Int. J. Exp. Path. (1999), **80,** 71–76

Current Status Review

The role of cell cycle-mediated events in Alzheimer's disease

ARUN K. RAINA*, MERVYN J. MONTEIRO†,
ANDREW MCSHEA‡ AND MARK A. SMITH*

**Institute of Pathology, Case Western Reserve University, Cleveland, Ohio, †Department of Molecular Biology & Biophysics and Neurology, University of Maryland, Baltimore, Maryland and ‡Fred Hutchinson, Cancer Research Center, Seattle, Washington, USA*

Summary. The mechanism(s) underlying selective neuronal death in Alzheimer's disease remain unresolved. However, recently, we and others showed that susceptible hippocampal neurones in Alzheimer's disease express markers common to cells in various phases of the cell cycle. Since neuronal maturation is associated with effective escape from the cell division cycle, emergence out of quiescence may be deleterious. Here, we review a number of current findings indicating that disregulated ectopic re-activation of cell cycle-mediated events, akin to neoplasia, represent an important early pathway associated with neuronal death and, more importantly, one that involves virtually the entire spectrum of the pathological events described in Alzheimer's disease.

Keywords: Alzheimer's disease, APP, mitosis, neoplasia, oxidative stress, presenilin

Recent observations demonstrate evidence of attempted re-entry into the cell division cycle in neurones in Alzheimer's disease (AD). Progression through the cell cycle is associated with many features associated with AD including the activation of signal transduction pathways, cell phase dependent kinases and transcriptional activation, that leads to cytoskeletal alterations and increases in mitochondrial metabolic activity and DNA replication. Rather than completing the cell cycle, we propose that there is an abortive progression that has been shown to be related to cellular dysfunction, oxidative stress, a possible protective response to apoptosis, end stage phenomena and neuronal death.

Cell cycle abnormalities in Alzheimer's disease

Adult neurones are generally thought as being effectively precluded from division and reside in a state of terminal differentiation. Thus, it is surprising that vulnerable neurones in AD, a neurodegenerative condition characterized by selective neuronal death, have certain phenotypic markers reminiscent of a cycling, rather than a quiescent nondividing, cell (Smith & Lippa 1995; Arendt *et al.* 1996, 1998; Vincent *et al.* 1996, 1997; McShea *et al.* 1997; 1999a,b). Though terminally differentiated neurones may lack the ability to complete the cell cycle, they may be able to proceed variously through to a point prior to the actual event of cellular division. Thus one might expect to observe a characteristic 'molecular phenotype' representing a neurone arrested in a transitional phase of the cycling process. Indeed, the presence of growth promoting genes such as CDK4 (Arendt *et al.* 1996; McShea *et al.* 1997) and CDK2 (A. K. Raina and M. A. Smith, unpublished observation) imply that vulnerable neurones in AD have emerged from quiescence. Additionally, the presence of p21, highly phosphorylated protein, Ki67, p107, mpm-2 other cell cycle-related proteins (Baumann *et al.* 1993; Nagy *et al.* 1997a,b; A. K. Raina and M. A. Smith, unpublished observation) suggest that the vulnerable neurones may be attempting to

Correspondence: Dr Mark A. Smith, Institute of Pathology, Case Western Reserve University, 2085 Adelbert Road, Cleveland, Ohio 44106, USA. Fax: +1 (216) 368 8964; E-mail: mas21@-po.cwru.edu

initiate the early phases of mitosis. However, as yet, there is no evidence suggesting a successful nuclear division nor chromosomal condensation in AD implying that the neurones do not complete mitosis (M-phase) and that the proteins associated with exit from the cell cycle such as p16 are apparently upregulated (McShea *et al.* 1997).

Cell cycle, τ, phosphorylation and NFT

The increased phosphorylation status of the microtubule-associated protein τ associated with the genesis of neurofibrillary tangles is a poorly understood phenomena (reviewed in Bramblett *et al.* 1993). In fact, similar, if not identical, forms of phosphorylated τ proteins are generated in mitotic cells (Brion *et al.* 1985, 1994). This mitotic τ phosphorylation is mediated by the primary regulators of cell-cycle progression, i.e. cyclin dependent kinases (CDKs), and it is of note that several of these CDKs have been localized *in vivo* in AD and phosphorylate τ *in vitro* similarly to that found *in vivo* (Freeman *et al.* 1994; Arendt *et al.* 1995, 1996; Gartner *et al.* 1995; Alcorta *et al.* 1996). Increased phosphorylation during mitosis coincides with microtubule reorganization and it is therefore perhaps not surprising that AD is invariantly associated with microtubular abnormalities. Furthermore, taxanes, i.e. taxol that stabilize polymeric tubulin result in an increase in mitotic specific phosphoepitopes, due to growth arrest in a phase of the cell cycle just prior to mitosis, induction of p21 and the initiation of chromosomal condensation, i.e. features shared with neurones in AD.

Oxidative stress in Alzheimer's disease

There is a significant body of evidence that the degeneration of senescent neurones is associated with oxidative stress (e.g. Smith *et al.* 1994a,b, 1996, 1997). The brain responds to oxidative assaults in part through the activation of NF-κB (Schreck *et al.* 1991) and by the induction of specific antioxidant enzyme systems. For example, haem oxygenase-1 (HO-1) is associated with degenerating neurones (Smith *et al.* 1994b; Castellani *et al.* 1995, 1996) and specific induction of HO-1 (an inducible isoform), but not HO-2 (a constitutively produced, noninducible isoform), parallels the regional susceptibility seen in neuronal degeneration (Premkumar *et al.* 1995). Interestingly, and suggestive of a mechanistic connection, markers against cytoskeletal alterations (i.e. abnormal τ), oxidative stress (i.e. HO-1) and cell cycle alterations (e.g. p16) all show complete overlap in degenerating neurones in AD (McShea *et al.* 1997).

Cell cycle and oxidative stress

The suggestion that cell cycle abnormalities are linked to alteration of the redox state (via oxidative or even reductive stress) find support from the following studies. First, free radicals and free-radical generators delay cell replication time (Curcio & Ceriello 1992) whereas, N-acetylcysteine, an antioxidant, suppresses proliferation and DNA synthesis of PC12 cells (Ferrari *et al.* 1995). Second, accumulation of p53 is synchronous with oxidative stress and causes blockage of the cell cycle at G1 (reviewed in Stewart 1994; Sugano *et al.* 1995). Third, prior to mitosis, there is both division of organelle nucleoids and organellokinesis such that during late S, G2 and mitotic phases, mitochondrial proliferation is most evident (Barni *et al.* 1996). While these events are critical for the high energy demands required for actual cell division, in cells where the cell cycle is interrupted, such excess mitochondrial concentrations represent sources of varied homeostatic imbalances, especially those of calcium (Sousa *et al.* 1997) and the redox state. Notably, there are significance increases in mitochondria in vulnerable neurones in AD (Hirai *et al.* 1998) and such neuronal populations are the same as reported to have cell cycle abnormalities and consequent oxidative damage in AD. Therefore, it seems likely that arrest of the cell cycle, at a point where mitochondrial mass is highest, poses an elevated and possibly a chronic oxidative threat to the cell, far beyond the blunting capacity of endogenous antioxidants.

APP, presenilins and cell cycle regulation

Genetic-linkage studies have established that mutations in at least three genes, the amyloid precursor protein (APP) located on chromosome 21, and the two homologous genes, presenilin 1 and presenilin 2 (PS1 and PS2) located on chromosome 14 and 1, respectively, are associated with early onset AD (reviewed by Hardy 1997). The exact mechanisms by which mutations in these genes are involved in AD etiopathogenesis has not been resolved. However, one hypothesis that is gaining increasing support is that the mutations in each of the three genes predispose cells to apoptosis. Since progression of the cell cycle and apoptosis are thought to be intimately linked (Evan *et al.* 1995; Meikrantz & Schlegel 1995; Evan & Littlewood 1998) this could in part account for the ectopic expression of cell cycle regulated proteins as discussed above.

The expression, phosphorylation, and metabolism of APP, have all been shown to be cell-cycle related

(Suzuki *et al*. 1994; Oishi *et al*. 1997). Therefore, any perturbation of the cell division cycle, as appears occurs in AD, could lead to misregulated expression of APP, including the generation of subsequent proteolytic products. The precise function of APP is not known. APP is a single pass transmembrane protein expressed at the cell surface, with a short C-terminal region located in the cytoplasm (reviewed by Selkoe 1996). APP is proteolytically cleaved by unidentified proteases, resulting in the accumulation and aggregation of proteolytic fragments (of varying length, between 1 and 43 residues) into extracellular senile plaques which are a characteristic lesion of AD-affected brains (reviewed by Hardy 1997). The relationship between presence of these so-called amyloid-$\beta\gamma$ (Aβ) plaques and their toxicity in AD is long-standing, but controversial, subject (see Hardy 1997; Neve & Robakis 1998). Recently new evidence obtained by Yamatsuji *et al*. (1996) has suggested that mutations in APP proteins increase apoptosis of neuronal cells by G-protein signalling.

Human presenilin (PS) proteins 1 and 2 are approximately 67% homologous, and although their function is unknown, recent studies have also implicated the proteins in regulation of apoptosis and in development. Mice disrupted in the PS1 gene die at birth and show defects in the central nervous system and axial skeleton (Shen *et al*. 1997; Wong *et al*. 1997). Interestingly, the Sel-12 gene of *Caenorhabditis elegans*, which is homologous to human presenilins, is involved in Notch-based signalling indicating that presenilins may play a role in cell fate determination (Levitan & Greenwald 1995). The PS proteins are expressed at very low levels which has lead to difficulties in determining their localization in cells. Nonetheless, the majority of reports indicate that presenilins are localized to the endoplasmic reticulum (ER) and Golgi, consistent with secondary structure predictions of a multiple transmembrane protein (see Kovacs *et al*. 1996; Janicki & Monteiro 1997). However, some reports have indicated that presenilins are localized to the cell surface (Dewji & Singer 1997) and also to the centromere and centrosome of dividing cells (Li *et al*. 1997). The binding of presenilins to centromeres and centrosomes have led to speculations that presenilins are involved in chromosome segregation and cell division, although direct evidence for this role has not been obtained so far.

There is substantial evidence that presenilins are involved in the regulation of apoptosis (Deng *et al*. 1996; Guo *et al*. 1996, 1997; Vito *et al*. 1996a,b; Wolozin *et al*. 1996, 1998; Janicki & Monteiro 1997; Zhang *et al*. 1998). Overexpression of presenilins in terminally differentiated neuronal cells results in perturbation of calcium homeostasis, oxidative stress, and increased vulnerability to apoptosis induced by a variety of agents (reviewed by Mattson *et al*. 1998). More interestingly, a common aspect of the transgenic expression of different FAD presenilin mutations is that they all increase sensitivity of cells to apoptosis compared to the expression of wild type presenilin genes. A similar increase in apoptosis is found following overexpression of the FAD PS2(N141I) mutant compared to wild type presenilin 2 in dividing HeLa cells (Janicki & Monteiro 1997), suggesting that the mechanisms of presenilin induction of apoptosis in dividing cells and postmitotic neurones may be similar. More recently, by BrdU labelling studies of transfected HeLa cells, we have shown that the overexpression of both presenilin 1 and 2 proteins results in G1 phase arrest of the cell cycle (S. Janicki and M. J. Monteiro, unpublished observation), consistent with the well established notion that apoptosis is preceded by cell cycle arrest (reviewed by Elledge 1996). Cell cycle arrest induced by PS overexpression is potentiated upon expression of the FAD PS2(N141I) mutation compared to wild type PS2 (S. Janicki and M. J. Monteiro unpublished observation). The potentiation of cell cycle arrest by the FAD PS2 mutant is revealing since it might have been predicted that such a mutation would decrease cell cycle arrest to account for the increased reexpression of cell cycle regulated proteins in AD. Although this possibility is still not excluded by the transfection results in HeLa cells, we suggest alternative explanations.

One possibility we propose is that in AD, neurones are attempting to re-enter the cell cycle but are blocked from entry at the G1/S phase of the cell cycle by presenilin (and possibly APP) mutations. This blockage at G1 would result in accumulation of cell cycle proteins as is seen in AD. Another possibility is that presenilin and APP mutations induce proapoptotic cascades which result in the upregulation of cyclin-dependent kinases, since the latter appear to play dual roles in controlling cell proliferation as well as in death signalling (see Park *et al*. 1998). The induction of cyclin-dependent kinase inhibitors, such as p16, p19, p21 and p27, in AD may reflect a protective response elicited by the cells to these death stimuli. There is good evidence for upregulation of cyclin dependent kinases in cultured neurones upon insult by a number of apoptotic inducing agents (Park *et al*. 1998). Interestingly, p16, p21, and p27, which are inhibitors of CDK4 and CDK6 kinases protect neurones from these apoptotic insults (Park *et al*. 1998), and many of these same proteins are reexpressed in AD neurones (Ledesma *et al*. 1992; Smith & Lippa 1995; Ardent *et al*. 1995, 1996; Vincent *et al*. 1996, 1997; Nagy *et al*. 1997a,b; McShea *et al*. 1997, 1999a,b).

Cell cycle, growth factors, and oncogenic stimulation

Given the apparent importance of mitotic mechanisms in AD, one is left with a key remaining question, namely to determine what factor(s) stimulate the neurones to re-enter the cell division cycle. A likely explanation is the inappropriate expression, or release, of a growth or cellular differentiation factor(s), and, in this regard, it is noteworthy that elevated levels of nerve growth factor (NGF), transforming growth factor-1 (TGF) and basic fibroblast growth factor (bFGF) are found in AD (Gomez-Pinilla *et al.* 1990; Crutcher *et al.* 1993). Indeed, in AD, there are significant alterations in signal transducing pathways that respond to growth factor stimulation such as the ras and mitogen-activated protein kinase pathways (McShea *et al.* 1999a). More importantly, signalling leading to emergence from quiescence, increased phosphorylation of τ, re-expression variously of a developmental phenotype, and dysregulation of cell-matrix homeostasis (M. A. Smith, unpublished observation), is in effect homologically similar to proximal events associated with neoplastic transformation. Indeed, ectopic mitogenic signalling is frequently associated with early events in neoplasia lending the possibility that a neoplastic-type transformation occurs in neurones in AD.

Conclusions

In summary, mitotic neurones and degenerating neurones in AD, effectively share a cell-cycle related phenotype. Whether re-entry into the cell cycle reflects a primary pathogenic pathway or, is instead, a protective response to cell death stimuli, remains to be determined.

Acknowledgements

Work in the authors' laboratories is supported by funding from the Alzheimer's Association (MAS) and by the NIH/NIA (MAS and MJM).

References

Alcorta D.A., Xiong Y., Phelps D., Hannon G., Beach D. & Barrett J.C. (1996) Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. *Proceedings Natl. Acad. Sci. USA* **93,** 13742–13747.

Arendt T., Holzer M. & Gartner U. (1998) Neuronal expression of cycline dependent kinase inhibitors of the INK4 family in Alzheimer's disease. *J. Neural Transm.* **105,** 949–960.

Arendt T., Holzer M., Grossmann A., Zedlick D. & Bruckner M.K. (1995) Increased expression and subcellular translocation of the mitogen activated protein kinase and mitogen-activated protein kinase in Alzheimer's disease. *Neuroscience* **68,** 5–18.

Arendt T., Rodel L., Gartner U. & Holzer M. (1996) Expression of the cyclin-dependent kinase inhibitor p16 in Alzheimer's disease. *Neuroreport* **7,** 3047–3049.

Barni S., Sciola L., Spano A. & Pippia P. (1996) Static cytofluorometry and fluorescence morphology of mitochondria and DNA in proliferating fibroblasts. *Biotech. Histochem.* **71,** 66–70.

Baumann K., Mandelkow E.M., Biernat J., Piwnica-Worms H. & Mandelkow E. (1993) Abnormal Alzheimer-like phosphorylation of tau-protein by cyclin-dependent kinases cdk2 and cdk5. *FEBS Lett.* **336,** 417–424.

Bramblett G.T., Goedert M., Jakes R., Merrick S.E., Trojanowski J.Q. & Lee V.M.-Y. (1993) Abnormal tau phosphorylation at Ser^{396} in Alzheimer's disease recapitulates development and contributes to reduced microtubule bindings. *Neuron* **10,** 1089–1099.

Brion J.P., Octave J.N. & Couck A.M. (1994) Distribution of the phosphorylated microtubule-associated protein tau in developing cortical neurons. *Neuroscience* **63,** 895–909.

Brion J.P., Passarier H., Nunez J. & Flament-Durand J. (1985) Immunologic determinants of tau protein are present in neurofibrillary tangles of Alzheimer's disease. *Arch. Biol.* **95,** 229–235.

Castellani R., Smith M.A., Richey P.L., Kalaria R., Gambetti P. & Perry G. (1995) Evidence for oxidative stress in Pick disease and corticobasal degeneration. *Brain Res.* **696,** 268–271.

Castellani R., Smith M.A., Richey P.L. & Perry G. (1996) Glycoxidation and oxidative stress in Parkinson disease and diffuse Lewy body disease. *Brain Res.* **737,** 195–200.

Crutcher K.A., Scott S.A., Liang S., Everson W.V. & Weingartner J. (1993) Detection of NGF-like activity in human brain tissue: increased levels in Alzheimer's disease. *J. Neurosci* **13,** 2540–2550.

Curcio F. & Ceriello A. (1992) Decreased cultured endothelial cell proliferation in high glucose medium is reversed by antioxidants: new insights on the pathophysiological mechanisms of diabetic vascular complications *in vitro*. *Cell. Dev. Biol.* **28A,** 787–790.

Deng G., Pike C.J. & Cotman C.W. (1996) Alzheimer-associated presenilin-2 confers increased sensitivity to apoptosis in PC12 cells. *FEBS Lett.* **397,** 50–54.

Dewji N.N. & Singer S.J. (1997) Cell surface expression of the Alzheimer disease-related presenilin proteins. Proceedings. *Natl. Acad. Sci. USA* **94,** 9926–9931.

Elledge S.J. (1996) Cell cycle checkpoints: Preventing an identity crisis. *Science* **274,** 1664–1672.

Evan G.I., Brown L., Whyte M. & Harrington E. (1995) Apoptosis and the cell cycle. *Curr. Opin. Cell Biol.* **7,** 825–834.

Evan G. & Littlewood T. (1998) A matter of life and cell death. *Science* **281,** 1317–1322.

Ferrari G., Yan C.Y. & Greene L.A. (1995) N-acetylcysteine (D- and L-stereoisomers) prevents apoptotic death of neuronal cells. *J. Neurosci.* **15,** 2857–2866.

Freeman R.S., Estus S. & Johnson E.M. Jr (1994) Analysis of cell cycle-related gene expression in postmitotic neurons: selective induction of Cyclin D1 during programmed cell death. *Neuron* **12,** 343–355.

Gartner U., Holzer M., Heumann R. & Arendt T. (1995) Induction of p21ras in Alzheimer pathology. *Neuroreport* **6,** 1441–1444.

Gomez-Pinilla F., Cummings B.J. & Cotman C.W. (1990) Induction

of basic fibroblast growth factor in Alzheimer's disease pathology. *Neuroreport* **1,** 211–214.

Guo Q., Furukawa K., Sopher B.L. *et al.* (1996) Alzheimer's PS-1 mutation perturbs calcium homeostasis and sensitizes PC12 cells to death induced by amyloid beta-peptide. *Neuroreport* **8,** 379–383.

Guo Q., Sopher B.L., Furukawa K. *et al.* (1997) Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid beta-peptide: involvement of calcium and oxyradicals. *J. Neurosci.* **11,** 4212–4222.

Hardy J. (1997) Amyloid, the presenilins and Alzheimer's disease *Trends Neurosci.* **20,** 155–159.

Hirai K., Smith M.A., Wade R. & Perry G. (1998) Vulnerable neurons in Alzheimer disease accumulate mitochondrial DNA with the common 5kb deletion. *J. Neuropathol. Exp. Neurol.* **57,** 511.

Janicki S. & Monteiro M.J. (1997) Increased apoptosis arising from increased expression of the Alzheimer's disease-associated presenilin-2 mutation (N141I). *J. Cell Biol.* **139,** 485–495.

Kovacs D.M., Fausett H.J., Page K.J. *et al.* (1996) Alzheimer-associated presenilins 1 and 2: neuronal expression in brain and localization to intracellular membranes in mammalian cells. *Nature Med.* **2,** 224–229.

Ledesma M.D., Correas I., Avila J. & Diaz-Nido J. (1992) Implication of brain cdc2 and MAP2 kinases in the phosphorylation of tau protein in Alzheimer's disease. *FEBS Lett.* **308,** 218–224.

Levitan D. & Greenwald I. (1995) Facilitation of lin-12-mediated signaling by sel-12, a Caenorhabditis elegans S182 Alzheimer's disease gene. *Nature* **377,** 351–354.

Li J., Xu M., Zhou H., Ma J. & Potter H. (1997) Alzheimer presenilins in the nuclear membrane, interphase kinetochores, and centrosomes suggest a role in chromosome segregation. *Cell* **90,** 917–927.

Mattson M.P., Guo Q., Furukawa K. & Pedersen W.A. (1998) Presenilins, the endoplasmic reticulum, and neuronal apoptosis in Alzheimer's disease. *J. Neurochem.* **70,** 1–14.

McShea A., Harris P.L.R., Webster K.R., Wahl A.F. & Smith M.A. (1997) Abnormal expression of the cell cycle regulators p16 and CDK4 in Alzheimer's disease. *Am. J. Pathol.* **150,** 1933–1939.

McShea A., Zelasko D.A., Gerst J.L. & Smith M.A. (1999a) Signal transduction abnormalities in Alzheimer's disease: evidence of a pathogenic stimuli. *Brain Res.* **815,** 237–242.

McShea A., Wahl A.F. & Smith M.A. (1999b) Re-entry into the cell cycle: a mechanism for neurodegeneration in Alzheimer disease. *Med. Hypotheses* in press.

Meikrantz W. & Schlegel R. (1995) Apoptosis and the cell cycle. *J. Cell. Biochem.* **58,** 160–174.

Nagy Z., Esiri M.M. & Smith A.D. (1997a) Expression of cell division markers in the hippocampus in Alzheimer's disease and other neurodegenerative conditions. *Acta Neuropathol.* **93,** 294–300.

Nagy Z., Esiri M.M., Cato A.M. & Smith A.D. (1997b) Cell cycle markers in the hippocampus in Alzheimer's disease. *Acta Neuropathol.* **94,** 6–15.

Neve R.L. & Robakis N.K. (1998) Alzheimer's disease: a re-examination of the amyloid hypothesis. *Trends Neurosci.* **21,** 15–19.

Oishi M., Nairn A.C., Czernik A.J. *et al.* (1997) The cytoplasmic domain of Alzheimer's amyloid precursor protein is phosphorylated at Thr654, Ser655, and Thr668 in adult rat brain and cultured cells. *Mol. Med.* **3,** 111–123.

Park D.S., Morris E.J., Padmanabhan J., Shelanski M.L., Geller H.M. & Greene L.A. (1998) Cyclin-dependent kinases participate in death of neurons evoked by DNA-damaging agents. *J. Cell Biol.* **143,** 457–467.

Premkumar D.R.D., Smith M.A., Richey P.L. *et al.* (1995) Induction of heme oxygenase-1 mRNA and protein in neocortex and cerebral vessels in Alzheimer's disease. *J. Neurochem.* **65,** 1399–1402.

Schreck R., Rieber P. & Baeuerle P.A. (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO J.* **10,** 2247–2258.

Selkoe D.J. (1996) Amyloid á-protein and the genetics of Alzheimer's disease. *J. Biol. Chem.* **271,** 18295–18298.

Shen J., Bronson R.T., Chen D.F., Xia W., Selkoe D.J. & Tonegawa S. (1997) Skeletal and CNS defects in Presenilin-1-deficient mice. *Cell* **89,** 629–639.

Smith M.A., Taneda S., Richey P.L. *et al.* (1994a) Advanced Maillard reaction end products are associated with Alzheimer disease pathology. Proceedings. *Natl. Acad. Sci. USA* **91,** 5710–5714.

Smith M.A., Kutty R.K., Richey P.L. *et al.* (1994b) Heme oxygenase-1 is associated with the neurofibrillary pathology of Alzheimer's disease. *Am. J. Pathol.* **145,** 42–47.

Smith T.W. & Lippa C.F. (1995) Ki-67 immunoreactivity in Alzheimer's disease and other neurodegenerative disorders. *J. Neuropathol. Exp. Neurol.* **54,** 297–303.

Smith M.A., Perry G., Richey P.L. *et al.* (1996) Oxidative damage in Alzheimer's. *Nature* **382,** 120–121.

Smith M.A., Harris P.L.R., Sayre L.M., Beckman J.S. & Perry G. (1997) Widespread peroxynitrite-mediated damage in Alzheimer's disease. *J. Neurosci.* **17,** 2653–2657.

Sousa M., Barros A., Silva J. & Tesarik J. (1997) Developmental changes in calcium content of ultrastructurally distinct subcellular compartments of preimplantation human embryos. *Mol. Human Reprod.* **3,** 83–90.

Stewart B.W. (1994) Mechanisms of apoptosis: integration of genetic, biochemical, and cellular indicators. *J. National Cancer Inst.* **86,** 1286–1296.

Sugano T., Nitta M., Ohmori H. & Yamaizumi M. (1995) Nuclear accumulation of p53 in normal human fibroblasts is induced by various cellular stresses which evoke the heat shock response, independently of the cell cycle. *Japanese J. Cancer Res.* **86,** 415–418.

Suzuki T., Oishi M., Marshak D.R., Czernik A.J., Nairn A.C. & Greengard P. (1994) Cell cycle-dependent regulation of the phosphorylation and metabolism of the Alzheimer amyloid precursor protein. *EMBO J.* **13,** 1114–1122.

Vincent I., Rosado M. & Davies P. (1996) Mitotic mechanisms in Alzheimer's disease?. *J. Cell Biol.* **132,** 413–425.

Vincent I., Jicha G., Rosado M. & Dickson D.W. (1997) Aberrant expression of mitotic Cdc2/Cyclin B1 kinase in degenerating neurons of Alzheimer's disease brain. *J. Neurosci.* **17,** 3588–3598.

Vito P., Wolozin B., Ganjei J.K., Iwasaki K., Lacana E. & D'Adamio L. (1996a) Requirement of the familial Alzheimer's disease gene PSo**2**: for apoptosis. Opposing effect of ALG-3. *J. Biol. Chem.* **271,** 31025–31028.

Vito P., Lacana E. & D'Adamio L. (1996b) Interfering with apoptosis: Ca (2+) -binding protein ALG-2 and Alzheimer's disease gene ALG-3. *Science* **271,** 521–525.

Wolozin B., Iwasaki K., Vito P. *et al.* (1996) Participation of

presenilin 2 in apoptosis: enhanced basal activity conferred by an Alzheimer mutation. *Science* **274,** 1710–1713.

WOLOZIN B., ALEXANDER P. & PALACINO J. (1998) Regulation of apoptosis by presenilin 1. *Neurobiol. Aging* **19,** S23–S27.

WONG P.C., ZHENG H., CHEN H. *ET AL.* (1997) Presenilin 1 is required for Notch1 and DII1 expression in the paraxial mesoderm. *Nature* **387,** 288–292.

YAMATSUJI T., MATSUI T., OKAMOTO T. *ET AL.* (1996) G protein-mediated neuronal DNA fragmentation induced by familial Alzheimer's disease-associated mutants of APP. *Science* **272,** 1349–1352.

ZHANG Z., HARTMANN H., DO V.M. *ET AL.* (1998) Destabilization of β-catenin by mutations in presenilin-1 potentiates neuronal apoptosis. *Nature* **395,** 698–702.