T Cell Proliferation and Homeostasis: An Emerging Role for the Cell Cycle Inhibitor Geminin

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ABSTRACT: Thymic T cell differentiation to peripheral T cells aims to assist the generation of effector cells mediating adaptive immune responses. During this process, which takes place during embryogenesis and in adulthood, proliferation is coupled with changes in chromatin organization and transcription. Moreover, B and T lymphocytes start to proliferate and rapidly expand their numbers when activated following an encounter with an antigen. This expansion phase is accompanied by differentiation of naïve T cells and is followed by a period of population contraction, resulting in only a small fraction of the expanded population surviving and entering the memory cell pool. The kinetics of the expansion and contraction affect the speed of antigen clearance and the clinical course of disease. Molecules that are involved in the coordination of proliferation, chromatin reorganization, and transcriptional regulation are likely to play an important role in T cell generation, homeostasis, and disease. Here we review how cell cycle regulators affect lymphoid system development and homeostasis and discuss recent evidence implicating the cell cycle inhibitor Geminin in this process. Geminin has been shown to coordinate proliferation and differentiation by regulating cell cycle progression, chromatin organization, and transcription in the nervous system. In the immune system, progenitor T cell commitment and differentiation progresses normally in the absence of Geminin. However, Geminin is required for TCR response in vitro and T cell proliferation upon lymphopenia-induced proliferation, suggesting that Geminin might be an essential factor for T cell expansion during the immune response.

KEY WORDS: Geminin, Cdt1, T cell proliferation, T cell lineage commitment, licensing, genomic integrity, chromatin modulation, transcriptional regulation

ABBREVIATIONS

APC, anaphase promoting complex; APCs, antigen presenting cells; AP-4, activator protein 4; BAF, Brahma-associated factor; BMP4, bone morphogenetic protein 4; CDK, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; CHK1, checkpoint kinase 1; CUL4-DDB1, Cullin4 damaged-DNA binding protein1; DC, dendritic cells; DN, double negative; DP, double positive; DYRK1A, dual-specificity tyrosine-phosphorylated and regulated kinase 1A; ESC, embryonic stem cell; FOXP3, Forkhead box P3; HSC, hematopoietic stem cell; H3K27, histone 3 lysine 27; ICM, inner cell mass; IFN-γ, interferon γ; IL, interleukin; IRF-1, interferon regulatory factor 1; LCR, locus control region; LIP, lymphopenia induced proliferation; MCM, mini chromosome maintenance complex; MHC, major histocompatibility complex; NK, natural killers; ORC, origin recognition complex; PRC, polycomb repressive complex; RNAi, RNA interference; SCF-SKP2, Skp1-Cullin1-F-box protein Skp2; SP, single positive; TBP, TATA box binding protein; TBPL1, TBP like factor 1; TCR, T cell receptor; TGF-β, transforming growth factor β; TH, T helper; TIPT2, TATA-binding protein-like factor interacting protein 2; Treg, regulatory T cell

I. INTRODUCTION

Development of the immune system depends on the generation of the appropriate number of functionally specialized cells, capable of distinguishing self-antigens from foreign antigens. This process proceeds in an orderly fashion, so that multiple rounds of division and differentiation are required for the genera-
tion of mature cells from stem and progenitor cells. Moreover, innate immune responses require coordination of proliferation and differentiation of cells that secrete inflammatory cytokines to clear specific pathogens, whereas adaptive immune responses require the generation of highly specialized cells that eliminate pathogenic challenges.

T cells are central components of the immunological network, participating in cell-mediated immunity. Differentiation to the T cell lineage takes place in the thymus and depends on interactions of hematopoietic T cell progenitors with thymic stromal cells, which provide extrinsic signals that regulate chromatin reorganization and establish a T cell–specific transcriptional program. During T cell lineage commitment, thymic progenitor cells proliferate extensively to generate a large pool of cells that will undergo positive and negative selection. Much progress has been made toward identifying which are the critical factors regulating thymic progenitor proliferation, chromatin modulation, and lineage specification.

After maturation in the thymus, naïve T cells are exported to the periphery, where they encounter antigens. Naïve T cells are activated in secondary lymphoid organs after interaction with antigen presenting cells (APCs). Activation of naïve T cells leads to clonal expansion, heritable modification of chromatin, altered gene expression, and ultimately to generation of effector cells. The antigen-specific effector cells that persist following an infection form the memory compartment. This compartment has distinct properties compared to naïve T cells: reduced threshold of activation, increased lifespan, and enhanced capacity for response upon re-exposure to antigens. Thus, coordination of cell cycle progression with modulation of chromatin structure and transcriptional regulation is critical throughout T cell development, maturation, and differentiation.

In this review, we will present molecular pathways that control proliferation/differentiation decisions during T cell development in the thymus, and during peripheral T cell expansion, by regulating the cell cycle and transcriptional programs. Studies in the developing nervous system previously proposed that Geminin, a cell cycle inhibitor associated with chromatin reorganization activities, might balance neural stem cell self-renewal and differentiation decisions, operating as a molecular link controlling the cell cycle, epigenetic modifications, and transcription. Based on our recent findings, we discuss how Geminin might be incorporated in the molecular network that regulates T cell proliferation and lineage specification in the thymus and periphery and how it may participate in the regulation of T cell development, maturation, and homeostasis.

II. REGULATION OF THYMOCYTE PROGENITOR PROLIFERATION AND DIFFERENTIATION

A. T Cell Development in the Thymus

T cell development initiates with the migration of the thymocyte progenitors from the fetal liver or bone marrow to the thymus. These cells have been reported to commit to T cell lineage after instructive signals from Notch. As T cell progenitors differentiate, they gradually loose their ability to give rise to other cell types such as B cells, dendritic cells (DC), and natural killer (NK) cells. Immature double-negative (DN) progenitor cells constitute only 3–4% of the cells in the thymus and can be further subdivided into four developmental stages from DN1 to DN4, depending on the expression of c-kit, CD44, and CD25. Successful rearrangement and expression of a functional T cell receptor β (TCR-β), triggered by IL7Rα signaling, promotes DN3 progenitor cell proliferation and expansion. Rearrangement and expression of the TCR-α locus, along with CD4 and CD8 expression, determines the transition from the DN stage to the double-positive (DP, CD4+CD8+) stage. Positive and negative selection ensures that only cells expressing α and β TCRs that recognize self-MHC/peptide complexes with low affinity will survive and appropriately downregulate CD4 or CD8 to generate single-positive (SP) thymocytes.

B. Cell Cycle Progression of T Cell Progenitors

1. Proliferative Stages of Thymic T Cell Development

A small number of DN progenitor T cells in the thymus undergo approximately 10 divisions to generate
the big pool of DP T cells, the majority of which will be eliminated by negative selection.12,15 The earliest stage progenitors in the thymus, DN1 cells, are found in the G1 phase with a very low percentage of cells in the S/G2/M phases.16 The largest proportion of cycling cells was shown to be in the DN2 and DN4 stages. It has been proposed that cells undergoing TCR-β recombination are either dividing very slowly or exhibiting cell cycle arrest. After successful TCR-β rearrangement, progenitor thymocytes begin to expand with a high proportion of cells entering the S phase.14

2. Cell Cycle Regulators of T Cell Development

Central cell cycle regulators including cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors (CDKIs), which have been shown to control mammalian cell cycle progression, are also important in the regulation of progenitor T cell divisions (Table 1). The generation of mouse models has shed light on the role of cell cycle regulators in progenitor T cell proliferation. Although an essential role of cyclins D1 and D2 for normal T cell development has not been demonstrated, cyclin D3−/− mice were shown to have a deficit in the generation of DP cells.17–19 In the absence of cyclin D3, DN3/DN4 thymic progenitors failed to undergo the proliferative burst that generates the proper number of DP cells.17 In the absence of cyclin E, overexpression of cyclin E predisposes thymocytes for hyperplasia and malignant transformation.20 Deletion of cyclin A2 and B1 results in early embryonic lethality; therefore, their exact role in T cell development has not yet been addressed.21,22 However, the ablation of A1 and A2 cyclins in hematopoietic stem cells (HSCs) revealed their essential role in the proliferation of HSCs.23 It is possible that cyclins A are required for normal cell cycle progression of thymocytes. Consistent with an important role of cyclin D3 in thymocyte progenitor proliferation, CDK6-deficient mice showed pronounced thymic atrophy (Table 1). This study proposed that CDK6 is required downstream of Notch/Akt signaling for the transition from DN3 to DN4 cells. In contrast, knock out of CDK4 or CDK2 was shown to have no significant effect on T cell development.25,26

Mice deficient for the p27kip1 gene exhibit multiple organ hyperplasia, including an enlarged thymus.27 Generation of transgenic mice expressing p27kip1 in the T cell lineage resulted in a differentiation arrest of thymocytes at the DN stage, suggesting that p27kip1 is a negative regulator of progenitor T cell proliferation.28 Similarly, the combined deletion of the p16Ink4a locus and p27kip1 substantially increased lethality due to the appearance of T cell lymphomas, possibly due to overproliferation of progenitor T cells (Table 1).29

Cell cycle regulators play an important role in regulating cell cycle progression and normal T cell development. Deregulation of these mechanisms may result in aberrant T cell proliferation and development of T cell lymphomas (Figure 1).

C. Progenitor T Cell Differentiation: Role of Transcription Factors and Chromatin Modifiers

1. Different Transcriptional Programs Promote T Cell Lineage Commitment and CD4/CD8 Lineage Differentiation

Commitment to T cell lineage occurs at the DN stage, where the successive downregulation of genes for alternative lineages and upregulation of T cell–specific genes occurs. Several transcription factors and chromatin remodeling complexes act in a precise order to establish T lineage specification and subsequently regulate CD4 versus CD8 lineage choice. Notch signaling is one of the best-characterized positive regulators of T cell lineage development. Several studies using mouse models have proposed that Notch-dependent signaling is necessary and sufficient for T lineage commitment acting via downstream transcription factors such as Hes-1.30–32 Other transcription factors involved in early T cell development include PU.1 and GATA-3. Genetic ablation of PU.1 in the mouse embryo results in a block of T cell development prior to T cell commitment, suggesting that PU.1 plays a role in T lineage specification.33 PU.1 is downregulated at later stages of T cell development.34 Moreover, GATA-3−/− embryonic stem cells (ESCs) failed to give rise to thymocytes or mature T cells in mice
generated with the tetraploid blastocyst complementation system. Studies in mouse models proposed that after T cell lineage commitment, Runx and GATA3 transcription factors regulate CD4/CD8 lineage commitment. Gata3 is expressed in thymic CD4 SP cells and is essential for commitment to the CD4 but not the CD8 lineage. Runx1 is required for active repression of the CD4 gene in DN thymocytes, whereas Runx3 regulates CD4 repression in CD8 mature thymocytes. Mice deficient in Ikaros expression lack fetal T cell progenitors, whereas postnatal thymocytes show skewed differentiation toward the CD4 lineage. Subsequent studies showed that Ikaros family members directly regulate the CD8a gene during CD8 lineage commitment. Furthermore, Hox genes are expressed dur-

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Table 1. Mouse Models of Hox Genes, Cell Cycle Regulators, and Chromatin Modulators Involved in the Regulation of T Cell Proliferation and Differentiation

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Chapter 2

2. SWI/SNF and Polycomb Complexes Are Important Regulators of T Cell Lineage Specification

The establishment of the precise transcriptional program that leads to T cell lineage specification requires the spatial and temporal modulation of the chromatin structure of target genes. Chromatin remodeling complexes are important regulators of progenitor cell specification during T cell development, editing permissive or inhibitory marks in chromatin at different developmental stages. Targeting of Brg1, the ATPase subunit of the SWI/SNF chromatin complex, in the thymus highlighted a pivotal role of the protein in the successive stages of T cell development from DN to SP cells. Homozygous deletion of Brg1 was shown to dramatically reduce thymic cellularity, derepress CD4 expression in a fraction of DN cells, and block the transition from DN to DP. Brg1-deficient thymocytes showed defects in Wnt signaling, leading to c-kit and c-myc repression. Moreover, in the absence of Brg1, DN thymocytes induce p21Cip1/Waf1/Sdi1 with a subsequent arrest in cell cycle progression. These results suggest that Brg1-dependent chromatin remodeling regulates signaling-dependent survival (Wnt, myc pathway), cell cycle progression (p21Cip1/Waf1/Sdi1 induction), and lineage commitment (CD4 gene derepression) of T cells.

Polycomb-group (polycomb repressive complex [PRC]) proteins are organized as multimeric nuclear protein complexes, PRC1 and PRC2. PRC2 induces transcription repression by the introduction of di-methylation and tri-methylation modifications to histone H3 at lysine-27 (H3K27). Subsequently, PRC1 is recruited through recognition of methylated H3K27 and maintains transcription silencing by mediating monoubiquitination of histone H2A at

Figure 1. Studies in knock-out and transgenic mice demonstrating the in vivo role of cell cycle regulators, Hox genes, and chromatin modifiers in the regulation of progenitor T cell proliferation and differentiation. The generation of mouse models shed light on the factors required for normal T cell development (DN1–4, DP, CD4, CD8). The arrowheads depict the stages during T cell development in which CDK6, cyclinD3, p27, HoxA9, HoxB3, Bmi1, Eed, and Brg1 have roles in the regulation of proliferation and T cell specification.
lysine-119. Members of the PRC are known to play important roles in T cell lineage commitment. Bmi1 knockout mice have severely depleted thymi due to inhibition of differentiation beyond the CD4-CD8- stage. It has been suggested that during T cell differentiation, Bmi1 epigenetically represses the cell cycle inhibitor p19ARF, allowing normal progression at the DN3 stage. Furthermore, a hypomorphic allele of Eed, another component of the PRC2, has been shown to result in a partial block at the B-cell selection checkpoint.

Chromatin remodeling complexes and epigenetic modifications play a pivotal role in T cell development by the incorporation of extrinsic signals that coordinate cell cycle progression to lineage commitment.

III. REGULATION OF PERIPHERAL T CELL PROLIFERATION AND DIFFERENTIATION

A. Peripheral T Cell Populations

Naïve CD4 and CD8 T cells from the thymus are exported to the periphery, where they continue to proliferate and differentiate generating effector cells. Because thymic output is not sufficient to sustain the number of the short-lived naïve peripheral T cells, it has been proposed that peripheral expansion of T cells also contributes to the total number of peripheral T cells. Under steady-state conditions, the number and composition of peripheral T cells is maintained relatively constant by homeostatic mechanisms. However, both the number and composition of the various subsets of peripheral T cells are influenced by infections, age, and sex of the animal. During the course of an infection, naïve T cells that encounter antigens undergo a dramatic clonal expansion and generate effector cells, which exert their functions by the secretion of specific cytokines (expansion phase). In the second phase, following the clearance of the antigen, the majority of activated T cells are removed and only a small number of antigen-specific cells remain (contraction phase). A distinct subset of peripheral T cells, known as regulatory T cells (Tregs), suppresses and restricts the immune response. Surviving antigen-specific cells develop into memory cells, a differentiated subset of peripheral T cells, which are responsible for the clearance of the same antigen in case of subsequent exposure (memory phase).

B. Naïve T Cells

Naïve CD4 and CD8 T cells in the periphery are typically quiescent with a half-life of approximately 6 mo. Interactions with low-affinity self-antigen/MHC complexes contribute to the survival, maturation, and differentiation of naïve peripheral T cells. In addition, interactions with foreign-antigen/MHC have been shown to promote naïve T cell proliferation and differentiation to effector T cells. Activation and proliferation of naïve T cells is triggered by signaling through TCR and co-stimulatory molecules such as CD28, CD40, and OX-40. The duration of the cell cycle, frequency of cells entering the proliferative pool, and number of divisions depends on TCR engagement, co-stimulatory signaling, and a milieu of cytokines. Progression through the cell cycle and clonal expansion of naïve peripheral T cells is largely driven by interleukin 2 (IL-2). Both CD4 and CD8 cells proliferate shortly after antigenic stimulation; however, the rate of cell division for CD4+ T cells is much lower than that of CD8+ T cells. Moreover, the number of antigen-specific CD8+ T cells produced is larger than that of CD4+ T cells. Therefore, different subsets of naïve T cells have been shown to have different lifespans and proliferative potential.

C. Effector T Cells

Naïve CD4 and CD8 T cells in the periphery differentiate to T helper cells (Th) and cytotoxic T cells, respectively, and participate in specialized immune responses. T helper 1 (Th1) cells mobilize cellular immune responses, T helper 2 (Th2) cells participate in the humoral immune response, and cytotoxic T cells eliminate infected somatic and tumor cells. Differentiation of naïve T cells to effector cells involves the reprogramming of gene expression by epigenetic regulation of chromatin structure and
subsequent changes in the recruitment of active transcription factors. In the presence of IL-12, naïve T cells differentiate into Th1 cells, which express interferon γ (IFN-γ). IL-12 has been shown to activate STAT4 transcription factor, which in turn leads to interferon regulatory factor 1 (IRF-1) expression. Consistent with this finding, STAT4-deficient mice have impaired Th1 development. More recent data suggest that T-bet is also involved in regulating Th1-specific gene transcription. Furthermore, the Th1-dependent responses in vivo were impaired in transgenic mice expressing a dominant negative form of IκBα, implicating the NF-κB transcription factor in Th1 differentiation. Memory Th1 cell generation from Bmi1−/− effector Th1 cells was impaired, suggesting that the polycomb complex has roles in Th1 differentiation.

IL-4 promotes differentiation into Th2 cells that express IL-4, IL-5, and IL-13. Signaling through IL-4R was shown to activate STAT6, which is important for the activation of the Th2-specific differentiation program. In addition, GATA-3 and Ikaros transcription factors have been identified as essential activators of Th2 cytokine-specific gene expression. Moreover, it has been shown that GATA-3 physically interacts with Bmi1 and that this interaction prevents GATA-3 ubiquitination and degradation, facilitating Th2 differentiation. Even though epigenetic modifications in the IFN-γ gene and IL-4 gene are essential for the differentiation of naïve T cells to Th1 and Th2 cells, respectively, the protein complexes controlling this process have not yet been clarified.

**D. Memory and Regulatory T Cells**

The majority of activated T cells will perish after antigen elimination but a few cells will survive, forming the memory T cell compartment. Several studies have established that the interaction of T cells with antigen presented on APCs regulates both the overall expansion and total number of memory T cells generated. High expression of CD44 is a hallmark of memory T cells and studies suggest that memory T cells retain their effector function. However, little is known about the molecular mechanisms that regulate development, survival and maintenance of memory T cells. The transcriptional regulator IL-7 and interaction with Class II MHC molecules have been proposed to be important in peripheral memory T cell survival and function. It will be interesting to further elucidate the exact epigenetic mechanisms that control T memory cell specification and survival.

Tregs are essential for maintaining self-tolerance and immune homeostasis. CD25 expression characterizes T cell populations with suppressor activities; however, this molecule is not uniquely expressed exclusively in Treg populations. Several reports have established the forkhead box P3 transcription factor (Foxp3) as the master regulator of Treg differentiation. Moreover, transforming growth factor β (TGF-β) and IL-10 have been shown to have a critical role in the development of regulatory T cells. Several lines of evidence suggest that Foxp3 acts as a cytokine production suppressor, targeting genes such as IL-2, IL-4, and IFN-γ. Moreover, transforming growth factor β (TGF-β) and IL-10 have been shown to have a critical role in the development of regulatory T cells. The involvement of other factors in regulatory T cell specification requires further investigation.

**IV. GEMININ: REGULATION OF CELL CYCLE PROGRESSION AND CELL FATE COMMITMENT**

Geminin was initially characterized by two independent studies as a DNA replication inhibitor and a regulator of neuronal differentiation. In these initial studies, McGarry and Kirschner showed that Geminin is specifically degraded during mitosis and blocks initiation of DNA replication but not elongation. More importantly, they showed that overexpression of Geminin inhibited loading of the minichromosome maintenance (MCM) protein complex onto the chromatin, a complex essential for licens-
In parallel, Kroll et al.93 showed that Geminin is involved in early specification of the neuroectoderm during Xenopus embryogenesis.93

**A. Geminin an Inhibitor of DNA Replication Licensing That Regulates Maintenance of the Genomic Stability**

From yeast to humans, the initiation of DNA replication is controlled by the assembly of a multi-protein complex, called the pre-replicative complex, which is formed to specific regions of the chromatin known as origins of replication during the G1 phase.94,95 The formation of this complex licenses initiation of DNA replication during the S phase. The formation of the pre-replicative complex begins with the binding of the origin recognition complex (ORC) onto the chromatin and subsequent recruitment of the Cdc6, Cdt1, and the MCM complex proteins.96–98 The six-subunit ORC is believed to act as a scaffold for the assembly of the pre-replicative complexes in the G1 phase of the cell cycle.99 Cdc6 and Cdt1 were identified as essential factors in yeast, Xenopus, and mammalian cells for the loading of the MCMs onto chromatin and the initiation of DNA replication.97,98,100,101 The MCM complex consists of six proteins (MCM2–7), which are highly conserved across eukaryotes, carry an ATPase motif, and are believed to act as a DNA helicase complex.92 Following formation of the pre-replicative complex, an increase in the activities of the cyclin/CDK complexes, cyclin E/CDK2, and cyclin A/CDK2 trigger the initiation of DNA replication at the G1/S transition.102,103 After initiation of DNA replication, pre-replicative complexes are disassembled and licensing factors are inactivated in a temporally and spatially controlled fashion, whereas MCMs are believed to travel along chromatin with replisomes, as DNA replication helicases.

Several regulatory mechanisms ensure that the pre-replicative complex will not be re-activated in the same origin after initiation of DNA replication.104 Cdt1 is a central component of the pre-replicative complex and it is regulated by two mechanisms to ensure the timely and once-per-cell-cycle initiation of DNA replication. Cdt1 accumulates during G1, while following initiation of DNA replication it is targeted for ubiquitination and degradation by damaged-DNA binding protein1-Cullin4 (DDB1-Cul4) or cyclin-dependent phosphorylation and degrada-

mediated by Skp1-Cullin1-F-box protein Skp2 (SCF-Skp2).105–107 An additional mechanism operating in metazoa involves a protein inhibitor called Geminin. Geminin has been shown to bind Cdt1 and inhibit loading of MCMs onto origins of DNA replication.108 The interaction between Geminin and Cdt1 was shown to be remarkably strong, since 4M urea is needed to dissociate the complex.101 Inactivation of Geminin by proteolysis by the anaphase promoting complex (APC), nonproteolytic CDK-dependent ubiquitination, and cytoplasmic transport of Geminin ensure that Cdt1 will be available for a new round of licensing upon mitotic exit.

Experiments in tumorigenic cell lines and model organisms emphasize the importance of the regulation of Geminin and Cdt1, which ensures that re-initiation of replication will not occur within the same cell cycle (Table 2). Cdt1 ectopic expression caused re-replication in Xenopus and human cell lines.112–114 The combinatorial overexpression of Cdc6 and Cdt1 in human cell lines in the absence of functional p53 enhances re-replication.115 Moreover, the overexpression of Cdt1 in cancer and normal cell lines induced DNA damage and checkpoint activations that arrested cell cycle progression.116–118 Mouse fibroblasts that overexpress Cdt1 lead to tumor development when injected subcutaneously in mice.119 Furthermore, transgenic mice that overexpress Cdt1 in mouse T cells exhibit lymphoma formation in the absence of functional p53.120 Silencing of Geminin in normal human and cancer cells with intact p53 resulted in re-replication of the genome and activation of checkpoint pathways accompanied by arrest at G2/M transition, regardless of p53 activity.121,122 Consistently, knock-down experiments of Geminin in Xenopus embryos arrest cell cycle progression and activate checkpoint protein kinase 1 (Chk1).123 In agreement with these results, depletion of Geminin in *Drosophila* cells led to cessation of mitosis and asynchronous overreplication of the genome.124 An elaborate analysis of whole genome replication in *Drosophila* cells after RNAi silencing of Geminin proposed that heterochromatic regions are preferentially re-replicated in the absence of Geminin.125
Table 2. Effects of the Geminin silencing on cell cycle progression and differentiation

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Conversely, depletion of Geminin in HeLa cells did not cause arrest of the cell cycle or overreplication of the genome.\textsuperscript{112,126} Most intriguing were the findings that Geminin silencing arrested proliferation by induction of DNA re-replication only in tumorigenic cell lines and not in nontransformed cells.\textsuperscript{127}

**B. Geminin Involvement in Transcription and Development Processes**

Balanced regulation of proliferation and differentiation is essential during development and homeostasis. In addition to its role in regulating proliferation, Geminin was identified as a protein that promotes neuronal fate acquisition and differentiation.\textsuperscript{93} Injection of Geminin mRNA in Xenopus embryos induces expansion of the neural plate and ectopic neurogenesis at the expense of epidermis, in a process that suppresses bone morphogenetic protein 4 (BMP4) signaling and induces pro-neural genes.\textsuperscript{93} Similar results were obtained from experiments in *Drosophila*, in which ectopic overexpression of the Geminin homologue in *Drosophila* resulted in the formation of ectopic neuronal cells.\textsuperscript{128} Although these initial studies established a role for Geminin in neurogenesis, it was poorly understood how an inhibitor of DNA replication can affect the commitment and differentiation of neural progenitor cells.

Subsequent studies from Del Bene et al.\textsuperscript{129} and Luo et al.\textsuperscript{130} shed light on the molecular mechanisms through which Geminin might regulate the decision of progenitor cells toward proliferation or differentiation. They have proposed an antagonistic interaction between Geminin, Cdt1, and the homeobox-containing transcription factors Hox and Six3.\textsuperscript{129} Binding of Geminin to Six3 does not prevent Six3 binding to DNA but inhibits its ability to activate transcription, whereas Six3 does not permit binding of Geminin to Cdt1.\textsuperscript{129} Overexpression of Geminin in Medaka induces specific eye and forebrain defects due to reduced proliferation, premature differentiation, and enhanced apoptosis, whereas Geminin inactivation by morpholino experiments leads to expanded optic vesicles due to increased cell proliferation.\textsuperscript{129} This experimental evidence suggests that a direct, antagonistic interaction takes place between Geminin and Six3 to control cell proliferation and differentiation. Moreover, mouse Geminin was found to interact with HoxD10 and HoxA11, whereas GST pull-down assays further pinpointed several other Hox proteins (such as HoxA7, B7, C8, C9, and A10) to directly interact with recombinant Geminin.\textsuperscript{130} This interaction prevents Hox transcription factors from binding to DNA and inhibits their ability to activate transcription (Fig. 2).

Geminin might affect transcription by interactions with the basic transcriptional machinery. The TATA-binding protein-like factor-interacting protein isoform 2 (TIPT2), which is widely expressed in mouse embryonic and adult tissues, was shown to be a novel binding partner of Geminin.\textsuperscript{131} TIPT2 interacts with polycomb factors such as Scmh1, Mph2, Ring1B, the general transcription factor TATA box binding protein (TBP), and the related protein TBP-like factor 1 (TBPL1). TIPT2 acts synergistically with Geminin and TBP for the activation of TATA box-containing promoters, and with TBPL1 in the activation of the TATA-less NF1 promoter. Geminin and TIPT2 were detected in the chromatin near TBP/TBPL1 binding sites. These data suggest that both Geminin and TIPT2 bind to the basic transcriptional machinery and activate transcription synergistically (Fig. 2).\textsuperscript{131}

Furthermore, Geminin interacts with activator protein 4 (AP-4), a helix-loop-helix transcription factor, to restrict neuronal gene expression, such as PAHX-AP1 in nonneuronal cells.\textsuperscript{132} AP-4 and Geminin form a complex that attracts co-repressor SMRT and histone deacetylase HDAC3 and represses the transcription of dual-specificity tyrosine-phosphorylated and regulated kinase 1A (DYRK1A).\textsuperscript{132} This study proposed a novel mechanism through which Geminin may restrict neuron-specific gene expression in nonneuronal cells (Fig. 2).

In addition to its ability to modulate transcription by direct interaction with transcriptional factors, Geminin is able to modulate epigenetic marks and chromatin organization. Indeed, Luo et al. showed using pull-down experiments that Geminin interacts with the Scmh1 protein of the polycomb complex.\textsuperscript{130} Immunoprecipitation of Geminin from mouse embryonic extracts co-precipitated the Rae28 protein, suggesting interactions of Geminin with the PRC1.\textsuperscript{130} Apart from direct protein-protein interactions with various members of the polycomb
group, chip experiments suggest that Geminin binds to plzf-binding regulatory elements in the HoxD11 gene, which are required for Polycomb-mediated epigenetic silencing of Hox expression and regulation of axial patterning. These findings were further supported by ectopic expression of Geminin in the chick neural tube that resulted in a shift of the anterior transcription boundary of HoxB9 expression, indicating that Geminin is involved in suppression of Hox gene transcription (Fig. 2).

Geminin was also found to interact with SWI/SNF, an ATP-dependent chromatin-remodeling complex. It has been suggested that Geminin interacts with Brg1, the catalytic subunit of the SWI/SNF complex, regulating the timing of neurogenesis. Expression of Geminin was shown to inhibit SWI/SNF interactions with bHLH transcription factors, resulting in the suppression of neuron-specific gene expression and maintenance of neural progenitor cells in an undifferentiated state. An example of how Geminin interactions can regulate chromatin structure and lead to transcriptional activation of genes came from studies of the Sox2 transcriptional activation. Sox2 is a transcription factor associated with developmental plasticity of progenitor cells, and is involved in cell fate specification and the establishment or maintenance of chromatin architecture. Papanayotou et al. have identified two novel coiled-coil proteins, named as ERNI and BERT, as molecular partners of Geminin. They showed that competitive interaction between Geminin, ERNI, and BERT modulate the choice of heterochromatin proteins HP1α and HP1γ and regulate the ability of the SWI/SNF complex to activate Sox2 gene transcription through the N2 enhancer. Their study suggests that these interactions control the timely expression of the Sox2 gene, aiding the establishment of neural plate identity in avian embryos (Fig. 2).

These lines of evidence indicate that Geminin interacts with a number of transcription factors and chromatin modulators regulating transcriptional programs and epigenetic modifications, thus affecting neural progenitor self-renewal and differentiation. Geminin-deficient mouse embryos arrest at the eight-cell stage and fail to form the inner cell mass (ICM). These data and novel studies in the hemopoietic system support the idea that Geminin might have a general role in controlling progenitor self-renewal and commitment.

V. ROLE OF GEMININ IN THE REGULATION OF T CELL PROLIFERATION AND DIFFERENTIATION

A. Geminin Is Dispensable for Cell Cycle Progression of Thymocyte Progenitors

Geminin was shown to maintain progenitor cells and participate in fate determination decisions during neural development. A recent study from our laboratory has proposed a novel role for Geminin in the regulation of T cell proliferation, suggesting a more general role for Geminin in controlling proliferation and differentiation.9,140

Geminin mRNA is expressed in all stages during progenitor T cell development with higher expression detected from DN4 to DP transition coinciding with the higher proliferation rates of progenitor T cells. Geminin expression is nearly undetectable in resting naïve T cells, consistent with reports showing that Geminin is downregulated upon cell cycle exit. Its expression is dramatically increased upon entry of T cells into the cell cycle, for example, after TCR activation.

To obtain in vivo evidence of the role of Geminin during T cell development and homeostasis, we have generated mice in which exons 3 and 4, which encode the largest part of the coiled-coil domain, are flanked by loxP sites. The use of a transgenic line that drives Cre expression under the control of the promoter and locus control region (LCR) of the human CD2 gene results in the efficient inactivation of the mouse Geminin gene from the DN1 stage and onward.9,142

Mice lacking Geminin expression in the lymphoid lineage generate all the thymic subpopulations with only minor reductions in the DN1 and DN4 populations, suggesting that Geminin is not essential in the commitment and differentiation of progenitor T cells. The mild effect of Geminin in
progenitor T cell development was an unexpected result, given the high abundance of the protein in the thymus and the previously described role for Geminin in the early stages of mouse development and tumorigenic cell lines. Our data suggest that the role of Hox genes, SWI/SNF, and the polycomb complex during T cell differentiation does not require Geminin activity, contrary to what has been suggested for neuronal differentiation. Notch and Wnt/β-catenin pathways that have been shown to regulate cell cycle progression of thymic progenitors and instructing lineage commitment of progenitor T cells are mediated by multiple downstream effectors that may compensate for the loss of Geminin. Other central cell cycle regulators have been shown to be redundant for thymocyte proliferation as their activities can be compensated by members of the same family. Genetic ablation of cyclins D1, D2, and E in the mouse did not significantly affect T cell development. In different mouse models it was shown that the loss of one member of the cyclin D family can be substituted by the function of another member of the same family and prevent cell cycle progression defects. More-
over, some members of cyclin A and B families can partially compensate for the loss of members of the same family. Furthermore, cyclin A was shown to be redundant for normal cell cycle progression in some cells (fibroblasts) but not others (hematopoietic and embryonic stem cells). Our study suggests that similarly to other cell cycle regulators, the requirement of Geminin during cell cycle progression may be cell-type specific. Therefore, it is possible that in some cell types other pathways can act redundantly to replace Geminin’s function.

B. Geminin Depletion Leads to Pronounced Defects in T Cell Proliferation in the Periphery

In contrast to thymic T cell development, peripheral T cell populations such as naïve, memory, and regulatory T cells are significantly reduced in the absence of Geminin. One possibility is that the reduced numbers of peripheral T cell subpopulations are due to defects in the proliferative capacity of these cells. However, it would be interesting to examine whether Geminin inactivation also affects differentiation of peripheral T cells. The reduced cellularity and/or functionality of the peripheral T cell compartment may perturb germinal center formation, B cell maturation, and immunoglobulin class switching, leading to defects in humoral immunity of mice lacking Geminin in the lymphoid compartment. Therefore, these mice may prove valuable in the study of immune responses in the absence of efficient T cell help.

Moreover, T cells derived from mutant animals show reduced proliferation upon activation using various stimulants even though activation of cell surface markers following TCR-stimulation occurs normally. Geminin-deficient T cells show a dramatic proliferative defect upon adoptive transfer into lymphopenic mice. It is known that under conditions of T cell loss, space-driven expansion of T cells takes place, operating as a compensatory mechanism for the restoration of T cell numbers. Two mechanisms have been suggested to interpret this phenomenon. Interactions between T cells act as homeostatic sensors and inhibit proliferation when a large number of T cells are present. Alternatively, T cells are competing for access to limiting stimulatory ligands that includes cytokines and MHC/peptides. Naïve T cells undergoing lymphopenia-induced proliferation (LIP) acquire phenotypic and functional characteristics of memory T cells and eventually resemble central memory cells generated in response to foreign antigens. It has been suggested that T cells upon adoptive transfer undergo a rapid LIP, which is presumably driven by interactions with self-peptide MHC, IL-7, and IL-15. Further experiments are necessary to clarify how Geminin interacts with signaling triggered by cytokines and influences proliferation in lymphopenic conditions (Fig. 3).

BrdU pulse labeling experiments have suggested that cell cycle progression is defective in Geminin-deficient T cells in the periphery. Activated naïve T cells lacking Geminin showed an accumulation

Figure 3. Geminin dependent and independent processes during T cell development and differentiation.

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in the G2 phase, confirmed by increased levels of cyclin B1 and phosphorylation of cdc2 at tyrosine 15. A similar accumulation in the G2 phase is observed in knock-down experiments in tumorigenic cell lines, as a result of cell cycle blockage due to DNA damage initiated by re-replication events. In our experiments, we could not detect activation of DNA damage responses or increased DNA content in activated T cells that lack Geminin expression; however, this cannot be excluded since primary T cells are not a synchronized cellular population and therefore these changes may escape detection. Furthermore, we have shown that a significant fraction of activated T cells that lack Geminin expression withdrew from the cell cycle, suggesting that Geminin-deficient T cells show multiple defects (Fig. 3).9 One interesting possibility could be that peripheral T cells, in the absence of Geminin, may progress to senescence. Senescent cells are not responsive to mitogenic stimulation, and they show irreversible cell cycle exit and high expression levels of cell cycle inhibitors.152 Senescent T cells are developed in individuals infected with HIV and are implicated in reduced antiviral responses and age-related pathologies.153 Further studies of the peripheral T cell compartment of mutant mice are required in order to understand the possible involvement of Geminin in T cell induction, immune senescence, and the development of age-related pathologies.

C. Pathways May Act Redundantly to Geminin in Regulation of Cell Cycle Progression and Maintenance of Genomic Integrity

One of the main roles that have been attributed to Geminin is the regulation of normal cell cycle progression and maintenance of genomic integrity though Cdt1 regulation. Conditional inactivation of the mouse Geminin gene in the lymphoid lineage provides in vivo evidence supporting the idea that other mechanisms operate redundantly of Geminin in some cells. For example, cyclinE-A/CDK complexes are involved in Cdt1 phosphorylation and regulation.154,155 CDK-dependent phosphorylation of Cdt1 results in Skp2 recognition, ubiquitination, and subsequent degradation of Cdt1.107,156,157 In addition to this mechanism, an alternative pathway was found to regulate Cdt1 activity. Cul4-DDB1 E3 ubiquitin ligase targets Cdt1 for proteolysis after Cdt1 binds to PCNA onto chromatin.158,159 Re-replication in specific cell types is also prevented by the activity of CDK1 and cyclin A.160

The relative levels of expression between Geminin and Cdt1 have been postulated to be important for the maintenance of genomic integrity.161 Several studies have suggested that Geminin and Cdt1 expression levels are mutually regulated. Silencing of Geminin in Drosophila cells led to a downregulation of Cdt1, whereas in mammalian cell lines the overexpression of Cdt1 resulted in the induction of Geminin.124,162 How is Cdt1 expression regulated in the absence of Geminin during T cell development? Cdt1 protein expression is similar in WT and mutant thymocytes in which cell cycle progression is apparently normal. On the contrary, activated peripheral T cells show increased Cdt1 expression in the absence of Geminin, a deregulation that might explain the severe cell cycle defects of these cells both in vitro and in vivo.9 The activity of pathways that act compensatory to Geminin’s role may be critical for normal cell cycle progression and maintenance of genomic integrity. Differences in the activity of these pathways in different cell types may explain why Geminin is required for normal cell cycle progression in some cells but not others.140

D. The Role of Geminin Deregulation in the Promotion of Carcinogenesis and Leukemia

Several studies have established that the balance between Geminin and Cdt1 is important for the maintenance of genomic integrity. Ectopic expression of Cdt1 in Xenopus and human cell lines results in genomic lesions often combined with detectable re-replication of the genome, whereas Geminin and p53 can restrict re-replication caused by Cdt1 overexpression.113,114 Cdt1 expression is increased in dysplastic and cancerous lesions, whereas over-expression of Cdt1 in U2OS cells leads to clones resistant to senescence and apoptosis.115 These data promote the idea that Cdt1 deregulation triggers genomic instability and operates as an initiating event in carcinogenesis.115 Similarly, Geminin silencing has been shown
to have similar effects to Cdt1 overexpression in some cell lines, triggering activation of DNA damage response pathways (ATM/ATR dependent) and cell cycle arrest. However, several cell lines are resistant to re-replication possibly due to other pathways safeguarding genomic maintenance. For example, only after the silencing of both Geminin and cyclin A did primary cells exhibit re-replication, demonstrating the critical role of cyclin/CDK complexes in the maintenance of genome stability.

These lines of evidence propose that the deregulation of Geminin and Cdt1 expression results in the accumulation of genomic lesions that if not inhibited and/or repaired could lead to a breach of genomic integrity and promotion of malignant transformation. Could alterations in the balance between Geminin and Cdt1 trigger or promote malignant transformation of T cells resulting in the development of leukemia? The generation of transgenic mice overexpressing Cdt1 under the T cell–specific promoter was not sufficient to induce malignant transformation of T cells. However, when Cdt1 overexpression was combined with mutation in the p53 gene, mice showed abnormal T cell development, enlarged thymi, and development of thymic lymphoblastic lymphoma that caused death at 5.5 mo. These results are consistent with published studies in tumorigenic cell lines supporting the idea that Cdt1 overexpression should be combined with defects in anti-tumorigenic barriers in order to promote carcinogenesis.

A direct role for Geminin in malignant transformation induction has not been clearly demonstrated. Given the inhibitory role of Geminin in the regulation of DNA replication, it was initially thought that Geminin would act as a tumour suppressor gene. Therefore, it was surprising to find a positive correlation between Geminin expression and aggressive characteristics of cancer cells. Geminin expression was found to be elevated in cancer cell lines, breast cancer, colon carcinomas, oligodendrogliomas, astrocytomas, high growth fraction lymphomas, and B cell lymphomas. Furthermore, Hox genes that are regulated by Geminin during nervous system development have been shown to be involved in T cell leukemias. Ablation of Geminin in progenitor T cells resulted in the cell cycle arrest of activated peripheral T cells; however, increased tumorigenic incidence in mice that lack Geminin expression was not observed. It would be interesting to examine whether tumour suppression mechanisms, orchestrated for example by p53, participate in preventing tumor development.

E. Geminin: a Regulator of the Epigenetic Process Facilitating T Cell Differentiation?

Geminin interactions with members of chromatin remodeling and modifying complexes, such as SWI/SNF and polycomb, are important for embryonic patterning and neural specification. Although it is not yet fully understood how these interactions affect cell fate decisions, Geminin may have a novel, up-to-now unexplored, role as an organizer of the transcriptional program of stem/progenitor cells, through epigenetic control of target genes. This is supported by recent findings showing that knock-down of Geminin in ESCs impairs their ability to acquire the neural fate due to reduced expression of neural-specific genes, whereas overexpression of Geminin in ESCs promotes activation of neural gene expression. More importantly, overexpression of Geminin in ESCs correlated with a hyperacetylated chromatin configuration of neural genes such as NeuroD1 and Ebf2. Is it possible that Geminin may have a similar role in T cell lineage specification to the one observed during neural fate acquisition? It has been proposed that the epigenetic modifications dictating an open chromatin configuration of lymphoid-specific genes occurs much earlier than the initiation of the transcription of these genes and the commitment to the T cell lineage. Human HSCs were found to have many lymphoid- and myeloid-affiliated genes associated with acetylated H4 and H3 histones, which is suggestive of an open chromatin configuration. Moreover, the lymphoid-specific genes examined in HSCs were not found to bear repressive chromatin modifications such as H3K9me3 and H3K27me3. Differentiation of HSCs toward the T cell lineage in vitro correlated with reduced levels of acetylation in all non-T-specific genes. These results are consistent with the notion that lymphoid genes are “set” for expression in hemopoietic stem and progenitor cells before commitment to the T cell lineage. In this
case, a potential association of Geminin with chromatin modifiers in order to introduce active chromatin modifications for priming lymphoid-specific gene expression would occur in lymphoid-restricted multipotent progenitors that are generated in the fetal liver. This hypothesis could explain why the T cell differentiation program in the thymus remains undisturbed in the absence of Geminin.

On the other hand, it has been shown that the transition of naïve T cells to effector and memory T cells depends on the introduction of active epigenetic modifications in lineage appropriate genes and repressive chromatin modifications in lineage inappropriate genes.\textsuperscript{176,177} Increased levels of the activating modification H3K4me3, as well as high levels of H3 and H4 acetylation, were found in the IFN-γ, IL-4, and IL-17 genes, the signature cytokine genes of Th1, Th2, and Th17 cells, respectively.\textsuperscript{178–180} Conversely, induction of Th2 differentiation was associated with increased levels of the repressive histone modification H3K27me3 in the IFN-γ locus.\textsuperscript{180} Moreover, it has been demonstrated that Brg1-dependent chromatin remodeling of the IFN-γ locus is implicated in Th1 differentiation.\textsuperscript{181} High histone acetylation has also been found to correlate with expression of genes that regulate the differentiation of naïve CD8 T cells to memory cells.\textsuperscript{182} Together these studies suggest that epigenetic regulation of chromatin is critical in the generation of effector and memory T cells. Mice lacking Geminin in the lymphoid lineage presented reduced numbers of memory cells. Moreover, the generation of memory-like cells after transplantation of Geminin-deficient T cells in lymphopenic hosts was severely impaired.\textsuperscript{9} The defective differentiation of Geminin-deficient T cells to memory cells may be associated with the impaired epigenetic regulation of genes guiding this transition. Therefore, Geminin could act as an orchestrator of cell cycle control and epigenetic regulation, organizing the heritable gene expression profile of memory T cells. Further studies are needed to validate this hypothesis and to identify Geminin-dependent epigenetic modifications that drive generation of effector and memory T cells.

VI. PERSPECTIVES

Conditional inactivation of Geminin in the lymphoid lineage has provided insight into the role of Geminin in the regulation of T cell proliferation and differentiation. An absence of Geminin does not perturb the developmental program of T cell lineage in the thymus, but results in severe defects in peripheral T cell proliferation after TCR-dependent activation or during homeostatic expansion. The differential requirement of Geminin for cell cycle progression of progenitor and peripheral T cell populations suggests that different pathways and intrinsic mechanisms regulate immature and mature T cell proliferation. It would be interesting to investigate the cytokine pathway that acts through Geminin and regulates naïve T cell proliferation.

Moreover, the reduced number and impaired ability of T cell proliferation observed in mice that lack Geminin expression from the lymphoid system have been associated with autoimmune disorders such as multiple sclerosis, rheumatoid arthritis, and psoriasis.\textsuperscript{183} The immunological challenge of Geminin-deficient mice could potentially shed light on the mechanisms involved in the development of immune disorders.

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REFERENCES

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