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ASOCIACIÓN MADRILEÑA
PARA EL SÍNDROME DE
PRADER-WILLI



A MASTER REGULATOR OF COORDINATE GENE EXPRESSION IN PRADER-WILLI SYNDROME

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Genetic defects in Prader-Willi syndrome (PWS) lead to loss of function of a unique set of imprinted, paternally-expressed genes in chromosome 15q11.2 that encode proteins, snoRNAs and, in the mouse, microRNAs. PWS genes have enriched expression in neurons with most predicted to regulate other RNAs. However, the mechanistic relationships between imprinting, somatic expression of PWS genes and the phenotypic basis are unknown. Bioinformatic analyses of mammalian genome sequences using permutations of Nuclear Respiratory Factor-1 (NRF-1) consensus motifs revealed an unexpected high number of potential NRF-1 sites within the PWS imprinted region. In mouse, a total of 18 phylogenetically conserved NRF-1 motifs were identified within the *Ndn* promoter, U1 promoters, *Snurf-Snrpn* promoter and enhancer, a mini-CpG island between *Mkrn3* and *Magel2* and a cluster of four adjacent NRF-1 binding sites within *Mirh1* intron 2. Using chromatin immunoprecipitation we found that NRF-1 binds to open chromatin [acetylated H4 and dimethylated H3 (K4) histones] and unmethylated CpG regions containing NRF-1 sites of the active, paternally-derived allele in mouse brain and human neuroblastoma (NB) cells. However, NRF-1 was not associated with inactive chromatin at the sites located in the intergenic region between *Mkrn3* and *Magel2*, *Mirh1* intron 2 (NRF-1 cluster) and U1 promoters in mouse fibroblasts suggesting a neuronal specific function for these elements. To assess the significance of NRF-1 for the activity of PWS regulatory regions, mutations in NRF-1 binding sites were analyzed using dual-luciferase reporter assays. Luciferase (*Luc*) gene transcription under the control of the *Ndn*, *Snurf-Snrpn* and U1 minimal promoters was significantly reduced in the absence of NRF-1 binding sites. Interestingly, NRF-1 confers promoter activity *in vitro* for both the intergenic *Mkrn3/Magel2* mini-CpG island and *Mirh1* intron 2 NRF-1 cluster despite the fact that these structures are not promoters *in vivo*. Moreover, when cloned in the enhancer position of the pGL3-basic vector, the NRF-1 cluster dramatically increased promoter activity of *Ndn* and *Mirh1* in NB cells suggesting a broader regulatory role for this element. To demonstrate that NRF-1 is a master regulator of PWS-region genes, we used vector-mediated small interfering RNA (siRNA) to examine the effect of down-regulation of NRF-1 expression on the transcription of PWS imprinted genes in NB cells. Knockdown of NRF-1 expression with an average fold of 0.48 ± 0.04 resulted in significantly reduced mRNA levels of all PWS genes analyzed by regular and quantitative RT-PCR analysis. Intriguingly, down-regulation of NRF-1 also decreases transcription of PWS genes that don't possess NRF-1 binding sites within 5' regulatory regions (eg. *Mkrn3*, *Mirh1*). These results demonstrate that NRF-1 acts as a master transcription factor for PWS somatic expression. Further studies aim to identify long-range regulatory mechanisms mediated by NRF-1 in the PWS region by visualizing interactions between distant PWS regulatory regions.