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# Epigenetic effects of nano-sized materials

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ABSTRACT

The term epigenetics includes several phenomena such as DNA methylation, histone tail modifications, and microRNA mediated mechanisms, which are able to mold the chromatin structure and/or gene expression levels, without altering the primary DNA sequence. Environmental agents can exert epigenetic properties and there is increasing evidence of epigenetic deregulation of gene expression in several human diseases, including cancer, cardiovascular diseases, autism spectrum disorders, autoimmune diseases, and neurodegeneration, among others. Given the widespread use and dispersion in the environment of nano-sized materials, this article summarizes the studies performed so far to evaluate their potential epigenetic properties. Those studies highlight the ability of certain nano-sized compounds to induce an impaired expression of genes involved in DNA methylation reactions leading to global DNA methylation changes, as well as changes of gene specific methylation of tumor suppressor genes, inflammatory genes, and DNA repair genes, all potentially involved in cancer development. Moreover, some nano-sized compounds are able to induce changes in the acetylation and methylation of histone tails, as well as microRNA deregulated expression. We also provided a detailed description of currently available methodologies to evaluate epigenetic modifications. Standard protocols are currently available to evaluate cytotoxic and genotoxic effects of nano-sized materials. By contrast, there are at present no available standard protocols to evaluate the epigenetic potential of any given compound. The currently available methodologies offer different, but often complementary information to characterize potential epigenetic changes induced by exposure to nano-sized compounds. Given the widespread use and dispersion in the environment of nano-sized materials, at present and foreseeable in the near future, and in light of the indication of potential epigenetic properties here reviewed, more attention should be paid to unravel the consequences of such effects in future studies.



## 1. Introduction

A major concern applying to the use and handling of engineered nanomaterials (ENMs) is that their toxicity is likely to be due to physicochemical properties that standard toxicity screening tests are not able to evaluate. Several physicochemical factors have been related to biological responses; *e.g.*, size, surface area, high aspect ratio, charge, solubility, surface chemistry and reactivity. Progresses have been made in identifying priorities or minimal analytical characterization of ENM needed for hazard assessment in biological matrices (Bouwmeester et al., 2011). At present the OECD and the USEPA guidelines for the investigation of high production volume chemicals represent the reference model for assessing toxicity of ENM, although the OECD has published two documents specifically devoted to this subject: the first was published in 2009 ("Guidance Manual for the Testing of Manufactured Nanomaterials" (ENV/JM/MONO(2009)) and the second "Guidance Manual for the Testing of Manufactured Nanomaterials: First revision" was published in June 2010 (ENV/JM/MONO(2009)20/REV). These documents are considered to be living documents and subjected to regular review, revision and improvement as the state of the art advances. Therefore it is important to provide input for future development of methods which should be considered for future investigation, aiming anyway at improving our knowledge about potential effects exerted by ENM.

Recently there is growing interest in the potential that the environment may have not only against the genome (mutations) but also against epigenome (epimutations). The topic of how epigenetic processes can significantly modulate cellular behavior and potentially complex diseases risk, including cancer, especially in response to environmental chemicals, is just such an emerging issue (Preston, 2007).

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Epigenetics investigates heritable changes in gene expression occurring without changes in DNA sequence. Several epigenetic mechanisms, including DNA methylation, histone tails modifications, and non-coding RNA expression, that regulate gene expression through altering chromatin configuration, inhibition of translation, and degradation of RNA, can change genome function under exogenous influence (Cyr and Domann, 2011). It is well established that long-term toxicity of chemicals could be caused by their ability to generate changes in the DNA sequence through the process of mutagenesis. However DNA function and health could be stably altered by exposure to environmental agents without changing the sequence, just by changing the state of DNA methylation. The majority of environmental factors such as nutrition, or toxicants such as endocrine disruptors, do not promote genetic mutations or alterations in DNA sequence. However, these factors do have the capacity to alter the epigenome. Moreover some environmental factors are able to promote a phenotype or disease state not only in the individual exposed but also in subsequent progeny for successive generations (transgenerational inheritance). Epimutations in the germline that become permanently programmed can allow transmission of epigenetic transgenerational phenotypes (Skinner et al., 2010).

This has important implications on the way we assess the safety of chemicals, drugs and food and broadens the scope of definition of toxic agents (Preston, 2007; Szyf, 2011). *In vitro*, animal and human investigations have identified several classes of environmental chemicals able to modify epigenetic marks, including metals (cadmium, arsenic, nickel, chromium, and methylmercury), peroxisome proliferators (trichloroethylene, dichloroacetic acid, and trichloroacetic acid), air pollutants (particulate matter, black carbon, and benzene), and endocrine-disrupting/reproductive toxicants (diethylstilbestrol, bisphenol A, persistent organic pollutants, and dioxin) (Cheng et al., 2012; Christensen and Marsit, 2011; Szyf, 2011). Most studies conducted so far have been centered on DNA methylation, whereas only a few investigations have studied environmental chemicals in relation to histone modifications and non-coding RNA. Our current screening tests however are not properly addressed to detect agents that have epigenetic properties. In view of a broad environmental health research strategy aimed at protecting and improving human health we should advance our understanding of the potential epigenetic role of ENM.

## 2. Epigenetics: concept and mechanisms

Epigenetics, literally meaning "above genetics", comprises heritable modifications that alter gene expression levels without resulting from direct changes in the primary DNA sequence. Epigenetic mechanisms include nucleotide modifications, such as methylation and hydroxymethylation of cytosine, covalent modifications of histone tails, and nucleosome positioning. These mechanisms interact to determine chromatin folding and the relative accessibility of a given genetic locus to activating and suppressing transcription factors. Non-coding RNAs affecting gene transcription are also largely recognized as epigenetic mechanisms (Martín-Subero, 2011). A summary of major epigenetic processes is shown in Fig. 1.

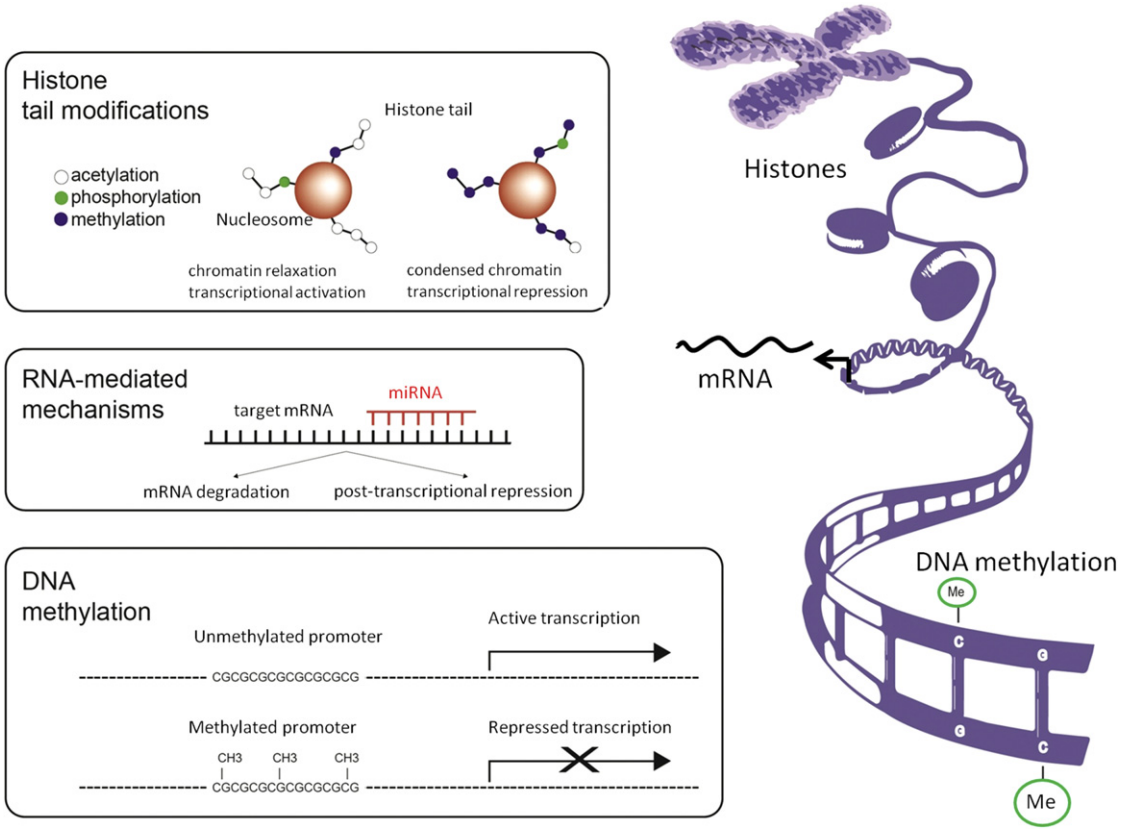


**Fig. 1.** Overview of the main epigenetic mechanisms that can undergo deregulation following exposure to nano-sized materials. Histone tail modifications lead to a more or less relaxed chromatin structure thus allowing or blocking the access of transcription factors to gene promoters. DNA methylation of the promoter regions is generally associated with gene silencing, while demethylated promoters allow the binding of transcription factors and the gene is transcribed into messenger RNA (mRNA). Following post-transcriptional processing the mature mRNA reach the cytoplasm. A microRNA (miRNA) can bind the target mRNA, leading to its degradation or blocking the access to the ribosome and translation into proteins.

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