Evidence of an Epigenetics System in Archaea

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ABSTRACT: Changes in the phenotype of a cell or organism that are heritable but do not involve changes in DNA sequence are referred to as epigenetic. They occur primarily through the gain or loss of chemical modification of chromatin protein or DNA. Epigenetics is therefore a non-Mendelian process. The study of epigenetics in eukaryotes is expanding with advances in knowledge about the relationship between mechanism and phenotype and as a requirement for multicellularity and cancer. However, life also includes other groups or domains, notably the bacteria and archaea. The occurrence of epigenetics in these deep lineages is an emerging topic accompanied by controversy. In these non-eukaryotic organisms, epigenetics is critically important because it stimulates new evolutionary theory and refines perspective about biological action.

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Epigenetics in Eukaryotes

In eukaryotes, chromatin protein (histones) and/or DNA undergo reversible chemical modification in response to a broad range of physiological and environmental triggers. These modifications lead to changes in gene expression that in turn alter cellular and organismal phenotypes. Such changes are important because they influence adaptive traits such as fitness and reproduction. Epigenetics thus has broad relevance to ecological processes. In eukaryotes, the components of chromatin epigenetic mechanisms include histones, histone modification writers, readers and erasers,[1–4] and histone remodelers.[5] DNA methylation epigenetic mechanisms include enzymes that deposit,[6] read,[7] and erase[8] CpG methylation. It is known that these epigenetic components are ubiquitous across eukaryotes because of their broad genomic conservation.

Epigenetics in Bacteria

In bacteria, DNA methylation is also ubiquitous although chromatin protein modification does not appear. Bacterial DNA methylation is fundamentally unlike that observed in eukaryotes. These differences raise questions about the extension of epigenetics to bacteria through this mechanism. Bacterial DNA methylation is site specific and not patchy as observed in eukaryotes. It occurs at adenine or cytosine residues located within canonical usually hexameric sequences. Methylation is relatively complete across bacterial genomes where all canonical sequences gain methylation. It is also intimately paired with DNA restriction where double-strand cleavage occurs at or near the canonical sequence. The so-called DNA restriction modification (methylation) systems are broadly distributed across the bacterial domain with innumerable examples of these highly conserved features. Occasionally, restriction or modification enzymes occur in an orphan genomic state without a matching paired gene and its activity. In the case of orphan modification, it has been suggested that they confer alternative functions such as stationary-phase phenotypes[9,10] but also that the methylases may be selfish genetic elements.[9–11] Although occasional references to these functions have been called epigenetic, the lack of underlying epigenetic mechanisms raises concerns about their relationship to true epigenetics. For example, variation in these orphan examples of DNA modification follows a stochastic pattern of heritability. That means that derivative (progeny) cells lose DNA methylation. A more common term encompassing this and other stochastic phenomena in bacteria is phase variation. Phase variation means that a phenotype and its molecular basis rapidly change at high frequency within a clonal population. This pattern contradicts the definition of epigenetics. Genes affected by phase variation include the *Escherichia coli* pili gene *pap*[12] and outer membrane protein antigen Ag43,[13] the Salmonella O-antigen chain length genes,[14] and others.[15] A second fundamental issue is that bacterial DNA methylation can undergo variation in pattern without a corresponding change in phenotype. This lack of connection between epigenetic state and phenotype implies that variation in bacterial DNA methylation has no causal role and may result from evolutionary drift.

Epigenetics in Archaea

Archaea constitute a distinct domain from bacteria and eukaryotes. However, they harbor many mechanisms found in eukaryotes that use proteins that have a common evolutionary origin. These range from proteins necessary for DNA replication, DNA repair, and RNA transcription to protein translation. Consequently, it has been proposed that eukaryotes arose

from archaea, the so-called 2-domain tree of life.[16] If this is true, then eukaryotic-like epigenetics may be evident in archaea. Early studies of methanogenic archaea belonging to the euryarchaeota phylum led to the discovery that they and other members of their phylum use histones to condense and organize their genomic DNA.[17] It was then of some disappointment that post-translational modification of histones was shown not to occur in these organisms.[18] Perhaps, then the evolution of histone modification occurred later with the appearance of eukaryotes. Recently, however, our group examined this question in a different archaeal phylum, the Crenarchaeota and the microbe called *Sulfolobus*. We found a eukaryotic-like epigenetic system that uses chromatin protein modification.[19–21] As eukaryotic epigenetics couples modification to phenotype and thereby affects traits such as biological fitness, our approach initiated with the production of evolved cell lines with improved fitness that were subsequently examined for epigenetic-like modifications.

Many Crenarchaeota belonging to the order Sulfolobales flourish in hot acidic habitats that are strongly oxidizing. Adaptive laboratory evolution over a 3-year period was used to test whether such organisms harbor additional thermoacidophilic capacity and to search for epigenetic-like modifications.[21] Three distinct cell lines derived from a single-type species were subjected to high-temperature serial passage while culture acidity was gradually increased. A 178-fold increase in thermoacidophile was achieved after 29 increments of shifted culture pH resulting in growth at pH 0.8 and 80°C resulting in cell lines named super-acid-resistant Crenarchaeota (SARC). Genome and transcriptome sequencing of 1 lineage identified a set of 8 nonsynonymous changes and a lack of transposition. Four multigene components of the SARC transcriptome implicated oxidative stress as a primary challenge accompanying growth at acid extremes. These components included accelerated membrane biogenesis, induction of metal resistance and an increased capacity for the generation of energy and reductant. These traits were subsequently evaluated for the involvement of an epigenetic process.

Interestingly, genome and transcriptome sequencing of the other 2 cell lines revealed that 1 line had no mutation whatsoever, whereas all strains had conserved, heritable transcriptomes implicated in acid resistance.[20] Relative to a similar passaged control produced in the absence of acid selection, the 3 evolved strains exhibited significantly enhanced genome stability. A mechanism that would confer these traits without DNA sequence alteration could involve post-translationally modified archaeal chromatin proteins. To test this idea, homologous recombination with isogenic DNA was used to perturb native chromatin structure. Recombination at upregulated loci from the heritable SARC transcriptome reduced acid resistance and gene expression in most recombinants. In contrast, recombination at a control locus that was not part of the heritable transcriptome changed neither acid resistance nor gene expression. Variation in the amount of phenotypic and expression changes across individuals was consistent with Rad54-dependent chromatin remodeling that dictated crossover location and branch migration. These data support an epigenetic model implicating chromatin structure as a contributor to heritable traits in the archaeal SARC lineages.

Native chromatin proteins in *Sulfolobus* are basic and highly abundant and undergo post-translational modification through lysine monomethylation. In all SARC lines, 2 chromatin proteins, Cren7 and Sso7d, were consistently undermethylated, whereas other chromatin proteins were unaltered.[19] This pattern was heritable in the absence of selection and independent of transient exposure to acid stress. The bulk of Sso7d was undermethylated at 3 contiguous N-terminal lysine residues but not at the central or C-terminal regions. The N-terminal region formed a solvent-exposed patch located on the opposite side of the binding domain associated with the DNA minor groove. By analogy to eukaryotic histones, this patch could interact with other chromosomal proteins and be modulated by differential post translational modification. Previous work established an epigenetic-like mechanism of adaptation and inheritance in *Sulfolobus*.[20,21] The identification of heritable epigenetic marks consisting of chromatin protein hypomethylation along with the broad conservation of at least one of these chromatin proteins (Cren7)[22,23] in all Crenarchaeota further supports the occurrence of an epigenetic process in Archaea.

If as predicted there is a protein network in which chromatin proteins interact with other proteins along the chromosome, then the protein interactions may be influenced by the methylation state and provide a means of communicating triggers that lead to epigenetic responses. If true, then archaea may offer a unique view of the origin of epigenetic process that predate the evolution of the nucleus and led to the rise of subcellular compartmentalization. As epigenetics is a critical component of multicellularity,[24,25] the study of epigenetic processes in archaea may shed light on the evolution of multicellularity.

Author Contributions

The authors contributed equally to the paper.

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