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Review

Phenotypic diversity and epigenomic variation – The utility of mass spectrometric analysis of DNA methylation☆

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ABSTRACT

Epigenomic variation may underlie phenotypic diversity that is not attributable to differences in genomic sequence. Such processes provide an organism the flexibility to respond to changing environmental cues within its lifetime, and perhaps its offspring's lifetime, and would therefore be expected to confer a selective advantage in evolutionary terms. Analysis of epigenomic variation within a population may be both a useful measure of developmental exposures and an indicator of future phenotype. A key molecular indicator of epigenomic variation in organisms is the chemical modification of DNA by methylation at specific nucleotide residues in the genome. Here we discuss how mass spectrometry can be utilised to provide quantitative analysis of DNA methylation patterns across populations. This article is part of a Special Section entitled: Understanding genome regulation and genetic diversity by mass spectrometry.

Contents

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1. Introduction

Phenotypic variation provides a substrate for evolutionary processes. A top down view of evolution calls upon natural selection to act on phenotype to select heritable biological traits that confer survivability and reproductive success. But how is phenotypic variation generated? Traditional models of genetic architecture assume a simple genotype to phenotype map and that hereditary phenotypic variation is the result of random genetic mutation, in a manner uncoupled from the environment. It is evident however that the relationship between genotype and phenotype is more complex, that genomic regulation depends on input from the environment and, that the molecular mechanisms underpinning hereditary can involve more than just DNA.

The generation of alternative phenotypes is a common evolutionary strategy for maximizing individual fitness in response to variable environments [1]. The programming of future life course trajectories in early development is a particularly notable case of phenotype plasticity in response to variable environmental signals and underpins the "Developmental Origins of Health and Disease" (DOHaD) paradigm [2,3]. Central to this model is the notion that predisposition to future health and non-communicable disease is influenced by variance in the environment experienced by the mother during pregnancy, with information transmitted across the placental boundary leading to *in utero* physiological programming of the fetus, or on infancy, in the expectation that the offspring will encounter a similar post-natal world. Organisms must therefore maintain a capacity for responding to environmental cues received if a range of phenotypes are to be generated and life-course decisions influenced. Rather than relying on random genetic changes, it is likely that evolution has in many cases selected for maintaining a capacity for phenotypic plasticity in response to the environment. Such phenotypic driven evolution has been incorporated in a number of conceptual evolutionary-developmental models including that of genetic assimilation (West–Eberhardt) and the Baldwin Effect [4,5].

The term epigenetics – a portmanteau of genetics and epigenesis – was first proposed by Waddington [6] to mean "above" or "over" genetics, in an attempt to bring together the then disparate fields of genetics and developmental biology. The subsequent growth in our understanding of development (in particular the processes of embryogenesis) has led to the gradual recognition that a type of inheritance distinct from the traditional trans-generational genetics is required at a somatic level to explain the stable maintenance of cellular differentiation patterns [7]. With this realization has emerged a modern definition of epigenetics which clearly recognises the importance of direct modification of the genome by the environment to yield stably heritable phenotypes resulting from changes in a chromosome, without alterations in the DNA sequence [8,9].

As reviewed by Jablonka and Lamb [10], epigenetic systems operate at multiple levels, and phenotypic variations that result from epigenetic systems may be acquired and manifest in a number of ways. Firstly, in a broad sense, they outline how epigenetics may be thought of as body to body transmission of information based on interactions between cells, organ systems, individuals, and other systems (for example mother and embryo interactions during development, social learning or symbolic communication). Secondly, at a cellular or molecular level, epigenetic systems concern the transmission from mother to daughter cell of phenotypes and information that is not dependent to DNA sequence differences. Cellular epigenetic systems are found across all taxa and act to maintain differing gene expression patterns, structural organisation and complex metabolic states. Indeed, it is cellular epigenetic inheritance systems that enable multiple phenotypes from the same genotype and the ability to pass on information from induced changes is essential for the development of multicellular organisms.

Concerning cellular epigenetics, Jablonka and Lamb [10] further outline four major mechanisms by which information transfer can be mediated:

1. Self-sustaining feedback loops – diffusible gene products that activate their own expression or activity and are transmitted to daughter cells during cell division, perpetuating their activity.
2. Structural inheritance – whereby existing cellular structures that act as templates for similar structures are distributed during cell division (e.g. prions).
3. Chromatin marking – proteins and chemical modifications attached to DNA that influence gene activity. Semi-conservative DNA replication allows the reconstruction of similar chromatin marks in daughter cells.
4. RNA mediated inheritance – small diffusible replicating RNAs which are inherited by daughter cells and perpetuate states mediated by RNA dependent processes.

While the various epigenetic systems are likely to interact and influence each other in a multitude of ways, epigenetics at a molecular level is frequently defined in terms of chromatin marking which has recently been the focus intense investigation enabled by the availability of second generation sequencing technologies. In this review we restrict our focus to chromatin marking, and in particular DNA methylation and its analysis by mass spectrometry.

At the molecular level, the induction of persistent state changes in the regulation of specific gene expression or the activity of functionally related genetic pathways may provide the necessary mechanism for generating phenotypic plasticity and programming within the lifetime of an individual [11]. There is an analogy here to epigenetic regulation of cell