Epigenetics and acquired tolerance to environmental stress

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Abstract

The unprecedented rate of increase in global temperatures is threatening to outpace the evolutionary adaptability of corals. With climate change endangering the very existence of coral reefs, the ability of corals to mitigate detrimental changes in their environment within their lifetime is becoming more critical than ever. A range of experiments and observations suggest that corals might be able to retain environmental memory from previous stress that provides increased resilience to recurrent events, and examples from other organisms suggest that such responses could potentially be transferred across generations. However, the underlying molecular mechanisms and the extent to which they can improve resilience and survivability in light of climate change have yet to be elucidated. This chapter provides an overview of the current knowledge on acquired tolerance to environmental stress in corals and the potential role of epigenetic mechanisms in this process. Based on the current evidence from corals and other organisms, I provide a theoretical model by which epigenetic mechanisms could confer transcriptional memory and, thus, promote acquired tolerance in these organisms.

Keywords

DNA methylation, histone modifications, non-coding RNAs, epigenetic crosstalk, transcriptional regulation, chromatin remodeling

9.1 A brief history of Epigenetics

Epigenetics has originally been defined as the study of heritable changes in the phenotype of an organism that are not caused by changes in its genetic code. The term was coined in the early 1940s when developmental biologists started looking into the mechanisms of cell differentiation during embryogenesis (Waddington 1942). The observation that totipotent cells differentiated and adopted different cell fates that were heritable and not reversible spurred the notion that non-genetic mechanisms must exist that control how the genetic components are translated to produce the phenotype. However, it was only in the early 1990s, long after the discovery of the DNA as the carrier of the genetic information and the identification of the first epigenetic marks, that the field of epigenetics evolved into the more general study of the mechanisms that convey spatial and temporal control of gene activity (Holliday et al. 1990). While different definitions still exist (Holliday et al. 1990; Martienssen et al. 1996; Bird 2007; Ptashne 2007; Deans and Maggert 2015), especially regarding the requirement of meiotic or mitotic heritability of epigenetic marks and the resulting phenotype, the general notion of these mechanisms underlying the regulation of the genotypic output and the integration of environmental cues to produce a phenotype is, in principle, unchanged. This looser definition, which does not require heritability across generations, expanded the scope dramatically from its former niche looking at embryonic development and cell differentiation, to a much broader field that studies how the

environment influences organism development and phenotypic responses. The understanding that genotypes cannot only produce different cell types from the same genetic information but also modify the phenotype of an organism, be it behavior, morphology, or physiology, in response to environmental conditions provided a mechanistic link between epigenetic mechanisms and phenotypic plasticity. However, the idea that such epigenetic changes are adaptive and might even be inherited across generations caused a hype and promoted a quasi-Lamarckian view to inheritance in the field (Deichmann 2016; Bird 2007; Maderspacher 2010) that still drives research activities in many areas, including coral research.

9.2 Epigenetic Mechanisms

Depending on the definition and, specifically, the requirement for heritability of the respective marks, different molecular mechanisms are considered epigenetic. The most common mechanisms that are referred to as canonical epigenetic mechanisms are DNA methylation and histone modifications . However, other mechanisms, such as small non-coding RNAs, have also been shown to induce phenotypic changes that can even be inherited over multiple generations in some organisms (Duempelmann et al. 2020) and should thus also be considered canonical even based on a stricter definition of the term that requires heritability (Fig. 1). Most of these mechanisms and their functions are being studied in model organisms that provide many functional tools to decipher their molecular mode of action. However, thanks to the advance of new sequencing technologies and the broader availability of molecular tools, these studies are also increasingly performed in non-model organisms such as corals.

9.2.1 DNA methylation

Likely the most studied epigenetic mark is DNA methylation, which involves the covalent addition of a methyl group to certain bases in the DNA. The most common DNA methylation mark is 5-methylcytosine (5mC) which is usually found in the context of CpG dinucleotides where methylation is symmetrically present on both strands (Heard and Martienssen 2014), but 5mC can also be found in other contexts in plants (Zhang et al. 2018), i.e., CHG and CHH where H represents any nucleotide but guanine. However, DNA methylation is not restricted to cytosines and can also occur on adenine (6mA) in some species, such as the nematode *Caenorhabditis elegans* (Greer et al. 2015; Ma et al. 2019). The addition of methyl groups to these bases is established and maintained by specific enzymes called DNA methyltransferases which are found across all domains of life (reviewed in (Lyko 2018). The methylation of cytosine, for instance, is catalyzed by DNA methyltransferases 1 and 3 (DNMTs), whereby several variants of DNMT3 exist, most notably DNMT3A and DNMT3B.

DNMT1 is responsible for maintaining existing methylation patterns during cell division and, thus, their inheritance from the mother to the daughter cell. This enzyme recognizes hemimethylated DNA and subsequently methylates the newly synthesized strand at the same positions. DNMT3s, on the other hand, are responsible for the addition of new methylation marks and thus likely the key enzymes responsible for the epigenetic integration of environmental cues. Other so-called non-canonical DNMTs exist that do not possess DNA methylation activity, such as DNMT2, which is implicated in the methylation of tRNAs (Lyko 2018). The putative roles of DNA methylation include transcriptional silencing of repetitive elements, the transcriptional regulation of genes through promoter methylation , the regulation of alternative splicing, and the inhibition of spurious transcription (Jones 2012; Zhang et al. 2018). However, most of the research on these functions has been conducted on model organisms, and only a few studies have been done on corals, e.g. (Liew et al. 2018;

Dixon et al. 2018; Dimond and Roberts 2020; Durante et al. 2019; Rodríguez-Casariego et al. 2020).

9.2.2 Histone modifications

Besides DNA methylation, histone modifications are considered the second canonical epigenetic mechanism. Although the molecular mechanisms underlying their heritability during mitotic divisions are not yet fully understood (Sandholtz et al. 2020), positional heritability of histone marks has been reported in yeast (Radman-Livaja et al. 2011) and vertebrates (Reverón-Gómez et al. 2018; Madamba et al. 2017). Histones are basic, i.e. positively charged, proteins that can interact with the negatively charged phosphate backbone of the DNA double helix, whereby ~150 bp of DNA are wound around a histone octamer to produce a nucleosome (Fig. 2). They are the main protein constituent of chromatin and control the degree of DNA packaging through the addition of chemical modification to specific residues of their protruding (N)-terminal tails (Fig. 2). Through this mechanism, histones control the accessibility of the DNA for regulatory proteins and the transcriptional machinery . They can either open a region to promote transcription or binding of regulatory proteins, such as transcription factors and the transcriptional machinery, or densely package the DNA to prevent access, resulting in transcriptional silencing of a region in the genome. The different chemical modifications, collectively termed the histone code (Strahl and Allis 2000), change how the histones interact with the DNA and other histones and include the acetylation, methylation, phosphorylation, or ubiquitylation of residues (Fig. 2). Some of these modifications, such as the acetylation of lysine residues, are correlated with transcriptional activation, while others, such as methylation, have more context-dependent functions (reviewed in (Lawrence et al. 2016)). For instance, depending on the core histone and the specific residue that is modified, methylation can either promote transcriptional activation, as is the case with the trimethylation of histone 3 at position lysine 4 (H3K4me3), or transcriptionally downregulate a locus as is the case for H3K27me3.

The addition of modifications is catalyzed by proteins called "writers" and removed by "erasers", while other proteins exist, termed “readers”, that can bind and recognize these modifications and attract other proteins to these sites (reviewed in(Zhang et al. 2015)) (Fig. 3). Importantly, the histone code is also regulated through the crosstalk with other epigenetic mechanisms, such as DNA methylation (Du et al. 2015). This crosstalk between the different mechanisms is performed by proteins that can recognize and bind (i.e., read) one modification, such as a specific histone modification, while having the ability to modify another (write), such as DNA methylation, for instance. Examples of such proteins are the de novo DNA methyltransferases DNMT3A and B, which possess a specific binding domain, termed PWWP domain, that can bind H3K36me3 while being able to catalyze the methylation of CpGs in the vicinity (Neri et al. 2017) (Fig. 3).

9.2.3 Non-coding RNA's

The field of non-coding RNA research is comparably young, and several new species of epigenetically active non-coding RNAs have been identified in recent decades. These include micro RNAs (miRNAs), piwi RNAs (piRNAs), small interfering RNAs (siRNA), snRNAs, and long ncRNAs, among others (Wei et al. 2017). Similar to the crosstalk between histone modifications and DNA methylation, non-coding RNAs can also act through or interact with other epigenetic modifications and their machinery. The piRNA-mediated silencing of repetitive elements in the germline of animals, for instance, is mediated through the interaction between the piRNA-Piwi protein complex with histone or DNA methylases that results in the transcriptional silencing of these regions in the germline (Holoch and Moazed 2015). Similarly, miRNAs have been shown to act through epigenetic modifiers, such as

histone methyltransferases, to epigenetically silence target genes via the modulation of chromatin structure (Yao et al. 2019).

9.3 Molecular mechanisms of epigenetic transcriptional memory in model organisms

The altered expression or responsiveness of genes to recurrent stimuli has been reported for a broad range of organisms, including single-cell eukaryotes like yeast and *Plasmodium*, as well as plants and animals, and they include a plethora of environmental stimuli, such as oxidative stress, heat stress, salt stress or nutrient stress, among others. These changes in expression or transcriptional responsiveness of certain genes have been shown to persist over days and weeks, and in some cases, even generations. Analyses of the mechanisms underlying these memory responses found correlations with several epigenetic marks, most prominently with histone modifications (Ding et al. 2012), but also with DNA methylation (Wibowo et al. 2016), and non-coding RNAs (Stief et al. 2014). Studies across a diverse range of organisms, including plants, yeast, and animals, implicate the histone modification H4K4me2 in the memory of previous stress exposures (D'Urso and Brickner 2017). For instance, experiments subjecting *Arabidopsis* to recurrent drought stress revealed that a subset of stress-response genes showed a decline of expression to pre-stress levels during recovery but superinduced expression during subsequent stress exposures (Ding et al. 2012). This changed behavior in expression suggested that these genes were somehow "primed "for faster induction in response to recurrent stress. Interestingly, these "primed" genes also exhibited localization of poised RNA Polymerase II (RNA Pol II) and increased H3K4 tri- and dimethylation levels in their promoter region that persisted for at least several days. The epigenetic priming of such genes is thought to be regulated through specific transcription factors that interact with epigenetic modifiers to control the expression of such "trainable genes" in response to recurrent but not to the initial event. For instance, Heat Shock Factor-like transcription factor HSFA2 was shown to bind the promoter of heat stress genes in *Arabidopsis* during recurrent heat stress. However, the observed effects on heat stress gene expression persisted longer than the binding of HSFA2, which suggests that its function is related to the epigenetic priming required for “memory” (Lämke et al. 2016). Similar observations of increased responsiveness of stress response genes during recurrent stimuli have also been made in yeast (Brickner et al. 2007) and human cell lines (Gialitakis et al. 2010). While most of these experiments monitored environmental “memory” over days, inter- and transgenerational memory have also been reported in plants (Wibowo et al. 2016) and animals (Perez and Lehner 2019). These effects were associated with changes in DNA methylation patterns of certain genes or regions in the genome or the expression of non-coding RNAs, such as siRNAs. Experiments in *Arabidopsis*, for instance, revealed that exposure of plants to hyperosmotic stress for two successive generations triggered an intergenerational stress memory that increased survival of offspring in response to hyperosmotic stress. This phenotype was shown to correlate with RNA-mediated differential methylation within the promoter region of stress response genes, and offspring from plants mutant for components of the RNA mediated DNA methylation, or DNA methylation removal machinery did not show the same enhanced survival. However, this "priming", which was inherited through the mother, was reset after a single generation without stress exposure (Wibowo et al. 2016).

9.4 Corals and phenotypic plasticity

The ability of organisms to phenotypically respond to the environmental conditions they experience, or undergo acclimatization, is an essential mechanism to mitigate adverse environmental effects within their lifetime. This ability becomes even more important during

times of fast and drastic changes that might outpace the species ability to adapt through evolutionary adaptation. Generally, it is assumed that drastic changes in the environment affect sessile organisms, such as plants and corals, more than mobile ones, as they cannot simply move away. Similarly, phenotypic plasticity may also be more critical for organisms with long generation times as their ability to adapt through natural selection might not be fast enough to mitigate rapid changes in their environment.

Reef-building corals form the structural foundation of one of the most productive and biodiverse ecosystems on our planet, coral reefs. However, these ecosystems are under threat due to climate change-driven increases in temperature and other anthropogenic stressors (Hoegh-Guldberg et al. 2017). Corals are so called holobionts, i.e. metaorganisms composed of several organisms, including the coral host, dinoflagellate symbionts and a diverse assemblage of associated microbes. These metaorganisms live close to their thermal maximum (Baker et al. 2008), a trait that was likely evolutionary beneficial in the stable climate of tropical oceans where these ecosystems flourish. However, due to the unprecedented speed in global temperature rise, these ecosystems and their inhabitants now face serious danger of extinction (Hoegh-Guldberg et al. 2017). In contrast to most other animals, corals share many traits with plants. They are also sessile, require access to light to support algal symbiont photosynthesis, reproduce clonally, and potentially become hundreds and even thousands of years old (Devlin-Durante et al. 2016). It is therefore conceivable that their inability to avoid changes in their environment by actively moving elsewhere might have selected for extensive physiological plasticity as an alternative means to mitigate changes in their environment, as known from plants (Schlichting 1986). The need for a mechanism that allows for phenotypic acclimation to environmental changes within the lifetime might be exacerbated by their comparably long generation times and longevity (Torda et al. 2017). Longer generation times reduce the adaptability of a species through natural selection, while longer life spans increase the probability that individual genotypes face dramatic changes in their environment at one time or another. In line with these assumptions, corals are notorious for their ability to adjust their growth morphology to the prevailing conditions, most notably to the current and light environment they experience (Todd 2008). This increased potential for environmentally driven colony morphology, which is in itself a form of phenotypic plasticity, might already reflect an inherent flexibility of these organisms in responding to their environment.

9.5 Acquired tolerance to environmental stress in corals

Much like their plasticity in growth morphology, experiments looking into the ability of corals to respond to environmental changes suggest that they possess the intrinsic potential to mitigate stress through phenotypic plasticity. More importantly, though, some studies suggest that corals that have been exposed to specific stressors might even be able to maintain a certain degree of enhanced tolerance towards these stressors over months and even years.

9.5.1 Acquired tolerance in adult corals

One of the most important studies in this regard came from Phuket, Thailand, where colonies of the coral *Coelastrea aspera* were reported to display differing bleaching patterns between east and west-facing sides of colonies during a bleaching event in 1995 (Brown et al. 2000). These intra-colony differences in bleaching susceptibility were attributed to differences in the amount of solar irradiance received by the east and west-facing sides of the colonies during the year. During spring, higher irradiance levels at the western side were thought to induce acclimation responses that protected these sides from bleaching during hot summers. Interestingly, this protection was retained over more than a decade, even when colonies had

been rotated so that the previously pre-exposed western sides ceased to receive the environmental cue for more than ten years (Brown et al. 2015). These findings suggested that previous exposures to stress triggered responses akin to environmental memory.

Changes in bleaching susceptibility in response to previous stress exposures were also reported from other regions across the globe, particularly after recurrent bleaching events (Pratchett et al. 2013; Hughes et al. 2019; Fisch et al. 2019; Gintert et al. 2018; Fox et al. 2021; Guest et al. 2012). The bleaching severity of three major coral genera (*Acropora, Pocillopora, and Porites*) in the Great Barrier Reef (GBR) was reported to be much lower than expected during the 2002 bleaching despite higher and longer temperature stress compared to the previous 1998 bleaching event. Similar observations were also made during the back-to-back bleaching events of 2016 and 2017 in the GBR. The colonies that survived the first bleaching event showed higher resistance despite more severe or longer heat stress (Hughes et al. 2019). Comparable observations were also made in the Florida Keys after the consecutive bleaching events of 2014 and 2015, where >3 times fewer colonies bleached in the second year despite higher and more prolonged temperature stress (Gintert et al. 2018; Fisch et al. 2019). These studies were further echoed by analyses of coral skeletons that showed a decrease of cores with 'stress bands' following successive bleaching events (DeCarlo et al. 2019) as well as reduced impact on growth during subsequent bleaching events (Clarke et al. 2019). In summary, these findings suggest that coral populations across the globe showed decreased bleaching susceptibility in response to recurrent stress events.

However, most of these studies did not track specific colonies over time or only analyzed coral skeletons. Therefore, factors other than environmental memory, such as changes in symbiont associations, selective mortality, or even genetic adaptation, cannot be entirely excluded as the cause for the observed decreases in bleaching susceptibility. Similarly, these studies do not allow differentiating between acclimation responses in the host or the symbionts, as was proposed for the case of *Coelastrea aspera* in Phuket (Brown et al. 2002). Conversely, a study on the great star coral *Montastraea cavernosa* on the effects of recurrent experimental bleaching found that increased bleaching resilience was associated with a change in symbiont type (Silverstein et al. 2015). Similar findings were made in other Caribbean coral species where increased bleaching resilience or recovery in response to recurrent bleaching events was best predicted by changes in the dominant symbiont strain (Grottoli et al. 2014).

Other studies using experimental pre-exposure treatments have found no, or contradicting effects of previous stress exposures on responses to recurrent events. For instance, a study on the coral *Acropora millepora* from the GBR found that symbionts showed increased ability to dissipate incoming light in response to recurrent stress, yet pre-exposure to the treatment did not affect the bleaching susceptibility of the coral host (Middlebrook et al. 2012). In contrast, a study on *Acropora aspera* by the same author found that colonies pre-exposed to temperature stress showed increased photoprotection and lost significantly fewer symbionts than colonies that were not pre-exposed (Middlebrook et al. 2008).

It should be noted, however, that lab experiments looking into the effects of pre-exposure treatments are often performed with recurrent stress events on shorter timescales in the range of days or weeks. While such shorter term acclimations to sub-bleaching pre-exposures are biologically relevant and have previously been proposed to promote beneficial effects during natural heat stress events (Ainsworth et al. 2016), it is questionable if these responses indeed reflect environmental memory that can persist over seasons and even years or if they are rather direct acclimation responses that only persist over shorter time frames.

9.5.2 Inter- and transgenerational acquired tolerance

Interestingly, pre-exposure and resulting subsequent environmental memory have not only been reported for adult coral colonies but have also been shown to affect coral offspring. Experiments on brooding colonies of the coral *Pocillopora damicornis* exposed to combined temperature and ocean acidification (OA) stress showed adverse effects on adult performance and resulted in smaller larvae. However, larvae from the high-temperature OA-treated parents showed reduced size-normalized respiration at ambient temperature but significantly higher respiration when exposed to the combined high temperature and OA treatment the parents were exposed to, suggesting some kind of phenotypic acclimation to the stressor (Putnam and Gates 2015). Similar potentially beneficial effects on *P. damicornis* larvae were also found when parents were pre-exposed to OA treatment alone, whereby offspring showed increased survival and growth during continued exposure (Putnam et al. 2020). While these studies suggest that corals might possess the ability to retain environmental information across a generation, it is yet unclear what the molecular mechanisms underlying this phenomenon might be. DNA methylation has frequently been proposed as the potential mechanism underlying inter- and transgenerational plasticity in other organisms and corals (Putnam et al. 2016) due to its higher potential for inheritance. However, while DNA methylation is broadly inherited in plants, its heritability in animals is usually low and restricted to few loci in the genome since the patterns are erased twice during development, first during early embryogenesis and then again during the development of the germline (Messerschmidt et al. 2014). Interestingly, though, it appears that corals are an exception to this, as a recent study suggests that corals broadly inherit parental DNA methylation patterns from both parents (Liew et al. 2020).

9.6 DNA methylation in corals

Current evidence for a role of DNA methylation in acclimation and phenotypic plasticity in corals comes from studies looking at changes in DNA methylation patterns in response to environmental changes (Putnam et al. 2016); (Liew et al. 2018; Dixon et al. 2018; Durante et al. 2019; Dimond and Roberts 2020; Rodríguez-Casariego et al. 2020; Putnam et al. 2016; Dimond et al. 2021). By nature, these studies are purely correlational as sophisticated technologies that allow for a site-directed modification of epigenetic marks do not exist for corals at the moment. Nonetheless, there is mounting evidence that DNA methylation patterns in the coral host respond to environmental changes in a treatment-specific fashion. Liew et al. (2018) showed that DNA methylation patterns in the coral *Stylophora pistillata* changed during long-term ocean acidification stress. Analysis of the differentially methylated genes showed enrichment of genes involved in the JNK pathway, whereby genes involved in its negative regulation displayed a pH-dependent increase in DNA methylation while genes involved in the positive regulation of the pathway exhibited a corresponding decrease. These changes, together with differential methylation of genes involved in cell cycle progression, insulin signaling, and organism growth, correlated with a significant increase in coral cell and polyp size. The authors concluded that the corals responded to ocean acidification stress, and the consequent impairment of calcification, with an increase in cell and polyp size that allowed them to maintain growth rates despite reduced calcification rates. A similar correlation between changes in DNA methylation patterns and phenotypic responses was also reported in *Acropora millepora* by Dixon et al. (2018) in response to colony transplantation. Analysis of the DNA methylation levels across loci revealed that DNA methylation patterns of transplanted fragments showed increasing similarities to the patterns of local colonies, a trend that was also observed in the coral *Porites astreoides* (Dimond and Roberts 2020). These studies strongly suggest that the local environmental conditions shape DNA methylation patterns in corals. Interestingly, these studies also found that phenotypic changes

showed a stronger correlation with changes in DNA methylation than with changes in gene expression (Liew et al. 2018; Dixon et al. 2018). These findings were echoed by a study looking at the correlation between DNA methylation patterns and phenotypic variation in stress response between clone mates (Durante et al. 2019). The authors concluded that DNA methylation contributed in part to the observed differences in bleaching susceptibility, however, most of the variation observed remained unexplained.

While correlations between environmental changes and DNA methylation patterns are evident across multiple studies (Liew et al. 2018; Li et al. 2018; Dixon et al. 2018), the mechanism, i.e., the mode of action underlying these correlations, is still poorly understood. This has created a lot of skepticism (Torda et al. 2017), not only in coral research but also in many other fields. Some of this skepticism can be attributed to misconceptions about how DNA methylation might act in regulating transcription. Studies in model organisms like *Arabidopsis* and mice suggest that DNA methylation can act directly on transcription by preventing transcription factors from binding to their regulatory elements, thereby providing a mechanism by which DNA methylation could act upstream of transcription. However, newer studies show that the regulation of gene expression through the methylation of regulatory regions is much more complex than previously thought. While some transcription factors and regulatory proteins appear to exhibit reduced binding in response to DNA methylation, others do not seem to be affected or are even found to bind to methylated binding sites preferentially (Yin et al. 2017; Héberlé and Bardet 2019; Harris et al. 2018). Furthermore, DNA methylation studies in invertebrates, including coral, revealed that highly methylated promoter regions, as found in plants and animals, are basically missing in their genomes and that DNA methylation is mainly restricted to gene bodies (Sharif et al. 2010). This does not necessarily rule out a potential direct regulatory role for DNA methylation as other regulatory elements, such as enhancers, exist that could allow transcriptional regulation through the modification of their methylation status. However, coral genomics is still in its infancy, and current genome annotations do not include any known and validated regulatory elements that would allow the study of such functions.

Nonetheless, clear correlations between gene body methylation and gene expression exists in eukaryotes (Zemach et al. 2010), whereby methylated genes show significantly higher expression levels than non-methylated genes . This positive correlation between gene body methylation and gene expression, however, has fueled the expectation that changes in gene body methylation have to correlate with respective changes in gene expression, whereby increases in DNA methylation are expected to result in increases in gene expression. However, analyses of DNA methylation and corresponding gene expression changes have so far failed to show any correlation (Dixon and Matz 2021; Dixon et al. 2018; Liew et al. 2018), thus feeding skepticism around the role and function of DNA methylation in gene regulation and phenotypic plasticity in corals (Torda et al. 2017).

The misconception here is the interpretation that gene body methylation itself can induce gene expression, which is not the case. Newer studies suggest that gene body methylation is actually a consequence of transcription. The consequent interpretation of the observed correlation between gene body methylation and transcription is thus that it is the increased gene expression that leads to increases in gene body methylation, and not the other way around. Studies in model organisms suggest that gene body methylation instead functions as a mechanism to improve transcriptional fidelity of genes by preventing the transcriptional machinery from erroneously binding to cryptic promoter sequences within the gene body (Neri et al. 2017). Such spurious transcription initiation events could result in the expression of partial transcripts and, in the worst case, partial proteins that could interfere with proper protein function. Analysis of the enzymatic machinery involved in the establishment of gene

body methylation in mice revealed functional interaction between the histone modifications H3K36me3 and DNA methyltransferase DNMT3B that link these mechanisms to create an epigenetic landscape conducive to high expression and bonafide transcription (Neri et al. 2017). Comparison of levels of spurious transcription between methylated and non-methylated genes in the coral *S. pistillata* suggest that this function of gene body methylation is indeed conserved in corals (Liew et al. 2018). This conclusion was further supported by similar findings in the sea anemone *Aiptasia pallida,* where methylated genes also showed reduced spurious transcription and association with H3K36me3 as shown for mice (Li et al. 2018; Neri et al. 2017).

9.7 Histone modifications in corals

Current studies employing nucleotide resolution of epigenetic marks in corals are limited to the study of DNA methylation. The studies on histone modifications available to date have instead used bulk assays based on enzyme-linked immunosorbent assays and western blotting to determine overall changes for the two histones H2A.X (Rodriguez-Casariego et al. 2018) and H3 (Roquis et al. 2021). These studies, however, showed that both histone variants respond to environmental changes. Western blot analysis of histone H3 in the coral *Pocillopora acuta*, for example, revealed unusual clipping in healthy colonies that was not present in bleached colonies (Roquis et al. 2021). Histone clipping has been observed in a broad range of organisms from yeast to mammals and has been proposed to be involved in the regulation of various processes (Azad et al. 2018). It is currently unknown what the function in corals could be, and additional experiments are needed to confirm this response and determine its function during bleaching. A more concrete model on the potential consequences of changes in histone modifications during stress comes from the study of the histone variant H2A.X in the coral *Acropora cervicornis*. Rodriguez-Casariego et al. (2020) reported that H2A.X showed significant decreases in phosphorylation ratio in response to increased nitrogen availability. H2A.X is a vital histone mark involved in DNA damage repair. Upon DNA double strand breaks it is phosphorylated and accumulates DNA repair proteins at the damaged site (Maréchal and Zou 2013; Sharma et al. 2012). The authors, therefore, concluded that the reduced phosphorylation ratio of H2A.X might reflect an impaired ability of the coral host to repair DNA damage. Consequently, the observed changes might not reflect a directed epigenetic response of the host to mitigate the effects of nutrient stress but rather a negative consequence resulting from phosphor depletion due to increased proliferation of the symbiont population. Such adverse effects of nutritional or environmental stress on the host phenotype mediated through epigenetic marks have also been observed in other animals, the most prominent example being the agouti mouse (Jirtle and Skinner 2007). These examples have contributed to an increasingly critical view on the role of epigenetic mechanisms in organismal acclimation as they call into question if the positive responses observed in other experiments are indeed specific acclimations responses aimed at mitigating stress. In other words, they question if the observed responses are just random epigenetic variations on which selection acts or if they are truly directed responses induced by the environment (Heard and Martienssen 2014).

Overall, studies on histone modifications in corals are currently very limited. The main limitation for more in-depth studies looking into the role of histone modifications in coral acclimation and acquired tolerance is the lack of protocols that allow overcoming the particular issues associated with working with these organisms. Extraction of high-quality chromatin required for chromatin immunoprecipitation (ChIP), which allows the position-specific analysis of histone modifications in the genome, is hampered by the difficulties associated with removing the coral skeleton as it requires additional dissolution steps that

impact chromatin quality and subsequent immunoprecipitation. However, studies in the anthozoans *Nematostella vectensis* and *Aiptasia pallida* suggest that the location and function of many histone modifications, including the interactions of H3K36me and gene body methylation, are conserved in anthozoans (Schwaiger et al. 2014; Li et al. 2018).

9.8 Non-coding RNAs in corals

Similar to histone modifications, research on non-coding RNAs in corals is sparse, with only two studies on miRNAs in the corals *Stylophora pistillata* (Liew et al. 2014) and *Acropora digitifera* (Gajigan and Conaco 2017) and another looking into potential long ncRNAs in the latter (Huang et al. 2019). However, more in-depth research is available from other anthozoans, including the sea anemones *Aiptasia pallida* (Baumgarten et al. 2018) and *Nematostella vectensis* (Praher et al. 2017; Fridrich et al. 2020). These studies, however, provide some mechanistic insight that suggests that at least some of the functions of miRNAs and piRNAs are evolutionarily conserved in cnidarians.

9.9 A model for epigenetic memory and acquired tolerance in corals

The transcriptional regulation of stress responses through epigenetic mechanisms initially gained a lot of traction in many different fields as it provides a mechanism by which epigenetic mechanisms can directly regulate the expression of genes. Many examples are found in the literature that report specific, persistent changes in stress response genes associated with phenotypic responses of organisms to stress (D'Urso and Brickner 2017). In plants, epigenetic adaptation to a range of stressors has been reported that are now investigated for agricultural exploitation to increase critical traits such as drought and salt resistance, among others (Kakoulidou et al. 2021). However, as with studies in corals, there is often little causal evidence for upstream functions of epigenetic mechanisms in these responses. By nature, epigenetic mechanisms themselves are not sequence-specific, i.e., they do not possess independent sequence-specific mechanisms that could direct the epigenetic machinery to a specific location in the genome where it should act and thus require other signals, such as transcription factors, RNAs, or the transcription complex itself to direct the machinery to the right locations in the genome. Therefore, a mechanistic model in which epigenetic marks do not initiate changes in transcription but rather "register, signal or perpetuate altered activity states", as Bird et al. (2007) proposed, appears to be the most parsimonious. Indeed, newer hypotheses in the field are increasingly focused on the role of transcription and transcription factors in regulating epigenetic mechanisms, also at the level of regulatory elements such as promoters and enhancers (Blattler and Farnham 2013).

While the genomic resources currently available for corals do not allow in-depth studies on the role of methylation on regulatory elements, there is still enough evidence to propose a potential model for epigenetically controlled transcriptional “memory” and acquired tolerance.

In this model, histone modifications and gene body methylation work in concert to “prime” genes for improved response during recurrent stress. The conservation of interactions between the histone modifications H3K36me3 and gene body methylation, as observed in corals and anemones, provides a molecular mechanism to prime genes for high induction and transcriptional fidelity. Similarly, studies in the sea anemone *N. vectensis* revealed conservation of the localization of the histone mark H3K4me2 (Schwaiger et al. 2014), which has been shown to coincide with poised RNA Pol II and transcriptional memory in a range of organisms (D'Urso and Brickner 2017). Furthermore, this study also confirmed the conservation of its co-localization with gene body methylation, suggesting that genes marked

by H3K4me2 are also often methylated, albeit in mutually exclusive regions of the gene (Schwaiger et al. 2014). The localization of H3K4me2 to primed genes is supposedly controlled by specific transcription factors that regulate transcriptional responses to recurrent stress events, such as the Heat Shock Factor A2 in *Arabidopsis*. While this family of heat stress regulating transcription factors is less diverse in invertebrates, with only a single copy of Heat Shock factor 1 (HSF1) found in coral genomes (Cleves et al. 2020), it has been recently studied in the coral model Aiptasia using CRISPR mediated knockdown. These experiments confirmed its essential role in regulating the heat shock response in Aiptasia larvae (Cleves et al. 2020).

Based on these combined findings, a model can be proposed in which specific transcription factors (e.g., HSF1) activate initial gene expression of downstream stress response genes (Fig. 4). Active expression of these genes leads to the recruitment of histone methyltransferases, such as SetD2, by the RNA Pol II complex and the subsequent trimethylation of H3K36 within the gene body. This histone modification is actively bound by DNMT3, which subsequently methylates the gene body at CpG positions. The methylation of the gene body reduces spurious transcription by preventing RNA Pol II from binding to cryptic promoters within the gene body of highly expressed genes which allows for high, bonafide expression. Notably, the methylation of these genes persists even after the initial stress has ceased, thereby preserving the epigenetic optimization for high expression. Activation of these genes further leads to the dimethylation of histone H3K4 in the promoter region through the association of specific histone lysine methyltransferases with the respective transcription factor, e.g., HSF1. The "priming" of the promoter with H3K4me2 leads to the recruitment of poised RNA Pol II. In this state, the gene is transcriptionally silent but ready to be induced and highly expressed as rate-limiting steps for transcription initiation are bypassed (D'Urso and Brickner 2017). The gene body remains methylated to promote full-length transcription and ensure high transcriptional output upon recurrent induction.

This epigenetic priming might allow corals to respond faster to changes in their environment, as the genes necessary for an efficient response are already optimized for fast induction and high and efficient expression. Primed genes might thus be expressed faster, be superinduced, or sensitized to respond at a lower stress threshold (Fig. 5, (Lämke and Bäurle 2017)). While it is somewhat abstract to imagine to what extent such priming might translate to improved stress response and increased survival, studies in plants suggest that such priming of stress response genes can indeed increase survival rates significantly (Friedrich et al. 2021; Wibowo et al. 2016). However, how long these epigenetic optimizations persist over time and to what extent they can increase resilience and survival in response to increasing temperature stress events in corals has still to be explored. Filling these knowledge gaps is not only essential for our understanding of the intrinsic mechanisms these organisms possess to respond to increasing temperature stress, but also to assess their potential for their exploitation in coral reef restoration as part of broader assisted evolution approaches (van Oppen et al. 2015). The increase of resilience via epigenetic priming through controlled stress exposure of corals, also termed environmental hardening (Putnam 2021; Hackerott et al. 2021), holds great potential for the improvement of current restoration strategies by increasing the stress resilience, and thus the survivability, of the coral stocks produced.

9.10 Future directions

Although current studies only provide indirect proof for a role of epigenetic mechanisms in acquired tolerance in corals, their combined results indicate that such mechanisms might exist at least in some corals. The main limitation of many studies to date is the lack of long-term

monitoring of individual colonies on multiple levels, including the host as well as the associated symbionts. At present, most studies do not allow excluding other factors as potential causes for the observed responses, such as selective mortality, a change of symbiont type, or a persistent acclimation response of the symbionts to name a few. Furthermore, even in cases where effects are observed, it is not clear if these responses are indeed "adaptive", i.e. specifically directed responses towards mitigating stress or if they are even beneficial to begin with. Future studies will require experimental designs that include monitoring and tracking of individual colonies on all levels of the holobiont over time to exclude other factors and to determine if and to what extent corals can acquire thermal tolerance through environmental memory. Colonies should also be monitored on several physiological levels, including growth and reproductive output, to determine if the responses are indeed adaptive and to identify potential tradeoffs. Ideally, these studies should be performed over one or more generations to also validate if, and to what extent acquired tolerance can be transmitted to offspring.

Besides providing additional insights into the prevalence of acquired tolerance in corals, such studies will also aid in identifying suitable study systems to experimentally dissect the role of epigenetic mechanisms in these responses and to provide essential information for the development and application of environmental hardening approaches in coral restoration. However, the development of applications for restoration will also require extensive work looking into the factors involved in stress priming (i.e. the different types of stimuli, their magnitude and duration, frequency, variability, and the rate of change required) to develop suitable protocols and approaches for coral reef restoration (Hackerott et al. 2021).

Similar to the validation and identification of clear cases of acquired tolerance, the molecular mechanisms that confer this "environmental memory" also have to be functionally confirmed in corals. While the few studies on epigenetic mechanisms available to date show strong correlations between epigenetic marks and phenotypic responses, they do not provide enough causal evidence as proof for epigenetic regulation as the underlying mechanisms for the acquisition of thermal tolerance. Future studies need to aim at providing functional proof. This will require the establishment of molecular biological techniques for corals that allow the sequence specific manipulation of the epigenome i.e. the DNA methylation status or the associated histone modifications of specific loci. Primarily, this means that protocols for stable genetic transformation are required, which presents a significant hurdle in corals due to their long generation times and comparatively difficult husbandry. However, other molecular tools, such as ChIP-seq protocols for the genome-wide study of histone modifications or HITS-Clip for the study of small RNA and target interactions, need to be developed. Given the current experimental limitations associated with working with corals, other model organisms, such as the sea anemones *Aiptasia* (Baumgarten et al. 2018; Baumgarten et al. 2015) and *Nematostella* (Röttinger 2021), can be used in the meantime. These models allow overcoming the technical difficulties associated with working with corals and help define, refine and test current models and hypotheses. Especially *Nematostella* offers a range of molecular tools (Röttinger 2021), including stable transgenesis (Renfer et al. 2010) and CRISPR-Cas mediated knock-out (Ikmi et al. 2014), that make it readily available to address some of these questions in a related organism. It should be noted though that many of these questions are still outstanding even in model organisms for which many of the required tools already exist. Arguably one of the biggest issues in many of these model organisms is the separation of genetic and epigenetic effects, which poses a significant problem as it is neigh impossible to exclude genetic differences or changes (i.e. mutations) as the source of the observed effects (Heard and Martienssen 2014). This is where corals, being clonal organisms,

might help overcome this critical problem. However, irrespective of all the potential problems and issues, understanding the mechanisms by which corals can respond to environmental changes and how these mechanisms can be harnessed to increase coral survival should continue to be a central focus of coral research.

Figures

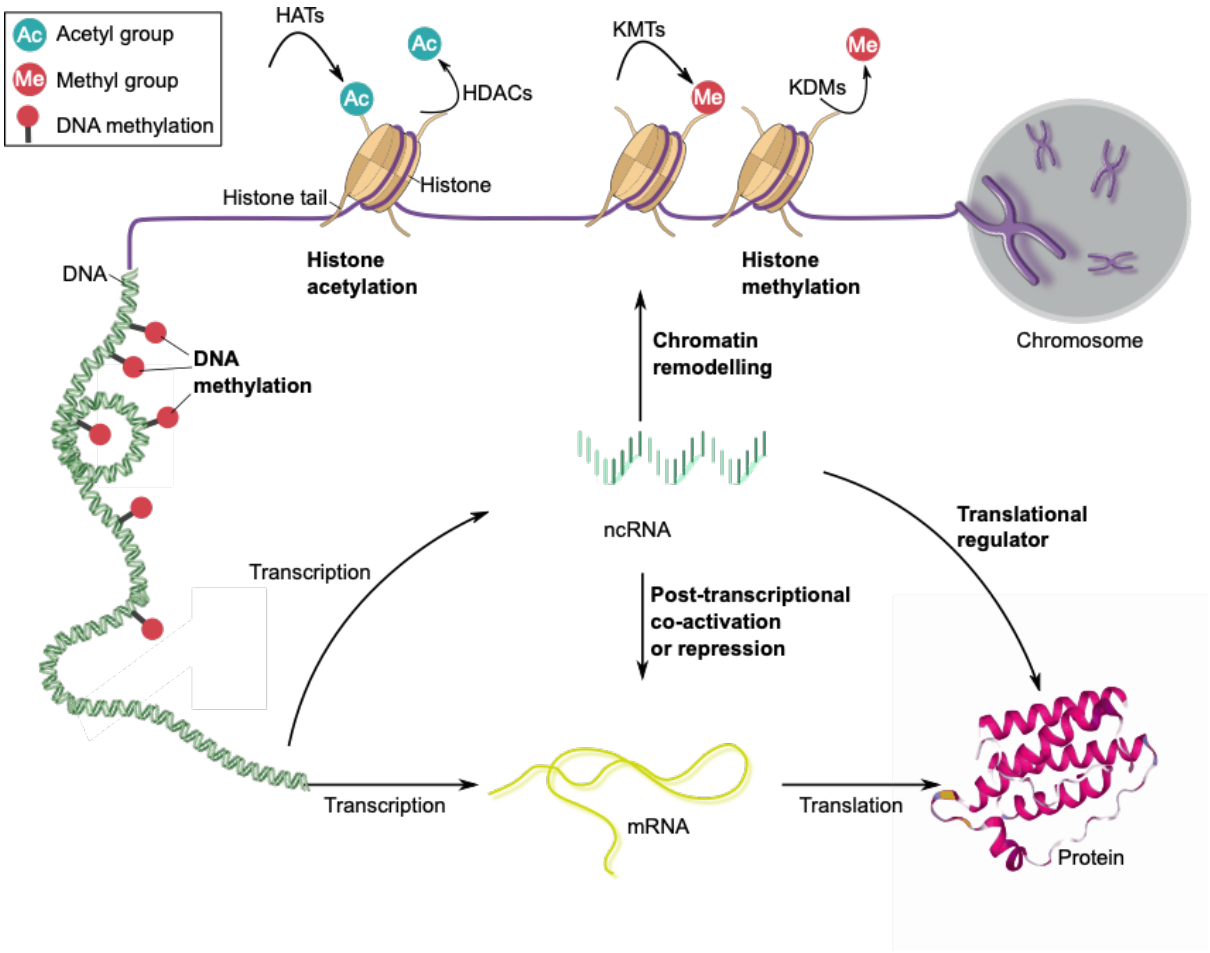

Fig. 1 Epigenetic mechanisms present in corals.

Histone modifications are covalent additions of chemical groups to specific amino acid residues in the N-terminal tails of the core-histone H2A, H2B, H3, and H4. These modifications are catalyzed by histone-modifying and can include the methylation or acetylation of lysine and arginine residues, among others. The chemical modification of the histone tails changes the chromatin structure by modifying how histones interact with DNA and each other. DNA methylation is the covalent addition of a methyl group to specific nucleotides in the DNA, most predominantly on cytosines in a CpG context. These methylation marks can be mitotically and meiotically inherited and modify how proteins, such as transcription factors and the transcription machinery, interact with the DNA. Non-coding RNAs encompass a range of different RNA species that can regulate gene expression at the level of translation or transcription through their interaction with the translational machinery or other epigenetic mechanisms, such as DNA methylation and histone modifications (adapted from Joosten et al. (2018)).

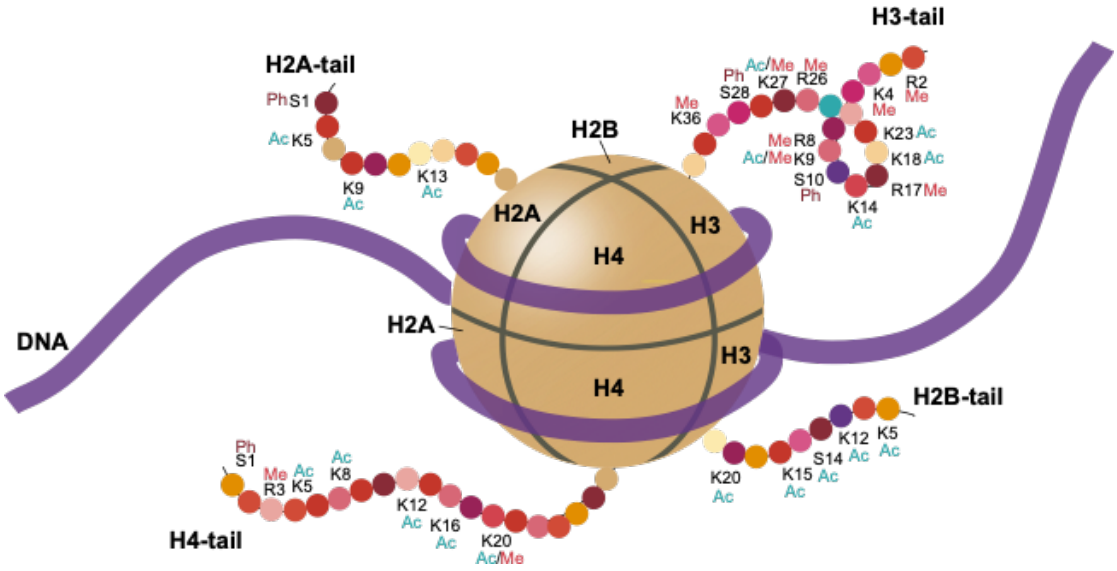

Fig. 2 Histone modifications and the nucleosome

A nucleosome is composed of a histone octamer, formed by the four core histones H2A, H2B, H3, and H4, and ~150 bp of DNA that is wound around it. The N-terminal tails of the histones contain specific amino acid residues that can be chemically modified through the addition of methyl, acetyl, phosphor, or ubiquitin groups.

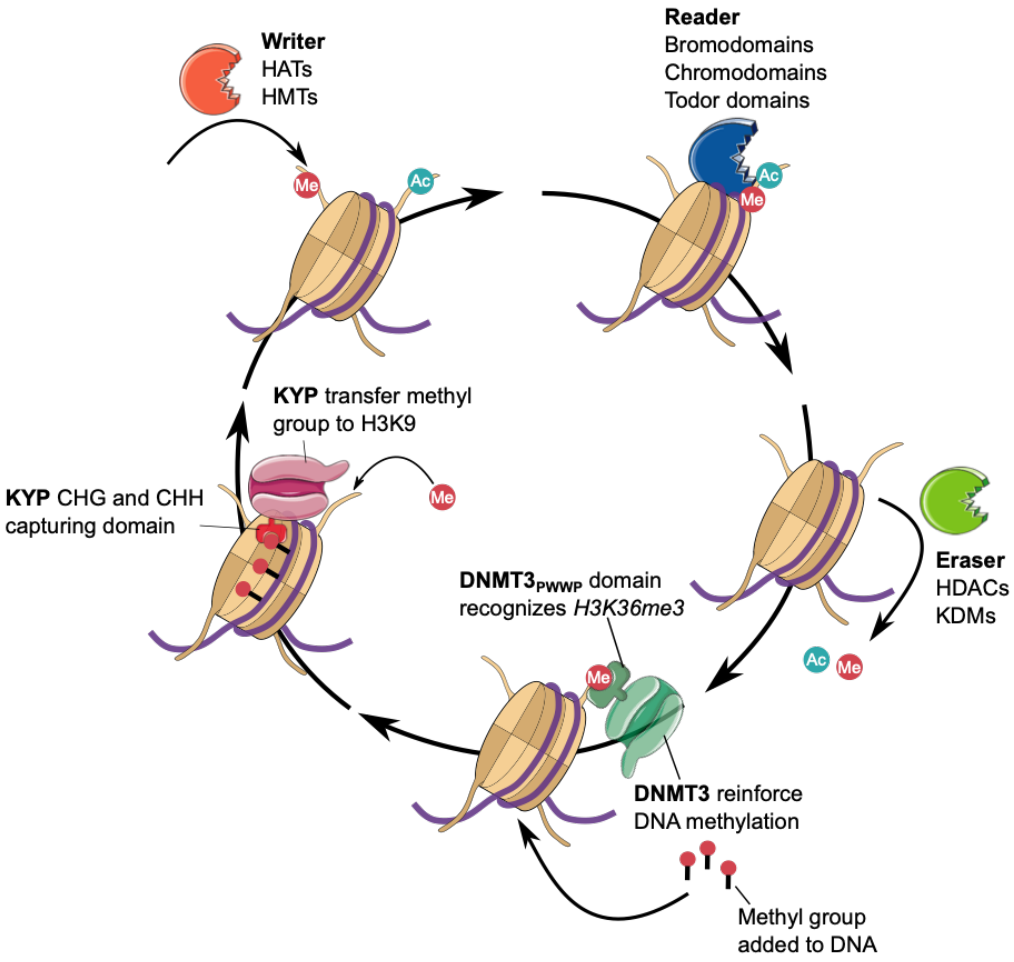

Fig. 3 Epigenetic "writers", "readers", "erasers" and crosstalk

Epigenetic mechanisms function through chemical modifications that are catalyzed and recognized by specific enzymes. The addition of chemical modifications is performed by "writers", enzymes that catalyze the covalent addition of a respective chemical group, such as methyltransferases that catalyze the addition of methyl groups or acetyltransferases that catalyze the addition of acetyl group. These modifications are bound, and therefore recognized, by effector proteins called "readers" and can also be removed by specific enzymes, called "erasers". Some enzymes can also bind and recognize one modification while being able to write another modification. Such enzymes can also link different epigenetic mechanisms, such as DNA methylation and histone modifications. One example is the *de no*vo DNA methyltransferases DNMT3B that can recognize and bind the histone modification H3K36me3 and methylate DNA at CpG positions in the vicinity.

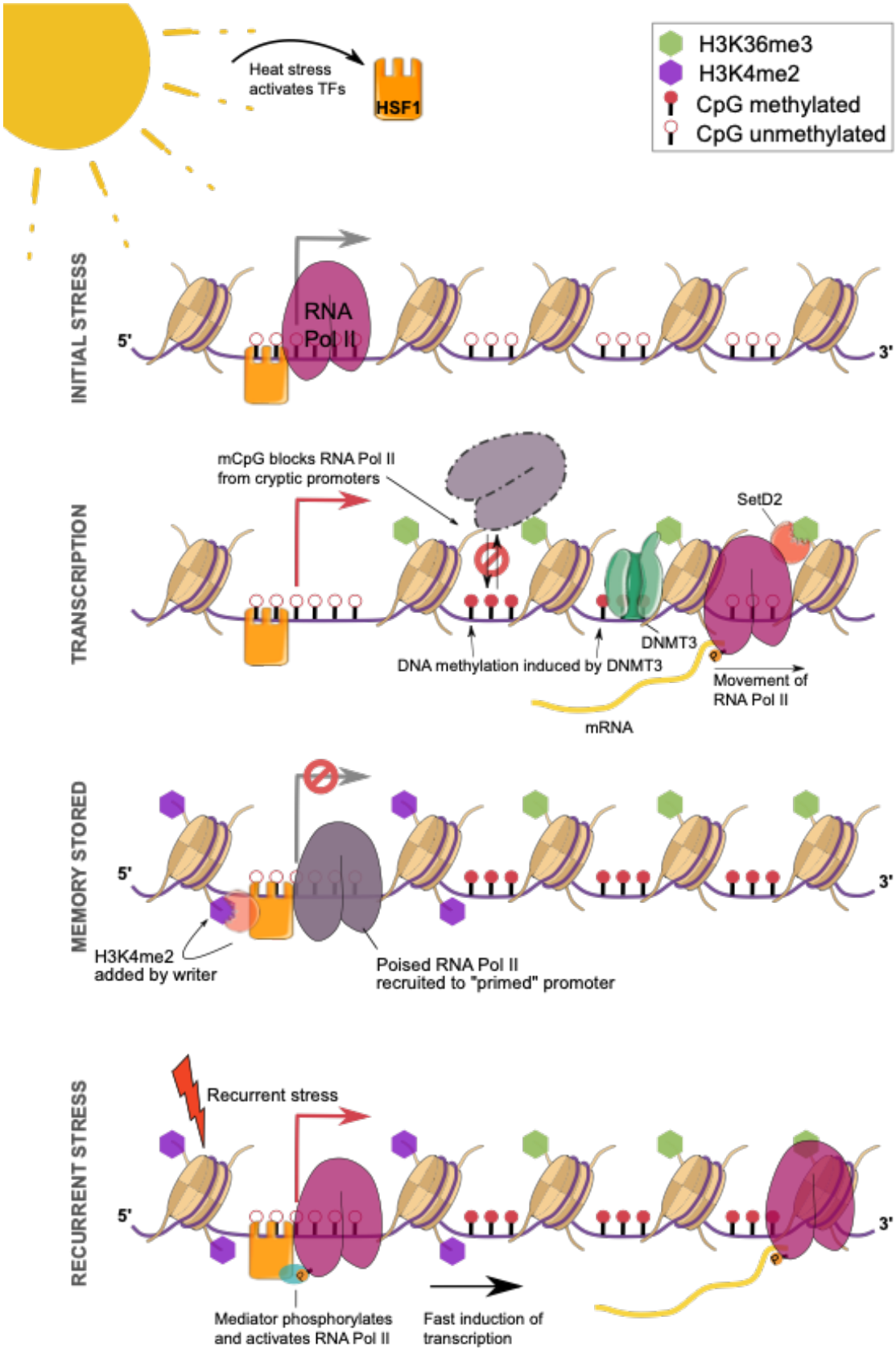

Fig. 4 Theoretical model for the epigenetic regulation of acquired tolerance

Initial heat stress triggers the expression of heat stress factors that activate downstream stress response genes. Active transcription of stress response genes leads to the association of the histone methyltransferase SetD2 with RNA polymerase II complex (RNA Pol II) and the subsequent trimethylation of histone H3 at position lysine 36 (H3K36me3). H3K36me3 is actively bound by the *de novo* DNA methyltransferase DNMT3B, which methylates CpGs in the gene body. Methylated CpGs prevent RNA Pol II from binding to cryptic promoters within the gene and inducing spurious transcription, thus promoting the expression of full-length transcripts. Interaction of a histone methyltransferase with the transcription factor at the gene promoter leads to the dimethylation of histone 3 at position lysine 4 (H3K4me2) and the recruitment of poised RNA Pol II (unphosphorylated). In this state, the gene is transcriptionally inactive but "primed" and ready to be induced. Upon recurrent stress, the transcription factor binds to the promoter of the "primed" genes and activates poised RNA Pol II through a mediator that phosphorylates RNA Pol II.

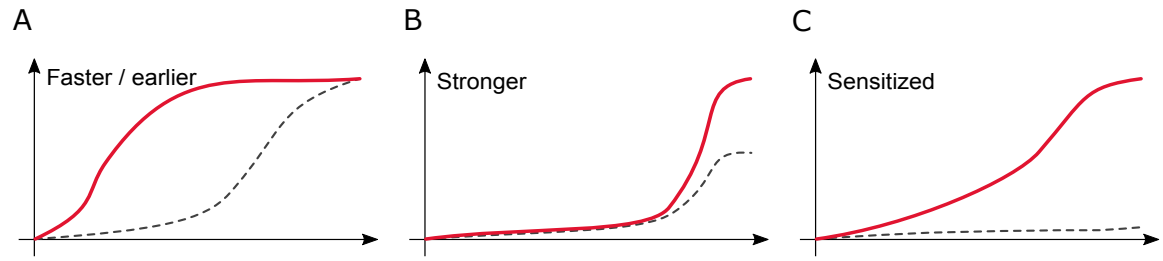

Fig. 5 Transcriptional memory and responses of "primed" genes

Transcriptional memory at primed genes can improve the response to recurrent stress events in different ways. "Primed" genes can respond A) faster, as rate-limiting steps have already been bypassed; B) stronger, as epigenetic optimization allows for high induction and expression; C) in a sensitized fashion requiring lower thresholds to trigger a response (adapted from Lämke and Bäurle (2017).

Acknowledgments

I would like to thank Dr. Kashif Nawaz for creating and providing the figures included in this chapter.

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