SUPPLEMENTARY TEXT :

Gfi1b and Ikaros bind to different sites in the SAT-B part of the γ-satellite sequence

Specific protein/DNA complexes could clearly be detected with MT-Gfi1b depending on the amount of protein added to the reaction (supplementary Fig. S3b, lanes 1, 2). These complexes were disrupted with an unlabeled SAT-B1 oligonucleotide or with an unlabeled oligonucleotide containing the Gfi1b binding site (supplementary Fig. S3b, lanes 5, 7). By contrast, these complexes could not be disrupted with an unlabeled SAT-A sequence, a “tg” repeat sequence or with an oligonucleotide containing the regular Ikaros binding site (supplementary Fig. S3b, lanes 3, 4, 6). This confirmed the specificity of the reaction between the SAT-B1 sequence and Gfi1b and indicated that the regular Ikaros binding site is not recognized by Gfi1b. In addition, the Gfi1b/SAT-B1 complex could be super-shifted with an anti Myc-tag antibody but not with an antibody against Ikaros (supplementary Fig. S3b, lanes 8, 9).

Similar to Gfi1b, Myc-tagged Ikaros protein showed a specific binding to the SAT-B1 sequence (supplementary Fig. S3b, lane 10) confirming previously obtained results (Cobb et al., 2000). The Ikaros/SAT-B1 complex is disrupted by competition with the SAT-A and SAT-B1 sites and by the ideal Ikaros binding site (“Ikaros BS”, supplementary Fig. S3b, lanes 12, 13, 14) but not by the “tg” repeat sequence or the Gfi1b BS (lanes 11, 15) indicating that Gfi1b and Ikaros do not recognize the same target sequence. MT-Ikaros/SAT-B1 complexes can be supershifted with an anti Myc tag antibody and are disrupted with an anti Ikaros antibody (lanes 16, 17). When both Gfi1b and Ikaros are incubated with the SAT-B1 site in the presence of anti Ikaros antibody, Gfi1b/SAT-B1 complexes are detected but Ikaros/SAT-B1 complexes are disrupted suggesting that Ikaros and Gfi1b bind independently to the SAT-B1 sequence (supplementary Fig. S3b, lane 19).

SUPPLEMENTARY FIGURES

Supp. Fig. S1

Vav-Gfi1b transgenic mice and target gene repression.

a.) Schematic representation of the vav-Gfi1b construct used to generate the transgenic mice that were originally described by Vassen et al. (2005). The Gfi1b cDNA was placed under the control of the enhancer and promoter elements of the vav gene. To terminate transcription, an SV 40 polyadenylation signal was inserted at the end of the Gfi1b cDNA (Vassen et al., 2005).

b.) Expression of the vav-Gfi1b transgene was detected by RT-PCR in bone marrow (BM), spleen (Sp) and thymus (Th) of the transgenic mouse line #7. Hind III digestion of RT-PCR products distinguishes between endogenous (wt) and transgenic (tg) Gfi1b expression. Higher levels of mRNA were apparent in all three organs of vav-Gfi1b transgenic mice (tg) than in wild type animals (wt). Endogenous Gfi1b mRNA was present in bone marrow and spleen of wt mice but was absent in thymus and liver (Li) from wt mice.

Supp. Fig. S2

Binding of Gfi1b to the different regions of the SAT-B part of the γ–satellite sequence.

a) –d) Electrophoretic mobility shift assays with the indicated radio labeled oligonucleotide probes (^{32}P-), the indicated competitors and in the presence of *in vitro* translated Myc tagged Gfi1b (MT-Gfi1b) with or without anti Myc tag antibodies (α-MT-AB). Asterisks denote non-specific signals obtained with empty (unprogrammed) lysate (see also Fig. 2).

SAT-B1, SAT-B2, SAT-B3 and SAT-A oligonucleotides were radio labeled and used in EMSA experiments with *in vitro* translated Gfi1b. Clearly, SAT-B1 and SAT-B3 sequences were able to form complexes with Gfi1b that could be supershifted with an anti Myc tag antibody (a, c, lanes 4, 5). In the case of SAT-B3, the complexes could be efficiently disrupted with the ideal site, SAT-B1, SAT-B2 and SAT-B3 but not with the unrelated CREB oligonucleotide as competitors (c, lanes 6-10). In the case of SAT-B1, competition was obtained with SAT-B2, SAT-B3 (a, lanes 7, 8), but was less efficient with the ideal site; as a control, the unrelated CREB sequence did not disrupt SAT-B1/Gfi1b complexes (a, lanes 6-10). With the SAT-B2 oligonucleotide or with a sequence covering the SAT-A part, no specific complexes with Gfi1b could be obtained (b, d, lanes 4, 5).

Supp. Fig. S3

Binding of Gfi1b to satellite sequences mediates repression of a reporter gene and is indepdendent of Ikaros

a) Gfi1b transregulates SAT-B1 dependent reporter genes

Reporter gene assay with constructs carrying the Gfi1b binding site (Gfi1b-BS) 5' of an SV40 promoter and the luciferase gene (Gfi1b-BS-Luc) or with constructs that contain one or two copies of the SAT-A or SAT-B1 oligonucleotide (see Fig. 2b) from the γ–satellite sequence 5' of an SV40 promoter driven luciferase gene (SAT-A-Luc, SAT-B1-Luc). All reporter constructs were cotransfected into NIH 3T3 cells along with expression vectors for Gfi1b or for fusion proteins of Gfi1b with the transactivation domain from the Herpes Simplex Virus protein VP16 or with vector alone (pcDNA3). The functionality of the expression constructs has previously been verified (Vassen et al., 2005).

b) Gfi1b and Ikaros bind independently to different sites of the γ-satellite sequence.

Electrophoretic mobility shift assay with a radio labeled SAT-B1 oligonucleotide (^{32}P-SAT-B1) and *in vitro* translated Myc-tagged Gfi1b or Myc-tagged Ikaros (MT-Gfi1b, MT-Ikaros) with the indicated unlabeled competitor oligonucleotides: SAT-A, tg-repeat, an ideal Ikaros binding site (Ikaros BS), the Gfi1b binding site from the Gfi1b promoter (Gfi1b-BS, see Fig. 2a) with or without anti Myc-tag antibodies (α-MT-AB) or an anti Ikaros antibody (α -Ikaros-AB). Asterisks denote non-specific signals obtained with empty (unprogrammed) lysate (see Fig. 2b).

Supp. Fig. S4

The association of Gfi1b with heterochromatic foci and its nuclear localization depends on the presence of its zinc finger domains. Transient transfections of NIH 3T3 cells with vectors for fusion proteins between GFP and Gfi1b or the indicated Gfi1b deletion mutants. Deletion of zinc finger 6 already abrogates the association of the Gfi1b protein with DAPI stained foci of heterochromatin.

Supp. Fig. S5

Real-time PCR analysis of CD3γ and Lymphotoxin-A expression using cDNA from previously described B-cell lines from wt and vav-Gfi1 transgenic mice (Vassen et al., 2005). Values were normalized by GAPDH expression levels and the the expression levels in vav-Gfi1 transgenic cells (tg) was set to 1.

Supp. Fig. S6

Western blots using either cytoplasmic (CE) or nuclear (NE) extracts from MEL75Acl.19 cells probed with antibodies against G9a or SUV 39H1 indicating the presence of both proteins in the nucleus of MEL75Acl.19 cells.

SUPPLEMENTARY MATERIAL AND METHODS :

ChIP-PCR primer :

Chr16-5’ US : GTTCTGCCTCCTACTTGCAAGAC

Chr16-5’ LS: CTGAAGATGGGCTGGGTCCTGCAG

Chr16-3’ US : CATATTGGCACTTCTATATAACTGTC

Chr16-5’ LS : GAATTGACGTAGAAGCAGATGATTAG

PLTA US: GCAGCTGCCAGCAGATGAGGTGGAC

PLTA LS : CAGAGGAATTATCTCTCTACCATTC

pCD3γ US : CCTTCCACCGTTCCATCTAGATGC

pCD3 γ LS : GAACTTCCTGAAATCTCCCTCTGG

Oligonucleotide sequences for gel shift analyses

Gfi1b-BS US : GATCCACAAATAATCAGATGAAAACAGGAGGG, Gfi1b-BS LS : GATCCCCTCCGATTTTCAATCTGATTATTTGTG, Ideal site US : GATCAAAATAAATCACAGCAT, Ideal site LS : GATCATGCTGTGATTTATTTT, CRE US : GATCCTTGG CTGACGTCA GAGAGAGCT, CRE LS : CTCTCTGACGTCAGCCAAG, SAT-A US : TATGGCGAGGAAAACTGAAAAAG GTGGAAAATTTAGAAATGT, SAT-A LS : ACATTTCTAAATTTTCCACCTTTTTCAGTTTTCCTCGCCATA, SAT-B1 US : AAACTGAAAATCATGGAAAATGAGAAACAT, SAT-B1 LS : ATGTTTCTCATTTTCCATGATTTTCAGTTT, SAT-B2-US : GAAAAATGACGAAATCACTAAAAA, SAT-B2-LS :GTTTTTAGTGATTTCGTCATTTT, SAT-B3-US : GAAAACTGAAAAT CACGGAAAATG, SAT- B3-LS :CTCATTTTCCGTGATTTTCAGTTT, Ikaros BS US : TGACAGGGAATACAC ATTCCCAAAAGC Ikaros BS LS : GCTTTTGGGAATGTGTATTCCCTGTCA.

Antibodies :

Anti-Histone H3 Lys9 di-methyl : 07-441 : upstate biotechnologies, anti-Histone H3 Lys9 tri-methyl (abcan), anti-Histone H4 Lys20 dimethyl : 05-672 : upstate biotechnologies, anti-Gfi1b (D-19) : sc-8559 : SANTA CRUZ, anti-Gfi1b (H-150) : sc-22795 : SANTA CRUZ, anti-Gfi1b (D-19) : sc-8559 : SANTA CRUZ, anti-G9A (C-15) : sc-22879 : SANTA CRUZ, anti-SUV39H1 : ab12405-50 : abcam, anti-GST (AB.1) : PC53 100UG : Calbiochem, anti-PIAS1 (H-175) sc-14016 : SANTA CRUZ, PE labeled anti –mouse CD71 : 553267 : PharMingen, APC conjugated anti-mouse TER-119 : 17-5921-82 : eBioscience.

Reporter gene assays :

$5x10^4$ NIH-3T3 cells per well were seeded in 12-well plates and transfected with 500ng of luciferase construct and 150ng of CMV-driven pCDNA-Gfi1b or pCDNA-herpes-simplex virus activator domain (VP16)-Gfi1b fusion protein expression plasmids as indicated. Transfections were performed using Rotifect (Roth, germany) according to the manufacturer's instructions. The amount of DNA was kept constant in each transfection by adding empty pCDNA vector where necessary. 30h later, cells were lysed at 4 °C in 25 mM Tris (pH 7.8), 2 mM 1,2-diaminocyclohexane-*N*,*N*,*N* ,*N* -tetraacetic acid, 10% glycerol, 1% Triton X-100, 2 mM dithiothreitol, 0.3 mM phenylmethylsulfonyl fluoride, and 2 μg/ml aprotinin. The luciferase activity was measured by luminometry after diluting the lysate 1:3 or 1:5 in 25 mm glycylglycine, 15 mM MgSO4, 15 mM K2HPO4, 4 mM EGTA, 40 μM ATP, 40 μM dithiothreitol, and 0.3 μg/ml luciferin. Expression of Gfi1b or Gfi1b mutants was confirmed by immunoblotting using Gfi1b-specific antibody as described previously (Vassen et al., 2005 and data not shown).