Prolonged Expression of c-fos Suppresses Cell Cycle Entry of Dormant Hematopoietic Stem Cells

By Seiji Okada, Tetsuya Fukuda, Kunimasu Inada, and Takeshi Tokuhisa

The proto-oncogene c-fos was transiently upregulated in primitive hematopoietic stem (Lin−Sca-1−) cells stimulated with stem cell factor, interleukin-3 (IL-3), and IL-6. To investigate a role of the c-fos in hematopoietic stem cells, we used bone marrow (BM) cells from transgenic mice carrying the c-fos gene under the control of the interferon-α/β-inducible Mx-promoter (Mx-c-fos), and fetal liver cells from c-fos-deficient mice. Prolonged expression of the c-fos in Lin−Sca-1− BM cells inhibited factor-dependent colony formation and hematopoiesis on a stromal cell layer by keeping them at G0/G1 phase of the cell cycle. These Lin−Sca-1− BM cells on a stromal layer entered into the cell cycle whenever exogenous c-fos was downregulated. However, ectopic c-fos did not perturb colony formation by Lin−Sca-1− BM cells after they entered the cell cycle. Furthermore, endogenous c-fos is not essential to cell cycle progression of hematopoietic stem cells because the factor-dependent and the stroma-dependent hematopoiesis by Lin−Sca-1− fetal liver cells from c-fos-deficient mice was not impaired. These results suggest that the c-fos induced in primitive hematopoietic stem cells negatively controls cell cycle progression and maintains them in a dormant state.

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IT IS WIDELY CONSIDERED that pluripotent hematopoietic stem cells (PHSC) are highly quiescent in normal steady state bone marrow (BM), and that dormancy plays an important role in their preservation. However, mechanisms involved in retaining PHSC at a dormant state are not well understood. Several cytokines are involved in initiation of cell cycle progression of the dormant PHSC. Those cytokines activate signal transduction pathways via their own receptors in the PHSC to induce expression of immediate early genes. Those early gene products function as transcription factors that regulate expression of target genes. Many transcription factors control proliferation and differentiation of PHSC. Although lineage-restricted transcription factors such as tal-1/SCL and rbtn2/LMO2 may be candidates as key regulator for differentiation of PHSC, widely or even ubiquitously expressed transcription factors may have a special role in maintaining PHSC in a dormant state.

The c-fos proto-oncogene, one of the immediate early genes, is transiently expressed on stimulation by external stimuli leading to cell cycle progression. Its product (c-Fos) forms a complex with the product of another proto-oncogene c-jun (AP-1) that regulates expression of AP-1-binding genes at their transcriptional level. Thus, c-Fos may play a key role in the transduction of signals induced by external stimuli. C-Fos is known to be critical for the G0/G1 transition and cell cycle progression in fibroblasts. The overexpression of c-fos in transgenic mice leads to a deregulated bone growth and results in sarcomas, and the overexpression in several cell lines leads to acceleration of cell cycle progression. On the contrary, overexpression of c-Fos negatively regulates cell cycle progression in some cell types. Thus, functions of c-Fos in cell cycle progression have remained open to question.

c-Fos is thought to be important in various differentiation processes as well as in development. Although the developmental capacity of PHSC lacking the c-fos gene appears to be fairly normal, functions of c-Fos in controlling proliferation and differentiation of PHSC are unknown. Inducible type transgenic mice are powerful tools to investigate the gene function at certain stages of development. Using the transgenic mice carrying the c-fos gene under the control of the interferon (IFN)-α/β-inducible Mx-promoter (Mx-c-fos mice), we have shown that c-Fos interferes cell cycle progression of mature B cells at the G1/S transition of the cell cycle. Furthermore, c-Fos induces apoptosis in pro-B cells and germinal-center B cells from Mx-c-fos mice. Thus, functions of c-Fos in regulating proliferation and differentiation of PHSC can be investigated using PHSC from Mx-c-fos mice.

Recent progress in the stem-cell biology revealed that PHSC and progenitors can be identified in BM cells, based on their surface marker profile. They lack lineage-specific antigens and express c-kit, H-2K, low level of Thy-1, and a high affinity to WGA. PHSC can be further isolated from committed progenitors by cell-surface staining with monoclonal antibody against Sca-1 or CD34, or by nuclear staining with supravital staining dye (rhodamine-123, Hoechst-33342). This method has made feasible studies on the nature of PHSC at the clonal level. We investigated the role of c-Fos in cell cycle progression of PHSC using primitive hematopoietic stem (Lin−Sca-1−) cells isolated from Mx-c-fos mice and also c-fos-deficient mice. We show here that the c-fos gene is transiently expressed in Lin−Sca-1− BM cells stimulated with stem cell factor (SCF), interleukin-3 (IL-3), and IL-6. The prolonged expression of c-fos inhibits Lin−Sca-1− BM cells from entering the cell cycle. The role of c-Fos in maintenance of PHSC in a dormant state is discussed.

MATERIALS AND METHODS

Mice. C57BL/6CrSlc mice were purchased from Japan SLC Co, Ltd (Hamasatsu, Japan). Transgenic mice carrying the mouse c-fos gene under the control of the Mx gene promoter (Mx–c-fos) and c-fos-deficient mice, provided by Dr E.F. Wagner (IMP, Vienna, Austria).

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GAAACCGAGAA-3

Total RNA was extracted from 1 A

Reverse-transcribed polymerase chain reaction (RT-PCR) analysis. Total RNA was extracted from 1×10⁶ Lin- Sca-1+ BM cells using an ISOGEN total RNA isolating kit (Waco, Tokyo, Japan). RNAs were reverse-transcribed using Superscript (Life Technologies, Grand Island, NY) and oligo(dT) (Pharmacia, Piscataway, NJ), in a final volume of 20 µL, and 1 µL of cDNA was used for PCR. After an initial 7-minute incubation at 95°C, 22 cycles of PCR were performed using the following conditions: c-fos cDNA, denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and polymerization at 72°C for 1.5 minutes; G3PDH cDNA, denaturation at 95°C for 1 minute, annealing at 60°C for 1 minute, and polymerization at 72°C for 1 minute. PCR primers for the cDNA amplification were as follows: the c-fos primers, 5′-TCTCGGTGTAACCGC-3′ and 5′-GGCGTT-GAAACCCGAGAA-3′; and the G3PDH primers, 5′-TGAAGGGTGTTGTTACGATTGGC-3′ and 5′-CATGAGGCGTATTGTC-GAACCAC-3′.

PCR products were separated on a 1.5% agarose gel, transferred onto a nylon membrane (Boehringer Mannheim, Mannheim, Germany) and fixed by cross-linking with ultraviolet irradiation and by baking at 80°C for 3 hours.

The probes on the filter were detected by hybridization overnight with the digoxigenin (DIG)-labeled probe at 42°C. Following hybridization, the filter was washed twice with 0.1 standard saline citrate (SSC)/0.1% sodium dodecyl sulfate at 68°C for 15 minutes. The probe on the filter was detected by sheep anti-DIG antibody conjugated with alkaline phosphatase. The antibody detection reaction was performed using the enhanced chemiluminescence cocktail of biotinylated monoclonal antibodies against lineage markers for 20 minutes at 4°C. FL cells isolated from c-fos cells were stained with a cocktail of biotinylated monoclonal antibodies against lineage markers with cold acetone for 10 minutes. The cells were then incubated with 2N HCl for 1 hour, followed by reaction with mouse monoclonal antibody to BrdU (Sigma) for 3 hours. Those cells were spread on a slide glass by sonication, Mountain View, CA) using Cell Quest software (Becton Dickinson). Those cells were incubated in hypotonic lysing buffer (0.1% sodium citrate, 0.01% Triton X, 0.1 mg/mL RNase, and 0.1 mg/mL PI). DNA content in each nuclei was analyzed on FACScan (Becton Dickinson, Mountain View, CA) using Cell Quest software (Becton Dickinson) for Macintosh (Apple Computer Inc, Cupertino, CA).

Biotinylated monoclonal antibodies against B220 (RA3-6B2), Mac-1 (M170), Gr-1 (RB6-8C5), CD4 (GK1.5), CD8 (53-6.72), CD3 (53-6.72), and TER119 were purchased from Pharmingen (San Diego, CA) and used to detect lineage markers. Fluorescein isothiocyanate (FITC)-conjugated anti-Sca-1 (Ly6A/E) antibody was purchased from Pharmingen. Biotinylated antibodies were visualized using Streptavidin-phycycoerythrin (PE; Pharmingen).

Isolation of Lin- Sca-1+ cells from BM cells or fetal liver (FL) cells. Total BM cells from Mx-c-fos mice and their littermates were stained with a cocktail of biotinylated monoclonal antibodies against lineage markers for 20 minutes at 4°C. FL cells isolated from c-fos-deficient embryos and their littermates on day 14.5 postcoitus were stained with a cocktail of biotinylated monoclonal antibodies against lineage markers except for anti-Mac-1 antibody, as described. After washing the cells 3 times with staining medium (phosphate-buffered saline with 3% fetal bovine serum [FCS] and 0.1% sodium azide), the cells were treated with streptavidin-conjugated immunomagnetic beads (BioMag; Perceptive Diagnostics, Cambridge, MA) for 30 minutes to remove lineage marker highly positive cells. The remaining cells were collected and stained with FITC–anti-Sca-1 antibody and Streptavidin-PE at 4°C for 20 minutes. After washing, the cells were resuspended in staining medium supplemented with propidium iodide (PI; 1 µg/mL). Stained cells were analyzed by FACS Vantage (Becton Dickinson, San Jose, CA), and the Lin- Sca-1+ cells were sorted and used as a primitive hematopoietic stem-cell fraction. 

In vitro colony assay. Methylcellulose culture was performed using the modified method described by Iscove. Briefly, 1 mL of culture medium contained an adequate number of total BM cells or sorted Lin- Sca-1+ cells. Culture plates were prepared in 35-mm nontissue culture dishes (Beckton Dickinson Labware, Lincoln Park, NJ) and incubated at 37°C in a humidified atmosphere of 5% CO₂. The number of colonies was counted using an inverted microscope. When IFN-α/β was added in the culture on day 4, 100 µL of alpha-medium with 200 U of IFN-α/β was added to the methylcellulose culture dish.

Spleen colony assay. The spleen colony assay of Till and McCulloch was used. Freshly isolated or cultured Lin- Sca-1+ cells were injected into lethally irradiated mice (9.0 Gy total body irradiation). The spleens were removed on day 8 or day 12 after transplantation, fixed in Bouin’s solution, and macroscopically visible colonies were counted and scored as colony forming unit in spleen (CFU-S).

Coculture of Lin- Sca-1+ cells with stromal cells. PA-6 stromal cells support myelopoiesis. PA-6 cells (3×10⁶/well) were seeded in a 6-well plate (PRIMARIA, Beckton Dickinson Labware) 1 day before coculture. Five hundred sorted Lin- Sca-1+ cells were cultured on the PA-6 stromal layer with 3 µL of alpha-medium containing 10% FCS in the presence (200 U/mL) or absence of IFN-α/β.

Cell cycle analysis. Cell cycle analysis was performed as described by Nicoletti. Briefly, sorted Lin- Sca-1+ cells were cultured for 24 hours with SCI, IL-3, and IL-6 in the presence or absence of IFN-α/β. Those cells were incubated in hypotonic lysing buffer (0.1% sodium citrate, 0.01% Triton X, 0.1 mg/mL RNase, and 0.1 mg/mL PI). DNA content in each nuclei was analyzed on FACScan (Becton Dickinson, Mountain View, CA) using Cell Quest software (Becton Dickinson) for Macintosh (Apple Computer Inc, Cupertino, CA).

5-bromo-2-deoxyuridine (BrdU) incorporation assay. Sorted Lin- Sca-1+ cells were cultured with SCI, IL-3, and IL-6 in the presence or absence of IFN-α/β for 36 hours and pulsed with 10 µmol/L BrdU (Sigma) for 3 hours. Those cells were spread on a slide glass by Cytospin (Shandon Southern Instruments Inc, Sewickley, PA) and fixed with cold acetone for 10 minutes. The cells were then incubated with 2N HCl for 1 hour, followed by reaction with mouse monoclonal antibody to BrdU (Boehringer Mannheim, Indianapolis, IN). These cells were further incubated with F(ab')₂ fragment of anti-mouse Ig labeled with horseradish peroxidase (Nycod Amersham plc, Buckinghamshire, UK). The DAB kit (Nichirei, Tokyo, Japan) was used to visualize peroxidase, and counter staining was done using hematoxylin.

RESULTS

Expression of the c-fos gene in primitive hematopoietic stem cells stimulated with SCI, IL-3, and IL-6. To examine expression of the c-fos gene in primitive hematopoietic stem cells after stimulation, Lin Sca-1+ BM cells from Mx-c-fos mice and their control littermates were stimulated with SCI, IL-3, and IL-6.

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IL-6 in the presence or absence of IFN-α/β. Expression of c-fos was analyzed by RT-PCR with Southern blotting (Fig 1A). c-fos RNA was detected in the Lin-2 Sca-1+ cells from both Mx–c-fos and control mice at 1 hour but not at 7 hours after stimulation in the absence of IFN-α/β, thereby indicating transient expression induced in these hematopoietic stem cells. When the cells were stimulated with SCF, IL-3, and IL-6 in the presence of IFN-α/β (200 U/mL), a large amount of c-fos RNA was evident in the Mx–c-fos but not in control stem cells, even 7 hours after stimulation. When IFN-α/β was added to those cultures on day 0 and day 4 slowed down the colony formation and lowered the plateau level in the Mx–c-fos cultures but not in the control cultures. These observations suggest that prolonged expression of c-fos suppresses the onset of colony formation by primitive hematopoietic stem cells stimulated with SCF, IL-3, and IL-6.

To further analyze the inhibitory effect of c-fos on growth of the colony, we serially plotted growth rate of each colony through colony mapping study, as described by Ikebuchi. One hundred and fifty of Lin- Sca-1+ cells per dish were cultured with SCF, IL-3, IL-6, and Epo, and emergence of a new colony later showed the CFU-Mix and its subsequent rate of prolifera-
independent experiments. The data presented are representative of two
number of colonies was scored every 4 days. Results represent mean

c-Fos IN DORMANT HEMATOPOIETIC STEM CELLS 819

d (200 U/mL) was added on day 0 (s), or on day 0 and day 4 ( ). The
number of colonies was scored every 4 days. Results represent mean
and SD of four dishes. The data presented are representative of two
independent experiments.

-IFN-α/β
+IFN-α/β (day 0)
+IFN-α/β (day 0.4)

0 4 8 12 16 20
Days in Culture

Number of Colonies / 300 Cells

Mx-c-fos mouse

0 20 40 60 80

Days in Culture

Number of Colonies / 300 Cells

B

Control mouse

Fig 3. Kinetics of colony formation by primitive hematopoietic stem cells. Lin−Sca-1− BM cells (3 × 10^6) from Mx-c-fos mice or control littermates were cultured with SCF, IL-3, and IL-6 ( ). IFN-α/β (200 U/mL) was added on day 0 ( ), or on day 0 and day 4 ( ). The number of colonies was scored every 4 days. Results represent mean and SD of four dishes. The data presented are representative of two independent experiments.

tion were analyzed (Fig 4). Although the number of CFU-Mix was about 13 to 16 per dish in both Mx-c-fos and control cultures in the presence (200 U/mL) or absence of IFN-α/β, the onset of each colony was delayed in Mx-c-fos cultures but not in control cultures when IFN-α/β was added on day 0 of culture. When IFN-α/β was added to those cultures on day 0 and day 4, the onset of each colony was further delayed in the Mx-c-fos cultures. However, the proliferation rate of each colony developed in the Mx-c-fos cultures did not differ significantly from that in the control cultures in the presence of IFN-α/β. Because 200 U/mL of IFN-α/β can induce expression of the exogenous c-fos gene in BM cells from Mx-c-fos mice for more than 2 days,^{26,28} these results suggest that after entering the cell cycle c-fos does not inhibit growth of colonies derived from primitive hematopoietic stem cells.

Effect of c-fos on cell growth of primitive hematopoietic stem cells stimulated with various combinations of cytokines in the liquid culture. The combination of SCF, IL-3, and IL-6 provides one of the strongest signals for differentiation of stem cells and progenitors.^{1,36} Stimulation of SCF and IL-6 is required for expansion of immature cells^{48} and that of IL-3 alone supports differentiation of stem cells and progenitors in a cycling state.^{1,47} To examine which cytokine signals were inhibited by c-fos, we did short-term liquid cultures of stem cells from Mx-c-fos mice, using various combinations of cytokines by adding IFN-α/β (200 U/mL) on day 0 and day 4 of culture, and the number of nucleated cells was counted on day 7 of culture (Fig 5). When total BM cells were cultured with combinations of cytokines, cell growth in the Mx-c-fos cultures with SCF+IL-6 stimulation was suppressed in the presence of IFN-α/β. However, cell growth in Mx-c-fos cultures with SCF+IL-3+IL-6 or IL-3 alone was not suppressed, suggesting that the prolonged expression of c-fos inhibits cell growth of primitive hematopoietic stem cells. Indeed, cell growth in cultures of Lin−Sca-1− BM cells from Mx-c-fos mice but not from control littermates with all of the combinations of cytokines used was inhibited in the presence of IFN-α/β.

To confirm the suppressive effect of c-fos on cell growth of primitive hematopoietic stem cells, cell cycle analysis of Lin−Sca-1− BM cells stimulated with SCF, IL-3, and IL-6 in the liquid culture was done using fluorescence-activated cell sorter (FACS). As shown in Fig 6A, the percentage of cells in the S/G2/M phase was approximately 15% in Mx-c-fos and control cultures in the absence of IFN-α/β and in control cultures in the presence of IFN-α/β (200 U/mL) 24 hours after stimulation. In contrast, only 3.3% of the Lin−Sca-1− cells from Mx-c-fos mice were in the S/G2/M phase in the presence of IFN-α/β. Similar results were obtained in the case of BrdU incorporation assay (Fig 6B). Approximately 30% of cells were BrdU positive in cultures from Mx-c-fos and from control mice in the absence of IFN-α/β and in control cultures in the presence of IFN-α/β 36 hours after stimulation. However, only 5% of cells were positive for BrdU in Mx-c-fos cultures in the presence of IFN-α/β.

To examine the suppressive effect of c-fos on differentiation of hematopoietic stem cells, we performed the spleen colony assay.^{42} Five hundred Lin−Sca-1− BM cells were cultured with SCF+IL-3+IL-6 in the presence (200 U/mL) or absence of IFN-α/β. On day 4 of culture, cells were collected and injected into lethally irradiated mice. As shown in Table 1, generation of day-8 CFU-S but not day-12 CFU-S was suppressed in mice injected with these cultured cells from Mx-c-fos mice in the presence of IFN-α/β. The number of day-8 CFU-S and that of day-12 CFU-S reflect the number of hematopoietic progenitor cells and that of more primitive hematopoietic stem cells.^{42,49} respectively. Therefore, these results indicate that prolonged expression of c-fos suppresses differentiation of hematopoietic stem cells.
Effect of c-fos on hematopoiesis by primitive hematopoietic stem cells cultured on a stromal cell layer. To investigate the suppressive effect of c-fos on hematopoiesis by primitive hematopoietic stem cells on a stromal cell layer, Lin\(^2\)Sca-1\(^1\) BM cells were cultured on a layer of PA-6 stromal cells. Medium in the cultures was changed twice a week, and the number of nonadherent cells was counted by Trypan blue exclusion. As shown in Fig 7, the number of cells in both Mx–c-fos and control cultures exponentially increased and reached a plateau after day 12 of culture. When IFN-α/β (200 U/mL) was added to medium from the start of culture, the number of cells in Mx–c-fos cultures did not clearly increase. When addition of IFN-α/β in the medium was ceased after day 12 of culture, the number of cells increased and caught up to the control level in the Mx–c-fos cultures within 1 week. These data suggest that prolonged expression of c-fos to primitive hematopoietic stem cells also inhibits stroma-dependent hematopoiesis.

Factor-dependent and stroma-dependent hematopoiesis by primitive hematopoietic stem cells from c-fos–deficient mice. FL cells from c-fos–deficient mice was used as the source of primitive hematopoietic stem cells because of the osteopetrosis in c-fos–deficient mice.\(^{20,21}\) Kinetics of colony formation by Lin\(^-\)Sca-1\(^-\) cells was examined in the cultures with SCF, IL-3, IL-6, and Epo. IFN-α/β (200 U/mL) was added on day 0, or on day 0 and day 4. Graphic presentation indicates cell number changes in individual colonies that later became a mixed colony (CFU-Mix). The data represent colonies identified in two plates. The data presented are representative of two independent experiments.

DISCUSSION

In the steady state, the majority of hematopoietic stem cells reside in BM at a dormant state,\(^{1,49,50}\) and only a few stem cells supply all of the hematopoietic cells at a given time.\(^1\)\(^,\)\(^51\) Because hematopoietic stem cells in BM are always exposed to various forms of stimuli, mechanisms to maintain the stem cells in a dormant state no doubt exist. We found that c-fos negatively controls cell cycle progression of primitive hematopoietic stem cells. Initiation of cell cycling and subsequent proliferation of stem cells appear to require collaboration of early acting cytokines. Ogawa proposed that cytokines regulating proliferation of primitive hematopoietic progenitors may be separated into three arbitrary groups.\(^1\) The combination among these three groups such as SCF, IL-3, and IL-6 is one of the most effective stimuli supporting the early process of hematopoiesis.\(^1\)\(^,\)\(^36\) These stimulations transiently induce expression of c-fos in primitive hematopoietic stem cells (Fig 1). Because prolonged expression of c-fos inhibits G0/G1 transition of dormant hematopoietic stem cells in both cytokine-dependent (Figs 2 to 5) and stroma-dependent (Fig 8) hematopoiesis, downregulation of the
c-Fos may initiate G0/G1 transition. Indeed, cell proliferation began in the stem cell culture from Mx-c-fos mice whenever addition of IFN-α/β (200 U/mL) was added to the culture on day 0 and day 4. On day 7 of culture, the number of viable cells was counted by Trypan blue dye exclusion. Results represent mean and SD of four dishes. The data presented are representative of three independent experiments. Purified recombinant SCF was used in this experiment.

The inhibitory effect of c-fos on cell cycle progression of dormant hematopoietic stem cells was also shown in a mapping study (Fig 4). However, growth rate was not significantly affected by the expression after colony formation began. This is supported by the finding that colony formation by total BM cells was affected by c-fos much less than that by purified Lin- Sca-1+ BM cells because the majority of colony-forming cells in total BM are committed progenitors. These results suggest that endogenous c-fos negatively regulates cell cycle progression of dormant hematopoietic stem cells.

Embryonal stem cells and 3T3-type fibroblasts lacking the c-fos gene divide at a normal rate. Furthermore, c-fos-deficient mice are viable but do have osteopetrosis, as a primary pathology. Although they have extramedullary hematopoiesis in the spleen, B lymphopenia, and thymic atrophy, we have found that hematopoietic stem cells can appear normal, except for failure differentiation into functional osteoclasts. In the present study, we found that both factor-dependent and stroma-dependent hematopoiesis by primitive hematopoietic stem cells from c-fos-deficient mice are normal (Fig 8 and 9). Colony formation by stem cells stimulated with IL-3+IL-6+SCF or IL-3

### A Whole Bone Marrow Cells

<table>
<thead>
<tr>
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<th>IFN-α/β</th>
<th>Mx-c-fos</th>
<th>Control</th>
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<tbody>
<tr>
<td>SCF+IL-3+IL-6</td>
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</tr>
<tr>
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### B Lin- Sca-1+ Cells

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**Fig 5. Inhibitory effect of c-fos on cell proliferation by primitive hematopoietic stem cells.** Lin- Sca-1+ BM cells from Mx-c-fos or control littermates were cultured with SCF+IL-3+IL-6, SCF+IL-6, or IL-3. IFN-α/β (200 U/mL) was added to the culture on day 0 and day 4. On day 7 of culture, the number of viable cells was counted by Trypan blue dye exclusion. Results represent mean and SD of four dishes. The data presented are representative of three independent experiments. Purified recombinant SCF was used in this experiment.
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ified recombinant SCF was used in this experiment.

Table 1. Spleen Colony Formation by Lin−Sca-1+ BM Cells Following
Prolonged Expression of c-fos

<table>
<thead>
<tr>
<th>Mice</th>
<th>IFN-α/β</th>
<th>Day-8 CFU-S</th>
<th>Day-12 CFU-S</th>
<th>Day-8/Day12 CFU-S Ratio</th>
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<tr>
<td>Control</td>
<td></td>
<td>5.3 ± 1.5</td>
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<tr>
<td>+</td>
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<td>7.5 ± 2.9</td>
<td>10.8 ± 2.9</td>
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<tr>
<td>Mx-c-fos</td>
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<td>6.4 ± 2.5</td>
<td>10.3 ± 4.2</td>
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</tr>
<tr>
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<td>1.0 ± 1.4</td>
<td>13.7 ± 5.1</td>
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</table>

Five hundred Lin−Sca-1+ BM cells from Mx-c-fos or control
littermates were cultured with SCF + IL-3 + IL-6 in the presence (200 U/mL)
or absence of IFN-α/β. On day 4 of culture, those cells were collected
and injected into lethally irradiated mice. Spleens from the mice were
removed on day 8 or day 12 after the injection, fixed in Bouin’s
solution, and the number of macroscopically visible spleen colonies
was determined. Results represent mean and SD of 5 mice. The data
presented are representative of two independent experiments.
Hematopoietic stem cells with the c-fos expression in culture survived in a dormant state and entered the cell cycle after c-fos was downregulated. We propose that c-Fos plays the role of gatekeeper in cell cycle progression of dormant hematopoietic stem cells.

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Prolonged Expression of c-fos Suppresses Cell Cycle Entry of Dormant Hematopoietic Stem Cells

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