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**Molecular Basis of Cell and  
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## The CCAAT Enhancer-binding Protein $\alpha$ (C/EBP $\alpha$ ) Requires a SWI/SNF Complex for Proliferation Arrest\*

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**The transcription factor CCAAT enhancer-binding protein  $\alpha$  (C/EBP $\alpha$ ) is a tumor suppressor in myeloid cells and inhibits proliferation in all cell types examined. C/EBP $\alpha$  interacts with the SWI/SNF chromatin-remodeling complex during the regulation of differentiation-specific genes. Here we show that C/EBP $\alpha$  fails to suppress proliferation in SWI/SNF defective cell lines after knock-down of SWI/SNF core components or after deletion of the SWI/SNF interaction domain in C/EBP $\alpha$ , respectively. Reconstitution of SWI/SNF function restores C/EBP $\alpha$ -dependent proliferation arrest. Our results show that the anti-proliferation activity of C/EBP $\alpha$  critically depends on components of the SWI/SNF core complex and suggest that the functional interaction between SWI/SNF and C/EBP $\alpha$  is a prerequisite for proliferation arrest.**

Transcription factors involved in terminal differentiation are frequently associated with cell cycle arrest. A prototype transcription factor that induces differentiation and proliferation arrest is the CCAAT enhancer-binding protein  $\alpha$  (C/EBP $\alpha$ ).<sup>1</sup> C/EBP $\alpha$  regulates genes involved in differentiation of the liver, gut, skin, adipose tissue, and in the myelomonocytic lineage of the hematopoietic system (1–9). In addition, C/EBP $\alpha$  is a powerful inhibitor of proliferation in all cell types reported to date, including virally transformed or tumorigenic cell lines that lack various tumor suppressor proteins (10–21). Loss of C/EBP $\alpha$  functions have been linked to leukemogenesis, suggesting an important role for C/EBP $\alpha$  as a tumor suppressor (22–26).

Different models involving both transcriptional and non-transcriptional mechanisms have been proposed to explain how C/EBP $\alpha$  causes arrest of the cell cycle. Initially, C/EBP $\alpha$  was found to enhance the expression and protein stability of the cyclin-dependent kinase (cdk) inhibitor p21 (20, 27). Furthermore, C/EBP $\alpha$  was suggested to directly inhibit the activity of cdk through interaction with p21 (12). However, C/EBP $\alpha$  also causes proliferation arrest in p21-deficient fibroblasts (16), de-

manding additional mechanisms of proliferation inhibition. These may include the association of C/EBP $\alpha$  with cdk2 and cdk4 independently of p21 and the enhancement of proteolytic turnover of the cdk (28, 29). An alternative explanation for C/EBP $\alpha$ -mediated proliferation arrest proposes that C/EBP $\alpha$  counteracts the activation of E2F-dependent genes (14, 17, 19, 30). This mechanism is particularly attractive, as it was shown that both terminal differentiation and cell cycle arrest depend on E2F repression (17), linking both events to one factor. Iakovova *et al.* have recently suggested that C/EBP $\alpha$ -induced cell cycle arrest is caused by two different and competitive mechanisms that might change with age. In liver cells of young rodents, C/EBP $\alpha$  was found to be associated with cdk complexes, whereas in older animals a complex containing E2F4, the retinoblastoma protein (Rb) and the ATPase Brahma (Brm) prevailed as C/EBP $\alpha$  binding partners (30). Although this model combines different mechanisms of C/EBP $\alpha$ -induced proliferation arrest, it involves the tumor suppressor Rb that was previously shown to be dispensable for the proliferation inhibitory activity of C/EBP $\alpha$  (13, 14, 16, 17, 19). Accordingly, a unifying biological mechanism of how C/EBP $\alpha$  suppresses proliferation has yet to be found.

We have shown previously that the functional interaction between C/EBP $\alpha$  and the SWI/SNF chromatin-remodeling complex is essential for the activation of a set of myeloid and adipogenic genes and for cell differentiation (31). The ATPases hBrm and Brg1, which are essential components of the mammalian SWI/SNF core complex, are also involved in cell cycle regulation (32–37) and tumor suppression (38–40). We therefore investigated whether a functional SWI/SNF complex is required for C/EBP $\alpha$ -mediated proliferation arrest.

Here, we report that C/EBP $\alpha$  fails to induce cell cycle arrest in SWI/SNF-deficient cells. Proliferation arrest by C/EBP $\alpha$ , however, is re-established once a functional SWI/SNF complex is restored. Thus, our data suggest that C/EBP $\alpha$ -mediated proliferation arrest critically depends on a functional SWI/SNF complex.

### EXPERIMENTAL PROCEDURES

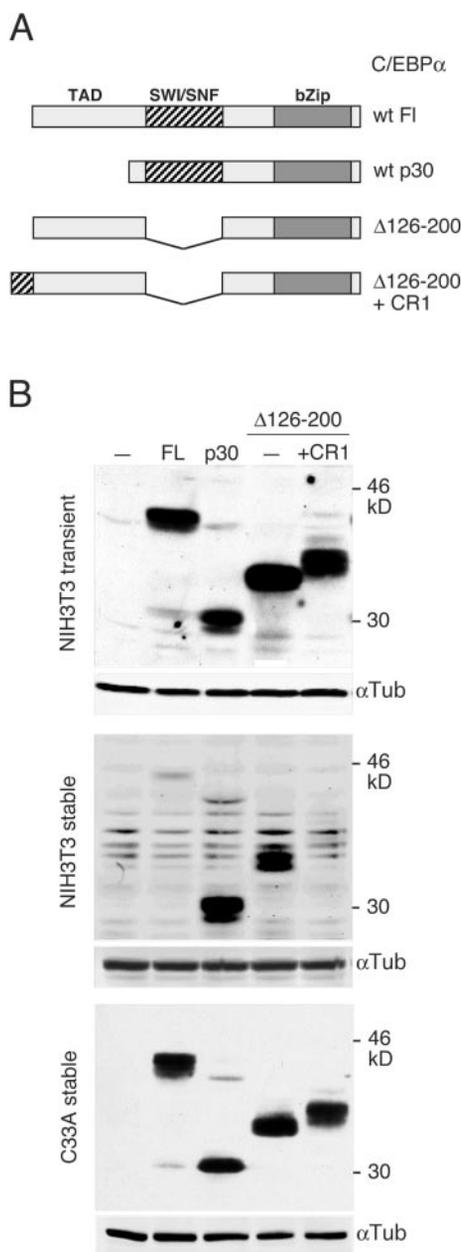
**Plasmid Constructs**—Rat C/EBP $\alpha$  constructs were cloned into pCDNA3 (Invitrogen). The generation of  $\Delta 126$ –200,  $\Delta 126$ –200 + CR1, and p30 have been described (31, 41). An EcoRI fragment containing the rat C/EBP $\alpha$ ER (21) was cloned into the pBABEpuro vector (42). EcoRI fragments of wild type or the ATP-binding site mutant of h-Brm (HA-tagged) (43) were cloned into the pCDNA3 and pBABEpuro vectors. The Brahma and Ini1-specific small interfering RNA (siRNA) oligonucleotides were designed as described in (44) and are as follows: Brahma upper strand, 5'-GAT CCC CAA AGG ACC TTG CCT GGC ATT TCA AGA GAA TGC CAG GCA AGG TCC TTT TTT TTG AAA-3', and Brahma lower strand, 5'-AGC TTT TCC AAA AAA AAG GAC CTT GCC TGG CAT TCT CTT GAA ATG CCA GGC AAG GTC CTT TGG G-3'; Ini1 upper strand, 5'-GAT CCC CCA CGG CCC CGG CCT GGT AAT TCA AGA GAT TAC CAG GCC GGG GCC GTG TTT TTG GAA A-3', and Ini1 lower strand, 5'-AGC TTT TCC AAA AAC ACG GCC CCG GCC TGG TAA TCT CTT GAA TTA CCA GGC CGG GGC CGT GGG

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<sup>1</sup> The abbreviations used are: C/EBP, CCAAT/enhancer binding protein; BCER, BALB/c mouse fibroblasts expressing the C/EBP $\alpha$ ER fusion protein; Brm, Brahma; Brg1, Brahma related gene 1; cdk, cyclin-dependent kinase; C/EBP $\alpha$ ER, C/EBP $\alpha$  estrogen receptor C/EBP $\alpha$ ER, C/EBP $\alpha$  estrogen receptor fusion protein; CR1, conserved region 1; HA, hemagglutinin A; hBrm, human Brm; Rb, retinoblastoma; siRNA, small interfering RNA.



**FIG. 1. Comparison of transient and stable expression of C/EBP $\alpha$  proteins in normal and SWI/SNF-defective cells.** *A*, schematic representation of the C/EBP $\alpha$  constructs that were used for transient and stable transfections. The major transactivation domain (TAD), the SWI/SNF binding domain (SWI/SNF), the DNA binding region, and the leucine zipper (bZIP) are indicated. CR1 represents the conserved region from C/EBP $\beta$  that also binds the SWI/SNF complex (47). *wt*, wild type. *B*, protein expression after transient (NIH3T3, top panel) or stable transfection (NIH3T3, middle panel and C33A, bottom panel) with the C/EBP $\alpha$  wild type and mutant constructs (FL, full-length C/EBP $\alpha$ ). Whole cell lysates were subjected to SDS-gel electrophoresis, blotted, and examined using a C/EBP $\alpha$ -specific antiserum. Equal protein loading was controlled by reprobing with an  $\alpha$ -tubulin-specific antibody ( $\alpha$ Tub) as shown in the small panel underneath each larger panel.

G-3'. The double-stranded oligonucleotides were cloned into BglII- and HindIII-digested pSUPER vector (44).

**Cell Culture**—Cells were incubated in 5% CO<sub>2</sub> at 37°C. NIH3T3 (American Type Culture Collection), C33A, SW13, and Phoenix A cells in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Invitrogen) and BCER (BALB/c mouse fibroblasts expressing the C/EBP $\alpha$ ER fusion protein) as described earlier (16). Transfected or retrovirally infected cells were cultivated with puromycin (2  $\mu$ g/ml) or G-418 (0.8 mg/ml).

**Transfection Methods and Colony Staining**—For stable expression,

**TABLE I**

*Suppression of colony formation by C/EBP $\alpha$  depends on SWI/SNF*  
Mean values of colony counts with standard deviation ( $\pm$ )  $n = 3$ . FL stands for full-length, and ND is not determined.

	NIH3T3	C33A	SW13
Empty vector	71.0 $\pm$ 3.6	143.3 $\pm$ 9.3	131.3 $\pm$ 8.2
C/EBP $\alpha$ FL	24.3 $\pm$ 4.9	124 $\pm$ 7	112.7 $\pm$ 8
$\Delta$ 126-200	53.3 $\pm$ 5	130.6 $\pm$ 15.9	ND
$\Delta$ 126-200 + CR1	24.7 $\pm$ 2.1	133.3 $\pm$ 11.9	ND
C/EBP $\alpha$ p30	75.3 $\pm$ 7.7	127.3 $\pm$ 6.8	105 $\pm$ 12.2

cells ( $5 \times 10^4$ ) were transfected with 5  $\mu$ g of DNA using calcium phosphate. Antibiotic-resistant colonies were pooled for protein expression analysis or fixed with 4% para-formaldehyde for 1 h and stained with Diff-Quick (Merz und Dade AG) to determine colony numbers. Gene Porter transfection reagent (Gene Therapy Systems) was used with  $1 \times 10^5$  BCER cells and 10  $\mu$ g of pSUPER-based vector plus 1  $\mu$ g pBABEpuro in siRNA experiments. After 1–2 weeks of puromycin selection, single clones were isolated and analyzed for Brahma or Ini1 expression by Western blotting. For transient expression,  $1 \times 10^6$  cells were transfected using calcium phosphate. 34–38 h later, cells were harvested.

**Reporter Assay**— $1 \times 10^6$  cells from C33A clones retrovirally infected with C/EBP $\alpha$ ER-pBABEpuro or with the empty pBABEpuro were transfected with 1  $\mu$ g of the C/EBP-responsive M82-luciferase construct as described earlier (45). Transfection of each clone was done in parallel with four plates, two of which received  $\beta$ -estradiol (1  $\mu$ M), whereas the other two received the solvent only. 36 h after transfection, cells were harvested for luciferase assay (45).

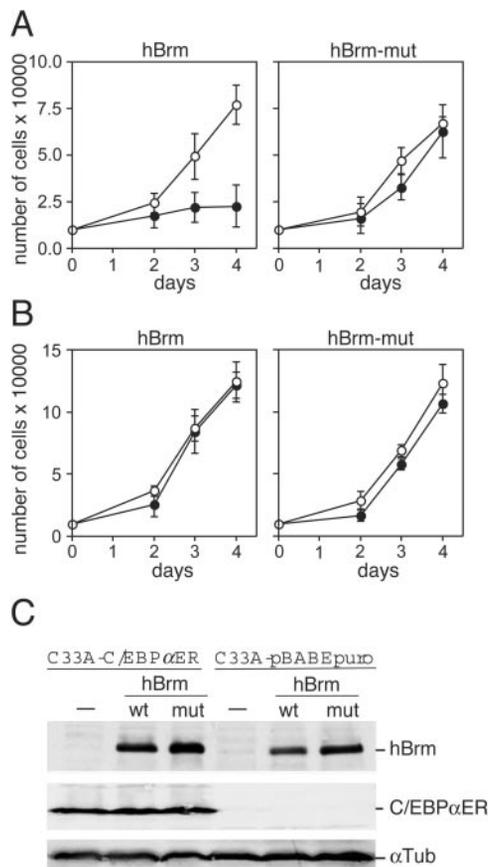
**Retroviral Infection**—Phoenix A cells were transiently transfected using calcium phosphate. 48 h after transfection,  $5 \times 10^5$  target cells were infected and selected as described (46).

**Growth Curves**—Cells ( $1 \times 10^4$  cells per well) were seeded in phenol red-free Dulbecco's modified Eagle's medium, and either  $\beta$ -estradiol (1  $\mu$ M) or solvent was added. Every 24 h cells were harvested, and the number of living cells was determined by trypan blue exclusion.

**Western Blotting**—Proteins were separated on 12, 10, or 8% SDS-polyacrylamide gels and blotted on polyvinylidene difluoride membrane (Immobilon-P, Millipore). Proteins were detected using antibodies against C/EBP $\alpha$  (14AA), Brm (N-19 and C-20), Ini1 (H-300), and  $\alpha$ -Tubulin (TU-02) (0.5  $\mu$ g/ml each, all from Santa Cruz Biotechnology Inc.), anti-HA (1:1000, Babco), and appropriate horseradish peroxidase-conjugated secondary antibodies (anti-rabbit, 1:5000 and anti-mouse, 1:5000, both from Amersham Biosciences, and anti-goat, 1:2000, from Santa Cruz Biotechnology Inc.) and detected by chemiluminescence (ECL, Amersham Biosciences). Alternatively, fluorochrome-conjugated secondary antibodies were used (goat anti-mouse IgG (H+L) and goat anti-rabbit IgG (H+L), both from Alexa Fluor, each in a dilution of 1:5000), and the blots were analyzed with the Odyssey-Imager (Li-COR).

## RESULTS

The anti-proliferative activity of C/EBP $\alpha$  and various C/EBP $\alpha$  mutants (Fig. 1A) was determined in a colony assay using mouse NIH3T3 fibroblasts and the SWI/SNF-defective human cell lines C33A and SW13 (43). As shown in Table I, full-length C/EBP $\alpha$  (C/EBP $\alpha$  FL) inhibits colony formation in NIH3T3 but not in SWI/SNF-defective cells. Removal of the SWI/SNF interaction domain in the center of C/EBP $\alpha$  (C/EBP $\alpha$   $\Delta$ 126–200) (31) largely abrogates the inhibitory effect on colony formation, suggesting a general contribution of SWI/SNF in C/EBP $\alpha$ -mediated proliferation arrest. Notably, when SWI/SNF recruitment of the internal deletion mutant was restored by adding the heterologous SWI/SNF recruiting domain of C/EBP $\beta$  ( $\Delta$ 126–200+CR1; CR1 was derived from the C/EBP $\beta$  N terminus; Refs. 31 and 47), the ability to suppress colony formation in NIH3T3 cells was also restored. In SWI/SNF-defective cells the same chimeric C/EBP protein, like the full-length C/EBP $\alpha$ , fails to suppress proliferation. The C/EBP $\alpha$  p30 isoform, which lacks the major N-terminal transactivation function, has been shown previously to be defective in the activation of genes and the suppression of proliferation (17, 41, 48). In a control experiment, C/EBP $\alpha$  p30 was expressed and

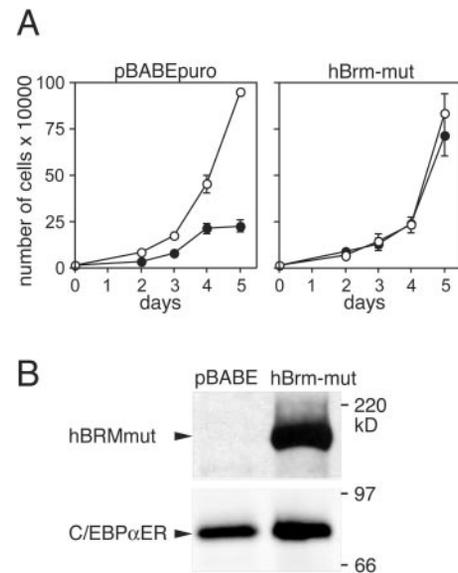


**FIG. 2. Restoration of C/EBP $\alpha$ -induced proliferation arrest in C33A cells by ectopic expression of hBrm.** C/EBP $\alpha$ ER-expressing cells (A) or control cells carrying empty pBABEpuro vector (B) were stably transfected with hBrm or dominant negative hBrm (defective in the ATPase function; Refs. 43 and 49) constructs as indicated. Pools of  $\sim 10^3$  independent colonies were examined for proliferation kinetics in the presence (closed circles) or absence (open circles) of estrogen (1  $\mu$ M). Mean values and standard deviation from triplicates are shown. C, protein expression controls from pools of transfected cells as shown in A and B. Whole cell lysates were examined by immunoblotting using either an HA tag-specific antibody to detect ectopic Brm expression (top panel) or a C/EBP $\alpha$  specific antiserum to detect the C/EBP $\alpha$ ER fusion protein (middle panel). Blots were re-probed with an  $\alpha$ -tubulin-specific antibody ( $\alpha$ Tub) as a loading control (bottom panel). wt, wild type; mut, mutant.

found to be unable to inhibit proliferation in both NIH3T3- and SWI/SNF-defective cells (Table I).

A comparison of C/EBP $\alpha$  protein expression levels after transient and stable transfection in NIH3T3 fibroblasts and SWI/SNF-defective cells further supports the idea that C/EBP $\alpha$ -induced proliferation arrest depends on interaction with SWI/SNF. As shown in Fig. 1B, C/EBP $\alpha$  proteins are expressed at equal levels in NIH3T3 cells shortly after transfection (2 days). After 2 weeks, however, only traces of full-length C/EBP $\alpha$  or C/EBP $\alpha$   $\Delta$ 126–200 + CR1 were found in NIH3T3 cells, suggesting counter selection, whereas expression of C/EBP $\alpha$   $\Delta$ 126–200 and p30 remained high. In contrast, expression from all constructs was maintained in the SWI/SNF-defective C33A cells (Fig. 1B) or SW13 cells (data not shown), indicating that, in the absence of SWI/SNF, C/EBP $\alpha$  expression does not restrain proliferation.

These results show that the SWI/SNF interaction domain of C/EBP $\alpha$  and a functional SWI/SNF complex are required for efficient induction of proliferation arrest. Furthermore, SWI/SNF-defective cells are the first cells identified that tolerate high levels of C/EBP $\alpha$  expression during proliferation.



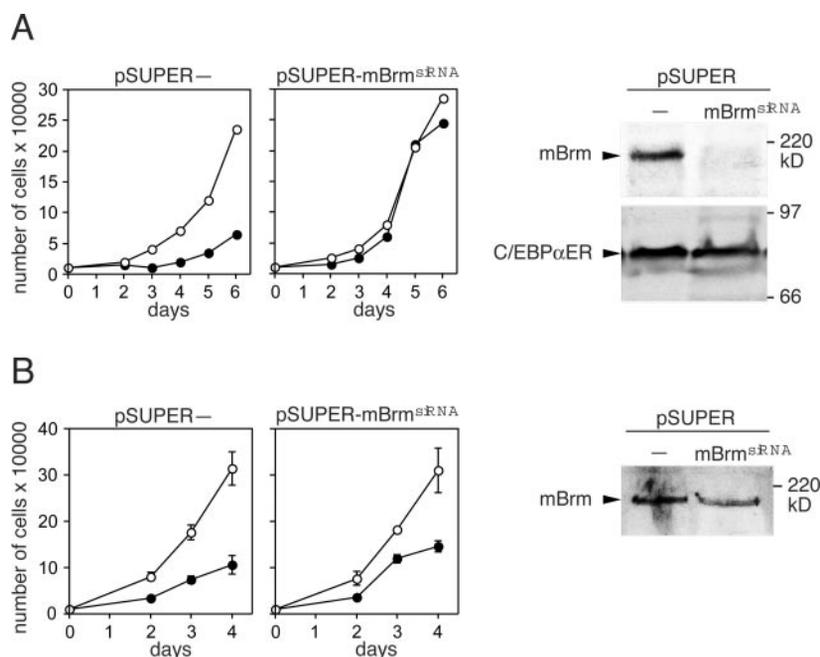
**FIG. 3. Abrogation of C/EBP $\alpha$ -induced proliferation arrest by a dominant negative hBrm mutant in BALB/c fibroblasts.** A, BCER cells were stably transfected with a dominant negative hBrm mutant (mut) (defective in its ATPase function; Refs. 43 and 49) or empty vector (pBABEpuro), as indicated. Pools of  $\sim 200$  independent colonies were examined for proliferation kinetics in the presence (closed circles) or absence (open circles) of estrogen (1  $\mu$ M). Mean values and standard deviations from duplicates are shown. B, expression control from pools of transfected cells. Whole cell lysates were examined by immunoblotting using an HA tag-specific antibody to detect expression of the hBrm mutant (top panel) or a C/EBP $\alpha$ -specific antiserum to detect the C/EBP $\alpha$ ER fusion protein (bottom panel).

In addition to the SWI/SNF defect, C33A and SW13 cells might have accumulated other mutations that may help to overcome C/EBP $\alpha$ -induced proliferation arrest. To examine this possibility, SWI/SNF-defective cells were reconstituted with intact hBrm and with a conditional version of C/EBP $\alpha$  (16, 21). First, the hormone-inducible C/EBP $\alpha$  estrogen receptor fusion protein, C/EBP $\alpha$ ER, was introduced by retroviral gene transfer into C33A cells. After confirming the expression and transcriptional function of the C/EBP $\alpha$ ER fusion protein by Western blotting and reporter assay (see "Experimental Procedures" for details) (45), proliferation was found to be insensitive to estrogen (data not shown). Stable expression of hBrm, however, rendered these cells sensitive to estrogen-induced proliferation arrest as shown in Fig. 2A (left panel). In contrast, expression of a dominant negative hBrm mutant (defective in its ATPase function; Refs. 43 and 49) instead of wild type hBrm was unable to restore the anti-proliferative C/EBP $\alpha$  activity (Fig. 2A, right panel). Fig. 2B shows that C33A cells expressing hBrm in the absence of conditional C/EBP $\alpha$  are also not estrogen-sensitive, ruling out the possibility that hBrm mediates estrogen sensitivity independently of the C/EBP $\alpha$ ER construct. Thus, failure of C/EBP $\alpha$  to arrest proliferation of C33A cells is due to the lack of the functional SWI/SNF ATPase Brm.

Interestingly, C33A cells are also defective in the tumor suppressor Rb (50), supporting previous studies (13, 14, 16, 17, 19) that have shown that a functional Rb is not required for the proliferation-suppressive activity of C/EBP $\alpha$ .

BALB/c mouse fibroblasts that stably express the C/EBP $\alpha$ ER protein were previously shown to respond to estrogen with proliferation arrest (BCER cells; Ref. 16). These cells were employed to independently assess the dependence on Brm and SWI/SNF for proliferation arrest in a different cell type. As shown in Fig. 3, expression of the dominant negative hBrm mutant abrogates estrogen-induced proliferation arrest in BCER cells. Thus, functional Brm, in conjunction with

**FIG. 4. Suppression of Brm expression by RNA interference abrogates the anti-proliferative activity of C/EBP $\alpha$ .** A, BCER cells were transfected with a mouse Brm-specific siRNA construct or empty vector (pSUPER) as indicated, both in combination with the empty pBABEpuro vector to permit antibiotic selection (ratio 10:1). A representative clone was examined for proliferation kinetics in the presence (*closed circles*) or absence (*open circles*) of estrogen (1  $\mu$ M). The *top right panel* shows loss of mouse Brm expression by Western blotting. Expression of the C/EBP $\alpha$ ER fusion protein is not affected, as shown below. B, recurring hormone sensitivity in long term cultivated Brm knock-down cells. The same clone as shown in A was cultivated for a prolonged time. Cells regained endogenous Brm expression after 3–5 weeks in culture, as shown in the immunoblot (*right panel*). Proliferation kinetics were examined in the presence (*closed circles*) or absence (*open circles*) of estrogen (1  $\mu$ M). Mean values and standard deviation from triplicates are shown.



C/EBP $\alpha$ , is also required in BALB/c fibroblasts to inhibit proliferation.

Similar results were obtained when Brm protein expression was knocked down by RNA interference in BCER cells, as shown in Fig. 4. A Brm-specific siRNA expressed from the pSUPER vector (44) diminished the Brm protein level and rendered BCER cells unresponsive to estrogen (Fig. 4A). Interestingly, knock-down of Brm was transient, and the protein reappeared after 3–5 weeks, probably because the interfering RNA, expressed from a non-selectable plasmid, was lost upon prolonged cultivation. As shown in Fig. 4B, the recurrence of endogenous Brm expression coincided with the restoration of C/EBP $\alpha$ -mediated proliferation arrest after estrogen induction. These results show that the transient down-regulation of Brm expression resulted in the reversible abrogation of C/EBP $\alpha$ -induced proliferation arrest.

Brm is an essential component of a subgroup of SWI/SNF complexes, but it might also display proliferation control activity independently of the complex. To determine whether the SWI/SNF complex is required for C/EBP $\alpha$ -induced proliferation arrest, we knocked down expression of Ini1/SNF5, another essential SWI/SNF component (51, 52), by RNA interference. Initially, 12 BCER clones were isolated that expressed reduced Ini1 protein levels, as examined by Western blotting (data not shown, and Fig. 5). Notably, all Ini1/SNF5 knock-down clones displayed significantly prolonged doubling times that might be due to enhancement of apoptosis in the absence of Ini1 (53). Nevertheless, the three clones that were further examined displayed strongly reduced to undetectable Ini1/SNF5 expression (a representative example is shown in Fig. 5) and also failed to respond to estrogen-induced proliferation arrest, whereas controls remained estrogen-sensitive (Fig. 5A). These results suggest that a functional SWI/SNF complex is required for C/EBP $\alpha$  to suppress proliferation.

#### DISCUSSION

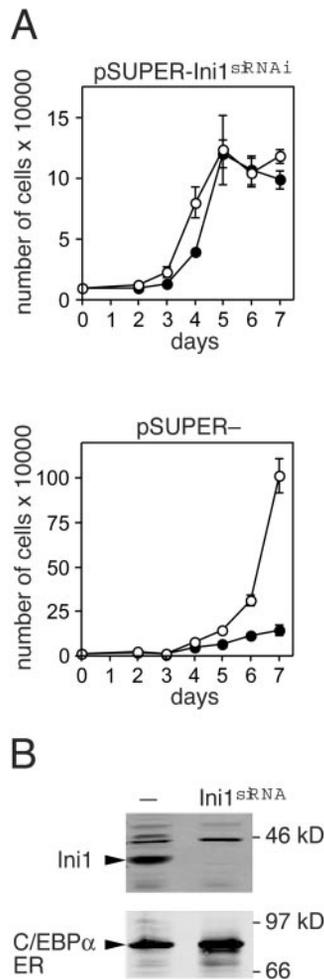
The full-length isoform of the transcription factor C/EBP $\alpha$  inhibits proliferation of many cell types, including cells that are defective in cell cycle control genes such as p53, Rb and related pocket proteins, or p21 (11–19, 21, 29). Genetic defects on both alleles that interfere with the expression of the full-length C/EBP $\alpha$  isoform were observed in a substantial fraction of

patients with acute myeloid leukemia, suggesting a tumor suppressor function for C/EBP $\alpha$  (22, 24, 26).

Various mechanisms that are difficult to reconcile have been proposed to explain how C/EBP $\alpha$  inhibits proliferation (see the Introduction). We have therefore carried out a functional analysis to examine the essential requirements for C/EBP $\alpha$ -mediated proliferation arrest. We find that, in the absence of a functional SWI/SNF complex, C/EBP $\alpha$  no longer suppresses proliferation. Consequently, the Brm-deficient C33A and SW13 tumor cells (43) are the first cell lines identified that tolerate stable expression of high levels of C/EBP $\alpha$  during replication. Importantly, we could restore the anti-proliferative C/EBP $\alpha$  function in C33A cells by ectopic expression of hBrm. This ruled out the possibility that other defects that might have accumulated in the tumor cell line contributed to the loss of the anti-proliferative activity of C/EBP $\alpha$ . The strict SWI/SNF dependence of C/EBP $\alpha$ -mediated proliferation arrest was also found in BALB/c mouse fibroblasts. Reducing functional Brm levels by RNA interference or through competitive ectopic expression of a dominant negative Brm mutant resulted in loss of C/EBP $\alpha$ -mediated proliferation arrest. Because the knock-down of Brm by siRNA in BALB/c cells was only transient, we also obtained a rigorous and conditional control that linked C/EBP $\alpha$ -mediated proliferation arrest to endogenous Brm expression. Furthermore, we did not detect any residual anti-proliferative activity of C/EBP $\alpha$  in the investigated cells in the absence of Brm/SWI/SNF. Hence, our data imply that Brm and/or SWI/SNF is involved in all the mechanisms proposed to mediate C/EBP $\alpha$ -induced proliferation arrest.

In addition to its function in the SWI/SNF complex, Brm might also be involved in the regulation of the cell cycle through other mechanisms. Although we can not rule out the possibility that multiple Brm-based mechanisms are simultaneously involved in C/EBP $\alpha$ -mediated cell cycle arrest, it is intriguing that elimination of Ini1/SNF5, another essential component of the SWI/SNF complex (51, 52), also abrogated the anti-proliferative C/EBP $\alpha$  activity. The data therefore suggest that a Brm-based SWI/SNF complex is involved in C/EBP $\alpha$ -induced proliferation arrest.

In a subset of mammalian SWI/SNF complexes, Brg1 is the ATPase subunit instead of Brm (49, 54). Brm and Brg1 are



**FIG. 5. Knock-down of Ini1/SNF5 expression abrogates the anti-proliferative activity of C/EBP $\alpha$ .** BCER cells were transfected with a mouse Ini1-specific siRNA construct or the empty vector (pSUPER) as indicated, both in combination with the pBABEpuromycin vector for antibiotic selection (ratio 10:1). Representative clones were examined for proliferation kinetics in the presence (closed circle) or absence (open circle) of estrogen (1  $\mu$ M). **A**, mean values and standard deviation from duplicates of Ini1 siRNA clones (top panel) or vector control (bottom panel) are shown. **B**, expression of Ini1 and C/EBP $\alpha$ ER in cells as shown in **A** was analyzed by immunoblotting using an Ini1-specific antiserum (top panel) or a C/EBP $\alpha$ -specific antiserum (bottom panel).

highly homologous proteins and were found to be at least partially redundant in the regulation of the cell cycle in several studies in cell culture (32, 35, 36, 55–57). However, the knock-out of Brg1 or Brm in mice produce different phenotypes that indicate differences in their function (33, 38). We and others have observed that C/EBPs may interact with both Brm- and Brg1-based SWI/SNF complexes to activate differentiation-specific genes (31, 47, 58). This raises the question of whether Brg1 is also able to cooperate with C/EBP $\alpha$  for its proliferation-suppressing function. In the present study, the down-regulation of Brm in BALB/c fibroblasts was sufficient to abrogate C/EBP $\alpha$ -mediated cell cycle arrest, although the expression of Brg1 was not affected by the Brm-specific siRNA (data not shown). This suggests that Brg1 can not substitute for Brm in the anti-proliferative C/EBP $\alpha$ -SWI/SNF complex, pointing at a Brm-specific role in proliferation arrest. Experiments designed to address a role of Brg1 directly, however, failed because C33A cells that stably express Brg1 could not be obtained, probably because of cell toxicity, as was also observed by others (32).

The possible existence of SWI/SNF complexes with different compositions that interact with C/EBP $\alpha$  brings up the question

of whether differentiation and proliferation are controlled by the same or by different C/EBP $\alpha$ -SWI/SNF complexes. Of note in this context are data from our previous study showing that C/EBP $\alpha$ -induced proliferation arrest and differentiation can be uncoupled from each other by human papilloma virus E7 oncoproteins from the high malignancy strains HPV16 and HPV18 (16). These oncogenes are also the only ones identified to date that overcome C/EBP $\alpha$ -mediated proliferation arrest, and they do so in a Rb/pocket protein-independent fashion. Furthermore, we observed that abrogation of C/EBP $\alpha$ -mediated proliferation arrest by HPV16E7 simultaneously enhanced C/EBP $\alpha$ -induced differentiation (16). A tempting hypothesis, therefore, is that E7 interferes with the function of a “proliferation control SWI/SNF complex” to the benefit of a “differentiation control SWI/SNF complex.”

Brm as well as Brg1 interact with the tumor suppressor Rb, and both are involved in Rb-mediated proliferation arrest (32, 34–37). C/EBP $\alpha$  can also interact with Rb (59), and a complex comprising C/EBP $\alpha$ , Rb, E2F4, and Brm was suggested to repress E2F target genes (30). However, a C/EBP $\alpha$  mutant that lacks its Rb interaction domain is still proliferation inhibitory (17). Furthermore, C/EBP $\alpha$  efficiently induces proliferation arrest also in Rb-deficient cells (13, 14, 19). We could restore the anti-proliferative potential of C/EBP $\alpha$  in C33A cells that lack functional Rb solely through ectopic expression of Brm. Therefore, our data support the view that Rb is not an essential requirement for C/EBP $\alpha$ -mediated proliferation arrest.

A particularity of C/EBP $\alpha$  is that full-length and truncated (p30) isoforms are expressed through differential translation initiation (41, 60). Several studies have demonstrated that the C/EBP $\alpha$  p30 isoform, which lacks the major transactivation domain, fails to suppress proliferation (17, 41, 48). Nevertheless, the C/EBP $\alpha$  p30 isoform can still interact with SWI/SNF (31). Therefore, a supplementary transcriptional activity may be required for effective induction of proliferation arrest by the full-length C/EBP $\alpha$ . Alternatively, additional functions, such as inhibition of E2F-mediated transcription (17), may reside in the transactivation domain that still have to be explored in greater detail. In summary, data presented here and elsewhere suggest that both proliferation and differentiation control by C/EBP $\alpha$  are tightly associated with SWI/SNF functions.

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