CMLS, Cell. Mol. Life Sci. 1425–1433
1420-682X/05/131425-09
DOI 10.1007/s00018-005-4553-1

CMLS Cellular and Molecular Life Sciences

Review

The regulation of embryonic patterning and DNA replication by geminin

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Received 10 December 2004; received after revision 27 January 2005; accepted March 2005
Online First 13 April 2005

Abstract. Geminin is a multifunctional protein. After DNA replication is initiated during a cell cycle, geminin binds to Cdt1, one of the key DNA replication licensing factors. This highly regulated interaction sequestrates Cdt1, thus preventing DNA rereplication in the same cell cycle. In addition, geminin directly interacts with Six3 and Hox homeodomain proteins during embryogenesis and inhibits their functions. The regulation of Hox function by geminin also involves a transient association with the Hox repressive Polycomb complex. The functions of geminin to obstruct key molecules of both cell proliferation and embryonic development suggest a competitive coordination of these two processes.

Key words. Geminin; Cdt1; rereplication; proliferation; Hox; embryonic development.

Introduction

There is no embryonic development without cell division. The two processes have to proceed in an extremely intricate and coordinated way in order to bring about distinct cellular patterns, the allocations of cellular fates, the positioning of progenitor cells, the establishment of germ layers, the formation of three-dimensional organs and the generation of their multiple connections within the context of an embryo. Therefore, it is not surprising that single proteins play multiple roles in the regulation of both the cell cycle and embryonic development. One example of such a protein, in which separate domains display such dual functions, was named geminin. Although we do not yet understand exactly how geminin executes its function, a lot has been learnt in the last years regarding its biochemistry, its expression dynamics, and its interaction with regulators of both development and the cell cycle. In this review we will give an overview of the growing field of recent publications concernig geminin.

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The pre-replication complex

The maintenance of genome stability and integrity from one cell generation to the other requires the accurate transmission of genetic material from mother to daughter cells. To ensure this, cells must replicate their DNA only once per cell cycle, and the duplicated chromosomes must be reliably separated during mitosis. In eukaryotes, replication initiates from multiple replication origins via a process that requires the successive loading of several proteins, the 'licensing factors', onto the chromatin at these origins (fig. 1 A, [1, 2]). First, six origin recognition complex (ORC) proteins start to bind during late mitosis, followed by two additional proteins, Cdc6 and Cdt1 [3–5]. Cdt1 is loaded onto chromatin probably via its intrinsic, non-specific DNA binding activity [6]. Its presence is an absolute prerequisite for the loading of the six minichromosome maintenance proteins MCM2–7, which form a hexameric, chromatin-associated complex [6–14]. Together, the ORC, Cdc6, Cdt1 and MCM proteins represent the pre-replication complex (pre-RC), which is required to initiate DNA synthesis.

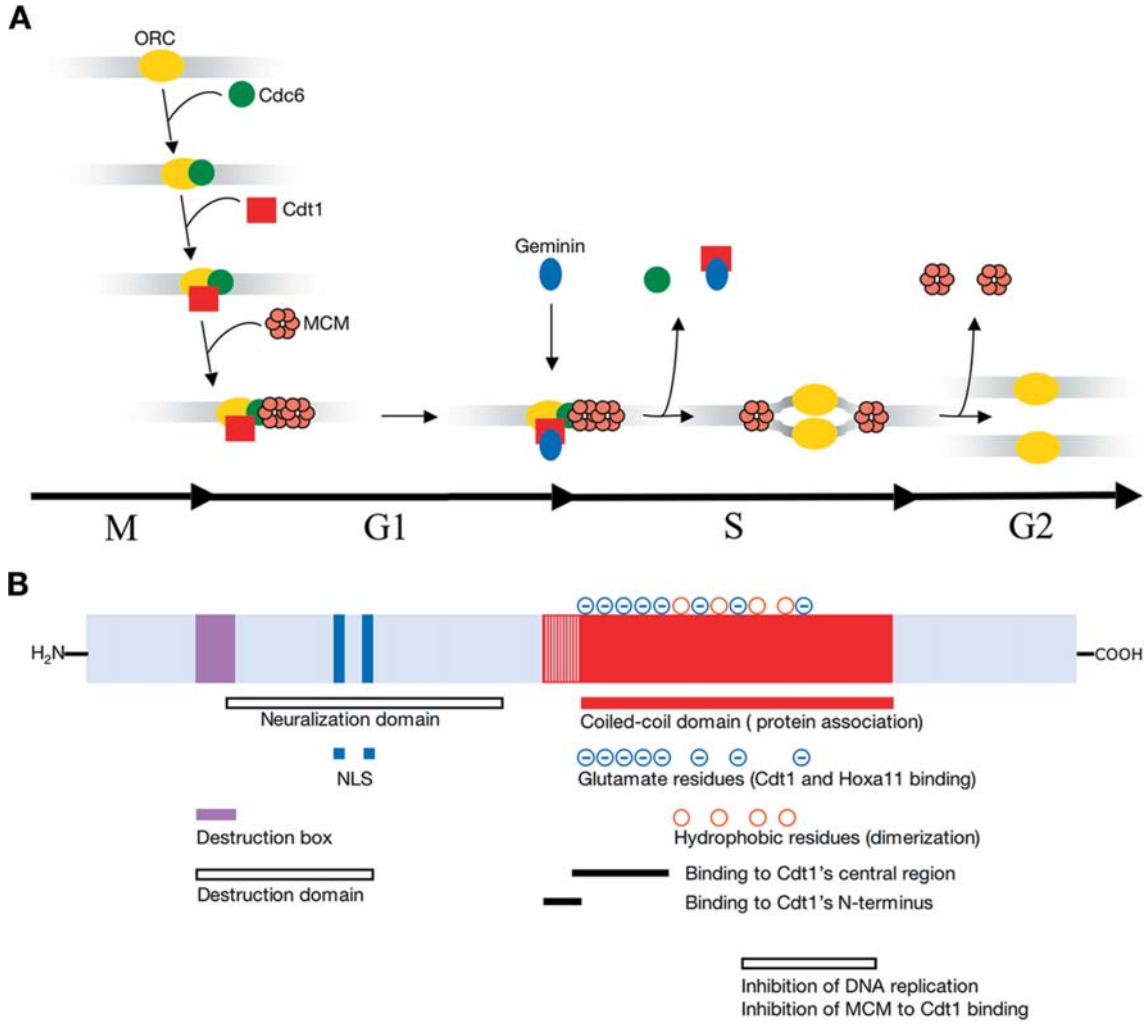

Figure 1. Structure and function of geminin. (*A*) Formation of the pre-RC. By the end of mitosis the licensing factors ORC, Cdc6, Cdt1 and MCM2-7 are successively loaded onto an origin of replication, and represent a pre-RC. At the onset of S phase geminin sequesters Cdt1, and thus rereplication is inhibited. (*B*) The structural and functional domains of geminin. For further discussion and references see text.

Once DNA replication is initiated, licensing factors have to be inactivated in order to prevent complex re-assembly during the same cell cycle. Cdt1, one of the critical factors, is inactivated by different species-specific mechanisms, which have in common to achieve a periodical fluctuation of nuclear Cdt1 protein levels during the cell cycle. Levels increase after the exit from mitosis, remain high during the G1 phase and decrease after the initiation of replication in the S phase [7]. In budding yeasts, Cdt1 is phosphorylated and exported into the cytoplasm at the G1/S phase transition, while in fission yeasts Cdt1 is degraded [14, 15]. In *Caenorhabditis elegans*, Cdt1 degradation through ubiquitin-mediated proteolysis involves the activity of the Cul-4 gene product, a member of the cullin family [16]. In mammals, Cdt1 is phosphorylated, then recognized by the F-box protein Skp2, polyubquitinated by an E3 ubiquitin-ligase, the SCF complex, and finally degraded by the proteasome [17–21]. Vertebrates and *Drosophila*, but apparently not *C. elegans* nor yeast, developed a conserved mechanism to block Cdt1, namely the association with a protein factor, geminin [10, 11]. The binding between geminin and Cdt1 inhibits the function of Cdt1 as a licensing factor, and thus prevents DNA rereplication. In the following chapters, we will review the biochemistry and biological function of geminin in the regulation of the cell cycle and of embryonic development.

The domain structure of the geminin protein

Geminin was discovered in a screen for complementary DNA (cDNA) encoded proteins selectively degraded by extracts prepared from mitotic but not by interphase

Xenopus eggs [22]. Except for B-type cyclins, cDNAs encoding geminin were most abundant. Following this initial finding, homologs of *Xenopus* geminin were characterized in most eukaryotes, including human, mouse, Medaka fish and *Drosophila*, but excluding *C. elegans* and yeast.

Several structural motifs were identified by inspection of the amino acid sequence of geminin (fig. 1B). The N-terminal portion was found to be homologous to the consensus destruction box of mitotic cyclins and functions as an ubiquitination signal [22]. A typical bipartite nuclear localization signal (NLS) consisting of two basic amino acid clusters, separated by seven residues, lies further towards the C-terminus of *Xenopus* geminin. Recently, geminin degradation was found to involve not only the destruction box, but also the NLS and the intervening region [23]. The central portion of geminin contains five heptad amino acid repeats predicted to form a coiled-coil, a structure commonly associated with protein dimerization, while the C-terminal end of geminin is poorly conserved.

A three-dimensional map of the full-length human geminin protein determined by electron microscopy down to a resolution of 17.5 Å suggests a key-like shape for the protein, with one head and two neck domains, a central body and a tail, with a hole between the head and neck regions [24]. The head represents the N-terminus of geminin, the central body domain corresponds to the coiled-coil regions, while the neck represents the DNA replication inhibition domain. The crystal structures of a truncated form of mouse geminin complexed with a truncated Cdt1, and of human geminin's coiled-coil region revealed the formation of a parallel coiled-coil dimer structure, with two extended α-helices packed together into a left-handed superhelix [25, 26]. The N-terminal regions of the coiled-coil domains of geminin are flexible, and are probably stabilized upon interaction with Cdt1. The coiled-coil domains are defined by repeats of seven amino acids. In the folded protein, charged residues are exposed on the outside of each helix, while hydrophobic residues remain inside, on the interface between the helices. Both the charged glutamate and the hydrophobic residues are crucial for geminin dimerization. Geminin dimers may go on to form tetramers, as indicated by crosslinking experiments [24].

Crystallography revealed three regions of geminin important for Cdt1 binding (fig. 1B, [25, 26]): first, the exposed, negatively charged glutamates which are essential for electrostatic interactions with Cdt1; second, the N-terminal part of the coiled-coil region, plus 10 upstream residues, which bind to the central region; third, the N-terminal flexible region of the coiled-coil domain, which contacts the N-terminal 100 amino acids of Cdt1.

Most studies up to now focused on the interaction between Cdt1 and geminin, on which we will take a closer look in a next chapter. However, evidence is accumulating that additional binding partners, in particular homeodomain proteins, interact with high affinity with geminin. We will review these studies in the last chapter of this review.

Cell cycle-dependent fluctuation of geminin levels

At the beginning of S phase, geminin accumulates in the nucleus [10, 11, 22]. Geminin dimers but not monomers bind free Cdt1 with high affinity in the nucleoplasma, thereby inhibiting the formation of novel pre-RCs during S phase [26, 27]. In addition, access of the MCM complex to Cdt1 is inhibited by the C-terminal part of the coiled-coil domain of geminin through steric hindrance within the chromatin [12, 25].

The nuclear presence of geminin protein is maintained throughout S and G2 phases until the end of mitosis [22]. Then, the nuclear geminin protein is inactivated through a number of different, possibly species-specific pathways. This inactivation is necessary to allow for the presence of free Cdt1, the prerequisite for licensing the next round of DNA replication. Experiments in mammalian cells and in *Xenopus* egg extracts suggested degradation by anaphase promoting complex-(APC)-mediated ubiquitination and proteolysis, via a process dependent on the conserved N-terminal destruction box sequence, and adjacent sequences [22, 23]. Inhibitors blocking APC-mediated ubiquitination, such as a D-box peptide, resulted in a stabilization of geminin [22]. A second mechanism leading to the inactivation of geminin involves transient poly-ubiquitination without subsequent proteolysis [28]. In a third pathway identified in *Xenopus* egg extracts, exclusion from the nucleus and reactivation following nuclear import regulates geminin activity [23, 29].

In addition to the posttranslational regulation of geminin levels, a transcriptional control mechanism involving E2F transcription factors was also identified [30, 31]. E2F factors need to dissociate from the complex with retinoblastoma (Rb) protein before they can function as transcriptional activators, rather than repressors. Since the tumor suppressor gene product Rb is inactive at the G1/S transition, and active at the end of mitosis, the Rb/E2F system normally supports the cell cycle dependent fluctuation of geminin protein levels. In murine Rb-null fibroblasts geminin levels were constitutively upregulated, but still DNA replication was not well controlled [30, 32, 33]. These cells, like some cancer cells, became apoptotic, with DNA damage and aberrant DNA replication after spindle-checkpoint activation. The reason could lie in the deregulation of further E2F targets, which include other members of the pre-RC complex [34]. These results demonstrate how an imbalance of cell cycle con-

trol proteins can influence the proliferative status of a cell. Multiple, partially redundant mechanisms underline the necessity to inactivate geminin at the transition from mitosis to G1 phase, in order to allow DNA replication licensing and normal cell cycle progression

The balance between geminin and Cdt1 levels

The proliferation of normal and tumor cells follows similar cell cycle phases, but differs with regard to regulatory mechanisms. A cell must pass different checkpoints, which control its progress through the cell cycle. A frequent cause of cancer is a malfunction of these checkpoints in G1, S or G2 phase. Since the function of geminin is to assure proper replication and progression through the S phase, it was suspected to be a tumor suppressor, rather than an oncogene. Expression analyses, however, pointed to an upregulation of geminin expression in tumor tissue and transformed cells [35–38]. Geminin protein was almost absent from normal breast, cervical and colon epithelia, but abundant in invasive carcinomas of the breast, cervix, colon as well as non-Hodgkin's lymphomas [39]. In sporadic invasive breast cancer, geminin levels correlated with tumor stages [38]. Already in early breast cancer geminin expression was increased, and high expression was associated with the development of metastasis and a poor prognosis [37, 38]. In rectal cancers, geminin expression levels decreased following chemo-radio treatment. The further use of geminin as a molecular marker for transformation and tumorigenesis is currently being investigated in several experimental studies.

In transformed cells, high levels of geminin correlate, through unknown mechanisms, with high levels of Cdt1 [35, 36, 40]. It appears likely that a balance between agonist and antagonist is an important checkpoint deciding on the growth parameters of a cell. In somatic cells, the levels of geminin and Cdt1 protein were largely complementary during cell cycle. Cdt1 protein accumulated only in G1, when geminin was not present [17, 22]. From S phase to mitosis a large proportion of Cdt1 was targeted for proteolysis, whereas geminin accumulated. In human HeLa cells and in *Xenopus* geminin and Cdt1 were present together on the chromatin during a narrow window of time at the G1- to S-phase transition [9, 27]. In several other transformed human cells, geminin was found to act on Cdt1, not only to prevent the formation of a pre-RC complex in S phase but also to protect a basal fraction of Cdt1 from ubiquitination and degradation in mitosis [41]. All observations point to the importance of a proper balance between levels of geminin and Cdt1, and other cell cycle regulators for the proliferation status of a cell. The quantitative variation of relative protein levels during the cell cycle requires sophisticated regulatory mechanisms. Their elucidation will also improve the understanding of the role of geminin and Cdt1 in tumorigenesis.

Experimental modulation of geminin levels

The highly controlled balance of cell cycle regulator levels can be experimentally perturbed by gain- or loss-of-function approaches. The forced expression of geminin by messenger RNA (mRNA) injection, the addition of a non-degradable form of geminin, or misexpression via the Gal4/UAS system in the *Drosophila* embryo, are all sufficient to hamper the assembly of a pre-RC [11, 22, 39, 42-44]. The loading of MCM proteins was then inhibited, whereas the chromatin association of ORC was not affected [22]. Such a blockage of the replication could be efficiently rescued by the expression of excess Cdt1 [10, 11, 44].

Transient overexpression of wild-type geminin in cell lines did not have major effects, and in particular did not arrest cell cycle progression [10]. However, stimulation of proliferation and accumulation of cells in the S phase were observed after stable transfections [37]. More severe alterations were observed following the expression of stabilized geminin protein in cultured cells, an effect that is dependent on the presence of endogenous Rb protein [31, 39, 42]. Under these conditions, MCM loading onto chromatin decreased, fewer origins were activated and consequently the cell cycle could not be completed. Rb-positive cells, such as U2OS or H1299, arrested at early S phase and activated the intra-S ATM/ATR checkpoint pathways leading to apoptosis [42]. Rb-null cells, such as Saos2 and BT549, arrested in late S and G2/M phases, and also underwent apoptosis. Primary fibroblasts arrested in G1 phase and contained insufficient licensed origins, possibly due to an inability to pass a 'licensing checkpoint'. Finally, HCT116 colon cancer cells (Rb+), expressing a stable form of geminin at almost physiological levels, arrested proliferation and activated the DNA damage checkpoint pathway, but without apoptosis [44]. As in the overexpresssion experiments, such phenotypes could be rescued by a corresponding increase of Cdt1 levels. In vivo, grafted cells containing non-degradable geminin gave less tumor formation in nude mice [44]. However, overexpression of Cdt1 alone resulted in rereplication, genomic instability and malignant transformation [40, 45]. Cdt1 and Cdc6 overexpresssion in several human cancer cell lines induced rereplication sensed as DNA damage with ATM/ATR and Chk2 kinase activation, with consequences depending on the induction of p53 phosphorylation [40]. In p53-positive cells rereplication stopped, and pro-apoptotic genes were induced. In p53 minus cells rereplication continued.

Experimental downregulation of geminin by antisense technology, dominant negative approaches, immunode-

pletion, RNA interference (RNAi), small interfering RNA (siRNA), or mutagenesis were found to create major problems for further cell divisions [23, 43, 46–49]. Effects included cell cycle arrests in G2, cessation of mitosis, formation of giant nuclei and partial polyploidy due to the occurrence of illegitimate rereplications. RNAi-mediated knock-down in transformed and primary human cells suppressed cell proliferation due to activation of the ATM/ATR and Chk1 kinase checkpoints, and led to partial rereplication of DNA, accumulation of cells in S phase without subsequent mitosis and development of giant nuclei [48]. Chk1 activation was independent of p53, and dependent on Cdt1 and Cdc6 expression. These phenotypes could be rescued when Cdt1 or Cdc6 were co-depleted. If the manipulated cells were treated with Chk1 inhibitors, progression through the cell cycle was forced and resulted in a 'mitotic catastrophe', associated with chromosomal breakage, chromosome fragmentation and finally death. In HeLa cells, depletion of geminin does not have such dramatic consequences, and no rereplication occurs, as Cdt1 levels in S phase are controlled by very efficient ubiquitination and proteolysis [18–21, 27]. It appears as if geminin is necessary to control normal cell cycle progression only when cells are deprived of other Cdt1 regulatory mechanisms.

Taken together, the detailed functional analysis of geminin in embryonic or cultured cells emphasized the complexity of different regulatory levels. Multiple mechanisms seem to exist to guarantee the proper progression of the cell cycle, but are not necessarily active in each cellular environment. Therefore, the modulation of geminin levels had dramatic effects in one context, but remained without consequences in others.

Geminin as a regulator of embryonic development

Simultaneously with its identification as a regulator of the cell cycle, geminin was recognized as a factor involved in embryonic patterning [50]. Indeed, geminin was first isolated in *Xenopus* using a functional screen to identify neuralizing activities by microinjection of mRNAs into one blastomere. In embryos geminin RNA is expressed in the dorsal, prospective neural ectoderm at the onset of gastrulation. Later, it is widely transcribed in the neural tube, the developing ectodermal placodes and their derivatives, and in neural crest-derived cells. Microinjection of geminin RNA led to an expanded neural plate at the expense of adjacent surface ectoderm. Such embryos developed hypertrophic and ectopic neural tissue, as indicated by an increase in the N-tubulin and X-ngnr-1 expressing domains, and increased density of primary neurons. While anterior markers seemed slightly reduced following geminin overexpression, posterior neural genes were induced in such embryos. BMP4 and epidermal keratin, both markers for future non-neural surface ectoderm, were suppressed. If neural tissue is induced by other neural inducers, such as chordin or noggin, geminin transcription was found to be concomitantly activated. Thus, geminin was recognized as a bona fide molecular marker, as well as an elicitor of neuralization in *Xenopus laevis*. The protein domain required for the neuralizing activity differed from the domain required for the control of replication (fig. 1B). Up to now, it remained unclear, however, what the biological significance of the two functions combined in one protein may be. New evidence underlines the role of geminin in other developmental processes [43, 49, 51].

In *Drosophila*, geminin mutants displayed a reduction of the dorsal-most peripheral neurons [43]. Ectopic overexpression of geminin resulted in the formation of ectopic neuronal cells, and led to a decrease in the size of eye imaginal discs as well as the size of adult eyes. The role of geminin in eye development was further substantiated in Medaka fish [51]. It was traced back to a direct interaction of geminin with Six3, a homeodomain protein known to be involved in eye development. A gain of Six3 function in Medaka fish caused larger or ectopic eyes, while knock-down of Six3 function with morpholino oligonucleotides resulted in a reduction of forebrain and eye structures [51, 52]. A strong interaction of overexpressed geminin with Six3 rendered the homeoprotein unavailable for its original function in eye development, and led to a reduction of eye tissue. This phenotype was associated with a decreased number of proliferative cells and premature neurogenesis in the optic vesicles. Along the same line of evidence, a reduction of geminin function using morpholino oligonucleotides was found to lead to enhanced Six3 activity, an increased number of mitotically active cells and hence larger eyes. Importantly, Six3 was able to rescue geminin gain-of-function phenotypes in a dose-dependent manner. Coinjection of anti-geminin morpholino oligonucleotides with Six3 RNA, at normally sub-effective concentrations, lead to enlargement of the optic vesicles. Altogether, these data suggest that the interaction between geminin and Six3 represents a bidirectional inhibition. The strong interaction between geminin and Six3 promoted a release of Cdt1 in vitro, thus rendering it available for the licensing of DNA replication. In contrast, the interaction between geminin and Six3 exibited such high affinity that it could not be competed by Cdt1. Competition of the bifunctional protein geminin for two regulatory networks may indicate a critical role for an appropriate balance between proliferation and differentiation.

The role of geminin in the hierachies or networks of homeobox genes is not restricted to Six3 and eye development. Recently, its interaction with vertebrate Hox and Polycomb proteins was reported [49]. In vertebrates, 39 Hox genes are organized into four clusters on four differ-

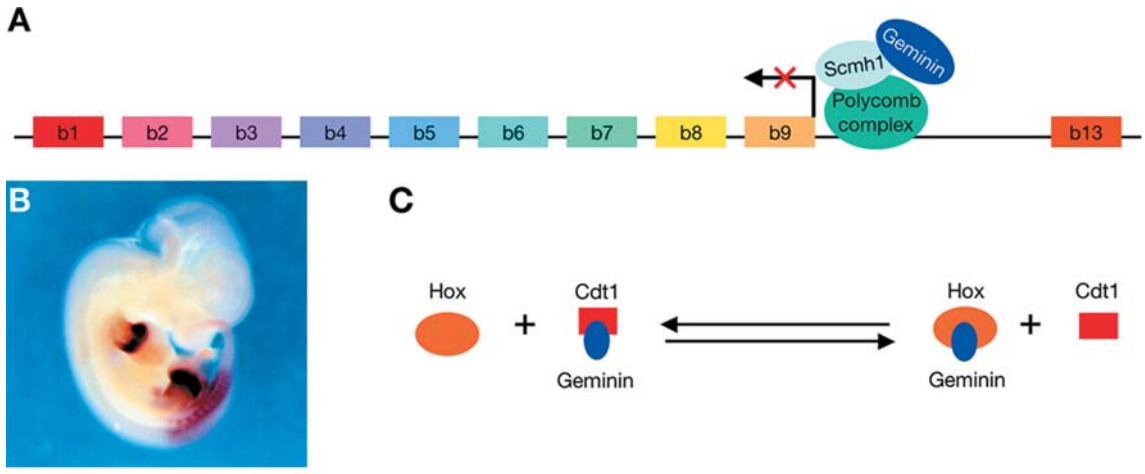

Figure 2. Inhibition of Hox transcription by geminin. (*A*) The Hoxb cluster consists of 10 genes as indicated. Polycomb complexes, also including the Scmh1 protein, bind to specific sites of the cluster and maintain repression of Hox genes depending on axial levels and embryonic stages. Geminin associates transiently with Polycomb proteins and thus functions as a transient Polycomb complex protein. (*B*) Hoxa11 expression in a mouse embryo on day 11.5. Note the restricted pattern in the limbs and the body axis (somites and neural tube), with a sharp anterior limit in the lumbar region. (*C*) Schematic representation of the equilibrium between geminin in proliferation and in developmental control. The Cdt1-geminin complex can be converted into a Hox-geminin complex depending on the availability of free ligands.

ent chromosomes (Hoxa, Hoxb, Hoxc, Hoxd). Thirteen paralogous groups can be defined based on sequence comparisons and their relative positions within the cluster, but one cluster contains only between 9 and 11 genes (fig. 2A). In developing vertebrate embryos, Hox genes specify anterior-posterior patterning, and are activated in non-identical, overlapping expression domains along the body axis (fig. 2B, [53, 54]). Hox gene transcription is activated sequentially, in colinearity with their distribution along the chromosome, suggesting a spatial and temporal coordination. The sequential opening of Hox genes occurs in cells with high mitotic activity, probably involving a process of chromatin remodelling [55, 56]. Every segment or single cell along the antero-posterior axis appears to have a distinct 'Hox code', defined by a specific combination of activated and repressed Hox genes [57].

Hox gene expression patterns established early in embryogenesis must be maintained and inherited during development. Thus, daughter cells receive a constellation of genes, in an 'on' or 'off' state. A part of this long-term cell memory system that maintains the repression of Hox genes is encoded by genes of the Polycomb group [58]. Polycomb proteins form dynamic multiprotein associations, the Polycomb complexes, which interact with chromatin remodelling proteins and associate with Hox regulatory DNA elements on the chromatin in order to repress their transcriptions [59, 60].

Mouse geminin protein interacted with several Hox proteins and with the Polycomb protein Scmh1 through their basic amino acid-rich domains [49]. The modulation of geminin levels led to a number of changes identified in vitro, in tissue culture cells and in embryos. Geminin acted on Hox gene transcription through Polycomb-mediated interaction (fig. 2A). It associated with the Polycomb complex in vivo and with chromatin within three Hoxd11 regulatory domains, regions reported to bind the Hox gene regulator PLZF and to associate with the Polycomb complex [60]. Unilateral overexpression of geminin in the neural tube of chick embryos repressed the activation of the endogenous Hoxb9 gene and shifted its anterior transcription boundary posteriorly. Co-expression of a dominant-negative form of Scmh1 de-repressed Hoxb9, suggesting that geminin behaved like a transient Polycomb complex member. These results demonstrated the in vivo presence, and highlight the biological significance of geminin in the regulatory context of Hox gene transcription.

Geminin protein also interacted directly with homeodomains of Hox proteins, thereby inhibiting the binding of Hox homeodomains to their target DNA and interfering with the function of Hox proteins as transcription factors. This could be demonstrated in vitro by band-shift and reporter gene assays, and also by interference with Hoxb7-dependent activation of the endogenous target gene FGF2 in melanoma cells [61]. The observed inhibition of Hox function by geminin at both transcriptional and protein levels may function individually at different embryonic stages and locations, or synergically to guarantee a complete block of Hox function.

Hox proteins could displace geminin from the Cdt1-geminin complex both in vitro and in primary cultured cells. Intriguingly, both Hoxa11 and Cdt1 associate with geminin via its coiled-coil domain. The importance of negatively charged residues in the coiled-coil domain of

geminin was also confirmed as being essential for binding to Hoxa11 [26]. Moreover, this interaction was stabilized by a second binding site located outside the geminin dimerization domain. Thus, by direct interaction with geminin, Hox expression could render Cdt1 available for DNA replication (fig. 2C). Such competitive interactions again suggest a mediator function of geminin between proliferation and pattern formation, similar to findings regarding Six3 and geminin in eye development. Effects of developmental control proteins in cell cycle are likely and, indeed, were previously reported, in particular also for Hox and Polycomb proteins. The Polycomb protein Mel18, for an instance, was categorized as a tumor suppressor gene and, as such, as a negative regulator of the cell cycle [62, 63]. By contrast, Hox genes were correlated with high mitotic activity, the triggering of cell divisions and tumor formation [55, 61, 64–66]. The mechanisms underlying the mitotic effects of Hox proteins could involve, at least partially, the competitive interaction of geminin with Cdt1 [67]. In addition, the early mitotic degradation of Hoxc10 by APC-dependent degradation also indicated connection to cell cycle progression [68]. Activation of the Hoxb cluster in the teratocarcinoma cell line P19 is dependent on DNA replication, and occurs in just one cell cycle [69]. Here, the chromatin must be organized such that a single round of DNA replication will de-repress the entire locus. Taken together, it is obvious that developmental functions must be tightly connected with decisions regarding cell divisions. Geminin is a protein that exemplifies the necessary integrative function, and thus represents a coordinator between developmental and proliferative control during embryogenesis.

Open questions and perspectives

Numerous regulators of the cell cycle have been identified, a collection that probably includes most of the major players. Their functional characterizations are so far advanced that the cell cycle must represent one of the best-understood regulatory circuits in biology. Obviously, enough questions to keep a large research community busy remain open. These include, for example, regulatory aspects in specialized cells, epigenetic mechanisms, the significance of protein modifications, the functions of splice variants, the importance of genetic redundancy and the dynamics of cell division. As in many other fields of biology, the phase of qualitative characterization will be completed by more quantitatively oriented studies in the future. The better the cell cycle as such is understood, the more interesting its relationship to other fields becomes. Undoubtedly, there must be highly interesting connections, dependencies and equilibria, which increase the complexity of the issue by orders of magnitudes.

For developmental biologists, studying the regulation of the cell cycle has always been an important topic, even though it remained a more or less separate discipline. In this review we have focused our discussion on one specific protein, geminin. The emerging concept from several studies indicates that geminin represents a mediator linking cell cycle control and control of embryonic development. However, many details of such a mediation are still unresolved. Open questions include the spectrum of geminin interaction partners, in particular among other homeodomain proteins, the dynamics, locations and timing of such interactions, their developmental consequences and the significance of the competition for Cdt1. It appears likely that in addition to geminin other cell cycle factors will also be identified which provide connections to a specific developmental circuitry. The more such entry sites are identified, the more we will have to switch from thinking about pathways to the consideration of a proteomic network.

Acknowledgements. The authors are grateful to M. Zeidler for critical comments on the manuscript and to P. Collombat for help with the figures.

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