Cancer as a script and possible implications on workings of genome

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There is a need for a genomic theoretical framework which would explain the increasingly vast and discreet genomic data in the context of phenomena observed in cell. The search for a genomic explanation to cancer lead to the concept of the genomic script which extends its influence over the workings of genome of a normal cell too. This framework explains multiple phenomenon like the development of an embryo, differentiation of cells, and genomic workings of cancer. It also shines light on the evolution of unicellular and multicellular organisms. Yet it remains a simple construct; a perennial loop with its adaptor loops constituting the genomic script.

Introduction

There is an urgent necessity for a genomic theoretical framework in biology. The more we discover about the genome the more discreet identities are identified (Bonetta 2005; Lango Allen et al. 2010; Yang et al. 2012; Randall et al. 2013). Though the studies are immaculately planned and executed, the results are difficult to explain in context of a bigger picture (Larder et al. 2011; Dastani et al. 2012). This makes it even harder when conclusions of a new study deconstruct the theoretical structure build by the preceding studies (Morris 2011; Whyte et al. 2013; Stepanenko et al. 2013). The completion of the human genome project has only increased our woes. There is no common theory that explains the massive genomic machinery which keeps surprising us at every turn. The state of affairs for the complex disease of cancer is the same. The number identified cancer genes keep rising (Futreal et al. 2004; Bonetta 2005; Ciccarelli 2010). There seems to be no “AHA It's you then” moment with any of the discovered cancer genes. Many other factors have been implicated in cancer increasing the complexity even more (Blagosklonny 2005; Kingsley et al. 2007). Nevertheless there are those who expect a few underlying principles forming the basis of cancer (Hanahan and Weinberg 2000; Markert et al. 2012; Aktipis and Nesse 2013; Vogelstein et al. 2013). They expect a common theme in cancer. A study reported two sets of genes which are highly expressed in neoplastic progression and undifferentiation (Rhodes et al. 2004). Functional networks of these genes show a characteristic pattern which indicates that the progression of cancer could

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be due to a change in gene networks (Figure 1 and 2). But another explanation would be that the change observed was the outward manifestation of a change occurring in an underlying process.

Simply put an alteration in this underlying process is reason why the gene networks have changed. That underlying process is the genomic script. Functional networks can be generated using STRING (Franceschini et al. 2013). Just pop the list of the genes in the web server. You can adjust the required confidence score and number of interactors shown to your liking. Avoid low confidence score or high number of interactors otherwise the resulting functional network will contain too many interactions. Other than that your are good to go. The neoplastic signature clearly shows sub-networks and the undifferentiated network shows a cohesive central network. This result is consistent even if you keep changing the values.

In this writing I present a framework of genomic script. I apply this framework to explain cancer progression. I extend this to explain certain other

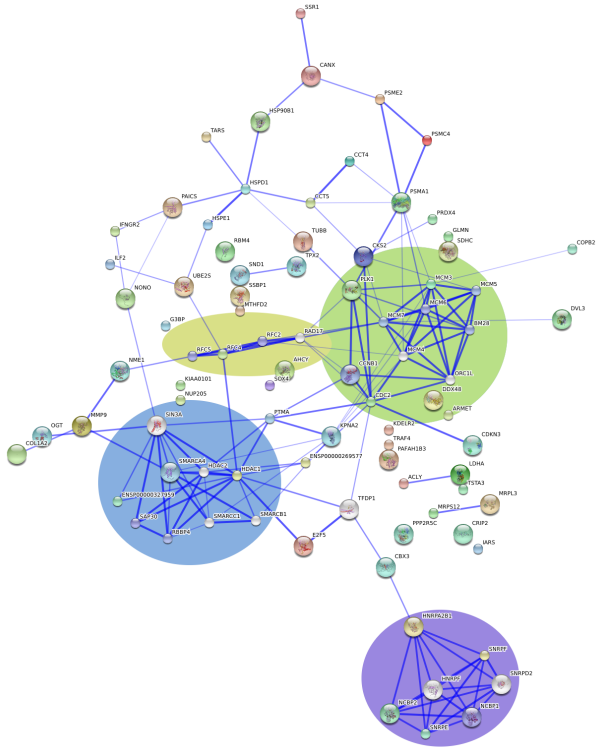

Figure 1: Functional network of neoplastic cancer metasignature genes.

This network shows distinct sub-networks.

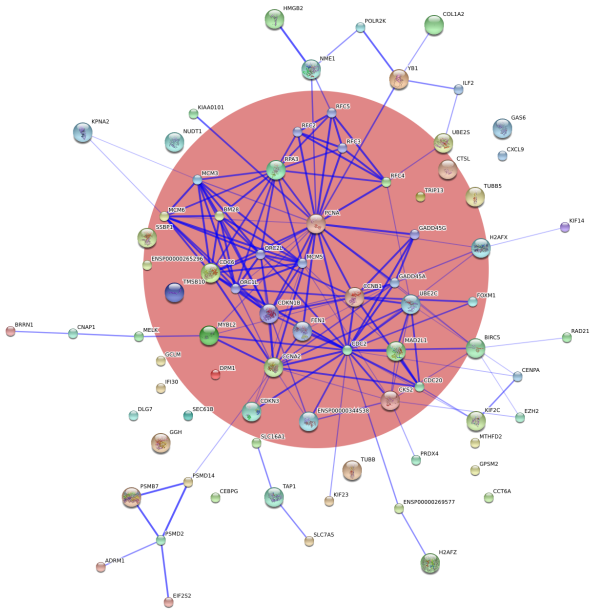

Figure 2: Functional network of undifferentiated cancer metasignature genes.

This figure shows a cohesive central network with no distinct sub-networks.

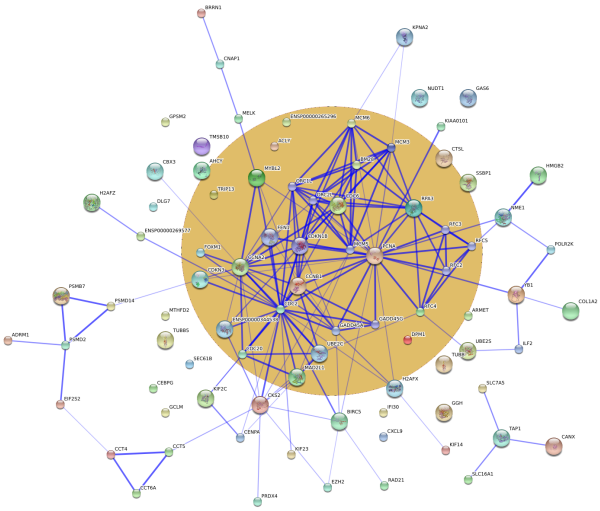

Figure 3: Functional network of combined cancer metasignature genes.

A central network is prominent. Both lists of the metasignature genes were combined to produce this functional network. This network is similar to undifferentiated network indicating genomic favour for progression from neoplastic state to the undifferentiated.

aspects like life in extreme habitats, antibiotic resistance and development of precision of function in cells of our body. But let me discuss the functional networks of cancer metasignature genes first. This would help us in grasping the concept of genomic script.

Cancer as a script

The metasignature genes are randomly located on the chromosomes and are not biased or limited to a specific chromosome indicating that the whole genome is accessed for expressing genes required for cancer generation and progression (Khan and Jamil 2008a).These genes are expressed in most of the normal tissues and are essential for various cellular processes which are important for survival of even normal cells (Khan and Jamil 2008b). The central network of undifferentiated metasignature dominates the network generated by combining the two lists of genes which distinctly shows the genomic tendency to favour the progression of cancer (Figure 3). Probably a script for cancer generation and progression does exist. The execution of this script causes the progression of a normal cell to a cancerous one.

A simple construct

Before venturing further let us equip ourselves with a standard but bare bones construct for this framework. This framework will be developed throughout the present writing. By the end I hope we will gain a complete picture of the concept of genomic scripts and its characteristics. So for the sake of explaining the functional networks we need to know what is a genomic script and what does it constitute. A *Genomic script* is a sequence of accessing genome which loops onto itself. It consists of a central loop and several side loops. For example a complete cell cycle which through generations loops onto itself could be considered a central loop of the script and those execution sequences of the genome which correspond to maintenance of cellular state are the side loops. The side loops may not be directly necessary for completion of the cell cycle. The result of an executed side loop is a sustained effect that we can observe in the cell. Let's say that execution of genome starts at point A and ends when it reaches point Z. This the central loop which is the *main agenda* for the genome. But in between execution gets stuck at points C and D where a loop is formed which delays reaching the destination of point Z till loop of C and D is complete. In this way, side loops delay the central loop controlling its execution. Between A and Z there could be other loops and those loops could in turn contain loops. Each loop transfers control to the other dependent loop only when it collapses. So the standard model is that in a cell a genomic script consists of a central loop and from it arise the side loops. It will become clear as we proceed with the present discussion that cancer is a type of genomic script and that genomic script may have a bigger role to play in the life of a cell.

Explaining functional networks

The sub-networks in the neoplastic functional network are similar to the smallest gear which rotates the fastest (Figure 1). These sub-networks or the side loops are executed more than the central loop (Figure 4). Hence they are observed prominently while the “large gear” or central loop is not. If sub-networks weaken or collapse into the central loop then the central loop would rotate faster since it is free of the time-delayed effect of the side loops. This might be the reason why a central network is prominent in undifferentiated and the combine functional network (Figure 5). The side loops consists of those genes which help in maintenance of the cell while the central loop is mostly cell proliferation related genes and as cancer cells proliferate they fail to build up cell differentiation associated characteristics which are usually observed in normal tissue cells resulting in their *undifferentiated* appearance. The tendency of the central loop is to go faster and tendency of the side loops is to delay it. In cancer weakening of side loops sends the central loop into a frenzy yielding non-stop divisions. So the best treatment strategy would be to trigger the cell to generate strong side-loops and the rest would be done automatically.

Role of gene networks

Progression of a cell from a healthy one to an undifferentiated cell is characterised by changes in gene network. These changes as observed in expression profiles are just snapshots of a highly dynamic genomic script framework which lies within the genome. In this framework, the gene network is modified avoiding collapse of the script. The genes, which are the constituents of the network, are moved

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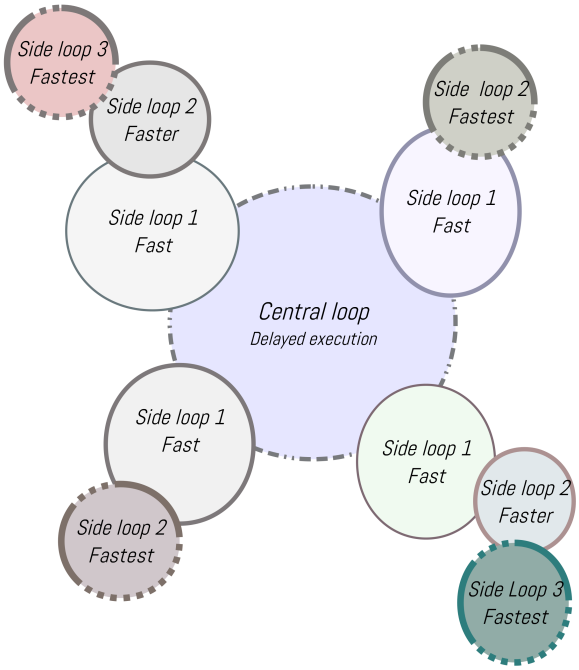

Figure 4: Central loop execution delayed by the influence of side loops.

Each circle represents a dynamic loop which is executed. The thick circles represent networks which are highly expressed and the thin circles represent reduced expression or loop execution. Each circle influences the other circle which it overlaps. So for continuous execution of the central loop the smaller side loops have to execute and collapse allowing the control to proceed. In the functional network of the neoplastic cancer metasignature genes, the side loops are stronger and execute faster hence they are prominently observed but the central loop is delayed which makes it inconspicuous in expression profiling. One has to imagine this and the next figure as dynamic constructs with constantly rotating circles indicating generation and collapse of loops.

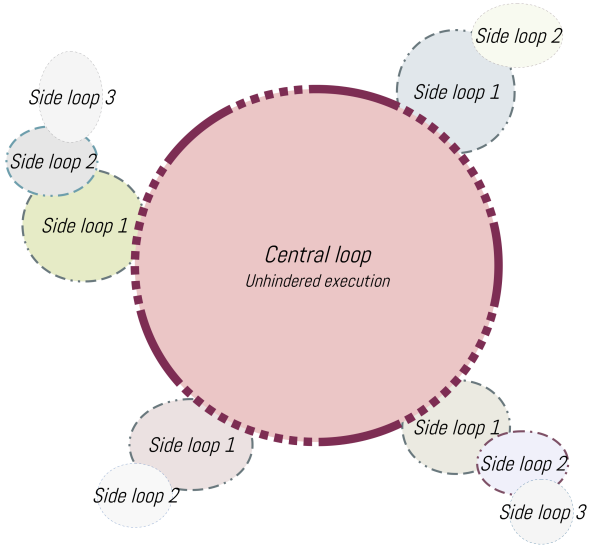

Figure 5: Central loop executes rapidly due to weak side loops.

The functional networks of the undifferentiated and the combined cancer metasignature genes show a prominent central loop because of the inability of weak side loops to delay it. The weak side loops are obscured and the central loop is detected prominently. Please see legend of figure 4 for further explanation.

or replaced till a new configuration is achieved. This is the shifting of genomic script. Gene networks act like tent pegs to the genomic script. Rearrangement of these "pegs" is essential to achieve a new route of execution. Execution of script establishes gene networks and in turn gene networks peg down the script. This interaction provides flexibility and robustness to the process. Individual genes could be targeted but the network could easily survive by rearranging its internal connections leaving the script unaffected.

Impact of genes on script

Varying expression of specific genes and their regulators within a network could alter the network's overall outcome. This alteration could be critical to the execution of the script since gene networks act as its pegs. In this way the genes and other genomic elements within a network indirectly but effectively impact the script. The concept of genomic script enables us to think of oncogenes and tumour-suppressor genes as potential locks on the cancer script. The varying expression of these genes could trigger or prohibit its execution. A study reports that varying expression of specific genes can trigger a change in the state of a select cancer cells *i.e.* from benign to malignant (Marjanovic et al. 2013). The switching of states is probably observed due to a shift in the genomic script nevertheless this study shows the impact of critical genes on the script. On the other hand the script influences the function of the genes recruited in the network. A recent study reports that certain cancer genes exhibit duality in their function under different experimental settings strengthening

the idea that the function of a gene is heavily influenced by the genomic context in which it is expressed (Giuriato and Felsher 2003; Stepanenko et al. 2013). This is further supported by findings of another study where researchers have reported certain disease related gene orthologs in plant, yeast and worm (McGary et al. 2010; Trapp et al. 2011). These studies show that execution of the script takes precedence over the function of an individual gene. All such candidate genes are important for conducting further studies since they expose the subtle relationship between genomic elements and the script.

The need for a new framework

Analyses of expression profiles and investigations into gene networks have generated interest in understanding a cell as a whole. Robust mathematical and bioinformatic approaches have been applied to analyse the expression data(Selimkhanov et al. 2012; Coulon et al. 2013; Mitra et al. 2013). If such is the state of investigations on networks then one might ask for the necessity for bringing in the concept of script. Networks, though informative, are diverse in their variety and vast in numbers. This has made it difficult for researchers to arrive at a core concept (Mitra et al. 2013). If we do not know what we are searching for then data has to be analysed in every possible way searching random relationships increasing wild goose chases which maybe theoretically valid but have no biological significance. In this scenario the concept of genomic script with its patterned structure would enable us to earmark potential areas evading blind alleys. For example if genome could be considered a database then relationships among genes and their regulators do not extend to the whole of DNA or the Genome but only up to the level of networks. On the other hand genomic script could increase the percentage of genome brought under the umbrella of sensible relationships. Simply put it should increase the number of jigsaw pieces falling into place enabling us to at least guess what could be in the areas which we know nothing of now. Thus if we were to consider that the networks of cancer metasignature genes have changed due to a change in an underlying script then the discreet randomness of the networks is transferred to patterned behaviour of a genomic script.

Genomic script does not require negation or tweaking of the concept of networks or any other established genomic elements nor does it demand to displace them. The beauty is in the way it accommodates the long standing ideas along with the latest research findings like the non-coding RNAs, super switches and networks etc., As a recent review declares that there are certain drawbacks in the way researchers study the complex biological systems which limit observation to static snapshots whereby the dynamic nature of the process is lost and the limitation of type of interaction investigated like protein-protein or protein-DNA reduces the complexity of the biological state (Mitra et al. 2013). These limitations would also restrict investigations in to this new concept of the genomic script owing to its complexity and dynamic nature. Nevertheless tell-tales for the existence of this framework are found in the cellular life itself. The observed cellular phenomena like development of an embryo, life at extreme conditions and cancer etc., could be understood basing on this concept which would in turn indicate that the genome is capable of a higher order functioning than previously thought. Here onwards I discuss the role of the framework in different scenarios and as we continue I hope it's importance would be established.

Observing genomic script

The case of developing embryo

An embryo of a vertebrate species exhibits different morphological features during the various stages of its development. These are similar to embryonic stages of other closely related species. Experts have expressed varying opinions regarding this phenomenon but generally agree that there is some kind of evolutionary significance to it (Richardson et al. 1997; Richardson and Keuck 2002; Hopwood 2007). I find a developing embryo to be a fascinating example for observing scripts in action. It could be assumed that cells of an embryo are passing through different stages of genomic script which belong to evolutionarily earlier but closely related species till it reaches its native script. The primitive versions of the script are locked in. At every step in evolution a distinct version of genomic script would have been deployed and tight controls would have been put in place so that only the right native script remains active. But in the case of cancer this lock

down is overcome. In a cancer cell, the collapse of the side loops probably opens the lock initiating the script that causes cancer. The tendency to survive and the no coordination policy with the surrounding tissue indicates that cancer is not the native script of the organism. So when the DNA is loosened it may be falling back to a primitive script.

The case of cell differentiation

Quiescence and senescence are terms describing states of growth arrest of a cell. While senescence is permanent quiescence is temporary but there are no hard boundaries for these states (Blagosklonny 2011; Rodier and Campisi 2011). Growth arrest is coupled with process of cellular differentiation which is a process where a stem cell becomes a specialised cell capable of performing a specific function (Metcalf 2007). The proliferation capabilities of a cell diminish as it progresses towards differentiation. In human body, cells of different tissues show different levels of growth arrest allowing them replenish the specialised cell population of that tissue accordingly. Growth arrest of a cell has been implicated in major complex biological processes like tumour suppression or promotion, tissue repair and ageing (Metcalf 2007; Rodier and Campisi 2011). Genomic script framework easily accommodates these states (Figure 6). A well differentiated cell has a specific pattern of strong side loops since they effect the function of the cell. Side loops push the cell towards differentiation. With each consecutive division, the side loops grow stronger acting as brakes to the cell division cycle by influencing the central loop to grind to a halt (Figure 6). After a limited number of divisions the cell enters the growth arrest phase. A specific pattern of side loops is achieved for each cell type and this pattern is refined achieving the precision in function of that cell. One of the many examples of precision in function is acid production by gastric parietal cells. The produced acid has a consistent pH of 1.5 to 3.5 (Lewin 1992). I'm sure other examples such as the functioning of cardiac pacemaker cells and muscle cells of the heart (Moorman and Christoffels 2003) and neurons and glia of the nervous system (Verkhratsky 2010) will come to the reader's mind.

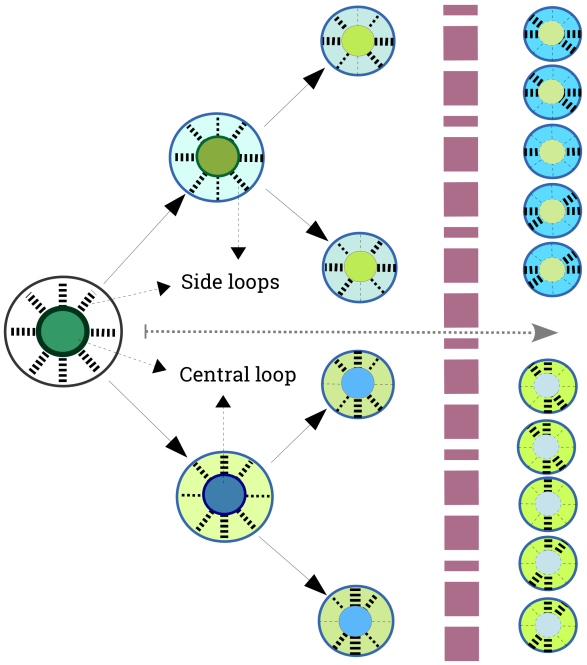

Figure 6: Progressive refinement of side loops halts the central loop during cell differentiation.

Each consecutive division before the cell enters growth arrest refines a specific pattern of side loops which helps to achieve precision in function yielding highly specialised cell. The side loop pattern of the progenitor cell is broken progressively in the consecutive divisions. Each circle represents a cell. Progressing from left to right is the progenitor cell yielding specialized cells. Each cell contains a central loop represented by a small circle in the centre. The side loops are represented by several lines reaching out from the centre to the edges of a cell. The halting of central loop and polishing of specific side loops is represented by progressive thinning and thickening of the lines in each circle.

The case of cancer like native script

Since cancer seems to be an alternate script, possibly a primitive one, that is executed there is a possibility that a script similar to cancer could exist as a native script in other organisms. This script would be beneficial to the organism enabling it to survive. By looking at general morphology of tumours, which ranges from being shapeless to exhibiting basic symmetry and the tendency of a cancer cell to lose all the trademarks of differentiation, we can look for similar scripts in organisms which evolved before there was a definitive symmetry for example sponges of the phylum Porifera. These organisms are potential candidates since they lack definitive body symmetry and exhibit primitive tissues with no organ development. An interesting point to note is that at the base of the kingdom metazoa or animalia lay the branching of Parazoa and Eumetazoa. While the

eumetazoans evolved into all the animals we know today, the parazoans which remain near to the branching point are represented by a single extant phylum Porifera (Müller et al. 2004; Van Soest et al. 2012). Could it be that genome of cancer cells has fallen back to a script similar to the organisms of that period in evolution? If it is so then sponges are ideal organisms to focus our studies on. The undifferentiated cancer cells show no distinguishable appearance which could mean that the cell's DNA has been freed further enabling the script to fall back to a script similar to organisms placed still lower in tree of life. Indeed this type of eukaryotic unicellular organism like behaviour has been observed in cells of canine transmissible sarcoma (Blagosklonny 2005). If a similar script is found it would be beneficial in studying the genomic characteristics of the script in cancer.

Evolution and the genomic script

Enabling unicellular life

Evolution is the copyright of prokaryotes. They are the pioneers who terra-formed this planet. My discussion here deals with prokaryotes. Eukaryotes have it easy in comparison to hazards faced by their predecessors. Every organism is adamant in holding on to its genomic state even if conditions are otherwise. Maintenance of this state enables cellular life to survive and propagate. Consider an extremophile like *Thermococcus gammatolerans*, in order to survive it had to develop the inherent resistance to radiation (Zivanovic et al. 2009). Even the rare and precise mutations do not confer this organism with the ability necessary to survive in such harsh environment. The organism survives on its own by responding aptly to the mutation that it's DNA had received or to any other environmental stimuli. It is here the genomic script comes into play providing a solid framework based on which the cell could respond in such a situation. If necessary the script would be shifted to balance any change in environment. The whole concert of moving gene-networks and re-routing script would be undertaken till the final desired effect is achieved. During the shift many routes would have been tried and many genes might have been dropped or recruited in gene networks. But once the required route that which confers radiation resistance to the cell is achieved it gets fixed allowing *T. gammatolerans* to consistently resist high radiation. Adaptation by a unicellular organism is basically due to side loops since they are related to the maintenance of the its cellular state. Hence side loops could also be called as adaptor loops.

Scripts in extreme and normal habitats

Singled celled organisms interact with the environment directly allowing their genomic scripts to shift routes to meet the demand. The two microbial domains of the accepted system of the three domains of life get fuzzier when the framework of genomic scripts is applied to them. For example where the majority of extremophiles are Archaea, Bacterial species are not far behind and if one can quote an archaean *T. gammatolerans* for radiation resistance another can quote a bacterium *D. radiodurans (Omelchenko et al. 2005; Zivanovic et al. 2009)*. It's the same when one quotes examples for mesophiles. Bacterial species dominate but one can state many mesophilic archaeal species which are being studied (Brochier-Armanet et al. 2008). So here I apply genomic script for differentiating between the organisms. Do bear in mind that any of the following types could a Bacterium or an Archaean. There is a broad difference between scripts of a mesophile and an extremophile. The script of extremophiles is a specialist script which has sacrificed its flexibility. This script evolved to perfection due to constant exposure to extreme but stable conditions of the environments like early earth biospheres and the hydrothermal vents. It holds up precisely to the demands of extreme habitats as described earlier in the case of *T. gammatolerans*. These organisms do not allow the script to fluctuate once the right route is achieved. So even a minor change in the environmental variables could kill the organism instantly. During evolution, genomes of extremophiles would have developed some kind of DNA binders or lockers which would help them maintain script rigidity.

If we leave the extreme habitats, normal habitats are occupied by unicellular organisms with flexible scripts which enable them to tackle the changing conditions. It has to be borne in mind that the

environmental variables of a normal habitat fluctuates within ranges which allow survival of high order eukaryotes. In another perspective it means that these stable conditions are the ranges that biosphere of planet earth settled in which turned out to be supporting the evolution of mesophilic multicellular organisms. In such a habitat the environmental stimuli appear and disappear intermittently and are not as consistent as they are in the extreme environments. The development of resistance to antibiotics in microbes is an excellent example where an immediate effect is observed indicating the flexible nature of their genomic script (Devirgiliis et al. 2013). This type of script allows mesophiles to explore new environments with more or less similar variables and the inflexibility of script inhibits extremophiles from leaving their habitats. From these derivations it is clear that during course of evolution the genomic script diverged into two broad possibilities. This might have happened not once but several times since we see a mixture of extremophilic and mesophilic species within domains of life. As we will see next the process of adapting to an environment may push the genomic script in to either of these paths. But both these routes of script evolution enable life to occupy the biosphere available to them completely.

Pulse is a better engine

Life on planet earth is firmly established. A major factor in the process of establishment would be the inherent adamant nature of the genomic script. If the scripts were not adamant they would continuous shift leading to an unstable genome which would result in extinction of organisms. So the script resists change to the maximum shifting only as a last resort. When generation of diversity results in organisms with new traits, these organisms in turn engrave the script which benefited them. They keep refining the script which leads to their increased ability in absorbing the stimuli like radiation which may cause mutations. Increasingly resisting the change inflicted by the environment tightens the “spring” of the script. But at a critical point the script gives in allowing the shift and depending upon the tension released the script is pushed to a new route causing an evolutionary pulse. This process repeats itself over time resulting in generation of the further pulses and depending upon favourable or unfavourable conditions of the environment at that time the results might vary from a minor change, resulting in repression or expression of a gene leading to a change in cellular metabolism, to a massive push which completely destroys the old identity of the organism yielding a new species. These pulses push the process of evolution forward. What happens if a strong pulse or may be multiple pulses occur in a conducing environment? Such a condition probably would have resulted in diversity explosions.

The greater the strain with which an organism resists the change the better the resultant push. If it were not for this torsion of the script, evolution would yield genomically weak organisms which would be easily be driven to extinction. Moreover this friction fills every adaptable niche with precisely adapted organisms. The aptness in adaptation is due to fine tuning of genomic script countering the nuances of environment. If a shift is successful then the script is stabilised and engraved. The external stimuli needs to be present till the script is stabilised. But if the stimulus is lost before engraving, the script could return to its earlier state. Then again if the new shift is established the organism gains a new trait and subsequent removal of the external stimulus after stabilisation might allow the newly gained trait to act upon the environment changing or even extending it. But it seems that this type of adaptation is not the aim of high order eukaryotes. Here the objective seems to be the maintenance of genome in a controlled state ensuring execution of the same script.

Scripts and evolution in tandem

With the understanding we have gained regarding the script up till now we can now paint with broad strokes the role of scripts in tandem with the process of evolution resulting in discernible consequences to cellular life. Early earth probably had pocket biospheres with primitive unicellular organisms (Figure 7). The pockets as seen in the figure are the organisms along with its environment. These pockets shrunk or expanded or merged with other pockets. The major event that tipped the scale for evolution of cellular life was the formation of the ocean. This single event provided an opportunity for merging the pockets and buffering their drastically different environmental conditions. This mega event brought organisms from different environments under similar conditions. Their genomic scripts which evolved in early pocket biospheres adapted to the new environment using varied coping mechanisms resulting in generation of all the possible

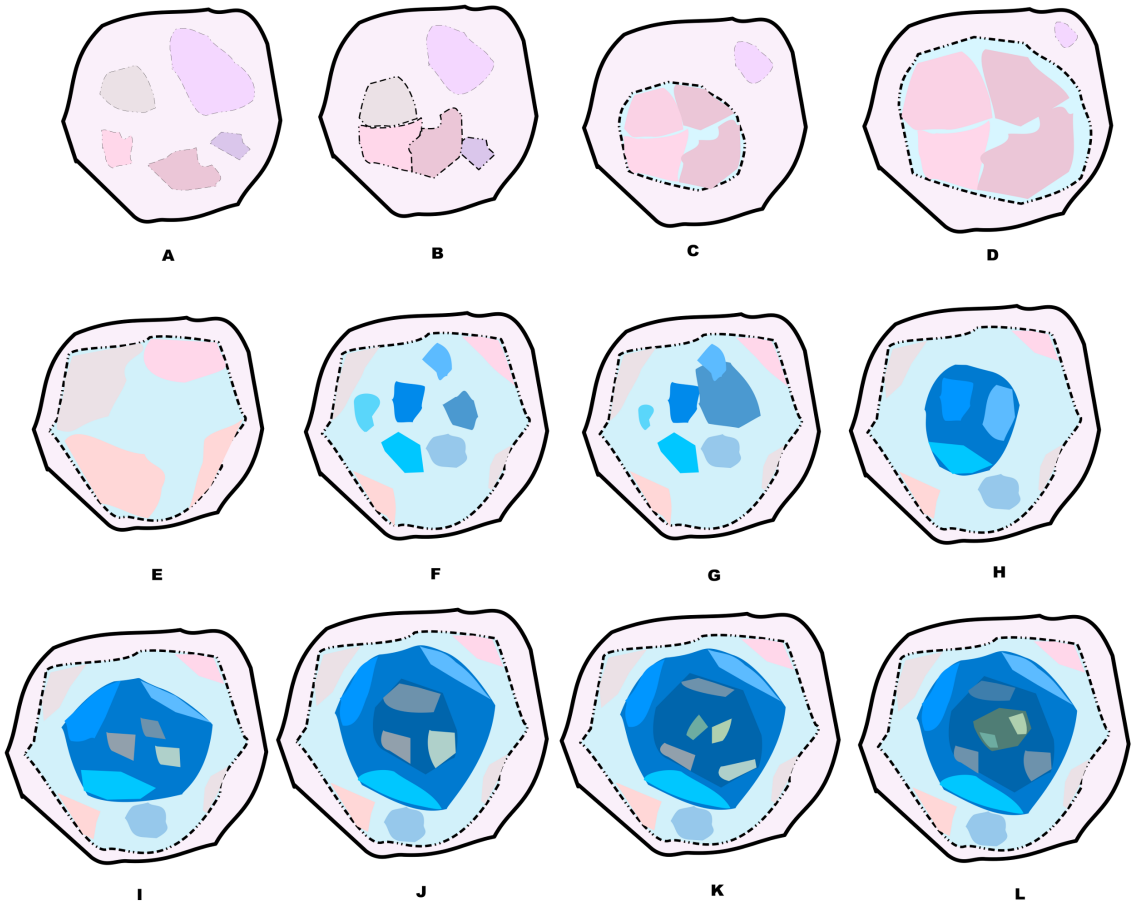

Figure 7: Evolution and genomic scripts in tandem.

Each figure represents the total space available and the dashed boundaries represent the biosphere. Each pocket of the biosphere is the environment, the organism and the genomic script taken together as one. Sometimes environment effects the cells and cells respond with resistance by absorbing stimuli or by shifting the script. The response then effects the environment there by changing it to increased suitability to the organism. This new environment may then provide fertile ground for further evolution. Figure A to C: Early earth may have had pocket biospheres with extreme conditions. These pockets contained life adapted to the extreme conditions of the given environment. As time passed these pockets could have amalgamated and some would have disappeared altogether. Figure D: shows an amalgamated pocket. Merger of the pockets with different conditions could have resulted in a new environment. Such merging of the pockets could have been greatly helped by the formation of the first ocean. Figure E: the pocket grows large enough to cover most of the available space. Organisms with rigid scripts move to the edges where conditions are similar to the preceding environments. The extended biosphere gives rise to the flexible script which enables the organisms to drift off from their mother habitats and explore. Figure F: newly available environmental freedom helps cellular experimentation probably propelling the birth of multicellularity. Figure G and H: these newly evolved pockets merge or disappear or remain as they are. Figure I: the merged pocket increases in size as much as allowed by the prevailing conditions. Again the well adapted species which favour rigidity remain confined to the edges of their environment. Figure J to L: these figures show the evolutionary process moving forward with a repeating pattern.

combinations of core cellular processes that can sustain life. Further environmental mergers such as this allow for new combinations to arise. This makes the suggestion which has been mentioned in a review that microbes are the guardians of metabolism to be utterly plausible (Falkowski et al. 2008). The scripts from the bygone era shifted to adapt to this new norm or were wiped out. The side loops or the adaptor loops would have played a major role in these circumstances. It was at this juncture that the genomic

script would have diverged into path of rigidity which enabled the organism to stay with the environments similar to the early pockets or into path of flexibility which enabled them to explore the recently made available but huge biosphere of the ocean. A point to note is that the decision of rigidity or flexibility forms a pattern when different species are faced with such a situation over and over again during evolution. In meantime, hectic cellular activity caused drastic changes in the atmosphere too (Kasting and Siefert 2002). This was another scale tipping event which threw open the land mass for future invasions.

Proceeding further in time the organisms adapting to their environment could have affected the formation of the localised environments. Similar to the early pocket biospheres, these pocket environment-organisms junta would have shrunk or expanded or merged. One has to keep in mind the role of the script which was continuously shifted and refined allowing the adaptation. This process would have repeated itself leading to formation of localised environments within the parent environments (Figure 7). The eukaryotic multicellular organisms might have evolved in such localised environments. Scripts were shifted by testing all the possible and plausible routes. Some of these routes might have resulted in a dead end while some others lead to multicellularity. It is interesting to note that genomic path that lead to the multicellular organisms as we know today shares the characteristics of both flexible and rigid scripts.

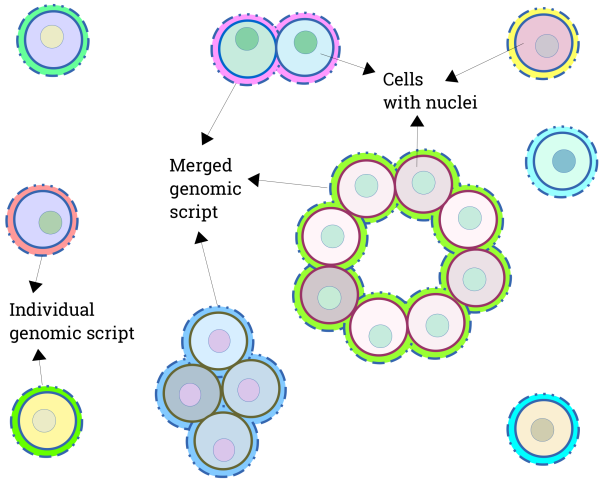

Figure 8: Multicellular organisms evolved by merging individual scripts.

Each cell is represented by a circle and its genomic script by the outer circle of each cell. Outer circle area has been shaded in different colours indicating different scripts. Simple multicellular organisms probably evolved by merging the individual scripts of their cells. The merged script acts like a single script compelling the individual cells into multicellular coordination.

Enabling multicellular life

When it comes to multicellular eukaryotes the script is relatively rigid. Flexible enough to respond to a few possible changes in the immediate environment. This limited flexibility could be due to locking of the genome. It is interesting to note that DNA binding proteins like histones are common in eukaryotes and certain Archaea (Luijsterburg et al. 2008) which also exhibit rigidity in their script. But why lock up the genome? One reason could be that emergence of multicellularity is dependent on it. Early simple multicellular organisms would probably have evolved due to merging of individual scripts giving rise to a merged script which would in turn compel the constituent cells to stay together (Figure 8). Successful cellular coordination which is necessary for implementing a merged script is made possible by the evolution of the communication networks. A script extends from a single genome to multiple genomes within a single cell or among different cells via these networks. For example the intra-cellular coordination between nuclear genome and mitochondrial genome (Woodson and Chory 2008; Finley and Haigis 2009),

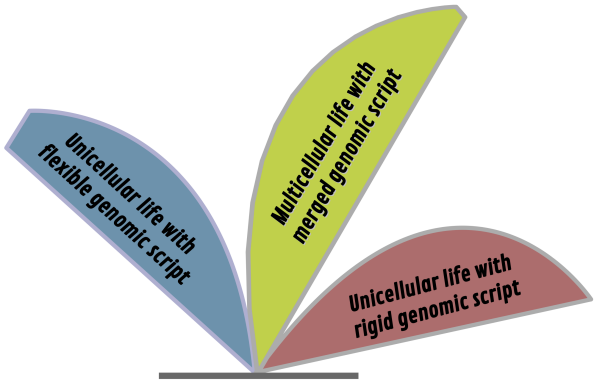

Figure 9: Genomic script diverged into three broad routes.

The genomic script seems to have diverged into three broad routes. The first diverging point resulted in rigid scripts and flexible scripts. The rigid script is a specialist script which is perfectly adapted to the habitat whereas the flexible script allowed exploration of the available biosphere. A third route which enabled the multicellular evolution was the merger of the scripts which necessitated the binding of DNA by placing tight controls over it.

cellular genome and viral genome (Gale et al. 2000), inter-cellular coordination between cells of complex multicellular organisms (Manz and Groves 2010; Geudens and Gerhardt 2011), coordination at cellular level between genomes of the host-endosymbionts (Hoffmeister and Martin 2003; Wrede et al. 2012) and host-parasite relationships. The DNA had to be bound if tight control over genome was to be achieved. Thus laying foundation for the development of complex multicellular organisms (Figure 9). The genomic script of eukaryotes branched further taking different routes. Each major route shift resulted in an evolutionary milestone such as evolution of vascular tissues in plants and evolution of exo or endo skeleton in animals.

Click is the genomic stable state

The locking of genome suggests that evolution of eukaryotic multicellular species is a tightly controlled process which might be responsible for reduction in DNA sequence variation from species to species. This would result in a distinct species at relatively short evolutionary distances. Genome of a cell finds itself in a stable state when environmental conditions are favourable, the side loops have adapted properly and the cell cycle goes on without a glitch. In such a state further change in genomic script is discouraged. In order to move out from a stable state an external pressure or an internal need must arise. So when an evolutionary pulse occurs it pushes the genome to a different state and quest for a stable state begins all over again. When this quest ends there is a high chance that a new species might be born. These genomic stable states are like the clicks of a regulating knob found on old radios for changing frequency bands or on old televisions for changing channels. When turned one can feel the tendency of the knob to snap into position with a click and It takes a nudge to move it from one click to another.

Now consider that the change in the genome is measured on an evolutionary scale whose units are standard units for variation in genome and not time. These units are arbitrary but necessary to drive home the point. In prokaryotes the genome holds onto its stable state over a wide range on the evolutionary scale owing to its flexible script and a free genome (Figure 10). So the “clicks” producing new species are widely spaced. On the other hand the increasingly bound genome of the multicellular organisms is prohibited to sway. Hence it occupies a short range on the evolutionary scale which allows for “clicks” with high probability of yielding a new species. One can observe the clear consistency in species in contrast to fluidity seen in unicellular evolution. For example this has made it possible for the existence of ape and man which share striking genomic similarity (Prufer et al. 2012) and yet remain individually distinct. High-order eukaryotes rarely change except in minor aesthetic details. If such a similarity would have existed between two species of prokaryotes it would be impossible to tell them apart. From the standpoint of evolution it can be said that all the high order multicellular eukaryotic species to be transient species but each and everyone of those is a completely distinct species. One will not be able identify a missing link species even if stared in one's face since

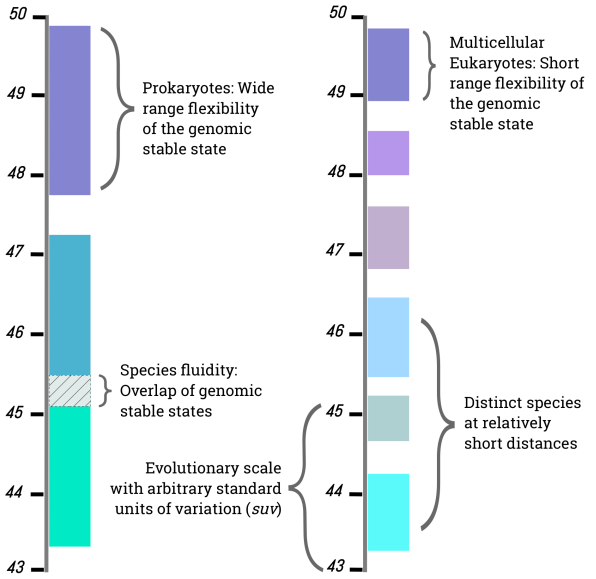

Figure 10: An evolutionary click represents the genome stable state.

The scale on the left represents prokaryotic genomic stable states and the scale on the right represents the eukaryotic genomic stable states. The scale itself has standard units for variation as the values. These are arbitrary and for representative purpose only. Each band on the scale represents a genomic stable state or a click where further change in the genome is discouraged. The bands are wider if the organism's genomic stable state is flexible and occur at longer distances on the evolutionary scale. The flexibility of the script enables prokayotic species to have wide clicks and sometimes two different species with highly similar genome may have overlapping stable states. In the case of multicellular eukaryotes the clicks are short and at the same time occur at shorter distances which allows distinct species to exist at short distances.

in its era it is a distinct species.

A rigid script can translate into a flexible script and vice versa under the influence of the evolutionary pulses and clicks. Consider an environment with organisms. When a localised pocket environment develops within this, it influences the scripts of the organisms to shift to new routes. This might result in an evolutionary pulse. As a result genomic script might deepen the genomic stable state of the organism by increasing in rigidity and forcing the organisms to stay with the previous environment or it might lose rigidity propelling the genome to move out of the click and search for a new stable state which would result in exploration and adaptation to the new environment.

Scripts of the one-way street

Binding of the genome would inhibit script flexibility. Encapsulating nucleus reduces the genome interaction to the environmental stimulus. Further enclosure by cytoplasm and cell membrane only increases this isolation. Controlled environment with only a few possible types of stimuli further ensures that the genomic script is maintained in a constant state without any unwanted shifts which reduces the possible occurrence of an evolutionary pulse to a minimum. Multiple copies of chromosomes would further ensure that the same script is executed. But this type of evolution has a drawback at the level of the organisms. If the organism is incapable of surviving in its environment it could be driven to extinction along with the responsible script. There is no possibility of shifting or readjustment of the script as was possible in the case of unicellular organisms. This one way street makes sure that only the successful script is propagated. Genomic script of any extant organism belonging to any branch on the tree of life could be successful. It does not matter from which pre-existing conditions that a successful script has evolved from. This caveat allows organisms with features radically different from one another which belong to multiple and distant phyla to flourish within the same environment. Coexistence of such nature would clearly help in building inter-species relationships as seen in the microbial ecosystems in oceans or any of the complex animal-bacterial interactions (Orcutt et al. 2011; McFall-Ngai et al. 2013).

A framework of genomic script

In the beginning

The central loop is not built in to *care* about the division of the cell. Nonetheless it's execution is kept in check by side loops till the necessary cellular functions are completed before the division. This seems to indicate that the central loop might have evolved even before cellular life came into existence in an acellular world. A world where DNA, RNA, proteins and the viruses were the key players (Woese 2004; Neveu et al. 2013). The first script which became the central loop would have evolved during this era. The inhospitable early earth might have contained pocket environments which became temporary biospheres due to intermittent availability of an essential environmental factor. Once this factor was lost the biospheres disappeared along with it leaving behind environments which were not conducive to life. If all conditions fall into place, these “now you see and now you don't” type biosphere could be found on any planet or their moons or a comet or a sufficiently large asteroid. Possibility of some kind of favourable environment has been suggested (Clark et al. 1999; Peplow 2006; Raulin et al. 2012). Such an environment could have been the playground where the (((DNA ↔ RNA) ← Protein) ← Virus) interactions occurred giving rise to the proto-central loop.

Side loops are basically unnecessary for this biomolecule-bioparticle cycle. This ready-made module of a simple central loop framework could have been enveloped resulting in primitive cells. The most important consequence of cellular life would be the generation of side loops which probably evolved in parallel with the process of cellularization. Though the side loops may change with time, the central loop does the work irrespective of time or organism which makes it aptly suited to be termed as a perennial loop. In a prokaryotic world, dynamic side loops were vital in dealing with the environmental challenges. But evolution of multicellular organisms required binding of the genome which ensured controlled execution of central loop and controlled enabling of a specific pattern of side loops.

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DNA is just a physical location

The DNA, which is the physical location of the script, is distinguished with addresses at specific locations like the promoter regions. The change in these addresses could cause a shift in the script. An address change could be a change in promoter region or the regulatory networks or the super switches or genomic expression effected by the increasingly important non-coding RNAs. The first script to evolve would have been a simple script probably with the execution addresses hard coded on the DNA itself resulting in a simple network. The function of this would have been to produce a single effect. It is possible that the first script is still active or at least exists as a relic in our genome. It would be interesting to see the function that this script performs. From its humble beginning probably in an acellular environment the genomic script has evolved into a complex entity which still does its job in the cells of our human body. One could imagine the colossal size of the genomic scripts by comprehending the complex genomic machinery of regulatory networks, repair mechanisms, replication mechanisms, transcription mechanisms and the massive networks of non-coding RNAs involved in executing, maintaining and shifting them. It has indeed come a long way through myriad of paths and decisions reaching its present state. It is extremely likely that these paths and decisions correspond to the branches and nodes of the tree of life.

Resilience of cellular life

As we have seen genomic script framework provides a unified theme for cellular activity across domains of life. It provides solid basis on which we could understand the responses of cell. The explanations we derive from this framework compel us to view the cell with a new perspective. It may have been by chance that life hit upon this planet but it was not just chance alone that helped it survived. Unicellular organisms were fighters in every sense of the word. Every option was thoroughly verified to find the best response. If any environment could be utilized for life, it was. By doing this they tamed the planet and extended the biosphere. They still hold the biosphere in check. They were not merely constituents in the process of evolution blessed by chance opportunity rather every step of evolution was a fight won by them. This *will* to be has made the cells of the complex higher organisms so resilient.

The genomic script framework holds on its own. No drastic changes or radical assumptions are required to make it work in different scenarios. A single concept which is capable of this type of durability is in itself a reason enough for arousing our curiosity in it. As far as I know there is no other concept at the genomic level which easily explains what we observe. However questions do remain like how did the central loop start in the first place? How are side loops generated and controlled? How can we study them with technology available to us? And how can we map the process of a genomic script? Answers to these and other such queries would be quite interesting. Further research and input of thought from critical thinkers is very much needed to unearth characteristics of the script in order to refine the concept and to put forward a new kind of unified perspective to the diversity of cellular life which was previously unheard of.

Conclusion

Coming back to where we started in the beginning, the framework of genomic script does indicate that cancer does indeed contain a few underlying principles. These principles appear to effect the functioning of genome of not only a cancer cell but a normal cell too. It is possible that different manifestations of those few principles of genomic script resulted in diverse cellular life as we know today and cancer seems to be just a type of it.

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