Protein Stability and Transcription Factor Complex Assembly Determined by the SCL-LMO2 Interaction

Determined by the SCL-LMO2 Interaction*5

From the "Institut de Recherche en Immunologie et Cancérologie and the Departments of "Molecular Biology, "Pharmacology, and "Biochemistry, Université de Montréal, Montréal, Québec H3C 2J7, Canada and the "Institut de Recherche Clinique de Montréal, Montréal, Québec H2W 1R7, Canada

Eric Lécyer,1,2, Simon Lariviére,3, Marie-Claude Sincennes,4,5, André Haman,1, Rachid Lahli1, Margarita Todorova,5, Mathieu Tremblay,5, Brian C. Wilkes,6, and Trang Hoang1,5,[*][**][‡][§][¶][1]

Gene expression programs are established by networks of interacting transcription factors. The basic helix-loop-helix factor SCL and the LIM-only protein LMO2 are components of transcription factor complexes that are essential for hematopoiesis. Here we show that LMO2 and SCL are predominant interaction partners in hematopoietic cells and that this interaction occurs through a conserved interface residing in the loop and helix 2 of SCL. This interaction nucleates the assembly of SCL complexes on DNA and is required for target gene induction and for the stimulation of erythroid and megakaryocytic differentiation. We also demonstrate that SCL determines LMO2 protein levels in hematopoietic cells and reveal that interaction with SCL prevents LMO2 degradation by the proteasome. We propose that the SCL-LMO2 interaction couples protein stabilization with higher order protein complex assembly, thus providing a powerful means of modulating the stoichiometry and spatiotemporal activity of SCL complexes. This interaction likely provides a rate-limiting step in the transcriptional control of hematopoiesis and leukemia, and similar mechanisms may operate to control the assembly of diverse protein modules.

Tissue-specific programs of gene expression are largely controlled by networks of interacting transcription factors that provide flexibility and specificity in gene regulation (1, 2). This theme finds particular relevance in the context of the hematopoietic system, where the generation of diverse cellular lineages is controlled by combinations of hematopoietic-specific and broadly expressed transcription factors (3–5). Regulation by networking is exemplified by the SCL/TAL-1 (hereafter referred to as SCL) transcription factor, which is essential for the establishment of the hematopoietic system and plays important functions at several branch points in the hematopoietic hierarchy (6).

SCL is a member of a subfamily of tissue-specific bHLH transcription factors that includes two other hematopoietic factors, TAL2 and LYL-1 (7), and two neurogenic factors, NSCL1/ nHLH-1 and NSCL2 (8). Transcription regulation by SCL requires its integration within multifactorial complexes (SCL complexes) containing the ubiquitously expressed bHLH factors encoded by the E2A gene (E47 and E12), LMO2 proteins, the LIM domain-binding protein Ldb1, and hematopoietic GATA family members (9–11). These complexes govern the switch between a proliferative state in erythroid progenitors and a commitment to terminal differentiation (12), and different variants of these complexes activate transcription of target genes such as c-kit, glycophorin A (GPA), and protein 4.2 in progenitor and erythroid cells (13–15). These observations are consistent with the view that subtle variations in the composition of transcription factor complexes modulate choices in cell fate. Interestingly, SCL complexes exhibit an all-or-nothing behavior in transcription activation requiring the simultaneous presence of each partner for robust target gene activation (13, 15). These complexes have, therefore, been proposed to form multi-input motifs within the hematopoietic regulatory hierarchy (5), a network feature that ensures specificity and flexibility in gene regulation. Despite these findings, the mechanisms governing the assembly of SCL-containing complexes on target gene regulatory elements remain ill-defined.

It has recently been shown that the SCL-LMO2 interaction is essential for hematopoietic cell fate specification (16–18), consistent with the phenotypic similarity of SCL−/− and LMO2−/− embryos, which exhibit early lethality due to a complete absence of blood cells (19–24). In addition to their important function during normal hematopoiesis, the SCL and LMO2 genes are the most frequent targets of chromosomal rearrangements in pediatric T-cell acute lymphoblastic leukemia (7, 25).

* This work was funded by grants from the Canadian Institutes of Health Research and the National Cancer Institute of Canada. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3 and Table S1.

1 Recipients of Canadian Institutes of Health Research studentships.
2 Present address: Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario M5S 3E1 Canada.
3 Recipient of the Canada Research Chair in Cell Differentiation and the Genetics of Acute Leukemias.
4 To whom correspondence should be addressed: Institut de Recherche en Immunologie et Cancérologie, Université de Montréal, P. O. Box 6128, Downtown Branch, Montréal, Québec H3C 2J7, Canada. Tel.: 514-343-6970; Fax: 514-987-5757; E-mail: Trang.hoang@umontreal.ca.

5 The abbreviations used are: BFU-E, burst-forming unit erythroid; bHLH, basic helix-loop-helix; CFU-GM/Meg, colony-forming unit granulocyte-macrophage/megakaryocyte; EMSA, electrophoretic mobility shift assay; Epor, erythropoietin receptor gene; F-LMO2, FLAG-LMO2; GFP, green fluorescence protein; GPA, glycophorin A; IB, immunoblotting; IP, immunoprecipitation; IRES, internal ribosome entry site; Ldb1, LIM domain-binding protein-1; LIM, Lin-Isle-Mec; LMO, LIM-only; MSCV, murine stem cell virus; NSCL1, neuronal SCL-1; SCL/TAL1, stem cell leukemia/T-cell acute leukemia-1; IF, immunofluorescence; PBS, phosphate-buffered saline; HA, hemagglutinin; RT, reverse transcription; GST, glutathione S-transferase.
Molecular Functions of the SCL-LMO2 Interaction

Furthermore, retroviral integration within the LMO2 gene, leading to a severe lymphoproliferative disorder, has been noted after gene therapy in one clinical trial (26). Finally, SCL genetically interacts with LMO1/2 to induce aggressive T-cell tumors in transgenic mice (7), underscoring the importance of the SCL-LMO2 interaction in vivo.

Despite this extensive body of evidence conveying the biological importance of the SCL-LMO2 interaction, it is not clear why this interaction is important at the molecular level. In addition, because LIM domain proteins, including LMO2, are known to be targeted for proteasomal degradation (27, 28), this raises the possibility that LMO2 degradation may represent a rate-limiting step in the assembly of SCL complexes. In the present study we show that SCL prevents the degradation of LMO2 via direct protein-protein interaction, allowing for the nucleation of multifactorial complexes with proper SCL/LMO2 stoichiometry.

EXPERIMENTAL PROCEDURES

Plasmids and Mutagenesis—A detailed description of plasmid construction methodology is provided as supplemental material.

Cell Culture, Infections, Transfections, Immunofluorescence (IF), and Protein Extraction—NIH 3T3, 293, ts20, and TF-1 cells were cultured as described previously (15, 29, 30). Primary fetal liver cells were isolated from E12.5 Epor−/− and SCLlacZ/WT embryos, sorted for TER119 expression, and cultured as described before (15). Fetal cell liver infections were performed by overnight co-culture on GP+E packaging cells stably expressing the viruses encoding SCL and SCL-M13. After infection, the cells were cultured in the presence of interleukin-3 (50 ng/ml) and steel factor (5 ng/ml) for an additional 24 h before performing further analyses. Infections of TF-1 cells with control retroviruses (MSCV-neo) or viruses encoding SCL and SCL-M13 were performed as detailed previously (15). Transient transactivation assays were performed as previously described (13, 15). Expression vector doses used in specific experiments are indicated in figure legends. In all samples the amount of total DNA was kept constant at 4.5 μg with pGem4. Proteasomal inhibitor (MG132, lactacystin) or cycloheximide treatments were carried out as described in figure legends from 4 h to overnight. After treatment, the cells were washed twice with cold PBS and instantaneously frozen on liquid nitrogen. Total cell protein extracts were then prepared by lysing the cells for 20 min at 4 °C with 400 μl of radioimmunoprecipitation assay buffer (10 mm Tris-HCl (pH 8.0), 140 mm NaCl, 1 mm EDTA, 1% Triton X-100, 0.1% SDS, 0.1% deoxycholate) supplemented with protease inhibitors.

EMSA, Pulldown Assays, IBs, and IPs—For EMSA, each binding reaction was performed using 10 μg of TF-1 or 293 cell nuclear extract in 20 mm HEPES (pH 7.5), 50 mm KCl, 1 mm dithiothreitol, 1 mm EDTA, 5% glycerol, 10 μg bovine serum albumin, 0.5 μg of dl-dC, and the GPA-84 probe (10,000 cpm) in a final volume of 20 μl. After 15 min at room temperature, protein-DNA complexes were resolved at 150 V on a 4% PAGE gel in 0.5× Tris-buffered EDTA at 4 °C for 4 h. Pulldown experiments were performed as previously described (13, 15). For IPs, 293 (10–60 μg) or TF-1 (500 μg) cell nuclear extracts were incubated overnight at 4 °C with 3 μg of antibody in 1 ml of IP buffer (20 mm Tris-HCl (pH 8.0), 137 mm NaCl, 1% Nonidet P-40, 10% glycerol, 1 mm EDTA). Protein complexes were precipitated by adding appropriately conjugated Pansorbin cells (Calbiochem) for 30–120 min at 4 °C, washed 3 times with 1 ml of IP buffer, and subjected to SDS-PAGE. After transfer on PVDF membranes, proteins were visualized by immunoblotting (IB) using ECL plus (GE Healthcare). The following antibodies were used for IP, IB, and IF analysis. The mouse anti-E2A (YAE), rat anti-GATA-1 (N6), rabbit anti-GFP (FL), goat anti-LMO2 (N-16), and goat anti-Ldb1/CLIM-2 (N-18) were all from Santa Cruz Biotechnology Inc (Santa-Cruz, CA). The BTL-73 and 2TL-136 mouse anti-SCL antiserum were provided by Dr. D. Mathieu (Institut de Génétique Moléculaire, Montpellier, France). The mouse anti-HA, anti-FLAG, and anti-P21 antibodies were obtained from Covance (Richmond, VA), Stratagene (La Jolla, CA), and BD Biosciences (Mississauga, ON, Canada), respectively.

RT-PCR Analysis—Total RNA was extracted from TF-1 or 293 cells as previously described (15). To eliminate contaminating DNA molecules, 500 ng of each sample was then subjected to digestion with EcoRI and DNase I in REact3 buffer (Invitrogen). After the nucleases were heat-inactivated for 10 min at 65 °C, the samples were subjected to reverse transcription using the Superscript first strand cDNA synthesis system (Invitrogen). The quantification of GPA, β-major, LMO2, and S14 and
S16 mRNAs was performed by real time PCR on a MX3000 apparatus (Stratagene, La Jolla, CA) using QuantiTect SYBR green PCR kit (Qiagen, Mississauga, ON, Canada) according to the manufacturer’s instructions and under the following conditions: 95 °C for 15 min followed by 50 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s. Oligonucleotides used for RT-PCR analysis were described previously (15).

Structural Modeling—The structure of the E47 homodimer-DNA complex (32) was obtained from the Brookhaven protein data base and was subjected to 1000 steps of conjugate gradient minimization. One of the E47 molecules was transformed into hydrogen SCL using homology modeling techniques. After the initial model was obtained, the side chains of the mutated amino acids were packed using SYBYL (Tripos, St. Louis, MO). In this procedure the backbone dihedral angles are held fixed, whereas the side chains of the individual amino acids are rotated one at a time until a sterically acceptable conformation was obtained. This structure was again minimized, at which time no major conformational changes were observed.

RESULTS

SCL and LMO2 Are Predominant and Specific Interaction Partners—The SCL and LMO2 interaction is essential for hematopoiesis. To determine what proportion of LMO2 is associated with SCL in hematopoietic cells, we performed quantitative IPs of nuclear extracts of CD34+ TF-1 cells with an antibody against SCL or control immunoglobulins. As shown in Fig. 1A, all of the SCL protein was precipitated with an anti-SCL antibody (lanes 2 and 4), whereas the protein remained in the supernatant with a control antisera (lanes 1 and 3). Under these conditions, most of LMO2 co-precipitated with SCL, and only 20% of LMO2 was found in the supernatant (lane 2). Furthermore, by performing successive SCL and Ldb1 IPs, we found that the majority LMO2 that did not come down with the SCL antibody was co-precipitated with anti-Ldb1 antiserum (data not shown). In contrast, all of LMO2 was recovered in the supernatant of control IPs (lane 1). Therefore, we conclude that the majority (~90%) of LMO2 is associated with SCL and Ldb1 in TF-1 cell extracts. In agreement with these findings, all three of these factors were found to exhibit overlapping nucleoplasmic localization patterns, as determined by IF analysis in TF-1 cells (Fig. 1B).

Together, our observations concur with the view that SCL, LMO2, and Ldb1 are predominant interaction partners in hematopoietic cells. SCL has recently been shown to exhibit exquisite specificity in comparison to other bHLH factors in inducing hematopoietic cell fate commitment in embryonic stem cells (17). SCL shares 64% identity within the bHLH domain with the closely related neurogenic factor NSCL1, and both proteins heterodimerize with the ubiquitous bHLH factor E47. Furthermore, LMO2, which is 59% identical to LMO1 within the LIM domains, interacts with SCL but not E47. To assess the specificity of the SCL-LMO2 interaction, we compared the binding properties of SCL and NSCL1 in pulldown assays. As expected, both SCL and NSCL1 interact with GST-E47 (Fig. 1C). Interaction with LMO proteins, however, revealed a striking difference. Both of the main isoforms of SCL (p42 and p22) associate equally well with LMO1 and LMO2. In contrast, NSCL1 was only weakly retained on GST-LMO1 columns and was not retained on GST-LMO2 columns. These results indicate that interaction with LMO2 is specific to SCL.

To ascertain whether this interaction specificity is also observed in transfected cells, we next performed IPs using nuclear extracts from 293 cells expressing SCL or HA-NSCL1, in combination with FLAG-LMO2 (F-LMO2), E47, and Ldb1. Increasing amounts of nuclear extracts were subjected to IP using anti-SCL, anti-FLAG, or anti-HA antibodies. In SCL-containing extracts, IP with both the anti-SCL and anti-FLAG

FIGURE 1. SCL predominantly and specifically interacts with LMO2. A, in hematopoietic progenitor cells most of LMO2 is associated with SCL. TF-1 cell nuclear extracts (500 μg) were subjected to IP using an anti-SCL antibody or control immunoglobulins. Samples of the precipitated (Pellet) or unbound supernatant (SN) fractions were analyzed by IB as indicated. The asterisks indicate Ig heavy chains. B, SCL co-localizes with LMO2 and Ldb1 in the nuclei of hematopoietic cells. TF-1 cells were double-stained with antibodies directed against SCL and LMO2 (upper panels) or with SCL and Ldb1 (lower panels). Red, SCL; green, LMO2 or Ldb1; blue, Hoechst-stained nuclei. C, SCL but not NSCL1, interacts with LMO2 in vitro. Pulldown experiments were performed with immobilized GST, GST-LMO1, GST-LMO2, GST-E47, and 35S-labeled SCL and NSCL1. Input samples (lanes 1) represent 10% of the amount used for the binding reactions (lanes 2–5). D, SCL but not NSCL1 interacts with LMO2 in transfected cells. Extracts of 293 cells (10, 30, and 60 μg) transfected with expression vectors (5 μg each) for E47, F-LMO2, Ldb1, GFP, and SCL (lanes 1–7), or HA-NSCL1 (lanes 8–14) were immunoprecipitated with the indicated antibodies and revealed by IB with antibodies listed to the right of each panel. Lane 1 represents input (I) samples (10 μg), and arrowheads point to specific bands. Asterisks indicate Ig light or heavy chains.
Molecular Functions of the SCL-LMO2 Interaction

antibodies efficiently precipitated SCL, F-LMO2, and E47 (Fig. 1D, lanes 1–7), showing that these factors associate in transfected cells. With HA-NSCL1 extracts, however, the anti-HA antibody brought down HA-NSCL1 and E47 but was unable to co-precipitate F-LMO2 (lanes 9–11). In agreement, the anti-FLAG antibody precipitated F-LMO2 but not HA-NSCL1 or E47 (lanes 12–14). Finally, co-expressed GFP protein, included as a negative control, was not precipitated with any of the antibodies. Because LMO2 and E47 only co-precipitate in the presence of SCL, we conclude that SCL acts as a bridging factor between its two partners. More importantly, whereas both SCL and NSCL1 are able to associate with E47, interaction with LMO2 is restricted to SCL.

Specificity of SCL in Hematopoietic Target Gene Activation and Assembly of Transcription Factor Complexes—To determine whether SCL also demonstrates specificity at the molecular level during hematopoietic target gene activation, we next performed transactivation assays of the c-kit and GPA promoters, two well characterized targets of SCL-containing complexes (13, 15). This approach previously revealed an essential requirement for each partner of the SCL complex in transcriptional regulation (13, 15). As suspected, although SCL-containing complexes efficiently activate the c-kit and GPA promoters, neither the neurogenic bHLH factor NSCL1 nor the myogenic factor MyoD is able to substitute for SCL function (Fig. 2A, left panels) despite being efficiently expressed in transfected cells (Fig. 2A, right panel). In contrast to SCL, other partners of the complex are functionally redundant with members of their respective families (supplemental Fig. S1).

To assess whether SCL is specifically required for the assembly of SCL complexes on DNA, we next performed EMSA using a probe derived from the GPA promoter (GPA-84), to which the SCL complex was previously shown to bind with high affinity (15). As shown in Fig. 2B, SCL and its partners, expressed in 293 cells, form a low mobility complex (arrowhead) on the GPA-84 probe (lane 5), which is comparable with that seen with endogenously proteins from hematopoietic TF-1 cells (lane 2) and is distinct from nonspecific complexes (asterisks) observed with untransfected 293 cell extracts (lane 3). This low mobility complex is supershifted by antibodies against SCL and its partners (data not shown; Ref. 13) and is not seen with 293 extracts lacking SCL but expressing the other partners of the complex (lane 4). Interestingly, NSCL1 was unable to substitute for SCL in nucleating the assembly of this complex on DNA (lane 6), in agreement with transactivation experiments. The expression of SCL and its partners as well as NSCL1 in 293 cell extracts was confirmed by IB (lanes 7–11). We conclude that SCL confers specificity in hematopoietic target gene activation and in the assembly SCL complexes on DNA.

SCL Interacts with LMO2 via Multiple Residues in the Loop and Helix 2—Schlaeger et al. (17) previously identified residues of the HLH domain that are critical for interaction with LMO2 and for the rescue of primitive erythropoiesis in SCL2/−/− ES cells. These include residues His-217 of the loop region and Phe-238 residing in Helix 2, which was also recently shown to be required for the hematopoietic inducing potential of SCL in zebrafish (18). It is not known, however, whether the same residues are required for the other molecular and biological functions of SCL, i.e. the induction of definitive erythropoiesis and the inhibition of lymphoid lineages. Therefore, to identify the determinants of SCL molecular specificity, based on comparisons with NSCL1, we first generated a series of SCL-NSCL1 domain swap mutants and tested their functional activity in transactivation assays. As expected, this approach revealed that specificity in transcriptional activation and interaction with LMO2 is imparted by the loop and helix 2 region of SCL (supplemental Fig. S2).

FIGURE 2. Non-redundant function of SCL in hematopoietic gene activation and complex formation on DNA. A, SCL is non-redundant with other bHLH factors in c-kit and GPA promoter activation. The kit-1146-Luc or GPA-84-Luc reporter constructs (1500 ng) were transfected into NIH 3T3 cells with expression vectors for E47 (150 ng), LMO2 (750 ng), Ldb1 (750 ng), and GATA-1 or -2 (150 ng) and the indicated amounts of SCL, NSCL1, and MyoD vectors (50–450 ng). Transactivation assays with kit-1146-Luc were conducted using GATA-1-containing complexes (upper graph), whereas GPA-84-Luc was assayed with complexes containing GATA-2 (lower graph). Results are expressed as fold activation relative to the reporter vector transfected alone, represent the average ± S.D. of triplicate determinations, and are representative of (n) independent experiments. Right panel, nuclear extracts of TF-1 cells (10 μg) or NIH 3T3 cells (10 μg) expressing SCL or HA-NSCL1 were analyzed by IB with the indicated antibodies. Expression of GFP in transfected cells was monitored as a control for equal loading. Arrowheads indicate specific bands. B, SCL is specifically required to assemble a high molecular weight complex on the GPA promoter sequences. EMSAs were performed using the GPA-84 probe (arrow), which covers positions −84 to −30 of the human GPA promoter (15), and nuclear extracts (10 μg) of TF-1 cells (lane 2) or 293 cells transfected with the indicated expression vectors (lanes 3–6). Note the low mobility complex containing SCL and its partners (arrowhead) (15), which is not formed in the presence of NSCL1. Asterisks indicate nonspecific complexes. Proteins expressed in the 293 cell extracts (10 μg) used for EMSA were revealed by IB with the indicated antibodies (lanes 7–10).
To further define the SCL-LMO2 interaction interface, we next systematically substituted patches of diverging residues of the HLH domain of SCL by those found at the corresponding positions in NSCL1. Comparison of HLH domains of SCL and NSCL1 reveals 15 amino acid differences (Fig. 3A). Intriguingly, mutations in single domain motifs (mutants M1 to M11) within the HLH domain had no effect on SCL function (Fig. 3A). It was particularly surprising to find that the N237S and F238Y substitutions (mutant M6, Fig. 3A) did not affect SCL activity, since Schlaeger et al. (17) previously showed that conversion of Phe-238 into a Gly residue, as found in the MyoD Helix2, disrupts SCL function during primitive hematopoiesis. They further showed that in the context of weakly active SCL chimeras harboring only the helix 2 of NC5L1 or MyoD, re-introducing Phe-238 partially restored the capacity of the chimeras to rescue SCL−/− ES cells (17). This suggests that Tyr and Phe residues at position 238 are functionally equivalent, whereas non-conservative changes (such as F238G) disrupt SCL function. This also indicates that additional residues within the loop and helix 2 of SCL contribute to SCL specificity and interaction with LMO2.

To further address this question, we performed phylogenetic comparisons of the HLH region of SCL orthologues from humans to fruit flies. This analysis reveals a near perfect conservation of residues, with the exception of Drosophila melanogaster SCL, which exhibits 13 amino acid differences compared with human SCL (Fig. 3A). Despite this divergence, which includes a F238L substitution, D. melanogaster SCL was found to be nearly as active as human SCL in transactivation assays (Fig. 3A), indicating that position 238 can accommodate an aromatic residues (Phe or Tyr) or a Leu residue. These observations are consistent with the idea that the loop and helix 2 of SCL contribute to SCL specificity and interaction with LMO2.
Molecular Functions of the SCL-LMO2 Interaction

**A.** Control (MSCV) → SCL → SCL-M13 → Epor+ TER119 Fetal Liver Cells → Real-time PCR → Colony Assays

**B.**

| CFU-Meg | CFU-GM | BFU-E |
|---------|--------|-------|
| CTB     | SCL    | SCL-M13 |
| SCL     | SCL-M13 | SCL-M13 |
| SCL-M13 | SCL-M13 | SCL-M13 |

**C.**

| Gene | mRNA Levels (fold over MSCV) |
|------|-----------------------------|
| GPA  | SCL                          |
|      | SCL-M13                     |
|      | SCL-M13                     |

**D.**

- CTL
- SCL
- LMO2
- PTP-1D

**E.**

- Red: SCL or SCL-M13
- Blue: Hoechst-stained nuclei

**FIGURE 4.** The SCL-LMO2 interaction is required for the stimulation of erythroid differentiation and endogenous GPA gene induction by SCL. A, day 12.5 Epor+ mice TER119 fetal liver cells, which are blocked at the pro-erythroblast stage, were infected with control retroviruses (CTL) or viruses encoding SCL or SCL-M13. Cells were assessed for the frequency of the different types of progenitors in methyl cellulose assays. In parallel, gene expression was assessed by real-time RT-PCR analysis. B, enforced expression of SCL stimulates the formation of erythroid (BFU-E) and megakaryocytic (CFU-Meg) progenitors, whereas no effect was observed on the number of myeloid (CFU-GM) progenitors. In contrast, transduction of the SCL-M13 mutant has no effect on the number of BFU-E and CFU-Meg progenitors. C, the SCL-LMO2 interaction is required for the induction of the endogenous GPA gene in primary fetal liver cells. Endogenous GPA mRNA levels were analyzed by real-time RT-PCR. SCL induces a 4-fold increase in GPA mRNA levels, whereas no effect was observed with SCL-M13. S16 was used as a normalization control. D, SCL and SCL-M13 were efficiently expressed in infected hematopoietic cells. TF-1 cells were infected in parallel with the same viral stocks as primary Epor+ cells, and nuclear extracts (10 μg) were analyzed by IB with the indicated antibodies. The limited number of infected primary Epor+ fetal liver cells precludes analysis by IB. E, SCL and SCL-M13 exhibit similar nuclear-plasmic localization in transfected 293 cells. Red, SCL or SCL-M13; blue, Hoechst-stained nuclei.

E-protein-dependent activation of the PTEs enhancer (Fig. 3B, right graph) (33). This type of configuration explains how the HLH domain can exert a nucleation function by simultaneously accommodating multiple protein interactions within larger protein complexes.

Taken together these results indicate that the SCL-LMO2 interaction is specified by four residues, His-217, Asn-226, Lys-234, and Phe-238 that appear to lie within an exposed surface of the SCL molecule. This interaction is required for assembling SCL complexes on erythroid gene regulatory elements and transcriptional activation, whereas it is dispensable for the inhibition of lymphoid E-protein target genes.

The SCL-LMO2 Interaction Is Required for Endogenous Erythroid Gene Activation and Erythroid Differentiation—Definitive erythropoiesis takes place in the fetal liver at day E12.5 in the mouse. To address the functional importance of SCL-LMO2 interaction in erythroid differentiation (34, 35), SCL or SCL-M13 were expressed in primary Epo receptor (EpoR)-deficient fetal liver cells by retroviral transduction (Fig. 4A). In the absence of EpoR signaling, erythroid progenitors are generated, but these cells are blocked in differentiation and do not express globin genes (36). Enforced expression of SCL leads to a 3-fold enhancement in the number of erythroid (BFU-E) and...
megakaryocyte (CFU-Meg) progenitors assayed in methylcellulose cultures (Fig. 4B) without affecting the number of granulocyte-macrophage progenitors (CFU-GM). Furthermore, SCL transduction induces GPA and β-globin expression by 2–3-fold in infected cells (Fig. 4C). In contrast, SCL-M13 transduction had no effect on GPA expression levels or on progenitor cell profiles despite being efficiently expressed in cells infected with the same virus stocks (Fig. 4D). Furthermore, IF analysis of 293 cells expressing SCL or SCL-M13 shows that the mutant protein exhibits normal nucleoplasmic localization (Fig. 4E), indicating that its lack of activity is not due to inappropriate subcellular distribution. Finally, SCL-M13 was also defective in inducing erythroid differentiation and GPA gene activation in transduced TF-1 cells (data not shown). We conclude that the stimulatory effect of SCL on erythroid differentiation at the onset of definitive erythropoiesis and its capacity to activate erythroid target genes in chromatin requires direct interaction with LMO2.

Interaction with SCL Enhances LMO2 Protein Levels—While conducting the above experiments we observed an intriguing correlation between SCL and LMO2 protein levels. First, we observed a modest but reproducible (~2-fold) increase in LMO2 protein expression in SCL-transduced TF-1 cells, as revealed after normalization to protein-tyrosine phosphatase-1P phosphatase levels used as a loading control, whereas LMO2 levels are unaffected in cells overexpressing SCL-M13 (Fig. 4D). Secondly, a similar correlation was seen in primary hematopoietic cells in which SCL protein levels are genetically reduced by lacZ insertion into one allele in the SCL locus (SCLlacZ/WT) (37). As shown in Fig. 5A, a striking decrease in LMO2 protein levels is observed in primary SCLlacZ/WT fetal liver cells, whereas LMO2 mRNA levels are unaffected. Significantly, this decrease correlates with reduced output in erythroid progenitors (data not shown). Third, we observed that LMO2 expression is barely detectable when transfected alone in various heterologous cell lines (Fig. 5B, lane 1; data not shown), whereas it is readily detectable upon co-expression of either Ldb1 or SCL/E47 (lanes 2 and 3) and is further augmented in the presence of all three partners (lane 4). In contrast, GFP expression from an independent vector is constant in all samples. A similar correlation in SCL and LMO2 expression was recently observed (17). Together, these observations suggest that LMO2 protein expression is enhanced in the presence of its interaction partners.

We next asked whether an intact SCL-LMO2 interaction interface is required for the enhancement of LMO2 expression. As shown in Fig. 5C, LMO2 protein is strongly expressed when either SCL or the active SCL-S6 chimera is present (lanes 3 and 5). In contrast, LMO2 is barely detectable when co-expressed with NSCL1 (lane 4) or SCL-M13 (lane 6). In this case the levels of the control GFP protein, translated from the same mRNA molecules as LMO2 via an internal ribosome entry site (IRES), are comparable in all samples. These differences in protein levels are not due to variations in LMO2 mRNA levels, as these were found to be comparable in transfected cell samples by quantitative real time RT-PCR analysis (Fig. 5D). We conclude that the effect of SCL on LMO2 protein expression is post-translational and requires the direct interaction of both factors.

Interaction with SCL Prevents LMO2 Proteasomal Degradation—Recent studies have shown that Ldb1 and LMO proteins are targeted for proteasomal degradation (27, 28, 38). To address the possibility that LMO2 may be degraded via the ubiquitin conjugation pathway, we made use of the ts20 cell line that expresses a temperature-sensitive ubiquitin-activating E1 enzyme (30, 39). The MSCV-F-LMO2-RES-GFP vector was transduced into ts20 cells, and both cytoplasmic and nuclear extracts were prepared from cells grown either at the permissive (34 °C) or restrictive temperatures (39 °C). As shown in Fig. 6A, LMO2 levels accumulate substantially in the nuclei of cells grown at 39 °C, thus providing genetic evidence that LMO2 is degraded by the ubiquitin conjugation system. Consistent with these findings, LMO2 expression in transfected 293 cells is

Molecular Functions of the SCL-LMO2 Interaction

FIGURE 5. LMO2 protein levels are modulated by SCL. A, reduced SCL expression perturbs LMO2 levels in vivo. Protein extracts from unsorted E12.5 fetal liver cells were isolated from wild type (WT) and SCLlacZ/WT mice and subjected to IB with the indicated antibodies. The fetal liver contained 70–80% TER119+ cells irrespective of the genotype. Note the concomitant decrease in SCL and LMO2 protein levels in SCLlacZ/WT cells. Normalized SCL and LMO2 mRNA levels, measured by real time RT-PCR analysis, are indicated below the panels. Note that LMO2 mRNA levels are unaffected in SCLlacZ/WT cells. B, expression of the LMO2 protein requires the presence of its interacting partners. 293 cells were co-transfected with the expression vector combinations (5 µg each) indicated at the top of the panels together with MSCV-GFP as a control. After 36 h nuclear extracts were prepared and analyzed by IB with the indicated antibodies. C, enhanced LMO2 expression requires direct interaction with SCL. 293 cells were transfected with the MSCV-F-LMO2-RES-GFP vector, which produces a bicistronic mRNA encoding F-LMO2 and GFP as well as vectors for SCL, NSCL1, or different SCL mutants (1 µg each). Note that SCL and the active SCL-S6 chimera enhance LMO2 protein levels, whereas NSCL1 and SCL-M13 do not. D, LMO2, SCL, and S14 mRNA levels in non-transfected 293 cells or cells transfected with the indicated combinations of expression vectors, as assessed by real time RT-PCR analysis. Shown are the average ± S.D. of two independent experiments performed in duplicate. ΔCT and relative quantifications (2^(-ΔΔCT) = ΔCTcontrol) were evaluated. Amplification was in the linear range (cycle threshold of 18 for LMO2, 14 for SCL, and 15 for S14).
Molecular Functions of the SCL-LMO2 Interaction

FIGURE 6. LMO2 degradation by the proteasome is prevented through interaction with SCL. A, LMO2 degradation requires a functional ubiquitin conjugation pathway. The MSCV-F-LMO2-IRES-GFP vector was transfected into ts20 cells, which harbor a temperature-sensitive allele of the ubiquitin-activating enzyme E1. Duplicate samples were kept either at the permissive (34°C) or restrictive (39°C) temperatures for 12 h, then cytoplasmic (C) and nuclear (N) extracts were prepared and subjected to IB. B and C, LMO2 is targeted for proteasomal degradation in transfected cells and in hematopoietic cells. The MSCV-F-LMO2-IRES-GFP vector was transfected into 293 cells. After 36 h the cells were treated with MG132 (12.5 and 25 μM), and lactacystin (10 and 20 μM) and total cell lysates were analyzed by IB. A control treatment was performed with Me2SO (0.01%). D, treatment of TF-1 cells with MG132 (25 μM) for 4 h stabilizes LMO2 and P21 expression. E, LMO2 nuclear extracts were subjected to IB with the indicated antibodies. The MSCV-GFP vector was co-transfected as a control for normal expression. E, the half-life of LMO2 is strongly increased in the presence of SCL. Transfections were carried out in 293 cells with an expression vector encoding HA-LMO2 alone (panels 1 and 2) or in combination with SCL (panels 3–6). Cells were then treated with cycloheximide (100 μg/ml) for the indicated times (0–300 min), and nuclear extracts were prepared. Samples were analyzed by IB (panels 1, 2, 3, and 5), and a portion of the extracts was subjected to IP with an anti-HA antibody before IB. Note the striking increase in the half-life of LMO2 after MG132 treatment (25 μM) or when SCL is present.

strikingly increased after treatment with either of two proteasome inhibitors, MG132, or lactacystin (Fig. 6B, lanes 3–6) but not in control untreated or Me2SO-treated cells (lanes 1 and 2). Again, GFP expression from bicistronic mRNA molecules is comparable in all samples. Furthermore, MG132 treatment of hematopoietic TF-1 cells increases endogenous LMO2 expression (Fig. 6C) as well as p21 levels, a protein known to be regulated by proteasomal degradation (30).

As seen with LMO2, expression of the LMO1 protein is also increased by treatment with MG132 or by co-expression of SCL (Fig. 6D). However, MG132 does not further increase LMO1 protein levels when SCL is present. As expected, the SCL-M13 mutant is inactive in this co-transfection assay. Taken together, our observations are consistent with the view that interaction with SCL protects LMO proteins from proteasomal degradation.

Finally, to directly assess whether the stability of LMO2 is influenced by SCL, we measured the half-life of LMO2 in the absence or presence of SCL through the use of cycloheximide, an inhibitor of de novo protein synthesis. When expressed alone in 293 cells, LMO2 is rapidly degraded and exhibits a half-life of 33 min (Fig. 6E, panel 1). As expected, LMO2 is stabilized after MG132 treatment, with its half-life increasing to 276 min (panel 2). Interestingly, co-expression of SCL leads to a similar increase in LMO2 half-life (255 min), and when the SCL-associated fraction of LMO2 protein is isolated via SCL IP, the half-life value increases to 497 min (panel 4). SCL expression is also stable in these experiments (panels 5 and 6). Finally, because most of the LMO2 pool is associated with SCL in hematopoietic cells (Fig. 1A), our observations are consistent with the view that excess LMO2 is degraded by the proteasome and that this degradation is prevented by interaction with SCL.

DISCUSSION

In the present study we define molecular determinants that control the regulatory activity and assembly of SCL complexes. We find that the SCL-LMO2 interaction provides biological specificity for the nucleation and function of these complexes during hematopoietic gene activation. Furthermore, we demonstrate that this interaction prevents LMO2 protein degradation, thus revealing an additional post-translational mechanism regulating SCL complexes during hematopoiesis.

The SCL-LMO2 Interaction Interface and the Control of Hematopoietic Gene Expression—The biological functions of the SCL and LMO2 genes are intimately intertwined during normal hematopoiesis (16, 19–24, 40) and leukemogenesis (7). Both factors also play important functions at different branch points of the hematopoietic cell hierarchy. Indeed, elegant gene complementation experiments in SCL−/− ES cells and morpholino-treated zebrafish embryos have shown the SCL-LMO2 interaction is required at the onset of hematopoiesis to specify mesodermal precursors to a hematopoietic fate (16–18). Therefore, SCL and LMO2 appear to act as commitment factors in hemangioblast development, thus placing them at the top of the regulatory hierarchy governing hematopoietic development (18). Conditional gene knock-out studies have also established that SCL plays an essential function in adult erythropoiesis and megakaryopoiesis (41, 42). Importantly, here we show that the SCL-LMO2 interaction is required for the stimulatory effect of SCL on erythroid and megakaryocytic progenitors. Because we find that SCL expands these lineages without affecting myeloid progenitor numbers, this suggests that SCL functions as an expansion factor within the erythroid and megakaryocytic pathways rather than a lineage commitment factor.

Our results suggest that the SCL-LMO2 interaction represents a primary nucleation event in the assembly of SCL complexes on DNA, a property that relies on the modularity of these
factors and their ability to simultaneously accommodate multiple protein interactions. Although Schlaefer et al (17) previously highlighted the importance of the His-217 and Phe-238 residues for the interaction with LMO2, our analysis further suggests that the SCL-LMO2 interaction interface involves additional residues located throughout the loop and helix 2 of SCL. Importantly, these residues lie in close proximity to each other in a three-dimensional model of the SCL/E2A heterodimer. We propose that heterodimerization with E2A, occurring via broadly conserved residues in the HLH domain of SCL, exposes the loop and part of Helix2 for interaction with LMO2. Because LMO proteins are likely constitutively associated with Ldb1, which has the capacity to self-dimerize and bridge the recruitment of additional LMO molecules, this enables subsequent assembly of multimeric complexes, as elegantly demonstrated in previous studies (10, 11, 43). The LIM1 and LIM2 domains of LMO2 have been shown to mediate specific protein interactions with Ldb1 and GATA factors, respectively (44, 45). This modularity, thus, enables the tethering of SCL complexes to DNA via different recruitment platforms, such as SCL-E2A heterodimers, GATA factors, or additional DNA-binding proteins, such as Sp1 (10, 11, 13, 15). Additional interactions of SCL complexes with co-factors, such as the ETO2 co-repressor, can further modulate the recruitment of chromatin remodeling and histone modification factors to target gene regulatory elements and, thus, control the transition from transcriptionally active to repressive states or vice versa (12, 46–49). These transitions in chromatin states likely provide epigenetic switches that regulate cell fate commitment and differentiation (12).

The all-or-nothing activity of SCL-containing complexes (13, 15) is reminiscent of enhanceosome complexes that control the expression of the interferon-β and T-cell receptor α genes, in which cooperative assembly of higher order complexes containing tissue-specific and signal-induced transcription factors on enhancer sequences is observed (2, 50–52). The function of these complexes also relies on architectural proteins, such as high mobility group I(Y) and LEF-1, and on interactions with chromatin remodeling complexes. The current study provides another example of how critical protein interactions can nucleate multifactorial complexes, thus providing a binary switch in transcription activation.

**Coupling Protein Stabilization and Protein Complex Assembly**—Proteins with short half-lives often represent rate-limiting components of biological processes. Over the past few years, ubiquitination and proteasomal degradation of transcription factors has emerged as a major mechanism in the control of gene transcription (53, 54). In some instances degradation serves to limit the activity of specific transcription factors, whereas high transcriptional potency is often directly correlated with high rates of transcription factor turnover (53–56). The underlying instability of LMO proteins likely prevents the assembly of higher order complexes in the absence of appropriate interacting partners, thus providing an efficient mechanism to control hematopoietic transcription factor codes.

This study demonstrates that LMO2 is subject to proteasomal degradation and that interaction with SCL stabilizes LMO2. It has recently been shown that regulated degradation of Ldb1 controls the stoichiometry of Ldb1-containing complexes during development (27, 28, 38). Several studies in *Drosophila* have also revealed that LIM-homeodomain and LMO proteins can be stabilized by association with Chip, the *Drosophila* orthologue of Ldb1 (43, 57, 58). Our finding that efficient LMO2 expression requires the presence of either Ldb1 or SCL, which belong to distinct protein families, suggests that protein stabilization by interacting co-factors represents a major mode of regulation of LIM domain proteins. This view is consistent with a recent study by Xu *et al* (28) who showed that Ldb1 and LMO2 can be stabilized through interaction with yet another category of partners, the single-stranded DNA-binding proteins. Similar mechanisms coupling protein stabilization to protein complex assembly likely operate to control a variety of functional modules within the cell.

Finally, different combinations of bHLH-LIM factor interactions may be utilized as a general strategy to control differentiation in many tissues. For instance, NSCL1 might interact with distinct LMO family members during neuronal development (59), whereas LMO proteins of the cysteine-rich protein family have been shown to enhance the myogenic activity of MyoD and to function as adaptors in multifactorial complexes that potently activate smooth muscle cell gene expression (60, 61). Therefore, we speculate that interactions between bHLH and LIM proteins have evolved to create codes that specify cell fates by enabling the formation of tissue-specific transcription factor complexes.

**Acknowledgments**—We thank members of the Meloche group for helpful suggestions and for the ts20 cell line.

**REFERENCES**

1. Tjian, R., and Maniatis, T. (1994) *Cell* 77, 5–8
2. Grosschedl, R. (1995) *Curr. Opin. Cell Biol.* 7, 362–370
3. Shvidasani, R. A., and Orkin, S. H. (1996) *Blood* 87, 4025–4039
4. Sieweke, M. H., and Graf, T. (1998) *Curr. Opin. Genet. Dev.* 8, 545–551
5. Swiers, G., Patient, R., and Loose, M. (2006) *Dev. Biol.* 294, 525–540
6. Lecuyer, E., and Hoang, T. (2004) *Exp. Hematol.* 32, 11–24
7. Begley, C. G., and Green, A. R. (1999) *Blood* 93, 2760–2770
8. Massari, M. E., and Murre, C. (2000) *Mol. Cell. Biol.* 20, 429–440
9. Visvader, J. E., Mao, X., Fujiwara, Y., Hahm, K., and Orkin, S. H. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 13707–13712
10. Wadman, I. A., Osada, H., Grutz, G. G., Agulnick, A. D., Westphal, H., Forster, A., and Rabbitts, T. H. (1997) *EMBO J.* 16, 3145–3157
11. Grutz, G. G., Bucher, K., Lavenir, I., Larson, T., Larson, R., and Rabbitts, T. H. (1998) *EMBO J.* 17, 4594–4605
12. Goardon, N., Lambert, J. A., Rodriguez, P., Nissaire, P., Herblot, S., Thibault, P., Dumenil, D., Strouboulis, J., Romeo, P. H., and Hoang, T. (2006) *EMBO J.* 25, 357–366
13. Lecuyer, E., Herblot, S., Saint-Denis, M., Martin, R., Begley, C. G., Porcher, C., Orkin, S. H., and Hoang, T. (2002) *Blood* 100, 2430–2440
14. Xu, Z., Huang, S., Chang, L. S., Agulnick, A. D., and Brandt, S. J. (2003) *Mol. Cell. Biol.* 23, 7585–7599
15. Lahill, R., Lecuyer, E., Herblot, S., and Hoang, T. (2004) *Mol. Cell. Biol.* 24, 1439–1452
16. Mead, P. E., Deconinck, A. E., Huber, T. L., Orkin, S. H., and Zon, L. I. (2001) *Development* 128, 2301–2308
17. Schlaefer, T. M., Schu, A., Flitter, S., Fisher, A., Mikkola, H., Orkin, S. H., Vyas, P., and Porcher, C. (2004) *Mol. Cell. Biol.* 24, 7491–7502
18. Patterson, L. J., Gering, M., Eckfeldt, C. E., Green, A. R., Verfaillie, C. M., Ekker, S. C., and Patient, R. (2007) *Blood* 109, 2389–2398
19. Warren, A. J., Colledge, W. H., Carlton, M. B., Evans, M. J., Smith, A. J., and...
Molecular Functions of the SCL-LMO2 Interaction

Rabbitts, T. H. (1994) Cell 78, 45–57
20. Robb, L., Lyons, I., Li, R., Hartley, L., Kontgen, F., Harvey, R. P., Metcalf, D., and Begley, C. G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7075–7079
21. Shivdasani, R. A., Mayer, E. L., and Orkin, S. H. (1995) Nature 373, 432–434
22. Porcher, C., Swat, W., Rockwell, K., Fujivara, Y., Alt, F. W., and Orkin, S. H. (1996) Cell 86, 47–57
23. Robb, L., Elwood, N. J., Elefanty, A. G., Kontgen, F., Li, R., Barnett, L. D., and Begley, C. G. (1996) EMBO J. 15, 4123–4129
24. Yamada, Y., Warren, A. J., Dobson, C., Forster, A., Pannel, R., and Rabbits, T. H. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3890–3895
25. Rabbits, T. H. (1998) Genes Dev. 12, 2651–2657
26. Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., McCormack, M. P., Wulfraat, N., Leboulch, P., Lim, A., Osborne, C. S., Pawliuk, R., Morillon, E., Sorensen, R., Forster, A., Fraser, P., Cohen, J. I., de Saint Basile, G., Alexander, I., Wintergerst, U., Frebourg, T., Aurias, A., Stoppa-Lyonnet, D., Romana, S., Radford-Weiss, I., Gross, F., Valensi, F., Delabesse, E., Macintyre, E., Sigaux, F., Soulier, J., Leiva, L. E., Wissler, M., Prinz, C., Rabbits, T. H., Le Deist, F., Fischer, A., and Cavazzana-Calvo, M. (2003) Science 302, 415–419
27. Ostendorff, H. P., Peirano, R. I., Peters, M. A., Schluter, A., Bossenz, M., Scheffner, M., and Bach, I. (2002) Nature 416, 99–103
28. Xu, Z., Meng, X., Cai, Y., Liang, H., Nagarajan, L., and Brandt, S. J. (2007) Genes Dev. 21, 942–955
29. Kros, G. H., Ge, H., Lefrancois, M., Charron, F., Romeo, P. H., Jolicoeur, P., Kirsch, I. R., Nemer, M., and Hoang, T. (1998) J. Exp. Med. 188, 439–450
30. Coulombe, P., Rodier, G., Bonneil, E., Thibault, P., and Meloche, S. (2004) Mol. Cell. Biol. 24, 6140–6150
31. Calkhoven, C. F., Muller, C., Martin, R., Kros, G., Pietsch, H., Hoang, T., and Leutz, A. (2003) Genes Dev. 17, 959–964
32. Ellenberger, T., Fass, D., Arnaud, M., and Harrison, S. C. (1994) Genes Dev. 8, 970–980
33. Tremblay, M., Herblot, S., Lecuyer, E., and Hoang, T. (2003) J. Biol. Chem. 278, 12680–12687
34. Aplan, P. D., Nakahara, K., Orkin, S. H., and Kirsch, I. R. (1992) EMBO J. 11, 4073–4081
35. Hoang, T., Paradis, E., Brady, G., Billia, F., Nakahara, K., Iscove, N. N., and Kirsch, I. R. (1996) Blood 87, 102–111
36. Wu, H., Liu, X., Jaenisch, R., and Lodish, H. F. (1995) Cell 83, 59–67
37. Elefanty, A. G., Begley, C. G., Metcalf, D., Barnett, L., Kontgen, F., and Robb, L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11897–11902
38. Hiratani, I., Yamamoto, N., Mochizuki, T., Ohmori, S. Y., and Taira, M. (2003) Development 130, 4161–4175
39. Chowdary, D. R., Dermody, J. J., Jha, K. K., and Ozer, H. L. (1994) Mol. Cell. Biol. 14, 1997–2003
40. Silver, L., and Palis, J. (1997) Blood 89, 1154–1164
41. Hall, M. A., Curtis, D. J., Metcalf, D., Elefanty, A. G., Sourris, K., Robb, L., Goh, J. R., Jane, S. M., and Begley, C. G. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 992–997
42. Mikkola, H. K., Klintman, J., Yang, H., Hock, H., Schlaeger, T. M., Fujivara, Y., and Orkin, S. H. (2003) Nature 421, 547–551
43. Milan, M., and Cohen, S. M. (1999) Mol. Cell 4, 267–273
44. Deane, J. E., Mackay, I. P., Kwan, A. H., Sum, E. Y., Visvader, J. E., and Matthews, J. M. (2003) EMBO J. 22, 2224–2233
45. Terano, T., Zhong, Y., Toyokuni, S., Hiai, H., and Yamada, Y. (2005) Exp. Hematol. 33, 641–651
46. Meier, N., Krpic, S., Rodriguez, P., Strouboulis, J., Monti, M., Krijgsveld, J., Gering, M., Patient, R., Hostert, A., and Grosveld, F. (2006) Development 133, 4913–4923
47. Schuh, A. H., Tipping, A. J., Clark, A. J., Hamlett, I., Goyot, B., Iborra, F. J., Rodriguez, P., Strouboulis, J., Enver, T., Vyas, P., and Porcher, C. (2005) Mol. Cell. Biol. 25, 10235–10250
48. Xu, Z., Meng, X., Cai, Y., Koury, M. J., and Brandt, S. J. (2006) Biochem. J. 399, 297–304
49. Huang, S., Qiu, Y., Shi, Y., Xu, Z., and Brandt, S. J. (2000) EMBO J. 19, 6792–6803
50. Giese, K., Kingsley, C., Kirshner, J. R., and Grosschedl, R. (1995) Genes Dev. 9, 955–1008
51. Kim, T. K., and Maniatis, T. (1997) Mol. Cell 1, 119–129
52. Merika, M., and Thanos, D. (2001) Curr. Opin. Genet. Dev. 11, 205–208
53. Thomas, D., and Tyers, M. (2000) Curr. Biol. 10, 341–343
54. Lipford, J. R., and Deshaies, R. J. (2003) Nat. Cell Biol. 5, 845–850
55. Molinari, E., Gilman, M., and Natesan, S. (1999) EMBO J. 18, 6439–6447
56. Salghetti, S. E., Muratani, M., Wijnen, H., Futcher, B., and Tansey, W. P. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 3118–3123
57. Milan, M., and Cohen, S. M. (2000) Development 127, 3069–3078
58. Weihe, U., Milan, M., and Cohen, S. M. (2001) Development 128, 4615–4622
59. Bao, J., Talmage, D. A., Role, L. W., and Gautier, J. (2000) Development 127, 425–435
60. Kong, Y., Flick, M. J., Kudla, A. J., and Konieczny, S. F. (1997) Mol. Cell. Biol. 17, 4750–4760
61. Chang, D. F., Belaguli, N. S., Iyer, D., Roberts, W. B., Wu, S. P., Dong, X. R., Marx, J. G., Moore, M. S., Beckerle, M. C., Majesky, M. W., and Schwartz, R. J. (2003) Dev. Cell 4, 107–118