Rendiconti
Accademia Nazionale delle Scienze detta dei XL
Memorie di Scienze Fisiche e Naturali
124° (2006), Vol. XXX, P. II, pp. 283-289

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Introduzione al convegno

The subject matter of the seminar "Genetics and Epigenetics" is too vast to be covered in one morning, although its aim is to focus attention on the comparison between epigenetics and genetics. We are all familiar with the many phenomena and mechanisms with which the latter discipline deals, while only recently have we learned about epigenetics, in particular about its involvement in the processes of development.

The *concept* of epigenetics – and I believe also the *term* epigenetics – came into being between the 1940s and 1960s with Conrad Hal Waddington who characterised it as a sort of "amalgamation of epigenesis and genetics".

To begin with, I would like to point that according to Waddington and other researchers before him the term "epigenesis" no longer had the meaning attributed to it in the 18th century by Caspar Friedrich Wolff – as simple developmental processes and egg growth, opposed to preformism – but rather as development of embryonic structures starting from different earlier material.

We will now move on to briefly analyze firstly the nature of epigenetics phenomena or epigenetic control of development, and secondly, the ways through which this control is performed. B. K. Hall and W.M. Olson (2003) give broad definition of epigenetics, which embraces all the different processes which regulate the differential expressions of the gene. Heritable information represent the raw material from which organisms are modelled.

This kind of information is called "familial" or "maternal" if belonging to parental or nuclear-zygotic genes; other classes of hereditable information are considered "less familial", such as the product of maternal genotype and the various heritable molecular structures, which the zygotic genome can take on. These structures, due to genetic information distinct from the zygotic genome, are called "gene phenotype" or "epigenetic heredity system".

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Once the problem has been framed this way, one cannot avoid mentioning the old dispute between preformism and epigenesis or between nuclear and cytoplasmic control of development. Therefore, if on hand we must acknowledge that epigenetics has its roots in epigenesis, which always remains the true alternative to preformism in explaining (embryonic) development, on the other hand we can state that epigenetic control does not follow routes other than those of genetic information but is rather an important component of the selective control of genetic expression.

The early stages of development would therefore be regulated by a combination of hereditary factors pre-existing maternal cytoplasm and inheritable models of gene expression, under the influence of processes such as DNA methylation, (histonic) acetylation, phosphorylation etc., inheritance of cortical cytoplasmic factors, variations in chromatin 3 D structure and other processes of which more further on.

Therefore, if two distinct heredity system, actually exist, the conventional genetic one and an epigenetic one, we would be dealing with a *genetic duality*, as accepted by many biologists (although not by all of them), among whom I would like to mention Schmalhausen and Wright, both from Waddington's times, who suggested integrated developmental programs, like those proposed by Waddington himself. In more recent years, starting from the 1970s, many researchers, among which Rieder, King and Stanfield, Maclean, Hall and Olson, have subscribed to Waddington's theories. Medawar and Medawar also maintain that the term "epigenetics" refers to all processes which lead to the implementation of genetic instructions contained in the fertilized egg, therefore, according to these authors "genetics proposes and epigenetics disposes".

In turn, Maynard Smith (1990) maintains "the existing link between generations, in organism reproducing sexually, is provided by a second genetic system – the epigenetic system – in addition to that based on DNA sequence". In this context Eva Jablonka, who honours us with her presence today, deserves special mention: she is the author of many works dealing with the subject of the "two genetics", where the problems are confronted with open mind and presented in a clear and insightful way.

I would now like present an overview of mechanisms of epigenetic control on gene expression and development, according to B. K. Hall.

1) *A first mechanism (material epigenetic control*) concernes the embryo's early developmental stages which are controlled by the maternal cytoplasm; this means that early development is not primarily controlled by the zygotic nucleus, but rather by factors pre-existing in the cytoplasm, produced by maternal and stored in the egg during oogenesis. These factors are either long lived mRNA or structural proteins or their precursors. Cohen (1979) observed that in mammals, interactions between maternal and zygotic genomes, in addition to environmental effects in the uterus, influence embryonic development.

It has also been recently proved that in mice generated from nucleus transplant, the adult phenotype can be stimulated by cytoplasmatic events also due to interactions between maternal cytoplasm and zygotic genes. During the phase of the cytoplasmatic maternal control, development can even proceed in the absence of the sperm nucleus, i.e. in the eggs of parthenogenetic species or in artificially activated ones, especially of amphibians.

Sophisticated studies by Goldstein and Freeman (1997) on eggs of Cnidarians and Ctenophores have demonstrated the existence of epigenetic control on the specification of the (primary and secondary) body axes of the future embryo. The primary axis is specified in a time span between an early stage of oogenesis and the first cleavage; the secondary one in a time span also beginning at early oogenesis but ending with an advanced gastrula stage.

2) *A second case of epigenetic heredity is that of cortical (or cytoplasmatic) heredity*, which has been especially studied in ciliate protozoans, for example in *Paramecium Aurelia*. It as been demonstrated that, if a portion of cortical cytoplasm with opposed orientation between two *P. Aurelia* individuals are transplanted, the transplanted cilia sweep in a direction contrary to that of the host and this behaviour is transmitted to the daughter cells, originating from the division of the transplanted cell. This means that the direction of the ciliary sweeping is transmitted from one generation to another through the inherited subcellular organization of cortical cytoplasma: this happens notwithstanding that fact that cell DNA is unchanged, and neither cilia or basal bodies contain DNA particles.

3) *DNA methylation*. This process consist of adding a methyl group to cytosine residuals in the DNA to form 5-methylcytosine; it does not alter the message encoded in the DNA, but it influences transcription, thus reducing its activity. Although methylation does not causes DNA to develop a new function, it stabilizes or modifies the function it already performs. Therefore, methylation is an inheritable state with consequences on gene expression. Not all genes are equally methylated; for example, inactive tissue – specific genes are often not methylated, as are genes that provide the cellular base metabolism (the housekeeping genes); on the contrary, active tissue specific genes are methylated. Metylation patterns of specific genes can be studied along the embryonic development: for example, methylation patterns expressed in mice gametes and inherited by the zygote are not present at the blastocyst stage, when all DNA is demethylated. However, new patterns can appear later in development.

4) *Chromatin structure*. There are numerous examples of dynamic chromatin structures which control various aspects of development such as a) *heterocromatisation* of paternal chromosomes during gametogenesis in male mammals and some insect species; b) reduction (or elimination) of chromosomes in many nematodes,

in dipterans, in copepods and in some lampreys; c) proteinchromatin association which maintains the active state of homeobox genes during cell division.

5) *Genomic imprinting*. Imprinting occurs when two copies of gene (both maternal and paternal, or a gene with its allele) do not function in the same way during development. This phenomenon takes place on a singlegene level, and curiously the effects of maternal and paternal genomic imprinting are not the same: for example, ginogenetic mice, which posses two sets of maternal chromosomes but no paternal chromosomes, display the beginning of a normal embryonic development, whilst extraembryonic tissue are unable to develop normally; on the contrary androgenetic mice, which have two sets of paternal chromosomes but no maternal ones, display a normal develop of extraembrionyc tissues, but not of embryonic ones. These phenotype models are inheritable and produce mechanisms for the epigenetic control of development. It would seem that a sort of "cellular memory" regulates gene expression.

Back to the general theme, as previously introduced, I would like to quote here a broad-scope definition of epigenetics according to Müller and Olsson (2003): «In each traditional research area – molecular genetics, development, heredity, evolution – "epigenetic" refers a different issue. The molecular mechanisms of gene regulation, the embryonic generation of form, the transmission of information from one generation to the next, and environment – induced variation do not represent the same biological problem. However, these issues are linked through an integrative, evolutionary perspective. In this view, the epigenetic agenda emerges as the science focusing on the role of the nonprogrammed, regulatory, and modulating factors in biological processes. Whereas evolution was traditionally studied either from a genetic or from a phenotypic perspective, the common epigenetic agenda represent a new level of analysis, focusing on the casual interactions between genes, phenotypes, and the environment. This epigenetic agenda will be central for the formulation of integrative models in evolutionary developmental biology».

Therefore, from Waddington to the present, for many biologists, epigenetics (or epigenetic control) is the sum of genetic and epigenetic factors which operate on cells in order to selectively control the gene expression which produces growing phenotypic complexity during development. We are therefore dealing with two heredity systems, genetic and epigenetic; to consider the epigenetic system as non genetic would be a mistake, as it would be to consider them as two opposites operating one against the other. A simpler but less recent definition was provided by Lawrence (1990): epigenetic is the process that link genotype to phenotype, beyond the initial genetic activity.

Some researchers, mainly among those who are more at home with theoretical rather then whit experimental issues, have developed, especially in the last decade, very different views on epigenetics from the one sketched in the previous lines.

Since the beginning, I have emphasized how the vision directly inspired by Waddington's view insists on the dualism between the genetic control of development and the modulation of gene expression due to different factors, whose dependence on the genes is more remote or less specific. But precisely this dualism is disputed by the group of researchers lead by Susan Oyama (2000) whose theoretic position is currently known as the *Developmental System Theory*.

This anti-reductionist theory is opposed to those enduring dualistic interpretations in the study of ontogeny and the theory of biological evolution in general: we are dealing with a radical vision which identifies development with a blend of heterogeneous influences and interdependent levels, which can be distinguished from one other only for epistemic convenience's sake.

The main hypothesis behind this theory is that "transmission" between generations does not concern so much individual information items but rather the whole set of interactants in development, which includes: genes, cell mechanisms and structures, the extracellular environment, up to the widest framework of organic development, within which are found the maternal reproductive system, the parental cares, and the relationships with other aspects of the surrounding living and physical world. Thus, evolution would be a succession of developmental systems, and every explanation which gives causal priority to one factor rather the another would risk being partial.

Other authors, in particular E. Fox Keller (2003) adhere to this view. These authors are very critical towards the concept of genetic programme. They blame in particular: 1) its inability to elaborate mechanisms of DNA editing or repair, which should assure replication stability and consistency; 2) the dependence of genic function on the epigenetic web, which may damage regulation of single gene transcription; 3) the posit that proteins are simply and directly encoded in DNA, therefore undermining the very base of the gene concept as a functional unity located in the chromosome.

According to Oyama and her group, it would be totally arbitrary to consider the genome and its cellular environment as two opposites, just as the software of a computer, that is the programme we use to manage data, is contrasted to the hardware, that is the machine's structure, which allows the programs to run. Still according to the Developmental System Theory, even the dichotomy between organism and environment would be arbitrary. This idea is based above all on the fact that an organism (with its genome and the rest of its structure), in a sense, specifically modifies and adapts its immediate environment, a kind of "extended phenotype" of the organism itself, to use an insightful expression of Richard Dawkins, although elaborated in a different context.

It is obvious that if we accept the Developmental System Theory, the meaning of epigenetics itself widens, but at the same it becomes less precise, as the concept of genic expression, one of the strong basic concepts in traditional interpretations, becomes quite fuzzy. We should not be surprised then by the many negative reac-

tions to the Developmental System Theory. One should expect that even with these problems, as on many other questions a more reasonable and especially more productive interpretation may finally emerge from positions midway between those, perhaps too conservative, centred on the by now fashionable but not completely satisfactory notion of genic programme, and those, perhaps too revolutionary, which would even question the actual identity of the gene.

As a conclusion to this short introduction, it is probably safe to remember how deeper and wider knowledge in molecular biology has shaped a concept of the gene which is increasingly less similar to the "one-piece" DNA segment, which, apart from mutations, was considered to be the core of molecular biology in the years when the genic expression itself was elevated to a level of "central dogma of molecular biology".

That mechanism seemed to be prodigious in its elegance and reliability, but in retrospect it seems just a rough approximation of a much more dynamic and complex reality, both on the level of the macromolecules actually involved, as still more, on that of the processes in which they are involved in the dimension of individual development and biological evolution alike.

I would like to thank prof. Alessandro Minelli for his precious help in the bibliographical research and for reviewing the text.

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* Citations are limited for only some authors whose works regard the epigenetic problems.