Spatial link between nucleoli and expression of the *Zac1* gene

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Supplementary File 1

Methyl Specific PCR (MSP) assay

Genomic analysis demonstrated that *Zac1* contains a CpG island in exon 1 which extends to the promoter region, suggesting that methylation is the mechanism for silencing *Zac1* maternal allele expression (Smith, et al. 2002). DNA was isolated from cell cultures using Wizard Genomic DNA purification kit (Promega) and transformed using the CpG Genome Modification kit according to manufacturer specifications (Chemicon). MSP primers were designed using MethPrimer software (http://www.urogene.org/methprimer) to detect differentially methylated CpGs of the *Zac1* gene promoter previously described (Kamiya, et al. 2000) (Suppl. Fig. 1a; Sequence accession number AF314094; Primer sequence in Table 1). Methyl specific PCR (MSP) assay was performed to detect differentially methylated CpGs of the *Zac1* gene promoter in the MEF cell line (G7) and neurons (Neu) from mouse embryos (16 dpc). PCR products characterization and semi quantitative estimation of their concentration was done by capillary electrophoresis in a 2100-Bioanalyzer (Agilent Technologies). The correct identity of PCR products were confirmed by DNA sequencing. MSP assay of chemically modified DNA from MEF cells and primary neurons showed that the CpGs of the promoter region exists in both methylated (M) and unmethylated (U) forms, suggesting that *Zac1* expression should be monoallelic in both cell types (Suppl. Fig. 1b). To control the completion of the bisulphate-mediated C> T conversion and primer specificity we performed the same experiments with unmethylated DNA generated by PCR. Briefly, mouse genomic DNA was amplified with primers designed for the whole sequence of the *Zac1* gene. The new and unmethylated PCR products were subjected to

bisulphate C> T conversion and then to MSP as described above (Suppl. Fig. 1c).

Table 1. Details of the primers' sequences and annealing temperatures (T_a) used in the different PCR reactions

Gene	Sequence	T_a	Software designer/Source
Zac1: Fragment5 primers for RNA FISH	F 5'- TACTCCCCAGAATGGCTTTG-3' R 5'- CTTCCGCTTCCTCTTCCTCT-3'	55ºC	Primer3 (http://primer3.sourceforge.net/)
Zac1: Fragment4 primers for RNA FISH	F 5'- GGAAAACAGGAATGGGGTTT -3' R 5'- TCCCCTTCCTAGGCTACACA-3'	55ºC	Primer3 (http://primer3.sourceforge.net/)
Sf3b5: Primers for RNA FISH	F 5'- GGAGGATTCGGAACAAGTCA -3' R 5'- CCATCACTCTCGTGCAGTCT-3'	55ºC	Primer3 (http://primer3.sourceforge.net/)
Zac1: primers for MSP	Methylated CpG F 5'- GTAGTTATTTTTTTGGTTGGCGT-3'	55ºC	MethPrimer (http://www.urogene.org/methprimer)

	R 5'- CCCGACTAAATCAAAACTCGA-3' Unmethylated CpG F 5'- GTTATTTTTTTGGTTGGTGT-3' R 5'- CCCAACTAAATCAAAACTCAAA-3'	55ºC	
Zac1: primers for qRT-PCR	F 5'-AATGTGGCAAGTCCTTCGTC-3' R 5'-CTTTGCCACACTCAGCCTTC-3'	55ºC	Primer3 (http://primer3.sourceforge.net/)
rRNA: primers for qRT-PCR	F 5'-CGCGTCGTTGCTCACTCTTA-3' R 5'-CCATTCGCCATGAATGTCC-3'	55ºC	Primer3 (http://primer3.sourceforge.net/) (Kass, et al. 1987)
α-*Actin*: primers for qRT-PCR	F 5'-CGCGTCCACCCGCGAG-3' R 5'-CCTGGTGCCTAGGGCG-3'	60ºC	Primer3 (http://primer3.sourceforge.net/)
Gapdh: primers for qRT-PCR	F 5'-AACGACCCCTTCATTGAC-3' R 5'-TCCACGACATACTCAGCAC-3'	55ºC	(Simpson, et al. 2000)
Sf3b5 primers for	F 5'- GGAGCATCTGCAGTCCAAGT-3' R 5'- GGCCCATGTAGGAGCAGTAG-3'	55ºC	Primer3 (http://primer3.sour

qRT-PCR			ceforge.net/)

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