ISJ 12: 1-4, 2015 ISSN 1824-307X

VISIONS AND PERSPECTIVES

Invertebrate immunological memory: could the epigenetic changes play the part of lymphocytes?

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Accepted December 10, 2014

Abstract

Different hypotheses have been suggested for the neurological memory storage in vertebrates, either based on the structural induction of synaptic plasticity or on chemical modifications, *i.e.,* DNA rearrangement. For invertebrates, DNA rearrangements, and in particular the involvement of epigenetic mechanisms which in turn regulate gene expression, have been proposed. Based on the deep link existing among immune and neuroendocrine functions, it is argued here that epigenetic changes could represent the basis for explaining the numerous observations reporting hints of immunological memory in absence of lymphocytes.

Introduction

Memory is a wide term encompassing the storage of experiences into specific neurons and the trace of immune challenges into specific cells. Memorized experiences and pathogens are accumulated during the life (Rensing *et al.,* 2009), but the fine mechanisms building up memory are still open to debate.

Different hypotheses have been suggested for neurological memory (see for review, Peña de Ortiz and Arshavsky, 2001). One hypothesis proposes that the synaptic plasticity is the key event for memory consolidation, describing memory storage as the consequence of a structural modification. A second hypothesis considers a chemical modification*, i.e.,* the memory storage is not at the synaptic, but at the genomic level. In this second view the storage of information is based on somatic DNA recombination. Such memory storage mechanism working in the brain neurons is similar to that observed in lymphocytes of the immune system. In this context, it is appropriate to stress the similarities between immune and neuroendocrine system workflow. Studies by J. Edwin Blalock and colleagues have demonstrated in mammals a functional integration of the immune and the neuroendocrine systems. One of the most convincing findings has been that both systems share a series of endogenous and exogenous mediators that combat threats to the homeostasis of the organism (Weigent and Blalock, 1987). Based on these similarities, Habibi *et al.* (2009) surmized that DNA rearrangements could be present in immune and nervous systems in order to accumulate permanent information and allowing the creation of long-term memory.

A possible relationship between genome changes and memory formation could be also contained into epigenetic modifications, *i.e.,* alterations in the chromatin structure, which in turn regulate gene expression (Levenson and Sweatt, 2006).

The present contribution suggests to consider epigenetic modifications as one possible mechanism of immunological memory formation in invertebrates.

Epigenetics

The term epigenetics was coined by Conrad H. Waddington in 1942. Today, epigenetics has been defined as ‘‘the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence’’ (Wu *et al*., 2001). The well-studied epigenetic mechanisms are the following: DNA methylation, histone tail modification and microRNA (miRNA) or non-coding RNA (Tammen *et al*., 2013) (Fig. 1).

Current status on the immune and neuroendocrine systems in invertebrates

Already 20 years ago, experiments of my and other laboratories have shown that in molluscs, as in vertebrates, there is a close functional correlation between the immune and the neuroendocrine

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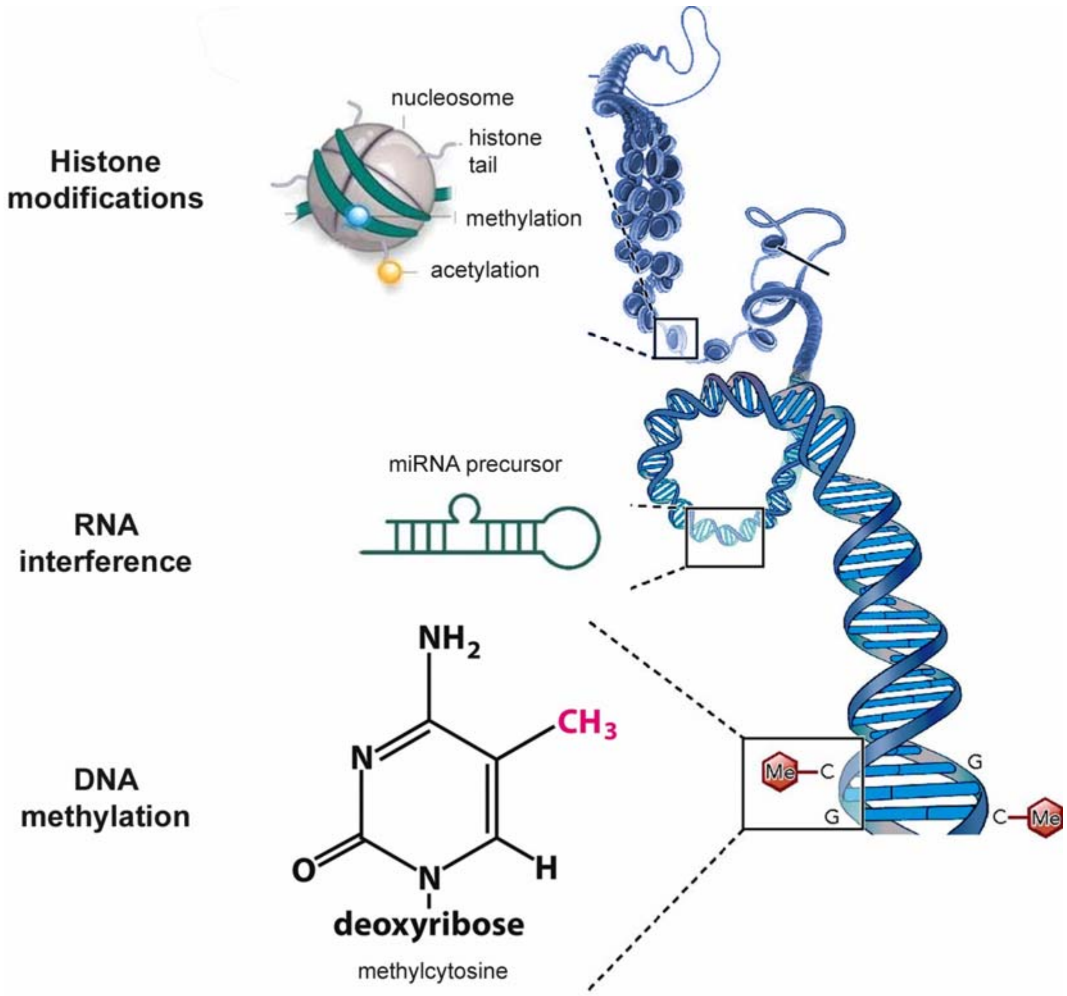

Fig. 1 Schematic summary of the epigenetic mechanisms acting on chromatin remodelling that are involved in gene regulation.

systems. Given the evolutive distance separating mollusca form vertebrates, we concluded that this relationship has a deep and ancient evolutionary root, predating the split between protostomian and deuterostomian lineages. It seems that nature has followed an economic strategy by reusing the same pool of signal molecules (neuropeptides, hormones and cytokines) preserved in an extraordinary way in the course of evolution (Ottaviani and Franceschi, 1997).

With regards the immune system, invertebrates present only the innate immunity and consequently there are no lymphocytes challenging the comparative immunologists focused on immunological memory studies. Despite the lack of a cellular substrate recalling the vertebrate lymphocytes, in literature several data in supporting the presence of immunological memory emerge (Cooper, 1969, 1976; Hostetter and Cooper, 1973; Karp and Hildemann, 1976; Hildemann *et al*., 1977, 1979a, b; Karp and Rheins, 1980). In our molluscan model, *Planorbarius corneus,* experiments performed on the humoral and cellular components, as well as on the bacterial clearance are in favor of the existence of some form of immunological memory (Ottaviani *et al*., 1986; Ottaviani, 1992). Repeated injections of bacteria *Staphylococcus aureus* and *Escherichia coli* into the foot of the mollusc induce specific and aspecific agglutinins. Specific agglutinins observed in direct-agglutination tests showed an increased titer after the second injection. Aspecific agglutinins evaluated in cross-agglutination tests showed no changes in titer after the second injection (Ottaviani, 1992). The *in vitro* bacterial phagocytosis experiments have shown a higher bacterial elimination across the entire time-range considered (30, 60, 90, 120, 150 min) in the snails that had already contacted the bacteria to be phagocytized (Ottaviani, 1992). The bacterial clearance experiments have revealed that after the second (14 days) and third (73 days) bacterial injections, clearance rates are faster (Ottaviani *et al.,* 1986). Also more recent investigations have provided further evidence of a potential immune memory in invertebrates, though the edge between real memory and the effect of immune priming is blurred, especially in short living species (Kurtz, 2004, 2005; Brehélin and Roch, 2008).

On the whole, a careful revision of the existing literature provides indications that from protozoans (Csaba *et al.,* 1984) to vertebrates, some types of memory may be present. The absence of a lymphocyte-based system comparable to those of jawed vertebrates and lampreys (Hirano *et al*., 2013), points towards alternative processes. The close developmental similarities recently observed between neurons and hemocytes in crustaceans (Benton *et al*., 2014) allow to speculate that the mechanisms described for neurological memory could be present also in invertebrates. With the exception of the data reported in *Drosophila* (LaFave and Sekelsky, 2009), mechanisms of somatic rearrangement of DNA are unusual and not occurring at loci hosting invertebrate immune genes. As an alternative mechanism, epigenetic modifications of hemocyte chromatin could be surmized, as it has been also proposed by Levenson and Sweatt (2006) for neurological memory.

Epigenetic effects in invertebrates

Recently, we have analyzed epigenetic modifications in neurons of the mollusc *Pomacea canaliculata* after injection of LPS (Ottaviani *et al.,* 2013). Following the treatment the phospho-acetylation of histone H3 correlates with the increase of c-Fos protein levels in the nuclei of the small ganglionic neurons. These findings show the highly conserved interactions between immune and neuroendocrine systems at a molecular level.

In contrast to the pattern of genome-wide DNA methylation in vertebrates, DNA methylation in invertebrates is relatively sparse (Bird et *al*., 1979; Suzuki and Bird, 2008; Field *et al*., 2004).

Epigenetics studies in molluscs suggest the presence of CpG methylation in *Mytilus edulis* (Bird and Taggart, 1980), *Donax trunculus* (Petrovic *et al*., 2009) and *Crassostrea gigas*, where DNA methylation has important regulatory functions (Gavery and Roberts, 2010). Moreover, in *Octopus vulgaris* methylation is involved in gene regulation during the development (Díaz-Freije *et al*., 2014). Beside DNA methylation, miRNAs present in metazoan are about 22 nucleotides in length and play a role in the control of animal development and physiology by altering the chromatin architecture (Ambros, 2004).

Conclusions

This article wants to trigger the attention of comparative immunologists towards those mechanisms widespread in metazoans that could explain numerous observations at present orphans of solid explanations. At present it is neither possible to state that immunological memory is present in invertebrates nor that epigenetic changes represent its basis. However, the presence of epigenetic mechanisms could represent a potential alternative to the lymphocyte-based memory in invertebrates, especially in consideration of the very few examples of somatic DNA recombination.

Acknowledgements

The author thanks Prof. M Mandrioli (University of Modena and Reggio Emilia, Italy) for the for the figure.

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