

## Perspective

# Geminin's Double Life

## Chromatin Connections that Regulate Transcription at the Transition from Proliferation to Differentiation

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Geminin, neurogenesis, cell cycle, differentiation, Brg1, chromatin remodeling, neural bHLH, Hox, Six3, Polycomb

### ABBREVIATIONS

Gem	Geminin
Brg1	Brahma-related gene 1
Brm	Brahma
bHLH	basic Helix-loop-helix
ORC	Origin recognition complex
Mcm	mini-chromosome maintenance
TrxG	Trithorax group
Rb	retinoblastoma

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### ABSTRACT

During embryonic development, transitions between cellular programs regulating progenitor cell proliferation and differentiation must be precisely coordinated and temporally controlled to ensure that a proper number of cells are allocated to various structures. The novel coiled-coil protein Geminin was previously characterized as a dual function molecule with roles both in maintenance of genome integrity through regulation of DNA replication licensing and in control of neural cell fate during embryonic development. However, the mechanistic basis of Geminin's activities during embryogenesis and the connections to its cell cycle regulatory role were unknown. Recently, some of Geminin's activities in regulating transcription were shown to occur through interactions with Brg1, the catalytic subunit of the SWI/SNF chromatin-remodeling complex. During development of the nervous system, Geminin controls the transition from proliferating precursor to differentiated post-mitotic neuron by modulating interactions between SWI/SNF and bHLH transcription factors that are critical for neurogenesis. In other developmental contexts, Geminin mediates proliferative-differentiative transitions through interactions with Six3 and Hox transcription factors and Polycomb Group proteins. Interactions of Geminin with Polycomb and SWI/SNF complex proteins link its transcriptional activities to modulation of chromatin structure. Here we incorporate recent findings regarding Geminin's regulatory roles in coordinating proliferation and differentiation during embryogenesis.

### TRANSCRIPTIONAL CONTROL OF VERTEBRATE NEURAL DEVELOPMENT

Formation of the vertebrate nervous system involves multiple processes, including an initial induction of neural tissue from naive ectoderm, cell proliferation to form neural structures of the proper size, commitment to neuronal cell fates, cell cycle withdrawal and subtype-specific gene expression needed for neuronal function. The transition of cells from proliferating neural progenitors into post-mitotic, differentiated neurons represents a critical aspect of vertebrate neural development. This process involves extensive changes in gene expression that must be under tight temporal control and coordinated with other cellular processes, such as cell cycle withdrawal, so that neural structures of the proper size and structure are formed (reviewed in refs. 1–3).

Vertebrate neural development is controlled by several groups of transcriptional regulators. The initial neuroectoderm consists of multi-potent neural progenitor cells that can proliferate to generate the number of cells needed to form the entire nervous system. These neural progenitors express the SoxB1 subfamily transcription factors *Sox2* and *Sox3*, which maintain the neural progenitor state.<sup>4,5</sup> Subsequent to their formation, subsets of these neural progenitors become committed to particular neuronal fates, undergo cell cycle withdrawal, and differentiate. Basic helix-loop-helix (bHLH) transcription factors related to *Drosophila* Achaete-scute and Atonal, including the Neurogenins and NeuroD, are pivotal regulators of neuronal cell fate determination and differentiation, respectively. In mammals, three Neurogenin proteins (Ngn1–3) are present. These are expressed in proliferating neuronal precursors and are critical for neuronal cell fate determination, as evidenced in *Ngn1* and *Ngn2* mutant mice, which show deficits of cranial and spinal sensory ganglia and ventral spinal cord neurons.<sup>6–9</sup> Conversely, *NeuroD* is expressed in committed, post-mitotic neurons and is required for neuronal differentiation (reviewed in refs. 10 and 11).

## ACTIVITIES OF GEMININ IN REGULATING THE CELL CYCLE AND EMBRYONIC DEVELOPMENT

Geminin (Gem) represents another potential regulator of early neural development. Geminin is a novel coiled-coil protein without obvious sequence homology with other known protein families. Geminin was identified in two independent functional screens in *Xenopus laevis*, and subsequently characterized as a protein with roles in regulating both DNA replication<sup>12</sup> and neural cell fate during embryonic development.<sup>13</sup> In the former work, Geminin was shown to prevent reinitiation of replication within a single cell cycle to maintain chromosomal integrity and euploidy. To ensure precise genome duplication in each cell cycle, each origin of DNA replication must be 'licensed' before S phase begins. Licensing involves sequential assembly of a pre-replication complex (pre-RC), containing the origin recognition complex (ORC), Cdc6, Cdt1 and then the mini-chromosomal maintenance proteins (Mcm2-7) onto DNA. Geminin plays a key role in inhibiting replication licensing: Geminin levels rise during S phase and Geminin associates with Cdt1 during S, G<sub>2</sub> and M phases to prevent reinitiation of DNA replication. Degradation of Geminin by the anaphase promoting complex (APC) during mitosis (or nonproteolytic inactivation of Geminin that escaped degradation) then enables a new round of DNA replication to be initiated in the subsequent S phase<sup>12,14,15</sup> (reviewed in refs. 16 and 17).

Geminin was concurrently identified in a functional expression screen in *Xenopus laevis* embryos for molecules that could expand the early neural plate.<sup>13</sup> In both vertebrate embryos and *Drosophila*, Geminin plays a critical role in neural cell fate determination.<sup>13,18</sup> In *Xenopus*, *Geminin* is highly expressed at the onset of gastrulation in a territory that prefigures the future neural plate and that encompasses the proliferating neural progenitor cell population, a pattern similar to that of *Sox2* and *Sox3*.<sup>13</sup> Overexpression of *Geminin* in naive ectoderm can induce ectopic neural tissue at the expense of nonneural cells, suggesting a cell fate change. Conversely, loss of function of *Geminin* using dominant-negative or antisense morpholino oligonucleotide approaches inhibits neural tissue formation.<sup>13,19</sup> In *Drosophila*, overexpression of *Geminin* also induces ectopic neuronal cells, while Geminin mutant embryos show neuronal deficiencies.<sup>18</sup>

Geminin's ability to impact both the fidelity of DNA replication and neural cell fate was suggestive of a potential role in integrating cell cycle and transcriptional controls during the transition from proliferation to differentiation. Recently, such a role has indeed emerged and molecular mechanisms through which Geminin regulates the transition from proliferation to differentiation have been defined in several cellular contexts. Geminin interacts with Six3 and Hox transcription factors and can antagonize the transcriptional activities of these proteins to regulate cellular differentiation.<sup>20-22</sup> Geminin's ability to block Hox function is linked to interactions with Polycomb group proteins, which form multi-protein complexes and regulate chromatin structure to repress gene expression. We also recently found that Geminin interacts with Brg1, the catalytic subunit of a SWI/SNF chromatin remodeling complex, and we showed that Geminin's antagonism of Brg1-dependent transcription regulates the proliferative-differentiative transition during neurogenesis.<sup>19</sup> Together, this work links Geminin's transcriptional activities to interactions with protein complexes that regulate chromatin structure and indicates that Geminin regulates proliferative-differentiative transitions during several developmental processes. We summarize these recent findings and highlight some of their implications below.

## GEMININ-BRG1 INTERACTIONS REGULATE THE TRANSITION FROM PROLIFERATION TO DIFFERENTIATION DURING NEUROGENESIS

Changes in gene expression during embryonic development are accompanied by reorganization of the chromatin structure. In general, actively transcribed chromatin segments contain highly acetylated nucleosomes and are methylated on particular histone residues, while nucleosomes from transcriptionally inactive segments are frequently deacetylated and have an alternate pattern of methylation. Two broad classes of enzymatic activities modify chromatin structure to enhance transcription: chromatin-modifying proteins such as histone acetyltransferases (HATs) covalently modify histones, whereas chromatin-remodeling enzymes reorganize the nucleosomal structure of chromatin. Chromatin remodeling enzymes use the energy of ATP hydrolysis to relocate histone octamers to alternate positions to affect the access of specific loci to the transcriptional machinery.

SWI/SNF, one such chromatin-remodeling complex, consists of a total of 7–13 subunits and interacts with various sequence-specific transcription factors, HATs, and histone deacetylases (HDACs) to either activate or repress target genes. SWI/SNF complexes in mammalian cells include one of the two ATPase catalytic subunits, Brahma (Brm) or Brahma-related gene 1 (Brg1), but not both. Brm- and Brg1-containing complexes have similar in vitro biochemical activities but appear to have some target gene specificity in vivo.<sup>23</sup> Four core SWI/SNF subunits (Brg1 or Brm, SNF5/INI1, BAF155 and BAF170) are sufficient to remodel nucleosomes in vitro, while the overall protein composition complex shows some heterogeneity within different tissues, with different SWI/SNF complex variants associating with particular transcription factors to activate or repress specific transcriptional targets<sup>24-26</sup> (reviewed in ref. 27).

The SWI/SNF complex has been implicated as a critical transcriptional regulator of numerous cellular processes including cell proliferation and differentiation.<sup>28-30</sup> Previously, we found that the SWI/SNF chromatin-remodeling protein Brg1 was required for cell cycle exit and neuronal differentiation of neural progenitor cells.<sup>31</sup> In the absence of Brg1 activity, initial formation of the neural plate occurs normally. However, neural progenitors fail to exit the cell cycle and remain as proliferating progenitors. Consequently, the neural progenitor population increases transiently and differentiated neurons are not generated. At later stages, at least some of these neural progenitors undergo apoptosis (Seo S, Kroll K, unpublished data). Brg1 physically interacts with the bHLH transcription factors Ngn and NeuroD and mediates their transcriptional activities to induce neurogenesis. This requirement of Brg1 for neuronal differentiation is conserved in both mammalian cells and *Xenopus* neuroectoderm. These data demonstrate that chromatin remodeling by Brg1 and the SWI/SNF complex is essential to mediate the transcriptional activities of Ngn and NeuroD in activating their target genes during neuronal differentiation.

Recently, we also found that Geminin interacts with Brg1 physically and genetically and antagonizes Brg1's activity during neuronal differentiation.<sup>19</sup> *Geminin* is highly expressed in proliferating neural progenitors and downregulated before neuronal differentiation. Misexpression of *Geminin* blocks neurogenesis driven by Ngn and NeuroD. In contrast, Geminin variants unable to bind Brg1 lack this activity, suggesting that Geminin's activity to inhibit neurogenesis is dependent on its ability to bind Brg1. Reduction of Geminin activity results in premature neurogenesis. Finally, overexpression of *Geminin* prevents the association of Brg1 and proneural bHLH

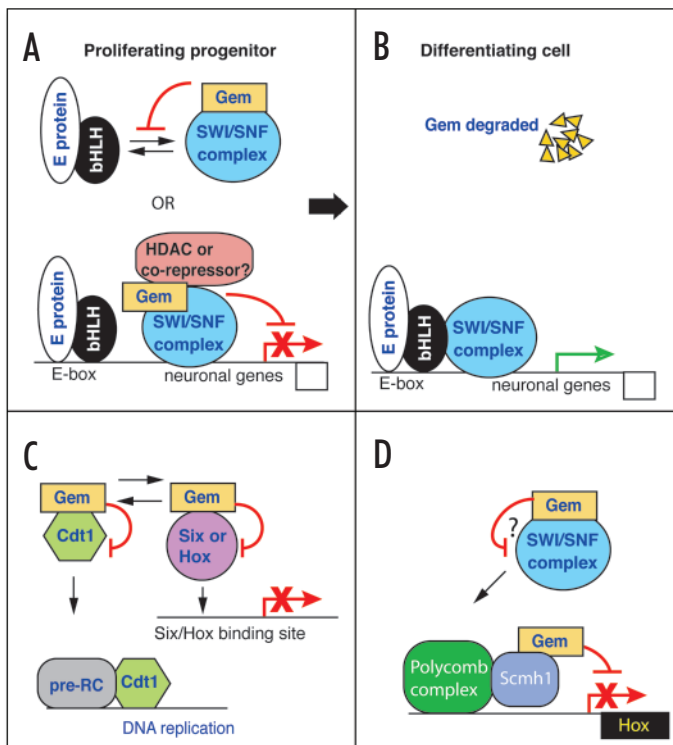


Figure 1. (A) High levels of Geminin, found in proliferating neural progenitor cells, can block coordinated activities of neural bHLH proteins and SWI/SNF at target genes, either by directly inhibiting bHLH-SWI/SNF interaction (top) or by associating with the SWI/SNF complex at bHLH target loci and recruiting HDACs or co-repressors to silence transcription (bottom). (B) Downregulation of Geminin levels occurs around the time of cell cycle exit, enabling productive bHLH-SWI/SNF transcriptional complexes to activate target gene transcription. (C) Geminin can bind to Cdt1 or to Six3 or Hox transcription factors in a competitive manner, either antagonizing Hox/Six3-dependent transcription or antagonizing Cdt1 association with pre-replication complexes (pre-RC) at the G<sub>1</sub>/S transition. (D) Gem can also negatively regulate *Hox* gene expression by interacting with Polycomb proteins at *Hox* enhancers. Since some SWI/SNF proteins contribute to TrxG activity to maintain *Hox* gene expression, Geminin's antagonism of SWI/SNF could provide an additional mechanism for blocking *Hox* transcription.

proteins, indicating that Geminin could inhibit neurogenesis by modulating the protein-protein interactions of Brg1. These data indicate that Geminin maintains neural progenitors in an undifferentiated state by antagonizing Brg1's function.

Based on these data, the models in Figure 1A and B depict how interactions between proneural bHLHs, Brg1, and Geminin could control the transition of neural progenitors to differentiated neurons. Proneural bHLH genes are expressed in committed neuronal precursor cells and drive neuronal differentiation, but they require chromatin remodeling activity from the SWI/SNF complex to induce target gene expression from a repressive chromatin context. In progenitor cells and committed precursor cells, Geminin is present at high levels and blocks the association of bHLHs and Brg1, preventing transcription of bHLH target genes and premature neurogenesis. In differentiating neurons, Geminin levels diminish, and neuronal differentiation driven by bHLH-Brg1 association and activation of bHLH target genes are permitted to occur. Therefore, Brg1 and the SWI/SNF complex provides chromatin-remodeling activities required for neurogenesis, while Geminin regulates the timing of neurogenesis within precursor cells (Fig. 1).

## HOW DOES GEMININ-SWI/SNF ANTAGONISM REGULATE TRANSCRIPTION DURING NEUROGENESIS?

The interaction between Gem and Brg1, described above, provides a mechanism through which Gem can integrate transcriptional changes at the intersection between proliferation and differentiation during neurogenesis. However, the mechanistic basis of Geminin's ability to antagonize Brg1 still needs to be clarified. Geminin interacts with domain II of Brg1. Brg1 domain II is highly conserved among SNF2 family proteins but its function has not been intensively studied.<sup>32,33</sup> Domain II is known to be a binding site for the SWI3/MOIRA/BAF155 subunit of the SWI/SNF complex.<sup>34</sup> In *Drosophila* embryos, deletion of domain II from Brm caused a small but reproducible decrease in the size of the BRM complex, suggesting that this complex lacks one or more subunits.<sup>35</sup>  $\beta$ -catenin has also been shown to interact with Brg1 through domain II.<sup>36</sup> Therefore, domain II might be a platform for protein-protein interactions in Brg1 and may modulate SWI/SNF complex function by changing the subunit composition of the SWI/SNF complex or mediating its interaction with other sequence specific transcription factors. By binding to Brg1 domain II, Geminin may cause a change in the composition and/or the remodeling activity of SWI/SNF complex. Alternatively, or in addition to this, Geminin may regulate protein-protein interactions between Brg1 and other sequence specific transcription factors. Indeed, even though the bHLH interacting domain(s) within Brg1 has not been determined, Geminin can block the interaction between proneural bHLHs and Brg1 in committed neuronal precursor cells.

Geminin may also recruit additional corepressors or HDACs to the regulatory regions of bHLH-Brg1 target genes to suppress their expression. In the competition model shown in Figure 1A (top), association of Geminin with chromatin is not required to antagonize Brg1's function. However, a fraction of Geminin is found on chromatin.<sup>37,38</sup> In addition, Geminin binds the regulatory elements of the *Hoxd11* intron and 3' untranslated region (UTR) and associates with Polycomb repressor complex proteins at these sites.<sup>20</sup> Geminin has also been reported to interact with an HDAC-associated activity,<sup>22</sup> so Gem could potentially repress transcription by recruiting HDACs to Polycomb-regulated loci. Therefore, Geminin may recruit transcriptional corepressors or HDACs to bHLH-regulated target loci to suppress their expression, as well as blocking bHLH-Brg1 associations (Fig. 1A, bottom). Chromatin immunoprecipitation (ChIP) experiments for bHLHs, Brg1, Geminin, and HDACs at representative bHLH target loci are needed to understand mechanisms by which Geminin regulates neuronal gene expression.

## OTHER TRANSCRIPTIONAL REGULATORY ACTIVITIES OF GEMININ DURING EMBRYONIC DEVELOPMENT

In addition to its Brg1-dependent role in neuronal differentiation Geminin impacts nervous system development in a Brg1-independent manner. For example, the N-terminus of *Xenopus* Geminin (amino acids 38-90) is sufficient for neural plate expansion and suppression of nonneural cell fates.<sup>13</sup> This activity must occur independent of Brg1-binding, which is mediated by a separate C-terminal motif. More recently we found that *Geminin* misexpression in *Xenopus* expands the territory that expresses general neural (*NCAM*) and neural progenitor (*Sox2*) markers and that Brg1-binding defective Geminin variants do not lose this activity (ref. 19; Seo S, Kroll K, unpublished data). *Sox 2* marks neural progenitors and maintains

progenitor properties, including self-renewal and multipotency, while preventing neuronal differentiation.<sup>4,5</sup> Geminin might therefore play at least two cooperative and complementary transcriptional regulatory roles in neural progenitor cells. Gem may both control the expression of genes required to generate and maintain the progenitor state in a Brg1-independent manner, while also acting through antagonism of Brg1 activity to block precocious transcriptional activation of bHLH target genes that mediate neuronal differentiation.

Geminin has also been shown to bind Hox and Six3 proteins and to regulate their transcriptional activities during body axis segmentation and retinal development respectively.<sup>20,21</sup> Geminin interaction with Hox and Six3 proteins prevents these transcription factors from activating their targets and also competes Geminin away from Geminin-Cdt1 complexes. Therefore, bidirectional competition between Gem-Cdt1 versus Gem-Hox/Six3 interactions can act as a cellular switch, coordinating transcriptional events with regulation of DNA replication and cell cycle progression. (Fig. 1C). Interestingly, Geminin can negatively regulate Hox activity both by directly interacting with and inhibiting Hox proteins (Fig. 1C) and also by negatively regulating Hox gene expression. For the latter, Geminin interacts with Polycomb complex proteins and exerts a "Polycomb-like" transcriptional repressive activity, associating with Polycomb-regulated cis-elements at Hox genes (Fig. 1D).<sup>20</sup>

Vertebrate Hox genes act combinatorially to define positional identity along the anterior-posterior body axis during embryonic development. Trithorax and Polycomb group proteins act in multiprotein complexes to respectively maintain activation or repression of Hox gene expression. They regulate chromatin structure to create a "cellular memory" of Hox transcriptional status that persists through many divisions (reviewed in refs. 39 and 40). SWI/SNF complex subunits (including Brahma, Moira/BAF155, and Osa) were identified in *Drosophila* as *Trithorax group* (*TrxG*) genes essential for maintaining *Hox* gene expression. Therefore, Geminin could block *Hox* transcription by interference with Brahma and Brg1 to negatively impact TrxG protein activity, as well as by exerting a Polycomb-like activity (Fig. 1D).

Geminin's antagonism of the transcriptional activities of Hox, bHLH, and SWI/SNF also suggests a role for Geminin in negatively regulating target genes controlled by these proteins. There is an extensive precedent for functional cooperativity between bHLH and homeodomain proteins in regulating developmental processes: for example, during neurogenesis and retinogenesis bHLH proteins drive neuronal fate and differentiation while homeodomain protein activities specify particular cellular subtypes.<sup>9,41-45</sup> This can involve direct transcriptional coordination between homeodomain and bHLH proteins bound to adjacent sites in target gene enhancers.<sup>46</sup> Therefore, it will be interesting to determine whether Geminin's interactions with both homeodomain proteins and SWI/SNF provide a dual negative regulatory mechanism at target genes coordinately regulated by bHLH, homeodomain, and SWI/SNF protein complexes.

## GEMININ, SWI/SNF, AND REGULATION OF THE CELL PROLIFERATION-DIFFERENTIATION TRANSITION

We found that, during neurogenesis, *Geminin* is highly expressed in neural progenitors and is downregulated prior to neuronal differentiation. This downregulation was required to enable transcriptional activation by coordinated neural bHLH-SWI/SNF activities and the

transition from proliferating neuronal precursor to differentiated neuron. Geminin and Brg1 both show enriched expression in neural tissues during embryonic development but also have widespread expression in other tissues in embryos and adult animals. Therefore, antagonistic Gem and SWI/SNF interactions may also act in other cellular contexts to regulate the proliferation to differentiation transition.

*Geminin* expression correlates strongly with the proliferative cell state in a wide variety of normal and cancer cells and embryonic and adult tissues.<sup>18,47-49</sup> *Geminin* is highly expressed in multiple cancer types, including B-cell lymphomas, invasive breast cancers, renal cell carcinoma, and colon and rectal tumors.<sup>49-52</sup> High *Geminin* expression levels often indicate malignancy or more aggressive neoplasms because *Geminin* expression specifically marks S/G<sub>2</sub>/M phase cells and therefore indicates cells with a shortened G<sub>1</sub> phase and increased rate of proliferation.<sup>48,50,51</sup> *Geminin*'s expression profile resembles that of cell-cycle regulated genes with oscillating expression (such as *cyclin A*) rather than that of genes that promote cell cycle withdrawal accompanying differentiation, such as the cyclin-dependent kinase (CDK) inhibitor *p21*.<sup>48</sup> Downregulation of *Geminin* expression during differentiation may be directly regulated by the Rb/E2F pathway. The Retinoblastoma (Rb) tumor suppressor forms repressive transcriptional complexes with E2F family proteins at genes that promote cell cycle progression, such as *cyclin E*. E2Fs activate *Geminin* expression in actively cycling cells, while Rb represses *Geminin* transcription, effects mediated by E2F binding in the Geminin intragenic region.<sup>53,54</sup>

Geminin also stimulates cell growth and proliferation in some contexts. For example, Geminin stimulates proliferation of both nontumorigenic mammary epithelial cells and anchorage-dependent and independent growth of breast cancer cells.<sup>49</sup> We also found that overexpression of *Geminin* variants deficient for Cdt1-binding in *Xenopus* embryos increases the number of mitotic cells marked by expression of phosphorylated histone H3 and the neural progenitor marker *Sox2* (Seo S, Kroll K, unpublished data). The "Polycomb-like" activity of Geminin is also suggestive of a role in progenitor cell maintenance. The Polycomb protein Bmi1 is critical for self-renewal and proliferation of hematopoietic and some neuronal progenitor cells and blocks premature cellular senescence during hematopoietic development.<sup>55-59</sup> Together, the data above indicate that *Geminin* expression is compatible with the progenitor cell state and can contribute to maintaining proliferating progenitors and blocking cellular differentiation, in a context and dose-dependent manner.

In contrast to these data for Geminin, Brg1 and several other SWI/SNF complex proteins have intrinsic tumor suppressor activity (reviewed in refs. 29, 30 and 60). Loss of function of the SWI/SNF subunits Brg1 or Snf5/INI1 predisposes mice to tumors.<sup>61-64</sup> In humans, specific Brg1 mutations are found in pancreatic, breast, lung and prostate cancer cells and Brg1 is mutated or downregulated in approximately 10% of all primary human tumors, supporting its role as a tumor suppressor.<sup>65-67</sup> Brg1's tumor suppressor activities have been linked to Rb: Brg1 and Brm interact directly with Rb and cells lacking both Brg1 and Brm fail to undergo Rb-mediated cell cycle exit. Additionally, reintroduction of Brg1 or Brm into transformed fibroblasts reverts the transformed state.<sup>68-70</sup> Snf5/INI1 is a critical suppressor of malignant rhabdoid tumor (MRT), an aggressive, usually lethal cancer of young children.<sup>71-73</sup> The majority of MRT tumors and cell lines have inactivating mutations in both alleles of the Snf5/INI1 gene and Snf5 loss is a critical determinant in the development of most, if not all, rhabdoid malignancies. Congruent

with these tumor suppressor activities, SWI/SNF complex activity is required to activate transcriptional programs underlying cellular differentiation in many cell types. Blocking Brg1 or SWI/SNF activity interferes with differentiation in retinal, myeloid, muscle, adipocyte, enterocyte, erythrocyte and neuronal cells.<sup>31,74-80</sup> Together, the observations above suggest that the antagonistic interactions between Geminin and SWI/SNF that we have observed during neurogenesis could also regulate the proliferative-differentiative transition in other cellular contexts.

Effects of both Geminin and SWI/SNF in regulating the proliferation to differentiation transition intersect with many other critical cell cycle and transcriptional control systems in a complex manner. Because of this, Geminin promotes cell proliferation and supports genome stability (by preventing overreplication) in some actively cycling cells, while antagonizing cell proliferation and promoting cellular differentiation in others. For example, in contrast to the findings above, Geminin acts as a proliferation antagonist and differentiation-promoting factor in the medaka retina.<sup>21</sup> Experimentally, both gain- and loss- of Geminin can result in cell cycle arrest: gain of function of Geminin can block association of Cdt1 with replication origins resulting in failure of replication initiation, while loss of Geminin can also arrest the cell cycle, due to activation of checkpoint controls following genome overreplication.<sup>48,81-84</sup> Geminin's activities are strongly influenced by aspects of the cellular context including the Geminin protein dosage, the availability and levels of protein partners, and the status of other regulatory proteins such as Rb and p53 (reviewed in Refs. 16,85). For example, Geminin depletion in HeLa cells does not cause chromosomal overreplication or cell cycle arrest because Cdt1 levels at S phase are regulated by a secondary redundant mechanism (efficient ubiquitination and proteolysis).<sup>37,86-88</sup> Interestingly, while some interacting proteins bind Geminin in a competitive manner (Cdt1 and Hox/Six3) through overlapping protein domains, some interactions (Gem-Brg1 and Gem-Hox/Six3/Cdt1 interactions, for example) might occur simultaneously as these involve distinct physical motifs in the Geminin protein. Therefore, Geminin may enable cells to monitor and physically integrate multiple cellular cues and transcriptional events at the transition from proliferation to differentiation.

## CONCLUSIONS

Geminin was initially characterized as a dual function molecule with the ability to regulate DNA replication via its central Cdt1-binding coiled-coil domain and to modulate neural cell fate, an activity for which Geminin's N-terminus was sufficient. Recently, demonstrations that Geminin interacts with the Hox and Six3 transcription factors, Polycomb complex proteins and the SWI/SNF complex have expanded its repertoire to include a role in regulating transcription and cell cycle progression at the proliferative-differentiative transition in several new contexts. It is perhaps surprising that such a small molecule should regulate numerous activities through interactions of different protein partners with distinct amino acid motifs. It will be essential to define the protein composition of Geminin containing- complexes involved in different cell cycle and transcriptional regulatory events and to determine how the interacting partners defined above are coordinately regulated by Geminin to regulate transcription and cell cycle progression. Collectively, these findings may underscore Geminin's critical roles in regulating and integrating cellular and transcriptional events at the proliferative-differentiative transition.

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