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Epigenetic Regulation of Growth and Stress Response in Oysters

Guilin Wang, Rudi Mai
Tropical Marine Fisheries Research Center, Hainan Institute of Tropical Agricultural Resources, Sanya, 572025, Hainan, China
Corresponding author: rudi.mai@hibio.org

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Abstract Epigenetics provides a new perspective to explain how the genome regulates biological phenotype without changing DNA sequences. As an important breeding shellfish, oysters (oysters) have their growth rate and stress resistance directly affect economic benefits and ecological functions. In recent years, research has found that epigenetic mechanisms such as DNA methylation, histone modification and non-coding RNA are widely involved in the growth and development of oysters and the environmental stress response. This study reviews the physiological basis of oyster growth and stress resistance, focusing on the role of DNA methylation in the regulation of key growth gene expression, the effect of histone covalent modification on nutritional metabolism and energy distribution, and the functions of non-coding RNAs such as miRNA, lncRNA, and piRNA in regulatory networks. In addition, we summarize how epigenetic mechanisms affect oyster adaptation to environmental stresses such as temperature, salinity, pathogens, etc., including the possible role of epigenetic memory in transgenerational stress resistance. Epigenetic regulation is an important frontier field for understanding the genetics and environmental adaptation of oyster traits, and has potential guiding significance for cultivating high-yield, stress-resistant oyster varieties.

Keywords Oysters; Stress resistance; DNA methylation; Non-coding RNA; Epigeneticss

1 Introduction

Oysters occupy an important position in the global aquaculture industry. In China, shellfish (including oysters) farming output accounts for about 70% of the total seawater farming output. Oysters not only have significant economic value, but are also a key species in marine ecosystems, maintaining ecological balance by filtering water bodies and building reefs. The rapid growth and strong stress-resistant oyster varieties are important goals pursued by the breeding industry. The growth rate directly affects the breeding cycle and yield, and stress-resistant determines the survival rate of oysters against environmental fluctuations and disease invasion. However, disease remains one of the biggest threats in oyster farming at present, with annual losses caused by diseases accounting for the first place in economic losses for aquaculture. For example, diseases such as "Pacific Oyster Death Syndrome" have caused large-scale oyster deaths. Therefore, improving the ability of oysters to resist diseases and stress resistance is equally important as promoting their rapid growth, and has become the key direction of aquatic scientific research.

Traditional genetic breeding methods play an important role in oyster growth and improvement of stress resistance traits, but relying solely on gene sequence variations is difficult to explain all phenotypic differences. In recent years, epigenetics has attracted attention as another layer of mechanism for genome regulation. Epigenetic regulation does not change DNA base sequences, but can hereditically affect gene expression and phenotype (Zhang et al., 2017). Studies have shown that most aquaculture traits are regulated by genetics and epigenetics, and the two should be studied in combination. For example, phenotypic plasticity caused by environmental factors is often mediated by epigenetic modifications, and its effects can be transmitted to offspring to some extent.

The rapid increase in the emphasis on epigenetics in the aquatic field is considered to be a new way to improve traits beyond genetic breeding. Some studies have reviewed new advances in marine environmental epigenetics and pointed out that epigenetics is of great significance in the adaptive evolution of marine invertebrates (Gawra et al., 2023). Therefore, in-depth study of the epigenetic mechanisms of oyster growth and stress resistance will

not only help reveal the molecular basis of trait formation, but also have practical significance for formulating new varieties cultivation strategies. This study explains the physiological and molecular basis of oyster growth and stress resistance, the main epigenetic types, the regulatory effect of epigenetic on growth and stress resistance, environmental interaction, and research methods and technical prospects, and reviews the research progress in the past five years.

2 Physiological and Molecular Basis of Oyster Growth and Stress Resistance

2.1 Major physiological processes and molecular pathways related to growth

The growth of oysters involves a series of physiological processes such as nutrient intake, energy distribution and tissue formation. Among them, filter feeding allows oysters to consume a large amount of plankton for growth, and digestive tissues such as the hepatopancreas play a key role in the transformation of nutrients. Endocrine axes such as growth hormone and insulin-like growth factor (GH/IGF) regulate the growth and development of shellfish, and their signals can promote protein synthesis and cell proliferation (Gawra et al., 2023). On the molecular pathway, studies have found that the expression of pathways such as ribosome biogenesis and nucleic acid metabolism in the high-growth lines of oysters is enhanced, suggesting that these pathways are closely related to rapid growth. Chitin formation (biomineralization) is also an important aspect of oyster growth, and the matrix proteins and ion transport channels secreted by the coat membrane determine the deposition rate and structure of shells (Rivière et al., 2013). Recent genomic analysis identified a batch of genes related to oyster shell formation and growth regulation. For example, several key genes controlling shell mineralization and body length were found in eastern oysters. The expression of these growth-related genes and pathways is affected by multiple factors, including nutritional level, temperature and developmental stage, and needs to be further studied in combination with an epigenetic perspective.

2.2 Key mechanisms of stress resistance: response to temperature, salinity, and pathogenic pressure

Oysters' stress resistance is reflected in their tolerance to environmental stress (such as temperature, salinity changes) and pathogenic infection. In terms of temperature stress, oysters can resist high-temperature damage through protective mechanisms such as heat shock protein (HSP). Studies have shown that sharply increasing water temperatures can induce high expression of the Oyster HSP gene, which helps protein fold and maintain cellular homeostasis. Under low temperature and dry dew (air exposure) stress, oysters tend to close both shells to reduce water dispersion and survive adverse periods by reducing metabolic rate to enter dormant state. Under salinity stress, broad-salt Pacific oysters can adapt to salinity changes by regulating the content of intracellular osmotic substances (such as free amino acids, glycerol betaine). When the ambient salinity drops sharply, oysters quickly accumulate amino acids to maintain cell osmotic balance; while under high salt conditions, free amino acids in the body are reduced to avoid cell dehydration. For pathogen invasion, oysters rely on innate immune mechanisms to resist infection (She et al., 2022). The hemolymph of oysters contains phagocytosis cells, which can actively engulf invading pathogens, such as *Vibrio parahaemolyticus* and *Zyxozoa* (shellfish parasites) that invade oysters. At the same time, oysters can produce humoral effectors such as antibacterial peptides and lysozyme to kill pathogenic microorganisms. When infection occurs, related signaling pathways such as NF-κB, TLR, etc. are activated, triggering the cascade expression of immune genes (Valdivieso et al., 2025). For example, in the study of the eastern oyster infection parasite *Perkinsus marinus*, high infection intensity induced a series of changes in the expression of immune response genes, accompanied by changes in the methylation level of the promoter region of some genes.

2.3 Trade-offs and interactions between growth and stress resistance

There is often a trade-off between growth and stress resistance traits. On the one hand, organisms distribute energy between growth and resistance to stress. When environmental conditions are good and food is sufficient, oysters will use more energy for growth and reproductive investments; while under harsh environments or pathogen threats, oysters tend to prioritize resources for maintaining homeostasis and immune defenses, which may temporarily sacrifice growth rate. Studies have observed that some fast-growing oyster strains have relatively weak stress resistance, and it is speculated that high growth rates may come at the expense of reducing partial

stress resistance. For example, experimental comparisons found that the survival rate of "Haida No. 1" breeding oysters that grew rapidly during the same period under low salt and high temperature stress was lower than that of slower-growing wild populations, showing a potential balance mechanism between growth and tolerance (Li et al., 2022). In addition, growth and retardation also affect each other through endocrine and signaling pathways. For example, nutrition and growth-related insulin signaling pathways and the KEAP1-Nrf2 pathway that responds to antioxidant stress may cross at the energy metabolism node. When environmental stress activates Nrf2-mediated antioxidant genes, it may inhibit the growth metabolic pathway of oysters to ensure survival priority. In essence, the trade-off between growth and stress resistance reflects the balance between biological resource allocation and survival and reproduction strategies. Understanding the genetic and epigenetic basis of this balance will help to simultaneously optimize oyster growth rate and environmental adaptability in breeding.

3 Main Types of Epigenetic Regulation of Oysters

3.1 DNA methylation and its role in gene expression regulation

DNA methylation is one of the most studied mechanisms in the epigenetic regulation of oysters. Typical mosaic methylation distributions exist in the oyster genome: the accumulation of 5-methylcytosine (5mC) can be detected in the gene coding region and promoter region, while the vast majority of non-CpG regions remain unmethylated. High-throughput sequencing analysis estimated that approximately 1.8% of cytosines in the entire genome of Pacific oysters had methylation modification. This methylation level is much lower than that of mammals, but is comparable to other invertebrates (such as insects). Oyster DNA methylation mainly occurs on CpG dinucleotides, showing a pattern of hypermethylation of gene bodies and hypomethylation of promoters. This methylation pattern is thought to be associated with constitutive and inducible expression characteristics of the gene. DNA methylation is generally regarded as a transcriptional repression signal: When the CpG island on the gene promoter is highly methylated, methylated binding proteins and chromatin remodeling complexes are recruited, making the local chromatin appear tightly, thereby inhibiting the binding of transcription factors and reducing gene expression (Li et al., 2024). In contrast, promoter demethylation is often associated with gene activation.

3.2 Regulatory functions of histone modification and chromatin remodeling

Covalent modification of histones is another important aspect of epigenetic regulation. In invertebrates such as oysters, although histone modification research is relatively limited, the conservative histone modification mechanism also exists in their genome. The lysine residues at the N-terminal tail of histones can undergo various modifications, including methylation, acetylation, ubiquitination, phosphorylation, etc. These chemical tags change the conformation of chromatin and thus affect transcriptional activity. For example, trimethylation of histone H3 lysine 4 (H3K4me3) is usually associated with transcriptional initiation activity, while H3K27me3, as a classic marker of transcriptional repression, is associated with gene silencing and pluripotency maintenance. There are also enzymes in the oyster genome that regulate these histone modification states, such as histone methyltransferase and demethylase, acetyltransferase (HAT) and deacetylase (HDAC). A class of homologs of lysine-specific histone demethylase 1 (LSD1) containing typical SWIRM domains and amine oxidase domains were identified in Pacific oysters. Functional studies show that after knocking down the Oyster *LSD1* gene, the two activity-related markers H3K4me1 and H3K4me2 are significantly increased in blood cells and are accompanied by upregulation of the hemocytic proliferation rate (Fellous et al., 2019). This means that LSD1 maintains the normal proliferation rhythm during oyster blood cell development by removing methyl groups on H3K4. In addition, when stimulated by bacteria, the transcription level of Oyster LSD1 rapidly declines, allowing the promoters of more immune-related genes to retain H3K4 methylation activity markers, thereby promoting the differentiation and release of antibacterial cells. This discovery reveals the mechanism by which histone demethylase regulates oyster immune function through chromatin state.

3.3 The role of non-coding RNA in regulatory networks

3.3.1 Research on miRNA-mediated inhibition of target gene expression and function

MiRNA is a small RNA about 20 to 24 nucleotides in length and can be paired with the 3’ untranslated region of the target mRNA, thereby triggering mRNA degradation or inhibiting translation. Hundreds of miRNA molecules

have been identified in oysters, which are widely involved in development, growth and stress response processes. For example, oyster miR-31, miR-8, etc. play a regulatory role in early shell development and larval metamorphosis; while miR-10, miR-1984, etc. have been reported to have significant changes in expression in immune and antioxidant reactions (Bonin et al., 2019). miRNAs perform functions by negatively regulating target gene expression. When oysters are subjected to environmental stress, the expression of many miRNAs is up-regulated or down-regulated, thereby changing the expression level of their target genes in response to stress. For example, some studies have found that Pacific oysters are significantly upregulated under high temperature stress, where miR-1 and miR-307 regulate the expression of antioxidant genes by acting on the Nrf2-Keap1 pathway and increase the resistance of oysters to oxidative stress. Another study on eastern oysters showed that after 30 days of exposure to an acidification environment with high pCO_2, some miRNA expression in oysters was altered, which may be related to the regulation of gene expression noise. In addition, in viral infection scenarios, small RNAs are also involved in host antiviral responses. When oysters are infected with the herpes virus OsHV-1, more than ten miRNAs will be differentially expressed, some of which can target immune pathway genes, and it is speculated that they regulate host antiviral responses during viral infection (Nahand et al., 2019).

3.3.2 lncRNA regulates growth and stress resistance related signaling pathways

Long-chain non-coding RNA is an RNA molecule with a length of more than 200 nt. It has no protein encoding function, but can affect gene expression through various mechanisms. In recent years, a large number of lncRNAs have been identified in oysters and many of them are found to be differentially expressed under different tissues, developmental stages or stress conditions. lncRNA can play a regulatory role at various levels of appearance, transcription and post-transcription, including acting as a molecular scaffold, bait or guide protein complex. In oyster growth, some lncRNAs have been shown to be associated with shell formation and energy metabolism. For example, the LncMPEG1 obtained by cloning in pearl oysters is a lncRNA that is specifically highly expressed in outer membrane tissue. Functional analysis showed that LncMPEG1 was the highest during the critical period of larval shell development. After exogenous interference reduced its expression, the crystal morphology of the oyster shell prism and nacres occurred abnormally, suggesting that the lncRNA regulates the biomineralization of the shell by affecting the secretion function of the outer membrane. Several lncRNAs have been reported to be significantly up-regulated or down-regulated during oyster gonad development, suggesting that they play a regulatory role in reproductive gonad maturation and gamete production. In terms of stress resistance, the review by Sun and Feng et al. pointed out that the differentially expressed lncRNA of bivalve shellfish (including oysters, abalone and mussels) is associated with multiple immune-related pathways, such as TLR signaling, lectin pathway, etc. Research by Lu et al. (2021) also found that abalone has a large number of lncRNA changes under bacterial and viral stimulation, which may participate in host defense responses by affecting classic immune pathways such as NF-κB.

3.3.3 The role of piRNA in genomic stability and stress adaptation

PIWI interactive RNA (piRNA) is a small RNA with a length of 24~30 nt that binds to PIWI subfamily proteins. It was first discovered in the germline and can mediate transposon silencing and genomic defense. There are also a large number of piRNA sequences in the oyster genome. In the methylation group analysis of hermaphrodites (such as the European flat oyster), there is evidence that gender switching involves extensive epigenetic reprogramming, where piRNA and PIWI pathways may play a role. Recent studies have found that piRNA is not only limited to germ cells, but is also expressed in somatic cells and exerts regulatory functions. Several piRNAs were identified in shellfish such as pearl oysters, and their expression changes under stress conditions such as hypoxia and high salt. It is speculated that the piRNA pathway may contribute to stress signal regulation and metabolic adaptation. A study of pearl oysters reported 69 highly expressed piRNA sequences, and analysis showed that these piRNAs targeted mRNAs of some metabolic and stress-related genes, suggesting that piRNAs may mediate rapid responses to environmental changes through mRNA silencing. In addition, studies compared the piRNA patterns of diploid and triploid Pacific oysters and found that the abundance and specificity of piRNA in triploid oysters were altered, some of which targeted spermatogenesis-related genes, which may be related to triploid infertility.

4 The Regulatory Effect of Epigenetic Mechanism on Oyster Growth

4.1 DNA methylation and activation or silencing of key growth genes

DNA methylation regulates the growth phenotype of oysters by affecting the expression status of growth-related genes. Breeding studies have shown that oyster hybrid offspring often exhibit growth advantages (hybrid dominance), which may be partly attributed to epigenetic regulation. For example, a study on the hybrid offspring of oysters and wild oysters in the "Haida No. 1" breeding line found that the overall DNA methylation level of hybrid oysters is lower than that of the parents, and this genome-wide hypomethylation state may be conducive to the widespread activation of growth-related genes and improve growth rate (Yang and Li, 2022).

At the specific gene level, DNA methylation can selectively silen or activate certain growth-critical genes. For example, if the methylation degree of the oyster's growth factors such as BMP, insulin-like growth factors, etc. increases, it will lead to transcriptional repression and hinder growth. Some genes involved in cell cycle and protein synthesis are also regulated by methylation. The study compared the oyster population with different growth rates and found that several growth-related gene promoters in the fast-growing group were demethylated and highly expressed, such as ribosomal protein genes and actin-related genes; while these gene promoters in the slow-growing group were highly methylated and low-expression (Tan et al., 2022). This shows that DNA methylation affects the growth rate of oysters by finely regulating gene expression. In addition to routine development, DNA methylation also plays a role in ploidy breeding. There are often differences in growth traits between long oysters (diploid oysters) and their triploid individuals. Studies have found that there are epigenetic differences in the genomic methylation maps of the two.

4.2 Histone modification regulates nutritional metabolism and energy distribution

Oyster growth depends on the accumulation and metabolic efficiency of nutrients, while histone modifications play a regulatory role in it by affecting the expression of metabolic enzymes and related pathway genes. Histone H3 acetylation is usually associated with high transcriptional activity. Under high nutritional conditions, the promoter of the key metabolic enzyme gene in digestive tissues such as oyster hepatitis and pancreas may enrich H3K9 and H3K27 acetylation markers, thereby enhancing the transcription of carbohydrate and lipid metabolic enzymes (Yang et al., 2023). This histone modification-mediated metabolic reprogramming needs to be verified experimentally. On the other hand, the cell proliferation and protein synthesis processes involved in growth are also regulated by histone modification. If the promoter region of genes that promote cell cycle progression is rich in activated histone markers (such as H3K4me3), it is conducive to rapid cell division and accelerated growth; if these genes are silent due to increased inhibitory modifications such as H3K27me3, it may lead to growth stagnation or even dormant state (Figure 1) (Fellous et al., 2019). Oysters often show growth stagnation when they encounter stress because the body preferentially triggers stress defense, and the growth-related genes may be temporarily turned off through histone modification changes.

4.3 Noncoding RNA-mediated regulation of growth signaling pathways

Non-coding RNA is also involved in signaling regulation related to oyster growth. In terms of miRNA, many miRNAs promote oyster growth by targeting growth inhibitors or cell cycle regulatory genes. *miR-8* is often thought to have a growth-promoting effect in animals, and it may accelerate cell proliferation by inhibiting the translation of certain growth-inhibiting proteins. This type of inference still needs experimental support in oysters (Wang et al., 2021), but preliminary analysis shows that the high expression of oyster *miR-8* is positively correlated with shell length growth. In terms of lncRNA, recent studies have shown that some lncRNAs can act as competitive endogenous RNAs, binding to microRNAs to relieve the inhibition of growth genes. An integrated analysis of BMC genomics identified several lncRNAs related to growth regulation in oysters, which are located close to or antisense to important growth genes, such as *FAS*, *CDC42*, which encodes the key enzyme of lipid synthesis, and *CDC42*, which regulates the cytoskeleton (Zhang et al., 2020). The expression of these lncRNAs is induced by culture temperature and bait abundance, and it is presumed that under different culture conditions, it plays a role by influencing the transcription of adjacent growth genes. For example, *LNC_012905* is located near the fatty acid synthetase gene, and the expression of both increases under high nutritional conditions, suggesting that the lncRNA may enhance the lipid synthesis pathway and promote the growth of oyster meat.

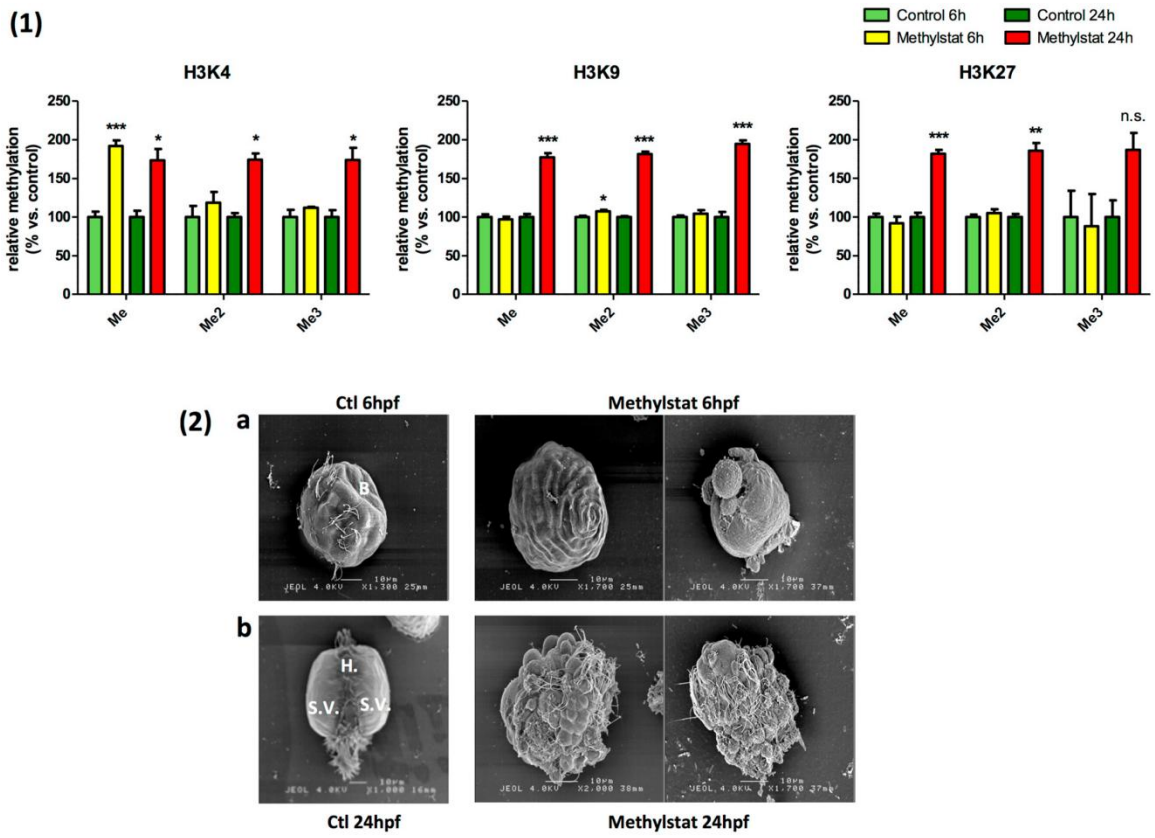

Figure 1 (1) Hypermethylation of histone lysine residues in presence of 10 μM Methylstat. Global H3K4 methylation increase in presence of methylstat, but only H3K4me1 and H3K4me3 are significant. Global H3K9 methylation increase in presence of methylstat, but only H3K9me2 is significant. H3K27me and H3K27me2 increase in presence of methylstat. Asterisk in the legend indicates significant variation of the indicated mark (One-way ANOVA; $p < 0.05$ was considered significant (<0.05 (*), <0.001 (**), <0.0001 (***)). (2) Abnormal development. Different phenotypes observed in control condition and under methylstat treatment at 6 h after fertilization (a) and 24 h after fertilization (b) (Adopted from Fellous et al., 2019)

5 The Role of Epigenetic Mechanism in Stress Resistance of Oysters

5.1 Dynamic changes in DNA methylation patterns under environmental stress

Environmental stress can cause reprogramming of the methylation state of the genome of oysters, thereby affecting the expression of genes related to stress resistance and the tolerance of oysters. Studies have shown that different stress types and stress durations will lead to dynamic adjustment of the methylation level of the whole genome of oysters. Taking dry dew (air exposure) stress as an example, Wang et al. put oysters in air for different times to measure the DNA methylation level of their cuff muscles and gill tissues. It was found that the overall methylation level gradually increased between 0.5 and 7 days of stress and reached its peak on day 7, which was about 10% to 15% higher than the control without dry dew; but when the dry dew continues to day 11, the methylation level drops again and approaches the initial state (Wang et al., 2021). This shows that the oyster genome has undergone a process of partial demethylation first with overall hypermethylation response and then partial demethylation as stress continues. It is speculated that the increase in early methylation may quickly turn off non-essential genes and concentrate resources on stress resistance, while the later demethylation may activate some chronic stress response genes to help survive for a long time (Li et al., 2024).

5.2 Histone modification regulates rapid response of genes related to stress resistance

When oysters are challenged by environmental challenges, histone modifications can turn on or off relevant genes by rapidly changing chromatin status, thereby achieving a rapid response to stress. Under hypoxia stress, organisms often use inhibitory markers such as H3K27me3 to activate hypoxia-resistant genes such as erythropoietin and heme oxygenase. In addition, histone modification changes will also be triggered when temperature changes are severely altered to regulate the activity of heat shock protein genes: high temperatures

will lead to an increase in the acetylation level of H3K9, the promoter of the *HSP* gene, thereby enhancing the expression of these protective molecules. Under continuous low temperature stimulation, the overall acetylation level of histone H3 in oyster cells will decrease, which will lead to a general reduction in non-essential gene expression, causing the body to enter a low metabolic pattern to survive the cold (Yang et al., 2023). After bacterial stimulation of oyster blood cells, their histone H3K4me2 levels increased significantly, corresponding to the upregulated expression of a variety of immune genes. This may be because the demethylase LSD1 is inhibited under immune stimulation and the active methyl marks on histones accumulate, thereby accelerating the generation of bactericidal effector molecules.

5.3 Non-coding RNA-mediated regulation of immune and antioxidant mechanisms

Non-coding RNA is also deeply involved in the immune and anti-resistance regulatory network of oysters, especially in pathogenic responses and oxidative stress. miRNA plays a key role in the oyster immune response. Many oyster miRNA target genes are important components of the immune pathway, such as signal transduction molecules or inflammatory mediators. By regulating these targets, miRNA carefully controls the strength and timing of the immune response. A typical example is oyster miR-223, which is known in humans to regulate granulocyte development and inflammatory response; when oysters are stimulated by bacteria, miR-223 expression is upregulated, which may promote the generation of bactericidal cells and enzymes by inhibiting factors that negatively regulate the immune response (Zhang et al., 2022). Secondly, lncRNA also plays a role in immune cell differentiation and disease resistance. Studies have found in shellfish such as abalone that pathogenic stimulation can cause hundreds of lncRNA expression changes, some of which are adjacent to or co-expressed with immune genes, suggesting that they may participate in the immune response by affecting the chromatin state of immune genes or acting as ceRNA to regulate immune-related miRNAs. In addition, long-chain non-coding RNA is also involved in the antioxidant regulation of oysters. Cells are usually activated when under oxidative stress to induce antioxidant enzyme expression. There is evidence that some lncRNAs can directly bind to Nrf2 protein, affecting their nuclear translocation efficiency; or complement the promoter region of Nrf2 downstream genes to form a three-strand structure of RNA-DNA, thereby changing the local chromatin state (Kundu and Basu, 2021).

6 Interaction Between Environmental Factors and Epigenetic Regulation

6.1 The influence of environmental factors such as temperature, salinity, pH on epigenetic markers

Environmental factors can affect the oyster phenotype by changing epigenetic markers, which has gradually been recognized in recent years. In terms of temperature factors, the epigenetic status of oysters is significantly different under different temperature conditions. Oyster seedlings cultured at high temperature showed a tendency to decrease genome-wide methylation levels and improve transcriptional activity, while increased methylation at certain sites was observed under low temperature conditions, accompanied by decreased expression of growth-related genes (Wang et al., 2020). The effects of salinity changes on the epigenetics of oysters are also worthy of attention. There may be systematic differences in the methylation map of the nearshore oyster population at the southeast coastal estuaries and the oyster population in the offshore high-salt environment. Experts compared DNA methylation in four geographical populations of Crassostrea virginica and found that although the genetic differences between populations mainly originate from DNA sequences, population-specific differences in DNA methylation were more significant than genetic variation (Suárez-Ulloa et al., 2019). Under experimental conditions, sudden salinity often causes changes in histone marking and miRNA profile of oyster stress response genes. For example, the promoter H3 acetylation level of some stress protein genes in oyster tissue under low salt stress is increased, which facilitates rapid expression of these genes to restore cellular osmotic pressure balance. In terms of pH factors, seawater acidification also has a subtle influence on the epigenetics of oysters.

6.2 Epigenetic memory: the mechanism of stress resistance formation across generations

Epigenetic memory refers to the fact that the epigenetic state of a organism changes and passes it to offspring after experiencing a certain environment, so that offspring also has certain adaptability when they are not exposed to

the environment. This phenomenon is more clear in plants, and it has also begun to attract attention in invertebrates such as oysters. Studies have shown that some environmentally induced epigenetic changes can be maintained over the course of oyster life and are not completely cleared when gametes form, thus passing on to the next generation (Figure 2) (Li et al., 2022). There were experiments that exposed parental oysters to low salt or high CO_2 stress, and then observed the phenotype of their offspring. It was found that the offspring born to parents who received stress treatment had a high survival rate under the corresponding stress conditions and showed some kind of "pre-adaptation" ability (Venkataraman et al., 2020). This suggests that oysters may have epigenetic memory mechanisms that transmit information from parental experiences through epitaphs coded in germ cells. DNA methylation is considered one of the vectors of this memory, as invertebrates such as oysters may not completely erase the methylation map of the parent during gametogenesis compared to mammals. Several sensitive molecular tests have found that the presence of specific methylation and ncRNA markers in oyster sperm and egg cells is associated with the parental environment. Studies have found that even if the pesticide dichloron is not exposed, the offspring of oysters has characteristic changes related to parental treatment on the DNA methylation map, and these changes are concentrated on development-related genes.

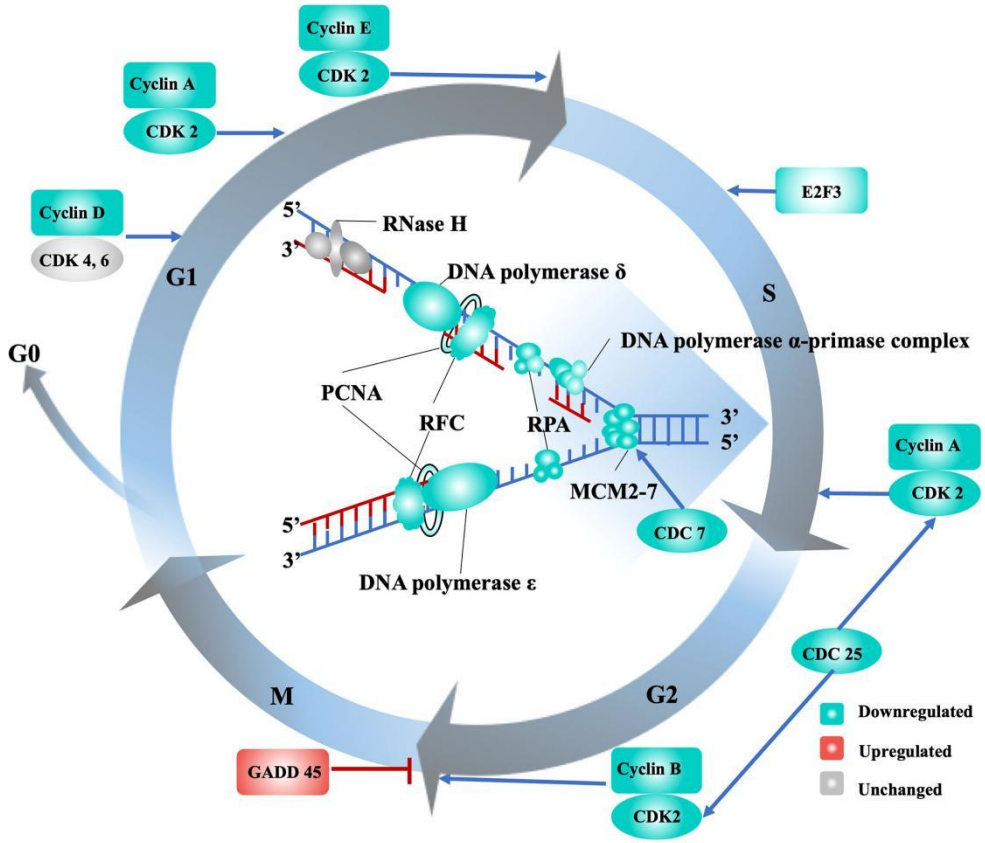

Figure 2 Transcriptional regulation of the cell cycle and DNA replication in eastern oysters under stress (Adopted from Li et al., 2022)

Image caption: The gene names are the following: CDC, cell division cycle-related protein kinase; CDK, cyclin-dependent kinase; E2F, transcription factor E2F3; GADD 45, growth arrest and DNA damage-inducible protein 45; MCM2-7, minichromosome maintenance proteins; PCNA, proliferating cell nuclear antigen; RFC, replication factor C; RPA, replication protein A (Adopted from Li et al., 2022)

6.3 Long-term impact of environmental changes on the adaptability of oyster populations

From a population and evolutionary perspective, environmentally driven epigenetic variation may be an important source of adaptation to environmental changes in oyster populations. Traditional concepts believe that adaptation mainly depends on natural selection of DNA sequence mutations, but recent studies have found that epigenetic mutations tend to be faster and more reversible, and may play a greater role in short-term adaptation. Johnson and

Kelly's study found that the degree of DNA methylation differentiation among different geographical populations of oysters in eastern even exceeds genetic differentiation, which means that the epigenetic drift caused by the environment may confer different phenotypic characteristics on the population, making it better adapt to local conditions (Johnson and Kelly, 2020). The research shows that epigenetic variation in disease-resistant oyster populations explains about 25% of disease-resistant phenotypic variations, while pure genetic variation explains only 14%. This suggests that epigenetics contributes more to this rapid adaptation (occurring within just over a decade) (Gawra et al., 2023). However, whether epigenetic adaptation can exist stably for a long time is also a question worth discussing. Without support for genetic variation, apparent markers may gradually recover after environmental stress is relieved, and adaptability may also subside.

7 Epigenetic Research Methods and Technological Progress

7.1 High-throughput sequencing and multi-omics integrated analysis technology

With the development of sequencing technology, methods for studying the epigenetics of oysters are becoming increasingly abundant. Whole genome methylation sequencing (WGBS) is widely used to map methylation maps of oysters, and can detect the distribution of 5mC in the genome with a single base resolution. Comparing the methylation differences of oysters with different growth performances by WGBS, key sites that affect growth can be localized. In the case of resource and cost limitations, simplifying representative genomic methylation sequencing (RRBS, MeDIP-Seq, etc.) is also an optional solution, focusing on the analysis of methylated hotspot regions such as CpG islands. In recent years, some studies have tried ChIP-Seq in pearl shells and successfully obtained the distribution of piRNA target sites bound by PIWI protein, providing reference for the subsequent expansion to oysters (Dellong et al., 2024). Another important direction is multiomics integration analysis, that is, obtaining methylation group, transcriptome, and even proteomic and metabolomic data of the same sample at the same time, and correlating different levels of information through bioinformatics to comprehensively analyze the epigenetic regulatory mechanism. This combination allows them to screen out candidates for epiregulatory key genes.

7.2 Epigenome editing and functional verification strategies

Epigenetic research is moving from correlation analysis to causal function verification. In order to verify the effect of an apparent marker on the phenotype, it is necessary to be able to modify the marker in a targeted manner. Currently, a cutting-edge technology is to use the modified CRISPR/Cas9 system to achieve epigenome editing, fusing dCas9 that does not cleave DNA with DNA methyltransferase (DNMT) or demethylase (TET), and directed recruitment of RNA to the target gene promoter, thereby manually adding or removing methylation modifications at this site (Morita et al., 2024; Qian and Liu, 2024). Similarly, DNA methyltransferase DNMT or demethylase TET can be knocked down or knocked out to evaluate the effect of changes in global methylation levels on oyster growth and survival. In addition, pharmacological methods are also important means. DNA demethylating agent 5-azacytidine (5-Aza) has been used to test the epigenetic function of oysters: After low dose treatment of pearl oyster larvae, it was found that its DNA methylation level decreased and induced an increase in the expression of some immune-related genes. This suggests that drug treatment can verify the relationship between methylation and traits.

7.3 Shellfish epigenetic database and bioinformatics tools

With the accumulation of research data, establishing a professional shellfish epigenetic database will greatly promote the development of this field. Currently, some public databases have included epigenetic data on oysters. The NCBI gene expression database (GEO) contains methylation sequencing data of different tissues of oysters and transcriptome data for stress treatment, which can be reanalysed based on this. The Chinese National Gene Bank also integrates the genomic and epitomical data of oysters to facilitate the retrieval of epigenetic status of specific genes under different conditions (Li et al., 2024). In order to promote data sharing, a resource platform with the nature of the "Aquatic Epigenetics Alliance" is being built internationally to summarize the apparent data and analysis results of fish and shellfish under various conditions. I believe that in the near future, an epigenetic information library specifically targeting oysters and other bivalves will appear, including their genomic

methylation maps, histone modification sites, and highly expressed ncRNA catalogs. Researchers can obtain the epigenetic status of target genes in different environments and developmental stages on these platforms in one-stop, thereby proposing more targeted hypotheses and designing experimental verification.

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Conflict of Interest Disclosure

The authors confirm that the study was conducted without any commercial or financial relationships and could be interpreted as a potential conflict of interest.

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