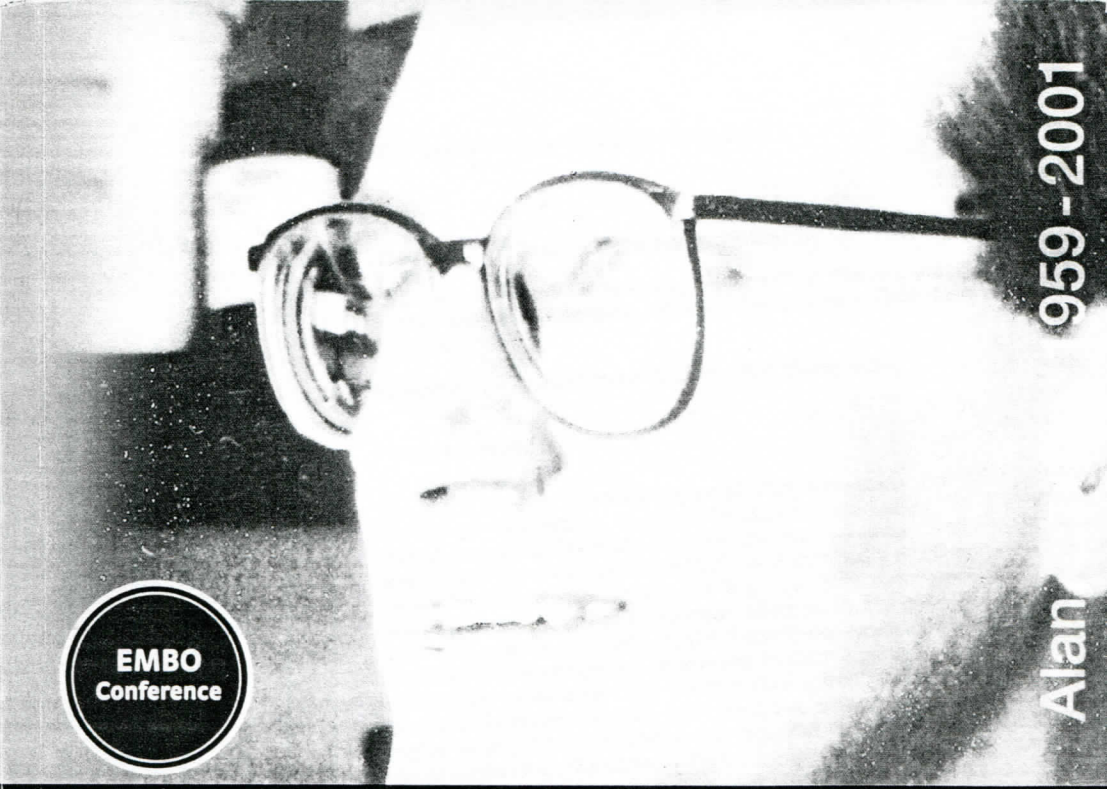

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Regulation of Cxcl12 gene transcription by DNA methylation

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Our aim is to study intertwining roles of DNA methylation and PARP proteins in regulation of Cxcl12 gene expression, important for diabetes attenuation. The experiments were done on mouse embryonic fibroblast (NIH3T3) and its counterpart PARP-1 knockout (PARP-/-) cell lines. Using real-time quantitative PCR we determined a much higher level of Cxcl12 mRNA in PARP-/- compared to NIH3T3 cell line. This is in accordance with our published results showing that PARP-1 has an inhibitory effect on Cxcl12 gene expression. Further, we treated both cell lines for 72 h with 30 μM 5-aza-2'-deoxycytidine (5AZA), known DNA methylation inhibitor. This treatment led to increase in Cxcl12 gene expression in NIH3T3. Finally, DNA methylation analysis of CpG island spanning the beginning of Cxcl12 gene was done by real-time methylation-specific PCR using two sets of primers. With both primer sets, much higher level of DNA methylation was detected in NIH3T3 cells compared to PARP-/- which could explain the differences in Cxcl12 gene expression. After the treatment with 5AZA, DNA methylation in NIH3T3 cells dropped between 20-30% which could account for the detected rise in Cxcl12 gene expression. In general, these results show that Cxcl12 gene transcription is regulated by methylation of CpG dinucleotides covered by used primer sets. Additionally, expression of Cxcr4, Parp-1 and Parp-2 gene was analyzed in control and 5AZA-treated cells. Since Cxcr4 is a Cxcl12 receptor it is not surprising that the Cxcr4 gene expression follows the same trend as Cxcl12 gene expression. Compared to controls, Parp-1 gene expression decreases an increase in treated NIH3T3 cells, while Parp-2 gene expression is increased in both cell lines treated with 5AZA. Higher Parp-2 gene expression in PARP-/- cells compared to NIH3T3 cells could be due to Parp-2 taking over the role of the missing Parp-1 in PARP-/- cells.