Paris: Intracellular Traffic and Neurodegenerative Disorders

Adapted from an original article by Jennifer Altman in Alzheimer Actualités, a newsletter published by the Ipsen Foundation.

3 July 2008. The inside of a cell is a very busy place—a microscopic world of molecules that interact dynamically in groups, in sequences, or in networks. To ensure that the correct sets of molecules talk to each other, the intracellular space is highly structured and special signals are employed to move specific molecules from one structure to another. At the Ipsen Foundation's 23rd Colloquium on Alzheimer's Disease, held 28 April 2008 in Paris, the focus shifted from the rogue proteins that characterize these diseases to the regulation of their movements around the cell. This shift comes with the growing realization that neurodegeneration is less a problem of toxic molecules per se but rather of the way these molecules disrupt the basic biological processes of the cell—their effects on the cell as a system. The meeting was organized by **Peter St George-Hyslop** (who is moving from the University of Toronto, Canada, to the University of Cambridge, U.K.), **William Mobley** (Stanford University School of Medicine, Palo Alto, California), and **Yves Christen** (Fondation IPSEN, Paris).

Each cell produces thousands of different protein and lipid molecules. Some of these are employed to maintain its own structure and function, and others are exported for use elsewhere in the body. As in a petrochemical plant, different steps in the production process take place in dedicated compartments, and signaling systems regulate the movement of molecules between compartments. But, unlike in the production line of a manufacturing plant, the cell may be making many different molecules at the same time, so labels and locations are needed to separate out these simultaneous processes.

In the cell, these processes take place in a network of microscopic tubules known as the endoplasmic reticulum (ER) and the Golgi apparatus, named after the Italian microscopist who first described it; it is usually referred to simply as "the Golgi." In these tubules, proteins are synthesized, folded, modified by the addition of sugars, and packaged for transport to their final destination. The tubules are all made of the same water-repellant fatty membrane that surrounds the cell, separating their interior from the watery cytoplasm. Many molecules are anchored into these membranes to provide a stable focus for chains of chemical reactions.

There is also traffic in the opposite direction: molecules are transported into the cell from outside, including worn-out or excess receptor and channel proteins from the outside of the cell membrane. Many are taken in by endocytosis, sinking into pits in the membrane that pinch off to form vesicles that drop into the cell, where they are known as early endosomes. These may deliver their contents to the Golgi for recycling or become more mature vesicles known as late endosomes, whose contents are passed to bodies called lysosomes, where they are destroyed.

The cells where all this processing takes place are only a fraction of a millimeter in diameter. The details of how proteins are moved around within a cell are being deciphered with high-powered microscopy combined with sophisticated techniques for labeling individual molecules, including fluorescent or "quantum dot" tags and antibodies tailored to adhere to a protein in a particular state of activation. Genetically

engineered mice and refined biochemical analyses are also providing detailed information. These painstaking methods are now being applied to investigating how molecules are moved around in neurons and how the proteins that characterize neurodegenerative diseases may disrupt one or more of these mechanisms.

In the Cell Body

Endocytosis is one way of limiting the responses of a cell to signals coming from outside. It inactivates the receptors that respond to the signal, or the transporter molecules that suck up the signal chemical by removing them from the cell surface. The family of tyrosine-kinase receptors, including those for epithelial growth factor (EGFR) and the transporter for the neurotransmitter dopamine (DAT), are regulated in this way. But there are subtle differences in the endocytosis of these two types of molecule (Alexander Sorkin, University of Colorado). Both the EGFR and the DAT proteins have sites to which the molecule ubiquitin—best known as labeling a protein for degradation—can attach. However, the EGFR can undergo endocytosis when no ubiquitin is attached, using a complex of specific helper molecules, whereas DAT requires the attachment of ubiquitin to activate a specific enzyme that triggers endocytosis (Miranda and Sorkin, 2007).

This is just one example of the precision of the labels required for sorting proteins into their correct pathways in the cell. Another signal is a complex of proteins known as the retromer, which helps to determine whether the protein inside an endosome gets recycled rather than destroyed (**Matthew Seaman**, Addenbrooks Hospital, Cambridge, U.K.). The retromer complex attaches to the membrane of the early endosome through a variety of proteins that includes the sorting receptors sortilin and SorLa (see more below). Endosomes carrying the retromer complex fuse with the Golgi and their contents are recycled; in cells lacking a main component of the retromer, the endosomes take the degradation path that ends in the lysosomes (Seaman, 2004).

A family of small proteins known as rabs, which assist in energy transfer between molecules promoted by the enzyme GTPase, are an essential part of the sorting process. Rab5 assists endocytic vesicles to fuse to form early endosomes, whereas rab7 is necessary for retromer function. Mutations in the gene encoding rab7 have been identified as a cause of Charcot-Marie Tooth type 2 neuropathy, but it is not known whether the mutations affect the interaction between retromer and rab7 (Seaman).

Looking at traffic in the other direction, rab1 is involved in the movement of vesicles containing newly synthesized and folded proteins from the endoplasmic reticulum to the Golgi. Yeast cells are being used as a model for investigating this traffic in Parkinson and Huntington diseases (**Susan Lindquist**, Whitehead Institute, Cambridge, Massachusetts). Some patients with Parkinson's have an overdose of the α-synuclein gene, leading to accumulation of misfolded α-synuclein protein in the dopamine neurons of the substantia nigra. The work in yeast cells has shown that this overdose blocks the movement of vesicles from the ER to the Golgi. Large-scale screening of yeast cells by asking "what makes them better?" has identified rab1 as a protection against this block, confirmed by testing in whole-animal models of Parkinson disease (Gitler et al., 2008). A similar strategy is being employed to find small molecules that protect against the effects of α-synuclein accumulation, which

also interferes with mitochondrial function and increases the production of destructive molecules containing charged oxygen ions.

The multiple effects of α-synuclein emphasize the many ways that an accumulation of a protein can disrupt cellular function. A similar range of effects have been identified in the type of frontotemporal dementia characterized by ubiquitinated inclusion bodies. Different mutations in the progranulin gene affect either the intracellular movement or the export of the growth factor progranulin, though how this leads to the formation of the ubiquitinated inclusion bodies, which do not contain progranulin, is not clear (**Christian Haass**, Ludwig-Maximilians University, Munich, Germany) (Exner et al., 2007).

Axonal Transport

Neurons have a particular problem with intracellular communication because the elongated axon and dendrites place many synapses millimeters or in some cases more than a meter from the cell body containing the synthesizing and degradation machinery. Special mechanisms are required for communicating the molecular requirements of the synapses to the nucleus and for directing the proteins synthesized in the cell body in response to these signals to the synapses that need them, particularly during synapse formation and the plastic changes associated with learning—processes that are badly affected in neurodegenerative diseases (**Kelsey Martin**, Brain Research Institute, UCLA). In both the sea slug *Aplysia* and the hippocampal area of the mouse brain, a protein named importin is produced in synapses during the establishment of long-term memory traces. Importins accompany transcription factors produced in the synapses along the axon and into the cell nucleus, where they enable new gene transcription.

How the proteins that are synthesized in response to the transcription factors get to the right place is another tricky question: they have to be targeted only to a few active synapses among thousands. It seems likely that some of the proteins required to stabilize synapses are synthesized on the spot: in *Aplysia*, localized messenger RNAs are being discoved in newly forming synapses (Martin and Zukin, 2006). One of these, named sensorin, seems necessary for synapse formation or stabilization, possibly by directing other mRNAs to sites where new protein is required. (Martin).

In axons, elongated microtubules form the rail lines along which molecules contained in vesicles are moved by motor proteins. The microtubules are stabilized by the protein tau, an aberrant form of which is found in the characteristic neurofibrillary tangles in Alzheimer disease. Tau also regulates the dynamics of the transport mechanism (**Eva-Maria Mandelkow**, Max-Planck Unit for Structural Molecular Biology, DESY, Hamburg, Germany). Kinesin, the motor protein that carries cargoes along the microtubules from cell body to axon terminals, moves up an increasing gradient of tau until, in the axon terminals, the concentration is so high that kinesin drops off the microtubule, depositing its cargo (**Erika Holzbaur**, University of Pennsylvania School of Medicine, Philadelphia) (Dixit et al., 2008). One of the early effects of the aberrant processing of tau in AD is that the concentration gradient reverses; another is the failure of the tau-assisted movement of mitochondria, so the synapses become starved of both new proteins and energy (Mandelkow).

The opposite, or retrograde, flow carries worn-out molecules to be broken down and recycled, and signal molecules such as growth factors from the terminals to the cell

body. These loads are ferried along the microtubules by dynein, which is activated by dynactin (Holzbaur). Mutations in dynactin are associated with the degeneration of motor neurons in a slowly progressing form of motor neuron disease. In contrast, mutations in the SOD1 gene, which produce rapidly progressing disease, affect the retrograde transport of vesicles (Ligon et al., 2005). Experiments are in progress to identify their cargoes, as these may be specific to the disease process. As an example of such specificity, the protein huntingtin is a part of the molecular motor needed for the retrograde transport of an essential growth factor, BDNF (brain-derived neurotrophic factor), in striatal neurons. DNA expansions in the huntingtin gene, which cause Huntington disease, increase the size of the huntingtin molecule, with the result that it falls off the microtubule and BDNF transport fails, leading to the death of the striatal neurons (**Frédéric Saudou**, UMR 146 CNRS, Institut Curie, Orsay, France) (Gauthier et al., 2004). The basal forebrain cholinergic neurons are another group of neurons that depend on the transport of a specific substance, in this case nerve growth factor, from axon terminals to cell body. Failure leads to loss of synapses and a breakdown in neural circuitry, an early manifestation of AD. Nerve growth factor, secreted by post-synaptic neurons, is taken up by endocytosis and transported in vesicles that carry a complex of signaling proteins on their outer surface (Mobley). The amyloid precursor protein (APP), the source of the amyloid-β (Aβ) peptide found in the plaques in brains of patients with AD, seems to be involved: mice producing too much APP have enlarged signaling endosomes that tend to block transport (Salehi et al., 2006). Early evidence suggests that misprocessing of APP within endosomes may play a defining role in disrupting normal endosomal size and retrograde transport of neurotrophic signals. These examples demonstrate clearly how failure in the transport of specific molecules can have catastrophic consequences for the whole neuron. Axonal transport could even be seen as the neuron's Achilles' heel because it is so vulnerable to disruption (Mobley).

APP Processing

As well as being the parent of Aβ, APP seems to have several roles in cell biology. The whole protein is located in the cell membrane at synaptic terminals, where it may act as an adhesion molecule, helping to anchor the terminal to post-synaptic neurons, particularly during the formation of new synapses (**Konrad Beyreuther** and **Stefan Kins**, Universität Heidelberg, Germany) (Soba et al., 2006). When in the membrane, it may be cleaved by the α-secretase enzyme to release soluble APPα, which is probably a neuronal growth factor, into the extracellular space (**Thomas Willnow**, Max Delbruck Center for Molecular Medicine, Berlin, Germany).

One hypothesis is that uncleaved APP, taken back into the cell by endocytosis, is usually recycled through the early endosomes and Golgi. Under certain conditions, a switch then sends it into the late endosomes, where the β- and γ-secretase cleavages are proposed to take place, producing Aβ and soluble APPβ (St George-Hyslop; Willnow). The switch has recently been identified as the endosomal sorting receptor SorLa or SORL1, a protein that combines with the retromer complex (see above) to direct APP into the recycling pathway (Seaman; St George-Hyslop; Willnow) (Nielsen et al., 2007). SorLa binds to APP through particular sequences in its tail, located inside the endosome membrane (Schmidt et al., 2007). When SorLa levels are reduced in mice, Aβ production increases and amyloid plaques form. There is some debate as to whether SorLa reduces the amount of APP that reaches the cell membrane and the production of sAPPα (St George-Hyslop; Willnow).

An exciting development is the discovery that some variants of the SorLa gene are associated with increased risk of late-onset AD (St George-Hyslop). Despite intensive searching, this is one of the few genes identified as a risk factor for late-onset AD besides APOE (see Alzgene). This raises the possibility that variants of other signaling proteins will turn out to be risk factors.

Not all agree on where either the α-secretase or β-secretase cleavages of APP take place. APP synthesized in the cell body is transported along the axon to the terminals in vesicles carried by kinesin, and some evidence indicates that the benign α-secretase cleavage takes place in these vesicles (Beyreuther) (Kins et al., 2006). The location of the β-secretase cleavage is more controversial: contrary to the position adopted by the SorLa hypothesis just described, some evidence points to this taking place in the early endosomes (**Lawrence Rajendran**, Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany), whereas other evidence was presented showing that APP and β-secretase are transported along axons in separate vesicles, so the cleavage at least does not take place in endosomes during the transport stage (Lindquist; Mandelkow). Maybe the location of processing varies with context and so with experimental conditions, or there may be a problem with the identification of the intracellular compartments involved.

Based on the hypothesis that the β cleavage takes place in the early endosomes, a β-secretase inhibitor is being designed with a tail that tethers it to the endosome membrane (Rajendran et al., 2008). In vitro, interestingly, this inhibitor not only reduces β-secretase cleavage but also enhances α-secretase activity, indicating that the two types of cleavage are in a chemical equilibrium. It also proves a principle that targeting drugs to a specific intracellular compartment is both possible and effective (Rajendran).

When β-secretase cleaves APP, it releases a form of soluble APP known as APPβ, leaving a stub of the molecule embedded in the membrane, where it is cut by γ-secretase to release Aβ. The compartment where this takes place is unknown. More has been discovered about how the production of Aβ42, the form of the peptide that aggregates into plaques, is promoted by mutations in presenilin-1, an essential part of the γ-secretase complex (Haass). Mutations slow down the rate of the enzyme's action, so that the site where cleavage takes place shifts along the APP molecule, increasing the amount of Aβ42 at the expense of the benign Aβ40. Some presenilin mutations produce a higher proportion of Aβ42 than others, and there is a clear correlation between Aβ42 accumulation and age of disease onset (Page et al. 2008). Some presenilin-1 mutations may also cause the misdirection of the APP stub and so enhance Aβ42 production (**Samuel Gandy**, Thomas Jefferson University, Philadelphia) (Gandy et al., 2007).

Another level of control in the traffic and processing of APP is through the process of phosphorylation, which provides a dynamic, moment-to-moment regulation of many intracellular chemical reactions. Phosphorylation is mediated by kinase enzymes, which add phosphate ions at particular sites on a protein, changing its shape and hence its reactivity. Many kinases are activated by extracellular signals such as hormones or neurotransmitters, and some of these pathways appear to be involved in regulating the amount of APP that reaches the cell surface and thus is inaccessible to β-secretase. Interestingly, this does not seem to involve the phosphorylation of either APP or β-secretase (Gandy) (Ikin et al., 2007). A mutation in any of these regulatory

kinases could have pathological consequences, which may be subtle but can be huge, as seen in the formation of neurofibrillary tangles. Two kinases regulate the attachment of the tau protein to microtubules; when one of them, MARK2, becomes overactive, tau is hyperphosphorylated and can no longer attach to microtubles. Instead, it diffuses rapidly through the axon into the cell body and dendrites, where it forms the aggregates that become tangles (Mandelkow) (Khlistunova et al., 2007).

Seeing the Cell as a System

Viewing the cell as a system gives a fresh perspective on the causes of neurodegenerative diseases. Rather than the aggregates of misfolded proteins that characterize these diseases being considered toxic in themselves, they can be seen as perturbing the high precision of intracellular functions (Lindquist). Although the details are not fully agreed upon, the cleavage of APP can be regarded as a question of which compartment it is delivered to. Mutated huntingtin kills cells indirectly by starving them of an essential supply of a growth factor—and so on.

This systemic view has considerable importance for therapy. By focusing attention on the chemical network that regulates the processing of proteins, many more potential target molecules come into sight—which are also candidates for genetic risk factors. Questions can be asked that are amenable to large-scale screens, such as the yeast screens described above (Lindquist) and a screen for rab signals that may be involved in directing the movements of APP and determining which cleavage path it enters (Rajendran). Appreciating the intricacy of the system will also help with designing therapeutic interventions as precisely as possible to avoid unintended consequences.

The time has come when we start to see neurodegeneration as a problem of cell and systems biology rather than just one of rogue molecules!—Jennifer Altman.

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