

HOW DO CELLS KNOW WHEN TO DIVIDE?

Cell proliferation: the far more ancient, and arguably far more *important* opposition to the core message of the Spice Girls' hit "2 become 1". Ever since the early stages of life on Earth, the ability of a cell to replicate itself has been as essential to life as the matter that makes up our being, despite the fact that the mechanisms at play in our body at this very moment make the mind boggle with their fine-tuned sophistication. Never mind *how* the cell divides, as even DNA replication alone calls for a gargantuan procession of processes, from the careful unzipping of the double helix, to the spindle fibres that pull at the code that breathes life into our population- and strikes the most delicate of internal balances. Whilst a small change in the DNA of a somatic cell might render it incapable of functioning, and ultimately lead to its demise, the far more terrifying thought is that of the complete opposite: that a somatic cell may be utterly changed, no longer able to serve its dutiful purpose to the organism that it comprises, and yet *live*. An uncontrollably proliferating cancerous cell can have the ability to divide and replicate at 6 times the rate of our somatic cells, and one can only assume that it has lost- or *abandoned*- the mechanism of regulating growth.

How do we know that cells regulate their division, as opposed to the simpler thought that the replication method consumes too much of the cell's time and resources? Professor Leonard Hayflick proved just this in 1962 with his experiment on the replication of somatic cells *in vitro*. Remarkably, a group of cells placed into a culture containing an excess supply of nutrients and resources began by proliferating vigorously, but eventually relinquished their ability to divide- a phenomenon coined by Hayflick as "cellular senescence"^[2]. The key to this discovery was the lack of limiting factors within the clinical environment, and the knowledge that cancerous cells are unaffected by this loss of ability to perform cellular mitosis, which provided the important clue: the cells' ability to divide is dissipating, one must assume, to provide some form of evolutionary advantage to the organism itself.

Though our somatic cells may have an internal clock, where time is replaced by number of divisions, a cancerous cell exploits a mutation in the gene that codes for telomerase to retain its ability to divide. The telomerase enzyme is responsible for turning back the clock, by adding to the number of telomeres (like the pages on a tear-away calendar, telomeres are sections of DNA removed each time the cell undergoes mitosis), and if the telomerase gene is overexpressed, for each page that is torn, another can be stuck back down; the cell never reaches its expiry date.

In evolutionary terms, cellular senescence developed in response to the risk of cancerous cells which take over part of an organism that is crucial to its survival. However, this variation indisputably came to prominence in the evolutionary timeline at a point where environmental factors would limit an organism's lifespan dramatically; the fitness cost of late-onset tumorigenesis would be relatively low^[1].

We, as a population, are outliving the underlying plan that was carefully constructed by thousands of generations of natural selection, hence the detrimental effects of a build-up of senescent cells has not been weeded out by evolution^[6] (I should note, here, that although I may describe the effect of natural selection as having a goal or purpose, evolution has no foresight, and as such cannot be expected to adjust the allele frequency of a population of organisms if the disadvantageous allele rarely presents itself as such, especially considering the extreme advantage in early life.) Although these fairly inactive cells may seem harmless, their inability to compete with tumour cells provides the perfect environment for the spread of cancer. Furthermore, they secrete certain factors that cause the surrounding tissue to become senescent too

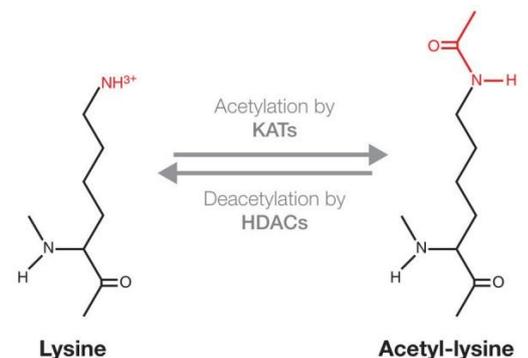
(imagine a zombie apocalypse, where the “living dead” spreads through a population), and studies that demonstrate the impact of senescent cells suggest their absence would noticeably increase our life expectancy^[15].

As it transpires, the genes in our cells are fighting a constant battle, the result of which is a carefully-orchestrated balancing act involving genes that suppress cell proliferation, and genes that promote it. To add another complexity, genes are not just *on* or *off*, a primitive light switch that definitively controls expression, but more like a dimmer switch, wherein the extent to which a gene is expressed can take any position in a whole spectrum. The umbrella term for the phenomenon that allows this *dimmer-switch*-style regulation is epigenetics, and it revolutionised the way the coding of life was viewed in the mid-20th century^[5]. The iconic double-helix structure of DNA, first deduced by Rosalind Franklin in 1952 via X-ray crystallography, is not actually the whole story; in reality, eukaryotic DNA is wound around many small proteins called histones, in order to ensure that the chromosomes- strands of DNA- fit into the nucleus^[7].

It may seem counter-intuitive that forming a complex between the DNA and a protein, termed chromatin, would make it “smaller”, but the positive charge of the histone proteins forms strong electrostatic forces of attraction with the negatively-charged phosphate groups in the sugar-phosphate backbone of the DNA, tightly holding the two components together. As such, the many coils that are formed can only be held in place with the help of proteins, and it is these coils that allow the DNA to be condensed.

Histone *modifications* are one of the possible changes that can be made to the chromatin, and will affect the way that the genes in the surrounding area are expressed- and in a slightly subtler way than modifications to specific genes directly. They involve different chemical groups being “tagged on” to the protein, and affect the genes that are within the range of the intermolecular forces formed by the modification^[4]. For example, a particular form of modification known as acetylation, which involves the addition of an acetyl group to an amino acid, in this case lysine, is a key factor affecting tumorigenesis.

Interestingly, it is not the modification itself that is detrimental, rather the imbalance of a group of modifications, as has been shown by studies that involve two opposing inhibitor drugs: one affects acetyltransferase, which controls the addition of acetyl groups; the other affects deacetylase, which is responsible for removing acetyl groups when necessary^[8]. When either of these key regulatory enzymes are inhibited from performing their purpose, the rate of cancer progression is dramatically changed, and as such, we see that



the miniscule “tags” that embellish DNA have the power to regulate cell proliferation^[9].

(10. Brock, T.)

But how can a cell *know* when it needs to proliferate? The key lies in considering the whole organism; there are no higher, god-like cells that exert control over all the other cells, so they must be exerting some sort of control over one another, with each tissue making up a huge civilization of cells that communicate in the most primitive, and yet amazing, way possible.

Firstly, some basics on cell division: in eukaryotes, the process of cell division can be split into four phases, known as G₁, S, G₂, and M. The G₁ phase is when the cell “commits” to dividing, and the remaining phases are all about the execution, with the G₂ phase acting as a gap between the DNA transcription in S phase and the mitosis itself in M phase (much like the G₁ phase does when the cell cycle restarts)^[16]. If the cell were to decide against cell division, in response to any number of factors, it enters a phase of quiescence (coined G₀), and is temporarily separate from the cycle of proliferation. Though, it should be

noted that G_0 can also be used to refer to another state of non-cycling in which the cell is not as poised to begin proliferating once again, and hence the G_0 states can be further classified into two distinct categories: quiescent (readily altered, due to its permissive epigenetic status) and senescent, or terminally differentiated (not readily altered, either due to the deliberate relinquishment of proliferation or because the cell has rolled to the end of a trough in the famous *Waddington's landscape*).

The transition from the G_1 phase heavily involves a specific protein called the retinoblastoma protein (pRB), which acts as a repressor of the transcription factors- E2F protein complexes- that trigger the transition into the S phase^[3]. E2F have target genes that they act to “switch on”- these are called activators and are most functional within the cell at the end of the G_1 phase, directly before the transition into S phase.

Clearly, mitosis involves the replication of DNA, in order that each daughter cell possesses a full set of chromosomes. E2F target genes comprise the majority of the DNA segments that code for the army of enzymes required for the DNA transcription^[11], and the activation of the enzyme genes is regulated by whether the E2F and their binding partner- “dimerization partner”- are bound to the site on the DNA^[12]. Dimerization- the process of two sub-units coming together to form one molecule- forms a complex (known overall as the transcription factor) that is essential to the induction of the target genes and, as such, E2F cannot act without the help of its specific dimerization partner.

The retinoblastoma protein represses E2F factors by binding to them to essentially deactivate them, thereby preventing the expression of any genes that are regulated by that transcription factor; not only that, but the pRB essentially converts the purpose of the E2F entirely, with the resulting complex of the aforementioned binding with pRB going on to actively prevent transcription. For example, when bound to E2F3a, the pRB is responsible for reducing the expression of the target gene directly by enlisting the action of enzymes responsible for chromatin remodelling (such as deacylase- as mentioned in the earlier section on epigenetics). The ability of the retinoblastoma protein to prevent the E2F transcription factors from prompting the cascade through the phases $G_1 \rightarrow M$ makes it an effective tumour suppressor. In fact, the human papillomavirus acts using a protein that binds to the pRB protein, essentially preventing it from repressing cell proliferation, and thus provides a path for the replication of the viral DNA. Therefore, even when the virus does not manage to be replicated to the extent that the cell is destroyed, the cell's pRB have been permanently inactivated, and as such the cell proliferation is not controlled, leading to cancerous masses of uncontrollably proliferating cells.

The phosphorylation of the retinoblastoma protein is key; before the transition, the E2F is bound to pRB, and so is trapped from involvement in the cell cycle. When the pRB is phosphorylated, it allows the E2F to separate, and thus it is free to not only “switch on” the target gene, but also to activate other factors that contribute to the cascade into the next phase. E2Fs themselves can indirectly (as it involves activating factors that then interact with the CDKs) activate cyclin-dependent kinases, which then- and this part really highlights the indescribable beauty of the cell's inner workings- are responsible for the phosphorylation of the pRB^[13] and, as such, the distinct processes come together to form one great cycle such that the end product can trigger the reaction that created it, and thus propagate cell proliferation without the need for additional molecules.

On a molecular level, the phosphorylation via the attachment of CDK to one of many phosphorylation sites contributes to a conformational change in the tertiary structure of the protein- initially held together by a multitude of bonds and forces- and so dramatically reduces the stability of the interaction between the protein and the E2F that it holds captive. Research done into the possibility of only one of these sites being “primary” in regulating the contact between pRB and E2F concludes that only once

several phosphate groups had accumulated on the molecule would the effect come to fruition.

Though the retinoblastoma protein- whose function is to inhibit cell proliferation- is present at all stages of the cell cycle, which may seem to make mitosis impossible, its varying phosphorylation allows it to change from being active to inactive as required, and this presents a small window of opportunity for the cell to evade the usual repression by pRB and divide.

Returning to the overarching idea, there must be some external factors that trigger the quiescent cell, which has a permissive chromatin environment (as mentioned earlier, essentially denoting that the modifications made to the DNA-histone complexes allow the cell to rapidly activate DNA transcription, the first step of mitosis), to return to the G₁ phase. The solution comes in the form of growth factors; small, naturally-occurring molecules that can be released by cells, and detected by others in order to stimulate changes in the cell that include proliferation and differentiation. These growth factors that are secreted will bind to a receptor that is integral to the cell's membrane- that is, the receptor is a protein that connects the outside world to the inner metabolism of the cell. Known as transmembrane signalling^[14], this phenomenon wherein external signals are transmitted into a cell relies heavily on phosphorylation, much like the processes discussed earlier. The exact mechanism of transmission varies between tissues but, in one specific situation, the binding of the growth factor (a specific form of a ligand) can trigger the phosphorylation of a particular amino acid (in this case tyrosine) within the protein. Coined *ligand-stimulated receptor phosphorylation*, the binding of the growth factor enables the phosphorylation of the C-terminal (the internal portion- characterised by the presence of a carboxyl group as opposed to an amine group) of the intrinsic protein, and stemming from there, modifications to other proteins and molecules within the cell can be made.

An example of a growth factor in humans whose signal is thus transmitted into the cell is insulin-like growth factor, which is a protein, secreted by the liver, that is associated with the proliferation of cancerous cells in breast cancer. In the cancerous cells, the receptor for this growth factor is either overexpressed (so there are many on the surface of the cells) or hyperphosphorylated (the receptors are overly "activated" for transmitting signals), both of which are advantageous to the cancer cells as it enables them to undergo mitosis more regularly, and outcompete somatic cells.

In summation, cells take into account a whole number of factors- from intrinsic DNA to intercellular signalling- when deciding whether it is appropriate to proliferate. Daisaku Iseda once said: "No one can live entirely on their own, nor can any society exist in isolation" and this is never more applicable than to the cells in our body, wherein survival is dependent on sending and receiving signals about the hostility of the environment that they inhabit, and, crucially, about whether they can undergo mitosis.

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