

Determining to Divide: How Do Cells Decide?

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“The best security for the fidelity of men, is to make interest coincide with duty.”

Alexander Hamilton

Abstract. A cell’s decision to divide must be regulated with the highest fidelity. Otherwise, abnormalities occurring in the replication of genetic material and cytokinesis would be incompatible with life. It has been known for almost a century that cells comprising a population undergo cellular division at extremely variable rates, even though genetically identical cell clones have been examined. Studies with T lymphocytes at the single cell level have revealed that the rate of cellular division is determined by the accumulation of a critical number of ligand-triggered interleukin-2 (IL2) receptors at the cell surface throughout the G₁ phase of the cell cycle. Thus, the cell “counts” the number of triggered IL2 receptors, and only decides to divide when the critical number has been attained. This information is then transferred to the cellular interior via intracellular sensors comprised of D-type cyclins, which ultimately determine when the cell surpasses the “Restriction Point” in late G₁, and which commits the cell irrevocably to initiate DNA replication. Beyond the R-point, the cell assembles a definite number of macromolecular pre-replication complexes (Pre-RCs) comprised of at least 6 distinct proteins at sites of the origin of replication on DNA. Complete assembly of the Pre-RCs is a prerequisite for their subsequent disassembly, which must occur before the initiation of DNA strand replication, and which occurs asynchronously throughout the S-phase of the cell cycle and only terminates when the entire DNA has been duplicated. Thus, the fidelity of the decision to divide is exquisitely regulated by macromolecular mechanisms initiated at the cell surface and transferred to the cellular interior so that the cell can make the decision in a quantal (all-or-none) fashion. The question before us is how this quantal decision is made at the molecular level. The available data indicate that the assembly and disassembly of a definite number of large multicomponent macromolecular complexes make the quantal decisions. Here, it is postulated that all fundamental cellular decisions, i.e. survival, death, proliferation and differentiation, are regulated in this fashion. It remains to be determined how the cell counts the signals it receives, and what the molecular forces are that dictate the behavior of macromolecular complexes.

Key words: cell cycle, Restriction point (R-point), interleukin-2 (IL2), Janus kinases (JAK), DNA replication, cyclin, cyclin-dependent kinases (CDK), retinoblastoma (Rb) proteins, quantal

Introduction

In science there are three fundamental questions; (1) What happens? (2) How does it happen? and (3) Why does it happen? For the most part, biologists are concerned with the first question, while chemists are concerned with the second,

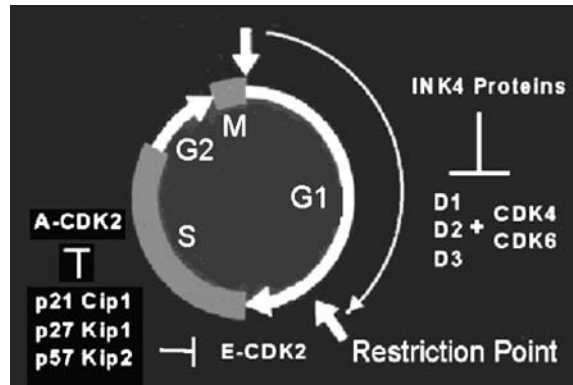


Figure 1. The Cell Cycle. Note that the relative length of each cell cycle phase is approximate to the duration of each phase. From [28].

and physicists are concerned with the third question. With regard to cell division, this discussion will focus on the first question, and will attempt to summarize as succinctly as possible the total knowledge we have as to how cells decide to divide. Most multicellular organisms are comprised of cells that are not in the process of dividing, while unicellular organisms usually are always dividing. Thus, in the parlance of cell biologists, most unicellular organisms are “in” the cell cycle, while cells making up multicellular organisms are not in the cell cycle. Therefore, for mammalian cells, one must first determine how “resting” cells decide to enter the cell cycle, after which one can focus on the question of how cells decide to progress through the cell cycle.

Traditionally, the cell cycle is divided into four phases by cell biologists as shown in Figure 1. There are two phases that are readily recognizable, the S-phase, which is the phase in which the cellular DNA is synthesized or replicated, and the M-phase, which is the phase when the cell undergoes mitosis or cytokinesis. Between these two phases are gaps (hence the designation as G-phases), where ordinary assays could not detect any characteristic activity. The G₁ phase begins after mitosis is complete, and leads up to S-phase, while the G₂ phase begins after S-phase is complete, and leads to the M-phase. There is also a phase designated G₀, which is reserved for cells that are resting and not “in” the cell cycle.

For unicellular organisms such as bacteria, yeast and protozoa, which are always “in” the cell cycle, the rate of movement through the cell cycle is dependent upon nutrients and temperature changes in the environment. Traditionally, the transitions from one phase to another have been thought to be regulated entirely internally, with no signals derived from outside the cell. By comparison, specific molecular ligands direct cells of multicellular organisms to leave the resting G₀-phase to enter the cell cycle and to proceed through the G₁ phase to the S-, G₂- and M-phases.

Studies focused on the behavior of individual cells have uncovered a perplexing, but apparently fundamental, principle that is common to all living cells. Cells behave

in a highly variable manner with respect to the time that they take to undergo a single traverse through the cell cycle. Even cells that are genetically identical, i.e. clones, or the progeny of a single cell, will exhibit variability in the timing of their cell cycle progression. Thus, a cloned population of cells can be stimulated by a ligand at a maximal concentration, and the cells comprising the cloned population will respond temporally in a highly variable fashion. Some cells will transit through the cell cycle very rapidly, while others will do so more slowly.

Such behavior was first documented for the growth of individual bacterial cells in 1932 [1]. Subsequently, over the last 70 years, eukaryotes from such diverse organisms as yeasts and protozoa, as well as all invertebrate and vertebrate cells, have been found to behave similarly: individual cell cycle times follow a normal distribution when examined as a function of division rate [1–5]. Thus, some cells divide very rapidly, whereas others divide slowly, with most cells distributed close to the mean [6]. As the mean cell cycle time of the cell population does not vary, it follows that the generation time of an individual cell is not passed on from one generation to the next. Otherwise, over time there would be a selection for the more rapidly growing cells, which clearly does not occur. Even so, some critical information must be passed on, in that the sibling cells resulting from a division have almost identical cell cycle times in the ensuing generation [4].

The Restriction Point in G_1 and the Growth of T Lymphocytes (T Cells)

Pardee and co-workers summarized the knowledge that had accumulated as of 25 years ago in a lucid and comprehensive review of what happens when cells of a population proliferate [5]. All of the variability in cell cycle times occurs in the G_1 -phase of the cell cycle, in that the S-, G_2 - and M-phases are fixed as to the time required for transit through these phases for a given cell population. Furthermore, 30 years ago Pardee defined the G_1 “Restriction Point” (R-point), as a point late in the G_1 phase, after which the external stimulus required to promote cell cycle movement is no longer necessary [7].

As a model system, Pardee utilized murine embryonic fibroblasts (MEF) and serum was used as a growth stimulus. Because the components of serum that provided the growth signals to the cell population had not yet been discerned, Pardee and his coworkers could not proceed further in their experimental dissection of the critical determinants of cell cycle progression. However, utilizing T lymphocytes (T cells) and armed with the new knowledge that T cells are stimulated to proliferate via a molecule with T cell Growth Factor (TCGF) activity termed interleukin 2 (IL2), we performed a series of experiments 20 years ago that showed that the rate-limiting step in T cell cycle progression is the interaction between IL2 and the IL2 receptor (IL2R), which controls the G_1 “R-point” [8]. These experiments were possible because we had defined the molecular determinants responsible for T cell cycle progression, and we could analyze the cell populations for the first time at the level of the individual cell.

We utilized cloned T cell populations [9], purified homogeneous IL2 molecules [10], monoclonal antibodies reactive with both IL2 [10] and IL2Rs [11], coupled with a quantitative radio-labeled-IL2 receptor binding assay [12]. Armed with these unique molecular reagents and single cell analysis via flow cytometry [13], we found that besides the affinity of the intermolecular reaction, only three parameters are important for determining the rate of cell cycle progression. To proceed beyond the R-point, the IL2 concentration, the IL2R density and the duration of their interaction determine the accumulation of a critical number of IL2/IL2R intermolecular interactions that signal a quantal (all-or-none) decision to initiate DNA replication [8].

The affinity of the IL2/IL2R interaction is quite high, such that the equilibrium dissociation constant (K_d) is ~ 10 pM, owing to a rapid association rate ($\kappa = 10^7 \text{ M}^{-1}\text{s}^{-1}$) and a relatively slow dissociation rate ($\kappa' = 10^{-4} \text{ s}^{-1}$). Thus the $K_d = \kappa'/\kappa = 10^{-4} \text{ s}^{-1}/10^7 \text{ M}^{-1}\text{s}^{-1} = 10^{-11} \text{ M}$ [12, 14]. Detailed kinetic binding and structure/activity studies revealed that the rapid association rate is attributable to IL2 binding to the α chain of the IL2R, while the slow dissociation rate is due to IL2 binding to the β, γ receptor chains [14]. Thus, when IL2 is bound to the heterotrimeric IL2R, a stable quaternary complex is formed.

More recently, assembly energetics of IL2 receptor signaling complexes have shown that the α and β IL2R chains can bind to one another in the absence of IL2, supporting the idea that an α, β complex probably exists preformed on the cell surface and serves as the initial interaction complex for IL2 on antigen-activated T cells [15]. Subsequently, the γ chain binds to the IL2/ α, β trimeric complex, thereby forming an active ligand/receptor complex capable of signaling the cellular interior. The energetics experiments indicate that the thermodynamic basis for the positive cooperativity in the formation of the stable quaternary signaling complex is achieved through compensation of loss in entropy by increasing enthalpically favorable receptor-receptor, and ligand-receptor, intermolecular interactions.

In other experiments, it was determined that after IL2 binding, the bound receptor complex is internalized and degraded by lysosomes with a half-time ($t_{1/2}$) of ~ 15 min [16]. Thus, as the $t_{1/2}$ for dissociation is three times longer (45 min), once the ligand binds to the receptor, it is essentially irreversibly bound and continues to signal until it is internalized and degraded. Accordingly, the IL2/IL2R system follows the "occupancy hypothesis" for signaling, in that once IL2 is bound and a stable quaternary complex with the receptor chains is formed, signaling persists [17].

Signaling to the cell interior occurs via the β and γ chains of the IL2R. With the formation of the stable quaternary IL2/ α, β, γ ligand/receptor complex, it is thought that the cytoplasmic domains of the β and γ chains are brought into closer proximity, which serves to approximate two already bound tyrosine-specific kinases, which are termed JAK-1 and JAK-3 [18]. These kinases then phosphorylate themselves as well as specific tyrosine residues on the receptor β and γ chains, which then serve as docking sites for molecules termed Signal Transducers and

Activators of Transcription-5 (STAT5) [19, 20]. Upon docking to the IL2R, these STAT5 molecules are phosphorylated by the JAKs, which activates them to undergo conformational changes that allow their dimerization and translocation to the nucleus, where they activate transcription of specific genes. The STAT5-promoted expression of cell cycle genes throughout the G_1 phase of the cell cycle then promotes progression to the R-point [21].

The duration of G_1 is determined by a critical number of triggered IL2Rs that must accumulate throughout G_1 because there are not enough IL2Rs on the cell surface to surpass the R-point immediately. Accordingly, new IL2Rs must be synthesized, transported to the cell membrane where they can bind IL2 and signal continuously during the G_1 interval. We were able to estimate the critical number of IL2/IL2R interactions necessary to reach the R-point from experimental data [16]. Antigen-activated T cells express a mean of 750 IL2R sites/cell, while the rate of receptor internalization and degradation is $t_{1/2} = 15$ min, which yields a rate constant $\kappa = \ln 2/15 \text{ min} = 0.0467 \text{ min}^{-1}$, which is necessary to achieve a constant number of receptors on the cell surface at steady state. Accordingly, the rate of new receptor synthesis necessary to maintain a steady number of receptors/cell is

$$R_{\text{syn}} = \kappa \times R \text{ at steady state} \quad (1)$$

$$R_{\text{syn}} = 4.67 \times 10^{-2}/\text{min} \times 750 \text{ R/cell} \quad (2)$$

$$R_{\text{syn}} = 35 \text{ R/cell/min} \quad (3)$$

where R represents the number of receptor sites. Inasmuch as 11 h (660 min) are necessary for 50% of the cell population to reach the R-point, a mean of approximately 23,100 IL2Rs must be triggered.

Thus, the variability of the cell cycle times of individual cells within the population is accounted for by heterogeneity in the number of IL2Rs expressed/cell. As can be seen in Figure 2, using single cell analysis of a cloned population of T cells, the IL2R density varies by much as $3 \log_{10}$. Accordingly, there are some cells

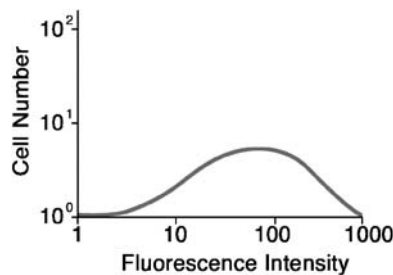


Figure 2. The Log-Normal Distribution of IL2 Receptors. Note that IL2 receptor density varies over three orders of magnitude, so that some cells have a 1000-fold higher density of receptors than others. Accordingly, when the IL2 concentration is low, only cells with the highest density of IL2Rs acquire the requisite number of triggered IL2Rs to pass the R-point, and undergo cell division. From [8].

within the cell population that have 1000-fold fewer IL2Rs than others, and there is a log-normal distribution of the IL2Rs within the cell population, even though it is a cloned cell population and therefore genetically homogeneous. Thus, if a cell expresses only 375 IL2Rs/cell, instead of 750 IL2Rs/cell, from the above equation we can calculate that 22 h will be necessary to reach the R-point, whereas if a cell has 1500 IL2Rs/cell, then only 5.5 h would be necessary.

The most important observation from all of these experimental findings is that a critical *number* of IL2/IL2R interactions is necessary for a quantal decision on the part of each cell to progress beyond the R-point. Thus, if either the ligand concentration *or* the IL2R density are low, the cell “counts” the number of triggered receptors, and waits until the requisite number of IL2/IL2R interactions has accumulated, before making the irrevocable decision to replicate DNA [16, 17, 22].

DNA Replication

Once the signals leave the cell membrane there is a complex series of biochemical reactions that ultimately culminate in triggering DNA replication. However, before moving the focus of our discussion from the cell membrane through the complex molecular cellular interior to the nucleus, it is easier to proceed directly to an examination of what is known about DNA replication in the nucleus before attempting to connect the cell’s outside with its inside to discern what happens when cells decide to divide. The accurate and timely replication of DNA is obviously essential to the faithful propagation of a cell’s identity. DNA replication and duplication of the chromosomes occurs only during the S-phase of the cell cycle, and during this phase the DNA is replicated exactly and only once.

At this time, there is only a simple framework in place to begin to understand how DNA replication is regulated. There are many missing elements, and many molecules and their functions yet to be discovered. Much of what we know has been discerned from genetic experiments with yeast cells, but when possible the general principles have been found to apply to other cells as well. In particular, *Xenopus laevis* (frog) and *Drosophila melanogaster* (fruitfly) have served as important species for analysis.

Biologists studying unicellular organisms that are always “in” cycle first described the “Cell Cycle”, and, as already mentioned, four distinct phases were recognized. However, when viewed from the regulation of DNA replication, there appears to be only a binary set of states in the cell, as depicted in Figure 3: the *assembly state*, and the *replication state* [23]. During the *assembly state*, which corresponds to the G₁ phase of the cell cycle, in IL2/IL2R-activated T cells, STAT5-promoted gene expression leads to the accumulation of proteins important to reach and surpass the R-point late in G₁. Subsequent to the R-point, the expression of the genes necessary for nucleotide synthesis and DNA replication occurs, which permits protein complexes termed Pre-replication complexes (Pre-RCs) comprised of at least 6 distinct proteins to be assembled at sites of the origins of DNA replication (Oris). As these

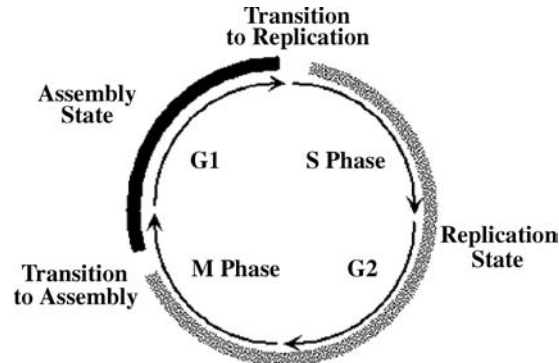


Figure 3. The binary states of DNA replication. Note that the inner circles that depict the cell cycle phases have been idealized and represented as of equal length, which is incorrect. From [23].

Pre-RCs are assembling, DNA replication is prevented from initiating. Then, during the *replication state*, which encompasses the S-, G₂- and M-phases of the cell cycle, the completely assembled Pre-RCs dissociate from the DNA, DNA strand duplication is initiated, and formation of any new protein Pre-RCs is prevented. Furthermore, DNA synthesis does not initiate at all sites simultaneously. Rather, individual “firing events” are temporally distributed continuously throughout the duration of the S-phase.

In addition to understanding the circumstances that distinguish the two cell states, it is also critical to understand the forces necessary for the cell to pass from one to the other. Thus, even though assembly of pre-replication protein complexes occurs only after the G₁ R-point, the complexes can only initiate DNA replication on activation of specific cyclin-dependent kinases (CDKs) that phosphorylate target proteins of the Pre-RC [24]. These activated CDKs thus promote the “transition to replication”, which is characterized by the disassembly of the Pre-RCs at the Oris, and the initiation of DNA strand duplication. However, even though CDKs are so important for the decision to initiate DNA replication, their substrates and how they promote dissociation of the Pre-RCs and DNA strand duplication regulation are largely unknown.

Moreover, even though many of the proteins that act at Oris have been identified, their biochemical mechanisms of action remain largely unknown [25]. For example, little is known about the forces that promote the disassembly of the Pre-RCs at the origins of replication, or whether the disassembly is coupled to the initiation of DNA strand replication. However, the process of DNA replication is carefully regulated, and, like the decision promulgated by a critical number of triggered IL2/IL2Rs to proceed beyond the R-point, it is apparent that the cell is capable of determining when and how to make a quantal decision to replicate DNA. The question is how the cell decides when to divide.

In this regard, as most studies focused on DNA replication have utilized autonomously proliferating cells such as yeast that are “in” a perpetual cell cycle, thus far external ligand-promoted DNA replication has not yet been studied, particularly in mammalian cells. However, the length of time that a cell remains in the G₁-phase (*Assembly phase*) of the cell cycle is determined by the accumulated number of critical ligand/receptor signals generated at the cell surface. Thus, it appears that in all cells regulated by mitogenic ligands and their receptors, the ligand/receptor signals ultimately are responsible for the timing of the onset of the assembly of the Pre-RCs at the *Oris*, and also for the timing of the initiation reactions that decide the onset of DNA replication.

Intracellular Sensors of Extracellular Signals

Somehow, knowledge of the critical number of triggered IL2/IL2R interactions that occur at the cell surface must be transferred to the inside of the cell in a reproducible, quantal fashion. From our calculation of a mean of 23,100 triggered IL2Rs necessary to surpass the R-point, and the fact that the mean number of IL2Rs expressed is only ~750 per cell, it is clear that the cell must continue to express the IL2R genes throughout G₁, and that the *serial* engagement of IL2Rs is necessary during G₁. It follows that there must be an intracellular sensor of the extracellular signals that receives the information of the number of triggered IL2Rs that have accumulated.

Detailed experiments have now shown that, in T cells, transcription of the gene encoding the G₁ cyclin D2 emanates directly the activation of STAT5 a/b by IL2-activated IL2Rs [26, 27]. Cyclin-D2 mRNA and protein appear first in early G₁, peaking after ~6 h of IL2 stimulation, followed by cyclin-D3, which shows a slower induction during G₁ to maximal levels as cells initiate DNA replication, and then remains high throughout S-phase [26]. The expression of both of these G₁ cyclins is essential for movement of the cell through the G₁ phase to reach the R-point and for the subsequent G₁-S-phase transition (*Transition to Replication*).

Accordingly, it has been postulated that the D cyclins serve as the *intracellular sensors* of the extracellular signals created by the IL2/IL2R interactions. Moreover, the G₁ R-point serves as the next intracellular quantal decision point. Thus, the question becomes how this intracellular quantal decision is made at the molecular level. The cyclin-dependent kinases (CDKs) are comprised of both regulatory (cyclin) and catalytic (kinase, cdk) subunits [28, 29]. Whereas the kinases (cdk4 and cdk6) that complex with the D-type cyclins are relatively long-lived proteins, the D cyclins are unstable, and their induction, synthesis and assembly with their catalytic partners all depend upon continuous signaling during G₁ via the IL2Rs.

Although it has not yet been demonstrated experimentally, the induction of cyclin D2 transcription by STAT5 is probably bi-allelic and quantal, such that the response elements (RE) of both cyclin D2 alleles are occupied by STAT5, and once signaling

begins, the transcription rate per allele remains constant as long as the STAT5-RE is occupied. Since there is a critical number of IL2-triggered IL2Rs that must be satisfied before cells will pass the R-point and make the quantal decision to begin DNA replication, this critical number of IL2-triggered IL2Rs must be eventually determined by the critical number of cyclin D2 & D3-cdk phosphorylation sites on the retinoblastoma (Rb) proteins, which bind to and block the transcription factors (designated E2F) regulating the key genes that control the expression of the molecules necessary for both nucleotide synthesis and DNA replication [30].

It has recently been shown that the D-type cyclins are important for replication of hematopoietic cells, in that the deletion of all three D cyclins or their catalytic partners, cdk 4/6, via genetic recombination results in embryonic lethality due primarily to severe anemia [31, 32]. However, the cells comprising the rest of the embryo apparently can replicate without the cyclin D/cdk complexes, and it appears that other extracellular ligands regulate cell cycle progression of the non-hematopoietic cells.

In IL2/IL2R-stimulated T cells, cyclin D2/3-cdk4/6 complexes phosphorylate Rb proteins throughout mid-late G₁, and not until all of the requisite Rb sites on the carboxy terminus of Rb have been phosphorylated can the next phase of signaling begin, i.e. the activation of cyclin E and cyclin A transcription. Thus, both cyclin E and cyclin A transcription is initiated after the cyclin D/cdk-mediated phosphorylation of the carboxy termini of the Rb proteins. Until recently, it was thought that the cyclin E, in combination with cdk2, initiates DNA replication, whereas subsequent activation of cyclin A and cyclin B-cdk1 complexes govern later events that terminate with mitosis. However, new data derived from cyclin E and cdk2 gene deletion studies indicate that the roles for these molecules may actually be solely mediated by cyclin A2-cdk1 [33]. Therefore, the kinase activity of cyclin A2/cdk1 may be the actual “spark” that completes the phosphorylation of the Rb proteins, thereby promoting their complete dissociation from the E2F proteins bound to DNA, which allows the transcription of all of the E2F-regulated cell cycle genes that are necessary for nucleotide synthesis and DNA replication. Alternatively, it may well be that cyclin E/cdk2 functions as the *intracellular sensor* for the extracellular signals that promote the proliferation of the non-hematopoietic cells, in that removal of cdk2 prevents cell cycle progression of MEF from cyclin D deficient cells [31].

Presumably, the dissociation of the cyclin/cdk-phosphorylated Rb from the E2F transcription factors and initiation of the transcription of all of the necessary E2F-regulated cell cycle genes constitutes the R-point. Once this checkpoint has been surpassed, the cell cannot revert to the pre-R-point G₁ state. It is irrevocably committed to proceed to S-phase. Moreover, it is this critical transcriptional activation of E2F-regulated genes that provides the gene products that comprise the Pre-RCs, as well as the gene products such as the DNA polymerases necessary for DNA strand duplication.

Even so, one is still left to explain how the cell decides to divide beyond the critical R-point. At each phase, the cell appears to be able to “count” the number

of triggered molecules, whether IL2Rs, phosphorylated Rb proteins, or assembled Pre-RCs, and this information is registered by a quantal decision once the critical requisite number of triggered molecules has accumulated. In situations where the IL2 concentration is low, or the IL2R density is low, the requisite number of IL2-triggered IL2Rs can still be registered, but the time required to reach this quantal decision point will be longer than if the intermolecular reaction had not been limiting. Presumably, exactly the same counting process occurs at each checkpoint during the progression through the various stages of the cell cycle where the information is transferred. Exactly how this counting process occurs is the question before us.

Other Examples of Quantal Decisions in Biology

It may well be that many, if not all, decisions in biology are made in a quantal manner. Developmental biologists speak of trying to understand how cell fate determinations are made [34, 35]. Thus far, they have identified cellular or soluble ligands that are responsible for directing some decisions, but they have not been able to analyze and quantify the cellular receptors for the various ligands identified at a single cell level, so that they have not come to realize that the quantal cell fate determinations occur as a consequence of the accumulation of a critical number of ligand-triggered receptors. Thus, they now understand that the cell fate determinations are ligand concentration-dependent, but they have not considered the role of the ligand-triggered receptor as the molecular vehicle for transferring the signal from an “analogue” concentration of ligand to a “digital” number of ligand-triggered receptors.

In the field of immunology a very convincing argument can be made for a “Quantal Theory” of how the immune system is able to discriminate between self and nonself, which is the basis for the phenomenon of tolerance (Smith, K.A. unpublished). A detailed series of experiments have accumulated over the past 20 years that indicate that the T Cell antigen Receptor (TCR) behaves in a fashion very similar to the IL2R. Thus, when activated by antigenic peptides, the TCR signals the activation of 3 distinct transcription factors which coordinately bind to REs in the IL2 gene promoter/enhancer, forming a macromolecular complex that activates IL2 gene transcription in a quantal fashion.

Studies have shown that when the TCR is activated maximally, the IL2 gene is expressed bi-allelically and at a constant rate, as long as the TCR continues to be triggered. As well, just as with the IL2R, the TCR must signal continuously for several hours before an adequate concentration of IL2 is generated that is then capable of binding to and activating the IL2R to initiate T cell proliferation, one of the hallmarks of antigen recognition and response. Therefore, the TCR also “counts” the number of triggered receptors that accumulate, thereby ensuring that the requisite number is attained, before the cell makes the decision to respond to the antigen. Another way of stating this is that the immune system does not recognize an antigen until the critical requisite numbers of TCRs have been triggered.

Conclusions

Molecular mechanisms have evolved so that cells are capable of making quantal responses to signals emanating from ligand/receptor interactions at the cell surface. In this manner, cells can regulate vital life processes with exquisitely high fidelity, so that errors are very infrequent. The molecular mechanisms that regulate the cellular decision to divide are perhaps among the most complicated, but also the most highly conserved and accurate examples known in biology today. Based on the knowledge that has accumulated thus far, it may be postulated that all fundamental biological decisions, such as survival, death, proliferation and differentiation are quantal. Deciphering how and why these decisions are determined at a molecular and atomic level is the task ahead. Clues as to these processes may be found in exploring the forces necessary to assemble, maintain and then disassemble macromolecular complexes of multiple proteins.

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