DALGARNO INSTITUTE


# Gene Environment Interactions & “Epigenetics”

**Dr. A.S. Reece**

**2010**

[A synoptic look at the emerging evidence of epigenetic impact on gene expression and how drug use factors into this impact.]

## Gene Environment Interactions & "Epigenetics"

Classically most people and many researchers have for a long time thought that our genes at birth are fixed, and that what happens to us after that is largely determined by the impact of our environment on our genetic make-up. Since Watson and Crick solved the macrostructure of the DNA helix in 1954, it has been thought that DNA (deoxyribonucleic acid) was transcribed to messenger RNA (ribonucleic acid, mRNA) which was then translated by the cell's protein translation machinery in the ribosomes into protein. Our body is made up largely of protein, and for a long time it was considered that this linear pathway from DNA to RNA to protein was a "one way street."

Several things have changed since those days. In 1964 Barker noticed that mothers who had high blood pressure in pregnancy, tended to be more likely to have babies who developed high blood pressure in later life. He therefore suggested that in utero effects could affect important events in later life. This "Barker hypothesis" has now been confirmed many times, albeit the exact mechanism was not well understood until recently [1-4]. Secondly the human genome was sequenced in about 2002, and found to consist of about 3 billion base pairs. What came as very odd was that only about 1% of this genetic code is occupied mainly with coding for proteins, which of course up until then had been thought to be the main business of the genome. It turns out that the other 99% of the genome, the "non-coding genes" are actually involved in gene regulation by various mechanisms as described below. It turns out that just as the human cerebral cortex is disproportionately massive compared to other animals, so also the level of "non-coding genes" in human beings is massively expanded upon that seen in other animals. This is thought to account for much of the variation between different people. Moreover, since most of the genes seen in man are also found in other mammals and have homologues seen in widely different life forms, this non-coding DNA (ncDNA) material is thought to account for the different expressions of those same or homologous genes in other organisms.

Thirdly in about 2006 it was shown that short pieces of RNA code of 18-22 bases could directly inhibit DNA transcription. This was known as RNA "interference" and the technique became abbreviated to RNAi. The short forms of RNA became known as short RNA (sRNA), short interfering RNA (siRNA) and micro-RNA's (miRNA). Since these RNA strands often had a hairpin bend in them they became known as hairpin interfering RNA's (hiRNA's). Special proteins were found to be responsible for cutting up the long segments of transcribed RNA into shorter segments which could be active in this way, including Dicer (1 and 2) and Argonaut (1 and 2). Furthermore the RNA itself can also be packaged by proteins known as RNA binding proteins, which also affect the behaviour of sRNA's, and so their gene silencing activity. Many thousands of siRNA's have been described of defined sequence. For some their activities are known. These sRNA's and ncDNA's are one of the major foci of current cellular and biological research. They are believed to have major roles in such key cellular processes as gene transcription, organism development and body and organ structure, cancer and ageing. These meshworks of interacting RNA's form a rich tapestry which facilitates gated external input to the genetic transcription machinery from the cytoplasm, and via the cytoplasm, the external cellular environment. This is believed to be one of the major loci of gene-environment interactions [5-11].

And finally it was learned just recently that DNA does not exist free in the nucleus but is actually bundled up as 178 bases and wrapped around sets of eight proteins called histone

octamers. There are four main histones, histones 1-4 and their variants. This wrapping process is very important as the DNA essentially cannot be used when it is tightly bound up, in much the same way that children are not able to play with their Christmas presents until they are freed from their wrapping paper. These histone proteins, which are of several different types can then be marked by various processes which indicate what is going to happen to that particular strand of DNA. Common processes include the adding (or removal) of a single carbon (methyl) group, the adding (or removal) of two carbon (acetyl) groups or various other chemical groups such as phosphates or short lipids. The acetylation steps are of particular relevance to the physiology of ageing as many studies have linked the classical histone deacetylases (known as sirtuins) with gene activation which has been shown to play a pivotal role in the aging process [12-14]. A classic activation signal is to triple methylate histone 3 on its third residue lysine 3 (H3K4me3), whilst a classic switch off signal is to triple methylate the 27$^{th}$ residue lysine (H3K27me3). When both these sites are trimethylated this is a pre-activation signal which tells the cell to "watch this space" as in stem cells getting ready for a major differentiation and phenotype transition [15]. All these phenomena can now be studied by rapid sequencing high throughput molecular techniques, some of them with high accuracy at the single cell level. There are other important DNA and chromosomal packaging techniques such as certain segments being located near the nuclear membrane where some of it is bound to the nuclear lamina. All these steps are regulated and therefore susceptible to control, and thus have the capacity to store important cellular information, and may therefore be thought of as forming part of the biological machinery for storing cellular memory.

Collectively these changes are known as epigenetic changes. It has been said that there is 100 times as much information stored in the human epigenome as in the genome proper!

Many environmental stimuli have been found to affect epigenetic expression. This includes nutritional status, hypoxia and drugs and poisons. It therefore seems inevitable that toxins such as addictive drugs can also affect these processes. Epigenetics is an area of active enquiry at the present time. It is believed that the epigenome is one of the key loci at which a *two way* gene – environment interaction occurs, which likely includes all of the mechanisms mentioned above.

Special mention is worthwhile of the role of immune stimuli in stem cell and genetic processes. It is well known in the popular culture that habitual users of addictive drugs frequently look awful, and it is possible to measure and therefore prove that drug dependent patients have a multitude of degenerative and age related processes to more severe degree than non-addicted patients; in other words drug addiction ages you in a more rapid way. This seems true whether one considers immunological, brain, heart, lung, liver or kidney related disorders, albeit the details of the most affected system varies a little with the different addictive substances. However all these addictions are marked by a generalized immune stimulation of the whole system, while at the same time showing evidence of being having a weakened or reduced ability to fight infections. In other words addictive patients are both immunostimulated and immunosuppressed at the same time. There are two major implications of this. Firstly all stem cells not only have receptors for immune active molecules (called "cytokines") on their cell membrane [16-21], but they hold within them major pathways which affect their growth and behaviour which are triggered

by them called the JAK-STAT (Janus activated kinase – signal transducer and activator of transcription) pathway. This is one of the major pathways within stem cells [22-24]. In most of them it powerfully suppresses stem cell replicative activity, but in the bone marrow myeloid series stem cell divisions are stimulated (at least initially), which is the basis for the well known white blood cell response to systemic infection.

A major pathway which is known to be responsible for limiting the growth of all cells is the shortening of the chromosomes with each round of cell division. When this end part of the chromosome, known as the telomere, becomes too short, it causes the cell to stop dividing, and usually to assume an old hypofunctional form known as a "cellular senescence". This is an old and exhausted type of cell, common in older people, and also associated with developing and fostering cancer development [25]. In other words short telomeres are known to induce a replicative crisis and trigger cellular senescence. It has recently been shown that telomere length can also trigger one of the immune stimulated genes called interferon stimulated gene 15 (ISG15) [26] prior to the crisis being reached in telomere length. In other words a chronically stimulated immune system can via ISG15 and this mechanism trigger the telomere length signalling machinery and replicative senescence so inducing cellular, and eventually organismal aging. Conversely telomere shortening can stimulate ISG15 which increases the levels of pro-inflammatory cytokines such as interleukin-6 which has been linked with immune stimulation, frailty, chronic illness and death [26], a profile well described in opiate [27-31] and other drug addicts. Similarly telomere dysfunction prior to the length crisis can also trigger premature tissue degeneration and cancer development [26]. This latter finding is of particular relevance to all the addictive drugs as they have all been shown to be mutagenic in various genetic and chromosomal assays [32]. This is particularly true for cannabis which has been shown to produce very severe telomeric dysfunction in sperm [33], obviously with severe disruption of the telomeric capping proteins (collectively known as "shelterin") binding activity.

These alterations are in addition to the effects of addictive drugs to suppress tissue growth, and to increase cell death directly.

***SUMMARY***

Hence many pathways exist by which the genes and the environment can impact each other. This is known now to be very much a "*two way street*", and the changes involved can occur relatively rapidly within short periods of time. As this is an evolving and developing area of medicine much is yet to be learned in this field, but we known enough now to state the following conclusions:

1) **Environment, and particularly toxins including addictive drugs, can affect genetic expression. This is known as gene – environment interaction;**
2) **These toxicities may occur at either the genetic or epigenetic level;**
3) **There are multiple pathways by which this may occur, particularly histone methylation and RNA interference;**

4) **Since they have been shown to occur in the germ cells (sperm) they are inheritable to the following generation;**
5) **Deleterious genetic and epigenetic changes are associated with foetal malformation, growth and tissue retardation, mental retardation, aging and cancer formation;**
6) **Addictive drugs have been shown to have direct toxicity on the genome, and likely also on the epigenome;**
7) **Since stem cells will also be affected by these changes, disorders in which stem cells play a prominent part such as ageing, cancer, senescence and ill-health, are also likely to be impacted by this direct and indirect genetic toxicity;**
8) **Immune changes are also likely to be particularly important, and are also largely deleterious. They are likely to occur through both direct and indirect mechanisms and to involve multiple pathways.**

## *References*


1. Barker DJ. The fetal origins of diseases of old age. European journal of clinical nutrition 1992;46 Suppl 3:S3-9.
2. Barker DJ. Fetal growth and adult disease. British journal of obstetrics and gynaecology 1992;99:275-6.
3. Barker DJB. Fetal and infant origins of adult disease. London: BMJ Publishing Group; 1992.
4. Robinson S, Walton RJ, Clark PM, Barker DJ, Hales CN, Osmond C. The relation of fetal growth to plasma glucose in young men. Diabetologia 1992;35:444-6.
5. Mattick JS. RNA as the substrate for epigenome-environment interactions: rNA guidance of epigenetic processes and the expansion of RNA editing in animals underpins development, phenotypic plasticity, learning, and cognition. Bioessays 2010;32:548-52.
6. Mattick JS, Amaral PP, Dinger ME, Mercer TR, Mehler MF. RNA regulation of epigenetic processes. Bioessays 2009;31:51-9.
7. Dinger ME, Pang KC, Mercer TR, Crowe ML, Grimmond SM, Mattick JS. NRED: a database of long noncoding RNA expression. Nucleic Acids Res 2009;37:D122-6.
8. Mattick JS, Mehler MF. RNA editing, DNA recoding and the evolution of human cognition. Trends Neurosci 2008;31:227-33.
9. Dinger ME, Pang KC, Mercer TR, Mattick JS. Differentiating protein-coding and noncoding RNA: challenges and ambiguities. PLoS Comput Biol 2008;4:e1000176.
10. Amaral PP, Mattick JS. Noncoding RNA in development. Mamm Genome 2008;19:454-92.
11. Liu C, Teng ZQ, Santistevan NJ, et al. Epigenetic regulation of miR-184 by MBD1 governs neural stem cell proliferation and differentiation. Cell Stem Cell 2010;6:433-44.
12. Blander G, Guarente L. The Sir2 family of protein deacetylases. Annual review of biochemistry 2004;73:417-35.
13. Guarente L, Picard F. Calorie restriction--the SIR2 connection. Cell 2005;120:473-82.
14. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. Cell 2005;120:483-95.
15. Marks H, Veenstra GJ, Stunnenberg HG. Insightful tales from single embryonic cells. Cell Stem Cell 2010;6:397-8.
16. Bhat R, Steinman L. Innate and adaptive autoimmunity directed to the central nervous system. Neuron 2009;64:123-32.

17. Boulanger LM. Immune proteins in brain development and synaptic plasticity. Neuron 2009;64:93-109.
18. Carpentier PA, Palmer TD. Immune influence on adult neural stem cell regulation and function. Neuron 2009;64:79-92.
19. Deverman BE, Patterson PH. Cytokines and CNS development. Neuron 2009;64:61-78.
20. Drapeau E, Nora Abrous D. Role of neurogenesis in age-related memory disorders. Aging Cell 2008.
21. Edelberg JM, Ballard VL. Stem Cell Review Series: Regulating highly potent stem cells in aging: environmental influences on plasticity. Aging Cell 2008;7:599-604.
22. Buecker C, Chen HH, Polo JM, et al. A murine ESC-like state facilitates transgenesis and homologous recombination in human pluripotent stem cells. Cell Stem Cell 2010;6:535-46.
23. Cherry CM, Matunis EL. Epigenetic regulation of stem cell maintenance in the Drosophila testis via the nucleosome-remodeling factor NURF. Cell Stem Cell 2010;6:557-67.
24. Kerr CL, Cheng L. Multiple, interconvertible states of human pluripotent stem cells. Cell Stem Cell 2010;6:497-9.
25. Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 2005;120:513-22.
26. Blagosklonny MV, Campisi J, Sinclair DA, et al. Impact papers on aging in 2009. Aging (Albany NY) 2010;2:111-21.
27. Reece AS. Chronic hepatitis as an important contributor to the immunosenescence of parenteral drug addiction. Addiction Biology 2008;14:214-26.
28. Reece A. S. Chronic Immune Stimulation as a Contributing Cause of Chronic Disease in Opiate Addiction Including Multi-System Ageing Medical hypotheses 2010.
29. Reece A. S. Chronic Opioid Agonism Encompasses a Neuroinflammatory disorder both in the Neuraxis and Systemically. British Medical Journal 2009;In Press.
30. Reece AS. Evidence of Accelerated Ageing in Clinical Drug Addiction from Immune, Hepatic and Metabolic Biomarkers. Immun Ageing 2007;4:6-15.
31. Brunton L.L., Lazo J.S., Parker K.L., eds. Goodman and Gilman's the Pharmacologic Basis of Therapeutics. Eleventh Edition ed. New York: McGraw Hill; 2006.
32. Li JH, Lin LF. Genetic toxicology of abused drugs: a brief review. Mutagenesis 1998;13:557-65.
33. Zimmerman AM, Zimmerman S., Raj A.Y. Effects of Cannabinoids on spermatogenesis in mice. In: Nahas G.G., Sutin K.M., Harvey D.J., Agurell S., eds. Marihuana and medicine. Totowa, N.J, USA.: Humana Press; 1999:347-58.