

Cell cycle-regulated repression of *B-myb* transcription: cooperation of an E2F site with a contiguous corepressor element

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ABSTRACT

B-myb belongs to a group of cell cycle genes whose transcription is repressed in G₀/early G₁ through a binding site for the transcription factor E2F. Here, we show that the *B-myb* repressor element is specifically recognised by heterodimers consisting of DP-1 and E2F-1, E2F-3 or E2F-4. Surprisingly, E2F-mediated repression is dependent on a contiguous corepressor element that resembles the CHR previously established as a corepressor of the CDE in cell cycle genes derepressed in S/G₂, such as *cyclin A*, *cdc2* and *cdc25C*. A factor binding to the *B-myb* CHR was identified in fractionated HeLa nuclear extract and found to interact with the minor groove, as previously shown by *in vivo* footprinting for the *cyclin A* CHR. The *B-myb* and *cdc25C* CHRs are related with respect to protein binding but are functionally clearly distinct. Our results support a model where both E2F- and CDE-mediated repression, acting at different stages in the cell cycle, are dependent on promoter-specific CHR elements.

INTRODUCTION

In mammalian cells, a specific set of cell cycle genes transcribed around the G₁/S border is regulated by factors of the E2F/DP family (for reviews see refs 1–3). The heterodimeric E2F/DP transcription factors frequently act as repressors in G₀/early G₁ owing to their association with pocket proteins of the pRb family. In late G₁, the pocket proteins become hyperphosphorylated and dissociate from the complex with E2F/DP, leading to the derepression of E2F-regulated genes. Several genes expressed in late G₁/early S, including *B-myb* (4,5), DHFR (6) and *E2F-1* (7,8), have been shown to be repressed through an E2F-mediated mechanism in G₀/G₁ and to be derepressed in late G₁. Although a plethora of E2F, DP and pocket protein family members has been identified, their precise role in the regulation of specific genes remains elusive (for reviews see refs 2,3).

Transcription of the *B-myb* gene in mouse fibroblasts greatly increases in mid-G₁ and reaches peak levels in S-phase (4). Structure–function analysis of the *B-myb* promoter identified an element close to the transcription start sites necessary for cell

cycle regulation (4). This element (CTTGGCGG) represents an E2F site, as shown by protein binding using cell extracts and recombinant proteins (9). Mutation of this E2F site leads to an up-regulation of transcription in G₀ cells (4), indicating that the interacting protein complex acts as a repressor. *In vitro* experiments with cell extracts suggested that the G₀ complex contains the p107 pocket protein (5). In contrast, free E2F is found in cell extracts throughout the cell cycle, and other higher order DNA-binding complexes are detected around S-phase entry (5). The function of the late G₁/S-phase complexes, however, remains unclear, because genomic footprinting of the *B-myb* promoter failed to show any protection of the E2F site later than mid-G₁ (9).

Cyclin A, *cdc2* and *cdc25C* exemplify a group of cell cycle genes whose transcription is up-regulated later than that of *B-myb*, i.e. in S-phase (*cyclin A*, *cdc2*) and G₂ (*cdc25C*) (for a review see 3). For all three promoters, repression of upstream activators via the ‘cell cycle-dependent element’ (CDE) has been established as the major regulatory mechanism (10–12). In addition, repression of the *cyclin A*, *cdc2* and *cdc25C* promoters is also dependent on a contiguous element, termed ‘cell cycle genes homology region’ (CHR) (12). As shown by genomic footprinting, both elements are bound by the repressor proteins in a periodic fashion, the CDE in the major groove and the CHR in the minor groove (12). The nature of the proteins interacting with the CDE and CHR elements remains at present unknown.

In the course of our studies, we noted a significant homology between the CHR in the *cyclin A*, *cdc2* and *cdc25C* and the region immediately downstream from the E2F site in the *B-myb* promoter, raising the question as to whether E2F-mediated repression might also be dependent on a CHR-like downstream element. In this manuscript, we show that this is indeed the case and identify a nuclear activity interacting with the *B-myb* CHR in the minor groove. We also show that this activity is related to but distinct from the factors interacting with the CHR of *cdc25C*, indicating that both E2F- and CDE-mediated repression is dependent on promoter-specific corepressors.

MATERIALS AND METHODS

Cell culture, DNA transfection and luciferase assays

NIH3T3 cells were kindly provided by R. Treisman (ICRF, London) and cultured in Dulbecco-Vogt modified Eagle’s

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medium (DMEM) supplemented with 10% fetal calf serum (FCS). HeLa cells were maintained in a medium containing DMEM and 10% calf serum. For synchronisation in G₀, NIH3T3 cells were maintained in serum free medium for 2 days. NIH3T3 cells were transfected by the DEAE dextran technique and determination of luciferase activity was performed as described (10). A SV40 promoter reporter construct was used to standardise the results.

Luciferase constructs

The *B-myb* constructs spanned the region from -301 to +100 relative to the major transcription start site of the mouse *B-myb* gene (4). The *cdc25C* constructs spanned the region from -290 to +121 (10). The promoter fragments were generated by PCR with compatible ends for cloning into the pXP2 luciferase vector (13). Mutations were introduced by PCR strategies as previously described (12). All PCR-amplified fragments were verified by DNA sequencing using the dideoxynucleotide chain-termination method (14) using Sequenase (USB). Ambiguous sequences and GC-rich stretches were verified by 'cycle sequencing' using *Tth* polymerase (Pharmacia).

EMSA

Nuclear extract (4 µg) or MonoQ fractions (0.5 µg) were incubated in 12 µl of a 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. ³²P-labelled probe (0.2 pmol) was added and the reaction mixture was incubated for another 20 min. All reactions were performed on ice. Probes were labelled by filling-in 5' overhanging ends of 4-7 bases. Samples were run on 4% non-denaturing polyacrylamide gels in 0.5× TBE at 4°C and 10 V/cm. Gels were exposed to X-ray films and quantitatively evaluated using a Molecular Dynamics PhosphorImager. The following double-stranded probes were used:

B-myb: 5'-GGCGCCGACGCACTTGGCGGGAGATAGGAA-GTTCTGTG, E2F site and CHR underlined. Mutations are indicated in the corresponding figures.

Cyclin A: 5'-TCAATAGTCGCGGGATACTTGAAGTCAAG, CDE and CHR underlined.

Cdc25C: 5'-ACTGGGCTGGCGGAAGGTTTGAATGGTCAA, CDE and CHR underlined.

The following antibodies were used: E2F-1 (Santa Cruz SC-251X), E2F-1/C (Santa Cruz SC-193X), E2F-2 (Santa Cruz SC-632X), E2F-3 (Santa Cruz SC-879X), E2F-4 (Santa Cruz SC-512X; also kindly provided by R. Bernards, Amsterdam),

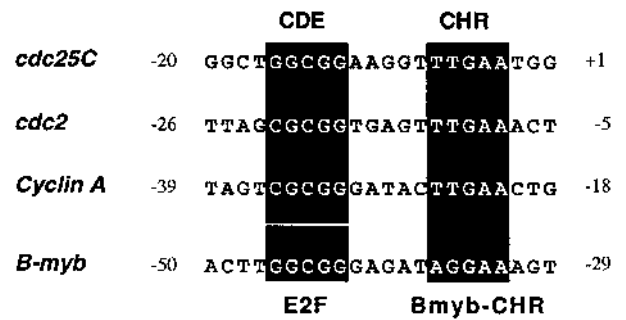


Figure 1. Alignment of *cdc25C*, *cdc2*, *cyclin A* and *B-myb* promoter sequences in the regions of the CDEs, CHRs and E2F site. Core sequences are highlighted by black boxes.

E2F-5 (Santa Cruz SC-999X; also kindly provided by N. La Thangue, Glasgow), DP-1 (obtained from N. La Thangue), DP-2 (Santa Cruz SC-830X), DP-3 (kindly provided by N. La Thangue). DP-2 and DP-3 antibodies are directed against homologous proteins (DP-2 is the human homologue of mouse DP-3; 15,16).

Fractionation of HeLa nuclear extract

Nuclear extracts were prepared from HeLa suspension cultures as described including protease inhibitors leupeptin, pepstatin A and aprotinin but omitting the dialysis step at the end of the procedure (17). Extract was diluted 10-fold with buffer A [50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 5% (v/v) glycerol, 0.5 mM DTT], centrifuged 10 min in a TLA 45 rotor at 45 000 r.p.m. and 4°C. The sample was loaded on a 1 ml Mono Q column equilibrated with buffer A and run with a flow rate of 1 ml/min at room temperature. Protein was eluted with a gradient of up to 1 M KCl in buffer A and monitored at 280 nm. Fractions of 1 ml were collected as soon as proteins appeared in the eluate.

In vitro DMS footprinting

For *in vitro* DMS footprinting the coding strand oligonucleotide was end-labelled, purified and annealed to the non-coding strand. Binding reactions were carried out as described above. Two microliters of 2% DMS was added, and the methylation reaction was stopped 3 min later by adding 2 µl of 60 mM β-mercaptoethanol. The samples were run on a 4% gel and transferred to ion-exchange paper. Both the shifted and the unshifted (free probe) bands were cut out, rinsed with TE buffer and eluted with TE buffer containing 1.5 M NaCl at 65°C. The eluted DNA was extracted with chloroform, precipitated and dissolved in water. Equal

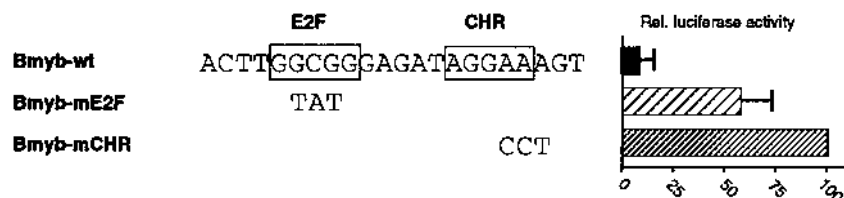


Figure 2. Activity in quiescent NIH3T3 cells of a wild-type *B-myb* promoter-luciferase construct, and constructs with mutations in either the E2F site (*Bmyb-mE2F*) or the CHR (*Bmyb-mCHR*). Values were normalised to the highest value (*Bmyb-mCHR*; relative activity 100 corresponding to 2.8×10^3 RLU). Values represent averages (\pm s.d.) from three experiments.

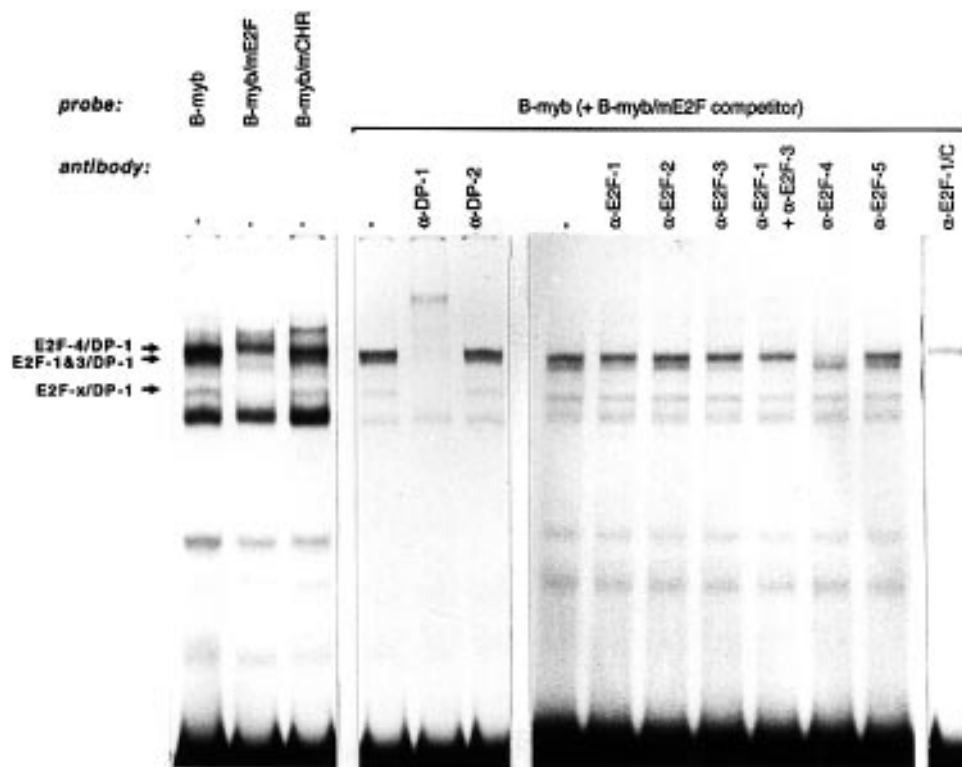


Figure 3. E2F/DP complexes of HeLa nuclear proteins with an oligonucleotide encompassing the *B-myb* E2F site and CHR, or probes with mutations in either site (as in Fig. 2) detected by EMSA. The right panel shows antibody supershift and extinction of specific complexes by anti-DP-1, anti-E2F-1, anti-E2F-3 and anti-E2F-4 antibodies. The α -E2F-1/C antibody is directed against the C-terminus of E2F-1 and cross-reacts with other E2F family members including E2F-3. Specific bands are indicated by arrows. The E2F site mutation gave rise to an unspecific band just above the location of the E2F-4/DP-1 complex. The antibody experiment was performed in the presence of a *B-myb* competitor oligonucleotide with a mutation in the CHR to reduce the background.

radioactive amounts of free probe and shifted complex were cleaved with 10% piperidine at 95°C for 30 min. The DNA was precipitated and loaded on a 15% denaturing acrylamide gel.

RESULTS

The *B-myb* promoter is regulated by E2F and a CHR-binding corepressor

The alignment of the proximal *B-myb* promoter with other cell cycle-regulated genes showed that the sequence 5 nt downstream of the E2F site (TAGGAA) closely resembles the CHR in *cyclin A*, *cdc2* and *cdc25C* (T/CTTGAA) found 5 nt downstream of the CDE in the latter genes (Fig. 1). This observation raised the possibility that an element similar to the CHR might also be involved in *B-myb* regulation and thus play a role in E2F-mediated transcriptional repression. We therefore investigated the role of the putative *B-myb* CHR in a functional assay by analysing the effect of a CHR mutation on the repression of the *B-myb* promoter in quiescent NIH3T3 cells. As shown in Figure 2, mutation of the CHR led to an ~10-fold increased activity in G₀ cells, and thus had an even stronger effect than the mutation of the E2F site (~6-fold increase in G₀) previously described to be involved in *B-myb* repression (4). Both mutations had only small effects in normally cycling cells (~2-fold; data not shown). These data demonstrate that the E2F site and a downstream located element resembling a CHR cooperate in the repression of the *B-myb* promoter in quiescent cells.

Interaction of E2F and DP family members with the *B-myb* promoter

In order to investigate the role of the CHR in further detail, we first asked which proteins interact with the *B-myb* E2F site and whether such interactions might be dependent on the presence of an intact CHR. The data in Figure 3 show that four specific complexes could be identified with HeLa nuclear extract (see labelling at the left margin). The formation of these complexes was totally abolished by a mutation in the E2F site, but not affected by the CHR mutation. All complexes contained DP-1 as shown by the complete supershift caused by a DP-1 specific antibody, while DP-2 (the human homologue of mouse DP-3; 15,16) could not be detected in any of the complexes. The slowest migrating complex was specifically extinct by an antibody against E2F-4 while the slightly faster migrating band represented two complexes containing E2F-1 or E2F-3. This is indicated by the fact that both the E2F-1 and the E2F-3 specific antibody alone led only to a partial extinction of this band, while the combination of both led to complete extinction. Furthermore, an antibody against the C-terminus of E2F-1 but cross-reacting with E2F-3 (α -E2F-1/C) also completely abrogated formation of this band. A fourth minor complex of faster mobility was identified, but none of the antibodies directed against the five known E2F family members affected this complex. Since this complex was extinct by the α -E2F-1/C antibody, it is likely that it also contains an E2F protein, either a novel family member or an unidentified variant of the known E2F proteins. Taken

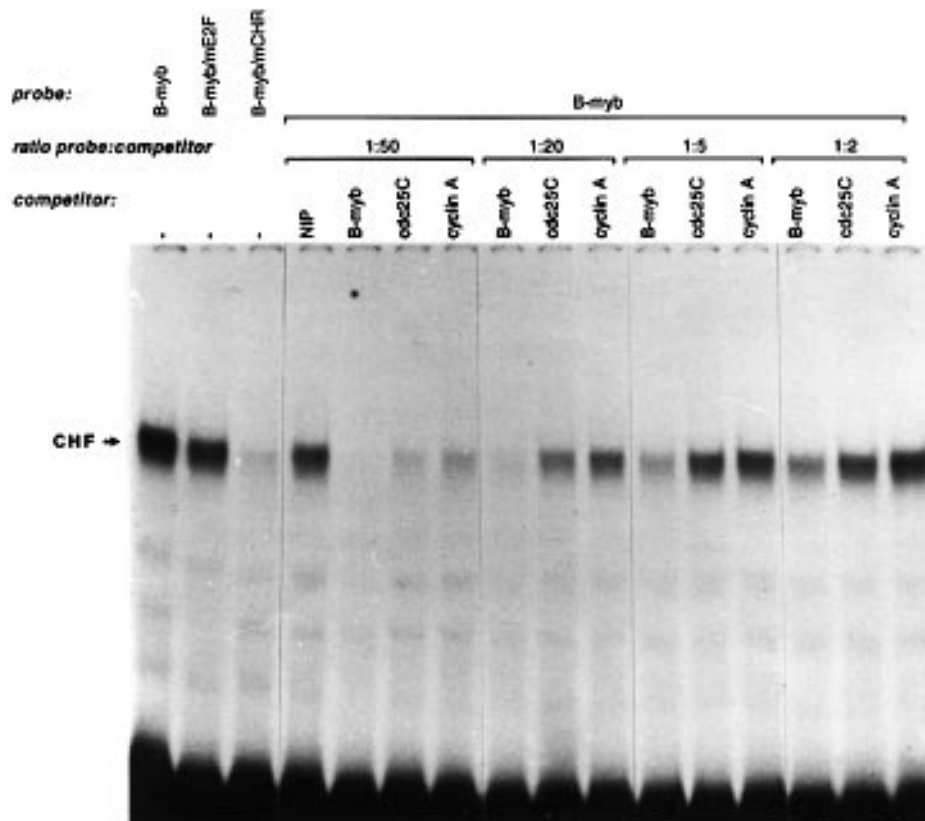


Figure 4. Detection by EMSA of an activity ("CHF") in Mono-Q fractionated HeLa nuclear extract interacting with the *B-myb* CHR. The probes were the same as those used in Figure 3. In addition, *cdc25C* and *cyclin A* competitors at different molar ratios were used to assess the affinity of the *B-myb* CHR-interacting factor for CHR elements in other promoters. NIP: unrelated oligonucleotide.

together, our results indicate that the *B-myb* promoter E2F site interacts mainly with E2F-1/DP-1, E2F-3/DP-1 and E2F-4/DP-1 complexes, and that the formation of these complexes occurs independently of the CHR.

Identification of a minor groove-binding factor interacting with the *B-myb* CHR

We next sought to obtain direct experimental evidence that the *B-myb* CHR indeed represents a protein binding site, and to investigate whether the adjacent E2F site might play a role in such interactions. To address this question we attempted to identify a *B-myb* CHR-binding activity in nuclear or whole cell extracts from different cell lines, but all attempts invariably failed (data not shown; see also Fig. 3). It is a well known fact that certain transcription factors are detectable by EMSA only after enrichment or partial purification from nuclear extracts, which prompted us to analyse fractions of HeLa nuclear extract obtained after MONO-Q FPLC. This attempt proved successful: fractions 8 and 9 (see Materials and Methods) contained an activity that bound to the *B-myb* promoter probe in a CHR-dependent, but E2F site-independent manner (Fig. 4; three left-most lanes). In addition, *in vitro* methylation protection footprinting of this activity showed a clear protection of two adenine residues within the *B-myb* CHR (Fig. 5), and hypermethylation of a third adenine located immediately downstream. This altered reactivity of the N3 position in adenine residues clearly indicates minor groove

protein interaction. This observation is therefore in perfect agreement with previous *in vivo* experiments demonstrating minor groove protection of the *cyclin A* CHR (12). In contrast, as expected, no protection of the E2F site was observed.

We also tested the potential interaction of the binding activity identified above with the *cdc25C* and *cyclin A* promoter by using appropriate promoter fragments at different molar ratios as competitors. The results presented in Figure 3 clearly show that the highest affinity was seen with the *B-myb* probe. *cdc25C* and *cyclin A* were also able to compete, but only at higher concentrations, which is clearly seen at a molar ratio of probe over competitor of 1:20. In contrast, an unrelated oligonucleotide competitor (NIP; 18) had no effect on complex formation.

The *B-myb* and *cdc25C* CHRs are functionally different

The results of the competition experiment described above suggest that the factor interacting with the *B-myb* CHR may be different from those binding to the *cdc25C* and *cyclin A* promoters. To test this hypothesis by a functional approach we constructed a *cdc25C* promoter molecule harbouring the *B-myb* CHR (Cdc25C-BmybCHR; Fig. 6). This construct showed an ~8-fold increase in activity when tested in quiescent NIH3T3 cells as compared with the wild-type *cdc25C* promoter (Fig. 3), and thus had a very similar effect as the replacement of the CHR or the CDE with an irrelevant sequence (data not shown; 10,12). In contrast, the increase in luciferase activity in normally growing

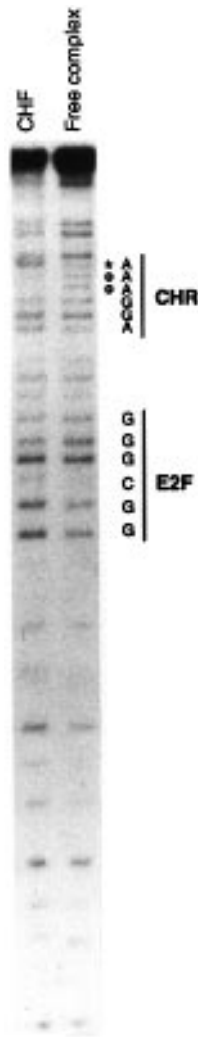


Figure 5. *In vitro* methylation protection footprinting of the CHR complex shown in Figure 4. Free probe: sample taken from the uncomplexed probe running at the bottom of Figure 4. A clear minor groove protection can be seen in the region of the CHR (two A residues marked by open circles), while the E2F site remains unprotected. In addition, another adenine in the CHR was found to be hypermethylated (indicated by an asterisk).

cells was only ~2-fold (data not shown), indicating selective deregulation of cell cycle-regulated transcription in G₀ cells. Based on these results we conclude that the *cdc25C* and *B-myb* CHRs are functionally not equivalent.

DISCUSSION

Even though the function of E2F as a transcriptional activator is now well established, there is a growing body of evidence pointing to a crucial role for E2F complexes in cell cycle-regulated transcriptional repression (for a review see 3). It is generally believed that E2F-mediated repression is a consequence of the association of E2F/DP heterodimers with pocket proteins (pRB, p107, p130) (19–28). This association not only blocks the activation function of E2F but also converts it to an active DNA-bound repressor. The pocket protein component is thought to establish physical contacts with other transcription factors bound to the promoter to be repressed, such as upstream activators, thereby blocking their function in establishing an active transcription complex. At least for pRB there is experimental evidence supporting this hypothesis (29,30), although other mechanisms may also apply (31). While it is clear from studies with artificial promoters that E2F-binding suffices to activate transcription, nothing is known about the sequences or elements required for transcriptional repression.

In the present study, we have used the *B-myb* promoter to address this question. This investigation was fostered by our observation that the region immediately downstream of the E2F site resembles a similarly located element previously shown to play an essential role in the CDE-mediated repression of *cyclin A*, *cdc2* and *cdc25C* (Fig. 1). As shown by the mutational analysis in Figure 2, our notion that this element, referred to as *B-myb* CHR, is functionally relevant was fully confirmed. Destruction of the *B-myb* CHR leads to deregulation in quiescent cells, as does the mutation of the E2F site itself. The *B-myb* CHR thus represents the first element identified to date that synergises with E2F in the establishment of transcriptional repression.

Protein binding studies showed that both the E2F site and the *B-myb* CHR are able to bind specific nuclear proteins, and that these interactions occur in a mutually independent fashion. Thus, E2F-1/DP-1, E2F-3/DP-1 and E2F-4/DP-1 major groove complexes are formed with the *B-myb* E2F site in the absence of an intact CHR, and a nuclear factor recognising the CHR in the minor groove was not dependent on the E2F site for DNA-binding. It thus appears that the cooperation of the two elements must occur at a level other than DNA-binding. It is possible that both factors synergise in the establishment of appropriate contacts with other promoter-bound transcription factors, perhaps by inducing a favourable DNA topology (as is often seen with minor groove-binding proteins; see e.g., 32). The answer to this question certainly has to await the purification, cloning and functional analysis of the CHR-interacting factor(s). The identification of such a factor in the present study, as shown in the experiments in Figures 4 and 5, clearly represents an important step in this direction.

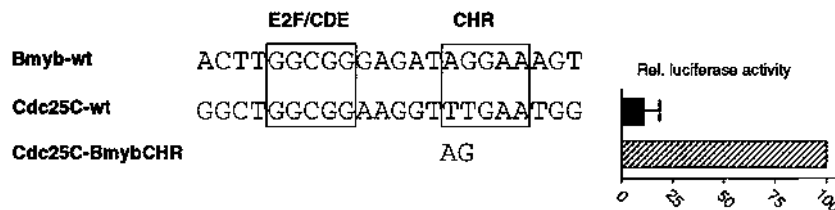


Figure 6. Activity in quiescent NIH3T3 cells of a wild-type *cdc25C* promoter-luciferase construct, and a chimaeric construct with Bmyb-CHR. Values were normalised to the highest value (*cdc25C*-BmybCHR; relative activity 100 corresponding to 3.2×10^3 RLU). Values represent averages (\pm s.d.) from three experiments.

The competition data in Figure 4, taken together with the functional analysis in Figure 6, suggest that different factors interact with the CHR in *B-myb* and *cdc25C*. In agreement with these results is the observation that the *cdc25C* CDE shows no interaction with E2F or DP family members, neither in nuclear extracts nor with recombinant proteins (N. Liu and K. Engeland, unpublished observation), while the *B-myb* E2F site does (9; Fig. 3). These data clearly suggest the formation of promoter-specific repressor complexes of E2F and CDF with different CHR-binding activities, and that it is the precise composition of these complexes that determines the timing of expression. This hypothesis is supported by our observation that the exchange of the region encompassing the E2F site and CHR in *B-myb* with the CDE-CHR module from *cdc25C* leads to a late induction of the *B-myb* promoter, similar to that of the wild-type *cdc25C* promoter (J. Zwicker and F.C. Lucibello, unpublished observation). Once CDF has been identified and its cDNA cloned, the questions relating to the mechanisms involved in the formation of promoter-specific complexes and their function in cell cycle-regulated repression can be addressed in detail, and these studies can be expected to unravel new mechanisms orchestrating the periodic expression of genes.

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REFERENCES

- 1 Nevins, J.R. (1992) *Science* **258**, 424–429.
- 2 La Thangue, N.B. (1994) *Curr. Opin. Cell Biol.* **6**, 443–450.
- 3 Müller, R. (1995) *Trends Genet.* **11**, 173–178.
- 4 Lam, E.W.-F. and Watson, R.J. (1993) *EMBO J.* **12**, 2705–2713.
- 5 Lam, E.W.-F., Morris, J.D.H., Davies, R., Crook, T., Watson, R.J. and Vousden, K.H. (1994) *EMBO J.* **13**, 871–878.
- 6 Means, A.L., Slansky, J.E., McMahon, S.L., Knuth, M.W. and Farnham, P.J. (1992) *Mol. Cell. Biol.* **12**, 1054–1063.
- 7 Hsiao, K.-M., McMahon, S.L. and Farnham, P.J. (1994) *Genes. Dev.* **8**, 1526–1537.
- 8 Johnson, D.G., Ohtani, K. and Nevins, J.R. (1994) *Genes Dev.* **8**, 1514–1525.
- 9 Zwicker, J., Liu, N., Engeland, K., Lucibello, F.C. and Müller, R. (1996) *Science* **271**, 1595–1597.
- 10 Lucibello, F.C., Truss, M., Zwicker, J., Ehlert, F., Beato, M. and Müller, R. (1995) *EMBO J.* **14**, 132–142.
- 11 Zwicker, J., Gross, C., Lucibello, F.C., Truss, M., Ehlert, F., Engeland, K. and Müller, R. (1995) *Nucleic Acids Res.* **23**, 3822–3830.
- 12 Zwicker, J., Lucibello, F.C., Wolfraim, L.A., Gross, C., Truss, M., Engeland, K. and Müller, R. (1995) *EMBO J.* **14**, 4514–4522.
- 13 Nordeen, S.K. (1988) *BioTechniques* **6**, 454–457.
- 14 Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
- 15 Girling, R., Bandara, L.R., Ormondroyd, E., Lam, E.W., Kotecha, S., Mohun, T. and La Thangue, N.B. (1994) *Mol. Biol. Cell* **5**, 1081–1092.
- 16 Wu, C.-L., Zukerberg, L.R., Ngwu, C., Harlow, E. and Lees, J.A. (1995) *Mol. Cell. Biol.* **15**, 2536–2546.
- 17 Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) *Nucleic Acids Res.* **11**, 1475–1489.
- 18 Engeland, K., Andrews, N.C. and Mathey-Prevot, B. (1995) *J. Biol. Chem.* **270**, 24572–24579.
- 19 Mudryj, M., Hiebert, S.W. and Nevins, J.R. (1990) *EMBO J.* **9**, 2179–2184.
- 20 Bandara, L.R., Adamczewski, J.P., Hunt, T. and La Thangue, N.B. (1991) *Nature* **352**, 249–251.
- 21 Chellappan, S.P., Hiebert, S., Mudryj, M., Horowitz, J.M. and Nevins, J.R. (1991) *Cell* **65**, 1053–1061.
- 22 Chittenden, T., Livingston, D.M. and Kaelin, J., W.G. (1991) *Cell* **65**, 1073–1082.
- 23 Helin, K., Lees, J.A., Vidal, M., Dyson, N., Harlow, E. and Fattaey, A. (1992) *Cell* **70**, 337–351.
- 24 Kaelin, W.G., Krek, W., Sellers, W.R., DeCaprio, J.A., Ajchenbaum, F., Fuchs, C.S., Chittenden, T., Li, Y., Farnham, P.J., Blunar, M.A., Livingston, D.M. and Flemington, E.K. (1992) *Cell* **70**, 351–364.
- 25 Helin, K. and Harlow, E. (1993) *Trends Cell Biol.* **3**, 43–46.
- 26 Beijersbergen, R.L., Kerkhofen, R.M., Zhu, L., Carlée, L., Voorhoeve, P.M. and Bernards, R. (1994) *Genes Dev.* **8**, 2680–2690.
- 27 Ginsberg, D., Vairo, G., Chittenden, T., Xiao, Z.-X., Xu, G., Wydner, K.L., DeCaprio, J.A., Lawrence, J.B. and Livingston, D.M. (1994) *Genes Dev.* **8**, 2665–2679.
- 28 Sellers, W.R., Rodgers, J.W. and Kaelin, W.G. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 11544–11548.
- 29 Bremner, R., Cohen, B.L., Sopta, M., Hamel, P.A., Ingles, C.J., Gallie, B.L. and Phillips, R.A. (1995) *Mol. Cell. Biol.* **15**, 3256–3265.
- 30 Weintraub, S.J., Chow, K.N.B., Luo, R.X., Zhang, S.H., He, S. and Dean, D.C. (1995) *Nature* **375**, 812–815.
- 31 Datta, P.K., Raychaudhuri, P. and Bagchi, S. (1995) *Mol. Cell. Biol.* **15**, 5444–5452.
- 32 Thanos, D. and Maniatis, T. (1992) *Cell* **71**, 777–789.