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Identification of imprinted loci by methylation-sensitive representational difference analysis: application to mouse distal chromosome 2.

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Abstract

Imprinted genes are distinguished by different patterns of methylation on their parental alleles, a property by which imprinted loci could be identified systematically. Here, representational difference analysis (RDA) is used to clone HpaII fragments with methylation differences on the maternal and paternal copies of distal chromosome (Chr) 2 in the mouse. Uniparental inheritance for this region causes imprinting phenotypes whose molecular basis is only partially understood. RDA led to the recovery of multiple differentially methylated HpaII fragments at two major sites of imprinted methylation: paternal-specific methylation at the Nesp locus and maternal-specific methylation at the Gnaxl locus. Nesp and Gnaxl represent oppositely imprinted promoters of the Gnax gene, which encodes the G-protein subunit, Galpha. The organization of the Nesp-Gnaxl-Gnax region was determined: Nesp and Gnaxl were found to be 15 kb apart, and Gnaxl was found to be 30 kb upstream of Gnax. Sites of imprinted methylation were also detected at the loci for **neuronatin** on Chr 2 and for M-cadherin on Chr 8. RDA was highly effective at identifying imprinted methylation, and its potential applications to imprinting studies are discussed.

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