Supplementary material

Material and methods

ES cell transfection and *in vitro* ES cell hematopoietic differentiation

ES cells (clone J1, wild-type or $SCL^{-/-}$ (3)) were maintained as previously described (3). $5x10^6$ $SCL^{-/-}$ ES cells were electroporated with 20 μg of linearized pEF1α-BirA (800 V, 3 μF). Stable clones were selected under puromycin (Sigma, 1μg/ml). A clone expressing high levels of BirA mRNA was chosen by Northern Blot analysis (not shown) and subsequently transfected with pEF1α-bioSCL. Stable clones were selected using puromycin (1 μg/ml) and zeocin (Invitrogen, 200 μg/ml). ES cells (wild-type or rescued $SCL^{-/-}$ cells) were subjected to *in vitro* hematopoietic differentiation. Primitive erythroid and definitive hematopoietic colonies were generated as described (1, 3). Colonies were scored on day 5 to day 7, then harvested and stained with May-Grunwald-Giemsa (according to standard protocols) or used to extract RNA (RNAeasy kit, Qiagen).

Semi-quantitative RT-PCR

cDNA was synthesized according to standard protocols. Primer sequences and conditions used for the PCR reactions have been described (3). Quantitation was carried out using the Phosphorimager (Storm, Molecular Dynamics).

Antibodies

Antibodies used for Western Blot analyzes, immunoprecipitation and co-localisation experiments: anti-SCL (2), -Ldb-1, -LMO-2 (generous gifts from Stuart Orkin) polyclonal rabbit antibodies, anti-SCL mouse monoclonal antibodies (kind gift of Karen Pulford); anti-ETO-2 (sc-9741 and sc-9739), E2A(E47) (sc-762), E2A(E12/47) (sc-349), HEB (sc-357), GATA-1 N6 (sc-265) or M20 (sc-1234), mSin3A (sc-994), N-CoR (sc-1609), Gfi-1b (sc-8559) and p300 (sc-585) (Santa Cruz); anti-SC35 and anti-GRB2 mouse monoclonal

antibodies (Sigma (S-4045) and BD Pharmingen, respectively); anti-Flag M2 antibody and anti-Flag M2 affinity gel (Sigma). For FACS analyzes, c-kit, Ter119 and CD71 antibodies were all from BD Pharmingen.

Note: the E2A(E47) and E2A(E12/47) antibodies used in Western Blot analyses typically recognise a single band and a doublet, respectively.

Online text

Biotinylated SCL rescues hematopoiesis from SCL$^{-/-}$ ES cells

The functionality of biotinylated SCL was tested in the SCL-null ES cell rescue assay whereby introduction of a wild-type SCL cDNA fully restores hematopoietic development from these cells (3). We co-transfected SCL$^{-/-}$ ES cells with the *E. Coli* BirA biotin ligase and a wild-type SCL cDNA fused to a biotin tag (hereafter named bio-SCL). Clones expressing biotinylated SCL were selected (not shown) and allowed to differentiate *in vitro* into hematopoietic lineages in a two-step assay (1, 3). Despite high levels of bio-SCL transcripts as judged by semi-quantitative RT-PCR from hematopoietic colonies (7 to 8.5-fold higher than endogenous SCL, Figure S1, top panels), rescue of primitive and definitive hematopoiesis was observed. Primitive erythroid colonies (Figure S1, left panel) and definitive erythroid, megakaryocytic and myeloid colonies (data not shown) derived from cells expressing biotinylated SCL looked morphologically identical to their wild-type counterparts. May-Grunwald-Giemsa staining showed normal terminal cellular maturation of bio-SCL rescued hematopoietic cells (Figure S1, MGG panels). Moreover, in colony assays, the number of hematopoietic progenitors derived from rescued ES cells was similar to that derived from wild-type ES cells (data not shown).

Heterodimerisation of SCL with E-proteins is required for interaction with ETO-2

To map the domain of SCL involved in interaction with ETO-2, we expressed a biotinylated bHLH domain of SCL (bio-bHLH) in MEL cells. Protein complexes were pulled-down with streptavidin beads and presence of ETO-2 was analyzed by Western Blotting. The bHLH domain of SCL appeared to be sufficient for interaction with ETO-2 (Figure S4). We then analyzed binding of ETO-2 to SCL variants defective in heterodimerisation (bio-FL) and DNA-binding activity (bio-RER) (2), and impaired for interaction with LMO2 (bio-H2(F-G)). No interaction between heterodimerisation-defective SCL (SCL-FL) and ETO-2 was detected. The bio-H2(F-G)/ETO-2 and bio-RER/ETO-2 interactions were preserved, suggesting that ETO-2 is not recruited to the SCL complex through LMO2/Ldb-1 and that the residues important for DNA-binding are not required for ETO-2 binding.

We also generated biotinylated SCL mutants where the SCL HLH domain was substituted by the corresponding region of E47 (bio-SES), MyoD (bio-SMS) and NSCL (bio-SNS). These variants heterodimerise with E12/E47 (4). We show that ETO-2 co-purified with bio-SMS, bio-SNS and, to a lesser extent, bio-SES (Figure S4). This suggested that HLH dimers of different nature can bind ETO-2 but that E47 homodimer may interact less efficiently than SCL/E47 or MyoD/E47 heterodimers with ETO-2.

Overexpression of E2A (wild-type and mutant DM) or ETO-2 did not affect cell proliferation and cell viability

To document possible effects of constitutive overexpression of E2A (wild-type and DM mutant) or ETO-2 on cell cycle, proliferation assays were performed on transduced, non induced MEL cells. They showed no significant differences in doubling times between populations (although MEL cells transduced with ETO-2 may have a slight and transient proliferative advantage) (Figure S6C). Moreover, analysis of the

percentage of GFP^+ cells in the unsorted transduced populations over time did not show dramatic proliferation advantage/disadvantage (Figure S6B). Annexin V assays on transduced cells did not show any apoptotic or anti-apoptotic effects of constitutive expression of E2A, DM or ETO-2 as compared to expression of GFP only (data not shown). Morphological inspection of the transduced, uninduced cells by MGG staining revealed no detectable difference between the populations (data not shown).

References

1. **Keller, G., M. Kennedy, T. Papayannopoulou, and M. V. Wiles.** 1993. Hematopoietic differentiation during embryonic stem cell differentiation in culture. Mol. Cell Biol. **13:**472-486.
2. **Porcher, C., E. C. Liao, Y. Fujiwara, L. I. Zon, and S. H. Orkin.** 1999. Specification of hematopoietic and vascular development by the bHLH transcription factor SCL without direct DNA binding. Development **126:**4603-4615.
3. **Porcher, C., W. Swat, K. Rockwell, Y. Fujiwara, F. W. Alt, and S. H. Orkin.** 1996. The T-cell leukemia oncoprotein SCL/tal-1 is essential for development of all hematopoietic lineages. Cell **86:**47-57.
4. **Schlaeger, T. M., A. Schuh, S. Flitter, A. Fisher, M. H., S. H. Orkin, P. Vyas, and C. Porcher.** 2004. Decoding haematopoietic specificity in the helix-loop-helix domain of the transcription factor SCL/Tal-1. Mol Cell Biol **24:** 7491-7502.

Figure legends

Figure S1. Biotinylated SCL rescues hematopoiesis from SCL$^{-/-}$ ES cells.

ES cells (wild-type, SCL$^{-/-}$ and SCL$^{-/-}$ expressing biotinylated SCL (bio-SCL)) were differentiated into primitive and definitive hematopoietic lineages *in vitro*. Levels of expression of SCL were analyzed by semi-quantitative RT-PCR in primitive erythroid colonies (left top panels) and definitive erythroid colonies (right top panels), relative to levels of expression of the HPRT gene and shown as ratios. Triangles represent decreasing numbers of PCR cycles. Morphology of primitive erythroid colonies was assessed by phase contrast microscopy (left panels). May-Grunwald-Giemsa staining of cells from hematopoietic colonies is shown (MGG panels). Meg: megakaryocyte; def: definitive; wt: wild-type; bio-SCL: biotin-tagged/biotinylated SCL.

Figure S2. Biotinylated SCL does not promote spontaneous differentiation of MEL cells

Benzidine staining of non-induced and DMSO-induced wild-type MEL cells and MEL cells expressing bio-SCL (wild-type or the H2(G-F) mutant). Percentages of benzidine positive cells are shown. bio-SCL=biotin-tagged/biotinylated SCL.

Figure S3. SSDP2 binds the SCL core complex

MEL nuclear extracts from cells expressing biotinylated SSDP2 were used to check interaction between SSDP2 and SCL or known partners (as indicated on the left) by streptavidin pull-downs and Western Blot analysis. IN=input of crude nuclear extract, PD= streptavidin pull-down, UN=unbound.

Figure S4. Mapping of the domain of SCL required for interaction with ETO-2

Nuclear extracts prepared from MEL cells transfected with BirA only or BirA and biotin-tagged SCL constructs (as indicated on top of the figure) were subjected to streptavidin pull-downs. Precipitated products were analyzed by Western Blot probed with □SCL antibodies, streptavidin-HRP conjugate (to check pull-down of bio-bHLH variant only), □ETO-2 and □E2A antibodies. IN: input of crude nuclear extract, PD: streptavidin pull-down, UN: unbound.

Figure S5. Histological staining and cell-surface marker analysis of day 12.5 fetal liver cells at day 0 and day 2 of erythroid differentiation.

MGG/Benzidine staining (upper panel) and FACS analysis (lower panel) of cells after 0, 1 and 2 days of differentiation are shown.

Figure S6.

A-Cell lysates were prepared from 293T producer cells transiently transfected with lentiviral expression constructs (E2A, DM, and ETO-2) and analyzed by Western Blotting for expression of E2A (wild-type and mutant DM, left panel) and ETO-2 (right panel). GFP: cells infected with GFP-only vector. MEL cells served as a control. Exogenous E2A (wild-type and DM) appears as a doublet absent from the sample expressing GFP only. Note the absence of endogenous ETO-2 in 293T cells (GFP lane, right panel). The bands corresponding to exogenous E2A (wild-type and mutant) and ETO-2 in the 293T samples immunoreacted with anti-Flag antibodies (not shown).

B-The percentage of GFP^+ cells in the unsorted MEL infected populations was first measured by flow cytometry analysis at 72 hours post-infection and regularly monitored over 23 days. The graph shown is representative of two independent experiments.

C-Proliferation of uninduced, GFP^+ populations was assayed over 6 days. The graph shown is representative of two independent experiments.

MEL

Protein ID	No of peptides	Biological processes	Remarks
E12/E47	10	transcription factor	known partner
E2.2	9	transcription factor	known partner
Ldb-1	5	transcription factor	known partner
SCL	11	transcription factor	
SSDP2	5	transcription factor	interacts with Ldb-1
ETO-2(MTG16)	6	co-repressor	
TIF1b=TPIM28	6	chromatin remodelling	
LAP2=thymopoietin	7	nuclear architecture	interacts with Rb
FACT 40	4	basal transcription	nucleosome shifting
SSRP-1	3	basal transcription	nucleosome shifting
RbAp46	3	co-repressor	interacts with Rb
HDAC class 1	4	chromatin remodelling	co-repressor
RUVB like1 (TIP49)	2	chromatin remodelling	co-activator
DEAD (p68)	7	ATP-dependent RNA helicase	interacts with CBP and p300
HSP70	17	chaperone	
EWS	4	transcription co-factor	interacts with basal machinery
Ku70/80	6	DNA repair	
TCP (TRIC)	2	chaperone	

L8057

Protein ID	No of peptides	Biological processes	Remarks
E12/E47	17	transcription factor	known partner
HEB	2	transcription factor	known partner
Ldb-1	8	transcription factor	known partner
SCL	21	transcription factor	
SSDP2	2	transcription factor	interacts with Ldb-1
ETO-2(MTG16)	5	co-repressor	
KAISO	2	co-repressor	
LCP-1	2	unknown	
DEAD (p68)	3	ATP-dependent RNA helicase	interacts with CBP and p300
HSP70	16	chaperone	

Table 1. Potential candidate partners of SCL identified by mass spectrometry analysis from MEL and L8057 cells.