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# The Clinical and Translational Implications of Evolutionarily Preserved Intracellular Signalling Pathways

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## Authors' contributions

*This work was carried out in collaboration between both authors. Author GSM designed and wrote the search protocol. Authors GSM and AB conducted the systematic search and interpretation and both authors produced the final manuscript.*

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## ABSTRACT

Despite the range of cell types found in the animal kingdom, only a limited number of signalling pathways are required to generate them. Furthermore the basic components and design of these signalling pathways remain largely homologous across the animal kingdom despite species being separated evolutionarily from one another for millions of years. This article explores the fundamental signalling pathways that have been evolutionarily conserved in the midst of millions of years of selection and environmental pressures. Knowledge of the development of these pathways may aid us in understanding various human disease processes and help to develop possible therapeutic targets. The review explores the role of these intracellular pathways and what relevance they may have to the understanding of disease processes.

*Keywords: Darwinian evolution; signal transduction; embryological development; intracellular pathway.*

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## 1. INTRODUCTION

Despite the myriad cell types found within the animal kingdom, only a limited number of signalling pathways are required to generate them. Consequently, there is a surprising level of homogeneity on the intracellular signalling level amongst a vast array of heterogeneous species which have been evolutionarily isolated from one another for millions of years. Knowledge of the development of these pathways may aid us in understanding various human disease processes and pathologies and help in the development of potential therapeutic targets.

The evolution of these pathways starts with the beginning of life itself. Fig. 1 shows a simple phylogenetic tree. Prokaryotic cells may be considered the simplest, self-sufficient forms of life. They contain many primal intracellular signalling pathways. However somewhere along the early evolutionary tree, two prokaryotic cells developed the potential to work together. The underlying reason for their ability to work together was probably due to a mutation resulting in altered cell surface molecules allowing two cells to bind together. Nonetheless such a mutation was 'selected for' (meaning it had a greater likelihood for success in survival and proliferation) under the environmental selection pressures present at the time. This resulted in the preservation and propagation of this characteristic and is the foundation of Darwinian theory ('survival of the fittest') [1].

In terms of developmental pathways, there are several main pathways responsible for early cell-to-cell interactions. These pathways are *Wnt*, *JAK-STAT*, *TGF- $\beta$* , *Notch*, *Hedgehog* and

receptor Tyrosine Kinase [2]. Cell cycle regulation is another very important area where massive conservation of proteins and pathways has taken place. The aim of this article is to collate and integrate all published information on intracellular pathways and to describe the fundamental signalling pathways that have been conserved throughout much of the animal kingdom. Furthermore it aims to investigate why certain pathways have been conserved and what relevance they may have to the understanding of disease processes in humans.

## 2. METHODOLOGY

A two-step process utilising a Medline/PubMed systematic search was conducted. The initial search was undertaken using elementary phrases including "intracellular signalling", "signalling pathways" and "developmental pathways". Only the most recent literature in the field was required so the time-window for the literature review was restricted to the past 30 years (1983-2013).

The resultant abstracts were analysed and appropriate papers were selected. The secondary search was performed by (i) using the reference lists of the chosen articles and (ii) by using PubMed weblink for related articles. The studies were selected if they were in the English language and included the appropriate topics. The search produced over 4000 published papers on the topic of the intracellular signalling and developmental pathways. All of the reports regarding intracellular signal transduction and physiological cascades or mechanisms of action were selected.

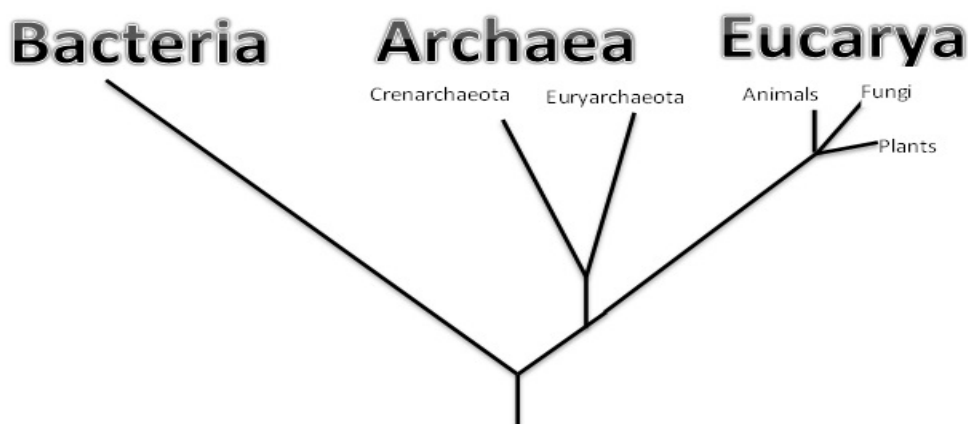


Fig. 1. A simplified phylogenetic tree

### 3. NOTCH SIGNALLING

The *Notch* pathway is a very important pathway in the regulation of genes and thus controls cell fate during development. All metazoans have this signalling pathway which is active at every stage of development. It functions at all stages of development in controlling differentiation, cell survival and proliferation. Thus, '*Notch*' gene in *Drosophila* is homologous to the *Glp-1* and *Lin-12* genes in *C. elegans*, *Xnotch* in *Xenopus*, *C-Notch-1* in *Gallus gallus domesticus* (Chicken) and *Notch1* and *Notch2* in mammals. In fact there are four different notch receptors in mammals. The *Notch* receptor is a transmembrane protein with an internal and external part. When a ligand (namely Delta and Serrate in *Drosophila*, and multiple Delta-like and Jagged ligands in mammals) binds to the external part, the internal part is proteolytically cleaved off and moves to the cell nucleus to affect the expression of various genes via the transcription factor *CBF-1* in mammals or *Su(H)* in *Drosophila*.

Defective *Notch* signalling is involved in many diseases and is often down-regulated in many cancers. Defective *Notch* signalling is involved in many diseases, such as Multiple Sclerosis, Tetralogy of Fallot, T-ALL (T-cell acute lymphoblastic leukaemia), Alagille syndrome, CADASIL (Cerebral Autosomal Dominant Arteriopathy with Sub-cortical Infarcts and Leukoencephalopathy) and many other diseases. By studying how this pathway evolved into its homologues in other species, it may provide valuable insights into these disease processes.

Experiments into illuminating point proteins along the transduction pathway in *Notch* signalling cascades may allow us to predict at what point defects in this pathway have resulted in each of these diseases processes. Such studies could allow therapeutic intervention to be focused and targeted to these specific points in the cascade. Thus there is much interest in elucidating the exact cascade mechanism in these developmental pathways. This interest has already led to the differential roles of the different notch receptors. It has been thought that the *Notch-1* may have a tumour-promoting role, whilst conversely, *Notch-2* may have a tumour-suppressive function in human breast cancer [3]. This suggests that suppression of *Notch-1* activity could present a novel therapeutic approach in treating breast-cancer. Notch may also control survival and cell fate decisions in

early T cells [4], hence having massive implications in deciphering the immune systems activities when dealing with infection.

### 4. HEDGEHOG SIGNALLING PATHWAYS

*Hedgehog* and *Wingless (Wnt)* pathways are involved in cell patterning in the development of the skeleton, nervous system, limbs, hair, lungs and gonads and are closely connected to one another as well as both being first discovered in *Drosophila*. *Hedgehog* binds to cholesterol to allow it to bind to a transmembrane protein called *Patched* on the cell surface. This reduces the inhibition of a receptor called *Smoothed* by *patched*. *Smoothed* then acts on a downstream transcription factor (*Ci* protein) which affects the transcription of various genes.

Defects in the regulation of hedgehog have been linked to small-cell lung cancer (SCLC) and pancreatic adenocarcinoma and studies into this developmental pathway have helped bring light on a potential intracellular molecular mechanism for these conditions[5]. This is a prime example of how such studies can aid our understanding of disease. More importantly however, the inhibition of *Hedgehog* in these cancer cells may provide novel treatment possibilities to some cancer types with synthetic HH blockers already synthesised [5,6].

### 5. THE JAK-STAT PATHWAY

In humans, the *JAK-STAT* signalling pathways play a vital role in principal cell fate decisions, regulation of the processes of hematopoiesis, differentiation, cell proliferation, and apoptosis. The pathway is the mechanism of transduction of a range of cytokines and growth factors. All of the known components of the mammalian *JAK/STAT* pathway have been uncovered by genetic analysis studies. Research carried out in a variety of animal models including *Drosophila melanogaster*, the fish *Danio rerio* and the nematode *Caenorhabditis elegans*, established the wide conservation of various *JAK-STAT* pathway components throughout the animal kingdom [7].

*Drosophila* in particular has been of particular significance, having homology with all of the components of the mammalian pathway. As a result, the majority of studies on *JAK/STAT* have focused on *Drosophila*. In *Drosophila*, there are 3 main ligands; *UPD*, *UPD2* and *UPD3* [8] in addition to the transmembrane receptor

*Domeless* (DOME) [9], a *JAK* kinase known as *Hopscotch* (HOP) and a transcription factor called *STAT92E* [10,11].

The *JAK/STAT* pathway is also involved in the downstream signalling of Epidermal Growth Factors. In *Drosophila*, *EGF-R* may promote cell survival by acting on ETS transcription factors (such as *prolactin*, *junB*, *cyclinD1*, *c-fos*, and *cdc2* [12]). *EGF-R* signals through the *Ras* pathway and hence functions to promote tumour cell survival after DNA damage such as radiation therapy [13]. This mechanism is a highly conserved signal transduction pathway responds to the ligands *Spitz* enhances cell survival and *Argos* antagonises *EGF-R* signalling, so therefore decreases cell survival factors [14]. It is chiefly due to these promotional attributes of the pathway that genetic changes that constitutively activate intracellular survival pathways often occur in cancer [15].

Even if homologs for each component of a particular pathway are conserved, it is not necessarily the case that the pathway will maintain the same function. However, many studies have provided evidence suggesting that the mechanism and function of the *JAK/STAT* pathway are conserved between vertebrate and drosophila, specifically in terms of cellular proliferation, the innate immune response and stem/germ cell development. By studying these proteins and their homologs between species, the evolutionary lineage may be mapped, including any mutations which have occurred, especially in terms of resultant disease processes. A good example of this is the *JAK2* mutation which has only recently been shown to be responsible for human polycythemia vera which produces a leukaemia-like haemocyte over proliferation in *Drosophila* [16,11]. Thus, further studies into the similarities between invertebrates such as *Drosophila* and vertebrates can help unveil the regulation of this pathway possibly revealing novel therapeutic targets.

## 6. WNT SIGNALLING PATHWAY

*Wnt* proteins play an important function in embryonic induction, the generation of cell polarity, the specification of cell fate, identification of cell-surface receptors and the mechanism of relaying extracellular signals to the cell nucleus. A number of studies have shown the presence of components of the *Wnt* signalling pathway in various organisms. *Wnt* signalling is implicated in early axis determination

in many organisms with very complex downstream signalling components. It involves a substantial number of different proteins which act as signalling molecules or complex regulatory elements in the pathway. This is in addition to the variety of *Wnt* extracellular ligands initiating these physiological processes.

*Wnt* signalling is complex and incompletely understood. A detailed exploration of *Wnt* signalling is considerably beyond the scope of this article. However it should be pointed out here that several components of *Wnt* signalling are implicated not only in the genesis of human cancer but also in the regulation of cancer progression. These downstream effects range from metastasis to tumour growth and cell death [17]. The canonical pathway is a subset of *Wnt* signalling and is mainly regulated at the level of  $\beta$ -catenin. It is intimately linked to the tumour suppressor protein adenomatous polyposis coli (APC) and so has an active role in colon cancer [18].

Although these have long been implicated as potential objectives for therapeutic agents, they have been historically difficult to target with existing drug discovery platforms [17]. Recent developments have changed this and preclinical models have been successful [19,20]. Recent research has focused at targeting the *Wnt* pathway at the level of ligand production. This novel class of compounds target the protein *porcupine* which is involved in *Wnt* secretion [21]. However since *Wnt* signalling is involved in cellular regeneration, a number of toxic effects of these agents remain a hurdle to overcome in their therapeutic potential [22]. These agents may cause immune suppression and anaemia [21,22].

## 7. TGF-B SIGNALLING PATHWAY

Transforming growth factor- $\beta$  (*TGF- $\beta$* )/*BMP/activin* superfamily ligands play a central role in regulating an extensive range of cellular responses. These include cell growth, cell differentiation, and the specification of developmental fate in a diverse range of organisms. Alterations in its signalling pathway are associated with a range of human diseases with cancer being the most well studied sequelae [23].

Fig. 2 illustrates the *TGF- $\beta$*  signalling pathway diagrammatically and shows how the *TGF-beta* ligand receptor interaction signals kinase

receptor complexes on the cell surface. These receptors phosphorylate cytoplasmic mediators called *SMADS*, which in turn enter the nucleus and cause transcription of a range of genes which have a tumour suppressive effect. In oncogenesis, malignant cells escape from the tumour-suppressive effects of *TGF-β* by mutational inactivation or deregulated expression of the molecular components in *TGF-β* signalling pathway.

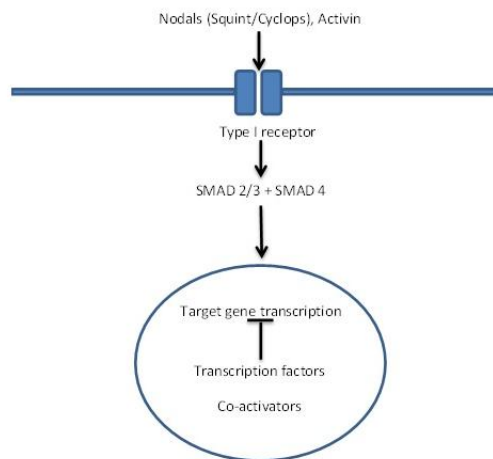
## 8. CANCER, EVOLUTION AND THE CELL CYCLE HOMOLOGIES

It is unsurprising that the most key molecular pathways which are evolutionarily conserved are those involved in the molecular machinery for reproduction and cell division. This is particularly the case in DNA replication. The accurate and reliable cell division and replication of DNA would seem to be the most important task of any organism, whether its aim is to reproduce as single cell organisms or to grow or regenerate as multicellular organisms. It is therefore vital that the process flows smoothly since there are many careful steps involved in the cell cycle in order for a cell to divide. It is due to the potential for error that several key checkpoints act as crucial milestones in cell division in order to ensure that everything has been completed before the next step may begin.

The stages of cell division are arbitrarily defined divisions given to the cell cycle and in reality this is a continuous process. However, it is nonetheless possible to see specific checkpoints and their respective molecular mechanisms highly conserved throughout the animal kingdom. Mutations resulting in altered proteins in these pathways can lead to devastating results, and although there are more levels of differentiation in terms of repair mechanisms between species, the pathways underpinning them are very similar. If damage to these pathways is beyond the scope of the organisms repair mechanisms, then apoptosis is the natural result (discussed later). However mutations can occur which can prevent this vital event (apoptosis) from happening, resulting to a living defective, rapidly dividing cell.

The term used to describe uncontrollably and rapidly dividing cells is of course cancer, and may be considered a novel example of Darwinian evolution [24]. This article previously dealt with Darwin's theory of survival of the fittest, where independent prokaryotic cells first made the leap to function together for mutual benefit (most

probably via an adhesion cell surface protein mutation), giving them a selective advantage over other self-autonomous cells. This analogy is useful when understanding cancer. In cancer it seems that the same principle applies, except in the opposite direction. Cancer may occur either due to increased proliferation, decreased cell death, or lack of differentiation (such as is the case in leukaemia).



**Fig. 2. A schematic of the TGF-beta signalling pathway. TGF-β= Transforming growth factor-β**

A cancer cell that has mutated so as to prevent differentiation has a selection advantage over all over cells in the body in terms of proliferation rate, since once a cell becomes committed, it no longer may divide (this is true for the majority of cell lineages). The competition for limited nutrients favours characteristics to increase the rate of nutrient arrival (e.g. angiogenesis) and nutrient uptake, (e.g. increased transmembrane transporters) [24]. These characteristics selectively favour cells resorting to the state of being 'self-centred' and producing agents to propagate the processes of angiogenesis and nutrient uptake,

Studies into whether there is functional overlap, and the significance of this overlap in various pathways are paramount to our understanding of the development of disease processes. This understanding brings us closer to the ultimate goal; to allow us to tailor treatments from 'blunt weapons' to fine-tuned 'magic bullets'. This is an analogy meaning that we may be able to eventually create treatments acting specifically on the abnormal disease pathway with 100% specificity with no adverse reactions or side

effects. We will now consider those pathways in the cell cycle which share the most inter-species homology and where such 'magic bullets' might be best targeted. The best way to assess evolutionary conserved pathways is to investigate which functions and features cells of a different species share.

As described above, the most important of these processes is that of cell division. For a cell to divide, there are 2 main points within the cell cycle of great importance, namely, S-phase entry (when the cell decides to initiate replication and DNA is replicated), and secondly M-phase entry (when the chromosomes begin to condense). The four main factors which control entry into M-phase are the mass of a cell, growth rate of a cell, the interval after the previous mitosis and the successful completion of S-phase.

The actual initiation of DNA replication is obviously a process that must be firmly regulated. After the growth phase of a cell, two main factors are required to enter S-phase (DNA synthesis phase), namely the Origin Replication Complex (ORC) [25] (in addition to the pre-Replication Complex (*pre-RC*) in G1 phase [26]) and the Replication Licensing Factor (*RLF*) [27]. These factors are essential for progression and commitment into S-phase. *RLF* can bind to chromatin during the G1-S transition and its function can be described quite basically as giving 'permission' for DNA replication to begin. As a result it dissociates once the replication has initiated. *ORC* comprises of 6 proteins which attach to the origins of replication of DNA [28]. They thus inform the DNA replication apparatus where to carry out its function after allowing the origins of replication to be recognized.

The *ORC* protein number in *archaea* varies greatly, and there are multiple *ORC* proteins in yeast. The other factor known to play a role in eukaryotes is Minichromosome Maintenance complex [29], *MCM*, a 6 subunit complex with 6 proteins and have sites for *cdc2* protein kinase phosphorylation, but how these proteins are conserved and interact remains unclear [30,31]. It may well be that these constituents are acted upon and up-regulated by factors involved in the G1-S transition, such as G1-S cyclises [30]. This is developmentally conserved across species suggesting that it has high importance.

Although the list of homologous proteins from *Drosophila* and mammals is incomplete, and few mammalian genes have been successfully cloned, both *ORC* and *MCM* are conserved in a

vast variety of creatures. Homology is shown to be present in yeast and various vertebrates in terms of *ORC2* with *MCM2*, *MCM4* in yeast and vertebrates (*DmMCM2* and *DPA* respectively in *drosophila*). However, the homologue of *ORC1* is yet to be discovered in *Drosophila*. But why may studies of these genes in different species prove useful? Well in terms of prognosis of any disease, it is always better to detect a pathology developing earlier rather than later in order to aid possible treatment. Thus the need for biomarkers of specific pathologies is paramount. *Ki67* and *PCNA* are older biomarkers which have been used for this purpose, but have limited use for many types of cancer.

Replication factors in yeast have been used to investigate their effectiveness as biomarkers to detect cancer. Due to the homologies described above between different organisms, they may be directly transferable to detect human cancers. The (*MCM*) family of proteins tend to be present in higher levels in tumour cells, being a great improvement on the previous markers *PCNA* and *Ki67* [32]. It was mentioned above that these *MCMs* have phosphorylation sites for *cdc2* protein kinase. Other members of the *cdc* family such as *cdc7* kinases also show higher transcription levels in cancerous cells than normal ones and could also act as potential tumour markers in the future [32]. This provides an example of how evolutionary studies into pathway development may aid the development of therapeutic techniques and understanding of the pathologies of current diseases.

The entry into the S-phase (phase of DNA replication) of the cell cycle is slightly more complex. Several other factors have an involvement, namely *Cyclin D-Cyclin* dependent kinase, *Cyclin E-Cyclin* dependent kinase heterodimers, transcription factor *E2F* and Retinoblastoma protein (Brody 1999) (see Fig. 3). The *p21* family of proteins negatively regulate *Cyclin-Cdk* heterodimers, however *myc* dependent pathways upregulate *Cyclin E-cdk2* heterodimer.

In mammalian systems, Retinoblastoma (*Rb*) protein also interacts with this regulation, however *cyclin D-cdk* and *Cyclin E-cdk* heterodimers down regulate *Rb* by phosphorylating it (this pathway is simplified into a schematic in diagram 3). *Rb* downregulates *E2F* transcription factor. Whereas *Cyclin E-cdk* has blocked *Rb*, *E2F* levels rise since they are no longer inhibited by *Rb* and allow it to perform

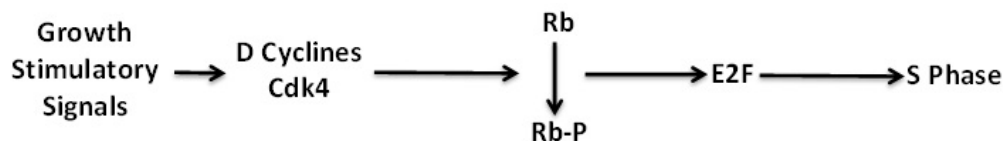
its function, i.e. to up-regulate those genes which code for proteins involved in S-phase activities, (including cyclin E, allowing a positive feedback loop). This is balanced by *Rb*'s interaction with *Cyclin D-Cdk* which also promotes S-phase progression by mainly being involved in the G1-phase of the cell cycle [33]. Although *Cyclin D-Cdk* does play a role in the *E2F/Rb* pathway [34], it is redundant in *Drosophila*, just as it is in mammals due to the actions of *CycE/Cdk2* complexes. This redundancy was most probably reserved as a failsafe, which developed early in evolution and was selected for since preventing entry into S-phase is a crucial step and it is better that damage in 2 factors be required than that of one to prevent possible pathological proliferation.

For the transcriptional activator function of the *E2F* complex, an accessory protein is required (*DP*) which allows *E2F* –*DP* interaction with *p53* in mammals [35]. *p53* is known to regulate cell division (via *E2F*[36]), but curiously there is no *p53* or *Cyclin A* (involved in S-phase function) homologue in *Drosophila*, whereas in mammals *Cyclin A* particularly is known to interact with *cdk2* to act with *E2F-1*. In *Drosophila* there is no *p21* gene (except for the primitive *dacapo*), *Rb* (although *Rb* is a critical switch in mammals, regulating S-phase entry, it's homologue *Rbf* in *Drosophila* is not so important and acts only as an intermediary factor responsible for *cyclin E* induction of *E2F* [37]) or *myc* (with *dMyc* protein having merely 26% homology to human *c-Myc* and different function in invertebrates) interaction homologues in the transition to S-phase. In invertebrates the functions of these cell cycle transition homologues appear less important, indicating that this is an added level of complexity that is apparent and important only in higher order organisms. All in all there seem to be considerably more complexity in mammalian regulation of these steps leading to S-phase induction than is present in invertebrates, although the basic framework on which these extra regulatory molecules act is astonishingly similar.

An important question to address at this stage is if the primitive pathway works adequately well in invertebrates, then why the requirement for so much more regulation? The answer lies in the fact that invertebrates such as *Drosophila*, have very short life cycles, thus for larger creatures with more cells, more cell divisions and for those which live longer, more complex and additional regulatory pathways have evolved. However with all additional levels of complexity, there are often more steps which could go wrong. Indeed, it was soon discovered that the control of this *Rb/E2F* pathway, which is not evolutionary conserved, is indeed neglected to be conserved for a reason, and is altered in almost all human cancers [38].

After the S-phase has successfully been completed the cell enters G2-phase, a phase of growth once again before the entry into M-phase. The G2-M transition is another important site of regulation, as mentioned earlier. As described above, *cyclin A* is absent in invertebrates, although it plays an important role in stopping the activation of genes regulating transcription in the DNA replication phase. Low levels of *Cyclin A* stop the cell cycle in G2, and increased levels of *cyclin* determine the progression into M phase, the replication of DNA in the synthesis phase as well as in the G2-M transition. *Cyclin A* does not play any role in the S-phase of invertebrates like *Drosophila*, nor does *cyclin B*.

The *cyclin B cdc2* heterodimer [39] (previously referred to as maturation promoting factor [40]) phosphorylates cellular substrates to the condensation of chromosomes, the breakdown of nuclear lamina and the assembly of mitotic spindles [41]. Although *Cdc2* is required in early mitosis, it must be inactivated for the late mitotic events to proceed. The conserved *Cdc25* phosphatase homologue, *Wee* kinase, activates mitosis by dephosphorylating *cdc2* active kinase of the *cyclin/cyclin* dependent kinase *dimmer*[42], whereas *Warts* (known in Vertebrates as *LATS1*) down-regulates *cdc2*.



**Fig. 3. The Rb/E2F pathway. Modified from [38]. Rb= retinoblastoma gene, Rb-P= retinoblastoma tumour suppressor protein. D Cyclines/Cdk4= D-type cyclin-dependent kinases, E2F= E2F transcription factor, S Phase= Synthesis phase**

Once entering mitosis, the metaphase-anaphase progression is marked by the decrease of active MPF concentration (i.e. destruction of *Cyclin B/cdc2*) once the spindle is fully completed and stabilised, allowing the chromosomal partition of anaphase to occur. The metaphase-anaphase transition is a mitotic checkpoint. At this juncture the cell is able to monitor the integrity of its spindle before proceeding to inactivate MPF and initiate chromosome separation. The fact that *Cyclin B/cdc2* is degraded at the metaphase-anaphase transition suggests that MPF is the direct target of this checkpoint. All of these factors have homologues between vertebrates and invertebrates. Another important protein present in anaphase is a multi-subunit ubiquitin ligase named anaphase-promoting complex/cyclosome (APC) [43]. This factor is down-regulated in M and G1 phase by proteins of the fizzy and fizzy-related genes which are only active at these stages of the cell cycle and are also responsible for decreasing the concentrations of *cyclins A, B, and B3*. These three *cyclins* are otherwise stable in interphase and G1 but destroyed in mitosis.

## 9. APOPTOSIS AND CONSERVED PATHWAYS

Death is an inevitable end to all life forms, thus it is not surprising that similar pathways in programmed cell death are shared across many species. It is by studying the development of these pathways and the differences between species that we can better understand the functions of each of the components of these pathways. Regulated cell death (apoptosis) is a very important process for the cell and is especially significant for multicellular organisms, where cell death aids the neutralisation of infected cells and abnormal or defective cells. Abnormal cells could otherwise cause a variety of problems for the organism; from proliferating in the wrong place (anatomical and mechanical problems) to damaging other cells in the vicinity (e.g. by secreting detrimental agents). Hence apoptosis serves a vital function. It is distinct from necrotic cell death in that it is a controlled, directed and highly 'efficient' cell death process that is regulated by genes and histologically appears to be the compaction and packaging of cell organelles for 'neat' suicide.

Apoptosis is not always the result of pathology. Even in natural embryonic development, the digits are sculpted out of the hand itself by apoptotic destruction of the cells between them.

Comparative studies have shown that various specific genes and factors are present across the evolutionary tree in regulating apoptosis. An example of this is interleukin-1beta-converting enzyme (ICE) and CPP32 in mammals [44] which has homologs across many species, such as *caspase-1* in *Drosophila* and *ced-3* in *C. elegans*. In fact, *ced-3* also shares homology with *caspase-8/Flice* in mammals and *Death* related *ced-3/Nedd2*-like protein in *Drosophila* (Brody 1999). The infamous *bcl-2*, *bax* and related genes are similar to the colourfully named death executioner *Bcl-2* homologue in *Drosophila* (which acts similar in mammals by unknown mechanism(s) to counteract the apoptosis process). Additionally, *MORT1* in mammals has shared homology with the *Reaper* gene [45], *Head involution defective* gene [46], and the *Grim* gene in *Drosophila*.

Although there is no homologous *Reaper* protein in mammals, research shows that if *Reaper* protein is introduced into *Xenopus* eggs it will lead to apoptosis [47]. Thus protein which is no longer coded for in vertebrates still exerts its effects on other species due to evolutionarily conserved pathways between species. This is due to the downstream mechanism of action and associate proteins remaining the same between species and retaining the potential to be activated. This may allow new therapeutic mechanisms inducing apoptosis in rapidly proliferating cells such as cancer cells to be developed, should a method of uniquely identifying them and administering the protein be identified. Such therapeutic implications are far from today's research, and other areas offer more promise, but funding for such research will increase the physician's arsenal in dealing with disease. By conducting knockout studies on vertebrate and invertebrate model systems, the likely outcomes of genetic diseases can be seen and compared with human diseases.

## 10. RNAI: AN EXAMPLE OF CONSERVATION OF A PATHWAY WITHOUT ACTUAL ACTIVITY

The final point in this article and perhaps the most interesting aspect is to study why some conserved pathways like RNAi (RNA interference) are inactive in some species despite having amazing implications for the cell in terms of fighting viral infections. This is an area of great interest for evolutionists who would ask why then would such pathways be conserved. It appears to defy the principle of



highly conserved pathways being vital mediators of essential cell functions (thus justifying their evolutionary conservation) and so loss of these essential pathways should result in severe morbidity or death. However if the RNAi pathway is not active in the first place, then loss of it would not affect mammalian cells. In this case, why it has not been lost evolutionary long ago remains a mystery.

The RNAi pathway plays an important role in regulating development and maintenance of the genome. It is thought to have evolved as a primitive form of innate immunity against viruses. The RNAi pathway is initiated in the presence of dsRNA (double-stranded ribonucleic acid) which is a common by-product of viral replication, hence implying establishment of viral infection within the cell. The enzyme 'Dicer' cleaves this dsRNA into much shorter units. One of the cleaved strands of the double strand is then integrated into the RNA-induced silencing complex (RISC) and base-pairs with a messenger RNA (mRNA) molecule. This induces destruction of the mRNA by the catalytic component of the RISC complex called argonaute. This pathway has been well-studied, especially in invertebrates such as *Caenorhabditis elegans* and *Drosophila melanogaster*, as well as *Arabidopsis thaliana*, a type of flowering plant.

However, surprisingly this pathway is inactive in humans and thus does not offer innate immunity against viral infections. It is most likely conserved simply due to its genetic nature and robust location, but the answer remains a mystery. RNAi studies have shown that genes with similar functions are clustered in specific yet massive regions of individual chromosomes and alongside genes sharing transcriptional profiles [48]. If the mechanisms underpinning the activation of this pathway could be sufficiently understood, then activation of this pathway could aid in the treatment of many diseases, by knocking out defective genes with high specificity.

## 11. CONCLUSION

This review has provided an overview of evolutionary comparative intracellular signalling. It has used specific examples to highlight the importance of studying these pathways from an evolution viewpoint, in order to better understand disease processes in terms of selection pressures and selection advantages. Despite the

plethora of cell types in the animal kingdom, only a few conserved signalling pathways are required to generate them. It is impossible to cover all of the numerous important pathways in just one short article. However, a few distinct pathways that exemplify the themes of pathway homology have been described in detail in respect to their similarities in other species at different points on the evolutionary tree and their relevance to clinical disease entities.

Developmental pathways, cell cycle regulation, growth receptor signalling, apoptosis and RNAi have all been discussed in light of these common themes and it seems that although the basic framework of pathways remain the same, the regulatory mechanisms governing them can greatly vary in complexity between species. By studying the beginnings of such regulatory mechanisms and the need for increased complexity (driven by changing evolutionary selection and environmental pressures), we can better understand their role in health and disease. This understanding allows therapeutic techniques and treatments to be developed which allows better molecular management of diseases based on insights into disease pathology elucidated by these types of evolutionary studies.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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