Nucleic Acids Research, 1997, Vol. 25, No. 24 **4915–4920**

CDF-1, a novel E2F-unrelated factor, interacts with cell cycle-regulated repressor elements in multiple promoters

Ningshu Liu, Frances C. Lucibello, Kathrin Körner, Lawrence A. Wolfraim[+], Jörk Zwicker[§] and Rolf Müller*

Institut für Molekularbiologie und Tumorforschung (IMT), Philipps-Universität Marburg Emil-Mannkopff-Strasse 2, D-35033 Marburg, Germany

Received September 8, 1997; Revised and Accepted October 19, 1997

ABSTRACT

The *cdc25C*, *cdc2* and *cyclin A* promoters are controlled by transcriptional repression through two contiguous protein binding sites, termed the CDE and CHR. In the present study we have identified a factor, CDF-1, which interacts with the *cdc25C* CDE–CHR module. CDF-1 binds to the CDE in the major groove and to the CHR in the minor grove in a cooperative fashion *in vitro*, in a manner similar to that seen by genomic footprinting. In agreement with *in vivo* binding data and its putative function as a periodic repressor, DNA binding by CDF-1 in nuclear extracts is down-regulated during cell cycle progression. CDF-1 also binds avidly to the CDE–CHR modules of the *cdc2* and *cyclin A* promoters, but not to the E2F site in the B-*myb* promoter. Conversely, E2F complexes do not recognize the *cdc25C* CDE–CHR and CDF-1 is immunologically unrelated to all known E2F and DP family members. This indicates that E2F- and CDF-mediated repression is controlled by different factors acting at different stages during the cell cycle. While E2F-mediated repression seems to be associated with genes that are up-regulated early (around mid G_1), such as B-*myb*, CDE–CHR-controlled genes, such as *cdc25C*, *cdc2* and *cyclin A*, become derepressed later. Finally, the fractionation of native nuclear extracts on glycerol gradients leads to separation of CDF-1 from both E2F complexes and pocket proteins of the pRb family. This emphasizes the conclusion that CDF-1 is not an E2F family member and points to profound differences in the cell cycle regulation of CDF-1 and E2F.

INTRODUCTION

Transcriptional repression has turned out to be a major mechanism of gene regulation during the cell cycle. A group of genes has been identified whose transcription is blocked early during the cell cycle by complexes consisting of the transcription factor E2F and pocket proteins of the pRb family including B-*myb*, *E2F*-1, *p107* and *orc*-1. All these genes become derepressed prior to S phase entry. We have identified a second group of genes which is expressed later during the cell cycle and seems to be controlled by a different mechanism of transcriptional repression. When the *cdc25C* promoter, which is up-regulated in late S/G_2, was studied by *in vivo* footprinting and mutational analysis a novel repressor element, the cell cycle-dependent element (CDE), was identified. The CDE is occupied in G_0/G_1 and its occupation is lost in G_2, when *cdc25C* is expressed. That CDE-mediated repression plays a role in regulating other promoters as well was shown by the presence of functional CDEs in the *cyclin A* and *cdc2* promoters, which reach their maximum expression in S/G_2. These studies also led to the discovery of an additional element contiguous with the CDE, which is identical in all three promoters. This element was termed the cell cycle genes homology region (CHR). Mutation of either the CDE or the CHR in the *cdc25C*, *cdc2* or *cyclin A* promoter largely abolishes repression in G_0. These functional data were supported by the demonstration of G_0/G_1-specific protein binding to both the CDE and CHR in genomic footprinting. Interestingly, the CDE is contacted in the major groove of DNA while binding to the CHR occurs in the minor groove. Meanwhile, two other genes which are expressed late during the cell cycle and contain CDE–CHR modules in their promoters have been described, i.e. the centromeric histone H3 homolog *CENP*-A and the Polo-like kinase *PLK*.

In the present study we have identified the *cdc25C* CDE–CHR binding activity, CDE–CHR binding factor-1 (CDF-1). We provide compelling evidence that CDF-1 behaves *in vitro* as expected from the results obtained by *in vivo* footprinting and in functional assays. In addition, we show that CDF-1 also interacts with the CDE–CHR repressor elements in the *cyclin A* and *cdc2* promoters and provide an initial biochemical characterization of this novel activity.

MATERIALS AND METHODS

Cell culture, DNA transfection and luciferase assays

NIH 3T3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum. For synchronization in G_0 cells were maintained in serum-free medium for 2 days. HeLa cells were grown in S-MEM plus 5% newborn calf serum.

*To whom correspondence should be addressed. Tel: +49 6421 286236; Fax: +49 6421 288923; Email: mueller@imt.uni-marburg.de

Present addresses: [+]Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO 63110-1093, USA and [§]Howard Hughes Medical Institute, Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley, CA 94720-3204, USA

NIH 3T3 cells were transfected by the DEAE–dextran technique and luciferase activities were determined as described.

Sequence analysis and luciferase constructs

The *cdc25C* and B-*myb* promoter-driven luciferase constructs have been described elsewhere. Mutations were introduced by PCR strategies as previously described.

Protein binding assays

Electrophoretic mobility shift assays (EMSAs) were performed as described previously. Briefly, nuclear extract (4 µg) was incubated in 12 µl buffer containing 50 mM Tris–HCl, pH 8.0, 10% (v/v) glycerol, 0.2 mM EDTA, 1 mM DTT, 0.8% sodium deoxycholate and 1 µg poly(dA/dT) for 10 min. NP-40 was added to a final concentration of 1.5% and incubation was continued for another 20 min. ^{32}P-Labeled probe (0.2 pmol) was added and the reaction mixture incubated for another 20 min. All reactions were performed on ice. Probes were labeled by filling-in 5′ overhanging ends. The following double-stranded probes were used for EMSAs: *cdc25C*-wt, 5′-ACTGGGCT**GGCGG**AAGG*TTTGAA*T-GGTCAA (CDE bold, CHR italic); T1, T4, T7 (also referred to as *cdc25C*-mCDE), A8 and C9 are mutated at positions –19, –16, –13, –12 and –11 respectively as described; *cdc25C* –10/–7, 5′-ACTGG-GCTGGCGGA*cttg*TTGAATGGTCAA; *cdc25C* –6/–3 (also referred to as *cdc25C*-mCHR), 5′-ACTGGGCTGGCGGAAGGT-*ggtc*ATGGTCAA; *cdc25C* –1/+2, 5′-ACTGGGCTGGCGGAAG-GTTTGAA*ggt*TCAA; *cdc25C* –2, 5′-ACTGGGCTGGCGGAA-GGTTTGA*c*TGGTCAA. The sequences of all other oligonucleotides, including B-*myb*, have been described elsewhere. The random oligonucleotide contains an irrelevant sequence. DP-1 antibodies were kindly provided by N.La Thangue (Glasgow). All other antibodies were purchased from Santa Cruz (SC-251X, SC-632X, SC-879X, SC-512X, SC-999X and SC-830X). *In vitro* dimethylsulfate (DMS) methylation protection footprinting was performed as described.

Genomic footprinting of stable transfectants

For the generation of stable cell lines the wild-type *cdc25C* luciferase construct C290 and the CHR mutant C290mCHR5/6 (TTTGAA mutated to TagGAA) were inserted into the pAGLu vector, which contains a matrix attachment region (MAR/SAR), and introduced into NIH 3T3 cells by electroporation. Stably transfected clones were isolated under G418 selection and analyzed for luciferase expression in quiescent and growing cells. Clones with the expected expression pattern were expanded and analyzed by genomic footprinting as described with the exception that the first primer (P1) was specific for the luciferase gene (5′-GTAACACAAAGGAATTCAAGC).

Glycerol gradient centrifugation

HeLa nuclear extract was dialyzed against a buffer containing 50 mM Tris–HCl, 100 mM NaCl, 1 mM DTT and proteinase inhibitors. Two hundred microliters of the dialyzed extract were applied to 2 ml gradients of 5–20% glycerol and centrifuged at 45 000 r.p.m. for 18 h. Samples were collected in 20 fractions starting at the top of the gradient. Pocket proteins were identified by immunoblotting using a mixture of antibodies against pRb, p107 and p130 (Santa Cruz; SC-50-G, SC-250X and SC-317X).

RESULTS

Delineation of the *cdc25C* CHR

One important requirement for identification of CDE–CHR binding protein(s) is the establishment of correlations between the ability of such proteins to interact with mutated repressor elements and the function of such mutated elements in cell cycle regulation. We have previously defined the consensus sequence of the CDE as G/C G C/T G G/C (GGCGG in *cdc25C*). For the CHR, however, such information is not yet available. In order to delineate the borders of the CHR and to identify critical nucleotide positions we introduced a number of point, triple and quadruple mutations into the CHR of the *cdc25C* promoter and analyzed the function of these mutant constructs by measuring their repression in NIH 3T3 cells synchronized in G_0. The data in Figure 1 clearly show that the CHR extends from –7 to –2 and that all nucleotide positions in this region are crucial. In contrast, the nucleotide positions between the CDE and the CHR (–11 to –8, AAGG) and the nucleotides downstream of the CHR (≥–1, TGG…) can be altered without

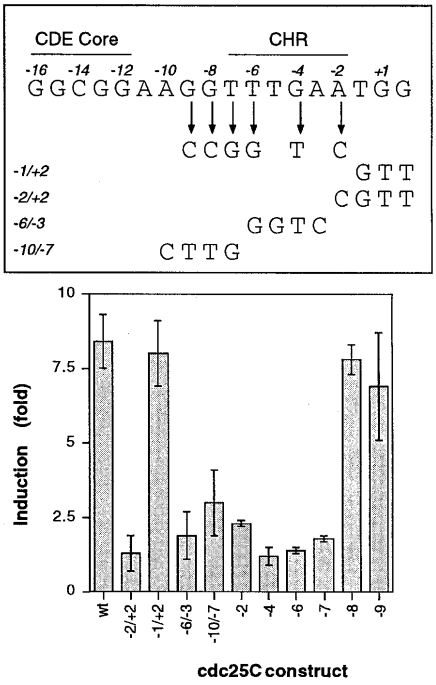

Figure 1. Structure–function analysis of the *cdc25C* CHR. *cdc25C* promoter constructs (based on C290; with mutations in the CHR region were analyzed for cell cycle regulation in NIH 3T3 cells. Positions –16 to –12 represent the CDE defined previously. Results of transient luciferase assays are expressed as the ratio of RLUs observed with growing cells relative to the activity in quiescent cells. The results shown summarize the data of four independent experiments using at least two independent preparations of plasmid DNA. Values represent averages; standard deviations are indicated by error bars. An SV40 reporter plasmid was included in each experiment to standardize the factor of induction (the SV40 reporter typically gave a 1.5-fold higher value in growing compared with quiescent cells).

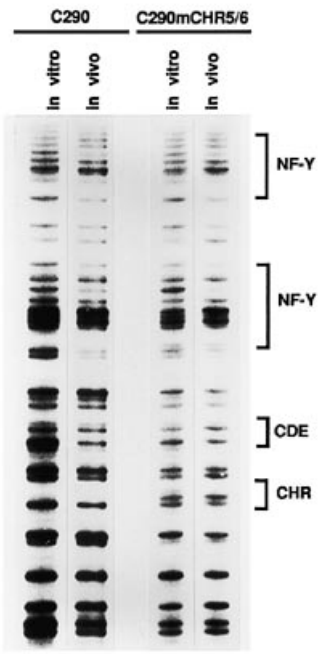

Figure 2. Detection of major groove contacts *in vivo* by genomic DMS footprinting in the *cdc25C* wild-type C290 construct and the CHR mutant C290-mCHR5/6 stably transfected into NIH 3T3 cells. Protection of two constitutively occupied NF-Y binding sites and the CDE are indicated. Note the loss of protection of the CDE in the case of C290-mCHR5/6, indicating cooperative binding to the CDE and CHR *in vivo*.

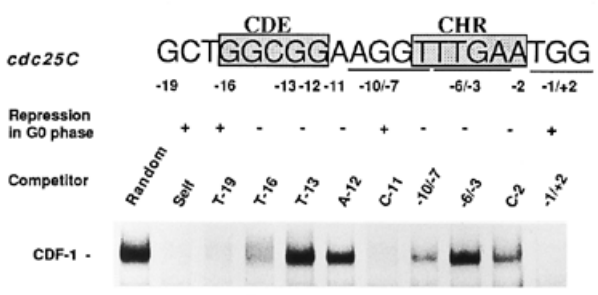

Figure 3. Ability of *cdc25C* CDE–CHR mutants to confer cell cycle regulation and to bind CDF-1. Functional data were obtained by transient transfection of luciferase constructs and analyzing their activity in G_0 cells. +, ratio mutant:wild-type <2; –, ratio mutant:wild-type >3. Binding properties were analyzed by EMSA using HeLa nuclear extract and the probe shown at the top in the presence of the competitors indicated above the autoradiograph (numbers refer to the positions in the *cdc25C* promoter; letters indicate the substituted nucleotide; e.g. T–19 contains a T at position –19 instead of a G).

detectable effects on repressor function. The *cdc25C* CHR can thus be defined as the sequence TTTGAA.

In vivo CDE occupation is dependent on an intact CHR

Previous data have clearly shown that the CDE and CHR in different promoters function in a synergistic way, since mutations in either element destroy repression in G_0. This could mean that the interacting factor(s) binds cooperatively to both elements. Obviously, this information is of great importance for the biochemical identification of such a factor(s). We therefore sought to clarify this question by genomic footprinting of a stably transfected NIH 3T3 cell line carrying a *cdc25C* promoter construct with an inactivating mutation in the CHR (*cdc25C*-mCHR5/6, TTTGAA changed to TagGAA). Figure 2 shows that the expected protection pattern was observed in a control line stably expressing a wild-type *cdc25C* promoter construct. In contrast, the cell line harboring the *cdc25C* promoter with the CHR mutation did not show any protection in the region of the CDE and the mutated CHR, while occupation of two constitutive upstream binding sites for NF-Y was unchanged in the mutant promoter. We therefore conclude that CDE occupation is dependent on an intact CHR, indicating cooperative binding *in vivo*. This conclusion is supported by the observation that insertion of either 5 or 10 bp between the CDE and the CHR in the *cdc25C* promoter abrogates repression (data not shown).

Identification of CDF-1

Electrophoretic mobility shift analysis (EMSA) of HeLa cell nuclear extract led to the identification of an activity that interacts in a cooperative fashion with both the CDE and the CHR of the *cdc25C* promoter (Fig. 3). In addition, binding of this activity to mutant repressor elements strongly correlated with the functional properties of these elements. Thus mutants T–19, C–11 and –1/+2, which all exhibited a wild-type-like repressor function, showed the same ability to compete in the binding assay as the wild-type sequence (self-competition). In contrast, six other mutants in the CDE–CHR region exhibiting decreased or impaired repression in G_0 cells also showed a diminished ability to compete for binding. The observed cooperative binding taken together with the correlations established by the structure–function analysis are in perfect agreement with the expected properties of the CDE–CHR binding factor. We refer to this activity in the following as CDF-1.

Interestingly, CDF-1 was detectable in HeLa nuclear extracts only in the presence of NP-40 and deoxycholate (unpublished observations), which might point to the presence in these extracts of an inhibitory activity interacting with CDF-1 and interfering with its DNA binding. For this reason the subsequent DNA binding studies with HeLa nuclear extract were carried out in the presence of these detergents.

In vitro footprinting shows that CDF-1 contacts the CDE in the major groove and the CHR in the minor groove

In order to obtain additional evidence that CDF-1 is the activity interacting with the repressor elements *in vivo*, we analyzed the interaction of CDF-1 with DNA by DMS methylation protection footprinting *in vitro*. We have previously shown that *in vivo* the CDE is contacted in the major groove, while the CHR is occupied in the minor groove. A very similar result was obtained by *in vitro* footprinting of the upper strand. Figure 4 shows specific protection of the four G residues in the CDE, indicating major groove contacts, and of the two A residues in the CHR, indicating minor groove contacts. The mode of interaction between CDF-1

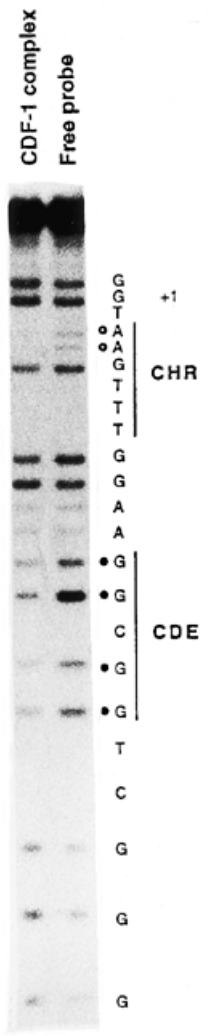

Figure 4. *In vitro* methylation protection footprinting of CDF-1 and free *cdc25C* probe after separation by EMSA. Open circles indicate minor groove contacts of adenines (N-3) within the CHR, filled circles indicate major groove contacts of guanines (N-7) in the CDE.

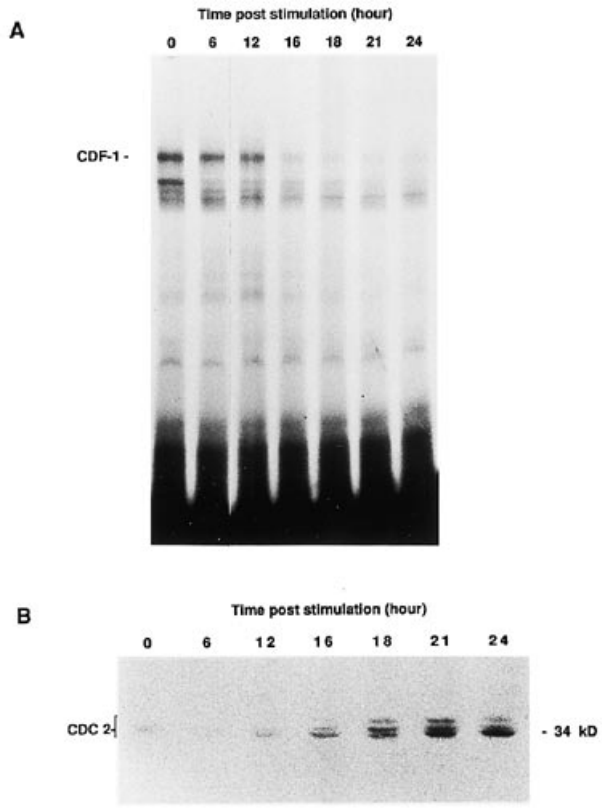

Figure 5. Cell cycle regulation of CDF-1. (**A**) Nuclear extracts from quiescent and serum-stimulated NIH 3T3 cells at different times after stimulation were analyzed by EMSA in the absence of detergents. The CDF-1 complex was identified by virtue of its co-migration with the HeLa complex and its lack of binding to *cdc25C* probes mutated in the CDE or CHR (not shown). The two bands below CDF-1 are NIH 3T3 specific, but also seem to be cell cycle regulated. The other bands are non-specific. (**B**) The same extracts as in (A) were analyzed for CDC2 expression by immunoblotting.

and the CDE–CHR *in vitro* is thus fully compatible with the observations made *in vivo*.

CDF-1 interaction with the CDE–CHR is cell cycle regulated

We next sought to establish that the activity identified above shows the expected pattern of cell cycle-regulated DNA binding. For this investigation we had to switch to NIH 3T3 cells, since the cell cycle in HeLa cells is deregulated. NIH 3T3 cells were serum deprived for 48 h, restimulated with 10% FCS and nuclear extracts were analyzed by EMSA using the *cdc25C* CDE–CHR probe in the absence of detergents. The data in Figure 5A clearly show CDF-1 activity (identified by its co-migration with HeLa CDF-1) in quiescent cells and at 6 and 12 h post-stimulation. At later time points between 16 and 24 h, however, CDF-1 binding activity was greatly diminished. This pattern was inversely correlated with expression of CDC2 (Fig. 5B), whose transcription is controlled by a CDE–CHR repressor module. Thus the kinetics of CDF-1 binding to DNA are in perfect agreement with its presumptive function as a cell cycle-regulated repressor.

CDF-1 interaction with multiple promoters containing CDE–CHR modules

Our previous studies have shown that functional CDE–CHR modules are present in different promoters, including *cdc25C*, *cdc2* and *cyclin A*. In addition, a similar configuration of binding sites is found in the B-*myb* promoter, where an E2F site with a sequence similar to the *cdc25C* CDE is located immediately upstream of a CHR-like element. It was therefore of obvious interest to investigate whether the CDF-1 activity identified above would interact with the repressor sites in these promoters. The experiment shown in Figure 5A provides a clear answer to this question. Both CDE–CHR-containing promoters, i.e. *cdc2* and *cyclin A*, bind the CDF-1 activity with a similar efficiency as the *cdc25C* promoter. In all three cases binding was dependent on cooperative binding to both the CDE and CHR, since mutation in either site impaired competition with the *cdc25C* probe. At an identical ratio of probe to competitor (1:20) competition by the B-*myb* promoter E2FBS–CHR module was insignificant (Fig. 6),

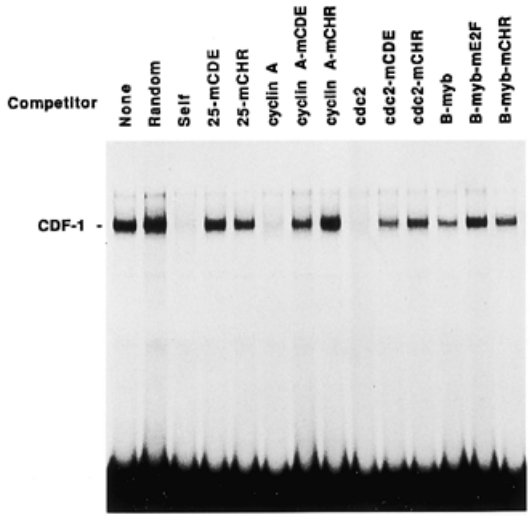

Figure 6. Interaction of CDF-1 with different promoters. EMSA was carried out with nuclear extract and the *cdc25C* CDE–CHR as probe in the presence of various competitors, including the *cdc25C* CDE–CHR (self-competition), the *cyclin A* CDE–CHR, the *cdc2* CDE–CHR and the B-*myb* E2FBS–CHR. Each competitor was used as the wild-type sequence and with inactivating mutations in the CDE, E2FBS or CHR (see Materials and Methods for details). The ratio of probe to competitor was 1:20 in each case. Random, irrelevant oligonucleotide competitor.

although some competition could be seen at higher competitor concentrations (data not shown). The fact that the CDF-1 activity shows a strong and selective interaction with all three CDE–CHR-containing promoters provides additional evidence for the relevance of the activity identified in the present study.

CDF-1 does not contain known E2F family members

In view of the similarity of the CDE with an E2FBS we sought to investigate whether the CDE–CHR binding activity identified above might contain known E2F or DP family members. For this purpose, EMSA was performed in the presence of antibodies directed against specific DP and E2F proteins. All of these antibodies have been shown to either induce supershifts or extinguish binding in different settings (17; unpublished observations). However, the results depicted in Figure 7 clearly show that none of the antibodies used affected complex formation, indicating that CDF-1 does not contain any of the known E2F or DP family members. This finding also implies that the *cdc25C* CDE–CHR sequence does not interact with E2F complexes present in HeLa nuclear extracts. In agreement with this conclusion we could not detect binding of recombinant GST–E2F or GST–DP proteins in any combination of the eight known family members to the *cdc25C* CDE–CHR sequence, whereas strong binding to the B-*myb* E2FBS was observed under the same conditions (K.Engeland and N.Liu, unpublished observations).

CDF-1 does not co-fractionate with E2F and pocket proteins

We finally asked the question whether CDF-1 might associate with, and thus be regulated by, pocket proteins of the pRb family. For this purpose we separated the nuclear protein (complexes)

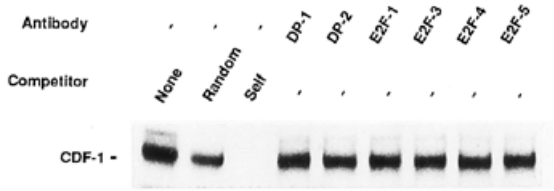

Figure 7. Effect of DP/E2F family-specific antibodies on CDF-1–DNA complex formation. The EMSA reactions were performed with HeLa nuclear extract in the presence or absence of different antibodies previously shown to be able to supershift or extinguish E2F complexes (see text).

from HeLa cells under native conditions by glycerol gradient centrifugation. Fractions were analyzed by EMSA for both CDF-1 and E2F binding activities (in the presence of detergent, which disrupts higher order complexes) and by immunoblotting for the presence of pocket proteins. The data in Figure 8 show that E2F binding activity was found in fractions 2–10, while pocket proteins (pRb, p107 and p130) were present in fractions 4–10, but not in fraction 2. Thus fraction 2 contains 'free' E2F, while fractions 4–10 contain the higher order E2F–pocket protein complexes. Strikingly, CDF-1 was detectable in fraction 2 and was not found in fractions 4–10, which contained the pocket proteins. This finding suggests that these proteins may not form complexes with the bulk of CDF-1.

DISCUSSION

The major goal of the present study was to identify the *cdc25C* CDE–CHR binding activity. Using specific EMSA conditions we were able to identify an activity (CDF-1) that fulfils the criteria expected of a CDE–CHR binding repressor. First, CDF-1 interacts in a cooperative fashion with the CDE and the CHR in

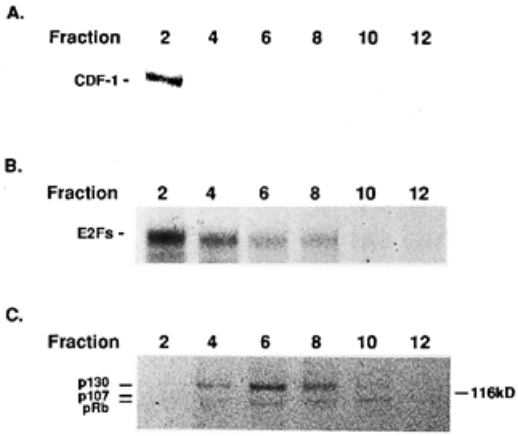

Figure 8. Glycerol gradient centrifugation of nuclear HeLa cell extract under native conditions. (**A**) EMSA using the *cdc25C* CDE–CHR probe to detect CDF-1 binding activity. (**B**) EMSA using the B-*myb* E2F binding site as probe to detect E2F binding activity. The EMSA was performed in the presence of detergent which disrupts higher order complexes. (**C**) Immunoblot of the same fractions using a mixture of antibodies against pRb, p107 and p130.

the *cdc25C* promoter (Fig. 3), which is in agreement with the CHR-dependent occupation of the CDE seen *in vivo* (Fig. 2). Second, CDF-1 interacts with G residues in the CDE (major groove) and with A residues in the CHR (minor groove) (Fig. 4). This protection pattern is identical to that found by *in vivo* footprinting. Third, the binding of CDF-1 to sequences containing mutated CDE or CHR motifs correlates precisely with the function of such mutated elements in cell cycle-regulated repression (Fig. 3). Fourth, the DNA binding activity of CDF-1 is clearly cell cycle regulated and is inversely correlated with expression of the *CDC2* gene, whose transcription is controlled by a CDE–CHR repressor module (Fig. 5). Fifth, CDF-1 binds with similar efficiency to all known CDE–CHR-regulated promoters, i.e. *cdc25C*, *cdc2* and *cyclin A*, but only weakly to the B-*myb* E2F site (Fig. 6). In each case this binding was dependent on the presence of intact versions of the CDE and CHR. Taken together these findings are compelling evidence that CDF-1 represents the CDE–CHR interacting repressor.

It is now well established that transcriptional repression plays a major role in control of cell cycle genes. One of the factors implicated in cell cycle-regulated repression is E2F, which can form DNA binding repressor complexes through its interaction with pocket proteins, such as pRb. Pocket proteins are endowed with repressor domains which are able to interact with and inhibit other promoter-bound transcription factors and thereby down-modulate transcription. Among the promoters controlled by transcriptional repression through E2F sites are *E2F*-1, *orc*-1 and B-*myb*. However, the mechanism of B-*myb* gene repression deviates from all models proposed for the action of E2F in that it requires a second element located directly downstream of the E2F site, termed the B-*myb* CHR or DRS. This observation is very similar to those made with other promoters, such as *cdc25C*, *cdc2* and *cyclin A*, which are periodically repressed through two cooperating elements, the CDE and the adjacent CHR. This raises the possibility that similar factors might be involved in repression of B-*myb* and the CDE–CHR-regulated promoters, such as *cdc25C*. In this study we present clear evidence that this is not the case: E2F complexes do not interact with the *cdc25C* promoter (Fig. 7), while CDF-1 does not bind the B-*myb* promoter under conditions where a strong interaction is seen with the *cdc25C*, *cdc2* and *cyclin A* promoters (Fig. 6). This conclusion is supported by our observation (not shown) that recombinant E2F complexes bind avidly to the B-*myb* E2F site but not at all to the *cdc25C* promoter. Interestingly, the E2F-repressed B-*myb* gene is up-regulated around mid G_1, while the CDF-1-controlled genes *cdc25C*, *cdc2* and *cyclin A* become derepressed later. These data strongly suggest that E2F and CDF-1 exert phase-specific roles in the repression of genes during the cell cycle.

E2F and CDF-1 not only differ in their target specificity but apparently also with respect to their regulation during the cell cycle. It is now well established that the function of E2F as a transcriptional repressor is dependent on its association with pocket proteins, which seem to endow the complex with an active repressor domain. In contrast, our results suggest that CDF-1 does not form complexes with pocket proteins. Thus pocket proteins co-migrated with a fraction of E2F but not with CDF-1 on native glycerol gradients (Fig. 8). This conclusion is supported by the observation that the CDF-1 complex seen in EMSA experiments with NIH 3T3 nuclear extracts (Fig. 5A) showed an unchanged mobility in the presence of detergents which disrupt higher order E2F complexes (data not shown). It cannot, however, be excluded that a minor fraction of CDF-1, below the detection limit of the experiments in Figures 5A and 8, forms complexes with pocket proteins, even though the physiological relevance of such a situation could not easily be explained. Our observations therefore suggest that the bulk of CDF-1 activity is regulated by other mechanisms, perhaps phosphorylation or interaction with another factor(s) blocking its DNA binding domain. The fact that CDF-1 is detectable in HeLa cell extract only in the presence of detergent (see Results) may point to the existence of such a putative inhibitory factor. These questions cannot be clarified at present, but have to await the purification and cloning of CDF-1. The present study should provide the basis for this future work.

ACKNOWLEDGEMENTS

We are grateful to J.Kaufmann for advice, to R.Treisman for NIH 3T3 cells, to Dr M.Krause for synthesis of oligonucleotides and to C.Schalk for FACS analyses. This work was supported by grants from the DFG (SFB397 and Mu601/9) and the BMBF. K.K. was the recipient of a fellowship from the Graduiertenkolleg ‘Zell- und Tumorbiologie’.

REFERENCES

1 Helin,K. and Harlow,E. (1993) *Trends Cell Biol.*, **3**, 43–46.
2 Müller,R. (1995) *Trends Genet.*, **11**, 173–178.
3 Zwicker,J. and Müller,R. (1997) *Trends Genet.*, **13**, 3–6.
4 La Thangue,N.B. (1996) *Biochem. Soc. Trans.*, **24**, 54–59.
5 Weintraub,S.J., Chow,K.N., Luo,R.X., Zhang,S.H., He,S. and Dean,D.C. (1995) *Nature*, **375**, 812–815.
6 Weinberg,R.A. (1995) *Cell*, **81**, 323–330.
7 Lam,E.W. and Watson,R.J. (1993) *EMBO J.*, **12**, 2705–2713.
8 Hsiao,K.M., McMahon,S.L. and Farnham,P.J. (1994) *Genes Dev.*, **8424**, 1526–1537.
9 Johnson,D.G., Ohtani,K. and Nevins,J.R. (1994) *Genes Dev.*, **8424**, 1514–1525.
10 Zhu,L., Xie,E. and Chang,L.-S. (1995) *Mol. Cell. Biol.*, **15**, 3552–3562.
11 Ohtani,K., DeGregori,J., Leone,G., Herendeen,D.R., Kelly,T. and Nevins,J.R. (1996) *Mol. Cell. Biol.*, **16**, 6977–6984.
12 Lucibello,F.C., Truss,M., Zwicker,J., Ehlert,F., Beato,M. and Müller,R. (1995) *EMBO J.*, **14**, 132–142.
13 Zwicker,J., Lucibello,F.C., Wolfraim,L.A., Gross,C., Truss,M., Engeland,K. and MYller,R. (1995) *EMBO J.*, **14**, 4514–4522.
14 Huet,X., Rech,J., Plet,A., Vié,A. and Blanchard,J.M. (1996) *Mol. Cell. Biol.*, **16**, 3789–3798.
15 Shelby,R.D., Vafa,O. and Sullivan,K.F. (1997) *J. Cell. Biol.*, **136**, 501–513.
16 Uchiumi,T., Longo,D.L. and Ferris,D.K. (1997) *J. Biol. Chem.*, **272**, 9166–9174.
17 Liu,N., Lucibello,F.C., Zwicker,J., Engeland,K. and Müller,R. (1996) *Nucleic Acids Res.*, **24**, 2905–2910.
18 Good,L. and Nazar,R. (1992) *Nucleic Acids Res.*, **20**, 4934.
19 Zwicker,J., Gross,C., Lucibello,F.C., Truss,M., Ehlert,F., Engeland,K. and Müller,R. (1995) *Nucleic Acids Res.*, **23**, 3822–3830.
20 Klehr,D., Maass,K. and Bode,J. (1991) *Biochemistry*, **30**, 1264–1270.
21 Pfeifer,G.P., Steigerwald,S., Mueller,P.R. and Riggs,A.D. (1989) *Science*, **246**, 810–813.
22 Bennett,J.D., Farlie,P.G. and Watson,R.J. (1996) *Oncogene*, **13**, 1073–1082.
23 Zwicker,J., Liu,N., Engeland,K., Lucibello,F.C. and MYller,R. (1996) *Science*, **271**, 1595–1597.
24 Weintraub,S.J., Prater,C.A. and Dean,D.C. (1992) *Nature*, **358**, 259–261.
25 Zamanian,M. and La Thangue,N.B. (1993) *Mol. Biol. Cell*, **4**, 389–396.
26 Sellers,W.R., Rodgers,J.W. and Kaelin,W.G. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 11544–11548.