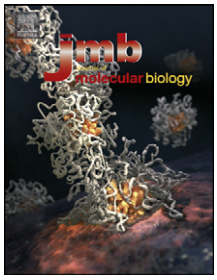

Unexpected Distinct Roles of the Related Histone H3 Lysine 9 Methyltransferases G9a and G9a-Like Protein in Myoblasts

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Abstract

Lysine methyltransferases G9a and GLP (G9a-like protein) are highly homologous and form functional heterodimeric complexes that establish mono- and dimethylation on histone H3 lysine 9 (H3K9me1, H3K9me2) in euchromatin. Here, we describe unexpected distinct roles for G9a and GLP in skeletal muscle terminal differentiation. Indeed, gain- or loss-of-function assays in myoblasts showed, in agreement with previous reports, that G9a inhibits terminal differentiation. While GLP plays a more intricate role in muscle differentiation,in one hand, both GLP gain and loss of function inhibit late steps of differentiation; on the other hand, in contrast to G9a, GLP overexpression promotes abnormal precocious expression of muscle differentiation-specific genes already in proliferating myoblasts. In agreement, transcriptomic analysis indicates that G9a and GLP regulate different sets of genes. Thus, GLP, but not G9a, inhibits proteasome subunit-encoding genes expression, resulting in an inhibition of the proteasome activities. Subsequently, GLP, but not G9a, overexpression stabilizes MyoD that is likely to be responsible for muscle markers expression in proliferating myoblasts.

Introduction

Cellular differentiation involves the coordinated activation and repression of specific subsets of genes, concomitant to significant changes in cellular metabolism. This is established by a network of transcription factors and epigenetic regulators, such as DNA methyltransferases, histone acetyltransferases, histone deacetylases, and lysine methyltransferases (KMTs). The latter are responsible for tri-, di-, or monomethylation of histone and non-histone lysine residues. Depending on the specific histone residue that is methylated, the degree of methylation can either positively or negatively regulate gene expression [1]. Thus, KMTs play key roles in transcriptional regulation during development and are also emerging as crucial players in the control of cellular differentiation, including myogenesis [2–6]. Among more than 50 different KMTs in mammals, histone 3 lysine 9 (H3K9)-specific KMTs of Suv39h family, G9a, G9a-like protein (GLP), Setdb1, and Suv39h are involved in the induction or maintenance of gene repression and heterochromatin formation (as reviewed in Ref [7]).

G9a (also known as KMT1C, EHMT2) and GLP (known as KMT1D, EHMT1) are two highly homologous H3K9 KMTs, bearing a catalytic SET domain and ankyrin repeats involved in protein–protein interactions [8,9] and methyl-lysine binding [10]. Although very similar, while the ankyrin repeat domain of G9a preferentially associates with H3K9me2, the ankyrin repeat domain of GLP preferentially associates with H3K9me1 [10]. Knockout of *G9a* or *GLP* genes in mice induces similar phenotypes, including embryonic lethality, and revealed that G9a and GLP are mainly responsible for mono- and dimethylation of H3K9 in euchromatin [11,12]. Although G9a and GLP can independently

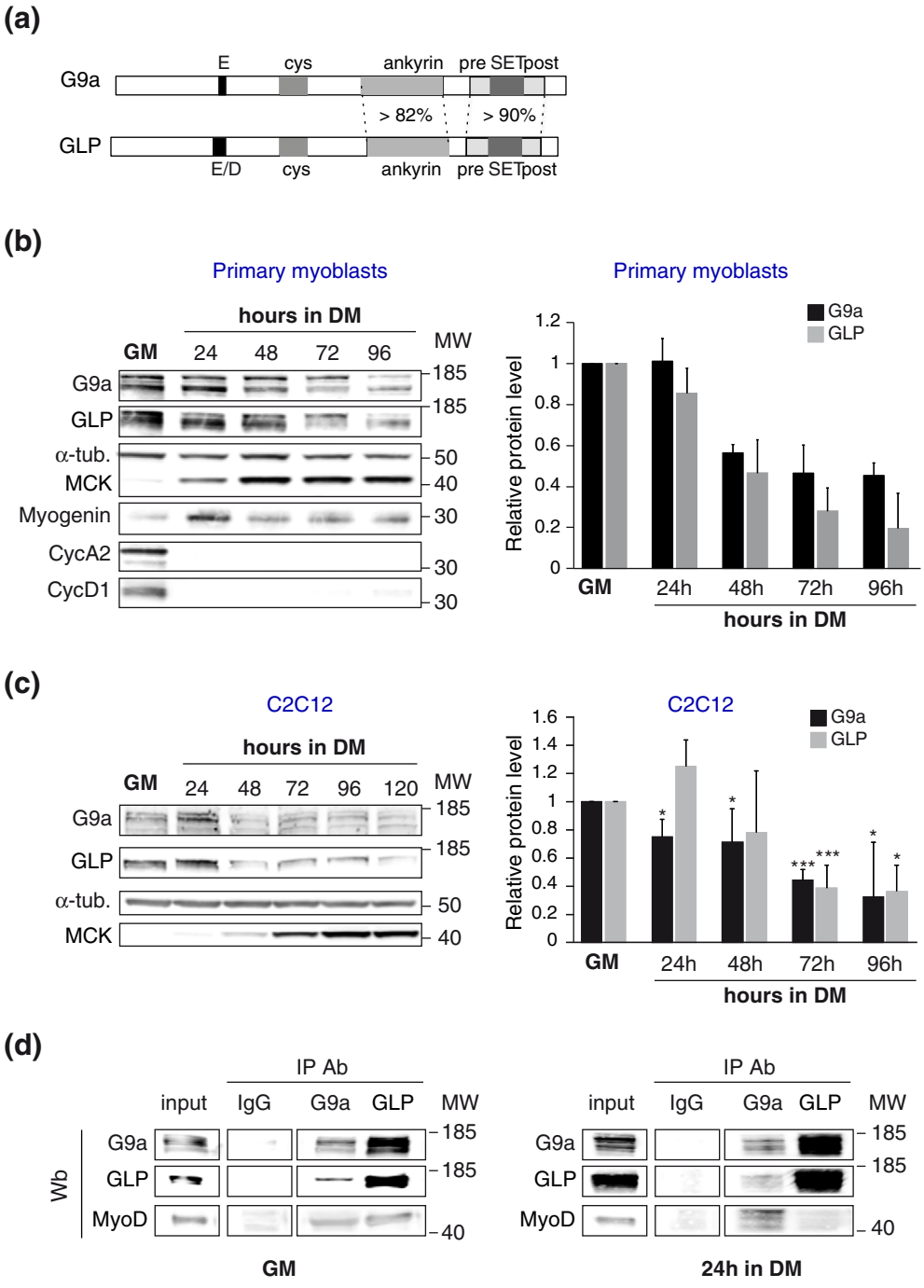

Fig. 1. The highly homologous G9a and GLP are similarly regulated during skeletal muscle terminal differentiation. (a) Schematic representation of G9a and GLP proteins. E: Glu-rich region; E/D: Glu/Asp-rich region; Cys: Cystein-rich region; Ankyrin: Ankyrin repeats; Pre: Pre-SET domain; SET: SET domain; Post: Post-SET domain. In the middle: % of homology for the most conserved domains. (b) Left: Representative WB analysis with the indicated antibodies on protein extracts from proliferating (Growth Media: GM) or differentiating (Differentiation Media: DM, for 24 h, 48 h, 72 h, or 96 h) primary myoblasts. Right: Quantification of WB signals using ChemiSmart system (Vilber Lourmat). GLP or G9a signals were normalized first to α-tubulin and then to protein level in proliferation. n = 2. (c) Left: representative WB as in B, but with protein extracts from C2C12 myoblasts. Right: protein quantification as in B with n > 3. * for p-value <0.05, *** for p-value <0.001. (d) Protein extracts from proliferating (GM) or differentiating (24 h in differentiation medium or DM) C2C12 myoblasts were subjected to IP using G9a, GLP, or irrelevant antibodies (IgG) and then analyzed by WB with the indicated antibodies. Molecular Weight is in kDa.