SUPPLEMENTARY INFORMATIONS

Experimental procedures

Cell culture and RA treatment: P19.6 cells were a gift from Pierre Chambon (Strasbourg, France). Cells were cultured in Dulbecco's modified eagle medium, supplemented with 7,5 % fetal calf serum, L-glutamine (2 mM), penicillin (100 U/mL) and streptomycin (100 μg/mL), and maintained at 37°C in a 5% CO_2 atmosphere. To induce differentiation, P19 cells were cultivated at density of 10^5 / mL on petri dishes for 24h to form aggregates. The culture medium was then replaced by medium containing all-trans retinoic acid (RA) (Sigma) at 0.5 μM. Fresh medium containing 0.5 μM RA was added every day.

Gene expression analysis : Total RNA was isolated from cells using TriReagent (Molecular Research Center). Reverse transcription into cDNA was performed using 2 μg of total RNA with Superscript II according to manufacturer's instructions (Life Technologies) and then quantified by real-time PCR. GAPDH primers were used to normalize cDNA quantities. One sample was treated the same as the others but without the enzyme for a negative control.

RNA in situ hybridization: DIG-labelled probes were generated using SP6 or T7 promoters from a linearized pGEM-T Easy vector containing the *Hoxb1* coding sequence. Probes were synthetised *in vitro* by SP6 or T7 RNA polymerases according to manufacturer instructions (New England Biolabs). Sample were then treated with DNAse, precipitated with LiCl, and eventually resuspended in H20 DEPC.

Before or after RA treatment, cells were trypsinised, diulted to 2.10^6 cells/mL and let on polylysine slides for 30 min at 37°C. After wash with PBS-DEPC, cells were fixed in 4% formaldehyde 15 min at room temperature. Hybridation was carried out with 5 μg of DIG probe, 16 to 18 hours at 50°C in hybridation mix (50 % formamide, 5X SSC pH 4,5, 1%SDS, ARNt yeast 50ug/mL, Heparin 50

μg/mL). Washes were performed three times for 15 min in at 50°C Solution I (50 % formamide, 5 X SSC pH 4,5, 1 % SDS), and repeated under the same conditions in Solution II (50 % Formamide, 2X SSC pH 4,5) at 50°C, and then in TBS 0,1 % Tween at room temperature. Detection was performed using an anti-DIG-rodhamine antibody (Roche), diluted 1/50 in TBS tween 0,1%, BSA 1%, Donkey serum 10%, overnigth at 4°C. Finally, after washes in TBS tween 0,1%, slides were mounted in prolong gold antifade reagent (Invitrogen) and analyzed by fluorescent microscopy.

Nascent strands purification : Total genomic DNA was extracted using the DNAzol reagent (Invitrogen) from 10^8 exponentially-growing P19 cells according to manufacturer's instructions. DNA was resuspended in TEN_{20} (10 mM Tris pH 8.0, 1 mM EDTA, 20 mM NaCl). After heat denaturation, DNA was layered on a 38 ml 5-30% neutral sucrose gradient in 10 mM Tris pH 8.0, 1 mM EDTA, 500 mM NaCl, and size fractionated by centrifugation in a Beckman SW28 rotor at 26,000 rpm for 20 hours at 4°C. 35 fractions of 1 ml each were collected, precipitated with ethanol, and resuspended in TEN_{20} in the presence of 1U/μL of RNasin (Promega). One-tenth of each fraction was analyzed by 1.2% alkaline agarose gel electrophoresis and visualized by EtBr staining and Southern hybridization with ^{32}P-labelled genomic DNA. Fractions from 500 to 1500 nt were treated with T4 polynucleotide kinase and λ-exonuclease as described (Gerbi and Bielinsky, 1997). After RNA removal by RNAse A treatment, DNA was purified by phenol-chloroform extraction, ethanol precipitation, and finally resuspended in 10 mM Tris pH 7.9. We confirmed that the quantitative PCR patterns we obtained were indeed due to RNA-primed nascent DNA by showing that alkaline treatment, which removes the RNA primers prior to the lambda-exonuclease treatment, prevented amplification by real-time PCR.

Real-time quantitative PCR: Quantification was carried out using the LightCycler (Roche) detection system with the QuantiTect SYBR Green PCR kit (Qiagen), or LC FastStart DNA master plus SYBR Green I (Roche), following manufacturers' instructions. Five five-fold dilutions of mouse P19 genomic DNA, with duplicates for the lowest concentrations, were used for the standard curve. PCR reactions with efficiencies of less than 90% were rejected. Nascent strand enrichment was calculated

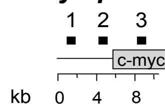
as fold enrichment over the background, whose level was determined by the average of values obtained for the four lowest enriched positions.

Chromatin immunoprecipitation: Briefly, cells were crosslinked with 1% formaldehyde in PBS for 4 min at room temperature, and lysed in LB buffer (20 mM Hepes 7.5; 2 mM EDTA, 1 mM EGTA, 10 mM KCl, 0.5% NP-40) supplemented with a protease inhibitor cocktail (Roche). Released nuclei were collected and washed in HEN buffer (20 mM Hepes 7.9, 150 mM NaCl, 1mM EDTA, 0.5 mM EGTA). Subsequently, crosslinked chromatin was dissolved in HEN+0.5%SDS and sonicated using a Bioruptor sonicator (Diagenode), producing DNA fragments ranging from 300 to 1500 bp in length.

Primers:

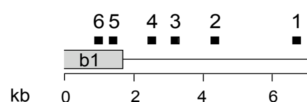
All primers are indicated 5' to 3' F: forward; R: reverse

c-myc primers



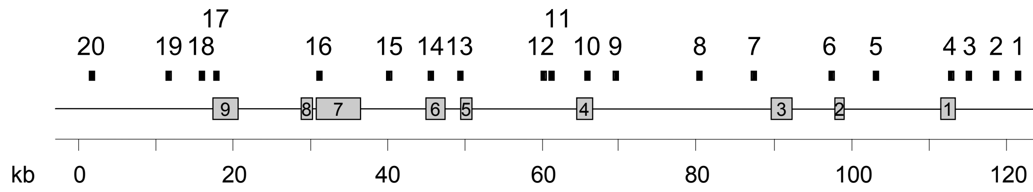
1	F R	cggcatgatttggacgtaa agcaacaggcaaggtattcaaca
2	F R	ttctgttttccccagcctta tcggctgaactgtgttcttg
3	F R	tttctcacctgtgccctaaccct agcccgactccgacctct

Hoxb1 primers



1	F R	agggctgtggtcttgg tctctactagatggcatggtt
2	F R	ccctactcggcaccatct cacacacccaaggagcaacca
3	F R	tgtgaaactggtgccattag cttcctcttgaacgcatctt
4	F R	ttgccctggaaactgtaaag aatttgccaacaacccatc
5	F R	gtcttgggacctggggaacac gggctcacactcaaacgcatc
6	F R	gctcaatgaaacgcaggt ggaagcccagttacttagga

HoxB primers



T : primer pairs located inside transcribed region

No.		Sequence	
1	F	agggctgtggtcttgg	
	R	tctctactagatggcatggtt	
2	F	ccctactcggcaccatct	
	R	cacacacccaaggagcaacca	
3	F	ttgccctggaaactgtaaag	
	R	aatttgccaacaacccatc	
4	F	gctcaatgaaacgcaggt	T
	R	ggaagcccagttacttagga	
5	F	gccatcctgtctacctta	
	R	aggctacacacagttggttc	
6	F	gtttgccaccaccacacagtc	
	R	cgccaatcgctagtcta	
7	F	gaagccgtatggagcctgaat	
	R	acagtgaggaacccgaaggtg	
8	F	aggagttgggttatttcagac	
	R	ggaatgttggctctatgga	
9	F	aacagcaaatct	
	R	ctcctctggtccccgttca	
10	F	aaaatctatctaccctaccct	T
	R	cggctcccttattgtc	
11	F	gatgcccagtgacacactctc	
	R	cgcctttgctttgatttttcc	
12	F	acaacattgccatgcctagat	
	R	atacttctgcccttactgag	
13	F	cggctcttacggctacaatta	T
	R	ttagtgcagggcagggact	
14	F	actcgtctggctatgcggac	T
	R	ctctccgaacacgctcttgtc	
15	F	gacaggaggcgttgtggtt	
	R	ctaatagcccaggagaccaat	
16	F	ccgctcctcattcgctccta	T
	R	cccagggactcgcacca	
17	F	ggcacgcccgagtacagtttg	T
	R	actttgatccggcctctcttt	
18	F	gcctcccagagcaccaca	
	R	ccgccttctcgcctacaatc	
19	F	cgaagcgaccctgacat	
	R	tctcttgcctcacaacaaatc	
20	F	gcctgcggaccgacctcaatc	
	R	cgcccagaggatgctcatt	