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Geminin in embryonic development: coordinating transcription and the cell cycle during differentiation

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1. ABSTRACT

Geminin was initially characterized as a bifunctional protein with roles in regulating the fidelity of DNA replication and in controlling cell fate during embryonic nervous system formation. More recently, Geminin's roles have expanded, encompassing regulation of cell proliferation and differentiation during retinogenesis, control of Hox transcription factor function during vertebrate axial patterning, and regulation of the timing of neuronal differentiation. Geminin interacts with homeodomain-containing transcription factors and with protein complexes that regulate chromatin structure, including Polycomb complexes and the catalytic subunits of the SWI/SNF chromatin remodeling complex, Brg1 and Brahma. Activities for Geminin in coordinating cellular events at the transition from proliferation to differentiation have recently emerged in multiple developmental contexts. This review will summarize Geminin's increasingly diverse roles as a developmental regulatory molecule.

2. INTRODUCTION

During development, embryos must generate the many cells needed to form various tissues, instruct these cells to acquire different fates, and organize them in the correct pattern. Proliferating precursors for each differentiated cell type must exit the cell cycle at the correct time and must coordinate this event with a large number of transcriptional and cellular changes accompanying differentiation. Molecular mechanisms regulating key aspects of embryonic development were elucidated over the past decades and often show striking conservation across metazoan organisms. But perhaps even more impressive than the embryo's ability to regulate each individual aspect of development is its precise integration of these activities with one another in time and space. Our understanding of how this is achieved is incomplete, but several molecular coordinators of cell proliferation, fate, differentiation, and/or tissue patterning during development have emerged in recent years (reviewed in 1-3). This review will focus on one such molecule, Geminin.

Geminin was initially characterized as a bifunctional molecule with two divergent activities that mapped to physically separate regions of the protein (4, 5). Geminin was identified in a screen for proteins degraded in a cell cycle-dependent manner and was found to undergo Anaphase Promoting Complex (APC)-dependent degradation at the metaphase to anaphase transition during mitosis (5). This work also defined a role for Geminin in regulating the fidelity of DNA replication. Initiation of DNA replication depends upon the step-wise formation of a pre-replication complex (pre-RC) consisting of the ORCs, Cdc6, Cdt1 and the mini-chromosome maintenance (MCM) proteins. These proteins are loaded onto chromatin at points of DNA replication initiation. Geminin interacts with and inhibits the function of Cdt1, preventing MCM loading to block DNA re-replication within a single cell cycle (6, 7). In many cell types, Geminin protein levels rise during the S and G2 phases of the cell cycle, when Geminin is active in inhibiting replication initiation and degradation of Geminin during mitosis then allows a new round of DNA replication initiation to occur in the subsequent S phase. This Geminin activity is critical for maintaining genome stability and euploidy in many cells. For recent reviews of Geminin's role in regulating the fidelity of DNA replication, see (8, 9).

Geminin was identified concurrently in an expression screen in *Xenopus laevis* for proteins that, when over-expressed, could affect formation of the embryonic nervous system (4). Over-expression of Geminin in embryos causes expansion of the neural plate and conversion of naive ectodermal cells into neural tissue, at the expense of non-neural cell types such as epidermis. These effects are manifested during the gastrula stages, indicating that Geminin can affect the early process of neural cell fate acquisition. Geminin is also required for neural cell fate, since reducing Geminin activity results in dose-dependent loss of neural marker expression and formation of epidermis within the prospective neural ectoderm. Some of Geminin's roles in formation of the embryonic nervous system may be conserved between vertebrates and invertebrates, since gain and loss of Geminin in *Drosophila* also causes formation of ectopic neurons or neuronal deficiencies respectively (10). An amino-terminal region (residues 38-90 of the *Xenopus laevis* Geminin L protein) is sufficient to convert uncommitted ectoderm to a neural fate in *Xenopus*. Conversely, Geminin's ability to bind Cdt1 and block DNA re-replication maps to a non-overlapping central domain of the protein (4, 5, 11).

Recently, additional roles in regulating development have emerged for Geminin, mediated by its interaction with an expanding repertoire of partner proteins. During chick embryogenesis, Geminin can bind to and antagonize the function of Hox homeodomain proteins that regulate axial patterning (12). Geminin can also regulate retinal cell proliferation and differentiation by binding to and antagonizing the activities of the Six3 homeodomain-containing transcription factor (13). Interestingly, these interactions may involve competition between Cdt1 and Six/Hox proteins for binding to Geminin, and this competition appears to be involved in regulating the transition from proliferating precursor to post-mitotic differentiated cell (12-14). Geminin can also regulate the transition from proliferating neuronal precursor to differentiated neuron, through its ability to antagonize the activities of Brahma related gene-1 (Brg1), the catalytic subunit of the multi-protein SWI/SNF complex. The SWI/SNF complex regulates chromatin structure to both activate and suppress transcription during proliferation and differentiation. Geminin interactions with Brg1 can antagonize associations between Brg1 and basic helix-loop-helix (bHLH) transcription factors required for neuronal differentiation (15-17). Geminin also interacts with the Polycomb Group (PcG) protein Scmh1 to affect transcription during axial patterning (12).

While many new activities and partner proteins have recently emerged for Geminin during embryogenesis, some common themes unite these findings. First, Geminin inhibits many protein activities, including transcription factors (Hox/Six3), chromatin regulatory proteins (Brg1), and the pre-RC protein Cdt1. Second, at least two of Geminin's interactions are with regulators of chromatin structure (Brg1 and Polycomb complexes). Geminin's ability to act at the chromatin level to regulate gene expression may represent a major (though still largely unexplored) theme for Geminin's activities. Third, Geminin is highly expressed in proliferating cells and acts in multiple contexts to regulate cellular transitions from proliferation to differentiation. Fourth, Geminin's activities in development show a strong dose-sensitivity, possibly related in part to competition between partner proteins for Geminin-binding. This feature may allow Geminin to act as a cellular "sensor" of levels of multiple partner proteins. Together, the recent work suggests a role for Geminin in coordinating and integrating changes in the cell cycle and transcriptional profile of cells at the transition from proliferation to differentiation during development.

3. GEMININ'S STRUCTURAL FEATURES

Geminin is a protein of approximately 33 kDa that shows a predominantly nuclear distribution in cells (4, 5). Defined structural features include an N-terminal destruction box (D-box) located at residues 23-31 in human Geminin; efficient degradation of Geminin was also found to require additional sequences just C-terminal to the D-box (Figure 1)(11). The N-terminus also contains sequences required for nuclear localization of the protein (Figure 1). A major feature of Geminin is an atypical leucine-zipper coiled-coil located in the central portion of the protein. Structural analysis has shown that this domain self-associates to form a parallel coiled-coil dimer (18-22). While this motif was initially predicted to encompass residues 110-144 using computer algorithms (4, 5), recent crystal structure data has extended the coil to include residues 94-150 (21). Within this coiled-coil domain, several leucine and isoleucine (L/I) residues are essential for Geminin homo-dimerization (Figure 1) (21). Interestingly, while the basic structure is that of a typical coiled-coil, formed by heptad amino acid repeats, the presence of unbranched amino acids and positively charged

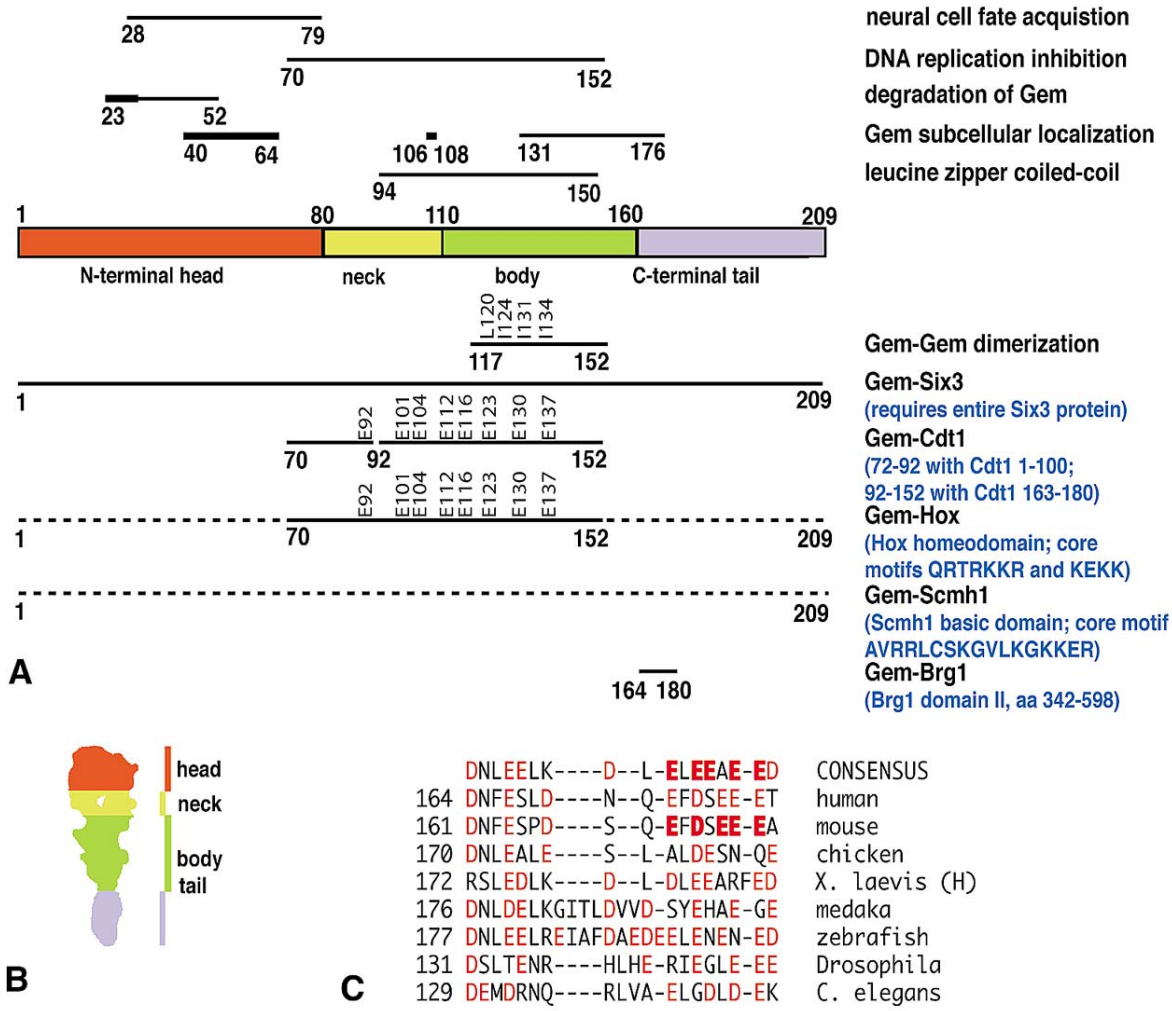

Figure 1. Schematized structure-function data and domain mapping for Geminin's protein-protein interactions. (A) At top, regions sufficient to carry out the initial activities defined for Geminin (neural cell fate acquisition and inhibiting the initiation of DNA replication) and other known protein motifs are mapped onto human Geminin. Within the sequences required for Geminin proteolytic degradation, the Geminin destruction box (D-box; aa 23-31) is denoted as a thick line (5, 11). Two bipartite nuclear localization signals, containing the motifs RTK+KRK (11) or KRK+KKAK (69, 70) have been described for *Xenopus* Geminin. The N-terminal region encompassing these motifs (aa 40-64) is demarcated by a thick line, with numbering adjusted for human Geminin. Note that only one of these motifs (KRK, amino acids 50-52) is well conserved across species; in human Geminin an alternate motif (RRK) located at amino acids 106-108 (thick line in the figure) was also shown to be required for nuclear localization (70). In some contexts, Geminin can also undergo Crm1-dependent nuclear export and amino acids 131-176 (thin line) are required for this activity (70). Based on data from (4, 5, 11, 19, 21, 22, 69, 70). At center, the Geminin domain structure is schematized after (19). Regions of Geminin interaction with various partner proteins are schematized below, with Geminin residues required for interaction indicated where known. Dashed lines denote Geminin interactions not yet mapped to a specific region of the protein. For example, Hox interaction requires the region/residues indicated by a solid line, as well as additional unmapped region(s) indicated by a dashed line (12, 21). Where known, Geminin-interacting motif(s) in partner proteins are denoted in blue text at the right. Data from: (12, 13, 16, 21). (B) Schematized shape of the Geminin oligomer, after (19). (C) The Brg1 interaction motif in Geminin (amino acids 164-180 for human Geminin) is shown for some Geminin orthologs, with acidic residues in red. Deletion of this motif or point mutation of amino acids shown in bold for the mouse Geminin protein strongly attenuates Geminin-Brg1 interaction (16). Alignment was performed using Jotun-Hein and MegAlign software (DNASTAR).

amino acids at some positions in the heptads may render the packing structure less stable than that found in other more typical coiled-coils (21).

The overall structure of full-length human Geminin has been characterized by electron microscopy and image processing to a resolution of 17.5 Angstroms and is proposed to form a tetramer or "dimer of dimers," based on cross-linking studies (19). The Geminin oligomer appears to have a key-like shape, with a flexible N-terminal

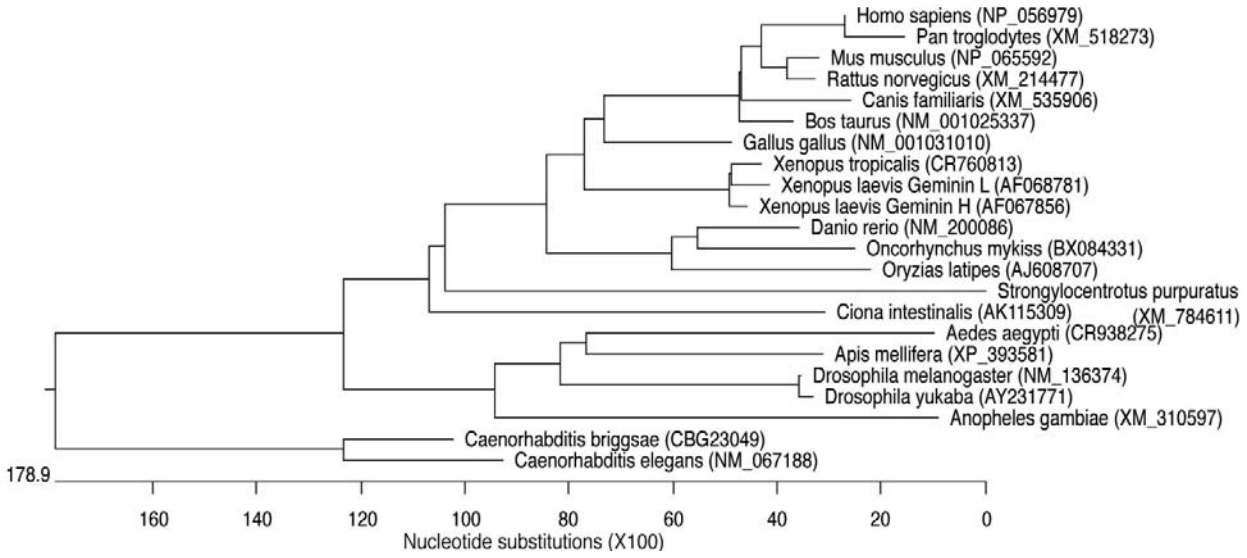

Figure 2. Phylogenetic analysis of Geminin orthologs. A Geminin protein sequence alignment was performed using Clustal V and MegAlign software (DNASTAR), and phylogenetic analysis is shown with units indicating the number of substitutions. Distance between any two sequences is the sum of horizontal branch length separating them. Genbank accession numbers are shown to the right of each species for one of the full-length cDNAs that was used to obtain each Geminin protein sequence.

"head" portion (amino acids (aa) 1-80), a neck (aa 80-110), a central body (aa 110-160) and a C-terminal tail (19). It is of interest that the activities initially defined for Geminin map to distinct physical domains in this structure: the N-terminal head encompasses the domain sufficient for Geminin's activity in regulating neural cell fate in *Xenopus laevis* (aa 28-79 in human Geminin) (4). Conversely, Cdt1-Geminin interaction and Geminin's activities in regulating the fidelity of DNA replication map to the neck and central body regions, which provide two separate Cdt1 interaction interfaces (11, 21, 22). Multiple negative charges on the surface of the central body of Geminin (within the Geminin coiled-coil) are required for interaction with positive charges in the central portion of Cdt1 (Figure 1)(21, 22). The C-terminal end of Geminin's coiled-coil can inhibit access of the MCM complex to Cdt1 through steric hindrance (22). The neck-like segment of Geminin is also involved in inhibiting DNA replication and provides a second interface required for interaction with the Cdt1 N-terminus (21). Residues of the head plus the neck appear to be flexible, with their structure probably stabilized by Cdt1 interaction (19).

Hox and Six3 transcription factors can compete with Cdt1 for binding to Geminin. Therefore, Geminin's Hox and Six3-interaction motifs are likely to overlap with Cdt1-interaction domain(s) or, alternatively, Hox or Six3 interaction may alter Geminin's conformation to inhibit Geminin-Cdt1 binding (Figure 1)(12, 13, 21). Indeed, both Geminin-Hox and Geminin-Cdt1 interactions require the same acidic residues in the Geminin coiled-coil, since mutation of these amino acids abolishes interaction (21). Geminin also exerts a "Polycomb-like" activity and binds to the PcG protein Scmh1, although the Geminin regions or motifs required for these interactions have not yet been defined (12). Finally, Geminin interacts with Brg1 through a non-overlapping motif in the Geminin C-terminal tail; this Geminin motif contains many acidic amino acids. Some of these acidic residues are required for Geminin-Brg1 interaction, since their point mutation abolishes or strongly attenuates Brg1 binding for the mouse and *Xenopus* Geminin proteins (Figure 1C, bold) (16). As is shown in Figure 1, several Geminin interactions involve electrostatic interactions between acidic residues in the Geminin central body or C-terminus with basic amino acid-rich regions or motifs in interacting partners (12, 16, 21).

4. GEMININ EXPRESSION DURING EMBRYOGENESIS

4.1. Geminin in the metazoa

Since Geminin's initial identification in *Xenopus laevis*, orthologs have been cloned from numerous other vertebrates, multiple arthropods, the tunicate *Ciona intestinalis*, the echinoderm *Strongylocentrotus purpuratus*, and the nematodes *Caenorhabditis elegans* and *C. briggsae*. A phylogenetic comparison is shown in Figure 2 for species where a known or predicted full-length Geminin protein is currently available. The mammalian Geminin orthologs shown have 74-99% amino acid sequence identity to each other. Human Geminin has a 31-46% identity to the non-mammalian vertebrates surveyed here, a 24-25% identity to *Ciona* and *S. purpuratus* Geminin, 18-19% identity to various arthropod Geminin proteins and 14% identity to *C. elegans* or *C. briggsae* Geminin. Despite having a low percentage of amino acid identity to vertebrate Geminin orthologs, *Drosophila* and *C. elegans* Geminin retain key features of vertebrate Geminin. These include cell cycle-regulated degradation, the ability to regulate the fidelity of DNA replication through Cdt1 interaction, and (for *Drosophila* Geminin) a role in nervous system development. Therefore, these appear to represent

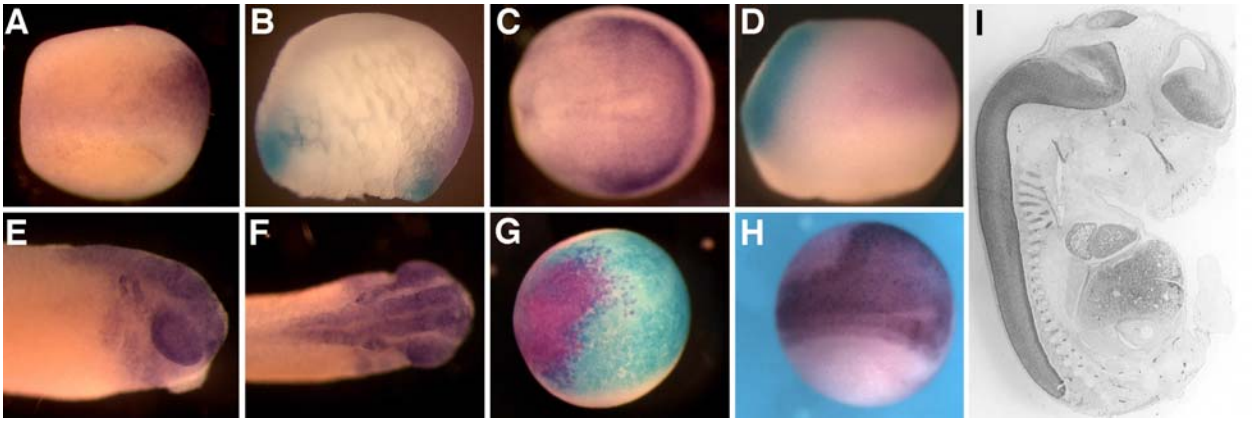

Figure 3. Geminin expression during embryonic development. (A-F) In situ hybridization for *Geminin* (purple or pink) in *Xenopus laevis* embryos at (A-B) early gastrula (st. 10+ to 10.25), (C-D) late gastula (st. 12.5), and (E-F) tailbud (st. 28). In B, D, expression of *Geminin* in prospective neurectoderm (pink stain) is shown relative to the mesodermal gene *Brachyury* (blue stain); in B, a saggital slice through the embryo is shown. (A-B) are side views with dorsal to the right, (C) is a dorsal-facing view and (D) is a side view, both with anterior to the right. (E-F) Expression of *Geminin* in the brain, eyes, and other neural-derived structures of later stage embryos is shown in side (E) and dorsal (F) views. (G) Over-expression of mRNA encoding the Geminin N-terminal fragment (*Xenopus laevis* Geminin L aa 38-90; marked by pink stain) is sufficient to suppress epidermal keratin expression (blue stain). (H) Geminin variants (including full length Geminin, its N-terminus, or a C-terminal fragment, aa 142-216, shown here) are sufficient to expand expression of the neural progenitor marker *Sox2* at late gastrula stages. *Geminin* mRNA was injected into one bilateral half (oriented up and lineage labeled by *Beta-galactosidase* mRNA coinjection; blue spots). This resulted in expansion of *Sox2* expression (purple) relative to the uninjected side. (G) is an animal hemisphere view and (H) is a dorsal view with anterior facing right. (I) Immunostaining to detect the *Geminin* expression pattern in a section of an e12.5 mouse embryo.

true Geminin orthologs. Extensive homology-based searches have not revealed *Geminin* orthologs in the genomes of uni-cellular eukaryotes including the budding and fission yeasts *Saccharomyces cerevisiae* and *S. pombe*, suggesting that Geminin is likely to be specific to metazoan (multi-cellular) organisms. This is supported by previously observed differences in regulation of DNA replication: high levels of cyclin dependent kinases (CDKs) at G2 and M can also block DNA re-replication within the cell cycle. However, inhibiting this CDK activity stimulates re-replication in yeast (which lack Geminin) but not in higher eukaryotes (which contain Geminin as an additional safeguard)(23-25). It is tempting to speculate that, in higher eukaryotes, the presence of at least two partially redundant mechanisms for regulating the fidelity of DNA replication could have favored Geminin's acquiring additional regulatory roles during embryonic development.

4.2. Expression during embryonic development

Geminin shows widespread expression in proliferating cells, with enrichment in neural or neural-derived tissues, in some cells of the adult gonad or germline, and in endoreplicating cells (in *Drosophila* and *C. elegans*) (4, 10, 16, 26-29). During *Xenopus laevis* embryogenesis, maternal stores of *Geminin* mRNA are present from oocyte stages onward, and zygotically-expressed *Geminin* is most highly expressed in presumptive neural tissue from the onset of gastrulation (Figure 3, A-B) (4). Throughout gastrulation, *Geminin* expression in *Xenopus* marks the future neural plate and neural precursor population, an expression pattern similar to the SoxB1 class transcription factors *Sox2* and *Sox3* (4, 16). At late gastrula and neural plate stages, *Geminin* expression is strongest in an anterior neural territory encompassing the sensory placodes, presumptive neural crest and neural plate. *Geminin* is also found in trunk neurectoderm and is expressed at slightly higher levels in the midline and lateral edges of the neural plate. At later embryonic stages, *Geminin* is highly expressed in brain, eye, otic and olfactory structures and in a dorsal region of the tailbud contiguous with the dorsal neural tube (Figure 3, E-F)(4). Expression of *Geminin* in the embryonic CNS of the Japanese medaka fish (*Oryzias latipes*) appears similar to that found in *Xenopus*, with expression enriched in anterior presumptive neurectoderm at early neurulation and later in the optic vesicles, forebrain (prosencephalon) and overlying head ectoderm, prospective optic tectum and midbrain-hindbrain boundary. *Geminin* is also expressed in neural and retinal structures at later stages, most highly in the proliferating marginal cells of the retina and less intensely in the subventricular zone, and also in the dorsal diencephalon and the cortical layer of the optic tectum (13). While a detailed characterization of *Geminin* expression during mammalian embryogenesis has not yet been published, immunostaining or *in situ* hybridization to detect *Geminin* expression in mouse embryos indicates a similar enrichment in the forming nervous system and eye (Figure 3 and K.L.K, unpublished data).

A striking feature of *Geminin* expression is that, in almost all cellular contexts defined to date, *Geminin* expression correlates strongly with actively dividing, progenitor cell states, while being down-regulated prior to or coincident with cell cycle arrest (10, 27-29). For

example, during neurogenesis in *Xenopus laevis*, analysis of sections through the neural plate between late gastrula and neurula stages revealed a strong transcriptional down-regulation of *Geminin* in the deep layer cells that differentiate into primary neurons at that time. *Geminin* is, however, retained in superficial cells that are refractory to becoming primary neurons but represent a neuronal precursor pool for later secondary neurogenesis (16). Likewise, during *Drosophila* embryogenesis *Geminin* expression correlates with dividing or endoreplicating cells and with a proliferative or precursor cell state in multiple tissues (10). This is seen in the G2 regulated cell cycles of early embryonic divisions (cell cycles 14-16) and also in the peripheral and central nervous systems, where *Geminin* is present in dividing cells but absent in their cell cycle arrested neighbors or derivatives. In the eye imaginal disc, *Geminin* is enriched in the undifferentiated, asynchronously dividing cells anterior to the morphogenetic furrow and also in cells posterior to the furrow that remain undifferentiated and may be G2 arrested. *Geminin* is also expressed in the endoreplicating tissues of the embryo and adult, including the gut and the nurse and follicle cells of the adult ovary. Therefore, as in vertebrates, *Geminin* marks undifferentiated, dividing and endoreplicating tissues in the embryo and the adult (10).

In non-embryonic cellular contexts, *Geminin* also strongly correlates with proliferating cells. For example, *Geminin* is highly expressed in many cancers including lymphomas, invasive breast cancers, renal cell carcinoma, and colon and rectal tumors (29-32). High *Geminin* levels often mark more aggressive neoplasms because *Geminin* specifically labels S/G2/M phase cells and therefore indicates cells with an increased rate of cell cycle progression and shortened G1 phase (28, 30, 31). *Geminin's* expression profile resembles other 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*(28). These data are consistent with Geminin's activities in regulating the transition from proliferation to differentiation in multiple precursor cell populations during development.

4.3. Regulation of Geminin expression and activity

In *Xenopus*, *Geminin* expression is among the earliest markers of presumptive neural tissue, marking the future neural plate at the beginning of gastrulation. Consistent with this, *Geminin* expression is induced by signals from the dorsal mesendoderm (the Spemann organizer) that induce neural tissue, including the Bone Morphogenetic Protein (BMP) signaling antagonists Noggin and Chordin. Conversely, *Geminin* is not expressed in response to molecules involved in later processes of neurogenesis such as *Xenopus* Neurogenin-related 1 (X-ngnr1). This suggests that Geminin acts upstream of X-ngnr1 and may also reflect the ability of X-ngnr1 to drive formation of differentiated neurons, while *Geminin* is highly expressed in neuronal progenitors but is down-regulated during neuronal differentiation (4). As expected, *Geminin* expression is suppressed by activation of BMP signaling, which is sufficient to promote epidermal and suppress non-neural cell fates (4, 33).

Recently, cis-sequences responsible for regulating *Geminin's* expression in neural tissue during early embryonic development were defined. Introduction of 5' sequences from either the human or the *Xenopus geminin* gene into transgenic *Xenopus* embryos drives reporter expression in a pattern that mimics that of endogenous *Geminin* from early gastrula to tadpole stages (33). Within the human 5' sequences, two cis-regulatory elements were defined that are both sufficient and required to recapitulate *Geminin's* neural-specific expression during gastrulation. Each of these cis-elements contains binding sites for the transcription factor Tcf, which can mediate Wnt signaling, and for Vent homeodomain proteins, transcriptional repressors that mediate BMP signaling. Mutation of these sites showed that they make unique contributions to regulation of *Geminin* expression in gastrula neurectoderm: regulatory sequence constructs with mutated Vent sites still drive neural-specific expression at early gastrulation, when *Geminin* appears in the presumptive neural plate. However, by late gastrula stages reporter expression in embryos carrying these Vent-mutated constructs is ubiquitous (33). These data suggest that BMP signaling through the identified Vent sites restricts *Geminin* expression to the dorsal side of the embryo, but is not required for initial establishment of the neural-specific expression pattern. Conversely, mutation of the identified Tcf sites abolishes neural-specific expression at both early and late gastrulation. Consistent with these results, *Geminin* 5' regulatory sequences and endogenous *Geminin* are positively regulated by Wnt signaling and negatively regulated by BMP signaling. Sequence alignments defined similar cis-elements containing Tcf and Vent sites in several other *Geminin* orthologs, suggesting that these motifs could represent a conserved mechanism for regulating neural-specific expression of *Geminin* during gastrulation (33).

Geminin expression is also regulated by Retinoblastoma (Rb)/E2F activities, consistent with observations that *Geminin* is highly expressed in proliferating precursor cells but is down-regulated before or coincident with cell cycle exit accompanying differentiation. The Rb tumor suppressor forms repressive transcriptional complexes with E2F proteins at genes promoting cell cycle progression, such as *cyclin E*. *Geminin* may be directly downregulated by Rb/E2F during differentiation, as E2F activates *Geminin* expression in actively cycling cells, while Rb can repress *Geminin* transcription. These effects are mediated by E2F association with binding sites in an intragenic enhancer distinct from those described above, and located in the first intron of the *geminin* gene (34, 35).

Finally, in addition to transcriptional regulation of *Geminin* expression, multiple post-transcriptional mechanisms exist to control Geminin activity in cells. As previously described, cell-cycle-regulated ubiquitination and degradation of Geminin protein by the APC appears to be a major mechanism for controlling the oscillating Geminin levels seen in cycling somatic cells (5). This mechanism may not be used to control Geminin activity in all embryonic contexts, however; during early *Xenopus* embryogenesis, a major pool of Geminin becomes

ubiquitinated without undergoing degradation (36, 37). Reactivation of this pool of Geminin requires nuclear import, suggesting control of Geminin sub-cellular localization as another means of controlling Geminin activity (38). Finally, Geminin is phosphorylated on multiple residues (39, 40). This feature could allow further regulation of Geminin activity, although its functional relevance has yet to be demonstrated.

5. ROLES FOR GEMININ IN VERTEBRATE EMBRYOS

5.1. Neural cell fate

In *Xenopus laevis*, over-expression of Geminin causes neural plate expansion, marked by disorganized and expanded expression of neural and neuronal markers, including a neuron-specific tubulin isoform (*N-tubulin*), *Otx2*, *Pax6*, *Delta-1* and *Synaptobrevin* (4). Expansion of neural-specific gene expression is predominantly the result of a cell fate change, as it is accompanied by suppression of non-neural markers (*Epidermal keratin*, *Bmp4*) (Figure 3, G-H)(4). Geminin can also expand the neural plate even when cell proliferation is inhibited by hydroxyurea/aphidicolin treatment of embryos, demonstrating that neural plate expansion does not depend upon proliferation. Geminin's effects on neural development are dose dependent: very low doses suppress epidermal gene expression, while higher doses are required for inducing neural markers including *N-CAM*. In addition, Geminin doses subthreshold for neural marker induction expand the *Twist*-expressing cranial neural crest, whereas Geminin doses that induce neural markers suppress neural crest. Over-expression of the Geminin N-terminal domain (residues 38-90 of *Xenopus laevis* Geminin L) is sufficient to elicit most of these effects in embryos (4).

Geminin loss of function in *Xenopus* has been performed using both antisense oligonucleotides and a rescuable dominant negative molecule consisting of the Geminin dimerization domain (4, 16). Both reagents have similar effects on neural development: expression of neural markers is suppressed, while *Epidermal keratin*-expressing cells appear in the territory fated to form the neural plate. Geminin loss-of-function effects are dose-dependent. During early *Xenopus* embryogenesis, severe or complete reduction of Geminin, including maternal mRNA stores, arrests cells in G2 at the mid-blastula transition, when embryonic cell cycles normally acquire gap phases, become asynchronous, and acquire additional checkpoint controls (41). This arrest phenotype requires Chk1 and therefore involves activation of checkpoint pathways (41). By contrast, partial interference with zygotic (but not maternal) Geminin activity results in embryos exhibiting a neural to epidermal cell fate change without discernable effects on cell cycle progression, as described (4, 16). Finally, reductions of Geminin that are insufficient to block initial neural cell fate acquisition instead alter the timing of neuronal differentiation during *Xenopus* primary neurogenesis (16).

The mechanism by which Geminin regulates neural cell fate remains unknown, but may involve down-regulation of BMP signaling required for epidermal cell fate. Geminin can suppress *Bmp4* expression by gastrula stages and *Bmp4* co-expression blocks Geminin's ability to suppress epidermal cell fate. These data suggest that Geminin's down-regulation of *Bmp4* at least partially accounts for its ability to modulate neural cell fate (4). As mentioned above, Geminin's neural cell fate promoting activity is physically separated from Geminin's effects on the fidelity of DNA replication, which localizes to a non-overlapping Geminin domain. The two activities are also functionally separable: the Geminin N-terminus has no apparent effect on the cell cycle, while the Geminin central region cannot modulate neural cell fate (4, 5, 11).

5.2. Neurogenesis

While Geminin can regulate neural cell fate at gastrulation through its N-terminal domain, the Geminin C-terminal domain was recently shown to regulate later neurogenesis, when neuronal precursors exit the cell cycle and differentiate. Two-hybrid screening identified Brg1 and Brahma (Brm), catalytic subunits of the SWI/SNF chromatin remodeling complex, as Geminin-interacting proteins (16). This interaction was confirmed both biochemically and by genetic analysis of interactions between Geminin and Brm (the single fly ortholog of vertebrate Brg1 and Brm) in *Drosophila*. Interestingly, Brm and Geminin interact in an antagonistic manner in both the *Drosophila* eye and wing, and vertebrate data supports this antagonistic relationship. Interaction was mapped to Brg1/Brm domain II (which is highly basic) and to an acidic C-terminal motif in Geminin (Figure 1). Deletion or point mutation of this Geminin motif abolishes Geminin-Brg1 interaction for both the *Xenopus* and mouse Geminin proteins (16). This acidic amino acid-rich region is relatively well conserved among Geminin orthologs (Figure 1C), consistent with physical or genetic interactions between Geminin and Brg1/Brm in both vertebrates (mouse, *Xenopus*) and invertebrates (*Drosophila*). While an activity was not previously attributed to the Geminin C-terminus, this represents a distinct structural domain of the Geminin protein (19).

Brg1 and *Geminin* expression patterns are consistent with a role for their interaction in neural tissue. In *Xenopus, Brg1* expression is ubiquitous through gastrula stages and then becomes enriched in neural tissues including the brain, spinal cord, and eye (15, 16). A similar expression pattern is also seen in mouse embryos and (while *Brg1* homozygous null mice are embryonic lethal) some heterozygotes exhibit the neural tube defect exencephaly, again suggestive of a role in neural tissue (42, 43). As described above, *Geminin* is highly expressed in presumptive neural tissue during gastrulation but is restricted to proliferating neural precursors while being down regulated prior to neuronal differentiation in the territory that gives rise to primary neurons (16).

Requirements for a Geminin-Brg1 interaction were analyzed by gain and loss of function approaches in *Xenopus* embryos and murine P19 embryonic carcinomal cells (16). During embryonic development, proliferating neural progenitor cells expressing *Geminin* and *SoxB1*

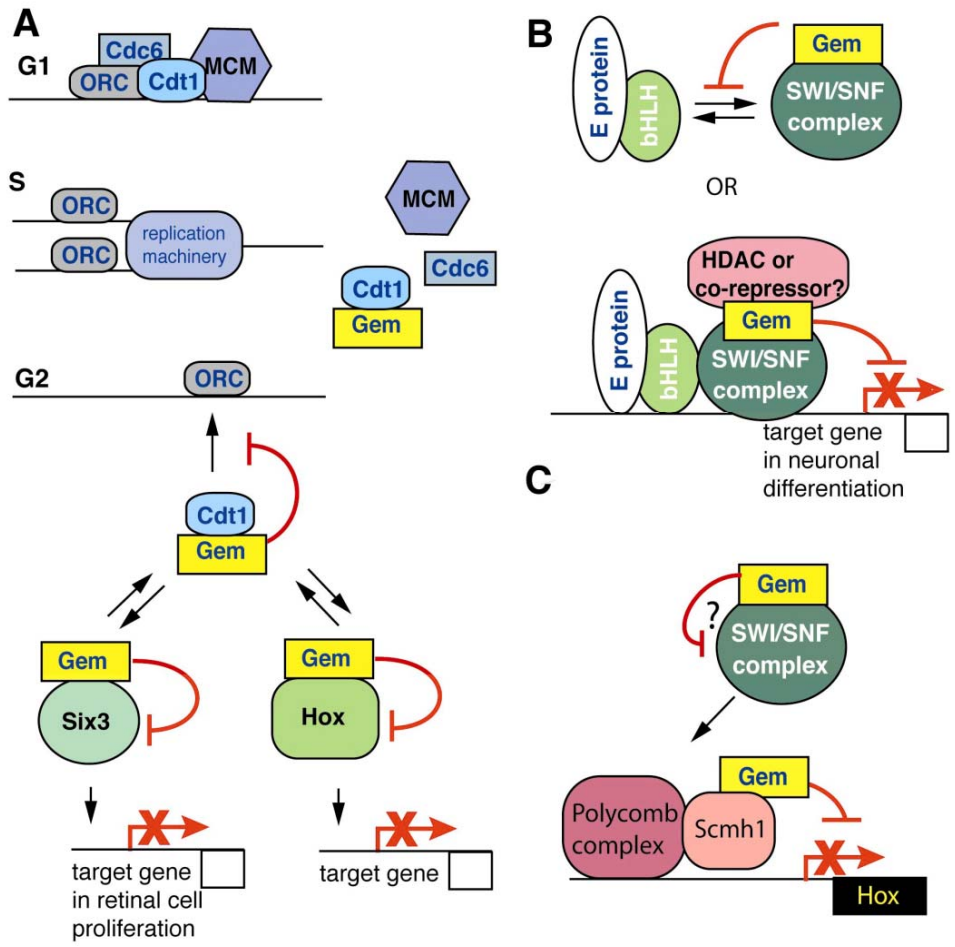

Figure 4. Models for Geminin activities in embryonic development. A. Geminin acts in S and G2 phases to block re-replication of DNA within a cell cycle by binding to and antagonizing the pre-RC protein Cdt1 (top). Geminin can also bind to and block the activities of Six3 and Hox transcription factors regulating retinal cell proliferation and axial patterning and these interactions are competitive with Geminin-Cdt1 binding (bottom). B. Geminin also binds to Brg1 and Brahma, catalytic subunits of the SWI/SNF chromatin remodeling complex, and antagonizes their ability to promote neural bHLH dependent target gene transactivation during neuronal differentiation. Geminin may either antagonize bHLH-dependent transcription by directly blocking Brg1-bHLH interaction off of the chromatin (top) or Geminin may associate with SWI/SNF at bHLH target gene loci, potentially recruiting HDAC or co-repressor activities to repress transcription. C. Geminin also represses Hox gene expression by interacting with the Polycomb complex protein Scmh1 at Hox gene enhancers. Cell cycle regulatory proteins are shown in blue, and proteins denoted in green are thought to activate transcription in the developmental context shown, while those in red shades repress transcription.

family transcription factors are initially formed; subsequently, some of these cells commit to a neuronal fate and differentiate. These activities are regulated by basic Helix-loop-helix (bHLH) transcription factors, including the Neurogenins (Ngns 1-3 in mammals or x-Ngnr1 in *Xenopus*) and NeuroD (44, 45). Brg1 interacts with Ngn and NeuroD and is required for their ability to transactivate target gene expression (15). Therefore, Brg1 loss of function in either *Xenopus* or P19 cells can block neuronal differentiation (15). Interestingly, this effect is phenocopied by over-expression of Geminin variants that can bind Brg1: Geminin over-expression causes excessive neural progenitor cells marked by *Sox2* or *NCAM* to be formed and failure of these cells to differentiate into neurons (16). The Geminin C-terminus is sufficient to exert this effect, but mutating the Brg1-binding site in either Geminin or its C-terminal domain abolishes Geminin's differentiation-inhibitory activity. The ability of Ngn and NeuroD to activate target genes and drive neuronal differentiation is also blocked by over-expression of Geminin or its C-terminal region, dependent upon Geminin's ability to bind Brg1 (16). Geminin loss of function also supports these findings: during *Xenopus* primary neurogenesis, dose-dependent reduction of Geminin to levels that enable neural precursors to form results in precocious neuronal differentiation. Likewise, in P19 cells, siRNA-mediated reduction of Geminin potentiates the ability of subthreshold NeuroD2 levels to

induce neuronal differentiation (16). Together, these results indicate that Geminin can bind to and antagonize Brg1 activity to maintain the neuronal progenitor population and regulate the timing of neuronal differentiation (16).

How does Geminin's interaction with Brg1 suppress neural bHLH-dependent transcription? This could occur through Geminin's ability to directly block Brg1-bHLH interactions needed for target gene transcription (Figure 4)(16). Geminin's binding site in Brg1, domain II, is an interaction site for the SWI3/MOIRA/BAF155 subunit of the SWI/SNF chromatin remodeling complex and deleting domain II from *Drosophila* Brm decreases the size of the SWI/SNF complex (46, 47). These data suggest that Brg1 domain II may be a protein-protein interaction scaffold. Therefore, Geminin's interaction with Brg1 domain II may affect the subunit composition of the SWI/SNF complex or its associations with transcription factors including the neural bHLH proteins. Alternatively or in addition, Geminin could associate with chromatin at bHLH target genes, repressing transcription by recruiting co-repressor or histone deacetylase activities to these loci (Figure 4). Consistent with this model, Geminin associates with chromatin, can also negatively regulate transcription by chromatin-association through Geminin-Polycomb complex interactions, and Geminin can bind an HDAC-associated protein (12, 14, 48). Identification of target genes regulated by neural bHLH, Brg1, and Geminin activities is needed to further define how Geminin and Brg1 regulate transcription during neurogenesis.

5.3. Axial patterning and control of Hox activity

A role for Geminin in controlling *Hox* gene expression and activity has also been defined (12, 14). Vertebrate *Hox* genes are arranged in four clusters and are expressed in overlapping patterns along the anterior-posterior axis of the embryo, with combinatorial expression of different *Hox* gene subsets defining unique positional identities. After establishment of *Hox* expression patterns, these are maintained through many subsequent cell divisions by the *Trithorax Group (TrxG)* and *Polycomb Group* genes. *TrxG* and *PcG* genes respectively maintain activated or repressed states of *Hox* gene expression by forming multi-protein complexes that regulate chromatin structure (reviewed in 49, 50).

Geminin can associate directly both with Hox proteins and with the Polycomb Group protein Scmh1 (12, 14). Geminin motifs required for Polycomb complex interactions have not yet been mapped but a basic amino acid-rich Geminin-interacting motif was defined for Scmh1 (Figure 1)(12). Geminin-Hox interaction requires two clusters of basic amino acids within the Hox homeodomain and acidic residues within the Geminin central coiled-coil (Figure 1). The same Geminin residues are critical for interaction with both Hox proteins and Cdt1 interaction, such that Hox-Geminin and Cdt1-Geminin interactions are competitive (Figure 4)(12, 21). Levels of Geminin, Cdt1 and Hox proteins in cells could potentially define whether Geminin-Cdt1 versus Geminin-Hox interactions prevail, acting as a switch between Geminin's roles in controlling the fidelity of DNA replication versus antagonizing Hox-dependent transcription in proliferating and differentiating cells (12). Hox proteins can displace Geminin from the Cdt1-Geminin complex, but Cdt1 cannot compete Geminin away from Hox. Interestingly, a similar competition for Geminin binding was observed for Six3 and Cdt1 during retinogenesis, as described below (13).

Through its interactions with both Hox and Polycomb proteins, Geminin can negatively impact Hox function and expression to regulate anterior-posterior axial patterning. Geminin over-expression represses *Hox* expression, shifting the boundary of *Hoxb9* posteriorly. Conversely, siRNA reduction of Geminin derepresses *Hoxb9* gene expression, so that *Hoxb9* is expressed one somite length anterior to its normal A-P boundary. Over-expression of the Scmh1 domain sufficient to bind to Geminin has an effect similar to reducing Geminin activity, also de-repressing *Hoxb9* expression. Therefore, Geminin exerts effects that are similar to those of Polycomb complexes in repressing *Hox* expression (12).

How might Geminin antagonize Hox expression and function? Geminin can directly bind to Hox proteins and block their ability to activate target genes (Figure 4). For example, Geminin binds to Hoxb7 to block activation of its target gene *FGF2* in a melanoma cell line. siRNA reduction of Geminin in this system restores *FGF2* expression. Geminin is not detected by chromatin immuno-precipitation and so appears to block Hox protein activity without interacting with DNA in this case (12). However, in addition to blocking Hox protein activity, Geminin can also block Hox gene expression. This activity involves Geminin's association with chromatin through Polycomb Group protein-mediated interactions. For example, Geminin interacts with enhancers of the *Hoxd11* gene that are repressed by recruitment of Polycomb Group protein complexes (Figure 4)(12).

Geminin's defined interactions with Hox, Polycomb Group, and SWI/SNF complex proteins suggest potential connections between these activities. For example, SWI/SNF complex subunits (including Brahma, Moira/BAF155 and Osa) were identified in *Drosophila* as *TrxG* genes required to maintain active *Hox* gene expression and to oppose Polycomb-mediated repression. Therefore, Geminin could potentially block *Hox* gene expression both through Polycomb complex interactions and also by interfering with Brahma/Brg1 to negatively affect TrxG activity (Figure 4). Geminin's antagonism of homeodomain-containing Hox and bHLH family transcription factors also suggests a role for Geminin in repressing target genes controlled by both types of proteins. bHLH and homeodomain transcription factors coordinately regulate multiple developmental processes, including neurogenesis and retinogenesis (51-56), and this sometimes involves direct binding of bHLH and homeodomain proteins to adjacent sites in target gene enhancers (57). Geminin could potentially repress these target genes by antagonizing the transcriptional activities of both homeodomain and bHLH proteins.

5.4. Eye development

Geminin can also regulate cell proliferation and

differentiation during medaka retinogenesis by binding to and antagonizing the function of the homeodomain protein Six3 (13). Geminin interacts with Six3 and the closely related Six6, but not with Six2. Six3 and Geminin interact in a manner that requires both full-length proteins and bi-directionally inhibit each other's functions (Figure 4). Six3 can compete Cdt1 away from Geminin or block Cdt1-Geminin interactions, whereas Cdt1 cannot displace bound Six3 from Geminin. When bound to Six3, Geminin can antagonize the ability of Six3 to activate target genes that stimulate cell proliferation in the retina. Conversely, in its Cdt1-bound state, Geminin can maintain genome fidelity by preventing Cdt1-mediated initiation of DNA replication (13). Therefore, the balance of Geminin, Six3 and Cdt1 levels may act as a proliferation-differentiation switch in this context as well (Figure 4).

The effects of these Geminin, Six3 and Cdt1 interactions were analyzed by gain and loss of function in the medaka CNS and retina (13). In this system, over-expressing Geminin leads to dose-dependent reductions in size of the eye and rostral forebrain, effects resembling loss of Six3 function. Geminin over-expression or Six3 loss of function also affects proliferation in a similar manner, with cells that normally express Six3 failing to proliferate and instead undergoing apoptosis. Gain of Geminin or loss of Six3 activity also has similar effects at the molecular level, resulting in dose-dependent reduction of expression of the retina-specific homeobox gene *Rx2*.

Results from Geminin loss of function experiments also support an antagonistic Six3-Geminin relationship: Geminin loss of function increases the number of mitotic cells (marked by phosphorylated histone H3), enlarges the retina and optic vesicles, and expands the domain of *Rx2* expression (13). This closely resembles the effects of Six3 over-expression. Increasing Six3 activity can also block the effects of over-expressed Geminin, rescuing Geminin gain of function phenotypes described above. Finally, subthreshold reductions of Geminin or increases in Six3 fail to elicit a phenotype; however, combining these subthreshold perturbations causes synergistic effects in enlarging the optic vesicle and *Rx2*-expressing territory (13). In conclusion, antagonistic interactions between Geminin and Six3 appear to play a role in regulating cell proliferation and differentiation and Six3 target gene transcription during retinogenesis.

How does Geminin antagonize Six3 activities? Geminin cannot displace Six3 from DNA and Geminin interacts with a Six3 variant point mutated in its DNA binding domain and unable to bind DNA. Furthermore, co-injecting DNA binding-deficient Six3 rescues Geminin over-expression phenotypes, suggesting that DNA binding is dispensable for Six3's ability to antagonize Geminin. Together, these data are most compatible with Six3 antagonism of Geminin by direct interaction and sequestration of Geminin protein, which could affect the Geminin pool available for Cdt1 interaction (Figure 4). In this way, bidirectional competition between Cdt1 and Six3 for Geminin binding could coordinate control of DNA replication with transcriptional changes occurring at the transition from proliferation to differentiation.

6. GEMININ IN INVERTEBRATES

6.1. *Drosophila* embryogenesis

How do the activities described for vertebrate Geminin compare to those of its invertebrate orthologs? A *Drosophila* Geminin ortholog (DmGeminin) was defined by sequence comparison to *Xenopus* and human Geminin. Although the primary amino acid sequence of DmGeminin is highly diverged from its vertebrate counterparts, it shares a similar domain structure. Furthermore, key features of vertebrate Geminin are conserved in DmGeminin, including the ability to inhibit DNA replication in *Xenopus* egg extracts by inhibiting MCM binding to chromatin, to interact with the *Drosophila* Cdt1 ortholog (Doubleparked), and to influence neuronal cell fate (10).

Three loss of function alleles (all P-element insertions) have been described for *DmGeminin*. Two alleles are embryonic to third instar larval lethal (with 40% of embryos dying before hatching), whereas one allele is adult viable and partially female sterile. Mutation of *DmGeminin* results in phenotypes in the eye, endoreplicating tissues of the gut, ovarian follicle cells, and nervous system (10). Some defects are clearly associated with DmGeminin's role in controlling the fidelity of DNA replication or other cell cycle-related Geminin activities during mitosis. Other phenotypes may reflect bona fide Geminin requirements for regulating embryonic development.

Trans-heterozygous embryos carrying the strongest *DmGeminin* mutant allele over a deficiency in the region show no obvious cell cycle defects until cycles 14-16; at this time increased numbers of mitotic cells are apparent, consistent with either premature entry into mitosis or delay of cells in mitosis. Some mitotic cells show anaphase defects including chromosome bridges (10). These embryonic defects are distinct from those seen in *Xenopus* embryos depleted for maternal Geminin (41), as *Drosophila* embryos mutant for *DmGeminin* do not show G2 arrest at either early or late embryonic stages, even through the maternal DmGeminin supply is likely to be exhausted at later embryonic stages. Early stage embryos mutant for *DmGeminin* do not show DNA over-replication, although this may merely reflect persisting activity of maternally provided Geminin. At later stages, over-replication of chromosomal DNA is seen in the endoreplicating tissues of the gut, the ovarian follicle cells, and the nervous system (10). Also in keeping with a requirement for DmGeminin in regulating the fidelity of DNA replication and preventing over-replication, *Drosophila* S2 cells reduced for Geminin activity show excessive DNA replication (up to 8N ploidy) (58).

While many embryonic defects described above appear strictly related to Geminin's roles in regulating DNA replication, gain and loss of DmGeminin in the nervous system may support additional roles in regulating neuronal cell fate or neuronal differentiation as seen for vertebrate Geminin. For example, over-expression of *Drosophila* Geminin in leads to formation of ectopic neurons (10). The epidermal location of these ectopic

neuronal cells is most consistent with ectopic neurogenesis rather than inappropriate migration of peripheral nervous system neurons. These data suggest that Geminin's ability to regulate neural or neuronal cell fate may be conserved in invertebrates. A fraction of *DmGeminin* mutant embryos also show a striking reduction in the dorsal-most peripheral neurons. This effect may be limited to a fraction of embryos because of persisting maternal DmGeminin activity at the time that neuronal specification occurs (10). Further analysis is needed to determine whether these effects reflect a DmGeminin requirement for neural development or whether they are secondary to Geminin requirements for DNA replication.

Another potential developmental role for Geminin is in the *Drosophila* eye. DmGeminin over-expression in the eye-antennal imaginal disc dramatically decreases both numbers of S phase cells and the size of both the eye discs and adult eye (10). A severely roughened eye is obtained. The severity of the resulting rough eye phenotype cannot be accounted for strictly by the 40-50% decrease in S-phase cells observed when DmGeminin is over-expressed posterior to the morphogenetic furrow of third instar discs. For example, a much less severe rough eye phenotypic is obtained upon expression of the CDK-inhibitor p21 in this territory, although this almost completely abolishes S phases. Quinn *et al*. suggest that ectopic neuronal differentiation may explain the severity of the eye defects obtained in DmGeminin over-expressing embryos (10). Together, results for DmGeminin suggest that its activities in regulating the fidelity of DNA replication and in nervous system development may resemble activities of its vertebrate Geminin orthologs.

6.2. *C. elegans* Geminin

Recently, a *C. elegans* Geminin ortholog (called GMN-1) was also identified based on sequence similarity to *Drosophila* Geminin (26). Like other Geminin orthologs, GMN-1 associates with Cdt1, inhibits interaction between Cdt-1 and Mcm6 in a dose-dependent manner and reduces the amount of Mcm6 associated with chromatin. GMN-1 also inhibits DNA replication licensing in *Xenopus* egg extracts, and this inhibitory effect is overcome by Cdt-1 over-expression (26). Consistent with these activities, charged residues in the Geminin coiled-coil that are needed for Cdt1 interaction in vertebrates are well conserved in the *C. elegans* and *C. briggsae* Geminin orthologs. These data suggest GMN-1 is an ortholog of vertebrate Geminin proteins with similar activities in maintaining the fidelity of DNA replication.

Developmental roles for *C. elegans* Geminin were assessed by RNAi-based reduction of Geminin levels, resulting in sterility in about 20% of animals (26). Animals with reduced Geminin levels have aberrant germ line development: oogenesis is impaired and partially differentiated germ cells accumulate in the proximal arm of the gonad. Germ cell nuclei in the distal gonad of sterile worms (where mitosis occurs) are enlarged and have misshapen nucleoli. Some somatic cells are also affected in *C. elegans* with reduced Geminin activity: chromosomal bridging is seen in intestinal cells, which normally endoreplicate and become multinucleate (with 30-34 nuclei). This appears to reflect a defect in coordinating endoreduplication with the nuclear division cycle (26). The sensitivity of these endoreplicating cells to loss of Geminin activity may be similar to observed phenotypes in endoreplicating cells in *Drosophila* (10).

Additional developmental activities for *C. elegans* Geminin are suggested by its interactions with Hox and Six3/6-related proteins: GMN-1 interacts with the product of the *nob-1* gene (orthologous to vertebrate posterior group homeodomain transcription factors Hox9-13) and with the product of *ceh-32*, a Six3/6-related gene (26). The Brg1-binding motif defined for mouse and *Xenopus* Geminin is also relatively well conserved in *C. elegans*, including its key acidic residues (Figure 1C). This suggests the potential for interaction between GMN-1 and psa-4, the *C. elegans* ortholog of vertebrate Brg1/Brm and yeast SWI2. In *C. elegans,* SWI/SNF complex (and psa-4) function is required for asymmetric division of T blast cells to define the hypodermal (epidermal) versus neural cell lineages (59, 60). Therefore, this SWI/SNF activity could be regulated by GMN-1 to affect neural development in *C. elegans* as a parallel to the vertebrate case. Yanagi and colleagues did not observe phenotypes involving neuronal development or control of *Hox* expression or activity (26). However, since many neuronal genes are refractory to RNAi-mediated knockdown by feeding or injection (61, 62), these negative results may reflect technical limitations rather than dispensability of GMN-1 for these aspects of *C. elegans* development. At present, it is clear that at least some of Geminin's roles in regulating the fidelity of DNA replication and its interactions with key transcription factors involved in development and tissue patterning are conserved between vertebrates and nematodes.

7. PERSPECTIVES

Together, the data above defines some common features for Geminin activity in various developmental contexts: competition of Hox/Six3 versus Cdt1 for Geminin binding may allow cells to integrate proliferative and transcriptional controls to coordinate transitions from precursor to differentiated cell for multiple cell types. Also, although Geminin binds some protein partners competitively (for example Cdt1 and Hox or Six3), some interactions utilize a distinct Geminin domain (Geminin-Brg1, for example) and therefore may occur non-competitively or simultaneously with Geminin's Cdt1, Hox or Six3 interactions. This feature may increase Geminin's ability to monitor cellular levels of multiple protein partners to coordinate different cell cycle and transcriptional regulatory cues.

Other major themes for Geminin activity in development are Geminin's ability to antagonize transcription and to interact with protein complexes that regulate chromatin structure. Geminin interactions with Brg1, Hox or Six3 directly antagonize the ability of these transcriptional regulators to activate target genes, suggesting that this may be a generalizable role for

Geminin. Geminin's ability to interact with Polycomb complex proteins and to exert a Polycomb-like repressive effect provides an additional, mechanistically distinct means for Geminin to block transcription, through activity on the chromatin at some target genes (Figure 4). Finally, Geminin's ability to antagonize transcription through Polycomb and SWI/SNF complex interactions suggest that Geminin may more directly regulate chromatin structure to influence transcription. It will be interesting to determine whether interactions of Geminin with SWI/SNF or Polycomb Group complexes affect nucleosome remodeling, covalent histone modifications, or recruitment of chromatin modifying enzymes such as HATs/HDACs to some target genes.

In the developmental contexts described above, Geminin's effects on cell fate and differentiation show a strong dose and context dependence. Perhaps this is not surprising, given the dynamic, cell cycle-regulated control of both Geminin's activity and that of protein partners such as Cdt1. Geminin promotes cell proliferation in some cell types (29), while blocking cell cycle progression to promote cellular differentiation in others. For example, in the medaka retina Geminin can act as a proliferation antagonist and differentiation-promoting factor (13), while in neural precursor cells Geminin is needed to maintain the precursor cell population (16). Geminin requirements in regulating the fidelity of DNA replication also vary between cell types and are strongly influenced by levels and mechanisms of Cdt1 regulation and also by the status of cell cycle regulatory and checkpoint proteins including Chk1, ATM/ATR, Rb, and p53 (5-7, 10, 11, 28, 41, 58, 63-65). For example, loss of Geminin function in HeLa cells does not cause chromosomal over-replication or cell cycle arrest because Cdt1 levels in S phase are regulated by the redundant mechanism of efficient ubiquitination and proteolysis (48, 66-68). By contrast, loss of Geminin function in other cell systems can lead to cell cycle arrest in G2, to accumulation of cells in mitosis with chromosomal bridging or other overt chromosomal abnormalities, or to accumulation of cells in S phase with formation of giant nuclei and no subsequent mitosis. Effects on chromosomal ploidy are likewise variable, ranging from minimal or no apparent over-replication of DNA, to partial genome re-replication, or to the generation of excessive genomic DNA levels per cell. Together, these findings suggest that complex mechanisms control Geminin's activities in regulating both the fidelity of DNA replication and events during embryonic development.

Geminin was initially identified based on two divergent and apparently unrelated activities in regulating the fidelity of DNA replication and in cell fate control during embryonic development. It has recently become evident that Geminin plays not two but multiple key roles in cellular and developmental control, through interactions with an expanding repertoire of partner proteins. New connections have emerged between Geminin's transcriptional regulatory activities during development, regulation of chromatin structure, and Geminin's role in maintaining chromosomal euploidy through control of DNA replication. While much remains to be learned, many contexts now exist for continued exploration of Geminin's developmental biology.

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