Journal of Cellular Biochemistry 96:962–970 (2005)

Remembering the Cell Fate During Cellular Differentiation

Guo-Ling Zhou, Li Xin, De-Pei Liu,* and Chih-Chuan Liang

National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100005, PR China

Abstract Higher eukaryote contains several hundreds of different cell types, each with a distinctive set of property defined by a unique gene expression pattern, even though every cell (with minor exception) shares the common genome. During cellular differentiation, the committed gene expression pattern is set up and propagated through numerous cell divisions. Therefore, cells must have evolved some elegant and inherent mechanisms to remember their expression states for the requirement of the stability of differentiation and development. Here we speculate a hypothetically cellular memory mechanism. In this hypothesis, the cell–cell variation during cellular differentiation may result from the inherent stochastic gene expression. The evolution of histone and distant regulatory sequences change the parameters of expression stochasticity. S-phase-dependent gene activation and epigenetic marks on chromatin provide means to discriminate transcriptionally active and repressive states. Eventually, mitotic memory mechanisms have been developed through which these expression states are transmitted through numerous cell divisions. J. Cell. Biochem. 96: 962–970, 2005.
© 2005 Wiley-Liss, Inc.

Key words: cellular differentiation; stochastic gene expression; histone; regulatory element; epigenetic memory; replication; mitosis

Cellular differentiation is achieved through selective gene expression that means switching on a relatively small number of genes and switching off a relatively large fraction of genes in a cell. Meanwhile, in order to reach the fixation of the differentiation direction and the progress of the programmed development, cells must remember who they are. Early on the development, totipotent embryonic cells can produce a wide range of cell types. But this potentiality becomes less and less with the proceeding of development. Reprogramming the development is difficult and problematic [Reik et al., 2001], which is due to that cells have remembered their fate. Generating the different gene expression states and fixing them are pivotal processes during differentiation of multicellular organisms. In most situations, cells carry the same set of genetic material, the genomic DNA, during their entire life, and thus epigenetic information including nucleosome structure, regulatory elements and the interactions among DNA, histones and non-histone protein factors, may account for cell memory mechanisms during cellular differentiation.

In this review, we propose a hypothesis to explain cellular memory of higher eukaryotes. In this hypothesis, the cell–cell variation during cellular differentiation may result from the inherent stochastic gene expression. The evolution of histone and the distant regulatory elements change the parameters of expression stochasticity. S-phase-dependent gene activation and epigenetic marks on chromatin provide means to discriminate transcriptionally active and repressive states. Eventually, mitotic memory mechanisms have been developed through which these expression states are transmitted through numerous cell divisions.

Grant sponsor: National Natural Science Foundation of China; Grant numbers: 30393110, 30300192.

*Correspondence to: De-Pei Liu, National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100005, PR China.
E-mail: liudp@pumc.edu.cn

Received 6 June 2005; Accepted 7 June 2005
DOI 10.1002/jcb.20572

© 2005 Wiley-Liss, Inc.

STOCHASTIC GENE EXPRESSION AND THE PROGRAMMED CELLULAR DIFFERENTIATION

The evidences from substantial experimental studies on gene expression at single-cell level suggested that the stochasticity is an inherent nature of gene expression. Theoretical analysis implies that the stochastic effects on gene expression can explain a large amount of cell–cell variations observed in isogenic populations [Spudich and Koshland, 1976; McAdams and Arkin, 1997]. The direct experimental evidences have been obtained from two bacterial systems [Elowitz et al., 2002; Ozbudak et al., 2002]. Blake et al. also showed that in *Saccharomyces cerevisiae* transcriptional stochasticity contributes to the levels of heterogeneity within a eukaryotic clonal population [Blake et al., 2003]. Increasing noise in the transcription of a regulatory protein could result in the increased cell–cell variability in the target gene output [Paszek et al., 2005]. These results implied that, by building the initial asymmetries that are amplified by feedback, the stochastic expression could function in such processes as cellular differentiation and monoallelic expression. A considerable body of evidences from hematopoiesis gave a strong support on this conception.

Cellular differentiation involves two processes: selectively turning on just a subset of genes and non-coding RNAs, and (selectively and/or universally) turning off the others. Stochastic expression may lead to the generation of cell–cell variations in multicellular organisms. However, a central problem for the development of multicellular organisms is how to maintain the stability of the specific cell types. Muller-Sieburg et al. traced the behavior of individual, clonally derived HSCs through long-term, serial repopulation experiments, and found that daughter HSCs derived from individual clones are remarkably similar to each other in the extent and kinetics of repopulation. Moreover, daughter HSCs within a clone can give equivalent contributions to the myeloid or lymphoid lineages differentiation [Muller-Sieburg et al., 2002]. Thus, the differentiation of cell types from HSCs in adult bone marrow is largely predetermined. Stem cell heterogeneity is generated earlier in development, perhaps when stem cells seed to the bone marrow during development, suggesting that intrinsic mechanisms can preprogram the behavior of hematopoietic stem cells (HSCs).

The studies on α- and β-globin genes further suggest that there are certain mechanisms in higher eukaryotic cells through which the stochastic expression patterns can be fixed during cell differentiation. Although α- and β-globin keep in tightly balance, their expression patterns are consistent with the prediction of stochastic mode [Wijgerde et al., 1995; Trimborn et al., 1999]. de Krom et al. depicted a detailed characteristic of mouse globin expression patterns in the nucleus and cytoplasm of single erythroid cell. The analysis revealed that a significant proportion of erythroid cells, both in nucleus and cytoplasm, exhibit an imbalance of 2α- versus 2β-globin gene expression and show the stochastic combination patterns, which are established prior to transcriptional activation. More importantly, both active and repressive expression states are clonally inherited [de Krom et al., 2002]. These inherited stochastic patterns could not be explained simply by the randomness of molecular encounters or the fluctuation in the transitions between the conformational states of a macromolecule, or the amplification of feedback loops of transcription factor network. The globin genes are expressed in terminal committed cells and play no role in cell lineage commitment decisions. And in fact imbalanced expression of α- and β-globin genes has both functional and selective disadvantage. So this inherited stochasticity may reflect an inherent nature of cellular differentiation. It also implies that the differentiated cells have developed some important mechanisms to remember both active and repressive expression states.

REPLICATION-DEPENDENT GENE ACTIVATION, ESPECIALLY LONG-RANGE ACTIVATION AND CELLULAR DIFFERENTIATION

In yeast, no correlation was observed between replication timing and transcriptional activity [Raghuraman et al., 2001], whereas the obvious correlation between them was obtained in Drosophila [Schubeler et al., 2002]. More likely, the replication-dependent (RD) transcription may be correlated with cellular differentiation of higher eukaryotes. The compelling evidences suggested that S-phase is a critical period not only for the maintenance of the repressive heterochromatin state but also for the establishment of transcriptional competence in higher eukaryotes. The inheritance of the repressive

chromatin state during cell cycle can be achieved through a replication-coupled nucleosome assembly pathway and it is correlated with multiply self-reinforced mechanisms concerned to histone modification, DNA methylation, and some heterochromatin proteins. This process has been well discussed in several reviews [Richards and Elgin, 2002; Vermaak et al., 2003].

More and more evidences are being accumulated to explain the effects of replication on transcription. A common characteristic of many species during the early development is to experience a period of general non-permissive state of gene expression that is initiated at the end of gametogenesis and lasts until the zygotic gene activation (ZGA). ZGA occurs at the end of the first cell cycle, upon the completion of DNA replication, with which embryonic control of gene expression begins. The detailed studies at this very beginning stage revealed that 1-cell embryos are able to utilize enhancer-responsive promoters efficiently without an enhancer, whereas 2-cell embryos require an enhancer to achieve the same levels of gene expression [Majumder et al., 1993]. Moreover, the long-range activation of enhancer appears to depend on DNA replication. Forlani et al. demonstrated that, in both microinjection and transgene analysis, the gene activation by proximally inserted enhancer is present in early-arrested 1-cell embryos, a time at which the major ZGA has not occurred yet. However, in the same embryos, the long-range activation from the same distantly placed enhancer is not observed [Forlani et al., 1998]. Hence, it was proposed that the purpose of the transcriptional repression in the 1-cell mouse embryo is to delay cellular differentiation until the appropriate stage. At the 2-cell stage, some genes would acquire both the permissive transcription state and the concomitantly distant enhancing effect, and these events would depend on the passage of DNA replication. Recently, Fisher et al. provided another direct evidence for the requirement of DNA replication for the expression of vertebrate HoxB genes. They investigated Hox gene activation in two vertebrate systems-the embryogenesis of Xenopus and the retinoic acid-induced differentiation of pluripotent mouse P19 cells. The results showed that the first cell cycle following the midblastula transition in Xenopus and retinoic acid induction is necessary and sufficient for HoxB activation, whereas the succeeding cell cycles are necessary for maintaining the correct expression state [Fisher and Mechali, 2003]. Therefore, RD gene activation, particularly long-range activation, during development progress, especially at the very beginning stage of the development, may be closely related to cellular differentiation.

Distant regulatory elements seem to be a unique phenomenon in higher eukaryotes. For example, cellular differentiation in yeast is slight and most of its genes are constantly in the active state. Correspondingly, the yeast lacks the distal regulatory sequences within its genome. The regulatory sequences of the yeast are often located about a few hundred base pairs upstream gene promoters, which are called upstream activation sequences (UASs) [Martin, 2001]. But in higher eukaryotic genomes the distal regulatory sequences appear frequently, spreading over the genome even in the area of gene deserts. And they are located so far away from the regulated genes, in some cases working over from several tens to several hundreds even thousands kilobases [Nobrega et al., 2003; Spitz et al., 2003]. Moreover, the distal regulatory action is often associated with tissue- or development-specific genes, suggesting that the evolution of the distant regulatory sequences may be accommodated to the requirement of cellular differentiation. Therefore, the distant regulatory sequences of higher eukaryotes may provide another way to affect the transcription stochasticity and fix the cell fate.

Recent studies suggested that the looping mechanism might be an important mechanism for the long-range activation [Bulger and Groudine, 2002; Palstra et al., 2003; Eivazova and Aune, 2004; Murrell et al., 2004], though it still needs testing more gene loci. However, it brings about another problem-how can a distal regulatory element contact with the gene promoter over a long distance? RD transcriptional activation may provide a solution. It has long been suggested that DNA polymerases are immobilized by attaching to the large architecture, where they reel their templates and extrude newly synthesized DNA strand [Cook, 1999]. This replication model gives an opportunity for physically tethering the distant separated elements together. Transcriptional factors and structural proteins will bind to the new synthesized regulatory element easily, then trap the following synthesized regulatory element and gene promoter, and then they are

self-organized into a huge framework. The different transcription units located over a large chromatin region could assemble transcription complex within this framework and form a transcriptional factory. It has been observed that transcription in nuclei is proceeding in some specific localized sites, and that each site-a transcription factory contains 8–15 different polymerase II transcription units and ~5 different polymerase III transcription units, respectively. Transcription complex is a huge and sophisticated complex containing factors not only involved in transcription but also pre-mRNA processing, DNA repair and replication [Jackson et al., 1998; Pombo et al., 1999; Szentirmay and Sawadogo, 2000]. The assembling of the complex is highly dynamic [Misteli, 2001; Dundr et al., 2002; Kraus and Lis, 2003], and so attaching the transcription units to a large and relatively stable framework will dramatically increase the transcription efficiency. If the clustered transcription units are conducive to transcription regulation, their localized arrangement may be conserved on genome and even the functionally unrelated transcription units could be colocalized in nuclei. By genome-wide gene expression profile studies in human, *Drosophila*, and *C. eleganshave* [Lercher et al., 2002; Roy et al., 2002; Spellman and Rubin, 2002], the discoveries of many clustered gene orders have given much support for the above prediction. The recently observed phenomena that the clustered active genes and even the unrelated active genes dynamically colocalize to the shared sites of ongoing transcription also give some evidences for the above prediction (our unpublished data) [Osborne et al., 2004].

HISTONE H3 VARIANT UNDERLIES GENE EXPRESSION STATES DURING CELLULAR DIFFERENTIATION

Nucleosome, as structural unit of eukaryotic chromatin, contains two molecules of each of proteins–H2A, H2B, H3, and H4 forming an octamer particle around which 146 basepairs of DNA are wrapped. The linker or H1 class histone binds to the octamer near the point where DNA enters and exits the nucleosome and associates with linker DNA between nucleosomes to stabilize higher order chromatin structure. Although histones are among the most highly conserved proteins in terms of both structure and sequence, there still exist histone subtypes with the possible exception of histone H4.

A recent analysis has proved that the histones of higher eukaryote might evolved from a replication-independent (RI)-like H3 variant gene [Rooney et al., 2002]. All *ascomycetes* including yeasts and molds carry only one kind of histone H3 that belongs to RI-like H3.3-like histone [Baxevanis and Landsman, 1998]. This is because cell differentiation of yeast is slight, and so its heterochromatin is scarce. If so, RD-like H3 is indispensable for higher eukaryotic organisms and may contribute to the silencing of majority of genes in terminally differentiated cells, and its copy numbers will be in line with eukaryotic complexity. This also indicates that the occurrence of RI-like histone variants may affect transcription stochasticity and discriminate the different expression states for the cellular differentiation of higher eukaryotes.

The increasing evidences have been obtained to reveal the roles of histone variants in the maintenance of gene expression states. In *Tetrahymena thermophila*, there are two kinds of nucleus: micronucleus and macronucleus. Although these two nuclei have similar genetic components, only genes in the macronucleus are expressed in vegetative cells. Macronucleus contains two core histone variants called hv1 and hv2, which are present at about 15–20% of the amount of major core histone and are absent in micronucleus of vegetative cells [Allis et al., 1980]. The hv1 is a H2A variant that is consistent with the conserved H2A.Z variants found in multicellular eukaryotes. The hv2 is a H3.3-like replacement variant. It is synthesized and deposited in the macronuclei of nongrowing as well as growing cells in a RI manner [Bannon et al., 1983]. Waterborg found that histone acetylation and H3-K4 methylation, the marks of active chromatin state, are enriched in the alfalfa variant H3.2, where H3-K9 methylation, the mark of repressive chromatin state, is found primarily in the major form of H3-H3.1 [Waterborg, 1990]. These evidences hint that histone variants may play a certain important role in establishing and maintaining the different expression states during differentiation.

By using GFP-tagged versions of H3.3 and H3, it was proved that in *Drosophila* cells H3.3 is deposited at transcriptionally active euchromatic regions by RI pattern, while the major H3 is strictly incorporated into DNA during replication [Ahmad and Henikoff, 2002]. An

investigation of the distribution of H3.3 at *Drosophila* polytene chromosome revealed that H3.3 is enriched in all active chromatin and throughout larger transcription units [Schwartz and Ahmad, 2005]. Quantifying the relative abundance of histone modifications in H3 and H3.3 in *Drosophila* showed that transcriptionally active marks, such as di and trimethylation of H3-K4 and acetylation of H3-K9, 14, 18, and 23, are enriched in H3.3 variant. In contrast, H3-K9 dimethylation, the mark of the repressed genes, is enriched in H3 [McKittrick et al., 2004; Schubeler et al., 2004]. Based on these results, a nucleosome dynamic model has been proposed to explain the effects of chromatin dynamic structure on transcriptional regulation [Workman and Abmayr, 2004]. This model emphasizes that the difference of histone categories plays an important role in gene regulation.

It has long been noticed that transcription and DNA replication might be mechanistically linked in eukaryotic cells. Housekeeping genes undergo replication in the first half of S phase in all cell types, whereas the replication of many tissue specific genes, which are developmentally controlled, is late in most tissues but early in the expressed tissue during S phase. Maybe the cell cycle-related histone incorporation provides a possible pathway to determine and maintain the cell fate. In single-cell microinjection experiments, reporter genes that were injected into early-S-phase nuclei, carrying either housekeeping promoter or tissue-specific promoter, are almost ten-fold transcriptionally active than the same genes injected into late-S-phase nuclei. Strikingly, once established, these transcriptional states remain stable during the successive cell cycles. Chromatin immunoprecipitation (ChIP) analysis showed that early-injected DNA is packaged into chromatin that contains hyperacetylated histones, whereas late-injected templates are hypoacetylated [Zhang et al., 2002]. As is known, H3.3 is hyperacetylated [McKittrick et al., 2004] and the expression of H3 will peak after middle S-phase [van der Meijden et al., 2002]. The study also demonstrated that these two distinct deposition pathways are mediated by the distinct histone chaperones-CAF-1 and HIRA, which are contained in H3.1 and H3.3 complexes, respectively [Tagami et al., 2004]. So it is likely that the real reason for the inheritance of different expression states is that template chromatin contains H3.3 in early-S phase, but H3 in late-S phase. These distinct histones mediated by different disposition pathways can be transmitted through cell division and thus H3.3 variant may function as an active mark for the reactivation of active genes in the next G1 phase. The latest discoveries about the dependence of the deposition and removal of H3.3 on transcription activation and its deposition on transcribed genes suggest it may underlie the active state of gene expression [Chow et al., 2005; Schwartz and Ahmad, 2005].

M-PHASE: REMEMBERING THE CELL FATE

The inheritance of stochastic expression pattern of α-like and β-like globin genes through numerous cell divisions [de Krom et al., 2002], the maintenance of active state of HoxB genes after the S-phase-dependent activation during the proceeding cell cycles [Fisher and Mechali, 2003], and the fact that reporter genes injected in early or late-S-phase can remember their active or repressive states in the following cell cycles [Zhang et al., 2002] hint that cells can remain certain information of their expression states in inactively mitotic chromosomes and transmit them into next generation, rather than rebuilding gene expression patterns start from scratch in every G1 phase. During development, both stem cells and differentiated cells will undergo many rounds of cell cycles. In each cell cycle, leaving their epigenetic information in mitotic chromosomes may be a key process to remember the cell fate.

The inheritance of the repressive chromatin state during cell cycle can be achieved through a replication-coupled nucleosome assembly pathway. The duplicated epigenetic marks of repressive heterochromatin state such as DNA methylation and H3-K9 methylatin can be equally distributed into progeny cells through cell division. However, the mechanisms of remembering the active gene expression state during cell division have less been noticed.

Building the transcriptionally active state is a step-wise process during development. By studying two model systems-chicken lysozyme and β-globin gene clusters, it was found that they have acquired potentially active chromatin configuration prior to final activation [Forrester et al., 1989; Jimenez et al., 1992; Kontaraki et al., 2000]. The erythroid-specific hypersensitive sites (HSs) located in locus control region

(LCR) of β-globin gene cluster have appeared in three independent multilineage progenitor cells. In erythroid progenitor cell, a pre-ACH (active chromatin hub) substructure has already come into being among 5′HS-60/-62, 3′HS1 and HSs at 5′ portion of the LCR [Palstra et al., 2003]. Some active epigenetic marks such as histone hyperacetylation and H3-K4, K79 methylations have appeared at both regulatory elements and promoters of transcriptionally competent globin genes (our unpublished data). Coupling with the recruitment of diverse transcription factors and the formation of active histone code at transcripitonally active regions, the genes will be fully activated. But how does the epigenetic information, marking active transcription, mediate the maintenance of active expression state during cell division? The recent advances give much of understanding for it.

It has been noticed that some HSs, once formed, can be propagated through cell division, suggesting an inheritable mechanism [Weintraub, 1985]. The direct evidences come from the finding that both DNase I hypersensitivity sites and $KmnO_4$ HSs of certain active genes in interphase cells can be detected on mitotic chromosomes [Michelotti et al., 1997], suggesting that there exist some molecular memory marks on mitotic chromatin to maintain the previously active expression state. The latest advances validate this speculation. The comparative chromatin immnoprecipitation (ChIP) analysis revealed that the localized active histone modifications including acetylation at H3 and H4, di- and tri-methylation at H3-K4 and di-methylation at H3-K79 at distant regulatory elements and potentially or completely active genes persist when transcription is inactivated during mitosis (our unpublished data) [Kouskouti and Talianidis, 2005]. These results testify one kind of cellular memory mechanism during cell division through which the previously active expression state can be marked on mitotic chromatin in spite of transcription halt during mitosis. The reserved active histone modifications also provide a surface for the binding of histone modification enzymes and chromatin remodeling complex at mitotic exit [Chow et al., 2005], which is in favor of the rapid resumption of a large number of genes at onset of next cell cycle.

Using ChIP assay, Christova, and Oelgeschlager examined the association of TFIID, TFIIB, NC2, and RNA polymerase II with diverse gene promoters in asynchronous cells and synchronous mitotic cells. They found that TFIID and TFIIB remain associated with active gene promoters during mitosis to serve as a molecular bookmarking, whereas RNA polymerase II and NC2 are displaced [Christova and Oelgeschlager, 2002]. In addition, one erythroid-specific factor-NF-E2 is also detected on mitotic chromatin (our unpublished data). Why do the condensed chromosomes contain the above transcription factors besides active epigenetic marks in spite that almost all other transcription factors are displaced from M-phase chromosomes? In order to maintain the stability of differentiated cell types, the mitotic cells not only leave the imprint of previously active state but also facilitate the transcription reactivation after mitosis. The reservation of TFIID, as a transcription factor firstly interacting with gene promoter and then recruiting other basal transcription factors in transcription, is an economical means to resume the transcription. The active histone modifications including H3 and H4 acetylation, H3-K4 di- or tri-methylation will restore their levels at mitotic exit [Chow et al., 2005]. For globin genes, the retained NF-E2 may direct the restoration of histone acetylation at potentially active globin gene loci since it can recruit coactivator CBP with histone acetyltransferase activity [Francastel et al., 2001]. So it is worth further probing whether there are a series of specific transcription factors on mitotic chromosomes with the common functional features to recruit other coactivators or corepressors at mitotic exit since almost all basal transcription factors and coactivators such as histone modification enzymes and chromatin remodeling complex are abrogated from mitotic chromosomes [Gottesfeld and Forbes, 1997; Kruhlak et al., 2001; Chow et al., 2005].

According to the above results, we postulate that highly condensed mitotic chromosomes may reserve some active chromatin framework information through higher order organization. Since so many epigenetic information such as active histone modifications, histone variant H3.3, certain *trans*-acting factors (not only general transcription factors but also tissue- or development-specific transcription factors), and maybe certain skeleton factors that are contained in both transcription factory and chromosome matrix, are remained in mitotic

chromosomes, these marks may constitute a unique 3-dimensional chromatin structure containing previously active chromatin conformation.

Although this hypothesis is presumable, recently more and more informations are being accumulated to reveal a general mechanism of mitotic inheritance. The binding of TATA binding protein (TBP) to the promoter is a rate-limiting step in transcription regulation for all three eukaryotic RNA polymerases since it is chosen as a protein firstly binding to gene promoter in transcription. It has been observed that GFP-tagged TBP could be constantly associated with mitotic chromosomes [Chen et al., 2002]. Runx (Cbfa/AML) proteins are tissue-specific transcription factors that control hematopoietic and osteogenic lineage commitment [Lund and van Lohuizen, 2002]. Runx factors can bind to the specific sites on the genome, and are target to transcriptionally active subnculear foci. They may be necessary to maintain chromatin architecture of target genes in the interphase nuclei. Its level persists through the proliferation of the lineage-committed cells. Such developmental specific transcription factors undergo progressive changes in cellular localization during mitosis while retaining a punctate distribution, and they also experience a spatiotemporal redistribution that results in equal partitioning of the proteins into each of the progeny nuclei [Zaidi et al., 2003]. By the dynamic analysis of mitotic chromatin, it was found that mitotic chromosomes in general and ribosomal genes in particular, although highly condensed, are accessible to transcription factors and chromatin proteins and the exchange of these proteins are continuously proceeding [Chen et al., 2005]. Therefore, we can speculate that at least the partial skeleton structure of the transcription factory may be contained in the mitotic chromosome as the part of chromosome matrix since mitotic chromosomes reamin so many information of active transcription. This arrangement will lead to a similar global position pattern of chromosomes in nucleus, which can be inherited from one cell generation to the next. And this phenomenon has been observed [Gerlich et al., 2003]. So it is interesting to test whether the spatial vicinity relationship of active chromatin structure could be conserved in mitotic chromosomes.

ACKNOWLEDGMENTS

We thank Dr. Xue-Song Wu for the critical review and suggestions.

REFERENCES

Ahmad K, Henikoff S. 2002. The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. Mol Cell 9:1191–1200.

Allis CD, Glover CV, Bowen JK, Gorovsky MA. 1980. Histone variants specific to the transcriptionally active, amitotically dividing macronucleus of the unicellular eucaryote, Tetrahymena thermophila. Cell 20:609–617.

Bannon GA, Calzone FJ, Bowen JK, Allis CD, Gorovsky MA. 1983. Multiple, independently regulated, polyadenylated messages for histone H3 and H4 in tetrahymena. Nucleic Acids Res 11:3903–3917.

Baxevanis AD, Landsman D. 1998. Histone sequence database: New histone fold family members. Nucleic Acids Res 26:372–375.

Blake WJ, M KA, Cantor CR, Collins JJ. 2003. Noise in eukaryotic gene expression. Nature 422:633–637.

Bulger M, Groudine M. 2002. Trapping enhancer function. Natl Genet 32:555–556.

Chen D, Hinkley CS, Henry RW, Huang S. 2002. TBP dynamics in living human cells: Constitutive association of TBP with mitotic chromosomes. Mol Biol Cell 13:276–284.

Chen D, Dundr M, Wang C, Leung A, Lamond A, Misteli T, Huang S. 2005. Condensed mitotic chromatin is accessible to transcription factors and chromatin structural proteins. J Cell Biol 168:41–54.

Chow CM, Georgiou A, Szutorisz H, Maia ESA, Pombo A, Barahona I, Dargelos E, Canzonetta C, Dillon N. 2005. Variant histone H3.3 marks promoters of transcriptionally active genes during mammalian cell division. EMBO Rep 6:354–360.

Christova R, Oelgeschlager T. 2002. Association of human TFIID-promoter complexes with silenced mitotic chromatin in vivo. Natl Cell Biol 4:79–82.

Cook PR. 1999. The organization of replication and transcription. Science 284:1790–1795.

de Krom M, van de Corput M, von Lindern M, Grosveld F, Strouboulis J. 2002. Stochastic patterns in globin gene expression are established prior to transcriptional activation and are clonally inherited. Mol Cell 9:1319–1326.

Dundr M, Hoffmann-Rohrer U, Hu Q, Grummt I, Rothblum LI, Phair RD, Misteli T. 2002. A kinetic framework for a mammalian RNA polymerase in vivo. Science 298:1623–1626.

Eivazova ER, Aune TM. 2004. Dynamic alterations in the conformation of the Ifng gene region during T helper cell differentiation. Proc Natl Acad Sci USA 101:251–256.

Elowitz MB, Levine AJ, Siggia ED, Swain PS. 2002. Stochastic gene expression in a single cell. Science 297: 1183–1186.

Fisher D, Mechali M. 2003. Vertebrate HoxB gene expression requires DNA replication. EMBO J 22:3737–3748.

Forlani S, Bonnerot C, Capgras S, Nicolas JF. 1998. Relief of a repressed gene expression state in the mouse 1-cell embryo requires DNA replication. Development 125: 3153–3166.

Forrester WC, Novak U, Gelinas R, Groudine M. 1989. Molecular analysis of the human beta-globin locus activation region. Proc Natl Acad Sci USA 86:5439–5443.
Francastel C, Magis W, Groudine M. 2001. Nuclear relocation of a transactivator subunit precedes target gene activation. Proc Natl Acad Sci USA 98:12120–12125.
Gerlich D, Beaudouin J, Kalbfuss B, Daigle N, Eils R, Ellenberg J. 2003. Global chromosome positions are transmitted through mitosis in mammalian cells. Cell 112:751–764.
Gottesfeld JM, Forbes DJ. 1997. Mitotic repression of the transcriptional machinery. Trends Biochem Sci 22:197–202.
Jackson DA, Iborra FJ, Manders EM, Cook PR. 1998. Numbers and organization of RNA polymerases, nascent transcripts, and transcription units in HeLa nuclei. Mol Biol Cell 9:1523–1536.
Jimenez G, Griffiths SD, Ford AM, Greaves MF, Enver T. 1992. Activation of the beta-globin locus control region precedes commitment to the erythroid lineage. Proc Natl Acad Sci USA 89:10618–10622.
Kontaraki J, Chen HH, Riggs A, Bonifer C. 2000. Chromatin fine structure profiles for a developmentally regulated gene: Reorganization of the lysozyme locus before trans-activator binding and gene expression. Genes Dev 14:2106–2122.
Kouskouti A, Talianidis I. 2005. Histone modifications defining active genes persist after transcriptional and mitotic inactivation. EMBO J 24:347–357.
Kraus WL, Lis JT. 2003. PARP goes transcription. Cell 113: 677–683.
Kruhlak MJ, Hendzel MJ, Fischle W, Bertos NR, Hameed S, Yang XJ, Verdin E, Bazett-Jones DP. 2001. Regulation of global acetylation in mitosis through loss of histone acetyltransferases and deacetylases from chromatin. J Biol Chem 276:38307–38319.
Lercher MJ, Urrutia AO, Hurst LD. 2002. Clustering of housekeeping genes provides a unified model of gene order in the human genome. Natl Genet 31:180–183.
Lund AH, van Lohuizen M. 2002. RUNX: A trilogy of cancer genes. Cancer Cell 1:213–215.
Majumder S, Miranda M, DePamphilis ML. 1993. Analysis of gene expression in mouse preimplantation embryos demonstrates that the primary role of enhancers is to relieve repression of promoters. EMBO J 12:1131–1140.
Martin DI. 2001. Transcriptional enhancers–on/off gene regulation as an adaptation to silencing in higher eukaryotic nuclei. Trends Genet 17:444–448.
McAdams HH, Arkin A. 1997. Stochastic mechanisms in gene expression. Proc Natl Acad Sci USA 94:814–819.
McKittrick E, Gafken PR, Ahmad K, Henikoff S. 2004. Histone H3.3 is enriched in covalent modifications associated with active chromatin. Proc Natl Acad Sci USA 101:1525–1530.
Michelotti EF, Sanford S, Levens D. 1997. Marking of active genes on mitotic chromosomes. Nature 388:895–899.
Misteli T. 2001. Protein dynamics: Implications for nuclear architecture and gene expression. Science 291:843–847.
Muller-Sieburg CE, Cho RH, Thoman M, Adkins B, Sieburg HB. 2002. Deterministic regulation of hematopoietic stem cell self-renewal and differentiation. Blood 100: 1302–1309.
Murrell A, Heeson S, Reik W. 2004. Interaction between differentially methylated regions partitions the imprinted genes Igf2 and H19 into parent-specific chromatin loops. Natl Genet 36:889–893.
Nobrega MA, Ovcharenko I, Afzal V, Rubin EM. 2003. Scanning human gene deserts for long-range enhancers. Science 302:413.
Osborne CS, Chakalova L, Brown KE, Carter D, Horton A, Debrand E, Goyenechea B, Mitchell JA, Lopes S, Reik W, Fraser P. 2004. Active genes dynamically colocalize to shared sites of ongoing transcription. Natl Genet 36: 1065–1071.
Ozbudak EM, Thattai M, Kurtser I, Grossman AD, van Oudenaarden A. 2002. Regulation of noise in the expression of a single gene. Natl Genet 31:69–73.
Palstra RJ, Tolhuis B, Splinter E, Nijmeijer R, Grosveld F, de Laat W. 2003. The beta-globin nuclear compartment in development and erythroid differentiation. Natl Genet 35:190–194.
Paszek P, Lipniacki T, Brasier AR, Tian B, Nowak DE, Kimmel M. 2005. Stochastic effects of multiple regulators on expression profiles in eukaryotes. J Theor Biol 233: 423–433.
Pombo A, Jackson DA, Hollinshead M, Wang Z, Roeder RG, Cook PR. 1999. Regional specialization in human nuclei: Visualization of discrete sites of transcription by RNA polymerase III. EMBO J 18:2241–2253.
Raghuraman MK, Winzeler EA, Collingwood D, Hunt S, Wodicka L, Conway A, Lockhart DJ, Davis RW, Brewer BJ, Fangman WL. 2001. Replication dynamics of the yeast genome. Science 294:115–121.
Reik W, Dean W, Walter J. 2001. Epigenetic reprogramming in mammalian development. Science 293:1089–1093.
Richards EJ, Elgin SC. 2002. Epigenetic codes for heterochromatin formation and silencing: Rounding up the usual suspects. Cell 108:489–500.
Rooney AP, Piontkivska H, Nei M. 2002. Molecular evolution of the nontandemly repeated genes of the histone 3 multigene family. Mol Biol Evol 19:68–75.
Roy PJ, Stuart JM, Lund J, Kim SK. 2002. Chromosomal clustering of muscle-expressed genes in Caenorhabditis elegans. Nature 418:975–979.
Schubeler D, Scalzo D, Kooperberg C, van Steensel B, Delrow J, Groudine M. 2002. Genome-wide DNA replication profile for Drosophila melanogaster: A link between transcription and replication timing. Natl Genet 32:438–442.
Schubeler D, MacAlpine DM, Scalzo D, Wirbelauer C, Kooperberg C, van Leeuwen F, Gottschling DE, O'Neill LP, Turner BM, Delrow J, Bell SP, Groudine M. 2004. The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. Genes Dev 18:1263–1271.
Schwartz BE, Ahmad K. 2005. Transcriptional activation triggers deposition and removal of the histone variant H3.3. Genes Dev 19:804–814.
Spellman PT, Rubin GM. 2002. Evidence for large domains of similarly expressed genes in the Drosophila genome. J Biol 1:5.
Spitz F, Gonzalez F, Duboule D. 2003. A global control region defines a chromosomal regulatory landscape containing the HoxD cluster. Cell 113:405–417.
Spudich JL, Koshland DE, Jr. 1976. Non-genetic individuality: Chance in the single cell. Nature 262:467–471.

Szentirmay MN, Sawadogo M. 2000. Spatial organization of RNA polymerase II transcription in the nucleus. Nucleic Acids Res 28:2019–2025.

Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y. 2004. Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. Cell 116:51–61.

Trimborn T, Gribnau J, Grosveld F, Fraser P. 1999. Mechanisms of developmental control of transcription in the murine alpha- and beta-globin loci. Genes Dev 13: 112–124.

van der Meijden CM, Lapointe DS, Luong MX, Peric-Hupkes D, Cho B, Stein JL, van Wijnen AJ, Stein GS. 2002. Gene profiling of cell cycle progression through S-phase reveals sequential expression of genes required for DNA replication and nucleosome assembly. Cancer Res 62:3233–3243.

Vermaak D, Ahmad K, Henikoff S. 2003. Maintenance of chromatin states: An open-and-shut case. Curr Opin Cell Biol 15:266–274.

Waterborg JH. 1990. Sequence analysis of acetylation and methylation in two histone H3 variants of alfalfa. J Biol Chem 265:17157–17161.

Weintraub H. 1985. Assembly and propagation of repressed and depressed chromosomal states. Cell 42:705–711.

Wijgerde M, Grosveld F, Fraser P. 1995. Transcription complex stability and chromatin dynamics in vivo. Nature 377:209–213.

Workman JL, Abmayr SM. 2004. Histone H3 variants and modifications on transcribed genes. Proc Natl Acad Sci USA 101:1429–1430.

Zaidi SK, Young DW, Pockwinse SM, Javed A, Lian JB, Stein JL, van Wijnen AJ, Stein GS. 2003. Mitotic partitioning and selective reorganization of tissue-specific transcription factors in progeny cells. Proc Natl Acad Sci USA 100:14852–14857.

Zhang J, Xu F, Hashimshony T, Keshet I, Cedar H. 2002. Establishment of transcriptional competence in early and late S phase. Nature 420:198–202.