously (Harper et al. 1995). Purification of GST–Cdk4 and immunoprecipitation of individual kinase subunits was performed as described (Harper et al. 1995). Gel filtration analysis of nondetergent lysates from NIH-3T3 cells was performed on a Pharmacia Superdex 200 column in 10 mM MOPS (pH 7.3), 25 mM β-glycerol phosphate 10 mM NaF, 2 mM EDTA at a flow rate of 0.25 ml/min.

To generate GST–Cdk4/Cdc37 associated p90 for microsequencing, GST–Cdk4/Cdc37 complexes were separated by SDS-PAGE, transferred to Immobilon membranes (Millipore) and p90 sequenced by the BCM Protein Chemistry Facility. The amino-terminal sequence PEEMQTDSGEVETFAFQAII displayed homology with the amino-terminus of human Hsp90β (identical residues underlined).

Antibodies

Polyclonal Cdc37 antibodies were produced in rabbits using His_6-Cdc37 and were affinity purified using GST–Cdc37 cross-linked to glutathione–Sepharose. Antibodies against Cdc2, Cdk2, Cdk4, Cdk6, and D-type cyclins were from Santa Cruz. Anti-Cdk7 was from Upstate Biotechnologies. Anti-Hsp90 monoclonal antibodies are from Stress Gen. Monoclonal antibodies against the p50 subunit of Hsp90 were from a previous study (Whitelaw et al. 1991).

Cell culture and immunoprecipitation

NIH-3T3 cells and early passage mouse embryonic fibroblasts were grown in Dulbecco's modified eagles medium (DMEM), 5%–10% fetal bovine serum (FBS), 37°C. At 70% confluence, cells were washed with PBS and lysed in binding buffer before centrifugation (20 min, 14,000*g*, 4°C). ML-1 cells were grown in suspension as described (Meyerson and Harlow 1994). Lysates (150 μl at ~2–4 mg/ml) were immunoprecipitated using the indicated antibodies in conjunction with 10 μl of protein A or protein G–Sepharose (1 hr, 4°C). Washed complexes were immunoblotted using 11%–13.5% SDS–polyacrylamide gels. Detection was accomplished using enhanced chemiluminescence (ECL; Amersham). [^{35}S]Methionine labeling was performed as described (Harper et al. 1995) unless otherwise noted. To generate nondetergent extracts, cells were suspended in 50 mM MOPS (pH 7.3), 25 mM β-glycerol phosphate, 2 mM EDTA and subjected to five rounds of passage through a 26-gauge needle before centrifugation at 14,000*g* for 20 min.

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In situ hybridization

Specimen collection, embedding, sectioning, and hybridization were performed as described (Parker et al. 1995; Sicinski et al. 1995). [α-^{35}S]UTP-labeled riboprobes were generated using pBluescript cDNA clones in conjunction with T7 or T3 RNA polymerase. Specimens were photographed by double exposure using darkfield illumination with a red filter and Hoechst epifluorescence optics.

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References

Aligue, R., H. Akhavan-Niak, and P. Russell. 1994. A role for Hsp90 in cell cycle control: Weel tyrosine kinase activity requires interaction with Hsp90. *EMBO J.* **13:** 6099–6106.

Baldin, V, J. Lukas, M.J. Marcote, M. Pagano, and G. Draetta. 1993. Cyclin D1 is a nuclear protein required for cell cycle progression in G_1. *Genes & Dev.* **7:** 812–821.

Bohen, S.P., A. Kralli, and K.R. Yamamoto. 1995. Hold'em and Fold'em: Chaperones and signal transduction. *Science* **268:**1303–1304.

Boschelli, F. 1993. Expression of pp60v-src in Saccharomyces cerevisiae results in elevation of p34CDC28 kinase activity and release of the dependence of DNA replication on mitosis. *Mol. Cell. Biol.* **13:** 5112–5121.

Brugge, J.S. 1986. Interaction of the Rous sarcoma virus protein pp60v-src with the cellular proteins p50 and p90. *Curr. Top. Microbiol. Immunol.* **123:** 1–22.

Brugge, J.S., E. Erikson, and R.L. Erickson. 1981. The specific interaction of Rous sarcoma virus transforming protein, pp60src, with two cellular proteins. *Cell* **25:** 363–372.

Coleman, T.R. and W.G. Dunphy. 1994. Cdc2 regulatory factors. *Curr. Biol.* **6:** 877–882.

Connell-Crowley, L., M.J. Solomon, N. Wei, and J.W. Harper. 1993. Phosphorylation independent activation of human cyclin-dependent kinase 2 by cyclin A in vitro. *Mol. Biol. Cell* **4:** 79–92.

Cutforth, T. and G.M. Rubin. 1994. Mutations in Hsp83 and cdc37 impair signaling by the sevenless receptor tyrosine kinase in Drosophila. *Cell* **77:** 1027–1036.

Desai, D., Y. Gu, and D.O. Morgan. 1992. Activation of human cyclin-dependent kinases in vitro. *Mol. Biol. Cell* **3:** 571–582.

Dey, B., A.J. Caplan, and F. Boschelli. 1996. The YDJ1 molecular chaperone facilitates formation of active p60v-src in yeast. *Mol. Biol. Cell* **7:** 91–100.

Doyle, H.J. and J.M. Bishop. 1993. Torso, a receptor tyrosine kinase required for embryonic pattern formation, shares substrates with the sevenless and EGF-R pathways in Drosophila. *Genes & Dev.* **7:** 633–646.

Durfee, T., K. Becherer, P.-L.Chen, S.-H. Yeh, Y. Yang, A. Kilburn, W.-H. Lee, and S.J. Elledge. 1993. The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. *Genes & Dev.* **7:** 555–569.

Elledge, S.J., R. Richman, F. Hall, R. Williams, N. Logsdon, and J.W. Harper. 1992. *CDK2* encodes a 33 kDa cyclin A-associated protein kinase and is expressed before *CDC2* in the cell cycle. *Proc. Natl. Acad. Sci.* **89:** 2907–2911.

Ferguson, J., J.Y. Ho, T.A. Peterson, and S.I. Reed. 1986. Nucleotide sequence of the yeast cell division cycle start genes CDC28, CDC36, CDC37, and CDC39, and a structural analysis of the predicted products. *Nucleic Acids Res.* **14:** 6681–6697.

Fisher, R.P., P. Jin, H.M. Chamberlin, and D.O. Morgan. 1995. Alternative mechanisms of CAK assembly require an assembly factor or an activating kinase. *Cell* **83:** 47–57.

Gerber, M.R., A. Farrell, R.J. Deshaies, I. Herskowitz, and D.O. Morgan. 1995. Cdc37 is required for association of the protein kinase Cdc28 with G1 and mitotic cyclins. *Proc. Natl. Acad. Sci.* **92:** 4651–4655.

Harper. J.W. and S.J. Elledge. 1996. Cdk inhibitors in development and cancer. *Curr. Opin. Genet. Dev.* **6:** 56–84.

Harper, J.W., S.J. Elledge, K. Keyomarsi, B. Dynlacht, L.-H. Tsai, P. Zhang, S. Dobrowolski, C. Bai, L. Connell-Crowley, E. Swindell, P.M. Fox, and N. Wei. 1995. Inhibition of cyclin dependent kinases by p21. *Mol. Biol. Cell* **6:** 387–400.

Hartson, S.D. and R.L. Matts. 1994. Association of Hsp90 with cellular Src-family kinases in a cell-free system correlates with altered kinase structure and function. *Biochemistry* **33:** 8912–8920.

Hunter, T. and J. Pines. 1994. Cyclins and cancer II: Cyclin D and CDK inhibitors come of age. *Cell* **79:** 573–582.

Hunter, T. and B. Sefton. 1980. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. *Proc. Natl. Acad. Sci.* **77:** 1311–1315.

Jakob, U. and J. Buchner. 1994. Assisting spontaneity: The role of Hsp90 and small Hsps as molecular chaperones. *Trends Biochem. Sci.* **19:** 205–211.

Kimura, Y., I. Yahara, and S. Lindquist. 1995. Role of the protein chaperone YDJ1 in establishing Hsp90-mediated signal transduction pathways. *Science* **268:** 1362–1365.

Kiyokawa, H., V.M. Richon, R.A. Rifkind, and P.A. Marks. 1994. Suppression of cyclin-dependent kinase 4 during induced differentiation of erythroleukemia cells. *Mol. Cell. Biol.* **14:** 7195–7203.

Matsushime, H., D.E. Quelle, S.A. Shurtleff, M. Shibuya, C.J. Sherr, and J.-Y. Kato. 1994. D-type cyclin-dependent kinase activity in mammalian cells. *Mol. Cell. Biol.* **14:** 2066–2076.

Meyerson, M. and E. Harlow. 1994. Identification of G1 kinase activity for Cdk6, a novel cyclin D partner. *Mol. Cell. Biol.* **14:** 2077–2086.

Morgan, D.O. 1995. Principles of CDK regulation. *Nature* **374:** 131–134.

Nathan, D.F. and S. Lindguist. 1995. Mutational analysis of Hsp90 function: Interactions with steroid receptor and a protein kinase. *Mol. Cell. Biol.* **15:** 3917–3925.

Ohtsubo, M., A.M. Theodoras, J. Schumacher, J.M. Roberts, and M. Pagano. 1995. Human cyclin E, a nuclear protein essential for the G1-to-S phase transition. *Mol. Cell. Biol.* **15:** 2612–2624.

Opperman, H., W. Levinson, and J.M. Bishop. 1981. A cellular protein that associates with the transforming protein of Rous sarcoma virus is a heat shock protein. *Proc. Natl. Acad. Sci.* **78:** 1067–1071.

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Pardee, A.B. 1989. G_1 events and regulation of cell proliferation. *Science* **246:** 603–608.

Parker, S., G. Eichele, P. Zhang, A. Rawls, A. Sands, A. Bradley, E. Olson, J.W. Harper, and S.J. Elledge. 1995. p53-Independent expression of p21Cip1 in muscle and other terminally differentiated cells. *Science* **267:** 1024–1027.

Perdew, G.H. and M.L. Whitelaw. 1991. Evidence that the 90-kDa heat shock protein HSP90 exists in cytosol in heteromeric complexes containing Hsp70 and three other proteins with Mr of 63,000, 56,000, and 50,000. *J. Biol. Chem.* **266:** 6708–6713.

Reed, S.I. 1980. The selection of *S. cerevisiae* mutants defective in the start event of cell division. *Genetics* **95:** 561–577.

Reed, S.I., M.A. de Barros Lopes, J. Ferguson, J.A. Hadwiger, J.-Y Ho, R. Horwitz, C.A. Jones, A.T. Lorincz, M.D. Mendenhall, T.A. Peterson, S.L. Richardson, and C. Wittenberg. 1985. Genetic and molecular analysis of division control in yeast. *Cold Spring Harbor Symp. Quant. Biol.* **50:** 627–634.

Schulte, T.W., M.V. Blagosklonny, C. Ingui, and L. Neckers. 1995. Disruption of the Raf-1–Hsp90 molecular complex results in destabilization of Raf-1 and loss of Raf-1-Ras association. *J. Biol. Chem.* **270:** 24585–24588.

Sherr, C.J. 1994. Cycling on cue. *Cell* **75:** 551–555.

Sherr, C.J. and J.M. Roberts. 1995. Inhibitors of mammalian G_1 cyclin-dependent kinases. *Genes & Dev.* **9:** 1149–1163.

Sicinski, P., J.L. Donaher, S.B. Parker, T. Li, A. Fazeli, H. Gardner, S.Z. Haslam, R.T. Bronson, S.J. Elledge, and R.A. Weinberg. 1995. Cyclin D1 provides a link between development and oncogenesis in the retina and breast. *Cell* **82:** 621–630.

Stancato, L.F., Y.-H. Chow, K.A. Hutchison, G.H. Perdew, R. Jove, and W.B. Pratt. 1993. Raf exists in a native heterocomplex with hsp90 and p50 that can be reconstituted in a cell free system. *J. Biol. Chem.* **268:** 21711–21716.

Tassan, J.P., M. Jaquenoud, A.M. Fry, S. Frutiger, G.J. Hughes, and E.A. Nigg. 1995. *In vitro* assembly of a functional human CDK7-cyclin H complex requires MAT1, a novel 36 kDa RING finger protein. *EMBO J.* **14:** 5608–5617.

Valay, J.-G., M. Simon, M.-F. Dubois, O. Bensaude, C. Facca, and G. Faye. 1995. The KIN28 gene is required for both RNA polymerase II mediated transcription and phosphorylation of the Rpb1p CTD. *J. Mol. Biol.* **249:** 535–544.

Whitelaw, W.L., K. Hutchison, and G.W. Perdew. 1991. A 50-kDa cytosolic protein complexed with the 90-kDa heat shock protein hsp90 is the same protein complexed with pp60v-src/hsp90 in cells transformed by Rous sarcoma virus. *J. Biol. Chem.* **266:** 16436–16440.

Whitesel, L., E.G. Mimnaugh, B. De Costa, C.E. Myers, and L.N. Neckers. 1994. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: Essential role for stress proteins in oncogenic transformation. *Proc. Natl. Acad. Sci.* **91:** 8324–8328.

Weinberg, R.A. 1995. The retinoblastoma protein and cell cycle control. *Cell* **81:** 323–330.

Xu, Y. and S.L. Lindquist. 1993. Heat shock protein hsp90 governs the activity of pp60v-src kinase. *Proc. Natl. Acad. Sci.* **90:** 7074–7078.

Yamaki, H., H. Suzuki, E.C. Choi, and N. Tanaka. 1982. Inhibition of DNA synthesis in murine tumor cells by geldanamycin, an antibiotic of the benzoquinoid ansamycin group. *J. Antibiot.* **35:** 886–892.

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Genes Dev. 1996, **10:**
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