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Transcriptional regulation of rat brain glycogen phosphorylase Benedikt Dolderer*, Stephan Verleysdonk and Bernd Hamprecht

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There are three known isozymes of the homodimeric glycogen phosphorylase (GP), liver (GP-LL), muscle (GP-MM), and brain (GP-BB). The observation that astrocytes from astroglia-rich primary cultures contain GP-BB, whereas astrocytes accompanying cultured neurons lacked this enzyme, led to the question of how the transcription of GP-BB is regulated. C6-BU-1 rat glioma cells were shown by both Western blot analysis and immunocytochemistry to express GP-BB. Since these cells express GP-BB and therefore must contain transcription factors for its transcriptional regulation, they were chosen as a model for this investigation. C6-BU-1 cells, however, turned out to be transfected by plasmids only with low efficiency. Therefore, lentiviral vectors were designed as more promising tools for the transfection of these cells. The lentiviral constructs were devised to contain two expression cassettes for integration into the host genome. Secreted alkaline phosphatase (SEAP) was placed under the control of a CMV promoter in order to serve as a measure for the efficiency of viral transfection. The second reporter gene, firefly luciferase (LUC), was put under the control of up to 4000 bp long segments of the 5'-untranscribed region of the rat GP-BB gene. Results will be communicated that were obtained when the LUC:SEAP activity ratios were used to serve in the identification of transcription factor binding sites.