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The DNA-binding subunit p140 of replication factor C is upregulated in cycling cells and associates with G_1 phase cell cycle regulatory proteins

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Abstract The DNA-binding subunit of replication factor C (RFCp140) plays an important role in both DNA replication and DNA repair. The mechanisms regulating activation of RFCp140 thereby controlling replication and cellular proliferation are largely unknown. We analyzed protein expression of RFCp140 during cell cycle progression and investigated the association of RFCp140 with cell cycle regulatory proteins in cell lines of various tissue origin and in primary hematopoietic cells. Western and Northern blot analyses of RFCp140 from synchronized cells showed downregulation of RFCp140 when cells enter a G_0-like quiescent state and upregulation of RFCp140 in cycling cells. Translocation from the cytoplasmic compartment to the nucleus did not account for the significant increase in RFCp140 protein levels observed in cycling cells. To investigate a potential association of RFCp140 with cell cycle regulatory proteins coimmunoprecipitation assays were performed. These studies demonstrated specific binding of RFCp140 to cdk4-kinase in hematopoietic and fibroblast cell lines. Additional coimmunoprecipitation studies revealed specific association of RFCp140 with cyclin D1, p21, proliferating cell nuclear antigen, and retinoblastoma protein. These findings link DNA replication and repair factor RFCp140 to G_1 phase cell cycle regulatory elements critically involved in cell cycle control.

Key words Replication factor C · RFCp140 ·Cell cycle · G_1 phase regulatory proteins

Abbreviations *cdk* Cyclin-dependent kinase · *PAGE* polyacrylamide gel electrophoresis · *PCNA* Proliferating cell nuclear antigen · *PHA* phytohemagglutinin · *Rb* Retinoblastoma · *RFC* Replication factor C · *SDS* sodium dodecyl sulfate

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Introduction

DNA replication is a highly controlled process which is regulated by a large number of proteins forming multiple protein-protein and protein-DNA complexes (reviewed in [1, 2]). Cell free in vitro replication systems have elucidated the fundamental processes: the first step consists of activation and binding of a multisubunit polymerase acces-

sory protein termed replication factor C (RFC) to the primed DNA template [3–5]. RFC is composed of five polypeptide subunits [5–7]. By virtue of its p140 subunit, RFC binds specifically to the primer template junction [4, 8, 9, 10]. A second polymerase accessory protein termed proliferating-cell nuclear antigen (PCNA) is then loaded onto the DNA in the presence of ATP [4, 8, 9, 11, 12]. This complex of primed DNA-RFC-PCNA can bind polymerase δ, resulting in efficient synthesis of the DNA leading strand during S phase (reviewed in [13]). The decisions of cells to enter the DNA synthesis (S) phase are made in late G_1 phase. At the START checkpoint (also known as the restriction point in mammalian cells) G_1 cyclins, their cyclin-dependent kinases (cdks) and their regulatory kinases and phosphatases are involved in integration of growth factor mediated signals that drive the cell cycle. Many checkpoints are deregulated in oncogenesis, and this is often due to changes in cyclin-cdk complexes (reviewed in [14]). In particular, the deregulation of the START checkpoint may allow cell proliferation to become independent of external growth control signals. Accumulation of genetic changes that contribute to the tumor phenotype may be the result of bypassing an abnormal START checkpoint thus allowing cells to replicate unrepaired mutations. The final common pathway in the G_1 phase is phosphorylation of pRb, the product of the retinoblastoma gene [15–19].

Upon binding of cyclin D to its partner cdk4 (or cdk6) a cyclin D/cdk4 (or cdk6) complex is formed which acts as a positive regulator for the cell cycle through phosphorylation of retinoblastoma (Rb) protein [20, 21]. Phosphorylation of pRb in late G_1 phase releases sequestered transcription factors for genes controlling the G_1/S transition [15, 17–19]. Despite detailed information of the mechanisms of replication, on one hand, and of the activation of G_1 cell cycle regulatory proteins, on the other, little is known about how these processes are linked molecularly. Recently we have shown that overexpression of the large subunit (p140) of RFC results in an accelerated G_1-S phase transition and in an increased cellular growth rate [22] indicating that RFCp140 is the limiting subunit of an active RFC complex in normal cells. Here we have investigated protein expression of RFCp140 during cell cycle progression and have identified cell cycle regulatory proteins that associate with RFC. Our analyses show that the large (p140) subunit of RFC specifically associates with cdk4-kinase. In addition, we detected specific complexes of p140 with cyclin D, p21, and Rb protein which are key regulatory elements of G_1/S phase transition. Together, our results indicate that G_1 phase regulatory proteins interact with the RFC complex.

Materials and methods

Cell culture and synchronization

All cells were grown in RPMI 1640 (Seromed, Munich, Germany) supplemented with 10% fetal calf serum (PAA, Cölbe, Germany) as described [23, 24]. NIH3T3, HL60, and K562 cells were obtained from the American Type Culture Collection (Rockville, Md., USA). The murine bone marrow stromal cell line +/+ 1.LDA11 was kindly provided by Dr. G. Derigs (University of Mainz, Mainz, Germany). Cells were synchronized in G_0 by serum starvation for 48 h (in RPMI medium with 0.1 % fetal calf serum). Cells arrested at the G_1/S phase boundary were obtained by incubation for 20 h with 3 µg/ml Aphidicolin (Boehringer, Mannheim, Germany). For synchronization in S phase, cells arrested at G_1/S were washed and reincubated in fresh medium for 2 h. A 20 h exposure to the microtuble inhibitor Nocadazole (0.12 µg/ml; Boehringer) caused cells to pause in the G_2/M stage of the cell cycle.

Isolation of human peripheral blood T-lymphocytes

T-lymphocytes were isolated essentially as described [25]. Briefly, neuraminidase-treated sheep erythrocytes were incubated with human peripheral blood mononuclear cells. Rosetted T-lymphocytes were separated by centrifugation over Ficoll-Hypaque density gradient (Biochrom, Berlin, Germany). Negative selection using mouse anti-CD19, anti-CD16, anti-CD56 (all purchased from Coulter, Krefeld, Germany) and anti-CD14 (Immunotech, Hamburg, Germany) antibodies and sheep anti-mouse beads (Dyna, Oslo, Norway) was performed according to the manufacturer's instructions.

Cell cycle analysis

Cells were grown to semiconfluency, trypsinized, and washed once in 10% FCS containing medium and twice in sample buffer (0.1% glucose in phosphate-buffered saline). Cell fixation was carried out in 70 % ethanol overnight, and fixed cells were stained with propidiumiodide solution (50 µg/ml) as described [26]. The stained cells were analyzed in a fluorescence-activated cell sorter (EPICS XL; Coulter). The percentage of cells in each phase of the cell cycle was determined by Multicycle software (Phoenix Flow Systems).

Antibodies

Preparation of anti-murine RFC-p140 polyclonal antiserum has been described elsewhere [22]. For preparation of anti-human RFC-p140 antiserum a peptide at the carboxy terminal region (amino acids 1124–1140) was designed. Peptide synthesis and immunization were carried out by Genosis (United Kingdom). Coimmunoprecipitation experiments were carried out using the agarose conjugated antibodies anti-cyclin D1 (rabbit polyclonal IgG raised against a peptide; Santa Cruz, Heidelberg, Germany), anti-PCNA (mouse monoclonal IgG_2a raised against recombinant PCNA, Santa Cruz), anti-cdk4 (rabbit polyclonal IgG raised against a mouse cdk4 peptide, Santa Cruz), anti-cdk4 (rabbit polyclonal IgG raised against a human cdk4 peptide, Santa Cruz), anti-Rb (mouse monoclonal IgG1 raised against a Rb-βgal fusion protein, Santa Cruz), anti-c-Kit (rabbit polyclonal IgG raised against a peptide, Santa Cruz). As controls we used agarose conjugated normal mouse IgG (Sigma), normal rabbit IgG (Santa Cruz) and rabbit preimmune serum. Anti-human RFC-p37 and anti-human RFC-p40 antisera were kindly provided by Dr. J. Hurwitz (Memorial Sloan-Kettering Cancer Center, New York, USA).

Immunoprecipitation and Western blot

Nuclear and cytoplasmic protein lysates were prepared as described previously [22]. Lysates were precleared by incubating with protein A/G agarose (Calbiochem, Cambridge, UK) for 45 min followed by a 15 min centrifugation at 500 *g*. Antibody coupled to agarose beads and 350 µl immunoprecipitation buffer (20 mM Tris/HCl; pH 7.0, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 0.2 mM sodium orthovanadate, 50 mM NaF, 1 µM oka-

daic acid) were added to a total of 150 µl clarified lysates and incubated for 2–16 h on a rotating platform at 4°C. Immunoprecipitates were washed three times with 1 ml immunoprecipitation buffer, resuspended in sodium dodecyl sulfate (SDS) sample buffer, and separated on 9% SDS polyacrylamide gels.

For Western blotting, protein lysates (100–400 µg per lane) or immunoprecipitates were subjected to SDS polyacrylamide gel electrophoresis (PAGE) and blotted to a nitrocellulose membrane (ECL membrane, Amersham) using a SDS electroblotting system (Bio Rad, Munich, Germany) for 4 h at a constant voltage of 50 V. The filter was blocked in blocking solution (Boehringer chemiluminescence kit) for 1 h, incubated with primary antibody (1:1000) overnight in blocking solution, washed twice in TBST and twice in blocking solution as described [22]. Appropriate secondary antibody (1:2000 horseradish peroxidase linked goat anti-rabbit or goat anti-mouse immunglobulin; Santa Cruz) was incubated for 1 h and specific proteins were detected using a chemiluminescence system (Boehringer).

For quantitative analysis of p140 protein levels densitometric scanning of autoradiographs was applied by using the Quanti-scan program.

Northern blot analysis

Total RNA was isolated by the method of Chomczynski and Sacchi [24] as described. RNA (20 µg) was subjected to electrophoresis on a 1% formaldehyde agarose gel and transferred onto nylon membranes (Hybond N, Amersham Buchler, Braunschweig, Germany). All hybridizations were carried out at 65°C for 16 h in a solution containing 1% bovine serum albumin, 7% SDS, 1 mM EDTA, and 0.5 M NaH_2PO_4 (pH 7.2). Filters were hybridized with human RFCp140 cDNA insert radiolabeled by random priming method.

Results

Expression of RFCp140 in cells synchronized at various stages of the cell cycle

To determine whether expression of RFC-p140 is regulated by the cell cycle, p140 protein levels were examined during progression from G_1 to G_2 phase and in a G_0-like quiescent state (Fig. 1A, and data not shown). For these experiments NIH3T3 cells (Fig. 1A) and other cell lines including K562 and +/+ 1.LDA11 cells (data not shown) were arrested in various stages of the cell cycle as described above. In comparison to nonsynchronized cells, the level of RFCp140 protein decreased significantly (20.5% of control, as estimated by densitometric scanning) when cells were arrested in G_0 by serum starvation (Fig. 1A, lane 2). In this experiment 76% of cells were in G_0, 19% in S phase, and 5% in G_2 phase as determined by cell cycle analysis. The persistent basal amount of p140 is most likely due to cells that remain in the cell cycle during the course of serum starvation. These results indicate minimal or no expression of p140 in cells which exit the cell cycle to enter a G_0-like quiescent state. Western blotting of RFCp140 from cells synchronized in late G_1 phase, S phase, and G_2 phase (Fig. 1, lanes 3–5) revealed that the abundance of p140 protein was the same as in asynchronous cells. Thus in cycling cells the abundance of p140 protein is kept at a high level which remains constant during progression through G_1, S, and G_2 phases.

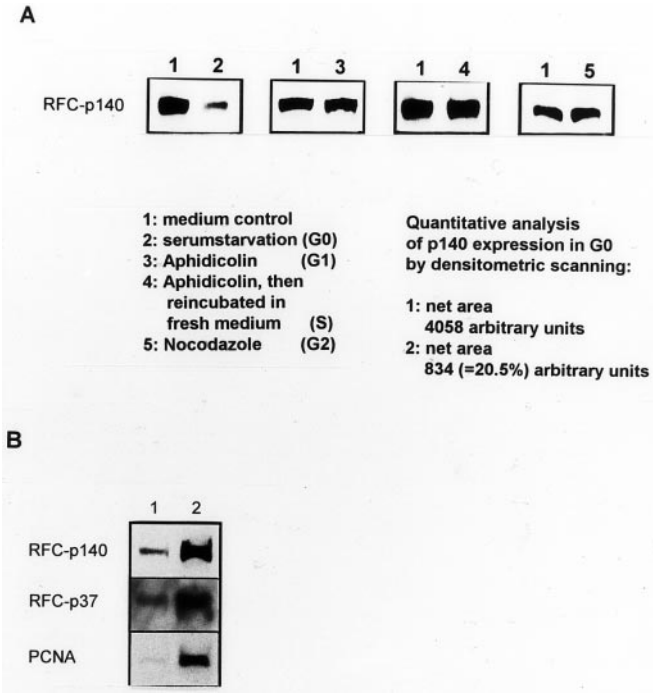

Fig. 1 **A** Expression of RFCp140 in nuclear lysates of cells synchronized at various phases of the cell cycle. NIH3T3 cells were synchronized at G_0/G_1 (serum starvation), G_1/S (Aphidicolin), S (Aphidicolin, then reincubation in fresh medium), and G_2/M (Nocadazole) stages of the cell cycle as described in the text. Cell cycle analysis was performed in parallel as described in the text, and the percentage of cells in G_0/G_1, G_1/S, S, and G_2/M phases was found to be greater than 70%. Exponentially growing cells (medium control) were used as controls. Nuclear protein lysates were made for assaying the expression of RFCp140 by SDS-PAGE and immunoblotting. To verify loading of equal amounts of protein, Coomassie staining of a control gel was performed. **B** RFCp140 protein expression in human peripheral blood T-lymphocytes is upregulated by PHA stimulation. T-lymphocytes were isolated from the peripheral blood of normal human donors as described in the text. Nuclear protein lysates were prepared from freshly isolated T-cells (*lane 1*) or upon incubation in medium containing PHA (10 µg/ml) for 48 h (*lane 2*). Protein lysates were subjected to SDS-PAGE and Western blotting was performed with antiserum recognizing RFCp140. Western blotting of RFCp37 and PCNA was performed in parallel using specific antibodies as described in the text. To verify equal loading, Coomassie staining of a control gel was performed

Upregulation of p140 expression upon entering the cell cycle

To demonstrate conclusively that downregulation of p140 protein abundance during serum starvation is due to cell cycle exit rather than to an artifact of serum starvation, we examined p140 protein levels in human peripheral blood T-cells with and without phytohemagglutinin (PHA) stimulation. Normal peripheral blood T-lymphocytes are in a quiescent G_0-like state and enter the cell cycle upon stimulation with PHA. When lysates of resting and PHA-stimulated T-cells were analyzed for the abundance of p140 protein, the result was similar to that obtained in cells with and without serum starvation: Resting T-cells (99.5% in

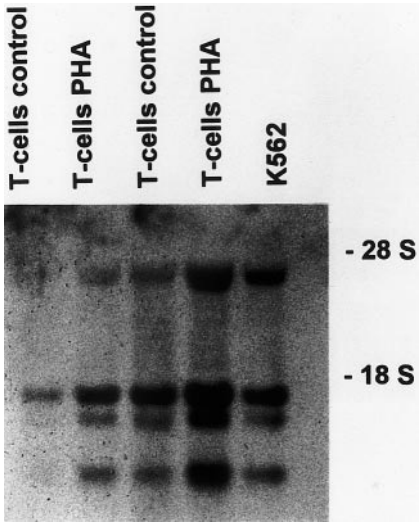

Fig. 2 Northern blot analysis of p140-specific mRNA levels in resting and PHA-stimulated human peripheral blood T-lymphocytes. T-cells were treated with PHA (10 µg/ml) for 48 h or left untreated. Untreated K562 cells were used as an additional control. Northern blotting was performed as described in the text. Equal loading was controlled by parallel methylene blue staining of the membrane. *Right margin*, markers for 28S and 18S bands

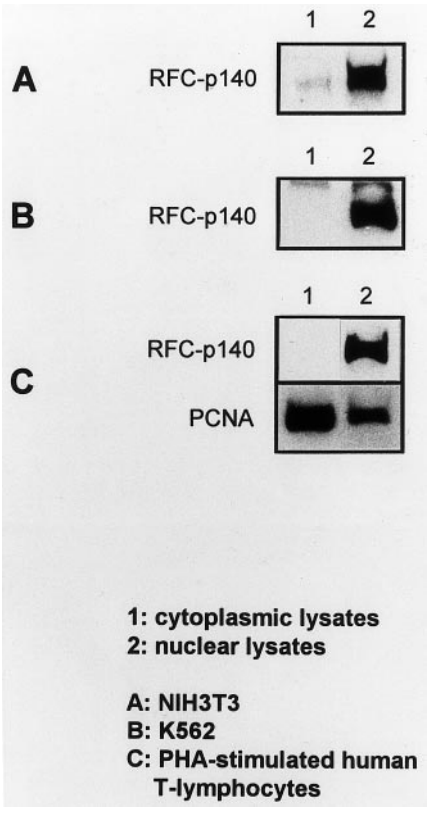

Fig. 3A–C Expression of RFCp140 in cytoplasmic and nuclear lysates. Cytoplasmic (*lane 1*) and nuclear (*lane 2*) lysates (400 µg per lane) from asynchronously growing NIH3T3 cells (**A**), K562 cells (**B**) and PHA-stimulated human peripheral blood T-lymphocytes (**C**) were analyzed by SDS-PAGE and Western blotting using polyclonal antiserum recognizing mouse (**A**) and human (**B**,**C**) RFCp140, respectively. As a control, Western blotting of PCNA was performed in parallel. Equal loading was verified as described above (data not shown)

G_0, 0.2% in S, and 0.3% in G_2) had very low amounts of p140 (Fig. 1B, lane 1), and accumulation of p140 began as cells entered the cell cycle (Fig. 1B, lane 2; 70.1% of cells in G_1, 24.9% in S, and 5.0% in G_2). Expression of the small subunit of RFC (p37) and of PCNA which is an essential cofactor of RFC [7] was examined in parallel. Both RFCp37 and PCNA levels increased significantly upon entering the cell cycle (Fig. 1B).

Analysis of p140-specific mRNA levels in resting and cycling human T-lymphocytes

To investigate whether cell cycle regulation of p140 occurs at the level of transcription or at the level of protein stability, Northern blot analysis was performed. Total cellular RNA was extracted from resting and PHA stimulated human peripheral blood T-cells. The level of the major p140-specific mRNA species (estimated length 4.5 kb) was strongly upregulated in cycling cells (Fig. 2). This result indicates that cell cycle control of RFCp140 includes regulation at the mRNA level.

Analysis of p140 protein abundance in cytoplasmic and nuclear lysates

Western blotting of RFCp140 in cytoplasmic and nuclear lysates from various cell lines (NIH3T3, K562, +/+ 1.LDA11) and from PHA-stimulated human peripheral blood T-cells showed that expression of RFCp140 can be demonstrated exclusively in the nuclear fractions of cells analyzed (Fig. 3, and data not shown). This result indicates that expression of RFCp140 in cycling cells is restricted to the nuclear compartment. To show that downregulation of p140 protein expression in G_0 phase is not due to a potential translocation from the nucleus to cytoplasm, we examined p140 protein levels both in nuclear and cytoplasmic lysates from asynchronous cells and from cells arrested in G_0 phase. This analysis demonstrated complete absence of p140 immunoreactivity in both nuclear and cytoplasmic lysates from G_0 cells (data not shown). Together, these data provide evidence that cell cycle dependent regulation of p140 protein abundance is not due to translocation between subcellular compartments.

RFCp140 associates with multiple G_1 phase key regulatory proteins

To identify regulatory proteins that specifically associate with RFCp140, immunoprecipitates of the cell cycle regulatory factors cdk4 and cyclin D were examined. Nuclear cell extracts of K562 and HL60 cells were immunoprecipitated with anti-cdk4 and anti-cyclin D antibodies and sepa-

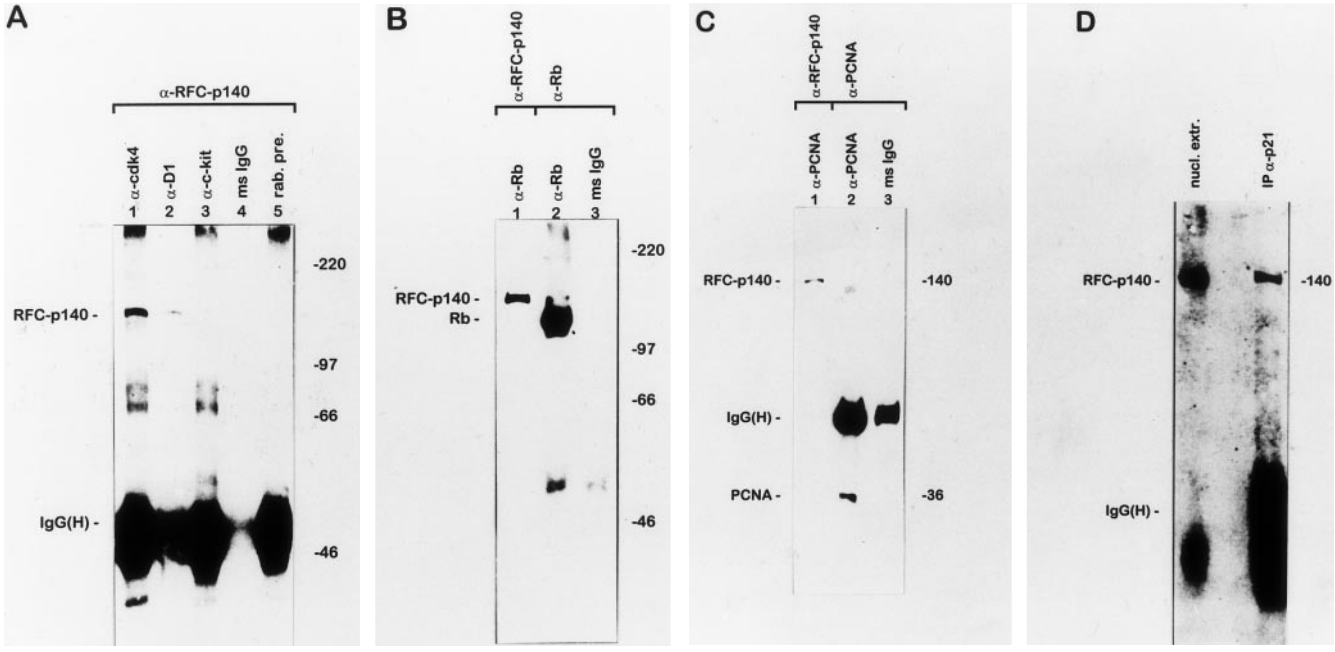

Fig. 4A–D RFCp140 associates with cdk4, cyclin D1, Rb, and PCNA in hematopoietic cells. **A** Nuclear lysates from HL60 cells (80×10^6 cells) were immunoprecipitated with anti-cdk4 antibody (*lane 1*), anti-cyclin D1 antibody (*lane 2*), anti-c-kit antibody (*lane 3*), preimmune serum (*lane 5*), and ms IgG (*lane 4*). The immunoprecipitates were analyzed by SDS-PAGE and immunoblotted with antiserum to human RFCp140. *Right margin*, molecular weight markers. Nuclear lysates from K562 cells (80×10^6 cells) were immunoprecipitated with anti-RB antibody (**B**), anti-PCNA antibody (**C**), and anti-p21(**D**) antibodies. Immunoprecipitates were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Western blotting was performed with antiserum to human RFCp140, anti-Rb antibody, and anti-PCNA antibody as indicated

rated by SDS-PAGE as described above. Western blot analysis with anti-RFCp140 antiserum revealed a single band with an apparent molecular mass of 140 kDa in these immunoprecipitates, but not in the respective control precipitates with anti-c kit antibody, rabbit preimmune serum and normal mouse IgG (Fig. 4A, and data not shown).

Additional immunoprecipitation–western blot experiments were carried out to investigate whether RFCp140 complexes with other G_1 cell cycle regulatory proteins. Nuclear extracts of K562 cells and HL60 cells were immunoprecipitated with anti-cdk4, anti-cyclin D, anti-Rb, anti-PCNA and anti-p21 antibodies. RFCp140 protein was readily detected in these immunoprecipitates (Fig. 4B–D, and data not shown). Specificity was controlled in precipitates of normal mouse IgG (Fig. 4B,C). To confirm positive immunoprecipitation of PCNA and Rb, Western blotting of anti-Rb and anti-PCNA immunoprecipitates was performed with anti-Rb and anti-PCNA antibodies. A single band with an apparent molecular mass of 110 kDa and 36 kDa, respectively, indicated specific immunoprecipitation of Rb and PCNA protein (Fig. 4B,C). Together, these data demonstrate that RFCp140 protein specifically complexes with cdk4, cyclin D, the retinoblastoma protein, the polymerase acessory protein PCNA and p21.

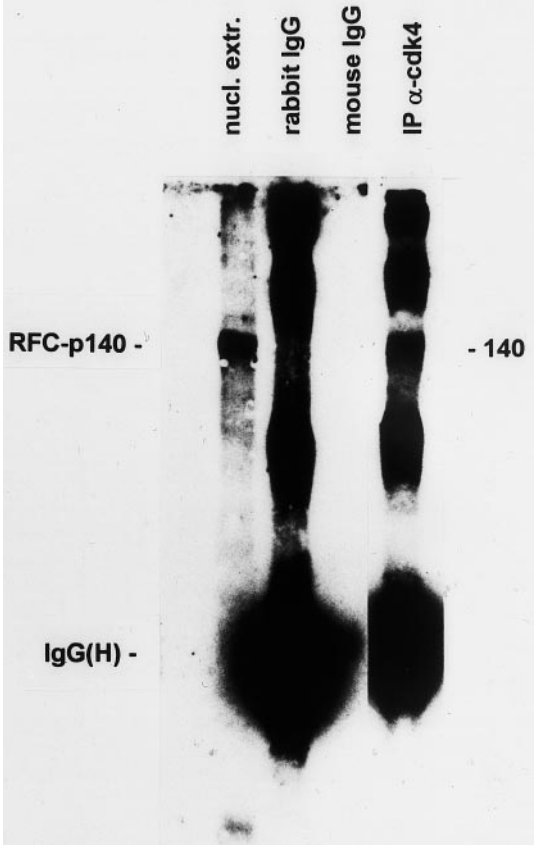

Fig. 5 RFCp140 associates with cdk4 in fibroblasts. Nuclear lysates from NIH3T3 cells were immunoprecipitated with anti-cdk4 antibody (*lane 4*), normal rabbit IgG (*lane 2*), and normal mouse IgG (*lane 3*). The immunoprecipitates and control nuclear extracts were analyzed by SDS-PAGE and immunoblotting with antiserum to ms RFCp140. *Right margin*, molecular weight position for p140

Additional immunoprecipitation studies were performed to investigate whether complex formation of p140 with cell cycle regulatory proteins can also be found in nonhematopoietic cells. For this analysis nuclear extracts of NIH3T3 cells were immunoprecipitated with anti-cdk4 antibody. Western blot analysis with anti-p140 antiserum showed a specific band with an apparent molecular weight of 140 kDa (Fig. 5) but not in the control precipitates with normal IgG (Fig. 5). These data show that association of cdk4 with p140 is not restricted to hematopoietic cells but can also be found in fibroblasts.

Discussion

RFC was identified initially in cell-free in vitro replication systems [4–6]. Many of its molecular interactions involved in DNA replication have been studied in yeast cells [8, 11, 27]. These investigations demonstrate that the large subunit of this multipeptide complex (RFCp140) is critical for DNA replication and cellular proliferation [27].

The studies outlined here describe several novel aspects of RFCp140 involved in cell cycle progression. First, the data presented demonstrate cell cycle dependent regulation of p140 protein and mRNA expression. Second, and more importantly, we show, for the first time, that DNA replication factor RFCp140 is directly connected to key regulatory elements of the cell cycle control via specific protein-protein interactions.

Several lines of evidence support the existence of a causal relationship between cellular proliferation rates and expression of p140. First, in comparison to slowly dividing cells, rapidly proliferating cells present higher levels of p140 mRNA [28]. Second, in vitro studies have demonstrated that efficacy of replication is correlated with RFCp140 protein abundance [6]. Third, overexpression of RFCp140 results in an accelerated cellular proliferation rate [22]. Our findings that mRNA and protein levels of p140 are downregulated in G_0 cells and accumulate when cells enter the cell cycle are in agreement with these results. Studies undertaken to investigate the molecular mechanisms behind downregulation of RFCp140 (H. van der Kuip, unpublished results) indicate that this pathway is not primarily regulated by protein kinase C isoenzymes but appears to be controlled by protein kinase A or G-kinase type activities. Since it has been shown recently that translocation between nucleus and cytoplasm may contribute to regulation of protein abundance of cell cycle factors [29], we performed Western blotting of RFCp140 in cytoplasmic and nuclear lysates in parallel. Expression of p140 was found exclusively in the nuclear fraction of both asynchronously growing and G_0 cells. This result indicates that transfer between cellular compartments is not involved in regulation of p140 protein expression. High levels of nuclear p140 expression throughout G_1, S, and G_2 phase are consistent with involvement of p140 in cellular functions beyond DNA replication. Indeed, a distinct version of the BRCT domain has been described in the large subunit of replication factor C [30]. This domain is likely to be involved in critical, yet uncharacterized, functions in the cell cycle control [30]. More importantly, experimental evidence has recently been provided that in addition to its role in DNA replication, p140 plays a pivotal role in coordinating interaction of multiple replication factors and auxiliary proteins required during DNA repair [10, 27]. Formation of these protein complexes is supported by the accessory protein PCNA which has recently been shown to be engaged also in binding to nuclear proteins involved in cell cycle control [31]. The first experimental evidence for physical association of PCNA with cell cycle regulatory factors was provided by Xiong et al. [32]. In these experiments PCNA was shown to coprecipitate with cyclin D1, and subsequent coimmunoprecipitation studies have demonstrated association of cyclin D1 with cdk2, cdk4, cdk5, and p21. It was proposed that there exists a quartenary complex of D-cyclin, cdk, PCNA, and p21, and that many combinatorial variations assemble in vivo [33]. As with PCNA, RFCp140 is an essential accessory factor for the δ polymerase which is required both for synthesis of DNA during replication and DNA repair. Coimmunoprecipitation experiments were performed to examine the possibility that RFCp140 may also associate with the above cell cycle regulatory proteins. Our results demonstrate that p140 specifically associates with cyclin D1, cdk4-kinase, PCNA, and p21. In addition, p140 was coprecipitated with Rb protein. Our data can be explained by participation of RFCp140 in formation of the above quarternary complex, although the experimental techniques employed do not formally rule out the existence of multiple pairwise interactions.

In contrast to cdk4 and PCNA, cyclin D1 is undetectable in G_0 mammalian cells [31]. Lack of D1 is the limiting factor in the formation of the cdk4–cyclin D1–PCNA complex in G_0 phase [31]. It has been suggested that cells need to inactivate the cyclin D1–cdk4 complex and dissociate the complex that D1 forms with PCNA to leave G_1 phase and start replication. In agreement with this hypothesis, D1 overexpression has been shown to inhibit DNA replication and DNA repair [34]. Together, these data suggest that cyclin D1 exercises its inhibitory function in G_1 by keeping PCNA in an inactive form. Binding of p21 to PCNA contributes to inhibition of replication [35]. It can be speculated that our experiments demonstrating association of RFCp140 with cyclin D1 and cdk4 indicate regulation of RFCp140 function in a similar manner. Thus, binding of RFCp140 to the quarternary G_1 complex would keep RFCp140 in an inactive form. It is also conceivable that p140 is a substrate for a G_1 phase cdk as cdk4. Preliminary studies analyzing phosphorylation of p140 suggest that p140 is phosphorylated at serine residuues (H. van der Kuip, unpublished results). The regulation of p140 phosphorylation by cdks will be an interesting subject for further studies. Together, the data presented here are in line with previous results on complex formation of PCNA with cell cycle regulatory factors and demonstrate, for the first time, physical association between DNA replication and repair factor RFC and G_1 phase regulatory proteins crucially involved in cell cycle control.

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