Alzheimer's disease

In search of γ-secretase

John Hardy and Alain Israël

When the race was on to identify the genes on chromosomes 14 and 1 which, when they contain mutations, lead to early-onset Alzheimer's disease, it was generally thought that they would encode enzymes involved in the processing of the β-amyloid precursor protein (APP). It is a derivative of APP, the neurotoxic peptide Aβ, that is implicated as the main agent of the disease. When candidate genes, called the presenilins, were cloned, their only known protein homologues were apparently involved in vesicle transport or the Notch developmental pathway in the nematode *Caenorhabditis elegans*. Subsequent work in transgenic mice and transfected cells, however, soon showed that mutations in presenilins all altered APP processing and caused increased levels of the 42-amino-acid β-amyloid derivative Aβ42. (See ref. 1 for references.)

Since then there has been intense interest in the relationship between Aβ production, the presenilins, the Notch pathway, vesicle transport and Alzheimer's disease. The latest findings appear in four papers in this issue[2–5], with further results being reported at a Keystone meeting in March. These studies do not demonstrate that Notch signalling is directly relevant to Alzheimer's disease. But they do show that presenilins regulate both APP processing and Notch signalling by influencing unusual protein cleavage events.

Receptors of the Notch family mediate cell–cell interactions that specify cell fate during development. They are large, single-pass transmembrane proteins (Fig. 1) and their ligands are expressed at the surface of neighbouring cells. How Notch signalling results in transcriptional activation of its target genes remains a mystery, but one model has it that proteolytic cleavage of Notch results in release of the intracellular domain[6–9], which translocates to the nucleus and associates with a DNA-binding subunit. A processing step responsible for releasing the intracellular domain takes place in or near the transmembrane domain[6]. So Notch undergoes proteolytic events that resemble those involved in cleavage of APP (where first α- or β-secretase, and subsequently γ-secretase activities, operate; Fig. 1).

Genetic evidence that presenilins are also involved in Notch signalling comes from both *C. elegans* and mice. In *C. elegans*, the presenilin gene *sel-12* facilitates the activity of the two Notch genes *lin-12* and *glp-1*, and reducing *sel-12* activity suppresses the effect of increased *lin-12* activity[10]. In mice, inactivation of presenilin-1 results in phenotypes associated with reduced Notch activity[11]. Finally, co-localization of Notch and presenilin in embryos of the fruitfly *Drosophila*[12], as well as a direct physical interaction between presenilins and Notch, has been observed in mammalian and *Drosophila* cells[13]. However, the exact role of presenilins in the Notch pathway has remained elusive, and these proteins have been tentatively attributed functions in either Notch processing directly, or in trafficking Notch to the correct compartment for that processing to occur.

The new work[2–5] emphasizes the connection between Notch and presenilins, and indicates that presenilins are required for the production of Notch proteolytic products implicated in signalling. Ye and colleagues (page 525)[2], and Struhl and Greenwald (page 522)[3] show that loss-of-function mutations in the *Drosophila* presenilin gene exhibit a lethal Notch-like phenotype. The amount and subcellular localization of Notch seems to be identical in wild-type and mutant embryos, implying that the defects actually result from the absence of Notch signalling activity.

Ye and colleagues analyse the Notch species in wild-type and mutant embryos, and conclude that some proteolytic cleavage event giving rise to low-molecular-weight, Notch-derived fragments is inhibited in the absence of presenilin. Struhl and Greenwald reach a similar conclusion. They introduce a Notch-derived transgene into *Drosophila*, one engineered so that activation-associated processing followed by nuclear translocation of the intracellular part of the receptor can provide a direct colorimetric assay for signalling[7]; in the absence of presenilin, no nuclear Notch activity is observed.

De Strooper *et al.* (page 518)[4] investigate the effect of presenilin on Notch processing by introducing a constitutively active form of murine Notch-1 (ΔE; see Fig. 1) into fibroblasts derived from presenilin-1-knockout mice. This construct had been previously used to identify a proteolytic cleavage site located in or near the transmembrane region of Notch. This site is constitutively cleaved in the ΔE construct, and ligand-inducibly cleaved in the full-length

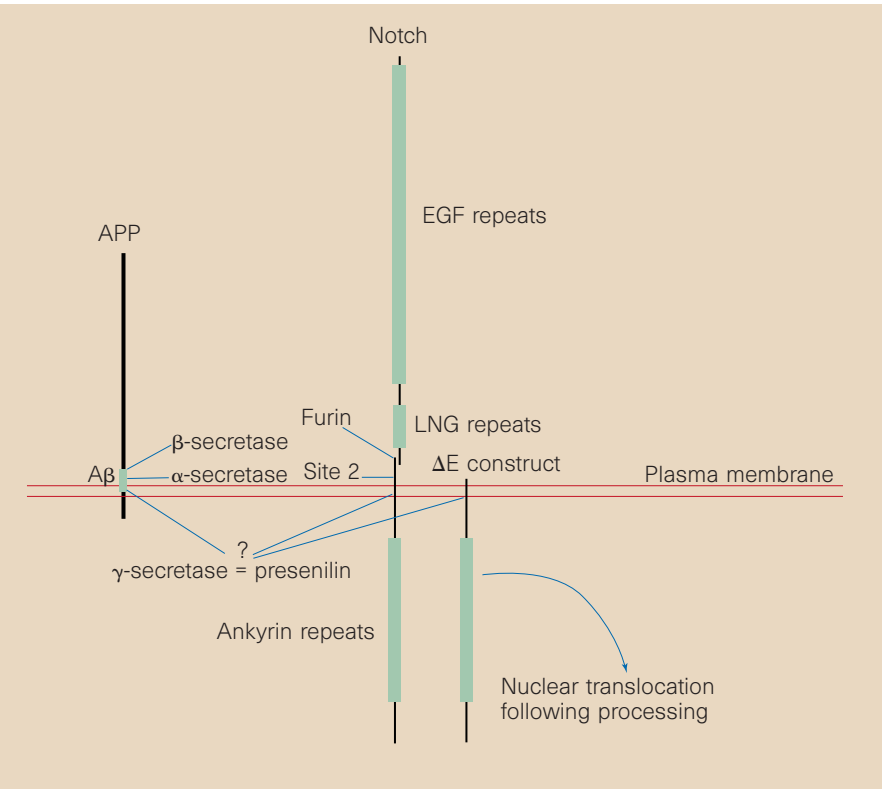

Figure 1 **Proteolytic steps involved in the maturation of β-amyloid precursor protein (APP) and Notch. APP is cleaved at either of two extracellular sites by α- or β-secretase, followed by intramembrane cleavage by γ-secretase. Successive cleavages by β- and γ-secretases release the amyloid peptide (Aβ), which lies between these two cleavage sites. Notch is first cleaved by furin in the trans-Golgi network: the two resulting fragments remain associated at the plasma membrane. A putative ligand-induced second cleavage is indicated (site 2). The last cleavage[6] releases the intracellular domain of the receptor, which translocates to the nucleus to activate transcription. The constitutively active ΔE construct discussed in the text is also shown. Three types of repeats present in the Notch molecules — epidermal growth factor (EGF), Lin-12/Notch/Glp-1 (LNG) and ankyrin — are indicated.**

receptor, and mutation of the site reduces cleavage and abolishes signalling[6]. Interestingly, this processing does not take place in presenilin-1-knockout cells. In addition, the processing of the ΔE construct is inhibited by a γ-secretase inhibitor designed from the relevant APP sequence[4,5]. Struhl and Greenwald similarly demonstrate that a ΔE-like construct is unable to signal in the absence of presenilin

So these three groups[2–4] all conclude that presenilin is required for release of the intracellular domain of Notch from the plasma membrane. Struhl and Greenwald, and De Strooper *et al.*, however, propose that presenilin-1 acts by facilitating the activity of the protease concerned or is the protease itself. This hypothesis is also adopted by Wolfe and colleagues (page 513)[5], who suggest that presenilin-1 might be able to cleave both APP and Notch. Their work involved mutagenesis of two transmembrane aspartate residues in presenilin-1, which completely abolished APP cleavage. (At the Keystone meeting, C. Haass reported similar data from mutagenesis of presenilin-2; the relevant aspartate residues are conserved in all known presenilins.) Wolfe *et al.* propose that these aspartates are the active site of proteases that can cleave APP or Notch inside the membrane.

The results of the mutagenesis experiments are provocative, but they do not conclusively show whether the presenilins are important in trafficking or in cleavage. They could be causing defects upstream of the cleavage events, either by altering trafficking of the substrates (APP and Notch), or by altering trafficking or activation of the protease or proteases involved in γ-secretase-type cleavages. Indeed, alteration of substrate trafficking is the interpretation favoured by Ye and colleagues[2]. They use a *Drosophila* Notch-derived construct similar to ΔE, which — contrary to the results of Struhl and Greenwald — they find can still signal in the absence of presenilin. So Ye *et al.* conclude that presenilin acts upstream of this processing step.

In this context, it is notable that, in humans, the apolipoprotein E genotype modulates the age of onset of Alzheimer's disease encoded by APP-717 mutations (which are believed specifically to affect γ-secretase cleavage) but not that of presenilin-encoded disease[14,15]. From this it would seem that these events are genetically distinct, a conclusion supported by other lines of evidence. At Keystone, R. Nixon reported that presenilin and APP mutations have different effects on the vesicular trafficking of APP; the function of *spe-4*, the 'forgotten' presenilin, seems more closely connected to vesicle trafficking in *C. elegans* testes than to proteolysis[16]; and most (but not all) studies in mammalian cells indicate that presenilins are located in the endoplasmic reticulum or in the Golgi, yet the processing events described above probably occur at the plasma membrane.

Nonetheless, the case is far from nailed either way. Both views remain viable — that presenilins are indeed γ-secretase, or that they instead directly traffic APP and Notch to the right cellular compartment for γ-secretase processing. Direct biochemical experiments will be required to distinguish between them.

Finally, a word of caution. Drugs targeting γ-secretase are seen as a possible treatment for Alzheimer's disease. Given the emerging role of Notch in the haematopoietic system[17], however, and the firm implication that Notch and APP may be processed by the same cellular machinery, such drugs may have unwanted immunosuppressive effects. □

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Supercooled water

Going strong or falling apart?

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For a tiny, almost spherical molecule made of just three atoms, water exhibits remarkably rich behaviour. In the crystalline state, water exists in at least 12, and possibly 14, forms of ice. Echoes of this polymorphism are to be found in the amorphous state as well. When prepared as an amorphous solid, or glass, by ultrafast cooling or by depositing vapour on a cold substrate, water is found in two forms: a high-density and a low-density form[1]. Similarly, it has been proposed that liquid water exists in two corresponding forms at low temperatures, as a result of a novel liquid–liquid phase transition[2]; however, decisive evidence for this transition is still lacking.

Not only can water exist in two glassy forms, but also in its approach to the glassy state, it appears to exhibit two drastically different behaviours. On page 492 of this issue, Ito, Moynihan and Angell[3] describe evidence that water changes character, from being a highly 'fragile' liquid at temperatures above 236 K to a 'strong' liquid near 136 K, its glass-transition temperature at atmospheric pressure (Fig. 1). They reach this conclusion by comparing kinetic and thermodynamic measures of a liquid's 'fragility', which are found to be consistent for all liquids they consider, except water.

In order to form a glass, a liquid has to be cooled below its freezing temperature, but without allowing crystallization to occur. A liquid 'supercooled' in this manner grows progressively more viscous. Viscosity controls how fast a liquid relaxes to equilibrium when it is perturbed; for example, when its temperature is altered. When the viscosity grows sufficiently large, to 10^{13} Pa s, the liquid 'falls out of equilibrium' and is trapped into a solid, but microscopically disordered, state — the glass. Yet the high viscosity at the glass transition is reached differently by different liquids. When displayed on an Arrhenius plot

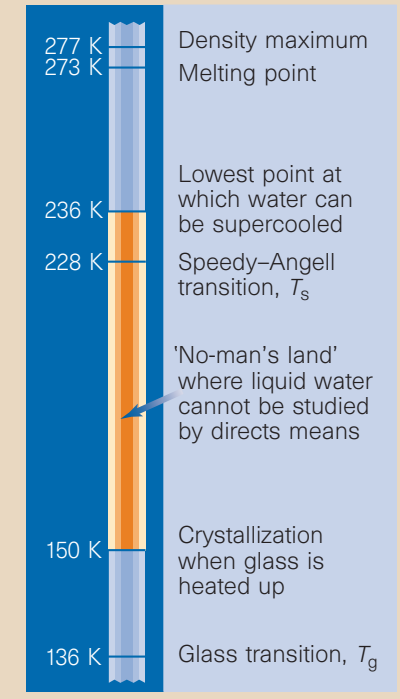

Figure 1 **The mysterious properties of water below 0 °C (273 K). (Modified from ref. 15.)**