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Melatonin seems to be a mediator that transfers the environmental stimuli to oocytes for inheritance of adaptive changes through epigenetic inheritance system

M.K. Irmak[a,*], T. Topal[b], S. Oter[b]

[a] *Department of Histology and Embryology, School of Medicine, Gulhane Military Medical Academy, 06018-Etlik Ankara, Turkey*
[b] *Department of Physiology, School of Medicine, Gulhane Military Medical Academy, Ankara, Turkey*

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Summary Possibility of inheritance of epigenetic modifications have led us to consider that adaptive geographic variations in humans may result from interactions between environmental factors and epigenetic inheritance system. In this system melatonin seems to be a mediator that transfers the environmental stimuli to germ cells (oocytes). While environmental factors produce modifications in the body, they simultaneously induce epigenetic modifications in the oocytes with the help of melatonin, and these changes are inherited to offspring. In this way, adaptive changes could be passed on to the next generation. This kind of heritable long-term changes is generally labeled biological adaptation. But, how can melatonin cause epigenetic changes in oocytes? We suggest that melatonin induces epigenetic modifications by affecting the nuclear melatonin receptors that can in turn change the superstructure of DNA. It was previously suggested that biological adaptation is limited to neural crest derivatives such as, craniofacial tissues, melanocytes, and structures related to stature, hair form and body proportions. Thus, inheritance of adaptive changes is possible only where environmental factors affect the neural crest derivatives, including the cells that produce the next generation.

Geographic variations result from biological adaptation

Recent analyses, particularly of mitochondrial DNA, have suggested that all human populations are descended from groups which migrated out of some part of Africa in quite recent times [1]. In this respect, all human groups of present-day are adapted to their ecological niche in various ways; their mere presence is proof for such a statement. As a result of adaptation, there is a great deal of variation from one geographic region to another in skin color, hair form, craniofacial morphology, stature, body proportions, and a host of less immediately obvious

* Corresponding author. Tel.: +090 312 3043533; fax: +090 312 3042150.
E-mail address: mkirmak@gata.edu.tr (M.K. Irmak).

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traits. The term adaptation is therefore interpreted to encompass those responses in the phenotype, which are produced by the action of the environment upon a given gene system, and is applied to those responses which improve some function of the organism or population in a specific environment. The population variation in skin melanin is a good example of the adaptive responses our species shows to an environmental stress. It is well known that the damage of ultraviolet rays to the skin is inversely proportional to the amount of melanin in the dermis since the melanin screens ultraviolet penetration. Given this effect of skin color, dark skin color in the high radiation areas is adaptive in the general sense. Understanding of how we have adapted, adjusted, and coped with our natural environment through time will be the key to explaining why we function the way we do.

Efforts for an appropriate description of adaptation have led to the use of a variety of words such as 'acclimatization' in reference to short-term changes, while long-term heritable changes are often labeled 'biological adaptation' [2]. Basically, all characteristics of a living organism must be explained by reference to its genetic substrate and how this structure has been and is interacting with the external environment. How do genetic material and environment interact to produce a more appropriate structure or function? The answer to this question is not known, but biochemical and developmental genetics have progressed to the point that some general mechanisms are now clearer. Unusual or extreme environmental conditions such as ionizing radiation or high temperature are known to increase mutation rate in germinal cells. It is a well-known fact that the vast majority of known mutations are inadaptive and they are almost always disadvantageous to the individual and to the population under the actually existing conditions [3]. This is precisely what would be expected. If adaptation is perfect, any other change is disadvantageous. Therefore usual environmental conditions must alter the structure of chromosomes in a predetermined direction by mechanisms other than mutations.

The idea of the inheritance of adaptive changes (biological adaptation) was explicitly rejected by August Weismann (1834–1914). He claimed that, starting from the fertilized egg, there are two independent processes of cell division, one leading to the body or 'soma' and the other — the germ line — leading to gametes that form the starting point of the next generation [4]. Of these two cell lines, the soma will die, but the germ line is potentially immortal. Weismann's central claim was that the germ line is independent of changes in the soma. If this is true, then adaptive changes in the soma cannot be inherited. But it is not clear why he thought it was true. He did point out that in most animals, the primordial germ cells that will give rise to the gametes are set aside early in development. The energy and material needed for the production of gametes are provided by the rest of the body, so there are opportunities for the soma to influence the germ line [5]. According to Darwin's and other versions of this theory, an environmentally modified part of the body liberates modified gemmules into the circulation. The modified gemmules then reach the germ cells and eventually participate in the formation of the corresponding modified part in the offspring. In this way, adaptive changes could be passed on to the next generation [5,6].

Inheritance of adaptive changes is possible through epigenetic inheritance system

It is commonly assumed that all heritable information resides in DNA base sequences, and that the only mechanism by which information is transferred from one generation to the next is through the semi-conservative replication of DNA. However, these assumptions are not correct. In addition to the DNA inheritance system underlying classical genetics, it is now recognized that variations can be transmitted between generations in other ways [5]. There are epigenetic inheritance systems (EIS), which enable organisms to acquire and transmit different phenotypes to the next generation [2]. The study of EIS is still in its infancy, but the types of mechanism that might underlie it were clearly envisaged at least 30 years ago. In 1974, it was suggested that information is carried not only in the primary structure of DNA, but also in its 'superstructure', or topology; DNA superstructure can be modified by interactions with other molecules, and subsequently the altered topology can be inherited [5,7]. The information is carried in chromatin as chromatin marks. Chromatin marks are the non-DNA parts of the chromosomes, for example binding proteins or additional chemical groups attached to DNA bases that affect the nature and stability of gene expression. An EIS is usually less stable than the genetic system, and more sensitive to environmental changes [7–9]. Perhaps the best characterized type of epigenetic modification is the methylation of DNA. The cytosines in DNA can be modified by the enzymatic

addition of a methyl group. The methyl group does not change the coding properties of the base, but may influence gene expression [3,10]. The inheritance of methylation patterns in eukaryotes is based on the fact that methylation occurs in CpG doublets or CpNpG triplets. Methylation is therefore symmetrical on the two DNA strands. Complementary base pairing means that a CpG (or CpNpG) on one strand is partnered by the same sequence but in the opposite direction, on the other strand. After replication, the parental strand is methylated, but initially the new strand is unmethylated. An enzyme, methyltransferase recognizes this asymmetrical state, and preferentially methylates the CpG of the new strand [9]. Mechanisms rather similar to those responsible for transmitting methylation patterns are thought to underlie the inheritance of DNA–protein interactions [5].

Studies have shown that differences in methylation patterns can be inherited, even through sexual reproduction [11]. During the formation of germ cells, genes subject to genomic imprinting are marked by methylation according to whether they are present in a sperm or an egg. In this way, the parental origin of the gene can be subsequently detected in the embryo; DNA methylation thus enables somatic cells to remember the parental origin of each of the two copies of the gene and to regulate their expression accordingly. In most cases, the methyl imprint silences nearby gene expression by enabling other proteins to bind and shut down the gene completely by further altering chromatin structure [2]. In vertebrates, imprinting is restricted to placental mammals, and all the imprinted genes are involved in fetal development [3,12]. Why imprinting should exist at all however, is a mystery.

It is clear that epigenetic variations are transmitted through meiosis and gametogenesis for a substantial number of sexual generations [5]. Organisms such as mammals, in which the germ line segregates early in development, can transmit to the next generation only those new variations that occur either before germ line segregation, or in the germ line itself [5]. As yet we do not know exactly what happens during gametogenesis — how marks are altered, what determines which marks are altered, whether marks that are altered usually, or rarely, leave 'footprints' of their previous nature, and so on. We need to know more about the processes occurring in the germ line in order to be able to assess the extent and persistence of inherited epigenetic variations.

Epigenetic inheritance occurs through oocytes

We thought for several reasons that, epigenetic inheritance must occur largely through the oocytes (maternal inheritance). First, the female contributes most to the zygote. Second, unlike spermatocyte, the mammalian oocyte is formed early in fetal life and may remain suspended at diplotene of the first meiotic prophase from 12th week of development, until several decades later (diplotene begins with puberty and is briefer and less distinct in male gametes) [3]. Third, oocytes are endowed with an exceptional capacity for epigenetic modifications [13], and in the diplotene stage they are more sensitive to environmental effects than in other stages [14]. This long interruption in continuity of the meiotic process, lasting from 12 to 40 years makes oocytes therefore more sensitive to epigenetic modifications [15]. Fourth, during the long and very active meiotic prophase of eucaryotic oocytes, diplotene chromosomes are arranged in decondensed, chromatin loops extending from the main chromosomal axis. These chromatin loops (lampbrush loops) being independent of each other [5,16] and insulating the genes within the domain from the regulatory influences of neighboring domains are more prone to epigenetic modifications [5]. Fifth, imprinted genes often cluster in large chromosomal domains, raising the possibility that imprinting is regulated by domain-specific mechanisms in diplotene stage of meiotic oocytes [17]. Sixth, while the paternal genome is relatively more important for development of the extraembryonic tissues, the maternal genome apparently has a greater influence on development of the embryo proper [13]. Previous considerations make it appear likely therefore that inheritance of epigenetic modifications occur mostly through female gametes (oocytes).

Transfer of environmental stimuli to oocytes by melatonin

We have looked for an appropriate mediator which can transfer the environmental information to the oocytes. In this respect, the hormone melatonin seems to be the most appropriate mediator which carries all the criteria mentioned. Actually, the best known hormone the secretion of which may be disturbed by almost all environmental factors

is melatonin [18]. Light and temperature are the main environmental factors affecting the secretory rhythm of melatonin [19]. Light affects melatonin secretion even in blind persons [20]. Besides visible light, certain ultraviolet wavelengths as well as extremely low frequency electric and magnetic fields affect the melatonin secretion [21,22]. Altitude and cold exposure both affect the secretion of the melatonin [23,24]. The released acetylcholine and noradrenalin from the nerve terminals also affect melatonin synthesis and secretion [25]. Besides these acute environmental effects, melatonin-based photoperiod time-measurement and circannual rhythm generation are long-term systems used to regulate seasonal cycles in physiology [26,27]. In this system, light acts exclusively through photoreceptor cells in the retina to control melatonin production. Melatonin is then secreted from pinealocytes into the peripheral blood and cerebrospinal fluid [28,29]. The decoding of the changes in melatonin signal duration that governs seasonal physiology depends on specialized melatonin target cells in the brain, pituitary and gonads. These cells express high affinity melatonin receptors to register the systemic signal, and to discriminate between short (6—10 h in summer) and long (12—16 h in winter) daily exposure to melatonin.

Ovarian follicular fluid is reported to contain much higher levels of melatonin compared to the serum suggesting an active uptake mechanism for the hormone [30]. The presence of melatonin receptors in maternal oocytes and their de novo synthesis in the early embryos point to important function of melatonin in development [31]. It affects the nuclear volume and metabolic behavior of oocytes even in the offspring [32,33]. Taken together, the mother's melatonin can transfer the environmental information to the oocytes and fetus, and this information is used, along with the information that is obtained after birth, to influence juvenile development [34]. The transfer of information from mother to oocytes and fetus by way of melatonin therefore shows that melatonin may act as a mediator of epigenetic inheritance system.

Induction of epigenetic changes by melatonin

How can melatonin cause epigenetic changes in oocytes? We suggest that epigenetic modifications may result from the interaction of melatonin with nuclear melatonin receptors (NMR) [35,36]. The binding of melatonin to these receptors is in the low nanomolar range, similar to the concentration of melatonin itself in the blood, suggesting that these receptors may be involved in nuclear signaling by the hormone [36—38]. Melatonin significantly increases the transactivating effects of these receptors [39,40], and nuclear melatonin receptors appear to have a functional role in DNA bending [35,41]. Melatonin may thus modify the superstructure of DNA by affecting the NMR. Accumulated experimental data unequivocally point to an important role of melatonin and NMR in cell differentiation [42]. Interestingly, unlike receptors for steroid, thyroid, and retinoid hormones, the interaction of NMR with coactivators can also occur in the absence of a ligand [43], this corresponding to constitutive activity of NMR. Germ cell nuclear factor (GCNF) as another member of the nuclear receptor superfamily may also contribute to epigenetic modifications in oocytes [44]. In the male, GCNF expression is postmeiotic; while in the female, expression occurs before the completion of meiosis, correlating with the long diplotene stage [45,46].

EIS is a genetic regulatory mechanism that is not available to bacteria, and it is thought to allow eucaryotes to maintain extraordinarily stable patterns of gene expression over many generations [11]. Epigenetic modifications induced possibly by nuclear melatonin receptors or GCNF may adopt local and specific folded conformations, which assemble proteins to form characteristic chromatin structures, thus contributing to EIS. The ability of a mark to fold and organize chromatin in this way (chromatin folding code) may affect transcriptional activity, time of replication, and recombination. The folding code may therefore act as an expression code because the proteins assembled in control regions determine the regulation of adjacent coding sequences [5]. However, the transition from an active to an inactive state or vice versa is normally the result of a developmental or environmental stimulus, and often seems to be a multistage process, rather than a simple switch [5,9]. In this model a type of epigenetic inheritance may occur, with a three dimensional, pre-existing structure directing the assembly of a new identical structure in the next generation. Since the chromatin-marking maintenance mechanisms are independent of the functional state of the gene, and the gene product is not required to have a specific regulatory role, this system is potentially much more flexible making

the genome far more fluid and responsive to the environment [5].

Inheritance of adaptive changes is limited with neural crest derivatives

We agree that geographic variations are due to direct environmental effects which, over many generations, became inherited [5]. In this system, pineal gland is an environmentally modified part of the body, and melatonin is a mediator (or gemmule as described by Darwin) liberated into the circulation and transfers the environmental stimuli to germ cells (oocytes). To explain how environmental influences on somatic characters could be transmitted to the next generation, we suggest that the melatonin affects the somatic parts of the organism as well as the oocytes. Multiple levels of melatonin action, from the hypothalamus and pituitary to gonads form a robust system highly sensitive to environmental effects [36]. In this system, melatonin acts as a mediator to produce epigenetic modifications in oocytes and contributes to inheritance of adaptive changes to the next generations. The response of organisms to a new environmental stimulus is likely to be epigenetic modification of many loci. The opportunities of variations following changed environmental conditions are therefore enormous [5]. At present it is difficult to know how common is the inheritance of adaptive modifications, because many changes are likely to have only small phenotypic effects, seen mainly as quantitative, rather than qualitative changes. If there are mechanisms through which adaptive changes can be inherited, why has more than a century of study of heredity failed to reveal sufficient cases of inheritance of this type? The main reason is that usually people have looked in the wrong type of organism, in the wrong place, and for the wrong type of change. Evidence for or against the inheritance of adaptive changes is not to be found in the type of experiment carried out by Weismann in the last century. He cut off the tails of mice for 22 generations and showed that it had no effect on the tail length of the progeny [5]. The type of character one should be looking at is a character that can be changed in cell lineages that can contribute to gametes. In many animals this means looking at characters induced either early in development, or at characters acquired in, and affecting, the germ-cell lineage itself [5]. Therefore, inheritance of adaptive changes (biological adaptation) occurs only if information from the somatic parts of the adult mammalian body is transferred to germ cells.

We previously suggested that biological adaptation is limited to those structures of neural crest origin [2] (such as, craniofacial tissues [47], melanocytes, and glands related to stature, hair form [48] and body proportions [49]). Thus, an experiment that is more likely to demonstrate biological adaptation is one in which an induced change affects the neural crest derivatives, including the cells that produce the next generation.

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