Trends in Genetics

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Review

Dealing with Gene-Dosage Imbalance during S Phase

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DNA replication perturbs the dosage balance between genes that replicate early during S phase and those that replicate late. If propagated to influence protein content, this dosage imbalance could influence cellular functions. In bacteria, mechanisms have evolved to use this imbalance to tune certain processes with the rate of cell growth. By contrast, eukaryotes buffer this dosage imbalance to ensure gene expression homeostasis also during S phase. Here, we outline classical and more recent studies describing how different organisms deal with this replication-dependent dosage imbalance, and describe recent results linking the eukaryotic buffering mechanism to replication-dependent histone acetylation. Finally, we discuss the possible implications of this buffering mechanism and speculate why it is specific to eukaryote cells.

Is Gene Expression Affected by Changes in Gene Dosage during Replication?

Growing cells double their content at each and every cell cycle to ensure the stable availability of organelles, proteins, genes, and metabolites. While distinct processes are involved in the production of different cellular entities, cellular functions depend on their relative abundances. For example, the rate of metabolic reactions depends on the relative concentrations of enzymes, as well as on the relative abundances of the associated substrates. How do cells coordinate their production processes to maintain homeostasis?

DNA replication presents a particular challenge in this respect due to its discrete nature. During replication, gene dosage does not increase continuously but doubles at a specific time. Furthermore, replication is not synchronized between genes, since different regions in the genome are replicated at different times during S phase. Therefore, gene dosage becomes transiently imbalanced during DNA replication. Does this imbalance propagate to perturb protein abundances and perhaps regulate cellular functions?

Modifying gene dosage through genome engineering typically leads to changes in protein abundance. For example, a systematic study of 730 different GFP-fusion proteins in budding yeast reported that protein abundance scaled with gene copy number in 80% of the cases [1]. The addition of a chromosome (aneuploidy) similarly doubles the amount of transcripts [2]. However, there are also clear examples of dosage compensation, the most studied probably being X-chromosome silencing in mammalian females [3], or the doubling of expression from the single X chromosome available to *Drosophila* males [4]. Recent work in *Drosophila* and humans further showed that, while most autosomal genes change in expression in proportion to changes in gene dosage, some genes are able to compensate for the changes in dosage [5,6]. What is the cellular consequence of gene dosage changes, occurring in every cell cycle, during DNA replication? Does it lead to increased expression, or is it subject to dosage compensation?

Trends

Gene dosage becomes transiently imbalanced during DNA replication.

In bacteria, expression increases immediately following replication, introducing growth rate-dependent biases that are capitalized for regulation.

In eukaryotes, the effect of changes in gene dosage during DNA replication on expression is buffered.

In budding yeast, buffering gene expression during DNA replication is chromatin based and is dependent on acetylation of H3K56 by Rtt109/Asf1.

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Bacteria: Imbalanced Expression as Readout of Cell Growth Rate

In bacteria, the increase in gene dosage during DNA replication is translated into higher protein expression. This was demonstrated in multiple studies that examined how the positioning of genes along the single bacterial chromosome influences their activity. Such position effects were first found when comparing isolated *Escherichia coli* strains in which the lac operon was transposed to different chromosomal locations [7], such that the lac operon expression level was higher the closer it was to the origin. Similarly, the enzymatic activity encoded by the *his* genes in *Salmonella typhimurium* also correlates with proximity to the replication origin [8]. A similar dependency was observed in other bacteria using different reporters, suggesting the generality of this phenomenon [9,10]. Still, gene-specific effects not detected in previous studies might exist [11]. Furthermore, reducing replication speed suppressed gene expression in proportion to the change in gene dosage, without changing growth rate or cell volume [12]. Interestingly, recent studies have shown that bacteria rely on this position-dependent modulation of gene expression to detect DNA replication stresses. For example, in *Streptococcus pneumonia*, a range of DNA-damaging antibiotics induces the competent pathway for scavenging foreign DNA, and this induction depends on the chromosomal positioning of competent genes relative to the origin of replication [13].

Since rapidly growing cells replicate their DNA more often than do slowly dividing ones, the dosage imbalance between early and late replicating genes increases in proportion to growth rate. This imbalance is amplified by the fact that rapidly growing bacteria initiate multiple rounds of DNA replication within each division cycle, all of which start from the same origin of replication [14]. In fact, measuring dosage imbalance between genes has recently been used to predict the growth rate of bacteria [15]. Can bacteria use this dosage imbalance as a generic readout of cell growth rate, regulating some process in a growth rate-dependent manner? Recent results suggest that this is indeed the case. In multiple bacteria, and in particular those that achieve rapid maximal growth, genes involved in transcription and translation are positioned close to the replication origin [16,17]. Perhaps more strikingly, bacteria, such as *Bacillus subtilis*, use this growth rate-generated dosage imbalance to activate the sporulation program in response to nutrient starvation [18]. By positioning the sporulation repressor close to the replication origin, and the sporulation activator away from it, any round of DNA replication represses sporulation. Yet, due to the connectivity of the sporulation circuitry, this transient repression is followed by a prolonged activation spike, which ends once cells re-enter replication. Therefore, sporulation is enabled only in slow-growing cells, where this next replication cycle is delayed.

Eukaryotes: Buffering Gene Dosage during DNA Replication

Bacteria translate gene-dosage imbalance during DNA replication into gene expression imbalance, and capitalize on this effect to regulate growth-dependent processes. By contrast, experiments in eukaryotic cells indicated a limited change in gene expression during S phase. This was first established in batch studies that quantified the overall rate of macromolecule synthesis (DNA, RNA, and protein) during the progression of the cell cycle. In fission yeast, the increase in mRNA synthesis occurs with a delay following completion of S phase [19–21], while, in budding yeast, mRNA synthesis increases continuously (in proportion to cell volume) as the cell cycle progresses [22]. Similar studies were also conducted in mammalian cell lines, which showed similar behaviors: some cell lines (e.g., HeLa, L5178Y mouse lymphoma) exhibited a step-wise increase similar to that seen in *Schizosaccharomyces pombe* [23,24], while others (e.g., mammary epithelia tumor cell lines or mouse fibroblasts) showed an exponential increase similar to that seen in *Saccharomyces cerevisiae* [25,26]. Notably, while these studies show that DNA is not limiting for transcription in all these cell types, the distribution of the transcription machinery could still be modulated during DNA replication, leading to preferential production of early replicating genes.

More recently, the question of whether gene expression increases immediately upon gene replication was revisited using modern tools that allow following the synthesis rates of specific genes during different phases of the cell cycle at single-cell resolution. For example, live cell tracking of MS2-labeled mRNA for reporters driven by the *CMVpr*– and *CCND1pr* promoters [27,28] was used to measure the intensity and number of individual transcription sites. These studies showed that transcription sites were clearly duplicated as cells entered S phase, yet transcription efficiency from either site was reduced upon doublet formation, maintaining approximately the same overall mRNA production rate as before S phase entry.

In a related study, single-molecule FISH (smFISH) was used to quantify the transcription rates of dozens of endogenous genes at single cell resolution [29]. Also here, gene transcription rates remained largely the same during S phase, although both replicated copies emerging during S phase were actively transcribed. Therefore, transcription was attenuated from both copies of the DNA, again to maintain stable transcription rates, compensating for the increased gene copy. This study further revealed that dosage compensation is achieved by reducing the frequency of transcription bursts (burst frequency), while the number of transcripts made at each burst (burst size) remained the same. Interestingly, this is in contrast with the mechanism used for adjusting gene expression with cell size, which is achieved by modulating burst size rather than burst frequency. A similar conclusion was reached in a study that applied smFISH to quantify transcription of *Oct4* and *Nanog* in mouse embryonic stem cells [30]. Compensation here was partial, with synthesis rates increasing by the 1.28- and 1.5-fold, respectively, upon duplication.

Finally, these gene-specific analyses were extended to examine possible biases in the expression of early- versus late-replicating genes on a genome-wide scale, using budding yeast [31]. Consistent with previous results, this recent analysis indicated clear dosage compensation, because the increase in gene dosage of early replicating genes was largely buffered when examining the respective mRNA levels or mRNA synthesis rates.

Chromatin-Based Mechanism for Buffering Gene Expression during S Phase

Thus, bacteria and eukaryotes have evolved distinct means for dealing with the gene-dosage imbalance generated during S phase (Figure 1A): while bacteria capitalize on this imbalance to regulate growth rate-related processes, eukaryotic cells are capable of buffering this imbalance to maintain expression homeostasis. How is buffering achieved at the molecular level? Is it a consequence of passive dilution of factors or an active mechanism?

To address this question, a published database of 165 chromatin-associated mutants of budding yeast was used to find mutants affecting specifically the expression of early replicating genes [32]. The acetyltransferase Rtt109, an enzyme structurally and functionally related to the mammalian p300/CBP [33,34], and its associated conserved histone chaperone Asf1, emerged as two candidates important for maintaining expression homeostasis during S phase [31]. Notably, in these mutants, changes in gene expression were correlated with gene replication timing. While the initial signal was low in these mutant lines, because the cells analyzed were not synchronized, the loss of S phase dosage compensation was verified in subsequent experiments focusing on S phase cells. In fact, in cells deleted of Rtt109, mRNA synthesis rates during S phase matched precisely the increase in DNA content during S phase. Rtt109, together with its co-chaperone Asf1, acetylates H3 on its internal K56 residue [35]. Furthermore, using mutants in which histones are mutated in lysine 56, mimicking either the acetylated or nonacetylated form, it was shown that the dynamic modification of this site during S phase is required for maintaining expression homeostasis, implicating this residue in the buffering mechanism.

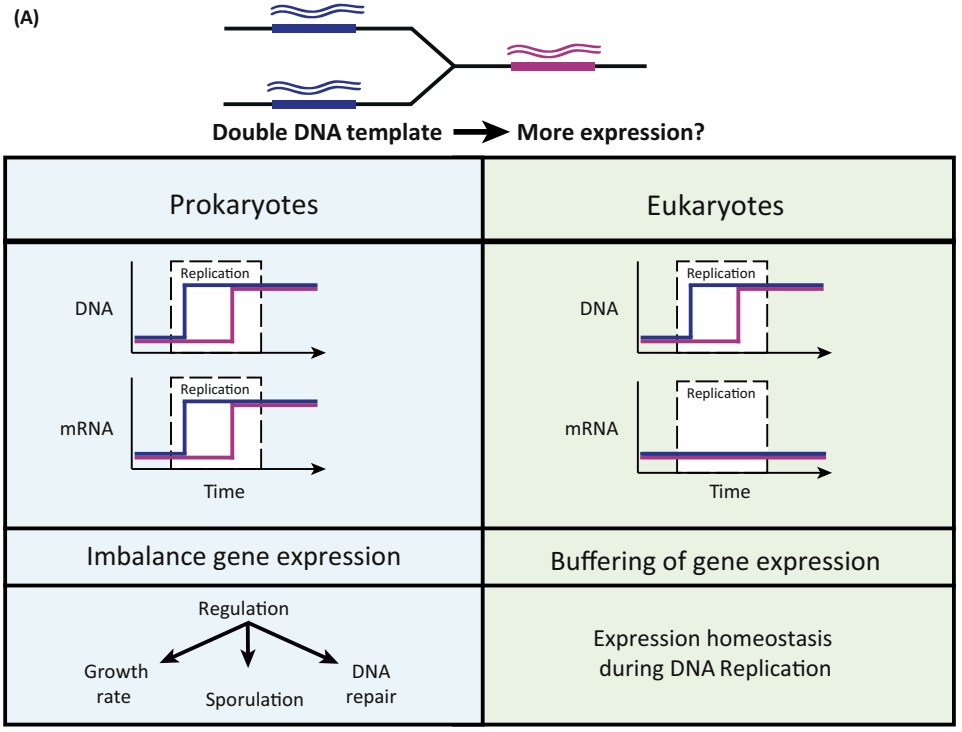

Figure 1. What Happens to Gene Expression during DNA Replication? (A) Regulation strategies for gene expression during S phase: during DNA replication, the gene dosage of replicated genes increases, hence making a double DNA template available for transcription. Given that genes replicate at different times, this may cause an imbalance in gene expression if it is not buffered. In prokaryotes, the expression of genes increases immediately following their replication. This causes an imbalance in gene expression that can be capitalized for regulating different cellular processes, such as growth rate and sporulation (see main text). By contrast, eukaryotes, from yeast to humans, buffer the changes in DNA dosage, ensuring expression homeostasis during DNA replication. (B) Acetylation of H3K56 by Rtt109 governs expression homeostasis in budding yeast: the histone acetyltransferase Rtt109, together with its co-chaperone Asf1, acetlyates newly synthesized histones on H3K56. These acetylated histones are incorporated into replicated regions, where H3K56ac serves as a mark for inhibiting expression. This mark is removed by the histone deacetylases Hst3/4 following completion of replication, alleviating this S phase-specific inhibition.

Outstanding Questions

How does H3K56ac reduce transcription efficiency during DNA replication?

What signaling pathways are upstream of the Rtt109-buffering mechanism?

Is there an extra layer of buffering in the level of mRNA translation?

Is expression homeostasis a general attribute of all cell types, or is it abrogated in specific cell types?

How conserved is the molecular basis of expression homeostasis?

What are the phenotypic effects of loss of buffering during DNA replication?

Why did expression homeostasis evolve only in eukaryotes?

Concluding Remarks and Future Directions

The ability to buffer changes in gene dosage during S phase requires that cells distinguish the replicated regions from the nonreplicated regions of the genome. H3K56ac, the chromatin modification identified as critical for this buffering, is indeed such a mark. Rtt109 acetylates newly synthesized histones, before their incorporation into DNA [36]. During DNA replication, these acetylated histones are incorporated for wrapping the newly synthesized DNA [37]. Indeed, the H3K56ac pattern mark during S phase closely matches the progression of replication, as was shown by the greater levels of H3K56ac that occur on newly synthesized DNA. Furthermore, the

dedicated H3K56ac deacetylases Hst3/4 are induced at the end of S phase to remove this acetylation when replication is completed [38].

Therefore, H3K56ac is ideally suited for marking replicating DNA in yeast. How is this mark interpreted to reduce transcription efficiency? One possibility is that H3K56ac itself impacts the stability of the interaction between RNA polymerase II and the DNA, thereby delaying the rate of transcription initiation or promoting pre-mature dissociation of the polymerase from the DNA, before the completion of synthesis of a full transcript. In addition, because H3K56ac promotes assembly of nucleosomes after replication, it may confer inhibition by creating an inaccessible chromatin state [37]. Alternatively, H3K56ac could signal to promote the recruitment (or function) of some transcription inhibitors. We note that, since H3K56ac is positioned in an internal site [35] and not on the histone tail, its contribution to factor recruitment is likely to be indirect by modulating the binding affinity and competition with the histones, as opposed to direct chemical recruitment [39]. An additional indirect effect may be through modulation of divergent transcription governed by H3K56ac [40] through competition on the transcription machinery, or through inhibition of genes by generating upstream antisense RNA. Such an indirect effect on binding affinity would also explain how such a mechanism could tune transcription by only approximately twofold, rather than a more severe shutdown, as would have been expected for enzymatic recruitment.

Furthermore, H3K56-acetylated histones are incorporated along the entire gene body, without preference for specific regions [31]. Given that the two copies of the genes are packaged by a mixture of both new and old histones, this raises the question of how uniform inhibition is achieved. This might be explained by the role of H3K56ac in changing higher-order chromatin structure, affecting accessibility to nucleosome-free regions [41].

Further studies are required for establishing the possible phenotypic effects of the observed buffering (see Outstanding Questions). The duration of S phase is relatively short and, even in rapidly dividing eukaryotic cells, lasts for only approximately 25% of the cell cycle. Furthermore, cellular mechanisms are mostly robust to changes in expression levels of individual genes. Still, there are some examples of diseases that are linked to copy number variation in specific genes [42,43]. For some of these genes, such as those encoding RAG proteins, it could be that even this short interval in the cell cycle can be detrimental. In addition, in stem cells, a twofold increase in the levels of Oct4 leads to different cell fates [44], suggesting the importance of expression homeostasis in these cells. It may also be that the buffering mechanism evolved primarily to protect cells that are arrested within S phase due to unforseen obstacles, such as nucleotide depletion or replication damage. In these cells, differences in expression between early- and late-replicating genes will accumulate, and could lead to deleterious effects, as observed, for example, for aneuploid cells carrying an extra copy of one chromosome [2]. Indeed, the overall expression imbalance under such situations could encompass approximately 20–30% of the genes, significantly more than the increase in the imbalance obtained when adding just one chromosome.

As we have discussed, all eukaryotic cells examined, from yeast to human, appear to buffer expression for gene dosage during S phase. By contrast, none of the bacteria species examined have shown such buffering. This adds an additional notable difference to the regulatory logics that function in bacteria and eukartyotes. One possible cause for this difference is that bacteria lack the chromatin structure that is used for marking replicated DNA for transcription repression. Alternatively, there is no clear separation of replication from the other stages in the cell cycle and, therefore, bacteria may use gene-dosage differences as means for regulating genes in a replication-dependent manner [45], which eukaryotes achieve by other means. Furthermore, DNA replication in bacteria is highly orchestrated, starting from a single origin and progressing

continuously. By contrast, in eukaryotic cells, DNA replication is more stochastic: it starts from multiple origins that are activated at different times during S phase and shows higher variability between cells [46]. This variability may be limiting for using the gene-dosage differences as means for regulation, but rather promotes the need for its buffering. A further source of variablity comes from the fact that, in mammalian cells (although probably not in yeast [47]), replication order is not constant but changes between cell types and during development. For example, highly expressed genes tend to be early replicating in higher eukaryotes [48]. This would further not only preclude the use of S phase-generated dosage imblance for general regulation, but also necessitate buffering.

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References

1. Springer, M. *et al.* (2010) A general lack of compensation for gene dosage in yeast. *Mol. Syst. Biol.* 6, 368
2. Torres, E.M. *et al.* (2007) Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science* 317, 916–924
3. Lyon, M.F. (1961) Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature* 190, 372–373
4. Baker, B.S. *et al.* (1994) Dosage compensation in *Drosophila*. *Annu. Rev. Genet.* 28, 491–521
5. Malone, J.H. *et al.* (2012) Mediation of *Drosophila* autosomal dosage effects and compensation by network interactions. *Genome Biol.* 13, r28
6. Schlattl, A. *et al.* (2011) Relating CNVs to transcriptome data at fine resolution: assessment of the effect of variant size, type, and overlap with functional regions. *Genome Res.* 21, 2004–2013
7. Beckwith, J.R. *et al.* (1966) Transposition of the Lac region of *E. coli*. *Cold Spring Harb. Symp. Quant. Biol.* 31, 393–401
8. Schmid, M.B. and Roth, J.R. (1987) Gene location affects expression level in *Salmonella typhimurium*. *J. Bacteriol.* 169, 2872–2875
9. Sousa, C. *et al.* (1997) Modulation of gene expression through chromosomal positioning in *Escherichia coli*. *Microbiology* 143, 2071–2078
10. Block, D.H.S. *et al.* (2012) Regulatory consequences of gene translocation in bacteria. *Nucleic Acids Res.* 40, 8979–8992
11. Joh, R.I. and Weitz, J.S. (2011) To lyse or not to lyse, transient-mediated stochastic fate determination in cells infected by bacteriophages. *PLoS Comput. Biol.* 7, e1002006
12. Chandler, M.G. and Pritchard, R.H. (1975) The effect of gene concentration and relative gene dosage on gene output in *Escherichia coli*. *Mol. Gen. Genet.* 138, 127–141
13. Slager, J. *et al.* (2014) Antibiotic-induced replication stress triggers bacterial competence by increasing gene dosage near the origin. *Cell* 157, 395–406
14. Cooper, S. and Helmstetter, C.E. (1968) Chromosome replication and the division cycle of *Escherichia coli* B/r. *J. Mol. Biol.* 31, 519–540
15. Korem, T. *et al.* (2015) Growth dynamics of gut microbiota in health and disease inferred from single metagenomic samples. *Science* 349, 1101–1106
16. Ardell, D.H. and Kirsebom, L.A. (2005) The genomic pattern of tDNA operon expression in *E. coli*. *PLoS Comput. Biol.* 1, e12
17. Couturier, E. and Rocha, E.P.C. (2006) Replication-associated gene dosage effects shape the genomes of fast-growing bacteria but only for transcription and translation genes. *Mol. Microbiol.* 59, 1506–1518
18. Narula, J. *et al.* (2015) Chromosomal arrangement of phosphorelay genes couples sporulation and dna replication. *Cell* 162, 328–337
19. Barnes, A. *et al.* (1979) Analysis of the significance of a periodic, cell size-controlled doubling in rates of macromolecular synthesis for the control of balanced exponential growth of fission yeast cells. *J. Cell Sci.* 35, 41–51
20. Fraser, R.S. and Moreno, F. (1976) Rates of synthesis of polyadenylated messenger RNA and ribosomal RNA during the cell cycle of *Schizosaccharomyces pombe*. With an appendix: calculation of the pattern of protein accumulation from observed changes in the rate of messenger RNA synthesis. *J. Cell Sci.* 21, 497–521
21. Fraser, R.S. and Nurse, P. (1978) Novel cell cycle control of RNA synthesis in yeast. *Nature* 271, 726–730
22. Elliott, S.G. and McLaughlin, C.S. (1978) Rate of macromolecular synthesis through the cell cycle of the yeast Saccharomyces cerevisiae. *Proc. Natl. Acad Sci. U.S.A.* 75, 4384–4388
23. Meltz, M.L. and Okada, S. (1971) Ribonucleic acid synthesis in the cell cycle of L5178Y mouse leukemic cells. Time of replication of the DNA template of rapidly labeled hybridizable RNA. *Exp. Cell Res.* 67, 90–96
24. Pfeiffer, S.E. (1968) RNA synthesis in synchronously growing populations of HeLa S3 cells. II. Rate of synthesis of individual RNA fractions. *J. Cell Physiol.* 71, 95–104
25. Killander, D. and Zetterberg, A. (1965) Quantitative cytochemical studies on interphase growth. I. Determination of DNA, RNA and mass content of age determined mouse fibroblasts in vitro and of intercellular variation in generation time. *Exp. Cell Res.* 38, 272–284
26. Skog, S. and Tribukait, B. (1985) Discontinuous RNA and protein synthesis and accumulation during cell cycle of Ehrlich ascites tumour cells. *Exp. Cell Res.* 159, 510–518
27. Yunger, S. *et al.* (2010) Single-allele analysis of transcription kinetics in living mammalian cells. *Nat. Methods* 7, 631–633
28. Yunger, S. *et al.* (2013) Zooming in on single active genes in living mammalian cells. *Histochem. Cell Biol.* 140, 71–79
29. Padovan-Merhar, O. *et al.* (2015) Single mammalian cells compensate for differences in cellular volume and DNA copy number through independent global transcriptional mechanisms. *Mol. Cell* 58, 339–352
30. Skinner, S.O. *et al.* (2016) Single-cell analysis of transcription kinetics across the cell cycle. *Elife* 5, e12175
31. Voichek, Y. *et al.* (2016) Expression homeostasis during DNA replication. *Science* 351, 1087–1090
32. Lenstra, T.L. *et al.* (2011) The specificity and topology of chromatin interaction pathways in yeast. *Mol. Cell* 42, 536–549
33. Wang, L. *et al.* (2008) Structure and chemistry of the p300/CBP and Rtt109 histone acetyltransferases: implications for histone acetyltransferase evolution and function. *Curr. Opin. Struct. Biol.* 18, 741–747
34. Das, C. *et al.* (2009) CBP/p300-mediated acetylation of histone H3 on lysine 56. *Nature* 459, 113–117
35. Davey, C.A. *et al.* (2002) Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 a resolution. *J. Mol. Biol.* 319, 1097–1113
36. Han, J. *et al.* (2007) The Rtt109-Vps75 histone acetyltransferase complex acetylates non-nucleosomal histone H3. *J. Biol. Chem.* 282, 14158–14164

37. Li, Q. *et al.* (2008) Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. *Cell* 134, 244–255
38. Celic, I. *et al.* (2006) The Sirtuins Hst3 and Hst4p Preserve Genome Integrity by Controlling Histone H3 Lysine 56 Deacetylation. *Curr. Biol.* 16, 1280–1289
39. Neumann, H. *et al.* (2009) A method for genetically installing site-specific acetylation in recombinant histones defines the effects of H3 K56 acetylation. *Mol. Cell* 36, 153–163
40. Marquardt, S. *et al.* (2014) A chromatin-based mechanism for limiting divergent noncoding transcription. *Cell* 157, 1712–1723
41. Watanabe, S. *et al.* (2010) Structural characterization of H3K56Q nucleosomes and nucleosomal arrays. *Biochim. Biophys. Acta* 1799, 480–486
42. Wellcome Trust Case Control Consortium Craddock, N. *et al.* (2010) Genome-wide association study of CNVs in 16,000 cases of eight common diseases and 3,000 shared controls. *Nature* 464, 713–720
43. Beckmann, J.S. *et al.* (2007) Copy number variants and genetic traits: closer to the resolution of phenotypic to genotypic variability. *Nat. Rev. Genet.* 8, 639–646
44. Niwa, H. *et al.* (2000) Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat. Genet.* 24, 372–376
45. Zhou, P. *et al.* (1997) Gene transcription and chromosome replication in Escherichia coli. *J. Bacteriol.* 179, 163–169
46. Bechhoefer, J. and Rhind, N. (2012) Replication timing and its emergence from stochastic processes. *Trends Genet.* 28, 374–381
47. Raghuraman, M.K. *et al.* (2001) Replication dynamics of the yeast genome. *Science* 294, 115–121
48. Schübeler, D. *et al.* (2002) Genome-wide DNA replication profile for Drosophila melanogaster: a link between transcription and replication timing. *Nat. Genet.* 32, 438–442