THE CELL SURVIVAL PATHWAYS OF THE PRIMORDIAL RNA–DNA COMPLEX REMAIN CONSERVED IN THE EXTANT GENOMES AND MAY FUNCTION AS PROTO-ONCOGENES

Joseph G. Sinkovics*

St. Joseph's Hospital Cancer Institute Affiliated with the H. L. Moffitt Comprehensive Cancer Center, Morsani College of Medicine, Department of Molecular Medicine, The University of South Florida, Tampa, FL, USA

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Malignantly transformed (cancer) cells of multicellular hosts, including human cells, operate activated biochemical pathways that recognizably derived from unicellular ancestors. The descendant heat shock proteins of thermophile archaea now chaperon oncoproteins. The ABC cassettes of toxin-producer zooxantella Symbiodinium algae pump out the cytoplasmic toxin molecules; malignantly transformed cells utilize the derivatives of these cassettes to get rid of chemotherapeutics. High mobility group helix–loop–helix proteins, protein arginine methyltransferases, proliferating cell nuclear antigens, and Ki-67 nuclear proteins, that protect and repair DNA in unicellular life forms, support oncogenes in transformed cells. The cell survival pathways of Wnt– β -catenin, Hedgehog, PI3K, MAPK–ERK, STAT, Ets, JAK, Pak, Myb, achaete scute, circadian rhythms, Bruton kinase and others, which are physiological in uni- and early multicellular eukaryotic life forms, are constitutively encoded in complex oncogenic pathways in selected single cells of advanced multicellular eukaryotic hosts. Oncogenes and oncoproteins in advanced multicellular hosts recreate selected independently living and immortalized unicellular life forms, which are similar to extinct and extant protists. These unicellular life forms are recognized at the clinics as autologous “cancer cells”.

Keywords: Caenorhabditis, cell survival pathways, ctenophores, de-differentiation, Drosophila, early multicellular eukaryotes, exosomes, malignant transformation, proto-oncogenes/oncogenes, reversed ontogenesis, tumor immunology, unicellular eukaryotes

Introduction

The presence and the molecular chemistry of essential ontogenetic and life-sustaining biological systems, the “cell survival pathways,” were compared in extant uni- and early multicellular organisms with those of evolutionarily advanced multicellular subjects, including vertebrate mammals, in particular Homo. The presumption that the basic molecular biology of extant organisms was acquired and inherited from extinct and extant predecessors was accepted. The natural occurrence of genome duplications and thus new gene formations, as well as gene losses, were taken into account. It was recognized that organisms evolving from ancestral-to-extant entities have operated these molecular biological pathways for hundreds of million years. The extant genomes exhibit a faculty for transformation of mature cells into those of unicellular life forms, which are characterized by incessant replication and extraordinary resistance to physicochemical insults. These biochemical reactions in these life forms appear to be sequentially acti-

vated beginning with anti-apoptotic features, and followed by the loss of inhibitory factors of the cell cycle. The metabolism of these cells continues without senescence and natural death. Genomic and proteomic studies indicate that the biochemical pathways operational in these life forms are derivatives of their ancient ancestors' ontogenetic and cell survival pathways. Characteristically, upon external interventions, a blocked pathway is readily replaced by another activated pathway. In a multicellular host, these unicellular life forms subvert the host's immune reactions and induce host cells to provide growth factors to them.

In addition to their major replicatory pathways often constitutively expressed, these life forms carry large numbers of different single point-mutations that could enable one of them to live and metabolize in almost any physicochemical environment. In the clinics, these life forms are recognized as autologous cancer cells and the malady of cancer is diagnosed. These interactions between host and its own parasitic life forms often result in the consumption and death of the multicellular host. The clinics design

* Corresponding author: Joseph G. Sinkovics; Cancer Institute, St. Joseph's Hospital, 3001 Dr. Martin Luther King Jr. Blvd., Tampa, FL 33607-6307, USA; Phone: 813-870-4255; Fax: 813-870-4825; E-mail: Sinkovi.Joseph@baycare.org

treatment protocols for the elimination of cancer cells, possibly without harm to the host (not yet achieved). The biologists view the phenomena of cancer as manifestations of an inherent faculty in the RNA–DNA genome for the sustenance of cellular life in the universe through inexhaustible mutability and the reversal of ontogeny/phylogeny in these subjects (referenced in [1]).

Unicellular and early multicellular eukaryotes express their cell survival pathways. Selected single cells of a multicellular host express the derivatives of these systems as proto-oncogenes

Several ontogenetic and vital molecular biological pathways are operational and shared in unicellular eukaryota (amoeba, including *Dictyostelia*), giardia, trichomonas, choanoflagellates, ciliates and paramecia, kinetoplastids (the *Trypanosoma*), the fungus *Neurospora crassa*, and the oomyceta *Phytophthora infestans*. The genomes of the first multicellular organisms, the urochordate tunicate *Ciona*; the Cnidaria, prominently including the sea anemone *Nematostella vectensis*, Hydra, *Hydractinia*, and Medusozoa; the Spongiae; the Strongylocentrotus sea urchins; and the protochordate *Branchiostoma floridae*, the representative of the amphioxus clade, conserved these systems. The most prominent ontogenetic and cell survival pathways commonly shared by these entities are those of the Wnt– β -catenin circuitry resulting in the intranuclear activation of the ancestral *tcf*TCF/*lef*LEF genes and gene product proteins. Their antagonists are the dickkopf (drosophila) gene product proteins. Their collaborators are the Hedgehog Hh, PI3K, MAPK–ERK, Ets, and JAK–STAT pathways (wingless/integrated, T cell factor/lymphocyte enhancer factor, phosphatidyl inositol kinase, mitogen-activated protein kinase, extracellular regulated kinase, retrovirus E26, Janus kinase, signal transduction, and activation of transcription). Further, frequently shared cell survival genes are those of the heat shock proteins (HSP), insulin-like growth factors (IGF), tumor necrosis factor (TNF α), transforming growth factors (TGF $\alpha\beta$), (decapentaplegic, drosophila), and their antagonists, and the inflammatory oncoprotein, nuclear factor kappa B (NF κ B) [1].

Many proto-oncogenes were discovered in advanced multicellular organisms. When rediscovered in unicellular eukaryotes, their original names were retained, even though the unicellular hosts had nothing to do, for example, with lymphocytes. The function these genes fulfill may change in different phases of their evolution.

As reported from the Observatoire Océanologique, Villefranche-sur-Mer, France, the Wnt– β -catenin pathway evolved very early for the segregation of embryonic germ layers. The *Ciona* embryo at its eight cell stage possesses four mesendodermal and four ectodermal precursor cells. In the 16-cell stage, β -catenin mRNA and β -catenin protein-dependent transcriptional activity is evident in full.

The GSK-3 (glycogen synthase kinase) protein interacts with cytoplasmic β -catenin. Accumulating cytoplasmic β -catenin eventually enters the nucleus, where it is directed to the ancestral *tcf* gene (T cell factor) DNA. The *tcf* DNA expresses β -catenin-binding domains, and when so activated, it encodes protein TCF. This interaction occurs only in mesoderm-precursor cells. β -Catenin performs two sequential binary fate switches for the separation of ectoderm and mesoendoderm, and mesoderm and endoderm. The ectodermally destined precursor cells express ephrin. By the 32-cell stage, the embryo has separated its ectodermal, mesodermal, and endodermal lineages [2]. Prominent other genes cooperating are those of the FGF–MAPK–Ets pathways [3], all becoming proto-oncogenes in mammalian vertebrate genomes.

The first mammalian *wnt* gene was discovered by R. Nusse and H. Varmus [4] in breast cancer-susceptible and MMTV-carrier (mouse mammary tumor virus, Bittner) mice. However, the MMTV genome possessed no recognizable oncogene in opposition to other retro- and reticulo-endotheliosis viruses, which incorporated various growth factor genes of their host cells, led by the Rous sarcoma retrovirus. Instead, MMTV inserted the DNA copy of its RNA genome into one particular gene of its host cell (gene *int-1*, for integration). Gene *int-1*, by itself, without MMTV gene insertion, proved to be oncogenic in mice (inducing breast cancers). Gene *int-1* turned out to be the mammalian homolog of the drosophila gene wingless, so the human *int-1* and *int-2* genes are mapped to chromosomes 12q14 and 11q13 [5, 6].

For distinction, the first *int-1* gene was renamed wingless-related integration site, “*wnt*/Wnt.” In the meantime, the vertebrate Wnt family grew to 19 members.

The drosophila protein Armadillo is the homolog of β -catenin; the drosophila homolog of GSK is zeste, and Wnt is a GSK3 antagonist (and *vice versa*). Healthy GSK phosphorylates β -catenin at serine/threonine residues, thus, marking it for degradation by ubiquitination. Elimination of its phosphorylation sites stabilizes β -catenin. Within the healthy GSK, APC (adenomatous polyposis coli), and Axin (homologous to Disheveled) complex, β -catenin is destroyed. Frizzled transmembrane proteins signal Disheveled. Homologs of the drosophila protein Arrow, the LRP (low density lipoprotein receptor-related proteins), are positioned adjacent to Frizzleds in the cell membrane. Their phosphorylated cytoplasmic tail interacts with the GSK3–Axin complex. When β -catenin escapes ubiquitination in the cytoplasm (due to the failure of the APC–Axin–GSK complex), it accumulates and eventually translocates into the nucleus to activate its target the *tcf/lef* genes (Fig. 1). These genes encode the high mobility group proteins TCF/LEF (T cell factor; lymphocyte enhancing factor) discovered in mammalian hosts. However, these are ancient genes functional in the early multicellular eukaryotes way before the appearance of lymphocytes; there they were and remained targets for β -catenin. In turn, these gene product proteins are DNA-binding and are targeting *wnt* genomic DNAs. Another of

Fig. 1. Pathway on the left is that of an active cell. The non-functional complex GSK/APC/Axin (see text) allows the accumulation of β -catenin in the cytoplasm and thus its entry into the nucleus, where it activates proto-oncogenes *tcf/lef*, which encode gene product potential oncoproteins TCF/LEF (see text). If the GSK/APC/Axin complex suffered loss-of-function mutations, the process continues constitutively expressed, and becomes irreversible. It renders the cell cycle non-responsive to negative control. The cell undergoes “malignant transformation”. If the fully functional GSK/APC/Axin complex switches itself on, phosphorylated β -catenin will be destroyed in the cytoplasm by ubiquitination and the cell rests (pathway on the right). Picture is drawn by Joerg Huelsken and is owned by Walter Birchmeier and Max Delbrück Center (MDC), Berlin, Germany. Copyright license issued by Josef Zens

their target is the promoter DNA of proto-oncogene *c-myc*. Wnt gene product proteins induce asymmetric mutagenesis in *Caenorhabditis elegans* and *Platynereis dumerilii*, the annelid worm. When the negative regulator (inhibitor) of Wnt, the *apc* gene, is loss-of-function mutated in stem cells, the cell cycles become uncontrollably incessant, and these cells grow as malignant tumors (reviewed by Nusse and Varmus) [4]. In the MMTV-Wnt1 mouse, the MMTV promoter drives Wnt1 transgene expression in breast tissue epithelial stem cells; *in vitro*, these cells form the malignant mammospheres. This effect is increased by injecting Wnt3 protein and is blocked by dickkopf protein [7]. The interaction of the hedgehog (Hh) pathway with that of Wnt consists of the liberation of smoothened (SMO) from patched (PTC) suppression, effected by the Hh ligands. The liberated SMO affects the expression of GLI (glioma) proteins, that translocate into the nucleus to bind and activate the *c-myc/c-Myc* and *wnt/Wnt* genes [8, 9]. Hh signaling is highly active in M.D. Anderson’s PTC-negative highly metastatic LoVo colon cancer cell line (commercially available).

The *Ciona* and the Cnidarians (*Nematostella*, *Hydra*, *Hydractinia*, *Medusozoa*) carry the β -catenin *int-1* (*wnt*) genes as physiological regulators of body axis plans in the past 600 my. In vertebrate mammalian hosts, the descendant genes *int-1* (*wnt*) and *int-2* have retained their

stem cell faculties and act as proto-oncogenes/oncogenes. When amplified, constitutively expressed, gain-of-function point-mutated, or fused, the vertebrate mammalian descendants of these ancient cell survival pathway genes are able to encode in selected cells of their hosts apoptosis-resistant and incessantly replicating cell cycles, and in their host cells, resistance to physicochemical attacks, bordering immortality. This latter process is recognized in medical clinics as the malignant transformation of the cell, the pathological entity, cancer [1].

Not only oncogenes but immunogens were also gained. The deprivation of the immune faculties of the host by parasites and cancer cells is alike

The molecular pathways evolving toward multicellular vertebrate hosts from sea urchins and the amphioxus to cartilaginous jawed fish (Chondrichthyes, Gnathostomata), to bony fish, amphibians and reptiles, aves, and mammals, including *Homo*, steadily advanced as to the complete installment of the individual elements of the adaptive immune system. These are the alloreactive MHC, VDJ, RAG, and RSS (recombination activating

Fig. 2. The subversion of the host by its malignant cells was recognized early in the case of chronic lymphocytic leukemia or lymphoma cell leukemia. These cells use interferon-gamma as their growth factor. Large fibroblasts under the effect of the chemokine ligand and receptor, stromal cell-derived factor (SDF-1; CXCL12; CXCR-4), promote the entry of these malignantly transformed B lymphocytes into their cytoplasm and thus provide a feeder layer, and protection from host immune factors for the lymphocytes. May-Günwald-Giemsa, haematoxylin-eosin, and coriophosphin-O stains. The phenomenon was first reported in 1962 [147], and elaborated on later with literature review [15–17]. Copyright license issued by Demetrios A. Spandidos, International Journal of Oncology

genes; recombination signal sequences). The genomics of the entire adaptive immune system were gradually inserted by retro- and herpesviral vectors reaching their full complementation in the Chondrichthyes and beyond [1, 10–12]. The malignantly transformed cells refrain from the expression of antigen-presenting MHC molecules, or express MHC class I-related ligands, that deactivate NK cell activating receptors NKG2D [13].

Malignantly transformed cells mobilize immunosuppressive host cells (Treg lymphocytes; myeloid derived suppressor cells; tumor-associated arginase-secreting M2 macrophages); keep dendritic cells immature and tolerogenic; create Th2 immunological environment in their hosts; and activate checkpoints (cytotoxic T lymphocyte antigens, CTLA4; programmed cell death, PD-1 ligands), whereby autoimmune or tumor cell-directed cytotoxic T cells kill themselves in the process of apoptosis (referenced in the example of pancreatic cancer by Nicole R. Murray *et al.* from the Mayo Clinic, Jacksonville, FL) [14].

IFN- γ and the chemokine stromal cell-derived factor-1 (CXCL12 $\rightarrow$ R4) direct leukemic lymphocytes to take up residence inside fibroblasts, where they replicate in a state exempt from host immune attacks (referenced in [15]).

The tumor cells enticed and subverted their host for their full support. In opposition, small compact immune T cells and the subsequently discovered large granular lymphoid (NK) cells attack human sarcoma cells [16, 17] (Figs 2 and 3).

The malignantly transformed cells enlist host factors for their replication (receptors and ligands of molecular growth factors, EGF, FGF, KGF, PDGF, and VEGF). Even erythropoietin (EPO) and its receptor are expressed and used as a growth factor by tumor cells, as found by the Ács brothers [18]. EPO is widely used by clinical oncologists for the treatment of malignancy-associated anemia. EPO is an inhibitor of apoptosis and an activator of proto-oncogene PI3K/Akt (oncogene in Jacob Fürth's AK mice incorporated by thymic retrovirus) and the MAPK proto-oncogenes [19].

In these events, the transformed cells of a multicellular host differ from the ancient single-celled eukaryotes, both activating their cell survival pathways, but the latter without outside antagonism or help. The potency to enlisting high mobility group proteins, protein arginine methyltransferases, intranuclear protein Ki-67, and proliferating cell nuclear antigens for accelerated replication was and remains available for the single-celled eukaryotes, as

Fig. 3. Male patient MDA#90641 with metastatic liposarcoma was treated surgically and received sarcoma viral oncolysate vaccination, while he remained in complete remission over 3 years. His case history was discussed at the 22nd Annual Clinical Conference of The University of Texas M.D. Anderson Hospital entitled “Immunotherapy of Human Cancer” and published [1, 148]. The patient’s blood circulated Immune T lymphocytes and large granular lymphoid cells referred to as NK cells reacting *in vitro* to cultured sarcoma cells; the NK cells released molecular mediators lysing the membrane of the sarcoma cells. Human NK cells attacking autologous and allogeneic tumor cells were visualized and recognized first, as preserved in original microphotographs, at M.D. Anderson Hospital in the late 1960s. This event of medical history is credited by Marshall Lichtman in the chapter reviewing the natural history of malignant lymphomas [149]. “In 1969 Joseph (József) Géza Sinkovics (b. 1924), a physician-scientist and Hungarian émigré, working in the Section of Clinical Tumor Virology and Immunology at the M.D. Anderson Cancer Center, observed that his unprimed lymphocytes killed allogeneic tumor cells *in vitro*. He also showed that the cells involved in attaching to and lysing tumor cells were large granular lymphocytes. Although his report was met with skepticism and rejection (a project site visitor proclaimed “There is no immune reaction without preimmunization”), this work was confirmed and expanded to indicate that unprimed lymphocytes also could kill virus-infected cells. He provided the first photographs and tumor cell growth-inhibitory graphs describing such cells. At about the same time (1970), Sinkovics described a lymphoproliferative disease, which in retrospect may have had a natural killer cell phenotype.” Lichtman cited the appropriate references. Copyright license issued by Schenk Buchverlag Budapest/Passau

their extant descendants also possess these inherited factors (referenced in [1]).

Some parasitic single-celled eukaryotes (Trypanosoma growing in liquid media) are able to recap their telomeres after each cell division. The protein enzyme telomerase (a reverse transcriptase, TERT) catalyzes its internal component RNA to form a template for telomere synthesis. Short telomeres induce more frequent antigenic changes in the parasites’ variant surface glycoprotein-encoding genes, whereas long telomeres stabilize the VSGs [20]. Thus, pathogenicity of the short-telomered parasites is increased in the infected host. Whereas, under conditions exempt from immune attacks and rich in nutrients, the parasites are afforded to lengthen their telomeres [21–23]. In a multicel-

lular host, subverted to rather support than to attack its parasite, the cancer cell, these cells express lengthened telomeres, thus, escaping senescence and gaining immortality. The telomeres’ T (TTAGGG) repeat-binding factors (TRF) between kinetoplastid promastigotes (Trypanosoma, Leishmania amazonensis), and vertebrate mammals, including Homo, are close protein homologs [24]. Observe in the Methods of the articles that cited plasmid construction, the isolation of genomic DNA, RNA analysis, techniques of transfections, aa sequence alignments, identification of the Myb-like DNA binding domains at the C-terminus of the proteins, chromatin immunoprecipitation, and Western, Southern and Northern blots [20, 23, 24]. By analogy to the trypanosomes, if in order to escape senescence, the malignantly transformed cells extend their telomeres in an immunoreactive multicellular hosts, they expose themselves as targets to more immune attacks.

The strategy of unicellular eukaryotic parasites and malignantly transformed cells of multicellular vertebrate hosts reveal many similarities: the ability of these cells to readily transspeciate (including reformatting their entire cytoskeleton); amoeboid locomotion; concealment of their antigenicity; masquerading “selfness”, extracting growth factors from the host, especially by converting macrophages from M1 to M2 entities; and establishing long-lasting host–parasite relationships (with the exception of acute leukemias). Using Naegleria and Theileria parasites as examples for this host–parasite relationship, the similarities with the host–tumor relationship were pointed out [25]. Posting the query “parasites inducing PD-1 ligands” to Google, the answer is “no items found”. This author expects that another similarity in the host–parasite and host–tumor relationships will be documented by finding PD-1-ligand inducer unicellular eukaryotic parasites that induce apoptotic death of immune T cells of their host by this mechanism. A crucial difference between the biology of individually living unicellular eukaryotes and selected malignantly transforming cells in a multicellular host, both mobilizing their cell survival pathways in stress, is that the former is on its own, whereas the host may either attack or support its transforming cells; for the latter cells, there is a host to interact with.

In semblance to the malignantly transformed cells, stand the fetal syncytiotrophoblasts of the placenta, which temporarily switch off the alloreactive transplantation immune faculties of the mother, in utilizing immunosuppressive mechanisms identical to those, that the malignantly transformed cell constitutively uses for its acceptance and support by its host [1].

Selected examples of proto-oncogenes deriving from ancient cell survival pathways

Choanoflagellates

In the pre-Cambrian era, the now over 600 myo single-celled eukaryotic choanoflagellates and the metazoans

derived from an extinct unicellular common ancestor, that utilized the postsynaptic density proteins, but without having a nervous system. Choanoflagellates *Monosiga* inherited the PSD proteins and sponges (demosponge *Amphimedon*) inherited both PSD proteins and choanoflagellate-like feeding cells [26]. In addition to vertical inheritance, and to endosymbiotic gene transfer from permanently implanted mitochondria and plastids, choanoflagellates acquired numerous horizontally transferred genes, most of them from algae and prokaryotes (bacteria). Most of these genes remain active in carbohydrate and amino acid metabolism [27]. The 41.6 Mb genome packs approx. 9200 intron-rich genes; these introns are much shorter than metazoan introns. Cell adhesion (cadherins, lectins, integrins) and some primordial immunoglobulin domains that characterize metazoans appear first in choanoflagellates. Of pathways ancestral to those of metazoans, Wnt is absent; the earliest elements of Hh, JAK–STAT, Notch, phosphotyrosine signaling, and homeodomain transcription factors (p53; Myc/Max; Sox, Tcf) are operational (Myc-associated X protein; SRY-related sex-determining region on Y chromosome high mobility group box). The DNA-binding helix–loop–helix leucine zipper transcription factor myc/Myc, and its binding partner Max, appear first in the choanoflagellates *Capsaspora* and *Monosiga* [28]. However, the exact origin of the p21-activated gene-product protein serine/threonine kinase (*pak*/PAK) is unknown; it is expressed in the choanoflagellate *Monosiga ovata* and in the sponge *Ephydatia fluviatilis*. Human cells (fibroblasts) transfected with the *Monosiga pak* gene undergo malignant transformation due to the constitutive expression of the Pak protein [29, 30].

Mammalian cells carry six PAK protein isoforms. The PAK proteins interact with GTPases (guanine triphosphatases) either in their upregulated or in gain-of-function point-mutated states. Antiapoptotic PAKs inhibit BAD (Bcl-2-associated death promoter) and caspases and activate VEGF and NFκB. PAK1/4 oncoproteins drive adenocarcinoma (breast, ovarian, colorectal, pancreatic prostate) cells. Short hairpin shRNAs can be created to inhibit (destroy) mRNAs, that are to be translated into PAK proteins in the argonautes of ribosomes. Cancer cells so treated stop replicating and stagnate; if Bcl-2 is coinhibited, the cancer cells die in apoptosis [31]. In the human genome, PAK phosphorylates and activates STAT5 and, thus, activates the leukemogenic (both myeloid and T lymphoid) pathways of JAK2. The *bcr*BCR/*abl*ABL fusion oncoprotein (breakpoint cluster region; Abelson mouse leukemia retrovirus oncogene) is a target of STAT5 [32].

Giardia

These low-branching protozoa lost their mitochondria and reformed their genomes from free-living to parasitic life style. The *Giardia* cell expresses three PI3K genes without an inhibitor like PTEN (phosphatase and tensin homolog deleted in chromosome ten), that is working in mul-

ticellular eukaryotes. In the infested host, *Giardia* PI3Ks render host dendritic cells (DCs) to be IL-12-devoid and IL-10-producer tolerogenic agents, thus, generating a Th2 immunological environment [33–35]. In the human genome, when the native PI3K inhibitor PTEN is disabled, the PI3K pathway extends to the activation of Akt–mTOR oncogenes (mammalian target of rapamycin) [36].

The Akt–mTOR pathway is prominently evident in Kaposi sarcoma (KS) coinduced by HIV-1 negative factor Nef and transactivator of transcription Tat, together with KSHV-8 ORF1-induced membrane glycoprotein K1. Glycoprotein K1 constitutively activates NFκB production, inhibits FasL → FasR-induced apoptosis, and suppresses PTEN, thus, liberating PI3K. The chain reaction of Akt (also known as protein kinase B) and mTOR activation follows. Several miRs align with the untranslated UTR region of the PTEN mRNA in various human cancers. In KS, it is miR-718 that inhibits the translation of PTEN mRNA [37].

Trichomonas

The avian myeloblastosis retrovirus Ets (E-twentysix) isolated from birds with leukemia (myeloblastosis) incorporated the host cell oncogene *myb*. In leukocyte physiology, *myb*/Myb is active in immature leukocytes and is silenced in mature cells. The constitutively expressed Myb proteins activate the cell cycle protein-chaperon heat shock transcription factor (HSF3) by DNA-binding. It is the promoter DNA of the *hsf* gene that expresses domains for binding Myb proteins [38]. This interaction is inhibited by the p53 protein.

The flagellated microaerophilic parabasalid trichomonas operates a functionally most complex ATP-binding cassette ABC transporter system [39]; its cytoplasm is well protected. The heavily duplicated trichomonas genome accepts and rejects horizontally transferred genes of prokaryotic derivation [40], carries dsRNA and totiviridae genomes [41–43], harbors inserted retrotransposons (Tc1/mariner, Kolobok) [44, 45], and practices morphological transformation of a flagellate entity into an amoeboid parasite [46, 47].

In the trichomonas cell, gene *ap65* (adhesin protein) expresses Myb protein-recognizing elements (MREs) and binds the cells' own Myb proteins. The activated adhesin protein of the *ap65b* gene is the mediator of cytoadherence, a 6-kDa hydrogenosomal malic enzyme (decarboxylating malate), essential for the pathogenicity of the trichomonas cell. Of the three tvMybs, the tvMyb3 protein shows 83% aa sequence identity with human c-Myb. Myb proteins are widely expressed in various avian and mammalian hosts and in plants [48–51]. The ciliates *Sterkiella* (also known as *Oxytricha*) and the ciliophora *Euplotes* both carry myb genes in their genomes [52]. Since the green alga *Chlamydomonas reinhardtii* operates the ancient *myb*-genes in the form of low CO₂ response regulators (*lcr*/Lrc), descendant *myb* genes are carried by

plants [53]. In *Arabidopsis*, Mybs are telomere-binding. Plant Myb genes are present in edible fruits (apples, oranges, peanuts, not considered to be oncogenic in the human genome). It is quite acceptable that the human *T. vaginalis* acquired its *myb*/Myb armamentarium evolutionarily and vertically, and not horizontally.

In the human genome, the *myb* genes are proto-oncogenes primarily involved in the induction of acute myeloid leukemia [54]. *myb*/MYB pathogenicity is highly suspected in some solid tumors (adenoid cystic carcinoma, non-small cell lung cancer), in which microRNA-195 by aligning to *myb*/Myb mRNA prevents its translation into the oncoprotein and melanoma [55–58]. The association of *myb*/Myb with human papilloma virus in squamous cell carcinoma of the uterine cervix is mutually promotional [59].

Spongiae

Like the *Ciona*, the swimming larvae of Porifera *Spongiae* metamorphose into a sessile organism. These two germ-layer (no mesenchyme) 600 myo multicellular organisms carry flagellated collar cells resembling choanoflagellates. The Great Barrier Reef demosponge *Amphimedon queenslandica* mobilizes the Wnt pathway for its transformation: Wnt expression dominating in its posterior, TGF β in its anterior pole (observe *Ciona*'s transformation, Hydra's regeneration of its cut off head directed by the Wnt pathway). The metamorphosing larva eliminates cells useless for the sponge by Bax contra Bcl2 type apoptosis [60, 61]. *myc*/Myc accelerates the cell cycles. Bcl-2 class molecules are antiapoptotic, and p53/63/73 and Bak (Bcl-2 antagonist killer) molecules are proapoptotic. The FasL $\rightarrow$ FasR and MDM (murine double minute) pathways, and the cyclin dependent kinase (Cdk) inhibitors Cip/Kip (cyclin kinase interacting protein, kinase inhibitor protein), are not yet installed (will appear in bilaterian eumetazoa). Eumetazoans and remarkably *Nematostella vectensis* operate the D, amoebas (*Dictyostelium discoideum*), the B, and choanoflagellates and sponges the A and E CDK subfamilies. The sponges' cell cycle rolls quite uninhibited, somewhat similar to that of the malignantly transformed cells of advanced eumetazoa. Ontogenetic and cell survival pathways Wnt, Hh, IGF, JAK–STAT, Notch, PI3K, Ras, NGFR (nerve growth factor receptor), and TGF β are installed. TLRs, interleukin receptors (Rs), chemokine Rs, and their ligands are operational. Sponges express alloreactivity [60–62].

The demosponge *Suberites domuncula* in the Adriatic sea possesses a tyrosine kinase that shows 55% similarity and 38% identity with the human Bruton kinase (BTK) [63]. The human BTK is a maturation factor of B lymphocytes. Its loss-of-function mutation causes X-linked agammaglobulinemia. Its gain-of-function mutations result in various malignant tumors of B lymphocyte lineages, including chronic lymphocytic leukemia (CLL) and mantle cell lymphoma. The small molecular inhibitor of the BTK protein, ibrutinib, by oral administration induces durable

complete remissions of these conditions. Further gain-of-function mutations of the *btk* gene result in resistance to ibrutinib [64–66].

Amphioxus

The *Branchiostoma floridae* of the Everglades and Tampa Bay is the representative of the world-wide amphioxus clade. Its innate immune system is based on 222 toll-like receptors (TLR), and chemokines and their ligands. The chitin-binding proteins contain future immunoglobulin variable domains [67, 68]. Its cells express 1363 LRRs (leucine-rich repeats), the precursors of the variable lymphocyte receptors of the lamprey, but there are no immunoglobulins. In its guts, amphioxus carries coelomocytes (like the sea urchin). The elements of the TNF system are functional as TRAIL/APO (tumor necrosis factor-related apoptosis-inducing ligand); in effect, the entire FADD system (FasL $\rightarrow$ FasR apoptosis stimulating fragment death domain) is represented [68]. The retrovirally originated recombination activation gene RAG1 is present and is functional with mouse RAG2 in recognizing mouse RSS (recombination signal sequences), that are not present in the amphioxus; RAG2 is absent [69]. A short sequence of APOBEC is present (apolipoprotein B-editing enzyme catalytic). No viral agents are known to parasitize the amphioxus, but retroviral and herpesviral genomic sequences remain inserted in its genome. Homeobox genes, *delta/notch*, *myc*, *snail*, *sox*, *twist*, and others for mesenchyme formation, for generation of various hormones, and retinoic acid and their receptors, are present in its 520 Mb genome [1].

The first nervous system consisting of peripheral single sensory cells (neurons) connected by γ -aminobutyric acid-circulating axons with the protochord is encoded by the achaete scute (ASC) gene complex (achaete, bristle; plate, drosophila). ASC homologs extend through crustaceans to insects and through fish, amphibians, reptiles, into two directions to aves, and to mammals. The human ASC homolog (HASH) with its promoter resides in chromosome 12q22–12q32. The amplified or gain of function point-mutated HASH is an oncogene; it encodes APUD (amine precursor uptake and decarboxylation) cells and their tumors, carcinoids, small cell undifferentiated carcinoma of the lung, gastrointestinal neuroendocrine carcinoma, neuroblastoma, and medullocarcinoma of the thyroid; transspeciates adenocarcinoma (colon, lung, prostate) into neuroectodermal carcinoma; and primarily induces esthesioneuroblastoma, the malignant tumor of the olfactory ganglion (the Atlas, [70]; referenced in [1]).

The ASH pathways are mRNA/miRNA-regulated. A shRNA-Asc/HT-29 (patient's initials) silenced HASH in colon carcinoma cells (very likely by aligning with the untranslated UTR-region of its mRNA, and thus destroying it). miR-375 is the inducer of ASC-like 1 homolog in small cell lung cancer. Transfected miR302b restored miR-375 and HASH expression in small-cell lung cancer cells. Wnt is signaling target ASC-like2 genes for activation [71, 72].

Neurospora crassa

The eukaryotic fungus of Barbara McClintock yielded the “one gene one protein” principle to Edward T. Tatum and George W. Beadle. It operates a polyADP-ribose polymerase PARP for restoring its damaged DNA. Several MAPK–ERK pathways are operational, including osmolarity-sensitive OS-MAPK [73]. In hyperosmotic stress, its class 2 phosphatases A2 (PP2A) act. Reactive oxygen species (ROS) is the activator of conidiation, circadian clock frequency, and protein phosphatase 2A [74]. However, the phosphatases PP2A dephosphorylate (thus inactivate) MAPK, resulting in growth-defective strains.

Thus, there is a discrepancy here. It is the loss-of-function-mutated PP2A phosphatases that allow MAPK overactivity up to its constitutive expression, resulting in incessantly upregulated hyphal cell proliferation and fusions in some strains (*App2A*; *Δcdc-14*; *Δcsp-6*; *Δpzl-1*, phosphatase; cell division cycle; cell shape phenotype; phosphatase Z-like). This process fails to respond to the inhibitor butyl-hydroxyperoxide t-BuOOH, Sigma [73, 75], thus, imitating chemotherapy-resistant neoplastic cells.

N. crassa cells are under the control of circadian rhythm. The light receptors Vivid, White Collar, and Frequency (WC activates the release of *frq* mRNA) activate the circadian rhythm clock proteins ATF and CREB (activating transcription factor, cyclic AMP-responsive element-binding protein) and the MAPK–OS-MAPK rhythmic pathways, thus, synchronizing cell divisions. The mitogen-activated kinases engage the clock-controlled genes (*cgc*) and some mitochondrial genes of the cells [73]. The protein products of genes *frq* and *catp* (clock ATPase) react with histones in the nucleosome. The *bd* (*band*) genes, ancestral to mammalian *ras* genes, are activated. Neurospora protein arginine methylase (PRM) remains conserved in all eukaryotes up to Homo. Some PRM-encoding genes (*amt*, ammonium transporter; *skb*, SH-kinase binding protein involved in asexual sporulation) may suffer deletions. *Δskb-1* results in hyperconidiation [76], as if *skb-1* acted as an eukaryotic tumor suppressor gene would. The mammalian/human cell cycle inhibitor *wee*/Wee kinase (small in Scottish) has its Neurospora homolog; it oscillates with other cell cycle components in circadian clock-dependent synchronized nuclear divisions [77].

The human neuroendocrine pineal gland receives adrenergic innervation from the hypothalamic suprachiasmatic nucleus (SCN). Photosensitive retinal cells transmit input into the SCN to activate the serotonin-melatonin circuit (through the enzyme aryl-alkyl-amine N-acetyltransferase, AANT). The Neurospora genome is devoid of melatonin synthesis. In the human malignant tumor pinealoma, MAPK, cryptochromes, CaMK (calmodulin-dependent kinase), and CREBs are related to those functional in the circadian rhythm of *N. crassa*. The pinealoma c-Myc and PRM [78] may have ancestral counterparts in the Neurospora. In the mammalian/human genomes, PRMs are chaperons to oncoproteins and are considered

to be proto-oncogenes coacting with NFκB and promoting epithelial-to-mesenchymal transitions (EMT). The papillary tumors of the pineal gland are unique in that the loss of PTEN allows the activation of the PI3K–Akt–mTOR pathway. Mitotic activity is extensive. Others are of mixed cell morphology with positivity for both cytokeratin and vimentin, and the neuroectodermal markers synaptophysin and chromogranin [79, 80]. Photosensitive DA-secreting (dopamine) cells appear first in the sensory vesicle of the Ciona larva, which are related to the amacrine cells of the mammalian (including Homo) retina and DA neurons of the hypothalamus [81].

Drosophila

Drosophila leukemia is frequently compared with the human disease, even though drosophila's hemolymph is not vascularized, but diffusely flows within tissues and organs. The hematopoietic cells are limited to three freely migrating lineages (macrophage-like phagocytic plasmacytes without immunoglobulins, crystal cells performing wound-healing with melanization, and lamellocytes able to engulf large invader parasites). However, Ig domain superfamily proteins (not immunoglobulins) are produced in response to bacterial infections. Hematocytes develop in the embryo for life (without any proliferation in adult life). These cells release antimicrobial peptides or kill engulfed bacteria and parasites by hydrogen peroxide or nitric oxide generation (ROS, NO). For migration to wound healing sites, or to bacterially infected regions, where epithelial cells release jun N terminal kinases (JNK) and form syncytia, the Rho family small GTPases, PI3K, and the JAK–STAT defensive pathways are also activated. Some PDGF/VEGF-like tyrosinases exist in ligand-to-receptor systems (platelet-derived growth factor, vascular endothelial growth factor) without platelets or blood vessels. The NFκB system is inhibited by protein Cactus, and activated by proteins Dorsal. The physiological ontogenesis and larva stage of the fruit fly utilize the Notch, Hh, and Wnt pathways [82]. Dramatic new observations clarify the underlying Wnt mechanism [83, 84]. Ras (rat sarcoma) gene activation in inflammatory processes precedes JNK and JAK–STAT activation. The drosophila histone methyltransferase trithorax (H3K4Me3) is homologous with the human MLL5 protein (mixed lineage leukemia), that frequently produces fusion oncoproteins, the inducers of aneuploidy in hematopoiesis [85]. The drosophila myelodysplasia/myeloid leukemia factor (dMLF, so-called even though drosophila does not have bone marrow, or granulocyte colony stimulating factor GCSF/CSF ligand and/or receptor, and produces no myelocytes/granulocytes) is a homolog of the human myeloid leukemia factor, hMLF1, a nucleophosmin fusion oncogene/oncoprotein at t(3,5)(q25.1,q34). The dMLF exerts its action through the Hh, RUNX (runt-related), and JNK pathways within the crystal cells [86]. In the human bone marrow, JAK–STAT mutations are the major causative factors for myelofibrosis and myelodysplasia with GCSF/CSF

as their promoter, and small molecular JAK-inhibitor ruxolitinib as their suppressor [87, 88].

Natural leukemogenesis in the drosophila is an inflammation-associated process, in which danger signals activate ancient cell survival pathways [82]. These pathways are those of the proto-oncogenes, which individualize and immortalize selected cells of a multicellular host-in-demise for prolonged survival under favorable conditions, for that particular cell population. In transplants, these cells prove their immortality. In the drosophila giant larva, the lethal giant larvae *lgl* tumor suppressor gene is null-mutated (a process not apparently inflammation-related), and cell differentiation is canceled. The undifferentiated larval cells replicate until the death of their host. Once established, they remain serially transplantable and kill healthy larvae [89].

In drosophila brain tumors, EGFR and PI3K/Akt are active. Two brain tumors of different biology arise after the inactivation of tumor suppressor genes *ldl* (low density lipoprotein) and/or *brat* (brain tumor). TGF β (decapentaplegic) is activated in *ldl*-tumors with increasing metastatic rate with passages, while *brat*-negative tumors maintained a steady metastatic rate with passages, reflecting to different stem cell biology. Ancient retroviral inserts express Sp1-binding sites (species-specific gene expression stimulatory proteins binding to GC box GGGCGGG motifs). In the drosophila genome, proto-oncogene expressions are intensified by Sp1-stimulated retroviral inserts [90–92]. In general, ancient retroviral inserts are activators of proto-oncogenes [1, 93].

Caenorhabditis

In the sea, Ciona (see above), and in the soil, Caenorhabditis (caeno, καίνος, fresh, new, recent, caenogenetic; rhabditis, ράβδος, rod, rhabditides, rod-like; rhabdoma, bundle of rods), embryos utilize the Wnt- β -catenin pathway in their ontogenesis. β -Catenin is the worm armadillo (WRM), expressing cyclin-dependent protein kinase (*cdk*/CDK) phosphoacceptor sites. Here, Wnt induces Frizzled to interact with its receptor LRP, as they recruit Dishevelled, and through this act, GSK β -induction. LRP is phosphorylated by CK (casein kinase) (Fig. 1). *cdk*/CDK and *scr*/Scr signaling converges with those of the Wnt pathway. This pathway is working in the endomesoderm blastome of the four-cell caenorhabditis embryo, for the proper rotation of the spindle complex onto anterior–posterior axis and for the production of an anterior mesodermal precursor cell and an endodermal (E) posterior cell by division, as shown in Craig Mello's laboratory by Kim et al. [94].

The starving larva in stress expresses *alk*/ALK (anaplastic lymphoma kinase) and TGF β gene-product proteins and enters the dauer (endurance) state: life without aging. One of the three *scd* genes (suppressor of constitutive dauer) fails to oppose it [95]. Another *scd* gene encodes the dauer pheromone. Genes showing gain-of-function activities encode insulin-like growth factor, TGF β , its opponent SMAD (signaling mothers against decapentaplegic, dro-

sophila; encoded by the *sma* genes in the Caenorhabditis), and a homolog of the mammalian anaplastic lymphoma receptor tyrosine kinase (WormBase website, <http://www.wormbase.org>) [95, 96].

Wild *C. elegans* strains (one living in an oasis in southern California) are able to develop dauer-resistance [95]. A dauer arrest factor-5 (*daf*) gene encodes Sno/Ski oncoprotein homologs (*v-ski*, Sloan-Kettering avian sarcoma retroviral isolates; Ski novel gene), which are present in all mammalian (including Homo) cells; these are the “Fussells”, which interact with TGF β signaling [96, 97]. Is the dauer state of the Caenorhabditis larva in any way related to human intrauterine and infantile leukemia – or sarcomagenesis?

The miR let-7 is essential for the differentiation of vulvar/uterine cells in the female caenorhabditis. This gene was cloned in Victor Ambros' laboratory and was found to be not protein coding. It forms a 70-nt hairpin dsRNA product and a ss 22 nt RNA. Identical conserved let-7 homologs exist in eukaryotes up to Homo (Craig Mello's Nobel Prize lecture) [98]. In its absence (let-7 null mutation), undifferentiated vulvar cells proliferate leading to the rupture of the organ with lethal outcome, as vividly shown in Amy Pasquinelli's lecture at the M.D. Anderson Hospital 2014 Fall Symposium “Illuminating Genomic Dark Matter” [99]. The undifferentiated vulvar cells are driven by lin/LIN-28, whose association with stem cell factors cMyc and Sox is well documented. The Let-7 Lin-41 antagonism has been well recognized earlier [100]. Cells mutated to proliferate, antagonize let 7. A major let-7 downregulator is the receptor for activated C kinase (RACK-1). RACK acts through the recruitment of the Argonaute mRNA-silencer RISC (siRNA-induced gene silencing complex) [101].

The Caenorhabditis genome revealed first the power of the primordial RNA and its numerous micro-derivatives over the late-comer DNA [102, 103]. Lin-28 docking on let-7 miRNAs abrogates the tumor suppressor ability of the latter. The assembly of Lin-28 on the terminal loop of let-7 precursors is stepwise [104]. Short loop-targeting oligoribonucleotide “looptomirs” preempt this assemblage and restore let-7's full maturation [105].

Reversal of ontogenesis to the stage of primordial stem cells is a physiological escape mechanism, so is dedifferentiation of mature somatic cells in multicellular hosts

The Medusozoa (*Turritopsis dohrnii*, *Hydra*, *Hydractinia carnea*) provide the proof that extant genomes retained the faculties to repeatedly regress into their ancestral life forms and to regenerate [106, 107]. Stress may elicit the process (the stress being a needle prick to the medusa in the laboratory). The mature end product of its ontogenesis (the medusa) reverses the course of its individual development by absorbing its tentacles and eliminating its adult cells

Fig. 4. The hydrozoa *Turritopsis nutricula* in stress undergoes the physiological process of “reverse ontogenesis”. The healthy medusa (a) becomes “unhealthy” (b) and assumes the life forms of the four-leaf clover (c), the cyst (d), and the polyp (e). Eventually, the polyps bud off healthy medusae (a). Reprinted from Tissue & Cell, 35/3, Carla E C, Pagliara P, Piraino S, Boero F, and Dini L, Morphological and ultrastructural analysis of *Turritopsis nutricula* during life cycle reversal, 213–222, 2003, with permission from Elsevier

through apoptosis, but retaining its embryonal pluripotent cells that take up the morphological and physiological features of stolons (the planula larva stage). Thereafter, the stolons transform into polyps (Fig. 4) [108]. Eventually, the polyp will again bud mature medusae. Here, the immature cells redifferentiated and returned into the service of the cell community (spectacularly illustrated by Günter Plickert et al., Jürgen Schmich et al.) [106, 107].

Some sponges repeatedly dedifferentiate into a colony of archeocytes (class “primordial stem cells”) and redifferentiate into the multicellular organism, thus, reaching the age of several thousand years for the colony (for the same individual cells or propagated clones thereof?) (for references [109]). The package of stem cells, the hydra (*H. magnipapillata/vulgaris*) experiences no age-related mortality and uses the Wnt pathway to regenerate its cut-off head. The ancestral stem cell gene *foxo*/FOXO encodes antisenesence longevity pathways. Human centenarians appear to have preserved the FOXO pathway but not over this limit (for references [109]). The supplement of primordial stem cells (PriSCs) is close to inexhaustible in the cnidarians and sponges, especially when reproducing asexually; restricted in annelids (segmented worms) and mollusks (clams and snails); and rudimentary in the drosophila and caenorhabditis, and in vertebrates and mammals [110]. The *t*(2,13)(q35,q14) fusion partner genes *pax*/Pax and *foxo*/FOXO (paired box, forkhead box O1) are oncogenes in the human host

causing alveolar rhabdomyosarcome in children and young adults [111, 112].

Are the tumor cell microvesicular exosomes distantly related to the ancient proto-cellular ribosomes?

The units “microvesicular exosomes” represent most diverse subpopulations. In general, exosomes are agents of intercellular communication. All cells, but excessively the dedifferentiated cancer cells, release exosomes. Examples here are Burkitt’s lymphoma [113], glioblastoma [114, 115] (Fig. 5), and prostatic adenocarcinoma [116] exosomes. Exosomes exert a wide range of activities depending on their complexity. The largest and most complex exosomes contain dsDNA oncogenes and oncoproteins. Mediocre exosomes are rich in all classes of RNAs from lncRNA to mRNA, miRNA, piwiRNA, shRNA, siRNA, and tRNA; they may operate argonaute units of ribosomes for RNA processing. Cancer cell exosomes practice mRNA destructions and exert the ability to eliminate those mRNAs that were to be translated into tumor suppressor proteins (for example RB, PT53, PTEN, etc.). Cancer cell exosomes convey immunosuppressive instructions to immune T and NK cells [117, 118].

Exosome formation, especially in cancer cells, may be viewed as the manifestation of an inherent faculty con-

Fig. 5. Glioblastoma cells release microvesicular exosomes in the blood stream of patients as shown and studied in the Massachusetts General Hospital and Harvard Medical School in Boston Massachusetts, by authors Johan Skog, Tom Wurdinger, Sjoerd van Rijn, Dimphna Meijer, Laura Gainche, Miguel Sena-Esteves, William T. Curry Jr, Robert S. Carter, Anna M. Krichevsky and Xandra O. Breakefield published in *Nat Cell Biol* 10, 1470–1476 (2008). Permission to show illustration from this material was issued by Xandra O. Braekfield. The incurable glioblastoma tumors are subjected to immunotherapy [150]

served in the extant genomes. Extant cells metamorphosing into the biological state of their deep-branching ancestors may descend to the low phylogenetic level of precellular, free-standing, DNA-free but RNA-rich ribosomes. These entities may be referred to as an ancient subclass of precellular organelles (precellular ribosomes), reappearing now in the form of extant exosomes. Some large exosomes contain both DNAs and RNAs, as extant ribosomes being generated in the nucleoli do. If exosomes are the current reproductions of the ancient precellular ribosomes, now they may also be generated in the nucleoli, the physiological site of origin of ribosomes. In contrast, it is a widely accepted view that exosomes derive from cytoplasmic vesicles. Be a Burkitt's lymphoma cell or a prostatic adenocarcinoma cell, their exosomes are visibly generated in cytoplasmic vesicles [113, 116, 119]. However, exosome genomics are characterized by the presence of all classes of microRNAs that must have derived from the nucleolus, the physiological site where ribosomes are generated.

The nucleolar genomes of extant eukaryotic cell nuclei are operating now by a strict DNA code but retained the ancient faculty of reproducing the protocellular ribosomes that may be recognized now as exosomes. This faculty is potentially expressed in every cell, but most prominently so in the cells undergoing a retrograde transformational event into their ancestral beings (the cancer cells). Indeed, cells undergoing the process of "malignant transformation" display active enlarged nucleoli, the present factories of extant ribosomes, and show other biomorphological changes in the cell preparing to malignantly transform; the example is the prostate adenocarcinoma cell. Here, it is again the proto-oncogene *myc/MYC* that initiates ribosome generation in the nucleoli [118]. In switching from the nucleolus to the cytoplasm, ribonucleic acid helicases and polymerases I and III release the precursor rRNAs in the process, with

the involvement of the helix–loop–helix heterodimer Myc/Max proteins, that bind the sequence E(CG), which is the 5'-CACGTG, CG-core E-box [120].

At the Symposium entitled "Illuminating Genomic Dark Matter" held at The University of Texas M.D. Anderson Hospital in Houston, TX on Oct. 9–10 2014, Sónia A. Melo presented her paper on "Cancer Exosomes and Extracellular microRNA Biogenesis". She reported miRNA replication occurring in cell-free human breast cancer exosome cultures with operational Dicer within argonaute (AGO2) units [121]. This author (J.G.S.) asked if it were possible to view cancer cell exosomes as "Precellular ribosomes in which Cech's ribozymes catalyze the replication of miRNAs". What this author had in mind was, that since extant bacterial (*E. coli*) extracts exerted ribosome-like function *in vitro* (serving Nirenberg and Matthaei in deciphering the genetic code) [122, 123], protocellular RNA-loaded, DNA-free ribosome-like units with an error-prone RNA code might have existed and functioned on the ancient Earth, even before the rise of unicellular eukaryotes. In order to identify exosomes as primordial ribosomal entities, 16S rDNA may not obligatorily be present in them; however, rRNA should be. This criterion so far is missing, or not well established [124], but when a class of microvesicles/exosomes containing rRNA is found [125], the evolutionarily reverse phylogenetic relationship of extant intracellular ribosomes and the current exosomes representing ancient precellular ribosome-like units will be acceptable. The trypanosoma–leishmania exosomes contain the core protein RRP5 (rRNA-processing proteins) [126].

Cell-free translation could be accomplished in crude and refined (free of detrimental enzymes) *in vitro* products of ribosomes constructed by using cloned genes. Cell-free protein synthesis is reproducible [127]. Naturally, in ribosomes, lncRNAs, some longer than 200 nt, structurally imi-

tate mRNAs, are transcribed by polymerase II, are polyadenylated, and possess ORFs (a primary ORF and additional ORFs in the UTR), and thus become polycistronic transcript codRNAs. These codRNAs encode (are translated into) polypeptides each well over 24 aa long [128].

The large RNA contents of the extant ribosomes allow the capacity of synthesizing polypeptides as a faculty conserved from the precellular past. The first enzymes and the viroids, persisting as fossils of the RNA-dominated world, must have been synthesized in these precellular ribosomes. Possibly, the first domain of life (Megavirales, now referred to as the fourth), predating the cellular domains, emerged and yielded the mimi- and mamaviruses, including the giant *Cafeteria roenbergensis* virus, possessor of the eight universal proteins [129]. Are the giant viruses recombinant products arising in precellular ribosomes [130]? In addition to their own basic genome and its product proteins, they now operate a genome much enlarged by genes horizontally received from archaeal, prokaryotic, and ancestral unicellular eukaryotic donors (the species *Acantamoeba*). The cnidarian *Hydra magnipapillata* and the oomyceta, *Phytophthora* in the phylum *Chromalveolata*, share RNAP (DNA-dependent RNA polymerase) genes with Megavirales [131].

The evolution of coenzymes as conceived in Harold B White's NIH Research Career Award in the 1980s (Fig. 6) [132] lends support to the possibility that precellular ribozyme-armed ribosomes existed and functioned in

producing ribonucleoproteins on the ancient Earth. Not only viroids but much more complex viral structures (DNA archaea prophages) might have been synthesized and further enlarged by fusions [130]. The primordial ribosomes with the large viruses within eventually became protocells encircled by double lipid membranes, in which soma and genome collaborated [133] and functioned in intracellular membrane-bound organelles [134]. The large viruses contributed to the formation of the eukaryotic nuclei (for references [1]). The protocells enriched their genomes by phagocytizing proteobacteria and/or cyanobacteria; the origin of proteobacterial mitochondria and cyanobacterial plastids. The primordial cell colonies existed as spheroplasms/spheroplasts (resembling Preisz's Pettenkoferia) [1, 135] and engaged in extensive exchanges (donations and receipts) of horizontally transferred genes [12]. Hypothetically (but possibly reproducibly in the laboratory), the ancestor of the extant fusogenic *Acholeplasma* phage carried out the ancient spheroplasmic/spheroplasmic cell fusions (referenced in [1]).

Living cells in the universe

The malignantly transformed mammalian (human) cells emerge as selfish individuals, independent from a cell community. They display extraordinary resistance to

Fig. 6. Early (in the early 1980s) foresight of protein enzyme evolution in the pre-cellular pre-DNA RNA-World in a ribozyme-armed ribosome-like unit. Reprinted from *The Pyridine Nucleotide Coenzymes*, Harold B White III, *Evolution of Coenzymes and the Origin of Pyridine Nucleotides*, 1–17 1982, with permission from Elsevier

Fig. 7. The fusion-oncoprotein-driven acute promyelocytic leukemia cells undergo complete re-differentiation in response to treatment with As₂O₃, or ATRA. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Cancer (Vol. 2, No. 9, Jun Zhu, Zhu Chen, Valerie Lallemand-Breitenbach and Hugues de The, How acute promyelocytic leukaemia revived arsenic), 2002

any physicochemical or biological (immunobiological, within its host) attacks. They move by amoeboid locomotion and practice cannibalism for subduing other cells by fusing with them. For this process, the transformed cell reactivates ancient retrotransposons [93] and expresses fusogenic retroviral Env proteins (for references [12]). The transformed cell divides in its young mature age and caps its telomeres, thus, escaping senescence and natural death. It prefers to express its oncogenes (*c-myc*) in G4 quadruplex formations (similarly to the ciliates *Oxytricha* and *Stylonychia*) [136, 137]. Under distress, it survives in the state of autophagy (recognized as tumor cell dormancy decades ago) and is able to recover from it. In its genome, measured sequential events prevent its apoptotic death as “tumor suppressor genes” (RB, PT53, PTEN, etc.) are methodically silenced or eliminated. Its incessant cell cycles (retinoblastoma RB kinases and cdk inhibitors by *cipCIP/kipKIP* switched off) are now constitutively expressed by amplified, gain-of-function point mutated, or fused genes. These genes encode the oncoproteins that are spared of cuts through their vital domains and are provided resistance to ubiquitination [138].

In the clinics, this process is recognized to be that of pathological carcinogenesis. In the processes of carcinogenesis, RNA ancestral to DNA appears to have regained its original superiority. The ancestrally inserted RNA → DNA transposons, the capsidless selfish replicon genetic parasites (as defined in Koonin’ group) [139], undergo events of silencing methylation. Demethylated and reactivated [93], these agents either reinsert themselves into proto-oncogenes, thus, activating them (Bittner MMT virus, Friend leukemia virus, Moloney leukemia virus-insertion sites), or act as templates for DNA synthesis (like in the Mauriceville plasmids). Introns, or bio-active micro-RNAs, working in spliceosomes and ribosomal argonautes, are fundamentally ingrained in every cell. Tumor suppressor and proapoptotic proteins are systematically eliminated. The oncoprotein MDM promotes the ubiquitination of the proapoptotic p53 protein, thus, immortalizing the transformed cell [140].

The production of the cell survival proteins (recognized as oncoproteins) is highly favored.

In addition to the major oncogenes and oncoproteins, the genomes of the transformed cells carry numerous point-mutated genes engaged in “house-keeping” and metabolism, as if the RNA–DNA complex prepared just one of many thousands of transformed cells to be able to fit and survive in a particular, but not foreseen and not precisely precalculated, physicochemical environment.

Are the selfish, highly individualized and immortalized malignantly transformed cells ever enabled to form another differentiated cell community? The HeLa cells fail to do so; the damage of chromothripsis appears to be irreparable and permanent [141]. However, all transretinoic acid (ATRA) induces the redifferentiation of *t*(15,17) RAR/PML (retinoic acid receptor/promyelocytic leukemia) fusion oncogene/oncoprotein-carrier acute PML cells (Fig. 7) [142]. This author refers to one of his patients with acute PML, who has entered and remains in complete remission for over 5 years on single ATRA therapy [1]. The ancient antagonism existing between the RA and fibroblast growth factor–Wnt–MAPK pathways, as expressed in the anterior and tailtip epidermis in the developing *Ciona* larva [143], is therapeutically replayed at the cancer clinics.

Further, this author reported the differentiation occurring in an established cell line of human chondrosarcoma cells under the effect of added T lymphocytes from a healthy donor. It is most unlikely that healthy fibroblasts coexisting with the sarcoma cells could outgrow the sarcoma cells. A gradual change of the large sarcoma cells to larger-than-normal fibroblastic cell morphology was observed (Fig. 8) [144]. Since then, it has become known that exosomes of dendritic cell-origin induce differentiation of mesenchymal stem cells [145]. As to chondrosarcoma, Gli oncoprotein (glioma-associated oncogene 1) activated in the end phase of the Hh pathway was found to be the major inducer of chondroblast dedifferentiation. Small interfering siRNA abolished Gli mRNA translation into oncoprotein, activated ERK (ex-

Fig. 8. In the late 1960s, middle-aged man (MDAnderson#73587) developed large chondrosarcoma in his right hemipelvis. His tumor was grown as an established tissue culture cell line #1459. His small compact immune T lymphocytes promptly killed his autologous tumor cells *in vitro*. Instead of small compact lymphocytes (immune T cells), healthy control (this author, JGS) yielded large granular lymphocytes, which attacked and killed these tumor cells. Project site visitors declared these latter reaction to be an “*in vitro* artifact”, since immune reactions without preimmunization were dogmatically unacceptable. Later, these large granular lymphoid cells were recognized to be “Burnet’s immune surveillance cells” or “natural killer” (NK) cells [16, 17]. At an even later time, small compact lymphocytes of the healthy donor still failed to kill these tumor cells, but coexisted with them, practicing emperipolesis, during which time some of the tumor giant cells gradually assumed the morphology of large fibroblast-like cells. It was proposed that as yet unidentified lymphokines induced tumor cell differentiation [144]. Springer, Pathol Oncol Res, Chondrosarcoma cell differentiation, Vol. 10, 2004, 174–187, Sinkovics J G. With kind permission of Springer Science+Business Media

tracellular signal-regulated kinase), stopped cell cycle progression, reduced Ki-67 expression, and induced either apoptosis or autophagy of the tumor cells [146]. The investigators induced the transition of autophagy into apoptosis; thus, it is not known if the autophagic cells could have possibly redifferentiated into mesenchymal stem cells or fibroblasts.

It is therefore possible that “malignantly transformed” individualized and immortalized cells may form a differentiated cell community in an environment favoring the selection of one particular genetic constitution over millions of others, that are unable to fit and perform so.

The ultimate bioengineer RNA–DNA complex endeavors the maintenance of aggressive living cells in the

universe. Is the cancer cell the product of supreme bio-engineering by the RNA–DNA complex, or a malformation constructed by “selfish biomolecules”? Is the event referred to at the clinics as “malignant transformation” of a cell tantamount to an escape mechanism, that is inherent in the successor descendants of the primordial RNA–DNA genome, for the sustenance of cellular life on Earth and in the universe?

Note added in proof

A Primeval Oncogenome. The Ctenophore genome dictates a much accelerated life style. The rapidly expanding ctenophore population represented by the comb jelly *Mnemiopsis leidyi* operates a ‘defective’ Wnt pathway without Axin, thus allowing the transfer of un-phosphorylated β -catenin from cytoplasm to nucleus for the activation of its target, the *tcf/TCF* complex; further, it lacks the Wnt inhibitor *dickkopf*. This pathway imitates the ‘defective β -catenin destructive complex’ and the ‘extinguished *dickkopf*’ of colon cancer cells. In addition to its upregulated ‘proto-oncogenic’ Wnt pathway, the ctenophore genome operates several *sox/SOX* pathways (referenced in the Appendix of [1]).

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