The role of epigenetics in maintaining genome stability

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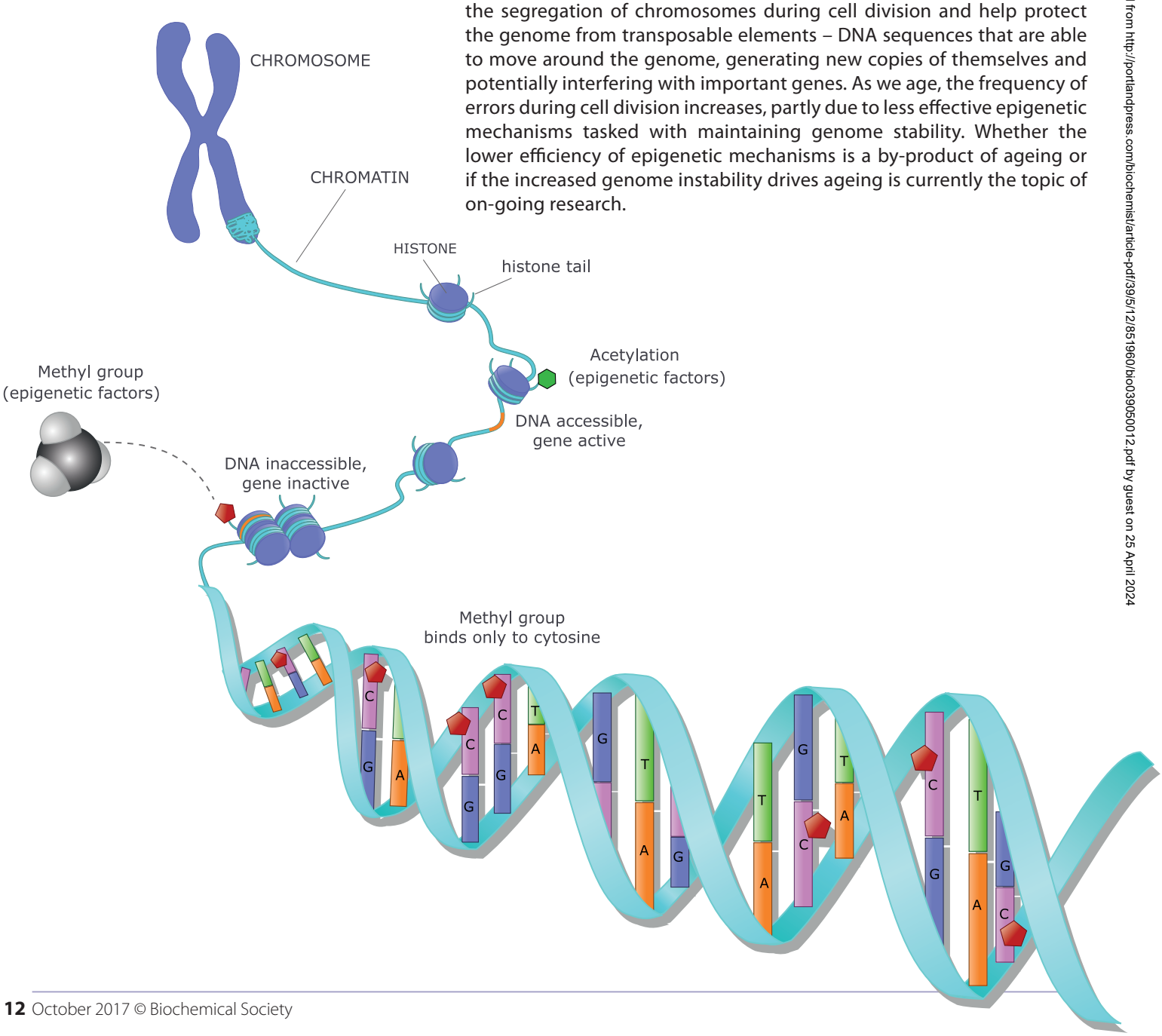
Epigenetic mechanisms play important roles in maintaining our genomes, helping to ensure that after every cell division each daughter cell contains an intact copy of the genome without the structural integrity of the chromosomes being compromised. They are also important for the segregation of chromosomes during cell division and help protect the genome from transposable elements – DNA sequences that are able to move around the genome, generating new copies of themselves and potentially interfering with important genes. As we age, the frequency of errors during cell division increases, partly due to less effective epigenetic mechanisms tasked with maintaining genome stability. Whether the lower efficiency of epigenetic mechanisms is a by-product of ageing or if the increased genome instability drives ageing is currently the topic of on-going research.



The data generated by the human genome project revealed the complexity of the human genome: approximately 20,500 protein-coding genes are scattered in a sea of 3 billion base pairs, wound into 23 chromosome pairs. During an organism's lifetime, most cells continually divide producing new daughter cells. The human body, for example, consists of an estimated 37.2 trillion cells, all of which trace their origin back to the single-cell zygote.

It is essential for human health that during each cell division, the genome is copied faithfully and a copy of each chromosome is passed on to the daughter cells. Protecting the genome's integrity is particularly important when germ cells are generated, as these cells provide the blueprint for the next generation. To facilitate this process, organisms have evolved a variety of mechanisms to ensure that the stability of the genome will be maintained despite its complexity. Among these mechanisms are several that belong to the field of epigenetics.

Epigenetics is concerned with phenotypic changes that are heritable (through mitosis or meiosis), but that are independent of changes in the DNA sequence. For instance, epigenetics includes the study of X inactivation in female mammals – how one of the female's two X chromosomes is selected and then maintained in a transcriptionally silent form to compensate for the difference in X chromosome copy number between males and females. It also includes the study of imprinting, whereby, for some genes, only one allele is transcriptionally active, either the copy inherited from the mother or the father depending on the specific gene in question. The molecular mechanisms responsible for these and other epigenetic phenomena are diverse and include DNA modifications (e.g. cytosine methylation), non-coding RNAs and alterations in chromatin structure – the way the DNA is packaged together with proteins in the nucleus. Decades of research have revealed that these epigenetic mechanisms play important roles in helping to maintain genome integrity.

Chromosome maintenance

One way that epigenetic mechanisms contribute to genome stability is by ensuring the integrity of the chromosomes themselves. Chromosomes have three features that are essential for faithful chromosome inheritance through cell division: the centromere, the telomere and origins of replication. All three of these features are required for a chromosome to be passed on to a daughter cell without damage, and all three are shaped by epigenetic mechanisms. Telomeres protect the ends of chromosomes and prevent the chromosomes from fusing end to end. To carry out this function, telomeres have a distinct chromatin structure, which along with specialized proteins is essential for protecting chromosome ends. The centromeres of chromosomes serve as the assembly point for the kinetochore, a complex of proteins where spindle fibres attach, ensuring proper chromosome segregation during cell division. Despite their essential function, the location of centromeres is not specified by DNA sequence. Instead, it is determined epigenetically through the presence of a specialized histone variant, CenH3, which only occurs at the centromere: wherever CenH3 is located, the centromere will form. Localization of CenH3 is not dependent on the underlying DNA sequence, illustrated by the fact that centromeres have been shown to move to new locations along the chromosome, thus encountering new DNA sequences (neo-centomere formation). Origins of replication are the sites along the chromosome where DNA replication is initiated. While the sites are determined by DNA sequence, *which* of the potential binding sites are selected is determined epigenetically, most likely by differences in the chromatin structure that makes certain sites accessible while keeping others inaccessible. By carrying out essential functions in centromere and telomere maintenance and by regulating access to potential origins of regulation, epigenetic mechanisms contribute to the maintenance of genome stability throughout an organism's life.

Protection from invading transposable elements

Epigenetic mechanisms also contribute to genome stability by regulating access to DNA sequences, in addition to the origins of replication discussed above. The human genome project revealed that the 20,500 protein-coding genes in our genome account for less than 1% of all the genome's DNA. The vast majority of the sequences in the human genome – and many other eukaryotic genomes – are repetitive sequences. Many of these repetitive sequences – likely more than 50% – are derived from so-called transposable elements. Transposable elements have the ability to move around in the genome. This movement can be through a 'cut and paste' mechanism, which leads to simple movement of the element, or through a 'copy and paste' mechanism, which generates additional copies of the element. Movement of transposable elements is risky for the organism's host genome: often the new insertions disrupt important genomic functions and have deleterious consequences. Thus, organisms employ a variety of mechanisms to protect their genome from transposable elements, and epigenetics plays a crucial role.

To prevent the unwanted movement of transposable elements, most organisms employ two different types of strategies, transcriptional and post-transcriptional silencing mechanisms. Transcriptional silencing prevents transcription of the transposable element sequences that are required for their movement. Transcriptional silencing is achieved by altering chromatin structure to block access by the transcription machinery to the transposable element sequences. This is achieved by the formation of heterochromatin, a specialized, transcriptionally inactive form of chromatin, over transposable elements. Heterochromatin blocks both transcription and movement of transposable elements. The heterochromatin is maintained at these sites through cell divisions through self-reinforcing feedback loops, meaning that as long as some of the biochemical markers of heterochromatin are retained, the enzymes responsible for making the mark can be recruited. If heterochromatin needs to be established 'from scratch', often small RNA molecules (described in more detail in the next paragraph) direct the necessary proteins to the correct location in the genome. Maintenance of the heterochromatin structure is essential for the stability of the genome, illustrating the importance of the epigenetic mechanisms that maintain the heterochromatin structure at appropriate locations in the genome.

Post-transcriptional silencing mechanisms are the second line of defence against the movement of transposable elements. These mechanisms kick in after the transposable elements have been transcribed (hence their name) to prevent their movement. Most organisms achieve this goal by utilizing a variety of RNA interference (RNAi) pathways. These pathways were discovered in the late 1990s/early 2000s and are characterized by the small, 20–30 nucleotide RNAs they generate. Generally speaking, small RNAs matching transposable element transcripts are produced. Through base pair matching, the small RNAs recruit enzymes to homologous RNAs that can cut or 'dice' the large transposable element RNAs into small fragments, thus destroying them. Transposable element transcripts are efficiently targeted by the RNAi pathways, ensuring that movement of transposons is minimized. Specialized pathways exist in the germline to protect the genome of the next generation. Furthermore, there is crosstalk between the RNAi pathways and the transcriptional silencing pathways to ensure that transposable elements that are transcriptionally active can be targeted for silencing via heterochromatin formation in the future. In plants, for example, small RNAs can direct the deposition of cytosine methylation and initiate the formation of heterochromatin. Thus, the interplay between chromatin and RNAi pathways efficiently protects the genome from movement of transposable elements.

Ageing reduces epigenetic mechanism efficiency

Interestingly, with ageing, increased genome instability is observed. This increased genome instability is characterized by increased occurrences of mutations, cells with incorrect chromosome number, loss of heterochromatin and mistakes in transcription. For example, as organisms age, the rate of chromosome segregation defects increases. Thus, more and more often, as organisms age, the two daughter cells that are generated after cell division are not identical, and instead, one cell is missing an essential chromosome, while the other has an additional one (aneuploidy). Chromosome segregation defects can occur for any chromosome, both sex chromosomes and autosomes, and, curiously, in humans, these segregation defects occur more frequently in males than in females of the same age. Centromeres are responsible for coordinating the segregation of chromosomes during cell division. It is currently unclear how epigenetic mechanisms that specify centromere identity contribute to the increased chromosome segregation defects that occur with ageing.

Other epigenetic mechanisms contributing to the maintenance of our genomes seem to break down with age as well. For example, the silencing mechanisms that keep transposable elements in check are less efficient in older animals. Heterochromatin is not maintained, and thus, heterochromatin seems to be lost gradually in older organisms. This loss of heterochromatin means that transcriptional silencing mechanisms that normally keep transposable elements in check are now leaky, allowing the transcription machinery to access some transposable elements. This change in accessibility leads to increased transcription from transposable elements as organisms age. With the increased transcription from the transposable elements, increased levels of transposable element movement have been reported, leading to further genome instability.

In organisms like humans that use cytosine methylation, DNA methylation levels also change with age. Because high levels of DNA methylation are a characteristic of heterochromatin, these changes likely contribute to the breakdown of heterochromatin with age. While it has been known for some time that DNA methylation changes with age, recently a subset of DNA methylation sites in the human genome have been identified that change with age in a predictable way. These DNA methylation changes are so predictable in how they increase or

decrease with age that they can be used to estimate the chronological age of the individual. It is currently unclear why these particular DNA methylation sites change in such a distinctive, predictable way and what processes control these changes. However, this finding illustrates the importance of epigenetic mechanisms such as DNA methylation to genome stability due to their role in heterochromatin formation and their role in ageing.

Future directions

As the above examples illustrate, epigenetic mechanisms play important roles in maintaining genome stability. They are essential for proper chromosome segregation, ensuring that after each cell division both daughter cells receive a complete set of chromosomes. Epigenetic mechanisms are also essential for ensuring that the correct fraction of the genome can be accessed by the transcriptional machinery in each cell type and for maintaining transposable elements and other repetitive sequences in an inaccessible, silent state. The links between these essential functions of epigenetic mechanisms and their break down with ageing are compelling. One question to be addressed in the future is that of cause versus consequence: is the breakdown of epigenetic mechanisms causing the various phenotypes associated with ageing, or are processes associated with ageing causing the observed decrease in efficiency of the epigenetic mechanism and the associated reduction in genome stability? Research in animal model systems such as mouse, the roundworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* will be critical for addressing this question as they allow precise genetic manipulation of the various epigenetic pathways, and their impacts on the organism throughout the lifespan can be studied in detail. ■

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