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COMMENTARY

The gap junction as a "Biological Rosetta Stone": implications of evolution, stem cells to homeostatic regulation of health and disease in the Barker hypothesis

James E. Trosko

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Abstract The discovery of the gap junction structure, its functions and the family of the "connexin" genes, has been basically ignored by the major biological disciplines. These connexin genes code for proteins that organize to form membrane-associated hemi-channels, "connexons", co-join with the connexons of neighboring cells to form gap junctions. Gap junctions appeared in the early evolution of the metazoan. Their fundamental functions, (e.g., to synchronize electrotonic and metabolic functions of societies of cells, and to regulate cell proliferation, cell differentiation, and apoptosis), were accomplished via integrating the extra-cellular triggering of intra-cellular signaling, and therefore, regulating gene expression. These functions have been documented by genetic mutations of the connexin genes and by chemical modulation of gap junctions. Via genetic alteration of connexins in knock-out and transgenic mice, as well as inherited connexin mutations in various human syndromes, the gap junction has been shown to be directly linked to many normal cell functions and multiple diseases, such as birth defects, reproductive, neurological disorders, immune dysfunction and cancer. Specifically, the modulation of gap junctional intercellular communication (GJIC), either by increasing or decreasing its functions by non-mutagenic chemicals or by oncogenes or tumor suppressor genes in normal or "initiated" stem cells and their progenitor cells, can have a major impact on tumor promotion or cancer chemoprevention and chemotherapy. The overview of the roles of the gap junction in the evolution of the metazoan and its potential in understanding a "systems" view of human health and aging and the diseases of aging will be attempted.

Keywords Gap junction · Connexin · Cell proliferation · Cell differentiation

J. E. Trosko (✉)
Department Pediatrics/Human Development,
College of Human Medicine, Michigan State University,
246 Food Safety and Toxicology Bldg,
East Lansing, MI 48824, USA
e-mail: James.Trosko@hc.msu.edu

> "Nothing in Biology makes sense except in the light of evolution" (Dobzhansky 1975)
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> "The hypothetical coordinating principle, which gets deranged in cancer, could belong to a class of genes exemplified by homeotic genes that determine tissue pattern formation in early embryogenesis." (VandenHooff 1989)

Introduction: the requirement of the leap of imagination is to bring one's feet back to earth

It has been said that the difference between the creative act in the arts and sciences is not the ability of either disciplines to make the leap of the imagination (both can do that because that is an attribute of being human), but, while the artist has no obligation to bring his/her feet back to earth, the scientist is obligated to make sure the feet touch the earth. Rarely, in any scientific publication, no matter how significant the science might be, are all the assumptions, logic and scientific evidence provided to the recipients of the new report. All too often, the complexities of the problem to solved, the techniques used, and the experimental results "drown" the recipients with reductionalistic details. Often, this prevents the information to be

seen in a larger light (hence, the references to the Dobzhansky quote).

In this "*Commentary*" about a topic involving a unique biological structure and its presumptive functions in the homeostatic process of metazoan development, an attempt will be made in the following to provide the abstract framework that has shaped the following attempt to integrate concepts of evolution, of stem cell biology, of the role of cell–cell communication in species survival and in normal development of the metazoan, as well in various disease processes. This integrative framework does not imply the construct is correct but that it provides the reader a "road map" of the thinking that has gone into the process, which includes the evolution of the germ line and somatic stem cells and of the gap junction gene family, that provides a means to survive, while at the same time, the mechanisms that characterize many of the aging and diseases of aging to be a part of "being human".

To begin, in order to understand one of those disease processes, i.e., carcinogenesis, the following are the basic assumptions, linked, by what is perceived to be logical, to other assumptions and reported observations or experimental facts.

Cancers are monoclonal or derived from a single cell. All cancers lack functional gap junctions, either because (a) they never express the connexin genes or (b) the connexin proteins are rendered non-functional by a mutation or by post-transcriptional/posttranslational modifications. There exists in each tumor "cancer-stem cells" and "cancer non-stem cells". Cancer stem cells metabolize via glycolysis or exhibit the "Warburg phenomenon". Cancer stem cells are, by nature, drug resistant due to the expression of drug transporter genes, not by induction of mutations by toxic agents. Cancers, with the exception of teratomas, are derived via the "initiation", "promotion" and "progression" processes. It will be impossible to prevent all cancers, since one can reduce the risk of a single cell to be "initiated" by reducing the exposure to "initiators", but it will be impossible to reduce the risk to zero. Initiation is assumed to be caused by a mutation. One cannot reduce to zero errors in normal DNA replication, therefore, a mutation or initiation of a cancer-related gene will occur, in spite of the elimination of exogenous initiators or mutagens.

Stem cells are likely targets to be the target cells for both teratomas and adult cancers. Stem cells are defined as those cells that can divide either by symmetrical cell division to produce two daughter stem cells or by asymmetrical cell division to produce one daughter stem cell and one progenitor cell destined to terminally differentiate, to senesce or to apoptose. These "initiated stem cells" are the likely progenitors for the "cancer stem cells". The "initiation" process is the result of preventing a stem cell from asymmetrical cell division under normal in vivo conditions, but they can "partially" differentiate when the 3-dimensional , in vivo microenvironment, changes, leading to "cancer non-stem cells". The promotion phase of cancer, which, in most non-childhood cancers, takes decades to occur, involves the clonal expansion of the initiated stem cell and the inhibition of the apoptosis of that initiated stem cell. Promotion can be interrupted or, in some cases, reversed. Once the "cancer stem cells" start to grow into tumors, the micro-environment changes, thereby causing some stem cells to divide asymmetrically and to partially differentiate into "cancer non-stem cells". The ratio of "cancer stem cells" to "cancer non-stem cells" in a tumor will be influenced by the endogenous and/or exogenous factors controlling symmetric versus asymmetric cell division of the cancer stem cells. Cancer prevention can occur via two mechanisms,: (a) reduction of the adult stem cell pools in specific organs during in utero development; or (b) by the induction of gap junctional intercellular communication in "pre-malignant cancer stem cells" that do not express the connexin genes or by preventing the down regulation of gap junctional intercellular communication (GJIC) by tumor promoters in initiated stems expressing their connexin genes. Cancer therapy must target the cancer stem cells by either transcriptionally expressing the connexin gene(s) in cancer cells, such as teratomas, and HeLa and MCF-7 carcinomas, or by inhibiting specific oncogene signaling pathways that render the connexin proteins non-functional. Finally, in utero modulation of the stem cell pools (increasing or decreasing the numbers of adult stem cells in specific organs) could affect the risk (increase or decrease) to any stem cell-based chronic disease later in life (The cellular mechanistic basis for the Barker hypothesis).

An attempt will be made to try to "close the circle" between stem cells, gap junctions, the Warburg metabolism in normal and cancer stem cells, the difference of mitochondria in normal and cancer stem cells stem cells and the differentiated cells. The observation that physical juxtaposition of the mitochondria and the gap junction plaques in differentiated cardiomyocytes [Forbes and Sperelakis, 1982] seems to support the idea of a close evolutionary causal link to support the idea that oxidative phosphorylation, gap junctional intercellular communication and differentiation were necessary. On the other hand, the link between normal adult stem cells, Warburg metabolism and cancer stem cells supports the stem cell hypothesis of the origin of cancer and the adult stem cells as the target cell for the "cancer stem cell".

It is this concatenation of ideas, assumptions and experiment findings that is the substrate on which this "*Commentary*" is based. While many of these separate statements are not yet universally accepted, and some of which are highly controversial, it is, at least, a starting point from which the scientific process of hypothesis testing and experimental design to test these hypotheses and assumptions can start.

The integration of molecular communication in the evolution of the metazoan or of the basis of systems biology

Whatever were the complex molecular events that happened to bring the "spark of life" on earth, it appears, that with the recent generation of a primitive "synthetic" life form (Gibson et al. 2010), it has ruled out the need for some "vital" force beyond natural physical/chemical principles. Given that during the evolution of the first living microorganisms, this primitive form had to survive to replicate the "instructions" in order that it could have offspring that also could survive that primitive Earth environment, as well as to be able to survive the earth's physical evolutionary changes. This real tension between trying to maintain the original genetic instructions, coded in nucleic acids, to be able to reproduce those strategies to cope with the physical necessities for life, and being able to be flexible enough to survive with the inevitable change of the physical environment, set the stage for life as we know it today. Billions of years later, that tension seems, in part, due to being able to resist and repair damage done to its genetic code and its ability to create a few mutational changes in a population. This allows for the possibility that during a severe environmental change, when most of the offspring with the origin genetic code would not survive, there could be a few that could adapt to the change and to carry on the species.

The physical chemical environment, with which the primitive life form had to cope, included gravity, temperature, radiation, atmospheric gases, mineral availability and other nutrient agents. Apparently, there was sufficient "slop" built into the original life form's genetic-coded biochemical functions to allow for a range of these physical /chemical factors, in which life functions are possible. For example, there was a limited temperature range of a DNA replicating enzyme to allow for a rather fidelity-producing genome. In brief, if that first living life form protected its DNA from most damage and repaired what little damage that did occur, then that organism would not have survived because the original genome coded proteins for an environment that no longer exists. If on the other hand, the first life form generated mutations so frequently, with or without DNA protection strategies, or with poor DNA repair (errors in repair) or DNA replicating (errors in replication) enzymes, that organism also would be unable to survive. Evolution had to strike a balance to allow these organisms to both survive as an individual but to be able to leave its offspring with survival adaptability, so as to perpetuate that species. In effect, these single cell organisms were "immortal" by being able to reproduce to maintain the species, yet they had to be controlled as individuals and population by constraints of temperature, gravity, nutrients, radiation, etc.

Whereas, a limited mutation strategy allowed the micro-organism to survive, an additional set of new genetic and phenotypic factors for the first multi-cellular organism had to appear. When two or more cells first appeared, they seemed to have acquired a new adaptive feature to survive a changing environment, namely, the genetic/molecular basis for the new phenotype, "internal growth control". Of course, this first multi-cellular metazoan was constrained by external temperature, nutrients, etc. for cellular replication; it had new mechanisms to control its ability to control unlimited replication, and not to be constrained by only external factors. A second new set of genetic and phenotypic factors had to appear, namely a mechanism to regulate gene expression for new phenotype functions, i.e., differentiation or "mortality" of somatic cells. Related to this new process of differential gene expression to create new phenotypes for survival, e.g., muscles, nerves, bone, liver, and kidney cells, the process of cell suicide or apoptosis appeared. With both differentiation and apoptosis designed to confer cell mortality or death to cells of the metazoan, a mechanism had to develop a process to ensure mechanism to ensure survival of "immortal" cells for both cell replacement and death of differentiated somatic cells for long term survival of the individual and for survival of the species. This introduced the creation of genes/phenotype of somatic (adult) and germinal stem cells. In addition to differentiation and apoptosis as means to "mortalize" individual somatic cells, the genes and phenotype of "senescence" also appeared.

Overall, the metazoan acquired these diverse phenotypes of growth control, differentiation, apoptosis, stem cell formation, and senescence within one individual, which had multiple cells containing the same genome, yet, differentially, expressing those genes. Remarkably, while individual cells of the metazoan could become "mortal", the organism itself could "age", yet maintain "immortal" somatic adult stem cells to provide for growth of the individual, wound or tissue repair/replacement. If ever the meaning of the term, "system biology" (Cornish-Bowden 2006), had substance, the integration of molecular information communication within cells, between cells of the same tissue/organ, between cells of different organs and between the organism and its physical/social and cultural environment, must be explained. Homeostatic control of that complex information flow, both positive and negative, in a dynamic physical/chemical, biological, social, and cultural environment, must be facilitated to maintain normal regulation of cell growth, differentiation and apoptosis and stem cells maintenance at appropriate times during development and normal aging processes.

One cannot ignore philosophical ideas to help to conceptualize what happens during the evolution of the metazoan. The terms of the "hierarchical" nature of the

living metazoan, where the organization of simple elements, such as atoms, leads to the emergence of new properties found in the molecules, which did not exist in the subunits of the molecules. In turn, organization of molecules into organelles, organelles into cells, cells into tissues, tissues into organs, organs into organ systems, and organ systems to organisms, organisms into societies, etc. (Trosko 1998). This happens because of the feedback of positive and negative molecular information via a "cybernetic process" (Brody 1973; Potter 1974). Therefore, what underscores the philosophical basis for the metazoans biological ability to facilitate molecular control of normal development and maintenance of health during the aging process leading to ultimate death of the individual but survival of the species?

The discovery of a "Biological Rosetta Stone" coincided with the discovery of a family of connexin genes whose functions integrated extra-cellular communication with intra-cellular communication pathways to regulate gap junctional intercellular communication and gene expression

The discovery of the meaning of the famous "Rosetta Stone" by Napoleon's cryptographer, ultimately led to the deciphering of the Egyptian hieroglyphics because of the association to two different sets of symbols to communicate the same reality. In this author's opinion, in biology, an equally significant discovery was made when an electrophysiological phenomenon of a low resistant transfer of ions between cells, seen in confluent population of cells, by Loewenstein and Kanno (1966), was associated with a anatomical structure of contiguous cells seen via a new technique, freeze fracture, the "gap junction". Equally important in the speculation that the low resistance transfer of ions and small molecular weight molecules between contiguous cells was via the anatomical structure on the plasma membrane was the presumptive roles it might play in regulating cell growth and differentiation of cells (Loewenstein, 1966). The gap junction was associated with regulation of both synchronization of electrotonic and metabolic functions of cells within certain tissues (Yamasaki and Naus 1996). The significance of this was missed for decades before in the study of development and physiological functions of metazoans. A mechanistic explanation of how the neighboring millions of cells could be different in morphology and function, yet all contain the same genes, was not even discussed with available scientific knowledge.

Then came the insight gained when these gap junctions were studied in normal cells compared to cancer cells. At that time, cancer cells were characterized by a loss of "growth control" or loss in "contact inhibition" (Borek and Sachs 1966), or by their inability to terminally differentiate and by their so-called "gain in immortality". Further, terms, such as "cancers as a disease of differentiation" (Markert 1968), "oncogeny as partially blocked ontongeny" (Potter 1978), or a disease of stem cells (Pierce 1974) were introduced to suggest that the target cells to initiate the carcinogenesis process were stem cells. Only later, were gap junctions linked to apoptosis (Wilson et al. 2000) and to stem cell phenotypes (Trosko et al. 2000). This insight has to be given to Werner Loewenstein, who noticed that, while normal cells that could (a) contact inhibit or have growth control; (b) could terminally differentiate; (c) were "mortal" and could senesce; but cancer cells, which (a) did not contact inhibit or have growth control; (b) could not terminally differentiate, but (c) were immortal, did not have functional gap junctions. While correlation does not automatically equate to causality, it does raise an interesting philosophical question, namely, Is the transition of a normal metazoan phenotype to a cancer cell phenotype, where the gap junction is non-functional, likened to the "de-evolution" to a unicellular phenotype, where the genes coding for the gap junction structure and functions in the metazoan are not found in the uni-cellular organism? This association is made even more tighter with the later discovery that gap junctions could function in normal cells to facility the transfer of the "death signal" for apoptosis in contact inhibited normal cells (Wilson et al. 2000). However, cancer cells, that do not have gap junctional intercellular communication, do not apoptose under normal condition (Hanahan and Weinberg 2000). In addition, it was shown that normal human adult stem cells did not have functional GJIC (Trosko et al. 2000), the maintenance of the stem cell's primitive state could be assured.

This might lead to a conundrum in that, if a normal stem cell, which immortal, does not have gap junctional intercellular communication, and is "undifferentiated", why is it not a cancer cell, which, also, does not have GJIC, does not terminally differentiate, and is immortal? The answer , while complex , is that a normal stem cell can be easily induced to express its connexin genes, form gap junctions, and to terminally differentiate and become "mortal", while the cancer cell cannot be easily induced to have growth control, to terminally differentiate or to apoptose or to "mortalize". Actually, a normal embryonic stem cell is actually defined as a cell that can form a teratoma when placed back into a adult microenvironment.

One last characteristic of cancer cells that are characterized by their inability to have functional gap junctional intercellular communication is that there are two classes of non-GJIC cancer cells. The first type includes cancer cells that do not express their connexin genes, transcriptionally. HeLa and MCF-7 cells seem to represent this class of cancer cells (Trosko 2003). More recently, the so-called "cancer stem cell" might be representative of this class class also (Trosko and Tai 2006). The second type would include cancer cells that express their connexin genes but the proteins and gap

junction channels are rendered non-functional but either a mutation or some posttranslational modification of the connexin protein by some oncogene (Trosko and Ruch 1998).

Gap junctions as the functional basis for its "Biological Rosetta Stone" role

Clearly, it could be argued that connexin genes or the gap junction functions play no bigger role during the evolutionary transition from the unicellular organism to the metazoan than any other metazoan gene or function not found in the unicellular organism's genome. Yet, in spite of the amazing absence of the inclusion of the role in gap junctions during carcinogenesis by current spokespersons for the current paradigm of molecular oncology and by developers of cancer chemopreventive or chemotherapeutic agents (Kelloff et al. 2006), there are a number of quite independent experiments linking gap junctions to normal growth control and differentiation in normal cells and the lack of GJIC in cancer cells (Trosko and Ruch 2002). One needs only to "Google" *gap junction and diseases* to find hundreds of scientific studies correlating the dysfunction of gap junctions associated with a wide spectrum of diseases, from birth defects, cancer, reproductive-, immune- and neurological-dysfunctions, cataracts, etc. In addition, the discovery that many non-genotoxic chemicals, by triggering various intra-cellular signaling mechanisms via oxidative stress, were associated with many toxic endpoints at the same time they reversibly inhibited gap junction function (Upham and Trosko 2009). Even agents, that induced inflammation and were associated with chronic diseases, could affect gap junction function (Trosko and Tai 2006). In addition, various oncogenes, that coded for proteins, could affect gap junction function strengthened the association between the need for growth control and functional gap junctions (Trosko and Ruch 1998). Further, agents that either prevented endogenous or exogenous agents from inhibiting gap junction function or those that enhanced gap junction function were shown to be cancer chemopreventive and chemotherapeutic agents (Trosko and Ruch 2002). Using antisense factors to connexin genes in normal cells could convert their phenotype to a tumor phenotype, while transfecting normal connexin genes into non-GJIC cancer cells restored cell growth (Trosko and Ruch 1998).

However, these correlation studies, while not convincing the scientific community of the fundamental role gap junctions play in regulating cell behavior in metazoans, the genetic creation of various connexin knockout and transgenic mice (Cruciani and Mikalsen 2005; Willecke et al. 2002), provided more convincing evidence of their roles in development and health maintenance (Lo 1996; Kelsell et al. 2001). Some of these studies showed the critical roles that connexin 26 and connexin43 played in development, as their knockout mice were unable to complete normal development. On the other hand the knockout 32, which, by allowing for normal development, predisposed the mice to a high spontaneous and chemically induced liver cancer frequency (Temme et al. 1997). It is also interesting to note that in a connexin32 dominant-negative rat, they seemed to be resistant to hepatic damage by hepatic cytotoxicants (Asamoto et al. 2004). It was the discovery that several human inherited diseases were associated with mutated connexin genes that provided addition strong evidence that specific dysfunctional connexins were associated with inherited diseases states (Dobrowolski and Willecke 2009). Charcot Marie-Tooth syndrome , erythrokeratoderma variabilis, non-syndromic sensorineural hearing loss; dominant zonular pulverant cataract are but a few of the reported genetic syndromes associated with inherited mutated connexins (Kelsell et al. 2001).

Coming back to the concept that the discovery of gap junctions and their fundamental roles in regulating homestatsis of cell proliferation, differentiation, apoptosis, immortality/mortality and stem cell maintenance can be viewed as a "Biological Rosetta Stone", the connection to the phrase, "Rosetta Stone" must be made. Clearly, When Jean-Francois Champollion recognized that the three languages carved into the basalt stone slab found by Napoleon's army in Egypt near Raschid (Rosette) were describing the same story in three languages (Demotic, Greek and Egyptian hieroglyphs), he was able to un-code the hieroglyphs for the first time, since he was able to understand the other two languages. Given the complexities of understanding the basic functions of cells, the homeostatic regulation of these mutually-exclusive cellular functions (e.g., cell proliferation, cell differentiation and apoptosis) had to be explained because they cannot occur at the same time in the same cell. The delicate regulation of these three cellular choices during development and maintenance of health at all stages of human development had to be resolved. It seems that, when Werner Loewenstein saw how the transfer of ions and small molecular weight molecules through a low-resistance membrane-associated protein channel, they could be used to synchronize both electronic and metabolic functions in specific arrangements of cells within tissues and to regulate cell proliferation and differentiation in the multi-cellular metazoan. It was only logical that the absence of that unique function of metazoans would lead to devastating consequences of cellular behavior leading to cancer.

The fact that, in a metazoan, such as the human being, which has each organ expressing specific connexins, it should be obvious that the normal function of specific

connexins allows for regional control of signals to bring about to specific cell differentiation and functions. Each type of connexin protein, coded by each of the 20 connexin genes, could be differentially regulated by endogenous or exogenous factors. Therefore, the "simplicity" of the gap junction channel, coded by a highly evolutionarily conserved family of genes, whose regulation is dependent on sensed intracellular alteration (change in pH, Ca++, activation of protein kinases, phosphatases, redox state, etc.), could either act as a "sink" or "source" of molecular information to regulate gene expression (Sheridan 1991).

Cell–cell communication as the rate-limiting step in the multi-stage, multi-mechanism process of carcinogenesis

While dysfunction of gap junctional intercellular communication has been implied earlier to play a role in many clinically-defined diseases, its role in the carcinogenesis process is best documented. First, cancer has been described as a "disease of differentiation" (Markert 1968), a "stem cell disease" (Pierce 1974) and as "oncogeny as partially blocked ontogeny" (Potter 1978). Cells within a tumor have been shown to have had a single cell origin, even though they could be genotypically and phenotypically unique, due to multiple changes that had occurred during the carcinogenic process (Nowell 1976; Fialkow 1979). An alternative hypothesis of the origin of cancers is that of the "de-differentiation theory" or the "re-programming of a normal somatic differentiated cell to a "iPS"-like embryonic stem cell" (Sell 1993).

It should be pointed out that, in reality, there are two types of tumors, embryonic-like tumors (teratomas) and adult-type tumors. The teratomas are interesting in that malignant teratoma cells, when transplanted back into the blastocyt of an appropriate animal, will contribute to the development of normal tissue of a normal animal. This implies that these teratomas are genetically normal but, due to some unknown factor, the genes of the original teratoma-originating cell were abnormally expressed or "epigenetically abnormal". During its growth as a teratoma in an adult animal, the microenvironment induces differentiation within the tumor to form disorganized collections of hair, bone, muscle, etc. cells. However, when the malignant teratoma cell is placed back into a normal blastocyte microenvironment, its abnormally-expressed normal genes are "re-programmed" to be normally expressed to contribute to a normal animal (Minsk and Illensee 1975). If the same type of transplantation is done with an adult, non-teratoma cancer cell, there is no normal re-programming to form a normal animal. That is because the adult cancer cell contains, not only several epigenetic alterations of normal genes, but most likely multiple irreversible gene and chromosomal mutations, which cannot be "re-programmed". Interestingly, one of the functional definitions of an embryonic or "iPS" stem cell is the ability to form teratomas when it is placed back into an appropriate adult animal.

Probably the most important concept that has come out of the adult cancer field is that of the "initiation", "promotion" and "progression" process of the multi-stage, multi-mechanism nature of carcinogenesis (Weinstein et al. 1984; Pitot and Dragon 1991). The operational definition of the initiation phase is an irreversible event in one normal cell of an organism after exposure to an agent (physical, chemical, biological). Next, operationally, when the "initiated" animal is exposed to an agent, which , by itself, is unable to induce "initiation", can cause a clonal expansion of the initiated cell to form, for example, a papilloma of the skin, an enzyme-altered focus of the liver, a nodule in the breast or a polyp of the intestine. Finally, when one of these promoted initiated cells acquires enough phenotypic changes to become invasive and metastatic, it has been converted to the progression phase.

While it has yet to be universally-accepted, the mechanisms of the three phases can only be accurately classified, operationally. Since initiation appears to be irreversible, the prevailing hypothesis is that the process that leads to initiation of a normal cell to an irreversible changed cell is mutagenesis. Both radiations (ionizing and non-ionizing UV light) have been associated with both experimental and epidemiological production of cancers; and, with the restoration of the role of viruses in the carcinogenic process (Zur Hausen 2003; Moody and Laimins 2010), it seems that certain viruses can be viewed as being potential "initiators" (Trosko et al. 2000). One source of "initiated" cells that seems to have been largely ignored is that of "errors in replication" or the origin of "spontaneous" mutations. Gene or point mutations can, in principle, originate from "errors in DNA repair" or from "errors in DNA replication". While UV light can induced genomic DNA damage, which if not repaired correctly, can lead to mutations and skin cancers, as in the case of the skin cancer-prone heredity syndrome of xeroderma pigmentosum (Brash et al. 1991). On the other hand, ionizing radiation does not seem to be an efficient inducer of point mutations but can damage genomic DNA to cause deletion mutations and chromosome aberrations (Trosko 2007). It does not seem to be an efficient "initiator" of cancers (Jaffe and Bowden 1987; Kaufman et al. 1987). Consequently, since cancers can appear in irradiated humans, its role in the carcinogenic process is still unknown. Epigenetic mechanisms play a role in carcinogenesis, and ionizing radiation can induce epigenetic intracellular signaling (Upham and Trosko 2005).

Promotion seems to be an epigenetic process, in that, mitosis, rather that mutagenesis, can best explain how a

single initiated cell can be clonally expanded to a pre-malignant lesion (Trosko 2001). In addition, promotion is brought about by agents and conditions that are non-mutagenic. Examples of promoters include, phorbol esters, DDT, TCDD, polybrominated biphenyls, Phenobarbital, pthalates, saccharin, growth factors, cytokines, wound healing and even cytotoxicity caused by many types of agents, even mutagens, non-genotoxic cytotoxicants and even viruses (Trosko and Chang 1988). In effect, many agents that can bring about chronic inflammation, such as solid particles, such as soot and asbestos, can be associated with the promotion process (Trosko and Tai 2006). Promotion has also been associated with the inhibition of cell killing by apoptosis (Bursch et al. 1992). Consequently, any agent that can cause an initiated cell to escape the suppressing mitogenic and apoptotic effect of surrounding normal cells will allow the initiated cell population increase.

Mitotic suppression of two types of cells that can proliferate (e.g., stem cells and progenitor cells) takes place by two different mechanisms. If all adult stem cells do not express their connexin genes and have functional gap junctions in their niche, they might be suppressed by some soluble extracellular communicating anti-mitogenic molecule produced by the terminally-differentiated daughter of the stem cell lineage (Trosko 2003). Consequently, if the "initiated" cell is the adult stem cell, it has to be promoted by agents that interfere with the anti-mitogenic soluble factor that prevents its ability to escape its niche environment. On the other hand, if the initiated cell is an early progenitor cells that has expressed their connexin genes and started to "partially" differentiate, but have not yet been "immortalized", they would be suppressed by gap junction-mediated contact inhibition. Promoting agents that can inhibit gap junctional intercellular communication between the initiated cell and its surrounding normal cells, it now can proliferate and not die by apoptosis. Chemical promoting agents seem to be able to inhibit GJIC, reversibly (Yotti et al. 1979). On the other hand many activated oncogenes can inhibit GJIC stable (Ras, Raf, Src, Neu, Mos) (Trosko and Ruch 1998).

Following this explanation to its logical conclusions, all cancer cells should be characterized by having no functional soluble inhibitory cell–cell communication or gap junctional intercellular communication, as hypothesized by Werner Loewenstein (Loewenstein 1966). Without the ability to have functional intercellular communication, they would be unable to terminally differentiate, to be mitogenically suppressed, to die by apoptosis or to "mortalize". However, on close examination, these non-communicating cancer cells will be cells, such as HeLa or MCF-7 (King et al. 2000; Momiyama et al. 2003), in that they do not express their connexin genes, have not connexin proteins of functional gap junctions. On the other hand, other cancer cells express their connexin genes, but their connexin proteins have been rendered non-functional by activated oncogenes, such as Ha-ras (De Feijter et al. 1990).

Since the promotion process is characterized as having species, gender, cell type-specificity, as well as needing threshold levels and regular, chronic exposures in the absence of anti-promoters (Trosko and Upham 2010a, b), and since the promoted initiated cell could be either one with no expressed connexins or one with expressed but non-functional gap junctions, chemoprevention will never be achieved by a universal agent. This is supported by the fact that the promotion mechanism, induced by an agent, such as phorbol ester that acts via activation of protein kinase C, will be different than the mechanism induced by DDT or phenobarbital, or by inflammatory cytokines or a hormone or growth factor. Although many of these tumor promoters induce oxidative stress induced signaling and anti-oxidants characterize many anti-tumor promoters or chemopreventive agents, not all anti-promoters can inhibit the promoting activity of all chemical promoters or of different oncogenes (Nakamura et al. 2005; Upham et al. 1997). While it is generally known that oxidative stress can cause macro-molecular damage, it has been assumed that promoters, by inducing reactive oxygen species (ROS), genomic DNA damage and mutagenesis are the mechanism of these promoters. However, while the targets for these ROS might include DNA, that DNA is probably mitochondrial, not genomic, DNA, for the evidence seems very clear that promoting chemicals (e.g. DDT, TCDD, Phenobarbital, PBB's) do not induce genomic DNA damage or mutations. If a compound produces enough ROS at high concentrations, it could damage genomic DNA. However, these cells would have incurred so much cellular damage they would die. Dead cells do not directly lead to cancer. Of course, real DNA damaging agents could contribute to the promotion phase by killing cells. The dying cells would induce factors to stimulate the compensatory hyperplasia of surviving initiated cells. In effect these death-inducing factors are "indirect" tumor promoters.

As the two types of initiated cells are stimulated to proliferate by the promotion process, additional changes, both mutagenic and epigenetic, are expected to happen. Once sufficient genetic/phenotypic changes occur to allow the initiated cell to stably overcome the suppressing effect of either a soluble anti-mitogenic factor or an agent that reversible inhibits GJIC (such as an activated oncogene), the initiated cell is now able to invade normal tissue and to metastasize. It is now independent of exogenous promoting factors or it has reached the progression phase. This phase, also, appears to be irreversible.

Lastly, the most efficacious strategy for cancer prevention would be the promotion phase, which can require

decades to occur in human beings. While one can, in principle, reduce the risk of creating initiated cells by too much exposure to real mutagens (i.e., UV light), one can never reduce the initiation event to zero. Even if one could reduce DNA damage to zero and prevent errors in DNA repair, there will always be a finite chance of errors in DNA replication of spontaneous mutations. All human beings have initiated cells in all of their organs. The older we get, the more of these spontaneous initiated cells accumulate.

Chemical modulation of gap junctional intercellular communication and epigenetic toxicology

The NRC Report, "Toxicity Toxicity Testing in the 21st Century: A Vision and a Strategy", pointed out the obvious, namely, current assumptions and practices to determine the toxicities of new chemicals were inadequate (NRC 2007). One of the "sacred cows" or assumptions of current approaches to test and interpret molecular and in vitro/in vivo assays to predict toxicities of chemicals was that the chemicals were genotoxic. When organisms, including human beings, were exposed to chemicals that were associated with various diseases (birth defects, cancer, cataracts, cardiovascular-, reproductive and neurological dysfunctions) and in which oxidative stress was measured, DNA lesions in the affected tissues and mutations in specific genes(i.e., oncogenes or tumor suppressor genes of cells of tumors) could be found. Therefore, it was only natural that the interpretation was that the toxic chemical induced DNA damage to cause mutations that, then, led to the disease.

An alternative hypothesis has been offered (Trosko and Upham 2005), that these chemicals, at realistic concentrations, only promoted pre-existing initiated cells (Trosko and Upham 2010a, b). The rationale for challenging this paradigm that chemical toxicants or chemical "carcinogens" is based by recognizing the limitations of all the in vitro assays for "genotoxicity", and that all chemicals, when interacting with all cells of the body, can, at non-cytotoxic concentrations, trigger intracellular signaling, which, in turn, can affect gene expression at the posttranslational, translational and transcriptional levels without damaging genomic DNA. This interaction is fundamentally "epigenetic", not mutagenic.

Since gap junctional communication is critically responsible for maintaining homeostatic control of cell functions in ALL tissues/organs, unscheduled modulation of GJIC during embryonic, fetal, neonatal, adolescent, mature and geriatric stages of life could upset how those cells and their gap junction functions behave. To cause uncontrolled stem cell proliferation or unscheduled loss of stem cells at critical stages of development in tissues having initiated stem cells could bring about dysfunction of any tissue/organ. This, in turn, would have non-adaptive consequences to the whole organism.

Many toxic chemicals have been shown to have multiple disease consequences. Other chemicals, such as thalidomide (Nicolai et al. 1997; Franks et al. 2004; Lenz 1988; Tseng et al. 2001) have also been shown to have pharmaceutical benefit under one set of circumstances yet have devastating toxic consequences under other circumstances.

Gap junction function, epigenetic regulation , stem cells and the Barker hypothesis

Since gap junction function can be enhanced and inhibited during NORMAL development by both endogenous factors (i.e., growth factors, hormones, cytokines) and exogenous factors (dietary components) to regulate normal embryonic/fetal, adolescent development (normal growth, sexual maturation, wound healing), it should be obvious that these factors are not either mutagenic or cytotoxic. This complex process of homeostatic control during development, which was beautifully described by Clem Markert (Markert 1984), could only be actuated by a carefully regulation of specific genes in the total genome of the cells. This has to be a deterministic, rather than a random stochiometric process controlling gene expression. Mutagenesis, a random process, could not be expected to control the exact expression of patterns of batteries of genes during normal development or the adaptive responses of cells to certain external signals.

Therefore, a major challenge has been proposed that chemical toxicants, at non cytotoxic concentrations in the body, must work by "epigenetic mechanisms". By that term, it is meant that the chemical, with or without metabolism, after entering the body and being distributed to various organs and tissues, will interact with the cells. That interaction must first be with the membrane, via membrane-associated receptors, ion channels, and membrane-associated physical changes (fluidity, membrane stress), which, in turn, will affect intracellular sensors, redox state, protein modifications, activation of signal transduction pathways and transcription factor modifications. In effect, epigenetic changes are alterations in the expression of genes at the transcriptional (methylation and acetylation of DNA and histones), translational (splicing of mRNA; micro-RNA interference) and post translation (phosphorylation, nitrosylation, glycosolyation of proteins) levels. Some have defined "epigenetic changes" that can lead to heritable changes (which is correct). However, other epigenetic changes can occur when cells are induced to die by apoptosis, to senesce without cell division, or to adaptively respond to stress-related signals without dying or dividing, such as in terminally differentiate cells.

The recent use of microarray technology to assess the differences in gene expression after toxic chemical exposures or in disease tissues can generate consistent "patterns" of gene expressions. One would not expect mutagens, which act randomly, to bring about consistent gene expression patterns. Even more serious in the interpretation of these micro-array studies is the fact that they have been done on populations of cells in control and treated or diseased tissues. In each sample population, there are always a few adult stem cells, many progenitor and terminal differentiated cells, as well as cells in different stages of the cell cycle, those that are dying by apoptosis, those that are stressed and invading cells (macrophages, neutrophils, etc.). Each of these express different genes in the control population and each cell type in the treated population will react to the toxicant differentially. Stem cells usually express drug transporter genes, while terminally-differentiated cells, such the hepatocytes, will express many drug metabolizing enzyme genes.

However, what has been missed to date is the fact that chemicals can influence the adult stem cell number, especially during early embryonic and fetal development. Chemicals, both endogenous and exogenous, can affect whether a stem cell will divide symmetrically or asymmetrically. Therefore, if a toxic chemical can cause the adult stem cell pool in any organ of a developing fetus to differentiate, prematurely, then that organ will have fewer stem cells needed for growth, wound repair, tissue replacement. In addition, if stem cells are targets for a chronic disease , such as cancer, this organ would have a lower risk to cancer, all other factors for carcinogenesis being equal (Trosko 2008a, b). Such an interpretation is possible to explain the radiation-induced breast cancer frequencies in the survivors of the atomic bombs who were exposed at a young age (Trosko and Suzuki 2009). This same control population, on the other hand, contributed to the long median life span of Japanese women and their high frequency of osteoporosis. The fact that these women were the offspring of Japanese women who were calorically-restricted, ate lots of soy-products, and drank green tea suggests that these factors might have had some effect of adult stem cells in various organs of the female fetuses. Genistein, a bio-active component of soy products, is known to induce differentiation of human breast stem cells (Hsieh and Chang 1999). If at the same time it did the same for bone stem cells, then a biphasic effect of a dietary compound on stem cells in two organs (breast and bone) would occur. It might explain the relatively low breast cancer frequency and high frequency of osteoporosis in Japanese women who have a long life span (Trosko and Suzuki 2009). Several studies have suggested that several chemicals could affect stem cell numbers, such as valproic acid (Bug et al. 2005).

The Barker hypothesis states that early effects in utero can lead to chronic diseases consequences later in life (Barker 2004). The possible modulation of stem cell numbers in specific organs during early development has to be viewed as an "epigenetic" effect. Even DNA micro-array results might be reflecting not only gene expression changes induced in specific cells by the toxicant, but, also, these results might reflect the alteration in stem cell numbers, which would effect the homeostatic control of all the differentiate cell types, hence changing the ratio of the cell types and therefore the net effect on the population's gene expression. Therefore, alteration in the quality and quantity of stem cells could bring about either disease sensitivities or disease resistance.

Paleochemistry of aerobic life, evolution of metazoans, appearance of stem cells, connexin genes, cancer and the Warburg hypothesis

In an attempt to start to integrate many concepts and experimental findings, a highly speculative framework seems to be emerging. In the spirit of using Dobhansky's admonition, admittedly as a "leap of the imagination", there now are a number of interesting and intriguing observations that are starting to support the Warburg hypothesis, namely, during the formation of a cancer cell, there seems to be a transition from oxidative phosphorylation to generate energy in an oxygen prevalent environment (Warburg 1956). Yet when one views the early paleochemistry of the ocean, where the origin of life occurred, it was an aneorobic world, where glycolysis was the means by which primitive cell–cell organisms generated energy (Saul 2008). After the oxygenation of the oceans and atmosphere, the toxic micro-environment created a situation where a new life form emerged that had the means to cope with an oxygen-rich environment and which forced the anaerobic organisms to live in specialized microenvironments. By no means, is the understanding of how all of the required heritable genetic factors came about. However, with the appearance of the multi-cellular metazoan, new phenotypes, with their attendant genetic factors, appeared.

Single celled organisms survived by being "immortal", namely by replicating symmetrically, they could multiply indefinitely if the temperature, gravitational factors, nutrients, radiation levels, etc. allowed the replication of their DNA for both the survival of the individual cell and for the species it represented. This included DNA repair and DNA replication enzymes that were not 100% in preventing errors in DNA repair or in DNA replication. If these enzymes were perfect in preventing mutations, the genome could not accumulate mutations that might be needed when the environment would change, thus preventing

both the individual and the species to survive. On the other hand, these genes and enzymes were restrained from generating too many mutations for either the individual cell or the species to survive.

When the evolutionary moment occurred to provide a group of cells to adhere, possibly because of the ability of organisms to synthesize collagen in an oxygenated environment (Saul and Schwartz 2007) and act as a single multi-cellular individual, new phenotypes of (a) growth control, (b) differential expression or differentiation of the total genome, (c) selective suicide or apoptosis of individual cells ; and (d) the appearance of a niche for harboring unique cells, namely germ-line and somatic/adult stem cells appeared. These stem cells helped the survival of the species, as well as providing the means to grow, to repair wounds. The trade-off of the ability of stem cells to symmetrically and asymmetrically divide was "mortality" or senescence of both somatic cells and the individual. This gave the metazoan alterative means, besides symmetric cell division, to be an adaptive strategy to survive an ever-changing environment.

This transition included the ability of some metazoan cells to divide either via symmetric cell division (to make two daughter cells that were phenotypically-alike) or asymmetrically (to have one daughter to be as its mother, while the other could differentiate). The ability of the metaozoans to generate muscle cells, nerve cells, hepatocytes, keratinoctyes and retinal cells gave the metazoans a unique means to adapt to their environments. By the same token, they had to create a special "niche" that allowed to the stem (both germ-line and somatic) cells to be sequestered from those factors that allowed the other somatic, non-stem cells to differentiate. That micro-environment seems to be a rather anaerobic environment (Csete 2005; Mohyeldin et al. 2010; Eliasson and Jonsson 2010; Panchision 2009; Silvan et al. 2009). This allowed for the survival of the species and for tissue repair/wound healing, and growth to reside in the individual that was destined to die.

Recently, in the stem cell field, it is now clear that embryonic and "induced pluripotent stem cells" or "iPS" cells have few mitochondria and metabolize via glycolysis, whereas the differentiated somatic cells, which are equipped with large numbers of mitochondria, metabolize via oxyidative phosphorylation (Armstrong et al. 2010; Prigione et al. 2010). From the classic observations that cancer cells seem to metabolize via glycolysis, a new look at the theories on the origin of cancers seems to be emerging. The two extreme hypotheses of the origin of cancer, the stem cell theory (Markert 1968; Potter 1978; Pierce 1974), or the theory of "de-differentiation" or re-programming of a normal differentiated cell (Sell 1993) needs to be critically re-examined.

One interpretation is that the normal adult stem cells are the targets for the initiation of the carcinogenic process (Trosko 2008a, b). Since these cells, which are naturally "immortal" have few mitochondria and since these normal cells metabolize primarily via glycolysis, when it is initiated, they are prevented from asymmetric division to terminally differentiate and from inducing their number of mitochondria to metabolize via oxidative phosphorylation. As it ultimately evolves to become a "cancer stem cell", it will remain, "immortal" (Tai et al. 2005). As the tumor growths, it generates new microenvironments, in part caused by angiogenesis-fed oxygenated cells and aneorobically starved cancer stem cells. In that tumor a mixture of "cancer stem cells" and "cancer-non stem cells", or as V.R. Potter (1978), call them, "partially differentiated" cancer cells, appear.

The alterative hypothesis is that the differentiated somatic cell, once "initiated", would "re-program" its genome to revert back to the "embryonic-like state". That would require a re-shuffling of methylation and acetylation patterns of the nuclear genome/histone proteins. Such alterations in methylation patterns have been shown with current microarray examination of normal and cancer tissues. However, even though the number and pristine nature of the mitochondria and mitochondrial DNA seem to be similar to the embryonic stem cells and "iPS" cells, derived from the differentiated somatic cells, but very different from the differentiated somatic cells, there seems to be a conundrum, namely, while it is theoretically possible to reprogram the genomic DNA, one cannot "reprogram" mutations of the mitochondria of the "iPS" cells. If this process of "re-programming" during carcinogenesis is similar/identical to the generation of 'iPS" cells, an explanation will be required for how the mitochondria in "cancer stem cells" and in "iPS" cells seem to have few mutations, whereas the differentiated somatic cells' mitochondria have many mutations. It is the opinion of this author, using Ockham's Razor, the stem cell theory of cancer seems more plausible.

Lastly, to try to complete the circle of reasoning, the role of gap junctions in early evolution of the metazoan (Revel 1988), their role in the homeostatic control of proliferation, differentiation and apoptosis in development (Trosko et al. 2000) seems to be linked to the suppression of the stemness gene, Oct4A, in adult stem cells, cancer stem cells (Tai et al. 2005). Since Oct4 is needed for stemness and low oxygen is needed for the stem cell in its niche, Oct4 turns out to be a redox-sensor gene/protein (Guo et al. 2004). In the presence of oxygen, Oct4 is repressed and its correlated behavior seems to linked to the hypoxia-inducible factors (Pathel and Simon 2008). Major physiological changes occurs right after birth and during wound healing when tissues are exposed to an influx of oxygen, which could

indicate a shift in stem cells being triggered to differentiate and proliferate in order for both growth and would healing take place. It seems that evolution from the single cell organism to the metazoan in an oxygen-rich environment was accompanied by the appearance of the connexin gene family with the Oct4 stemness gene and the HIF gene family, together with an oxygen-poor niche. Oxygen- triggers a dramatic shift in gene expression via redox-induced intracellular signaling (Upham and Trosko 2009), allowing for the induction of cell adhesion molecules, such as collagen. Cell adhesion is needed for cells to anchor themselves for gap junctions, of various channel sizes, to allow for specific gene regulation to create specific tissues and organ function .

Gap junctions, bystander effects and adaptive responses, and hormesis

In the context of this workshop, the question is; "In what manner does cell–cell communication play in the phenomenon of 'hormesis'". Given the definition of "hormesis" and assuming the validity of all the reports of a hormetic response to various physical, chemical and biological toxicants at multiple levels of the biological system (molecular, biochemical, cellular, physiological and organismic), and given the fundamental roles that cell–cell communication mechanisms play in regulating homeostasis controlling cell functions in normal development and in disease processes, it would seem that a hormetic response might affect cell–cell communication and vice-versa.

To begin, any biological response to a toxic agent exposure in a multi-cellular organism, such as a metazoan, must be "sensed" by the cell or a group of cells, which, in turn, must be sensed by neighboring cells if that interaction has altered the steady-state of that particular cell or group of cells. The reason is because if that external agent has caused sufficient perturbation in the delicate homeostatic signal pathways within the cell that controls the G0 state, the mitotic, differentiation, apoptotic or senescent options a cell has, then those new signals will cause the cell to react. In the metazoan, a major action of that single affected cell will be sensed by its neighbors. Therefore, conceptually, one would imagine that the dose of a physical agent or a concentration of a chemical agent that impacts that cell might (a) be insufficient to trigger a response, due to protective, homeostatic stabilizing mechanisms; (b) overcome a threshold level of protection and trigger a specific response (i.e., receptor-dependent mitogenic or differentiation signal); (c) overcome both a threshold and receptor-dependent signaling response (i.e., trigger receptor-independent oxidative stress response) to trigger some adaptive cellular response (apoptosis); or (d) overcome all protective and adaptive mechanisms and die by necrosis.

One of the early observations that cells could communicate with each other, via extracellular factors (hormones, growth factors, neurotransmitters, cytokines, chemokines) was the basis for the discipline of physiology. Later, the discovery of intra-cellar signaling mechanisms, triggered by these extra-cellular molecules, led to the control of gene expression, which, then, affects the cellular behavior. It wasn't until the discovery of gap junctional intercellular communication, that there was a mechanism to integrate all these communication processes. In between, signaling by extracellular substrates and cell-adhesion molecules (considered by some to represent extra-cellular communication mechanisms) was added to this complex homeostatic communication system.

After the discovery of the gap junction as the physical structure to facilitate the direct transfer of communicating ions and small molecular weight molecules, it was observed that either a negative or positive communication signal could be directly transferred from one cell to another via gap junctions. This was referred to as "metabolic cooperation". Later, it was shown that non-mutagenic, non-cytotoxic chemicals, such as tumor promoters, could reversibly inhibit "metabolic cooperation" when they reached a "threshold" level to inhibit gap junction function via their ability to trigger intracellular signaling to alter both the gap junction protein/function and the expression of genes (Yotti et al. 1979; Upham and Trosko 2009).

When it was shown that agents that inhibited gap junction function to trigger mitogenesis and inhibit gap junction-dependent apoptosis, there was a mechanism to explain tumor promotion in vivo (clonal expansion of an initiated stem cells by releasing its mitogenic repression and its potential death by apoptosis). [Clearly, not all apoptosis is triggered by gap junctional intercellular communication]. Extracellular triggering of the apoptotic signal could be triggered by soluble factors. The reverse could also happen when non-cytotoxic agents, such as chemopreventive compounds) can increase gap junctional intercellular communication (Trosko and Ruch 2002).

In the field of radiobiology, the observation of the adaptive response to an initial low dose exposure, followed by a higher dose, led to a lower biological response than to a single exposure that was equivalent to the total dose of the split doses. While it is difficult to explain the many observations, done under different circumstances, and measuring different endpoints at different biological levels, one could rationalize responses by noting that if the low dose exposure or chemical concentration could trigger intracellular signaling at a threshold level within killing the cell, gap junctional intercellular communication could be modulated and genes could be expressed. If under those conditions, any DNA damage or cytotoxicity might be prevented, while at the same time enhanced preventive

mechanisms could be induced to prepare for a subsequent dose/concentration. This might lessen the toxic effect of the second exposure.

In view of newer concepts in the field of adult stem cells and cancer stem cells, the "target cells" for radiation and chemical-induced cancers, might be intrinsically more resist than their progenitor or differentiated daughters because they might be more radio-resistant or toxic chemical-resistant because they express drug transporter genes (Shimano et al. 2003). Even the many reports of presumptive cancer chemopreventive and anti-oxidant chemicals have bi-phasic effects must give pause to the belief that there are simple explanations for preventive effects of the adaptive responses, since, on one hand, at low concentrations a given agent might be "protective" but at pharmacological levels, they could be harmful. Anti-oxidants can become prooxidants (Schwartz 1996). Along this line of thinking, the use of isolated "pure" bio-active compounds from their natural sources (fruits/vegetables) might not have any particular positive effects. In addition, applications of these natural supplements in cases of real deficiencies might provide some protection, whereas in non-deficient situations, supplementation might have no benefit or might even have detrimental effects (Nakamura et al. 2005). Example of this might be the results of the CARET and ATBC trials and in vitro assays on the effects of purified parent bioactive chemicals and mixtures of the parent and their metabolites (Duffield-Lillico and Begg 2004).

Another example comes from the observations of the "by-stander" effects, seen in some radiobiological studies (Wright 2004). The by-stander effect on a target cell, triggering a response on a distal cell, can do so via some soluble agent or a direct transfer, via gap junctions, from the target cell to non-target cells. How these effects might or might not be related to "hormesis" (Calabrese 2008) has not been tested. Clearly, in the case where cells had no functional gap junctions [92. Edwards et al, 2004], there were no by-stander effects. Even in the new field of stem cell biology cells, that are isolated on the basis of their ability to be resistant to toxic chemicals ("side-population" cells), had "stemness" characteristics and were resistant because they did not express gap junction function but did express drug transporter genes. Therefore, until specific experiments are designed to test if hormesis and cell–cell communication mechanisms are linked to any adaptive endpoint, it will remain speculative that low dose/concentrations exposures will be a real adaptive process to always protect the organism from the toxic effects of the exposed agents.

Last, is there any link between the CCN family of genes and the stem cells and gap junction family of genes? While both families of genes have been associated with cellular functions of growth control, differentiation and apoptosis, as well as with some of the same pathologies, strict testing, as to whether these two genes are either co-expressed or interact in stem cells or their differentiated daughters, has not been attempted. It seems the future of research in the stem cell field, connexin- and CCN-family of genes fields might be able to resolve this question.

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