

## **The last neuronal division: a unifying hypothesis for the pathogenesis of Alzheimer's disease**

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### **Abstract**

Alzheimer's disease is the third biggest killer in the developed world after cancer and heart disease. Although the first description dates back to almost a century ago, we still do not understand the cellular-molecular mechanisms that lead to the development of the brain pathology. There is no efficient treatment for the patients. And even the symptomatic relief comes too late, because there is no diagnostic method that could identify patients before severe clinical symptoms develop. Almost every aspect of Alzheimer's research, whether on pathogenesis, diagnosis, risk factors or treatment strategies, seem to spark controversy nowadays. Studies that apparently contradict one another or propose alternate explanations are published monthly. Patients' hopes are raised with the announcement of each new drug trial. Then hope is lost when the trials fail. Ironically, while progressive research is hampered by apparent contradictions and lack of funding, the old dogmas survive in the textbooks and no real progress is made in terms of therapy. The purpose of this review is to take stock of the most discussed and most strongly opposing views on the pathogenesis of Alzheimer's disease. These views shape the Alzheimer field currently and some will hopefully one day shape the diagnostic and therapeutic approaches to the disease.

**Keywords:** Alzheimer's disease • cell cycle • cyclins • amyloid • tau

### **Introduction**

Alzheimer's disease (AD) was labelled the 'silent epidemic' of the 20<sup>th</sup> century. The disease affecting the ageing population has become a major medical and social problem for western societies.

The clinical syndrome of slowly progressive dementia is associated with the gradual accu-

mulation of its diagnostic pathological features in the brain: the amyloid plaques and neurofibrillary tangles.

The main component of the AD-specific plaques is the short fibrillar  $\beta$ -amyloid peptide. The neurofibrillary tangles are composed of paired helical filaments of the hyperphosphorylated tau protein. The tau pathology, unlike  $\beta$ -amyloid deposition, is

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not specific for Alzheimer's disease (neurofibrillary tangles are found in progressive supranuclear palsy and subacute sclerosing panencephalitis).

Despite the century long search for clues for the origins of Alzheimer's disease, the scientific community is locked in debate punctuated by witty polemics [1] and media campaigns [2, 3].

## The amyloid cascade hypothesis

Although it was known that patients with Down's syndrome almost invariably develop Alzheimer's disease the pathogenic link between the two disease remained elusive [4] until the discovery of the amyloid precursor protein (APP) gene on chromosome 21, close to the region obligatory for Down's syndrome. The identification of an APP mutation associated with early onset familiar Alzheimer's disease [5, 6] was the prelude for the formulation of the "amyloid cascade hypothesis". Despite the subsequent recognition that APP mutations are extremely rare (less than a dozen families in the entire world) and that some APP mutations are associated with pathology other than AD [7, 8] or no pathology at all [9] amyloid remained the focus of Alzheimer's research. The identification of presenilin (PS1 and PS2) mutations associated with early onset AD [10–12] was 'naturally' followed by the search for links between the presenilins and amyloid [13] and the role of the presenilin mutations in the pathogenesis of AD is attributed, even in some recent reviews, solely to their effect on APP processing [14].

APP is a relatively small transmembrane protein and the normal function of the molecule is not entirely elucidated. The analysis of its primary structure led to the suggestion that APP might be a glycosylated cell surface receptor [15] while later studies showed that APP itself might be one of the cell surface mediators of  $\beta$ -amyloid toxicity [16]. The analysis of APP expression during brain development indicates that the protein is important for the maturation of CNS as it is involved in formation and maintenance of synaptic connections and neuronal plasticity [17]. Additionally APP plays role in stress response and in injury repair in the nervous system (reviewed in [18]).

APP is processed by three different mechanisms leading to the production of different cleavage

products. The processing of APP by  $\alpha$ -secretase (secretory pathway) results in the production of a single soluble (APPs) secreted fragment [19] and one non-amyloidogenic carboxy-terminal segment. The endosomal-lysosomal APP processing pathway leads to the accumulation of a complex set of APP fragments some of which are potentially amyloidogenic [20]. The third pathway leads to the secretion of the  $\beta$ -amyloid fragment as a soluble peptide [21] which has trophic effect on neurones and promotes neurite outgrowth (reviewed in [22]).

The main component of AD plaques is the insoluble  $\beta$ -amyloid. The central axiom of the amyloid cascade hypothesis was that this  $\beta$ -amyloid deposition is the primary cause of AD-related cell death, cytoskeletal pathology and synaptic loss. The mechanisms by which  $\beta$ -amyloid could be linked to the cellular pathology of AD varied over the years. The early claims of a direct toxic effect were based on *in vitro* experiments where the addition of fibrillary  $\beta$ -amyloid was found to be neurotoxic [16] or lead to the impairment of mitochondrial function [23] or  $\text{Ca}^{2+}$  homeostasis [24]. There was also speculation about the role of  $\beta$ -amyloid in increasing neuronal vulnerability to excitotoxicity, hypoxia or oxidative stress.

There are several difficulties with the 'amyloid cascade hypothesis'. The Alzheimer's patients where the disease is associated with mutations that alter the processing of APP and the production of  $\beta$ -amyloid represent only a very small minority (<5%). The majority of the patients (late onset sporadic AD) have no mutations on either APP or the presenilins.

Furthermore, many non-demented elderly individuals have large amounts of  $\beta$ -amyloid plaques in their brain without any functional consequence. Generally the amyloid deposits are not surrounded by any detectable tissue damage. From studies on human brains it is apparent that  $\beta$ -amyloid on its own is not harmful [25]. Damage to synapses and neurones is associated with the appearance of the tau pathology in the form of tangles, neuropil threads or distorted neurites in plaques [25–27].

The controversy over the toxicity of  $\beta$ -amyloid only deepened as toxicity experiments proved to provide inconsistent results, or were later found to have serious design flaws [28]. Most importantly the amyloid cascade hypothesis does not provide an explanation for the development of the tau pathology as a response to  $\beta$ -amyloid.

More recent theories claim that it is not the fibrillar  $\beta$ -amyloid, but the soluble oligomers of the peptide (the pre-fibrillar form of  $\beta$ -amyloid) are responsible for the disruption of synaptic plasticity and neuronal death [29, 30]. This theory at least offers a temporary solution for the problems caused by the earlier version of the amyloid cascade hypothesis. However, the evidence for the role of soluble  $\beta$ -amyloid oligomers in the causation of synaptic loss and subsequent dementia syndrome in patients is not fully convincing [31, 32]. The results of *in vitro* and animal studies would indicate that the process triggered by the  $\beta$ -amyloid oligomers does not resemble the slow, progressive development of AD-related tissue damage.

### **Disease-related changes of the tau protein and the formation of tangles**

As mentioned earlier, the tangles although characteristic and required for a post mortem diagnosis, are not specific for Alzheimer's disease. Early research showed that the accumulation of the tangles is associated with the development of the clinical symptoms in AD [33, 34]. As such the tau pathology was regarded more as a final common pathway of slow neuronal degeneration, and attention was focused on understanding the molecular-cellular mechanisms that could result in tangle formation.

The intracellular tangles that fill the neuronal cell bodies consist of paired helical filaments, made almost entirely from the hyperphosphorylated form of the tau protein. The same PHFs also accumulate in dendrites (neuropil threads) and in dystrophic neurites associated with senile plaques. The paired helical filaments are insoluble and persist even after the death of the neurone that contains them (ghost tangles).

The normal function of the tau protein is to stabilize microtubules and maintain the structural integrity of the cytoskeleton (reviewed in [35]). Phosphorylation alters the microtubule-binding properties of tau and favours self assembly into PHFs. Therefore the imbalance between phosphorylation (by kinases) and dephosphorylation (by phosphatases) was hypothesised to play a major role in the development of the tau pathology (reviewed in [35]) and the search for the enzymes involved, kinases and phosphatases, as well as the mechanisms that could alter their function intensified.

Over the years several kinases have been identified as responsible for the hyperphosphorylation of the tau protein. Extensive *in vitro* and *in vivo* work confirmed that the kinases involved in tau phosphorylation in AD are the mitogen activated protein kinases (MAPK), GSK3 [36, 37] cdk2 and cdk5 [38]. The hyperactivity of these kinases is paralleled by a reduction in the activity of phosphatases (specifically PP2A PP2B) [39, 40] leading to a loss of balance in favour of phosphorylation.

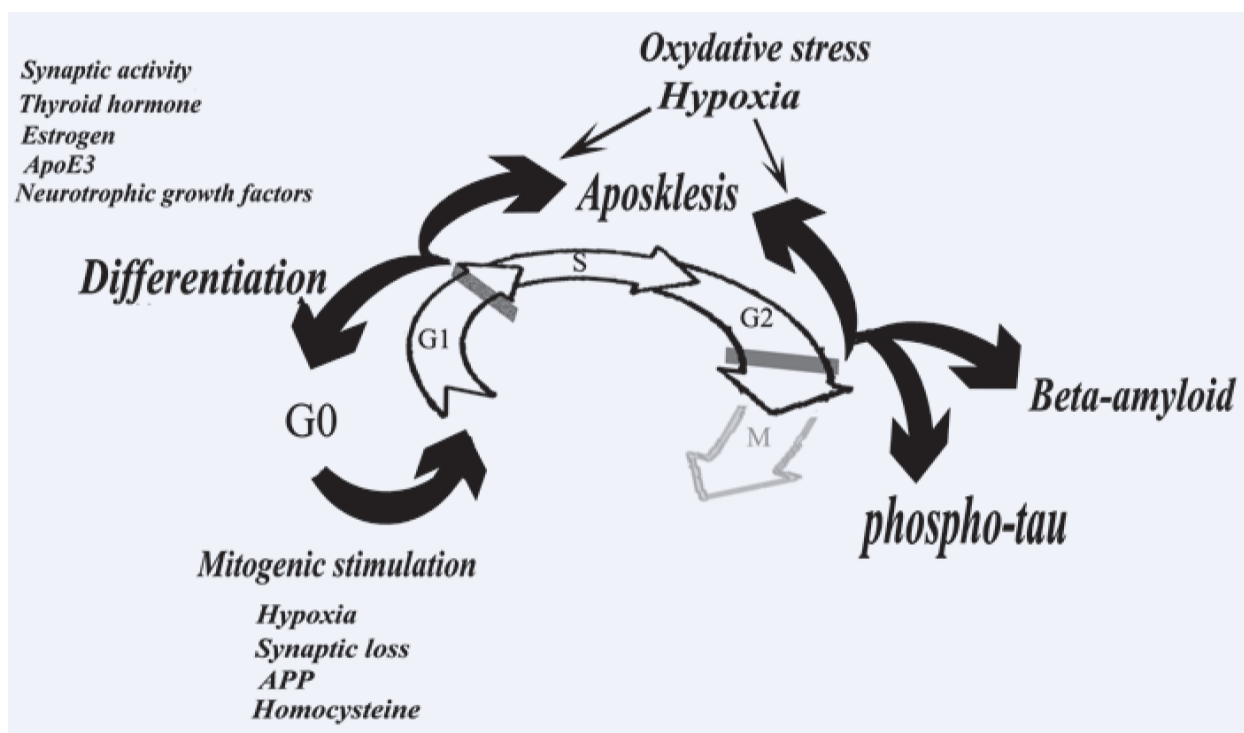
The effects of microtubule destabilisation and the accumulation of the insoluble PHFs in neurites and cell bodies it is not difficult to envisage: cellular transport is impeded leading to functional deficits and ultimately to neuronal death [41, 42].

### **The cell division cycle and Alzheimer's disease**

The latest, and inevitably the most controversial, theory for the pathogenesis of Alzheimer's disease postulates that the formation of the two seemingly unrelated pathologies, amyloid plaques and neurofibrillary tangles, is due to the re-activation of cell division-like phenomena in ageing neurones [43–46].

The theory explains the interesting parallels between the brain development and Alzheimer's disease. The cognitive and other functional changes in AD correspond to the reverse pattern of developmental events (reviewed in [47]). Alzheimer's devastates step by step the neuronal network responsible for higher cognitive functions [44, 48, 49]. This stepwise process follows a hierarchy affecting most severely brain regions that are ontogenetically, and phylogenetically, the most recent [48, 50].

In the developing brain the neurones are integrated in synaptic networks after the proliferation, migration and differentiation of neuronal progenitors have been completed. The cellular signals controlling connectivity and synaptic plasticity also represent the environmental cues that maintain neurones in a differentiated state and do not permit the reactivation of the cues leading to migration or proliferation. This dual role of morphoregulatory cues represents an inherent danger for neurones that retain a high degree of synaptic plasticity: aberrant neuroplasticity may lead to the reactivation of cell cycle-related phenomena, attempted proliferation



**Fig. 1** The neuronal cell cycle in Alzheimer's disease. Hypoxia, synaptic loss, homocysteine and, according to some authors, APP can represent mitogenic stimuli for adult neurones. The cell cycle re-entry (from G0 to G1) that follows however is not necessarily detrimental. If the G1/S regulatory checkpoints function adequately (genetic and epigenetic cues that aid differentiation and appropriate cell cycle control) re-differentiation is possible. The G1/S arrest may also serve as a pathway to age-related neuronal death. If the G1 control is inadequate neurones progress through DNA replication into the G2 phase of the cell cycle. At this stage all components of Alzheimer-type pathology, b-amyloid plaques and tangles are produced. Brain hypoxia or Oxidative stress may alter the outcome of cell cycle re-entry in neurones by inducing cell death (apoptosis).

and subsequently cell death in neurones (reviewed in [48]).

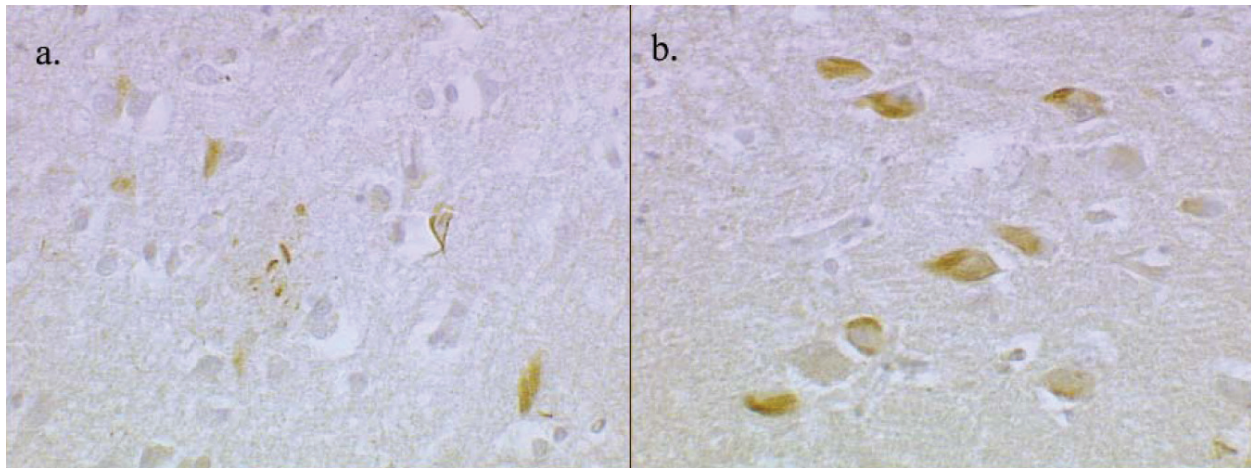
There is ample evidence to suggest that the pathogenesis of AD involves aberrant synaptic plasticity and sprouting together with a failure of neuronal differentiation and reactivation of the cell division cycle (reviewed in [48]).

The reactivation of the cell cycle in itself however, does not necessarily lead to cell death or pathology. Markers of cell cycle activation have been found in the neurones of healthy elderly people without any AD-related pathology [51, 52]. The difference between AD patients and healthy elderly controls was not primarily cell cycle re-entry, but the consequences of the reactivation of the division programme (Fig. 1).

In healthy elderly people the neuronal cell cycle re-entry is followed by the activation of cell cycle

control mechanisms that halt the progression of the cell cycle early (in the G1 phase) in these mitosis incompetent cells. The cell cycle arrest in the G1 phase can be followed, if appropriate regulatory cues are present by re-differentiation without any further consequences. Some regard this transitional re-entry into the cell cycle as a mandatory part of the synaptic remodelling process (reviewed in [48]). Alternatively, the G1 arrest may result in cell death and could be the mechanism by which age-related neuronal death occurs.

In AD, the G1/S regulatory mechanisms seem to be absent or fail and neurones are allowed to progress through DNA replication [46] into the late (G2) phase of the cell cycle [44]. The G2 phase, in normally dividing cells, represents the period of preparation for the mitosis. Neurones are not capable of cytokinesis and the cell cycle is arrested in



**Fig. 2** The expression of cyclin dependent kinase inhibitor proteins in Alzheimer's disease. Panel 2a. shows that the expression of p21<sup>cip1</sup> protein is associated with tangles and neuropil threads in plaques in some Alzheimer's patients. Panel 2b. shows a similar staining pattern for the p57<sup>kip2</sup> protein.

the G2 phase. Re-differentiation at this stage is not possible any longer. Naturally dividing cells arrested at the G2 phase usually die *via* apoptosis like mechanisms. Neuronal populations, on the other hand are highly protected against rapid apoptotic cell death. Even if the bcl-2 type anti-apoptotic proteins are not sufficient for protection [53], and the apoptotic cascade is initiated, the lack of the downstream caspases [54] leads to a drawn-out agony, apoptosis [55], instead of rapid apoptosis [56]. As a consequence neurones survive for long periods of time in the G2 phase of the cycle and the cellular mechanisms activated will inevitably change neuronal metabolism.

One of the most prominent features of the G2 phase (and generally of cell cycle phenomena) is the activation of kinase systems, required for pushing the cell through the division (reviewed in [57]). The MAP kinases initiate mitotic re-entry while the cyclin dependent kinases (such as cdk2) ensure the progression of the cell through the consecutive phases of the cycle. The activation of these kinases is associated with a downregulation of the phosphatases and a gradual destabilisation of the microtubule system in an attempt to prepare the cell for cytokinesis.

The destabilisation of the microtubules results in increased amounts of free tau in neurones. The concomitant activation of the kinases and downregulation of phosphatases will inevitably lead to the phosphorylation of this pool of tau not bound to

tubulin. This in turn prevents the re-attachment of tau to the microtubules and favours the formation of the paired helical filaments typical of the tangles. The same phenomena also provide an explanation for the presence of neurofibrillary tangles in some rare slow growing tumours (ganglioglioma).

The metabolism of other proteins is also changed by the G2-dependent phosphorylation. The phosphorylation of APP leads to changes in the processing of the protein, resulting in the predominant production of the  $\beta$ -amyloid fragment [58]. As the soluble  $\beta$ -amyloid oligomers accumulate in brain regions affected by tangle pathology [32] the fibrillar  $\beta$ -amyloid (plaques) is deposited in regions that these neurones project to [59].

Since neurones are not mitosis competent, cell cycle re-activation also elicits a strong drive to prevent or inhibit the process. Such an attempt results in the upregulation of the cell cycle inhibitor proteins, such as GSK3 and the cyclin dependent kinase inhibitors (Fig. 2) [43, 57, 60]. This attempted damage control, however, leads to further damage. GSK3 is one of the kinases responsible for the phosphorylation of tau aiding the development of tangles [60] and is also required for the maximal production of  $\beta$ -amyloid from APP [61].

Thus it appears, that the development of AD is a two-stage process. Morphodysregulation and subsequent cell cycle re-entry is the necessary first step, which, if followed by the G1/S regulatory failure and the progression of the cell cycle.

The reactivation of the cell cycle, DNA replication and subsequent cell cycle arrest leaves neurones with two alternatives. Either die, *via* a mechanism that resembles apoptosis [62] or survive and produce Alzheimer-type pathology [51, 53]. The balance between these two outcomes will depend on the inherent properties of the affected neurones, such as constitutively expressed caspases and other pro- and anti-apoptotic proteins. Changes in the neuronal microenvironment are able to tip this balance or accelerate the process itself.

Hypoxia has been found to activate cell cycle re-entry in neurones [63, 64]. However, hypoxia also alters the outcome of cell cycle reactivation leading to cells death rather than the accumulation of hyperphosphorylated tau and  $\beta$ -amyloid [65]. Due to this mechanism, in patients suffering from mixed Alzheimer's and vascular 'disease' the amount of AD-type pathology is relatively small [66] and hypoxic insults can unmask the presence of even sub-clinical Alzheimer's disease [67].

The similar effect of oxidative stress, in aggravating the consequences of cell cycle re-entry in neurones led to the formulation of the "two hit hypothesis", which postulates that both cell cycle re-entry and oxidative stress are necessary for the development of Alzheimer's disease [68]. The "two-hit-hypothesis" is probably valid in a much broader sense. Neurones arrested in the G2 phase of the cell cycle are vulnerable to all insults, including oxidative stress, hypoxia, excitotoxicity etc. Since some of these insults act themselves as mitogenic stimuli for neurones, they can in fact initiate the self-perpetuating circle of neurodegeneration.

In this context the cell cycle reactivation in neurones appears to be the key pathogenic mechanism responsible for the development of AD-type pathology and neuronal cell death. However, it is also evident that mitogenic stimulation on its own is not sufficient to cause AD [48] and neurones can be rescued by the arrest of the cell cycle in its early (before G1/S transition) stages. Cell cycle re-entry in neurones will cause degeneration only if the G1/S transition point is breached and DNA replication occurs [46, 69]. The regulatory deficit at the G1/S transition point therefore, is a necessary prerequisite of AD, although the weakness of this regulatory mechanism will never become evident without mitogenic stimulation.

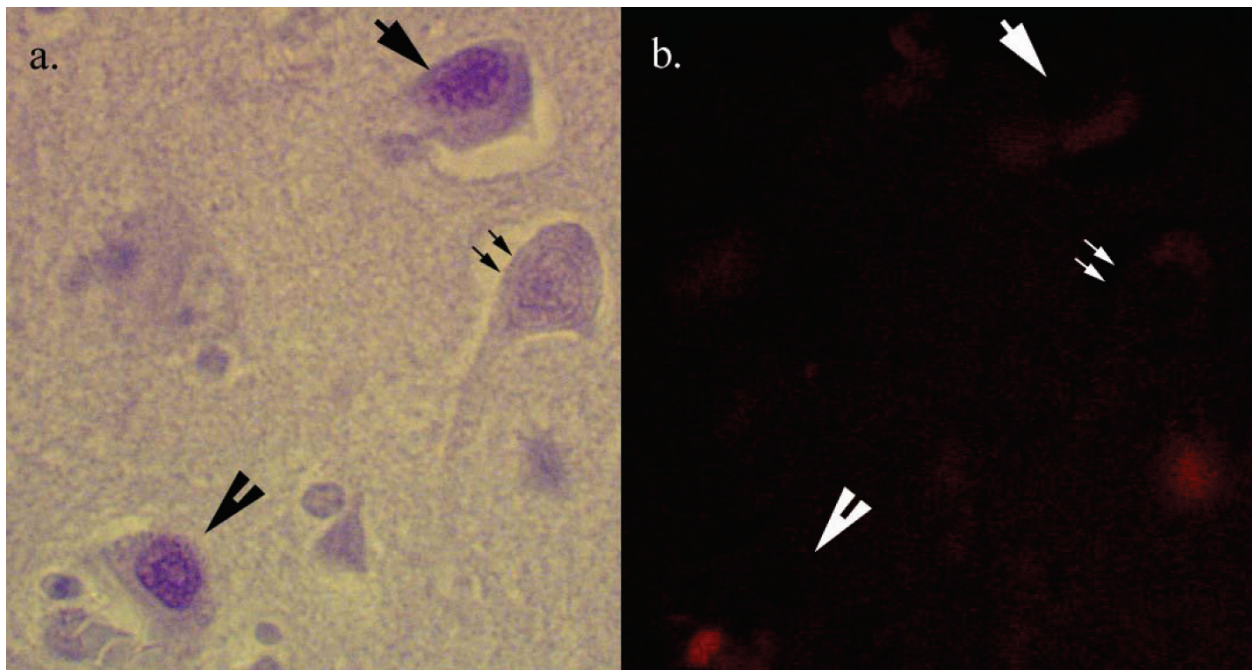
The cell cycle hypothesis provides the first comprehensive explanation for the cellular phenomena associated with Alzheimer's disease. It also explains the role of some of the factors associated with elevated risk of Alzheimer's disease. Homocysteine, the consequence of vitamin deficiency may act as a mitogenic factor for neurones, initiating the deadly cycle [70, 71]. Alternatively it may induce oxidative stress [72]. Low oestrogen levels in postmenopausal women and low education can contribute to morphodysregulation in neurones (reviewed in [48]). Depression would probably have a similar effect. However, the hypothesis also leaves many questions unanswered.

More research is needed to elucidate the nature of the G1/S regulatory failure. While the cyclin dependent kinase inhibitors seem to be expressed in neurones that have re-entered the cell cycle [73–75], they are not efficient in preventing cell cycle progression (Fig. 3). The cause of this loss of function remains to be explained.

The consequence of the G1/S regulatory failure is DNA replication in the neuronal populations affected. But how accurate is DNA replication in ageing neurones? It is possible to envisage that the accumulated DNA damage would inevitably result in somatic mutations that would further impede neuronal function.

Most importantly the ultimate the proof of the theory will come only if it will translate into improved diagnostic protocols and therapeutic approaches. In Alzheimer's disease the parallel development of diagnostic and therapeutic is a bigger necessity than in most conditions.

The pathology responsible for the clinical syndrome, the accumulation of neurofibrillary tangles in neurones, is not reversible. By the time the functional deficits due to tangle accumulation are detected by conventional cognitive testing the pathology has spread to much of the limbic areas involving the entorhinal cortex and hippocampus [26, 76, 77]. Therefore a diagnosis based on overt clinical symptoms and signs is usually too late for any treatment to be effective. The hope of early detection by imaging techniques is also stifled by the late appearance of morphological signs of the pathology [76, 77]. In view of the relatively late appearance of symptoms and signs the diagnostic procedure for Alzheimer's disease that could allow efficient treatment would need to detect the causes or early pathogenic events of the disease in its pre-clinical stages. Furthermore,



**Fig. 3** Double immunohistochemistry for cyclin B and p57<sup>kip2</sup>. Double immunohistochemistry for Cyclin B (marker of the G2 phase, labelled with DAB in panel a.) and the cyclin dependent kinase inhibitor p57<sup>kip2</sup> (labelled with vector red in panel b.) illustrates the heterogeneous expression pattern of these inhibitor molecules. The single arrow indicates a pyramidal neurone that has cyclin B in the nucleus and weak p57 cytoplasmic staining. Small double arrows indicate a pyramidal cell that has cytoplasmic p57 without cyclin B expression. Single large arrowhead indicates a pyramidal cell that has no p57 associated with the cyclin B expression.

specific and sensitive diagnostic methods are the prerequisite of successful drug development.

### Cell cycle in the diagnosis of AD: possibilities and pitfalls

The identification of a two-stage process that leads to the formation of AD-related pathology gives scope for simpler diagnostic solutions. While the presence of either mitogenic factors or G1/S regulatory deficiency makes the development of AD in an individual possible, the presence of both could allow an almost certain prediction of AD will development.

The nature of factors that could contribute to morphodysregulation and the subsequent loss of differentiation control and cell cycle re-entry are far from elucidated. From the theory proposed by Arendt however, it is apparent that the healthy balance between synaptic plasticity and a differentiated state might be disturbed by any harmful burden that

requires synaptic plasticity [48] to compensate for the damage. In other words, any damage to the neuronal network that retains synaptic remodelling ability could represent a mitogenic stimulus and could potentially trigger the development of AD. The epidemiological studies searching for the risk factors for AD support this possibility. Most of the epigenetic risk factors so far identified do not cause an overwhelming diagnostic challenge and can either be treated or prevented. The diagnosis of thyroid disease, diabetes, vitamin deficiencies, cardio-vascular disease are a routine part of the recommended diagnostic routine already. Just as the identification of socio-environmental factors, depression and possible toxic or harmful influences such as alcohol and exposure to electromagnetic fields.

The identification of genetic risk factors that could lead to loss of differentiation and cell cycle re-entry will be a much bigger challenge. The list of genetic polymorphisms that affect synaptic plasticity, and are found to be associated with AD, is growing by the day (reviewed in [48]) and the cal-

culuation of a compound risk from genetic profiling would be an almost impossible task.

There are indications that the G1/S regulatory deficit is not restricted to neurones in Alzheimer patients. Epidemiological studies indicate that sporadic AD is associated with certain types of solid tumours [78]. Additionally cell cycle-related changes were observed in lymphocytes and fibroblasts of Alzheimer's disease patients [79]. It is possible to envisage that the detection of the G1/S regulatory failure in peripheral cells, such as lymphocytes might prove of diagnostic or predictive value.

The attempt to detect the Alzheimer-type G1/S regulatory deficit however requires complex experimental procedures with live cells. The complicated nature of such tests by definition precludes these procedures from widespread diagnostic use. To complicate matters, the proliferation capacity of peripheral cells changes with age and can be altered by the use of widely used over-the-counter drugs such as NSAIDS.

The identification of the genetic factors responsible for the G1/S regulatory failure could provide an answer to this predicament. It will, however, be a long and difficult process to identify all important factors from the multitude of regulatory proteins responsible for the regulation of the G1/S transition point [57, 80].

### Does the cell cycle theory provide a therapeutic option?

It would be the most exciting development in Alzheimer's research if a remedy will be found that could stop the cell cycle re-activation in neurones. At first glance the task would seem simple enough in the era of targeted cell cycle inhibitor drugs. At closer examination however, this approach to Alzheimer therapy will pose several problems.

Cell cycle inhibitor drugs will have many undesirable side effects, just like current cancer treatments. Side effects such as hair loss, gastro-intestinal disturbances and blood disorders will limit the long-term use of cell cycle inhibitors drugs. Additionally, more often than not, the drug-induced cell cycle arrest is followed by the death of the cell. This is helpful in the treatment of cancers but, obviously, it would be disastrous for the nervous system.

The idea of prevention of cell cycle re-entry seems more attractive, but since the process is so intimately coupled to synaptic remodelling and neuronal plasticity, the stabilisation of synapses could actually backfire.

There were also suggestions that the inhibition of individual components of the pathogenic machinery, such as the GSK3, might be a useful in preventing the cellular pathology associated with AD. Since GSK3 might represent part of the innate neuronal damage-control system in response to mitogenic stimulation [57], reducing GSK3 expression or activity might actually speed up the process fuelled by the MAP kinases and cdk2.

Prevention of oxidative stress as well as hormone replacement therapy intended to prevent cell cycle re-activation seems a feasible approach. The reputation of these approaches however, has been tarnished by unsuccessful clinical trials.

Obviously the development of a new therapeutic approach based on the cell cycle hypothesis will not be easy. Most probably the heterogeneity of the factors that precipitate and drive the cell cycle in different patients will also mean that there will be no single drug for Alzheimer's disease. The likely scenario will probably include several drugs, each of which will be useful for a subgroup of patients depending on the mitogenic factors that are responsible for the activation of the process and the nature of the G1/S regulatory failure.

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