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The Architectural Organization of Human Stem Cell Cycle Regulatory Machinery

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Abstract

Two striking features of human embryonic stem cells that support biological activity are an abbreviated cell cycle and reduced complexity to nuclear organization. The potential implications for rapid proliferation of human embryonic stem cells within the context of sustaining pluripotency, suppressing phenotypic gene expression and linkage to simplicity in the architectural compartmentalization of regulatory machinery in nuclear microenvironments is explored. Characterization of the molecular and architectural commitment steps that license human embryonic stem cells to initiate histone gene expression is providing understanding of the principal regulatory mechanisms that control the G1/S phase transition in primitive pluripotent cells. From both fundamental regulatory and clinical perspectives, further understanding of the pluripotent cell cycle in relation to compartmentalization of regulatory machinery in nuclear microenvironments is relevant to applications of stem cells for regenerative medicine and new dimensions to therapy where traditional drug discovery strategies have been minimally effective.

Keywords

Human embryonic stem cells; reprogrammed pluripotent cells; histone gene expression; nuclear organization; chromatin structure

I. THE ABBREVIATED PLURIPOTENT CELL CYCLE

Human embryonic stem cells are uniquely committed to continuous proliferation. Within the blastocyst of the embryo as well as in culture, human embryonic stem cells repeatedly traverse the cell cycle and undergo successive symmetrical cell divisions that provide structurally and functionally equivalent progeny cells that retain pluripotency and refrain from gene expression associated with lineage commitment [1–26].

The abbreviated self-renewal cell cycle of human and mouse embryonic stem cells exhibits a short G1 period [27–39] with similar periods of time allocated to S phase, G2 and mitosis. While the rules that govern proliferation in human embryonic stem cells remain to be comprehensively established, several lines of evidence are consistent with linkage to

competency of the naïve transcriptome to support the stringent requirements of pluripotent cells to self propagate while suppressing expression of genes that are associated with lineage commitment and/or tissue specificity. From both fundamental biological and clinically relevant perspectives, understanding control of the abbreviated embryonic stem cell cycle can facilitate applications where obligatory relationships between proliferation and differentiation are operative. Wound healing, tissue engineering, cell replacement and mitigation of developmental aberrations illustrate where increased knowledge of mechanisms that mediate competency for proliferation and cell cycle progression in human embryonic stem cells will be instructive.

Complex and inter-dependent signaling networks that include extensive cohorts of regulatory factors are operative throughout the cell cycle in both human embryonic stem cells and somatic cells to promote proliferation, control movement through successive stages of the cell cycle and provide surveillance mechanisms that monitor genomic integrity, chromatin packaging and mitotic division [40]. Effectiveness of these processes is essential for immediate detection of errors in genome replication or checkpoint control Fig. (1).

During the G1 period of the cell cycle, a series of regulatory events are orchestrated that support exit from mitosis as well as competency for proliferation, cell cycle progression and initiation of DNA replication. Selective expression of genes and post-transcriptional control at a series of levels are necessary. The activity of cyclins, cyclin-dependent kinases, cyclin-dependent kinase regulators as well as signaling factors that monitor and control the regulatory cascades that culminate in initiation of DNA synthesis is a principal event in a G1 period of the cell cycle. The initial component of G1 is dedicated to establishing engagement in the proliferative process. Late in G1, the restriction point marks acquisition of growth factor independence for cell cycle progression [30; 31]. At this time, genes that encode the enzymology for deoxynucleotide metabolism are upregulated, reflected by activation of genes that include thymidine kinase, thymidylate synthetase, and dihydrofolatereductase. Subsequently at the G1/S phase transition, the initiation of DNA replication is accompanied by and functionally coupled with activation of histone gene expression [41; 42].

The obligatory relationship between histone gene expression and DNA replication ensures the availability of histone proteins to package newly replicated DNA as chromatin. The magnitude of this requirement is strikingly illustrated by biosynthesis of 2 ½ yards of DNA during the 8–9 hour S phase. Equally relevant to the mechanisms that monitor fidelity of DNA replication and support compensatory processes if necessary (e.g., editing), the G1/S phase transition is associated with a series of surveillance and checkpoint processes that, if required, delay the onset of proliferation, invoke DNA repair and/or default to apoptosis [30; 31].

The 2.5 – 3 hour abbreviated G1 phase in human embryonic stem cells, compared to the 8–12 hour G1 period in human somatic cells, appears to require the activity of the full complement of cell cycle regulatory factors Fig. (1). However, the signaling cascades that control critical steps during G1 that culminate in genome replication are confined to a brief period. Mapping the temporal sequence of events that occurs during G1 in human embryonic stem cells has established that regulatory activity which is restricted to the interval between completion of mitosis and the restriction point may be modified in human embryonic stem cells. In contrast, the regulatory events that support competency for genome replication, histone gene expression and DNA synthesis appear unaltered but occur rapidly following exit from mitosis. Perhaps the most compelling evidence for an obligatory relationship between the abbreviated cell cycle and pluripotency is transition from an abbreviated to an extended G1 period with the initiation of human embryonic stem cell

differentiation and reversion to an abbreviated cell cycle in human induced pluripotent stem cells [27–29; 34; 36; 38; 39].

II. ARCHITECTURAL ORGANIZATION OF CELL CYCLE REGULATORY MACHINERY IN A MINIMALLY ORGANIZED NUCLEUS

1. The Pluripotent Nuclear Landscape

There are unique features to the nuclear organization of regulatory machinery in human embryonic stem cells that are distinct from the nuclear regulatory landscape of lineage committed cells [6–9; 43–47]. The human embryonic stem cell genome is primarily packaged as euchromatin with minimal presence of heterochromatin. The functional significance remains to be established but considerations include: 1) a genomic organization that is poised for executing diverse options to support initial lineage-specific gene expression and subsequently tissue-specific gene expression; 2) a genomic organization that is compatible with a limited extent of architectural remodeling that can be accommodated during an abbreviated cell cycle; and 3) limited compartmentalization of regulatory machinery in nuclear microenvironments that characterize differentiated cells.

However, some parameters of subnuclear organization (e.g., X chromosome inactivation [48–54]) are established during the initial stages of development and others that include nucleoli and histone locus bodies are in place and functionally operative during the earliest stages of development in human embryonic stem cells. Obligatory relationships between these functionally organized and architecturally associated regulatory complexes with biological control are thereby indicated.

2. Histone Gene Expression as a Paradigm for Intra-Nuclear Compartmentalization in Cell Cycle Control

Characterization of the molecular and architectural commitment steps that license human embryonic stem cells to initiate histone gene expression is providing understanding of the principal regulatory mechanisms that control the G1/S phase transition in primitive pluripotent cells. Cell cycle control and histone gene expression is required to support the abbreviated cell cycle in human embryonic stem cells. As well documented in somatic cells, histone genes are not regulated by an E2F/RB switch but by a HiNF-P/p220^{NPAT} co-activation complex. The unique abbreviated human embryonic stem cell cycle with a short G1 [27–29] correlates with absence of an E2F/RB switch [55].

The S-phase specific expression of histone genes, that is temporally and functionally coupled with DNA replication, was the initial example of cell cycle dependent gene regulation and provides a paradigm for understanding signaling mechanisms operative at the G1/S transition [41; 56–60]. The induction of histone mRNA and protein synthesis at the initiation of S phase supports packaging newly replicated DNA into chromatin [57; 61–64]. Histone H4 gene transcription is activated at the G1/S phase transition and down-regulated during quiescence or differentiation [44; 65–76]. These transcriptional modulations occur concomitant with dynamic modifications in chromatin structure and *in vivo* occupancy of histone gene promoters [70; 75; 77–79] in somatic cells.

The increase in histone gene transcription early in S phase is mediated by cohorts of transcription factors that have been identified in somatic cells [42; 80–92]. The principal pathway that activates histone H4 genes at the G1/S phase transition in somatic cells is the cyclin E/CDK2 dependent phosphorylation of the p220^{NPAT} co-activation complex. The Cyclin E/CDK2/p220^{NPAT}/HiNF-P pathway defines a novel cell cycle transition point which we have designated the ‘S-point’. This concept has fundamental consequences for

understanding of cell growth control and cell cycle regulation, and may provide new avenues for manipulating self-renewal of human embryonic stem cells for therapeutic applications. S point-related cell cycle control mechanisms in the context of subnuclear organization can provide an understanding of the regulated assembly of the histone gene expression machinery at dedicated subnuclear domains (p220^{NPAT} foci or 'Histone Locus Bodies') in both naïve and pre-committed human embryonic stem cells.

3. Histone Gene Expression in Human Embryonic Stem Cells is Coupled to Architectural Localization of Regulatory Machinery in Nuclear Microenvironments

An indication of the contribution by subnuclear architecture to control of self-renewal in human embryonic stem cells is the focal organization and the CDK-dependent *in situ* phosphorylation of p220^{NPAT} during cell cycle progression in human embryonic stem cells Fig. (2). The number of p220^{NPAT} foci increases in G1 prior to the CDK dependent phosphorylation of p220^{NPAT} in S phase. This increase may render the p220^{NPAT}/HiNF-P histone gene regulatory complex poised for rapid activation by cyclin/CDK complexes to induce histone gene expression at the onset of DNA synthesis.

Spatial mechanisms for synthesis and processing of histone gene transcripts are different between human embryonic stem cells [28] and lineage-committed somatic cells [80; 81; 93–97]. For example, the p220^{NPAT} foci detected in the G1 phase of human embryonic stem cells do not colocalize with coilin. Although a subset of p220^{NPAT} foci co-localizes with coilin as S phase progresses, there are always some foci that have one but not the other protein. Therefore, p220^{NPAT} foci and Cajal bodies containing coilin are related but fundamentally distinct subnuclear entities.

In somatic cells, p220^{NPAT} and HiNF-P are associated with the two large human histone gene clusters on Chromosomes 1 and 6, as well as the unique U7 snRNP that cleaves the 3' end of nascent histone gene transcripts to generate mature non-polyadenylated mRNAs. The prototypical Cajal body component coilin interacts with U7snRNP, thereby providing structural linkage between Cajal bodies and the histone pre-mRNA processing machinery [98]. Current data suggest that at least a subset of p220^{NPAT} foci coincide with Cajal bodies and contain an integrated supramolecular architectural complex in which histone gene transcription factors, the co-activator p220^{NPAT}, histone gene clusters and the U7 snRNP related 3' end processing machinery are all associated contemporaneously. Recent results suggest that p220^{NPAT} and FLASH are necessary to maintain this structure during the cell cycle [96; 99].

While Cajal bodies and p220^{NPAT} subnuclear foci are relatively stable, they exhibit differences in their resident components depending on the species, cell type and/or cell cycle stage. For example, while coilin is considered a resident protein of Cajal bodies, there are coilin-negative residual Cajal bodies [100]. In *Drosophila*, coilin is absent and there is a functional distinction between Cajal bodies (CBs) and Histone Locus Bodies (HLBs) [101]. Similarly, a distinction has been made between cleavage bodies, which contain histone gene loci, and adjacent Cajal bodies [102]. Importantly, the cell cycle dependent organization of p220^{NPAT} foci is different between somatic cells and embryonic stem cells. The number of p220^{NPAT} foci double from two to four only upon entry into S phase in somatic cells [81; 94; 95; 103; 104]. In contrast, in human embryonic stem cells, p220^{NPAT} forms two subnuclear foci in G1 that double to four foci prior to the onset of S phase [29]. A viable basis is therefore provided to capitalize on this cell cycle related distinction in defining how human embryonic stem cells expedite G1 progression to accelerate the self-renewal cell cycle.

III. REPROGRAMMING PLURIPOTENCY LINKS HUMAN EMBRYONIC STEM CELL NUCLEAR STRUCTURE TO FUNCTION

The regulatory machinery for histone gene expression, the genes with associated transcription factors and coregulatory proteins, is organized and associated at punctate foci designated histone locus bodies at the onset of S phase when histone protein synthesis is initiated. This architectural association of the histone regulatory machinery in nuclear microenvironments, in human embryonic stem cells and in somatic cells, supports linkage of the subnuclear localization of histone regulatory complexes with cell cycle control during S phase. The restoration of an abbreviated G1 period in induced pluripotent stem cells, with organization of histone locus bodies immediately following completion of mitosis, provides persuasive support for a functional relationship between the intranuclear localization of transcriptional regulatory machinery and cell cycle control that is retained in human embryonic stem cells but modified to occur immediately in G1 for accommodation of the abbreviated G1 period. The extent to which the “simplified” nuclear organization of regulatory machinery that characterizes human embryonic stem cells is restored with reprogramming pluripotency remains to be established. However, it appears that critical components of cell cycle control exhibit conserved interrelationships between nuclear structure and function.

IV. REGULATORY INFRASTRUCTURE IN PLURIPOTENT CELL NUCLEUS: OPTIONS AND OBLIGATIONS

The abbreviated cell cycle in human embryonic stem cells provides a streamlined process for proliferation during initial stages of development where pluripotency is required. From a regulatory perspective, genes that mediate competency to proliferate and cell cycle progression, that are selectively expressed in a cell cycle dependent manner, appear to be constitutive. Other components of cell cycle control that are specific for human embryonic stem cells include preferential expression of regulatory factors.

The pluripotent cell cycle is abbreviated by a reduced G1 period, raising a series of mechanistic questions that require resolution. What is the extent that regulatory events which are confined to specific stages of the cell cycle become constitutive in pluripotent cells? How extensive is regulatory machinery for DNA replication organized and assembled immediately following mitotic division? Are unique cell cycle regulatory proteins involved at the transition from an abbreviated pluripotent cell cycle to an extended cell cycle in lineage committed cells? Can parameters of cell cycle and growth control be identified to account for properties of lineage committed stem cells which exhibit extended periods of quiescence and mitotic divisions that are generally asymmetric rather than symmetric? A compelling and clinically relevant series of questions relate to the properties of cancer stem cells that are primarily quiescent. How does proliferative dormancy of cancer stem cells contribute to resistance to both radiation and chemotherapy?

Beyond the biochemical mechanisms that are hallmarks of pluripotent cells, the parameters of nuclear organization that distinguish these primitive cells from lineage committed cells may offer insight into nuclear structure-gene expression relationships that can facilitate exploitation of pluripotent cells for tissue engineering and provide therapeutic targets in cancer stem cells. The minimal organization of regulatory machinery in nuclei of pluripotent cells may identify components of nuclear architecture that are fundamental to governing proliferation and cellular function in a broader context.

Architectural organization and compartmentalization of cell cycle regulatory complexes can be instructive for discriminating between regulatory processes that are operative in biology

and pathology. The assembly and activity of histone locus bodies when S phase is initiated rapidly following completion of mitosis in human embryonic stem cells, following an extended G1 period in lineage committed cells and again following completion of mitosis in reprogrammed pluripotent stem cells where an abbreviated G1 period is restored, points to cellular dependence on compartmentalization of cell cycle regulatory machinery. Whether there are variations in the mechanisms for such architectural organization of genes and cognate factors that support cell cycle and growth control that are operative in pluripotent and lineage committed cells remains to be established. However, it is realistic to anticipate that further insight into linkage of nuclear structure and gene expression will be mechanistically informative. From both fundamental regulatory and clinical perspectives, further understanding of the pluripotent cell cycle in relation to compartmentalization of regulatory machinery in nuclear microenvironments is relevant to applications of stem cells for regenerative medicine and new dimensions to therapy where traditional drug discovery strategies have been minimally effective.

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Fig. 1. Differences in cell cycle control between human embryonic stem (hES) cells and somatic cells. Competency for cell cycle progression in human somatic cells (left) is mediated by both an E2F/RB switch (R point) and the HINFP-P/p220^{NPAT} complex (S point), whereas hES cells have an abbreviated cell cycle with a short G1 and lack an E2F/RB switch (right).

Fig. 2.

Cell cycle dependent localization of p220^{NPAT} and HiNF-P at Cajal body related foci in somatic cells. (A) The micrographs show in situ immunofluorescence (IF) results for p220^{NPAT} (red) and HiNF-P (green); overlapping signals are yellow. (B) Rotational analysis reveals that IF signal overlap is non-random (C) Three-way colocalization of p220^{NPAT} (red), HiNF-P (green) and the Cajal body component coilin (blue) in somatic cells. Somatic and hES cells differ in the temporal assembly of p220^{NPAT} foci and their association with coilin. [Reprinted with permission from “Control of the Human Pluripotent Cell Cycle” by G.S. Stein, *et al.*, in *Stem Cells: From Bench to Bedside* (Second Edition), (A. Bongso and E.H. Lee, eds.) published by World Scientific Publishing Company, copyright 2010.]