CDC6 interacts with c-Myc to inhibit E-box-dependent transcription by abrogating c-Myc/Max complex

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Abstract The c-*myc* oncogene product (c-Myc) is a transcription factor that dimerizes with Max and recognizes the E-box sequence. It plays key functions in cell proliferation, differentiation and apoptosis. It is generally thought that c-Myc transactivates genes encoding proteins essential to cell-cycle progression by binding to the E-boxes that control them. The functions of c-Myc are also thought to be modulated by its associated proteins, several of which have recently been identified. In this study, we found that c-Myc directly bound in vivo and in vitro to the N-terminal region of human CDC6, a component of the pre-replication complex, and that both co-localized in cell nuclei. CDC6 bound to the C-proximal region of c-Myc, thereby competing with Max on the E-box sequence and changing c-Myc/Max heterodimer to a Max/Max homodimer. In consequence, the E-box-dependent transcription activity of c-Myc was abrogated. These results suggest that, in addition to its DNA replication activity, CDC6 also has a role as a transcriptional suppressor of c-Myc.

Key words: c-Myc; CDC6

1. Introduction

c-Myc is a transcription factor that is thought to regulate the expression of genes involved in the control of cell-cycle progression (see recent reviews, [1–6]). When c-Myc is complexed with Max, it recognizes the E-box sequence that controls the transcription of target genes. Many candidate target genes for c-Myc/Max have been reported based on the presence of E-boxes and other circumstantial evidence. However, the actual target genes, controlled by c-Myc/Max in vivo, remain poorly understood. Notably, the results of experiments with c-*myc*-negative (null) Rat-1 cells showed that of the many candidate genes for c-Myc/Max, only a few revealed a significant change in expression pattern as a consequence of the loss of c-Myc [6,7]. In general terms, it is thought that a chromatin remodeling complex binds to a coactivator or co-repressor, in order to bridge sequence-specific transcription factors and basal transcription factors (see a recent review, [8]). In particular, Max binds to Mad, antagonizing the activation function of c-Myc [9]. Mad further binds to mSin3, a corepressor recruited to the basal transcription factors [10]. In contrast, the mechanism by which c-Myc transactivates the genes in cells is not well understood. Recently, SNF5/INI1, a component of SNF2/SWI1 of chromatin remodeling complexes, was reported to bind to the C-proximal region of c-Myc to recruit it to the basal transcription factors [11]. TRRAP, a protein related to the ATM/PI3-kinase family, binds to the N-terminal transactivation region of c-Myc and recruits hGCN5, which possesses histone acetyl transferase activity [12,13]. Furthermore, TIP49 and TIP48, which possess ATPase/helicase activity, bind to the transactivation region of c-Myc [14]. All of these proteins are thought to remodel the chromatin, in order to activate gene expression linked to the action of c-Myc. We have recently reported that ORC1, a component of origin recognition complexes (ORCs) of DNA replication, competes with SNF5 for binding to the C-terminal region of c-Myc with SFN5, thereby abrogating the transactivation activity of c-Myc [15]. This transcription function of ORC1 has also been suggested by experimental systems in yeast and *Drosophila* [16–20]. In addition to the role of c-Myc as a transcription factor, an earlier study also showed that c-Myc is co-fractionated with DNA replication proteins in human HeLa cells [21]. Proof of a replication role of c-Myc, however, has not been obtained. In this study, we found that CDC6, a component of pre-replication complexes including ORC1, directly binds to the C-terminal domain of c-Myc, the region recognized by Max. CDC6 abrogated E-box-dependent transcription by interfering with the formation of c-Myc/Max complexes.

2. Materials and methods

2.1. Cells

Human HeLa cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% calf serum. Human HL60 cells were cultured in RPMI 1640 medium with 10% fetal calf serum.

2.2. Plasmids

An EST clone corresponding to human CDC6, clone ID#664627, was purchased from Genome Systems Inc. All of the plasmids used in this study were constructed by the insertion of PCR-based amplified fragments, starting from the first ATG, into the respective vectors. All manipulations were performed according to standard procedures; the details of the plasmid constructions are available upon request.

2.3. in vitro and in vivo binding assays

Glutathione-*S*-transferase (GST)-CDC6, GST deletion mutants of CDC6 (GST-CDCdls) and GST were purified from *Escherichia coli* BL21(DE3) transformed with pGEX-CDC6, pGEX-CDCdls and pGEX-6P-1, respectively, as described previously [22]. 2 μg of the purified GST-CDC6, GST-CDCdls or GST was first applied to a glutathione Sepharose 4B (Amersham-Pharmacia) in a buffer contain-

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ing 20 mM Tris–HCl (pH 8.0), 150 mM NaCl, 0.5% NP-40 and 1 mM EDTA. Subsequently, 0.1 μg c-Myc (released from maltose-binding protein (MBP)-c-Myc expressed in *E. coli*, as described previously [23,24]) was applied to the column. After extensive washing of the column with the same buffer as that described above, the proteins bound to the resin were recovered, separated in a 7.5% polyacrylamide gel containing sodium dodecyl sulfate (SDS), and blotted against an anti-c-Myc monoclonal antibody, 9E10 [25]. Co-immunoprecipitation was performed using human HL60 cell lysates in a lysis buffer (20 mM Tris (pH 7.5), 150 mM NaCl, 0.2% NP-40, 2 mM EDTA and 2 mM dithiothreitol (DTT)). After the cell extracts were immunoprecipitated with the anti-c-Myc antibody, the antigen–antibody complexes were immobilized on protein-G-Sepharose for 30 min at 4°C and washed extensively with the lysis buffer five times. The bound proteins were separated in a 7.5% polyacrylamide gel containing SDS, transferred onto a nitrocellulose membrane, and blotted with an anti-CDC6 antibody (N-19, Santa Cruz).

2.4. Luciferase assay

HeLa cells in a 6 cm dish were transfected with 1 μg of pCMV-β-gal, 0.5 μg of pEF-c-myc and various amounts of pCMV-CDC6-HA or pCMV-T7-S6 [15] together with 2 μg of p4xE-SVP-Luc by the calcium phosphate method [26]. Two days after transfection, whole cell extracts were prepared by addition of the Triton X-100-containing solution from a Pica gene kit (Wako Pure Chemicals Co. Ltd., Kyoto, Japan) to the cells. About one-fifth volume of the extract was used for the β-galactosidase assay to normalize the transfection efficiencies as described previously [22,27], and the luciferase activity due to the reporter plasmid was determined.

2.5. Indirect immunofluorescence

HeLa cells were transfected with pEF-c-myc together with pEF-CDC6-HA by the calcium phosphate precipitation technique [26]. Forty-eight hours after transfection, the cells were fixed with a solution containing 4% paraformaldehyde and 1% Triton X-100, and they were reacted with a mouse anti-c-Myc monoclonal antibody (9E10) and a rabbit anti-HA polyclonal antibody (Y-11, Santa Cruz). The cells were then reacted with a rhodamine- or FITC-conjugated anti-mouse or anti-rabbit IgG, respectively, and observed under a confocal laser fluorescent microscope.

2.6. Electrophoretic mobility shift assay

The binding reactions contained 0.5 ng of the radiolabeled E-box sequence (5′-CCCCCACCACGTGGTGCCTGA-3′), 25 mM HEPES (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 5% glycerol, 10 mM DTT, 0.1% NP-40, 0.5 mg/ml bovine serum albumin and the indicated amount of protein. A reaction mixture (15 μl) was incubated at 20°C for 20 min. The resulting protein–DNA complexes were resolved on 4% polyacrylamide gels containing 0.25×TBE (22.5 mM Tris, 22.5 mM H_3BO_4 and 0.63 mM EDTA) at 4°C.

3. Results and discussion

3.1. Interaction of c-Myc with CDC6 in vivo

We have previously reported that ORC1, a component of ORCs in DNA replication, interacts with c-Myc and abrogates the E-box-dependent transactivation activity of c-Myc by interfering with c-Myc–SNF5 interaction [15]. Since CDC6 is associated with an ORC complex, we examined the interaction between c-Myc and CDC6 under physiological conditions. A whole cell extract of human HL60 cells was immunoprecipitated with an anti-c-Myc antibody or non-specific IgG, and the precipitate was blotted using an anti-CDC6 antibody (Fig. 1A). The CDC6 was detected in the precipitate with the anti-c-Myc antibody but not in the precipitate with non-specific IgG (Fig. 1A, lanes 2 and 3). These results clearly indicate that c-Myc is associated with CDC6 in cells.

3.2. Co-localization of c-Myc with CDC6 in cells

To determine the cellular localization of CDC6 and c-Myc, expression vectors for CDC6-HA and c-Myc were co-transfected into human HeLa cells. Two days after transfection, the

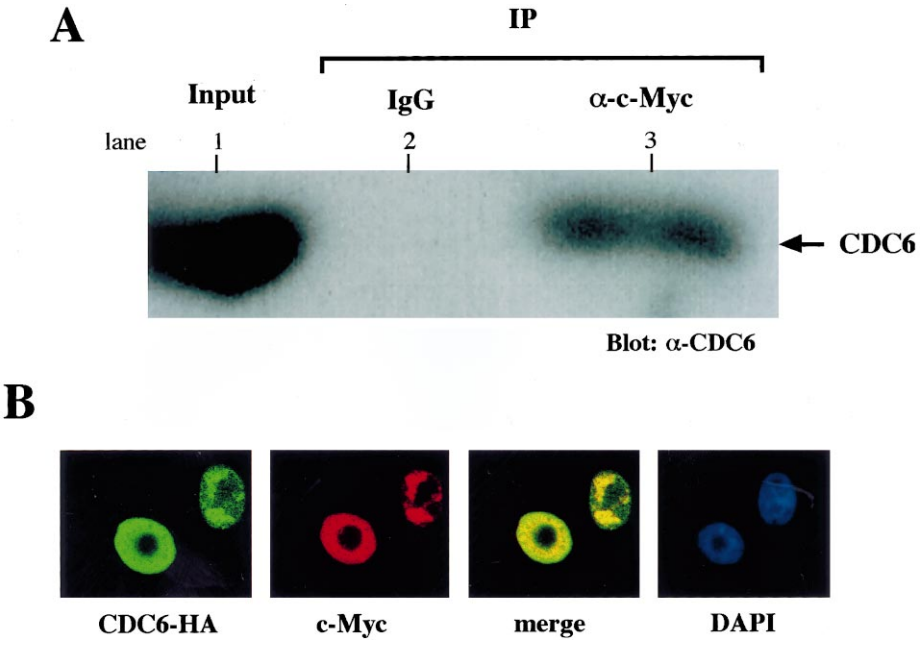

Fig. 1. Association of c-Myc and CDC6 in vivo. A: The extract of HL60 cells was prepared and subjected to an immunoprecipitation reaction using an anti-c-Myc or non-specific IgG (lanes 2 and 3, respectively). The precipitates were blotted with an anti-CDC6 monoclonal antibody. The 1/100 amount of the cell extract used for the reactions (lane 1, Input) was analyzed in parallel. B: HeLa cells were transfected with pEF-c-myc together with pEF-CDC6-HA by the calcium phosphate precipitation technique [26]. Forty-eight hours after transfection, the cells were fixed with a solution containing paraformaldehyde and Triton X-100, and they were reacted with a mouse anti-c-Myc monoclonal antibody (9E10) and a rabbit anti-HA polyclonal antibody (Y-11, Santa Cruz). The cells were then reacted with a rhodamine- or FITC-conjugated anti-mouse or anti-rabbit IgG, respectively, and observed under a confocal razor fluorescent microscope. The same cells were also stained with DAPI.

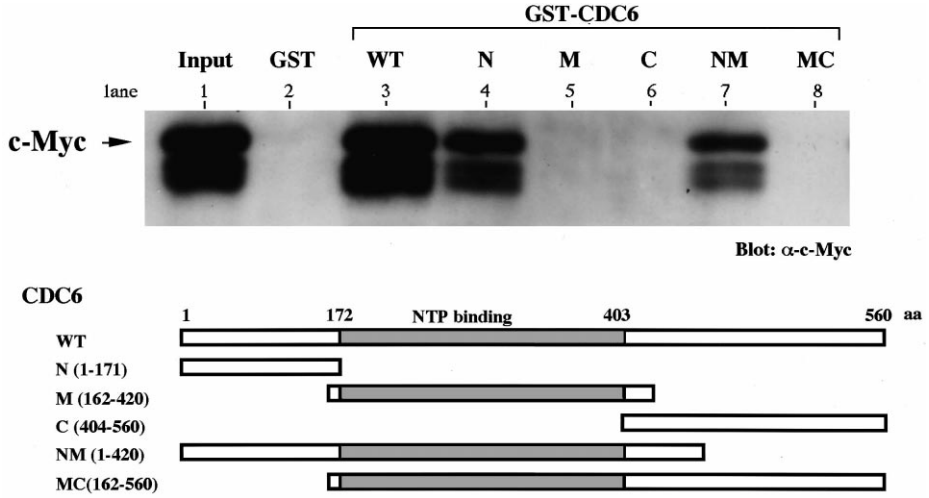

Fig. 2. In vitro binding activity of c-Myc and CDC6. GST fusion proteins of CDC6 and its various fragments were expressed as GST fusion proteins in *E. coli* and purified. MBP-c-Myc was also prepared as above and digested with PreScission protease to yield MBP-free c-Myc. The GST fusion proteins, or GST alone, were applied to the glutathione beads and incubated with c-Myc. After extensive washing, the proteins that had bound to the beads were analyzed in an SDS-containing polyacrylamide gel (7.5%) and blotted with an anti-c-Myc antibody (C-33, Santa Cruz). One-twentieth volume of the c-Myc used for the binding reaction was applied to the same gel (lane 1).

cells were stained with an anti-HA antibody and an anti-c-Myc antibody, and further with FITC- and rhodamine-conjugated secondary antibodies, respectively. Cell nuclei were also stained with DAPI. The results clearly showed that the CDC6-HA (green) and c-Myc (red) co-localized in nuclei, as shown by the yellow color (Fig. 1B).

3.3. Binding of c-Myc with CDC6 in vitro

Human CDC6 and its deletion mutants were expressed as fusion proteins of GST and affinity-purified by glutathione Sepharose resin. Full-size c-Myc was first expressed as a fusion protein to MBP, and prepared after cleavage of MBP by PreScission protease as described previously [23,24]. Subsequently, an in vitro binding assay, the so-called pull-down assay, was carried out by incubating GST-CDC6s and c-Myc under a high (150 mM) salt condition on glutathione Sepharose resin, and the bound protein(s) was detected by Western blotting with an anti-c-Myc antibody (Fig. 2). c-Myc possessed strong binding activity to wild-type CDC6 but not to GST (Fig. 2, lanes 2 and 3). As CDC6 contains an NTP-binding domain at the central part spanning amino acids #172–403 that is necessary for DNA replication activity, the five segments of GST-CDC4, N(1–171), M(162–420), C(404–560), NM(1–420) and MC(162–560), were prepared and used for an in vitro binding assay to c-Myc. The N-terminal segment, but not the segments containing the NTP-binding domain, bound to c-Myc (Fig. 3, lanes 4–8), indicating that c-Myc binds to the N-terminal region of CDC6 spanning amino acids #1–172. To determine the CDC6-binding region within c-Myc, various deletion constructs of c-Myc were expressed in *E. coli* as MBP fusion proteins and purified. MBP-c-Myc deletion mutants and GST-CDC6 (or GST-Max as a control) were mixed, and in vitro binding assays were carried out (Fig. 3). GST-Max and GST-CDC6 bound to MBP-c-Myc deletion mutants, but there was no binding to MBP itself (Fig. 3, lanes 4–27 and lanes 1–3, respectively). GST-Max could not bind to MBP-c-Myc mutants lacking the leucine zipper (Zip), N, M, HZ and LZ (Fig. 3B, lanes 7–12, 19–21 and 25–27). GST-CDC6 also showed the same binding patterns as those of GST-Max, except for one of the mutants, H1 (Fig. 3, lane 24). CDC6 does not bind to H1, which lacks only the first helix, spanning amino acids #368–382. These results indicate that CDC6 binds to the region containing all of the basic, helix-loop-helix and leucine zippers region of c-Myc.

3.4. Abrogation of E-box-dependent transcription activity of c-Myc by CDC6

Both CDC6 and Max bound to the C-proximal region of c-Myc. It is therefore possible that two proteins compete for binding to c-Myc. To assess this possibility, an electrophoretic mobility shift assay was carried out. Various combinations of recombinant GST-c-Myc-C, GST, Max and CDC6 were incubated with labeled oligonucleotides corresponding to the E-box as a probe. No nucleoprotein complex was observed in the incubation of the probe with CDC6 by itself, or with GST-c-Myc-C or GST (Fig. 4A, lanes 8–10). The formation of a Max/Max homodimer on the E-box probe was not affected by the addition of excess amounts of CDC6 (Fig. 4A, lanes 11–13). The c-Myc/Max heterodimer, on the other hand, was decreased by the addition of CDC6 in a dose-dependent manner, with a concomitant increase of the Max/Max homodimer (Fig. 4A, lanes 2–5). The results suggest that CDC6 interferes with the association between c-Myc and Max by a competitive binding to c-Myc. Since c-Myc/Max recognizes the E-box to stimulate gene expression [28], and since this interaction is abrogated by CDC6 as described above, we examined the effect of CDC6 on the E-box-dependent transcription activity of c-Myc. Luciferase activity was examined in HeLa cells transfected with 0.5 μg expression vector for c-Myc and 4xE-box-SVP-Luc, with or without expression vectors for CDC6. As a control, we also used an expression vector for the dominant-negative S6 fragment of SNF5, a component of the chromatin remodeling complex necessary for c-Myc transcription activity [11]. The results are shown in Fig. 4B. In the presence of an 'empty vector' (negative control), c-Myc bound to the E-box in the reporter plasmid and thereby activates the expression of luciferase. As previ-

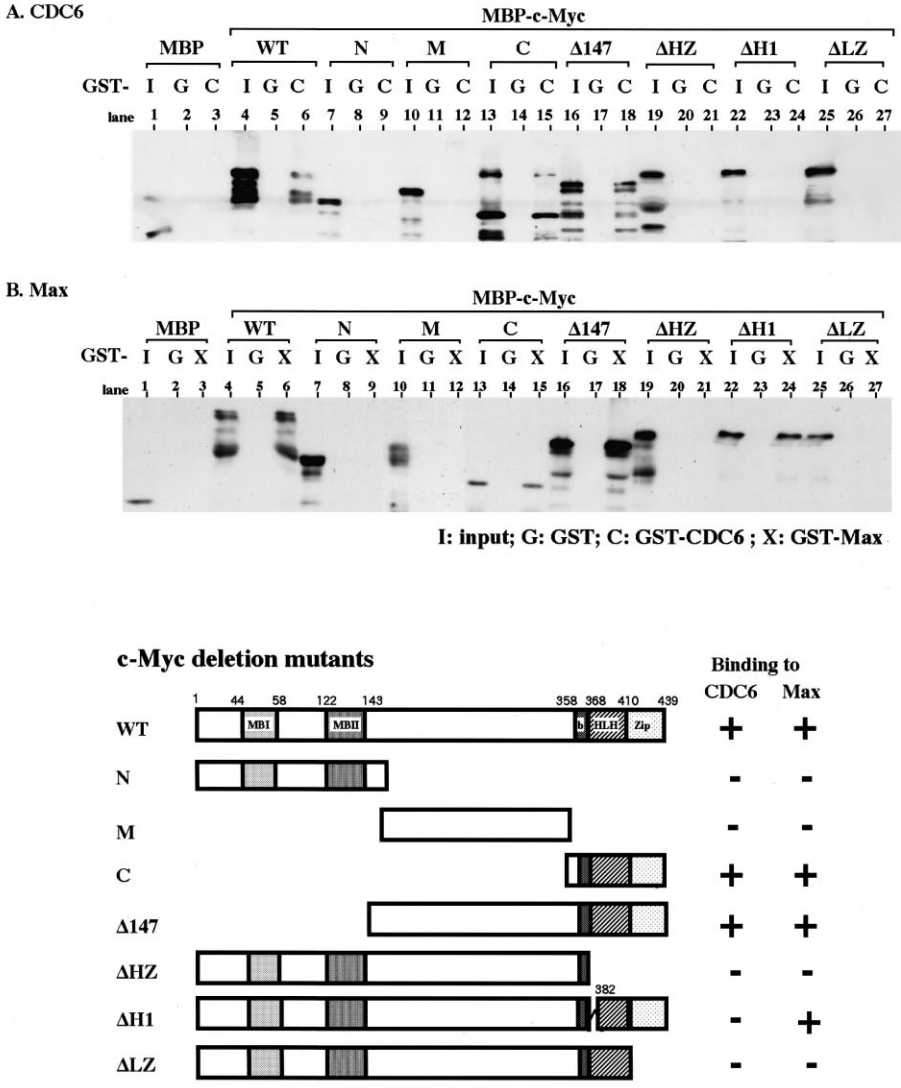

Fig. 3. Determination of the binding region of c-Myc with CDC6. The MBP or MBP fusion proteins of wild-type and various mutants of human c-Myc, schematically presented in the lower panel, were prepared in *E. coli* and incubated with glutathione beads coupled with GST-CDC6 (A) or GST-Max (B). Proteins that had bound to the beads were then separated by SDS–PAGE (10%) and blotted using an anti-MBP antibody (NEB). One-twentieth volume of the proteins used for binding reaction was applied to the same gel (I).

ously reported [11], the S6 fragment of SNF5 inhibited the E-box-dependent transcription in a dose-dependent manner. As for CDC6, it inhibited E-box-dependent luciferase expression in a manner similar to that of the S6 fragment (Fig. 4B). These results clearly indicate that CDC6 and Max are functionally competitive in the transcriptional activity of c-Myc.

In this study, we have found that (1) c-Myc interacts with CDC6 in vitro and in vivo, (2) the two proteins co-localize in HeLa cell nuclei, and (3) this complex inhibits E-box-dependent transcription by abrogating Max binding to c-Myc. This is the first report of CDC6 having a function in the modulation of transcription.

CDC6 is known to be a DNA replication factor necessary for forming a pre-replication complex [29–32]. An ORC first binds to the origin of DNA replication in the genome, then CDC6 makes contact with the ORC, and finally an MCM complex is recruited by the ORC–CDC6 complex to allow the initiation of DNA replication. In addition to this function in DNA replication, ORC and MCM have also been reported to play a role in transcription. On one hand, ORC is known to bind chromatin and to inhibit the transcription, and/or to remodel the chromatin structure in yeast and *Drosophila* cells [16–20]. On the other hand, MCM enhances the transcription activity of STAT-1α [33] and associates with RNA polymerase II holoenzyme in human cells [34]. Furthermore, CDC6, like ORC1 and like c-Myc itself, binds tightly to the chromatin during some stage of the cell cycle [32]. It is therefore not unreasonable to assume that CDC6 also has a transcription activity-linked c-Myc.

Many proteins that bind to the C-proximal region of c-Myc have been identified. These include Max [28,35,36], YY1 [37], Miz-1 [38], Nmi [39], BRCA1 [40], CBF/NF-Y [23], AP2 [41], cdr2 [42], SNF5 [11], MSSP [24] and ORC1 [15]. YY1, SNF5, MSSP and ORC1 have been reported to act as modulators of the transcriptional activity of c-Myc; all of the proteins except for SNF5 inhibit it. SNF5 is a component of the chromatin remodeling complex and ties c-Myc/Max with the basal transcription factors or with other chromatin remodeling proteins

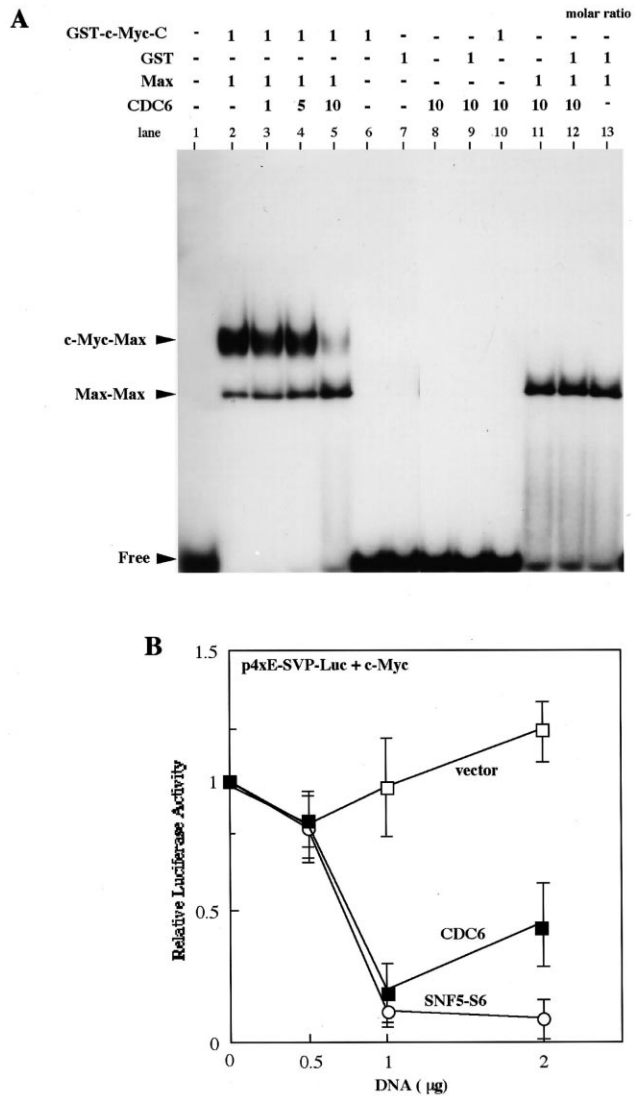

Fig. 4. Abrogation of the transcription activity of c-Myc by competitive binding between CDC6 and Max to c-Myc. A: Purified c-Myc (20 ng), Max (50 ng) and various amounts of CDC6 (see the molar ratio) were reacted with ^{32}P-labeled E-box oligonucleotides, and the bandshift assay was carried out as described in Section 2. DNA–protein complex was separated in 4% native polyacrylamide gel. B: HeLa cells were transfected with 1 μg of pCMV-β-gal, 0.5 μg of pEF-c-myc, 2 μg of p4xE-SVP-Luc together with various amounts of pCMV-CDC6-HA, pCMV-T7-S6 or the vector pCMV. After 48 h, the cell lysates were prepared and the luciferase activity was assayed. Relative luciferase activities to that of the cells transfected with p4xE-SVP-Luc and pEF-c-myc are shown.

possessing a histone acetyl transferase activity [11]. ORC1 competitively binds to c-Myc/Max and SNF5, thereby abrogating the transcription activity of c-Myc [15]. MSSP, a single-strand binding protein [43], makes a ternary complex between c-Myc and Max and this complex is released from the E-box sequence, thereby inhibiting the transcription activity of c-Myc [24]. We have found here that CDC6 competes directly with Max for binding to c-Myc. The mechanism of the inhibition of c-Myc transcription activity by CDC6 is therefore different from those by ORC1 and MSSP.

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References

[1] Henriksson, M. and Lusscher, B. (1996) Adv. Cancer Res. 68, 109–182.
[2] Ryan, K.M. and Birnie, G.D. (1996) Biochem. J. 314, 713–721.
[3] Hoffman, B., Liebermann, D.A., Selvakumaran, M. and Nguyen, H.Q. (1996) Curr. Top. Microbiol. Immunol. 211, 17–27.
[4] Dang, C.H. (1999) Mol. Cell. Biol. 19, 1–11.
[5] Prendergast, G.C. (1999) Oncogene 18, 2913–3016.
[6] Obaya, A.J., Mateyak, M.K. and Sedivy, J.M. (1999) Oncogene 18, 2934–2941.
[7] Bush, A., Mateyak, M., Dugan, K., Obaya, A., Adachi, S., Sedivy, J. and Cole, M. (1998) Genes Dev. 12, 3797–3802.
[8] Kingston, R.E. and Narlikar, G.J. (1999) Genes Dev. 13, 2339–2352.
[9] Ayer, D.E., Kretzner, L. and Eisenman, R.N. (1993) Cell 72, 211–222.
[10] Laherty, C.D., Yang, W.M., Sun, J.M., Davie, J.R., Seto, E. and Eisenman, R.N. (1997) Cell 89, 349–356.
[11] Cheng, S.-W.G., Davis, K.P., Yung, E., Beltran, R.J., Yu, J. and Kalpana, G.V. (1999) Nat. Genet. 22, 102–105.
[12] McMahon, S.B., Van Buskirk, H.A., Dugan, K.A., Copeland, T.D. and Cole, M.D. (1998) Cell 94, 363–374.
[13] McMahon, S.B., Wood, M.A. and Cole, M.D. (2000) Mol. Cell. Biol. 20, 556–562.
[14] Wood, M.A., McMahon, S.B. and Cole, M.D. (2000) Mol. Cell 5, 321–330.
[15] Takayama, M., Taira, T., Tamai, K., Iguchi-Ariga, S.M.M. and Ariga, H. (2000) Genes Cells 5, 481–490.
[16] Bell, S.P., Kobayashi, R. and Stillman, B. (1993) Science 262, 1844–1849.
[17] Foss, M., McNally, F.J., Laurenson, P. and Rine, J. (1993) Science 262, 1838–1844.
[18] James, T.C. and Elgin, S.C. (1986) Mol. Cell. Biol. 6, 3862–3872.
[19] Huang, D.W., Fanti, L., Pak, D.T., Botchan, M.R., Pimpinelli, S. and Kellum, R. (1998) J. Cell. Biol. 142, 307–318.
[20] Pak, D.T., Pflumm, M., Chesnokov, I., Huang, D.W., Kellum, R., Marr, J., Romanowski, P. and Botchan, M.R. (1997) Cell 91, 311–323.
[21] Studzinski, G.P., Shankavaram, U.T., Moore, D.C. and Reddy, P.V. (1991) J. Cell. Physiol. 147, 412–419.
[22] Mori, K., Maeda, Y., Kitaura, H., Taira, T., Iguchi-Ariga, S.M.M. and Ariga, H. (1998) J. Biol. Chem. 273, 29794–29800.
[23] Taira, T., Sawai, M., Ikeda, M., Tamai, K., Iguchi-Ariga, S.M.M. and Ariga, H. (1999) J. Biol. Chem. 274, 24270–24279.
[24] Niki, T., Izumi, S., Saëgusa, Y., Taira, T., Takai, T., Iguchi-Ariga, S.M.M. and Ariga, H. (2000) Genes Cells 5, 127–140.
[25] Evan, G.I., Lewis, G.K., Ramsay, G. and Bishop, J.M. (1985) Mol. Cell. Biol. 5, 3610–3616.
[26] Graham, F.J. and Van der Eb, A.J. (1973) Virology 52, 456–467.
[27] Taira, T., Maëda, J., Onishi, T., Kitaura, H., Yoshida, S., Kato, H., Ikeda, M., Tamai, K., Iguchi-Ariga, S.M.M. and Ariga, H. (1998) Genes Cells 3, 549–565.
[28] Lüscher, B. and Eisenman, R.N. (1990) Genes Dev. 4, 2025–2035.
[29] Leonhardt, H., Rahn, H.P. and Cardoso, M.C. (1999) Crit. Rev. Eukaryot. Gene Expr. 9, 345–351.
[30] Zannis-Hadjopoulos, M. and Price, G.B. (1999) J. Cell. Biochem. 32–33, 1–14.
[31] Boddy, M.N. and Russell, P. (1999) Front Biosci. 4, D841–D848.
[32] Quintana, D.G. and Dutta, A. (1999) Front Biosci. 4, D805–D815.
[33] Zhang, J.J., Zhao, Y., Chait, B.T., Lathem, W.W., Ritzi, M., Knippers, R. and Darnell Jr., J.E. (1998) EMBO J. 17, 6963–6971.
[34] Yankulov, K., Todorov, I., Romanowski, P., Licatalosi, D., Cilli, K., McCracken, S., Laskey, R. and Bentley, D.L. (1999) Mol. Cell. Biol. 19, 6154–6163.
[35] Blackwood, E.M. and Eisenman, R.N. (1991) Science 251, 1211–1217.
[36] Blackwood, E.M., Lüscher, B. and Eisenman, R.N. (1992) Genes Dev. 6, 71–80.

[37] Shrivastava, A., Saleque, S., Kalpana, G.V., Artandi, S., Goff, S.P. and Calame, K. (1933) Science 262, 1889–1892.
[38] Peukert, K., Staller, P., Schneider, A., Carmichael, G., Hanel, F. and Eilers, M. (1997) EMBO J. 16, 5672–5686.
[39] Bao, J. and Zervos, A.S. (1996) Oncogene 12, 2171–2176.
[40] Wang, Q., Zhang, H., Kajino, K. and Greene, M.I. (1998) Oncogene 17, 1939–1948.
[41] Gaubatz, S., Imhof, A., Dosch, R., Werner, O., Mitchell, P., Buettner, R. and Eilers, M. (1995) EMBO J. 14, 1508–1519.
[42] Okano, H.J., Park, W.-Y., Corradi, J.P. and Darnell, R.B. (1999) Genes Dev. 13, 2087–2097.
[43] Negishi, Y., Nishita, Y., Saëgusa, Y., Galli, I., Miyajima, N., Tamai, H., Kihara, F., Iguchi-Ariga, S.M.M. and Ariga, H. (1994) Oncogene 9, 1133–1143.