Cell Cycle Control and Paley's Watch

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ABSTRACT

In the past few years a revolution has occurred in our understanding of cell biology, caused by research in cell cycle control. This paper reviews the contemporary understanding of the cell cycle, showing that it is an eloquent verification of the Paley's watch argument. Paley argued that the design obvious in a watch is proof of a watchmaker, and likewise the design evident in the human body is proof of a creator. When proposed by Paley, his use of the classic design argument for God was somewhat premature. The knowledge of human physiology has vastly increased since Paley, and contemporary discoveries have eloquently confirmed his thesis. An excellent example is the extremely complex, intricate, highly co-ordinated cell cycle control system. This study reviews some of the various factors and mechanisms involved in controlling the cell cycle. Specifically examined are the cyclin-dependent kinase (Cdk) system, the ras protein, signal transduction pathways, p53 and other cell control repair systems, homeotic genes, protein degradation, especially the ubiquitination system, apoptosis, and control by the destruction of inhibiting proteins. These topics lie at the heart of understanding cell cycle control, and defects in any one of these systems can cause numerous problems. A major focus today is the relationship between cell cycle control and cancer.

INTRODUCTION

A classic proof for a Creator, and thus the creationist worldview, was provided by St Thomas Aquinas in what are now known as the cosmological and teleological arguments. All these proofs argue that the existence of a complex living structure demands an intelligent designer. Many contemporary scientists have concluded that this thesis, elaborated by Paley who compared the human organism with a pocket watch, has been refuted by recent research in biology. In actuality, this thesis has been eloquently supported by contemporary biological research showing that the evidence was actually comparatively weak in Paley's day.[1]

In the middle 1800s, Theodore Schwann and other naturalists wrote extensively about the then-scientific view that cells were simply structuralist substances which originated by the 'theory of free formation'.[2] In other words, cells were then considered unorganised substances that became organised through a process of spontaneous generation somewhat like what occurs when a drop of water falls: the individual drops rapidly become 'organised' into new small drops.

Scientists now realise that the cell is enormously more complex than the entire human body as understood in the early 1800s when Paley wrote. We also realise from the study of the cell cycle and signal transduction system that the cell's complexity is a closer parallel to the watch than first thought. The cell contains master clocks and a set of secondary clocks all of which are co-ordinated in a critical way in order for the cell to function adequately.

This review summarises some of the systems which are critical in controlling the cell's internal clock which in turn controls protein production and, ultimately, the various stages of the cell cycle system. These details reveal a level of intricacy, complexity and design that far surpasses the examples Paley used in his analogy. A major limitation of this paper is that only some of the highlights of the cell cycle can be reviewed briefly. A multi-volume work is required just to fill in the major details as presently understood — and researchers now recognise that they are just beginning to scratch the surface. When the book about the cell is completed, a score of volumes the size of the **Encyclopaedia Britannica** could not contain a complete

account of how it functions. The cell's communication system is of such complexity that the cell world is constantly humming

> *'with messages of incredible precision of such degree that we cannot help but marvel at the monitoring and self-correcting devices for detecting and rectifying errors . . .*[3]

THE CELL CYCLE

One complete cell division cycle, including both cell growth and cellular differentiation, is called a **growth cycle.** The growth cycle length varies enormously — from hours to years — according to the cell type, if it is normal or cancerous, and many other factors. Most cells must constantly divide to replace those that are diseased, damaged by trauma, old age or internal malfunction. The lining of the digestive tract is completely replaced in about three days, and bone marrow cells divide every two to three days to form new blood cells. At the other extreme, most neurons last the lifetime of the organism.

The first step in studying the growth cycle is to delineate the major normal growth cycle stages.[4] The length of each, called **generation time,** can vary enormously, but given a 24-hour growth cycle as a basis for comparison, an estimate of the duration of each part of the cycle is as follows:

(1) The **G-l period** (G meaning gap or growth) begins with the completion of the previous cycle and finishes with the start of DNA duplication. This stage involves the growth of the cell and synthesis of new proteins and enzymes to produce differentiation. To achieve this requires about 9.5 hours.

(2) The **S period** (S meaning *synthesis)* is the time during which synthesis of a complete new set of DNA and histones is completed, and requires about 10.5 hours. It usually is identified by incorporating tritium-labelled thymidine into the newly synthesised DNA.

(3) The **G-2 period,** the second gap or growth stage, is the time when the cell prepares to divide. The G-2 stage extends from the end of the completion of DNA synthesis to the beginning of the actual physical cell division or mitosis. The total time of this period is about three hours. The G-l, S and G-2 stages are together called *interphase.*

(4) The **M period** (M meaning *mitosis)* commences when the physical cell division occurs, resulting in two identical daughter cells (total time, one hour). This stage is divided into prophase, metaphase, anaphase, and telophase. Telophase is culminated by **cytokinesis,** or the physical cell division that results in two daughter cells.

Cells caused to stop at one stage in the cell cycle, such as those lacking in peptide growth factors, do not go beyond mitosis and are said to be in the *resting* or G-0 stage. These G-0 cells can be stimulated to re-enter the cycle by growth factors that are diffused or communicated to the inside from the outside of the cell membrane. Many factors can stop cell growth, including a large family of growth inhibitory agents and contact inhibition.[5]

Variations in the cell growth cycle can result in major changes in cell morphology and function. Significantly, cancer cells have greatly different cell cycles from the normal cells from which they originated. One major difference is that cancer cells have short G-1 growth stages; consequently they typically reproduce at a far faster rate compared to the normal cells around them. A short G-l time also impedes cell differentiation. The longer the G-l period, the more time the cell has to produce the needed structures for proper differentiation. Undifferentiated cells cannot carry out the functions of the normal cell from which they originated.[6]

A major cause of cancer is mutations of the genes that encode the components of cell cycle checkpoints which ensure both the orderly progression of cell development and growth, and also integrate DNA repair with cell cycle progression.[7] Most anti-cancer drugs work by interfering with cell growth, and many researchers believe that controlling cell cycle is the key to curing cancer, ideally affecting cancer cells but not normal cells.[8]

In order for a cell to move from one phase to the next, various cyclin proteins must activate one or more **cyclin-dependent kinases** (Cdks). Cdks consist of a cyclin regulatory subunit and a Cdk catalytic partner which are controlled by a wide variety of regulatory factors[9] (see Figure 1). **Cyclins** are a large family of proteins that are critical in

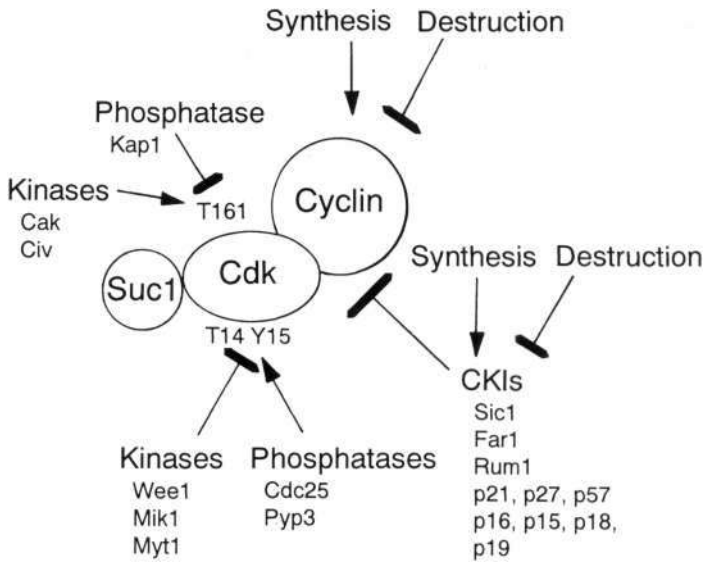

Figure 1. *Some factors involved in the regulation of cyclin-dependent-kinases (CDKs) which appear to be involved in all phases of control of the cell cycle. Arrows: activating events. Stopped lines: inhibitory events. Genes known to be involved are listed below. Cyclins and Cdk inhibitors (CKIs) are regulated by ubiquitin-directed proteolysis. This is only a partial picture of the systems.*

cell cycle control, and their levels oscillate enormously during the normal cell cycle.[10] Cyclins were discovered at Cambridge University in the early 1980s by Tim Hunter and his co-workers. They coined the term *cyclin* because it accumulated in large amounts during mitosis, then dropped precipitously soon after.[11] Mitotic cyclins are high during mitosis, and low during all other cell cycle stages. The term *cyclins* still refers to this protein family's rapid oscillation during the cell cycle.

Functional cyclins are destroyed very rapidly by highly specific proteases, rapidly lowering the cyclin level. Cyclins specifically activate a regulatory enzyme called a kinase that adds phosphate groups to other proteins. **Kinases** are enzymes which catalyse the transfer of a phosphate group from one substrate to another, commonly from ATP to the protein substance that is activated. The protein kinases usually transfer a phosphate group to a specific serine, threonine or tyrosine residue. This process, called **protein phosphorylation,** is one of the most important mechanisms by which extracellular signals cause biological responses in cells. The reverse of protein phosphorylation, **dephosphorylation,** is also a major mechanism. Both are used to control the cell cycle and other systems in eukaryotic cells.[12]

Cdk regulators directly or indirectly respond to feedback from numerous cellular checkpoints, including those that monitor the genome integrity, the mitotic spindle assembly and cell size.[13] Cdks are also controlled by **inhibitors,** and cells which are unable to produce the proper amount and type of cyclin-dependent kinase inhibitors are prone to neoplastic transformation.[14] All of the cyclin genes are normally transcribed only during the proper stage of each cell cycle, then mRNA is translated and the protein rapidly degraded so that each cyclin protein type exists only during one part of one stage of the cell cycle.[15]

THE SEARCH FOR THE MASTER REGULATOR

Cell cycle regulation involves a large number of controls. Hardly two dozen were known in the 1970s, but hundreds have now been identified.[16] A question that has been asked since the turn of the century is *'does the cell have a master regulator?'* In 1979 it was discovered that **Maturation Promoting Factor** (MPF), a complex which is present in all meiotic cells from yeast to humans, could induce G-2 arrested oocytes to move into the M-phase of the cell cycle. At the G-2 to M transition point, a cdk-cyclin maturation promoting factor complex induces mitosis by phosphorylating a specific set of structural and regulatory proteins. These include those that control the chromosomes' condensation, spindle assembly and nuclear breakdown so they occur at the proper time, place and extent.[17] While MPF could not be called a master regulator, it is the closest to this concept that researchers have yet found.

Other transitions in the cell cycle are controlled by a series of regulators which turn the cell cycle both on and off at various stages, including at the end of the G-l, S, G-2, and M stages, plus the stages existing in between these four major cell cycle divisions. Cell cycle progress can be stopped in response to conditions which include lack of essential nutrients, inadequate physical development for a particular stage, and even signals from nearby cells.

Speculation also exists, based on a number of studies, that an internal cell cycle clock exists which is independent of the events and processes that it controls.[18] Further, mutations can either prevent the cell from moving on to the next cycle or allow it to prematurely move to the next cycle. Consequently, cells may divide at smaller than normal sizes, or not divide when the normal size is reached, dividing either when the cell is considerably larger than ideal, or not at all. A key finding of past research on the effect of mutants is that there exists a clear *'underlying unity and order to cell cycle control'.*[19]

CELL CYCLE CONTROL MECHANISMS

Two major aspects of cell cycle control are the **alternation** and, secondly, the **completion** stages.[20] The alternation problem deals with understanding the mechanisms which ensure that all of the relevant processes are completed before the next appropriate process begins. This is achieved by **checkpoints,** development stages at which the cell cycle process is halted in order to evaluate or repair some condition. The system is somewhat like shutting down an assembly line to evaluate the quality of each item of concern according to a pre-existing quality control checklist, and not allowing the line to start again until all of the relevant points have been evaluated and approved.[21] If the chromosomes are not duplicated properly or are damaged, the next stage, mitosis, is blocked. If this checkpoint mechanism is defective, such as from a mutation causing a non-functional blocking protein, this stage will not be arrested. Consequently, the cell may enter mitosis with damaged chromosomes.

Many cells spend most of their life as haploids, and remain as such unless and until fertilisation occurs. This specific checkpoint varies according to the organism. For example, it occurs at the end of G-l for sea urchins, at the end of G-2 for clams, and at the last M stage, teleophase, for frogs. Fertilisation switches off the mechanism which arrests the cell cycle so as to allow progress to the next cycle. This control is due to the large transient amounts of calcium contained in the fertilising sperm. This finding reveals that cell cycle control is not only by protein structures, primarily hormones, but also minerals.[22] Evidently this specific control signal is universal in Animalia. Consequently, similar structural mechanisms must be utilised in all Animalia in order for calcium to function as the means to override the checkpoint, at least at the end of G-l, G-2 and M stages in the cell cycle, depending on the organism.

Understanding the cell cycle requires an extensive

knowledge of the many elaborate chains of command existing both inside and outside the cell. Hormonal signalling to all body cells, both local and distant, is achieved by the endocrine system and is called **long-range.** Conversely, **local** or **short-range** signalling is accomplished by the **paracrine system,** often called **autocrine** communication. This control is achieved by the chemical properties of the hormone which do not allow local hormones to travel very far without being destroyed by the blood. Extracellular commands are carried by hormones and prostaglandins to the specific cell receptors located on the cells' membrane surface. If the correct receptors exist on the cell, a series of proteins in the cell will carry the signal to the cell's nucleus.

Proteins convey information by their physical shape and their chemical charge pattern.[23] A major means of activating or inactivating proteins is to *change* their conformation (physical shape) by adding or removing a specific phosphate group, usually one on the protein's surface. For membrane bound proteins, the phosphate is added to the cytoplasmic side. Specialised kinase enzymes add these phosphate tags, a process often compared to an 'on and off' switch. The kinase functions as a messenger which conveys a signal by catalysing the addition of a phosphate group onto the next member of the series.

The 'off' switch is the dephosphorylation of the protein. This is achieved by protein phosphatases that remove the phosphate, thereby restoring the particular protein phosphorylation system to its basal stage. Most protein kinases possess a 30 to 32 kD catalytic domain that is very similar throughout the animal kingdom. Protein phosphorylation mediated by protein kinases is one of the most common means of controlling enzyme activity and the functions of other proteins such as hormones. It also controls the many stop and start points in the cell cycle. Probably the next most common cell cycle control factors are temperature and nutrient deficiencies.

Cell cycle control involves **sensors** that evaluate a specific condition, and **effecters** which respond to the information obtained by the sensors. This system is well known at a coarse level, and includes sodium chloride, water, and calcium level sensors. Hunt has concluded that all cell sensor-effecter systems use protein kinases in order to effect regulatory protein phosphorylation.[24] A secondary effecter system uses modifications of the electrical properties of the membrane to open or close ion channels as a means of control. There are a large number of phosphorylating enzymes that are specific to the proteins that they phosphorylate. Also, there are numerous kinds of ionic channels which differ in function and type of ions that they respond to. Some effecters cannot operate unless primed by another receptor — for 'y' to operate, x must first be stimulated to prime 'y'.

A major focus in current cell cycle research is on understanding the steps in the command chain that run from the sensor to the effecter. Problems at this level have been implicated as a major factor in loss of cell cycle control and thus cancer. This method is used both to initiate and amplify a signal. A number of steps in the chain vary, but they are generally so numerous that all of the steps needed to send biochemical messages to penetrate the cell membrane and reach the nucleus are called a *map.*

Most cell cycle research has been completed on yeast, revealing an intricate, sophisticated system. In Hunt's words,

> *'an idea of the complexity in higher eukaryotic cells is hinted at by the list of cydins: Al, A2, Bl, B2, B3, C, Dl, D2, D3, E, F, and G and an equally long list of Cdc2-like protein kinase subunits.'*[25]

And the list has increased greatly in the decade since then. However, similar principles appear to operate in all cells.

Both the cyclin and protein kinases are encoded by DNA, and consequently maintaining both translational and transcriptional control are critical to achieve cell cycle regulation.[26]

Secondary control is achieved by modifying the activity of existing enzymes and proteins by reversible protein phosphorylation and non-reversible specific proteolysis. A feedback system is necessary to initiate both translation and transcription at the proper time so as to control the amount and type of regulatory proteins produced. The specific proteins must be produced at the proper time so that the cell can complete the preparation necessary to enter the next stage at the appropriate time. Furthermore, the cyclins must accumulate at the correct cell cycle stage and in the necessary amount in order to move the cell through the next cycle at the proper time. Cell cycle stages are broad divisions, and within each stage exist numerous steps, all of which must be individually controlled. The sophistication of this enormously complex system reveals that a human-made watch is crude and simple by comparison, and the cell eloquently speaks to design far more than our knowledge of the human body in the late 1700s when Paley wrote.

THE RAS PROTEIN AND SIGNAL TRANSDUCTION

A critical first step of many message chains involves a protein called **Ras** that contains 188 or 189 amino acid residues.[27] The Ras super family consists of about 50 proteins, most of which are very similar in structure and differ primarily in a region consisting of 20 amino acids near the carboxyl end. They help to control processes ranging from the structure of the cell's internal skeleton to its chemical secretion system. Day concludes that Ras is *'integrated into almost every aspect of the cells behaviour'.*[28]

Since the Ras family is quite similar in organisms ranging from brewers' yeast to mice, the protein type is fundamental to eukaryotic life. The viral form of Ras affects both protein synthesis and the cell cycle in infected cells.

Not until late 1980 was it discovered how viruses cause the command chain to send out false information and consequently influence the development of carcinoma.[29]

Early research indicated that Ras forms only one step in a complex chain which passes information from the receptors on the outside of the cell membrane to the nucleus to switch genes either on or off in order to initiate or terminate the production of a specific protein. Disabling Ras impedes or blocks the information from entering the cell's nucleus.

A series of protein links controlled by phosphate molecules and kinases, act in sequence to cause a phosphate group to bond to the next member in the series. The normal inactive state Ras protein is guanine diphosphate (GDP) bound, and the active state is guanine triphosphate (GTP) bound. Flipping the Ras switch initiates the kinase message chain, eventually causing the information to reach the cell's DNA.

This system is called a **signal transduction pathway** from the word **transducer,** meaning a device which converts one form of energy into another. Signal transduction is a process of moving biological information within the cell, such as from a cell membrane receptor to the cell nucleus. Ras is one of possibly hundreds of signal pathways used in the cell, and many more are discovered each year. However, the *'total mass of certain signals in the body is hardly more than a billionth of a gram'.*[30] To identify many of these took decades of work and cost many millions of dollars.

Jamming the Ras protein in the off state by the use of antibodies influences a number of changes in the cell, including causing the cell to take on a physically bloated appearance.[31]

Ras is usually initiated via one of the many hormone receptors which protrude from the cell membrane's outer surface. When the epidermal growth factor hormone docks into a membrane's receptor, this stimulates it, causing the receptor's tail to be able to bond to phosphate groups, a condition called a *sticky tail.* This change then initiates a reaction between the **activator protein** in the cell cytoplasm and the Ras protein, turning Ras on.

This step in turn initiates another protein called *Raf,* which is also normally free-floating in the cell cytoplasm. *Raf then* triggers the 'go-between' kinases by binding to them, a step which eventually activates the proteins that control gene transcription. The end result is the production of new protein.

The protein phosphorylation and dephosphorylation system is commonly used for signal transduction to control numerous cellular processes. The target proteins are typically phosphorylated at a specific tyrosine, threonine or serine site by protein kinases. To reset the system, the phosphate group must be removed by a specific protein phosphatase. Protein phosphatases are now classified according to their substrate specificity, their dependency on metal ions, and even their sensitivity to an inhibitory agent.[32]

Most phosphatases require metal ions. A major exception is the protein **phosphatase PP1,** which is found in both cytosol myofibrils and the nucleus and helps to regulate the G and M targeting subunits. Both protein kinases and phosphatases are in turn controlled by other regulatory systems. The complexity of the system is illustrated by the fact that over 40 protein tyrosine phosphatases have already been characterised, and at least as many or more undiscovered ones probably exist. Each one possesses a similar 230 amino acid catalytic domain in addition to other domains that are probably essential for regulation of enzymatic activity and subcellular localisation.

Evidently many of the estimated 200 kinds of receptors on the cell surface are able to initiate the Ras chain reaction. This includes nerve growth factor and human growth hormone receptors. The human growth hormone increases the rate of cell division, and nerve growth factor causes the differentiation of undifferentiated cells into nerve cells. Because Ras is involved in both cases, a mechanism must exist to distinguish between human growth hormone and nerve growth factor. Possible mechanisms include the length of activation time: nerve growth factor may cause *sustained* Ras activation, and human growth factor transient activitation.[33] Others speculate that the effect of a hormone on Ras depends upon the cell *type* and its specific *development stage* when triggered.[34]

Mutations in the human Ras gene contribute to, or are involved in, about a third of all carcinomas. Since Ras protein genes are in virtually all organisms, it is assumed that Ras must be a critical protein type essential for normal function.

In the late 1980s it was experimentally determined that the Ras protein influences **G protein** production, a guanine nucleotide binding protein, named such because it links up to guanine as one step in the cell's internal signalling system. G proteins are located in the cell membrane, and are initiated by a structural change to carry a message via the command chain to the inside of the cell. Specifically, guanine triphosphate (GTP) binds to this protein to carry the signal.

Seven G Protein types have been identified, all having three distinct protein subunits and molecular weights of about 100,000. The guanine chains cause the production of a second enzyme messenger, another system which functions as a regulatory system. Both mutated Ras proteins and interruption of the G control system can disrupt control to cause rapidly dividing cancer cell reproduction.

Ras proteins have been found recently to have a more complex role in controlling cell behaviour. They are now known to function not only as part of a switch to trigger cell division, but also are critical in coordinating differentiation in a wide variety of cells. Ras can be initiated by a **signalling protein** produced by a nearby cell, conveying information by the paracrine system. A mutation producing a faulty Ras protein which is then inappropriately turned on causes problems. The focus now is on determining the details about specifically *how* this on and off switching

system functions — a critical concern, because cancer cells not only lose the cellular control that exists in normal cells, but also do not differentiate. Evidently Ras is involved in differentiation at ***all levels,*** even in gross body development, ensuring that the limbs, brain, eyes, and other structures all develop in the appropriate body location. Other Ras-related proteins, specifically *Rho* and *Rac,* regulate the development of cytoskeleton organisation, and yet others (including *Ral* and *Rap)* have functions that are still being determined.[35]

METHODS OF CONTROLLING THE CELL CYCLE TO CONTROL CANCER

Since cancer often involves mutations which jam Ras in the 'on' position (guanine triphosphate bonded), suggested treatments include injecting tumours with a virus that carries an anti-sense Ras gene to block the uncontrolled cell division and possibly the other effects of cancer. Another possibility is to chemically inactivate Ras without adversely effecting the cell in other ways.

The drug limonene blocks the Ras system at the outer membrane level, consequently inhibiting tumour growth. Conversely, limonene may also act by preventing the cell from attaching a **molecular address** to newly created Ras proteins, preventing them from being located properly to function as receptor sites. (After proteins are produced on the endoplasmic reticulum, a molecular address is attached which facilitates the proteins travelling to where they are needed.[36] These labels generally consist of sugars and other relatively simple molecules.[37] They are attached to the compound and are recognised by a receptor on the structure for which it is intended.)

The cell cycle can also be modulated using protein phosphatase inhibitors such as nodularin (a cyclic peptide), okadaic acid (a ionophore-like polyether derivative of a C38 fatty acid compound), cantharidin (a terpenoid), and calyculin A (a phosphorylated polyketide). Use of protein phosphatase inhibitors to block the cell cycle, though, is fraught with difficulties because many of these inhibitors have multiple actions, and while achieving the desired result may also have unfavourable side-effects. For example, suramin sodium salt interferes with the action of G proteins by blocking their interaction with intracellular receptor domains, consequently inhibiting the cell surface binding of the growth factor.[39]

All cells also have **autoreceptors** which are sensitive to the signal that the cell itself produces. These provide immediate feedback, thus serving as a self-regulation system, increasing the signal supply if too small and reducing it if too high. If the cell's autoreceptors are inhibited, the cell usually transmits an excessive number of signals.

CYCLIN-DEPENDENT KINASES

A critical means of regulating the cell cycle is by controlling the transfer of chemical energy to proteins. This control is somewhat akin to regulating an electrical appliance by controlling its power with an on-and-off switch. A large class of enzymes which transfers chemical energy to cellular proteins is the **cyclin-dependent kinases** (Cdks). They all function by activating various cyclin-dependent kinase proteins (see Figure 1). The mechanisms which cyclins use to control the cell cycle are complex, but have recently been partly delineated.[39] The critical role that cyclin plays in the cell cycle was first indicated by the discovery of a correlation between the rise and fall of cyclin concentration and the transition from the cell growth stage to the DNA replication stage. A cyclin-dependent kinase complex called **cyclin A-Cdk2** controls DNA replication. Defects in the cyclin A-Cdk2 gene are correlated to cancer, most notably thyroid cancer.[40]

Understanding the cyclin A-Cdk2 complex is important, because it is a potential model for other cyclin-dependent kinases. Specifically, the Cdk2 contains an amino acid T-loop which blocks the active site. The cyclin A operates to open the T-loop, which is the first step required to allow the enzyme to become active. It initiates this by binding to the enzyme, causing the T-loop to move about 2 nanometres. A part known as the 'PSTAIRE helix' is then caused to rotate 90°, allowing the amino acids in the active site to assume the conformation required to bind proteins.[41]

The PSTAIRE region is a highly conserved domain of 16 amino acids which determines specificity, allowing only certain cyclins to bind to its Cdk.[42] Analysis of the cyclin A-Cdk2 crystal structure indicates that cyclin binds primarily to one amino terminal lobe of Cdk. Especially prominent in this binding are two cyclin α-helices which clamp to the middle of the PSTAIRE helix by hydrophobic interactions, and to either end of the PSTAIRE by hydrogen bonds.[43] This causes the 90° rotation which effects changes in the amino-terminal loop packing and results in melting the a L12 helix and reorientation of the PSTAIRE helix. This moves a side chain of the catalytic residue GLU-51 into the active site.

The specific change in conformation which facilitates the enzyme being able to carry out its catalytic function is a modification of the ATP-binding site, so that the ß-y phosphate bonds on the ATP are now in a position which is favourable to transfer energy. This system appears to function as a double lock, that is, a gate must be removed and the active site conformation must be altered for the enzyme to become active. Before movement, the active site shape makes it difficult for proteins to bind, and after movement binding becomes very effective.

HOMEOTIC GENES

Homeotic genes or **homeogenes** are critical in controlling the cell cycle and differentiation of almost every part of an organism. They establish the specific developmental pathway by which an embryonic segment is

directed to develop a distinct adult phenotype. The homeotic gene family consists of several hundred genes which determine the general plan for the organism's major morphological subdivisions - the head, limbs, thorax, and abdomen. Research has found that the ***same genes*** associated with the formations of fins in fish also develop paws in mammals. A mutation in a homeotic gene can cause an appendage to develop into the homologous appendage of a ***different*** segment.

One homeotic gene called a **homeobox** consists of a highly conserved sequence of 180 base pairs that codes for about 60 amino acids called the **homeodomain.** All homeodomain proteins are either transcription factors involving specific DNA sequence recognition and binding, or proteins that influence other transcription factors. The identification of homeotic and homeobox genes derives from observations about the results of mutations in these genes. So far, hundreds of animal genes have been found to have a homeobox function, which is to turn off or on large numbers of genes. Many are also homeotic in function, and it is these genes which are critical for cellular differentiation. So far in humans only 38 out of the estimated 100,000 genes have been confirmed to produce proteins that have a homeotic function.

The *Hox* genes, all of which determine position identity along the body axis of a wide range of animals, consist of a cluster of at least nine tightly linked genes with the same transcription orientation.[44] If a single *Hox* gene is excised from the embryo, the part it controls does not develop normally, but develops like the section closest to it. The homeobox protein enables the *Hox* proteins to bind to specific areas of the DNA molecule involved in initiation or control of mRNA transcription, and consequently protein production.

The *Hox* gene family — of which 39 members are known in mammals — is most critical at the time of development when the vast majority of differentiation occurs, namely during embryonic development. The *Hox* gene is virtually identical in all vertebrates and controls the development of all major structures. The *Hox* protein seems to initiate differentiation according to a temporal sequence. The *Hox* genes are clustered in groups of eight to 11, and are scattered on different chromosomes. As with the cell cycle, the timing of the gene activation is critical — if the gene is initiated or switched off too early or too late, major structural deformities can result. Conversely, control of structural development can also be achieved by timing the switching of the *Hox* gene on and off. If the 'on' timing is fast, an animal with a short neck, for example, will result. If it is slow, an animal with a longer neck, such as a giraffe or a swan, will be produced.

Right and left asymmetry is also critical in development because the two halves of an organism are not necessarily identical. The liver is on the right side and the heart tilted to the left in a human, for example. Reverse organ development often causes cardiac ailments and other major health problems. At least three proteins are known to control left-right orientation of development. These proteins appear very early in embryo development, one on the right side, and the other two on the left of the embryo's chest.[45]

Early research revealed that the difference, for example, between a complex butterfly wing and a simple shoefly flapper, is not because the more developed structure necessarily has more genes, but an important factor is that the same genes are initiated in *different* ***patterns.*** This would argue against a hierarchy, from the morphologically similar fruit-fly to the apparently more complex butterfly — differences arise because of different initiation patterns of the genes.

PROTEIN DEGRADATION

Another critical aspect of cell cycle control is **protein degradation.** Now that many of the basic mechanisms of cell cycle initiation, cyclin-dependent kinases and the signal transduction system are understood, a current major focus of research is to understand the protein degradation system. Proteolysis was first determined to be critical in the early 1980s, especially due to the work of Tim Hunter of Cambridge.[46] The cell cycle, as is true of all systems which require the initiation of each process in a series, also requires that each step reach a termination point before the next step begins. In the cell cycle, this termination point is achieved by the regulation of the regulatory proteins, which usually involves termination of the production of certain proteins and the destruction of the remaining ones (see Figure 2).

For example, the protein known as p40Sicl (Sic 1) must be degraded in order for its Cdk partner to be activated and consequently for DNA replication to be initiated. Pagano *et al.*[47] also found that the mammalian Cdk inhibitor p27 is degraded via what is known as the ubiquitin pathway. Many other examples exist of the destruction of inhibitor protein by ubiquitin to achieve cell cycle control. Proteolysis must occur at *every* cell cycle transition point, and is often regulated by the ubiquitin system.[48]

Ubiquitin is a small polypeptide consisting of 76 amino acids that is found in all eukaryotes.[49] Its main role is to tag protein for degradation and recycling. It binds covalently to its target by a process called **ubiquitination** or **ubiquitinylation.** It is the main, but not the only, pathway for protein degradation. Ubiquitin is found either free in the cytosol, or covalently bonded to protein. Ubiquitin regulates the destruction of a wide variety of proteins including not only cyclins but also kinase inhibitors, and even the putative chromosome-tether proteins.[50]

Also involved is **proteasome,** a nonlysosomal multicatalytic proteinase complex made up of 15-20 proteins found throughout the cytosol in all eukaryotic cells. They associate with other proteins to form a complex called 26S **proteinase** which catalyses ubiquitin-dependent protein degradation. They have broad substrate specificity and

require ATP for energy.

The three major cellular proteolysis systems are:

(1) the ubiquitin system,
(2) the lysosomal and vascular systems, and
(3) physiological and pathophysiological cellular proteolysis.[51]

Lysosomes (Greek for *loosening bodies)* are spherical vesicles that contain digestive enzymes that break down unwanted cellular debris and bacteria. They fuse with the identified materials, then incorporate them inside their sack in order to break them down.

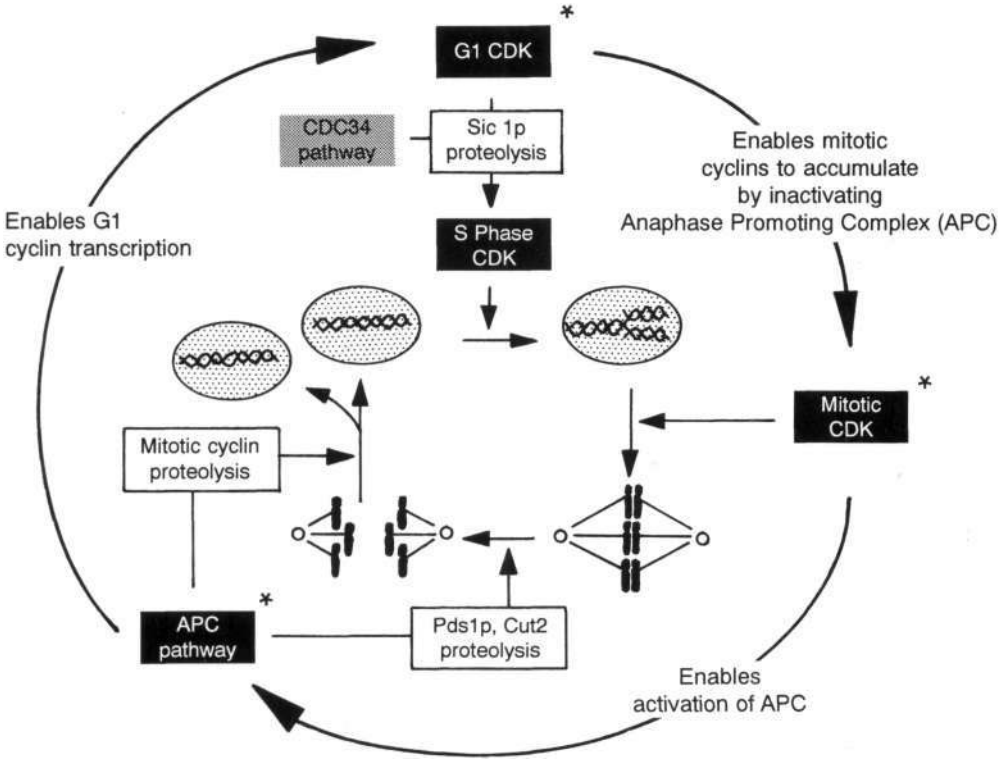

Figure 2. *The basic role of proteolysis in a composite eukaryotic cell cycle. The chromosome cycle is shown in the middle with interphase nuclei above and mitotic (metaphase and anaphase) arrangements below. Regulatory states (*) — each regulatory state has two functions: to stimulate a chromosomal event such as replication (above), alignment (right), or segregation (bottom), and to initiate the transition to the next regulatory state (curved arrows around the outside). The anaphase inhibitors Pdsp1p and Cut2 are ubiquinated to undergo proteolysis. APC: anaphase promoting complex.*

The rash of papers on the ubiquitin-triggered protein destruction system indicates that proteolysis is responsible for turning on and off the proteins that play critical roles in cell cycle regulation. A malfunction of this system blocks cell cycle progress, and is probably involved in cancer cell growth. Evaluation of tumor cell lines has revealed that abnormal protein destruction, as well as both premature and delayed protein destruction, may both be critical. Researchers may argue about which step is more important but, like a mechanical watch, scores of parts are all critical: even if the smallest gear was removed, it would render the watch non-functional.

Kirchner[52] discovered that the ubiquitin-mediated proteolysis system requires an attachment point to tag cyclins for destruction. He has identified a specific 9-amino-acid sequence recognition site which he found is necessary for cyclin destruction. When ubiquitin binds there, it functions as a marker, tagging the protein it binds with for destruction by the proteolytic system. Ubiquitin thus serves not only as a clean-up crew, tagging damaged, malformed cell proteins or cellular debris for destruction, but as an essential part of the cell cycle control system.[53]

By artificially modifying the recognition site on cyclin, researchers have found that ubiquitin is no longer able to control cyclin, and consequently, cells cannot move on to the next part of the cell cycle.[54] Inactivation of cyclin-dependent kinases is necessary for the cell to move out of mitosis, for example, and modification of the critical area of cyclin prevents ubiquitin from inactivating it. As a result, the cell is prevented from moving on to the next cell cycle stage.

Ubiquitin has also been found to control mitosis by regulating the cyclins that activate kinases in other parts of the cell cycle, such as the S phase (the DNA replication stage). After ubiquitin tags a specific cyclin, it is abruptly degraded, a process that is timed to occur when the cell begins to copy its DNA. Regulation thus occurs during the entire cell cycle, and not just at the end of one cell-cycle stage.[55,56]

A complex timing system is also critical in the symphony of the numerous complex biomolecules that are required for the cell cycle system to function. An important step in cell cycle research is to locate and understand the function of the master clock. One clock probably triggers a cascade of others, and each one is dependent upon the previous set to function. Research on the proteolytic system has not yet revealed the existence of a single master clock.

DESTRUCTION OF INHIBITING PROTEINS

Protein destruction can be stopped by inhibitory proteins that bind to, for example, Cdks, and protein production can be initiated by the destruction of inhibitory proteins. Cdks can also be caused to go into remission by inhibitory

proteins, and proteolytic destruction of the inhibitory proteins allows Cdks to function. With one exception, all known mammalian cell cycle protein levels correlate positively with mRNA levels. The only exception is p27 which declines in the presence of constant amounts of mRNA. These correlations are all critical in understanding the complex role of the many proteins and compounds involved in the cell cycle.[57] Protein p27 is a cyclin-dependent kinase inhibitor that exists at peak levels during the G-0 and G-l stages (quiescence and pre-replicative phases) of the mammalian cell cycle. As the cell cycle is entered, the level of p27 decreases.

Ubiquitin also controls the destruction of most other proteins necessary for cell cycle regulation. Indications also exist that, at least during certain cell cycle stages, other proteins in addition to cyclin must also be destroyed before the next cell cycle step can occur. One of these proteins has been labelled a 'putative chromosome-tether protein'.[58] The discovery of these new mechanisms raises important questions, including: What is the specific mechanism that orchestrates all of these control aspects of cell cycle?

The process of adding ubiquitins to proteins to label them for destruction involves a 'bucket brigade' which passes ubiquitin along a series of enzymes, called E1, E2 and E3, and then finally to the target protein. So far, only a single El type has been discovered, although a variety of E2 and E3 enzymes have been found. The many steps that exist in this mechanism likely serve the important function of *control.* Other factors are indicated in degrading proteins at the specific time necessary in the cell cycle. Since E2s are active throughout the cell cycle, researchers have pin-pointed a novel E3 as having the needed temporal specificity to control the cell cycle. This E3 contrasts with the others in that it is a larger unit, probably a complex of many separate proteins.[59] Its importance in orchestrating the next stage of the cell cycle at the proper time is also indicated by the fact that it becomes active for a short period in the middle of mitosis and then is rapidly inactivated.

The E3 complex has been determined to consist of a complex of at least three proteins — Cdcl6, Cdc23 and Cse 1. Exactly how these necessary complexes function to allow the continuation of mitosis is unknown. Other researchers have found that Cdcl6, Cdc23 and Cdc27 proteins form a complex which serves a regulatory role, specifically during the meiotic stage of the cell cycle.[60] Further research has found that the Cdcl6 and 27, and probably also Cdc23, are directly involved as components of the E3 complex.[61] It is likely that the Cdc 16-23-27 protein complexes are all involved in cyclin-degradation. It has been suggested that the E3 complex functions to ensure that *'all the chromosomes are arranged on the spindle before giving the signal to start anaphase.'*[62]

CO-ORDINATION OF THE CELL CYCLE

The last concern that must be considered in understanding the cell cycle is a system which will co-ordinate the numerous systems delineated above. One candidate is the 14-3-3 family of proteins.[63] The 14-3-3 protein family was named according to its electrophoretic mobilities, and has been discovered to consist of at least five major mammalian forms and 10 others in non-mammals. Research has implicated this family in many diverse functions, including a critical role in signal transduction pathways, cell cycle regulation, exocytosis and binding to oncogene and proto-oncogene products.[64] The 14-3-3 protein is a good candidate to serve the role of a major co-ordinator of multiple signal pathways, because 14-3-3 binds to numerous kinases to provide a mechanism by which the multiple signal transduction pathways can be simultaneously co-ordinated.

A pathway co-ordination system serves as a switchboard, sending the appropriate messages to the appropriate system in the cell cycle at the proper time. Some evidence also exists that 14-3-3 proteins act independently as signal transduction pathway mediators, controlling the association of various kinases, indicating a higher level of control function. Role co-ordination is also indicated by the evidence which shows that the 14-3-3 structure allows two kinases to bind simultaneously to it. Xiao *et al.*[65] speculate that this structure also provides a scaffold on which other proteins can interact.

Liu *et al.*[66] have identified a bundle of nine antiparallel helices that forms a palisade around an amphipathic (a molecule having both hydrophilic and hydrophobic groups, such as wetting agents, and membrane lipids, notably phosphoglycerides) groove which is large enough to accommodate a tenth helix. They propose that binding to an amphipathic helix allows 14-3-3 to interact with a diverse variety of cellular proteins. The dimer interface residues and the putative ligand-binding surfaces have been discovered to be invariant among vertebrates, yeast and plants, suggesting that they are critical structures and have a similar role in a wide variety of life. In support of this, Liu *et al.*[61] found that a yeast 14-3-3 protein was able to activate mammalian RAF-mediated signalling.

Another central unit which co-ordinates the cell cycle and other activities is the hydroxyl group at position 3 on the inositol ring of phosphoinositides which, in the words of Downward is *'switched on by a huge number of extracellular stimuli'*, thus functioning as a key control mechanism.[68] One of these compounds involves the lipid kinase phosphatidylinositol-3-OH kinase (PI(3)K) which phosphorylates the hydroxyl groups at position 3. At least two other protein kinase activities may also be regulated by phosphatidylinositol-3-OH kinase, including the protein kinase C family and p70s6k, a ribosomal protein S6 kinase which is activated in response to numerous mitogens (compounds that stimulate cells to undergo mitosis).

Arguing against a central role for any one signal transduction protein is the fact that a mutation of this protein would be lethal to the cell, and that several transduction

signal pathways exist which could be compared to a telephone system in a large nation. If a line in a certain area fails, alternate routes can be used to ensure that downtime is minimised. Conversely, as in a phone line system, some lines are more critical than others, although alternate lines may exist. In the case of cells, this may allow a cell to avoid depending on any single signal system.

Another example of the inner complexity of the kinase signal transduction system is **protein kinase B** (PKB), a serine/threonine kinase named for its sequence analogy to both protein kinases A and C.[69] PKB is activated by the range of stimuli which includes insulin, platelet-derived growth factor, epidermal growth factor, basic fibroblast growth factor and others. Conversely, protein kinase B is inhibited by the phosphatidylinositol-3-OH kinase inhibitor wortmannin. Researchers have also identified protein kinase B as a critical element of the growth factor-induced signal transduction pathway, and also the phosphatidylinositol-3-OH kinase as a possible oncogenic conversion site. The co-ordination of the cell cycle and the events which relate to it probably involves thousands of structures, and signal transduction pathways in normal cells may be many times as complex as this, as appears evident from the studies reported here.[70,71]

CONCLUSIONS

Detailed comparisons between the watch and the cell cycle reveal that both have an intricate structure which must work in complete harmony, posing a major problem for evolution. How could the system evolve and yet be highly functional so that the animal can survive and successfully compete with other animals while it is evolving? If the system is functional as in bacteria, what need is there for it to evolve into a more complex one? Understanding the cell cycle has revealed that it is similar in all eukaryotes and prokaryotes, except that it is more streamlined in the latter. The average *E. coli* bacterium contains over a million base pairs, revealing a complexity in this so-called simplest form of life that is staggering.[72] Humans have over 3 billion base pairs, revealing a complexity that could be as much as 3,000 times greater.

An appropriate analogy is that the difference between the inanimate structures naturally found in the soil and a bacterium is equivalent to an abacus in comparison to an IBM main-frame computer. Conversely, the difference between a bacterium and a human being is somewhat comparable to the difference between an IBM main-frame and several Cray supercomputers. The same principles and many components are utilised in both.

Evolutionists assume that DNA evolved, thus it once existed as crude structures in so-called primitive organisms, and later as more finely tuned structures in the so-called more advanced organisms. Clearly though, as noted by Guttenplan and Oratz, *'there is not always a relationship between the complexity of the organism and the size of the DNA'*[73] although generally the amount of genomic DNA is much greater in higher organisms. Whereas a bacterium codes for about 2,000 proteins, a human being can manufacture about 100,000, which is a huge increase in complexity/information. This analogy is useful, but limited: a human being is not just a more complex bacterium. A human is much more than that, as extensive and intensive research is now revealing daily.

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