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Previews

Sorting out Receptor Trafficking

Establishing and maintaining the proper spatial distribution of integral membrane proteins is a functional necessity of all cells. This is particularly obvious in the case of neurons, where the polarized distribution of receptors and ion channels in a wide variety of specialized membrane domains underlies the neuron's ability to receive, process, and transmit information. However, the identification of determinants of neuronal polarity—the targeting signals that act as molecular zip codes and the sorting machinery that recognizes the encoded sorting signals and trafficks the proteins to their proper subcellular destinations—remains an elusive goal.

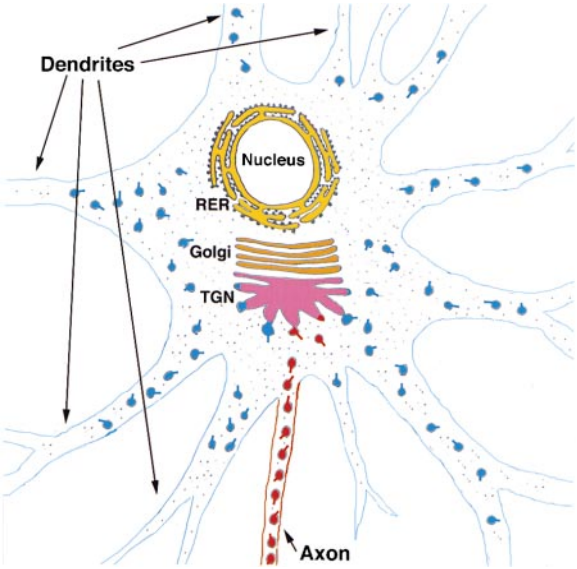
For over 2 decades, polarized expression of membrane proteins has been extensively studied in kidney epithelial cells, where selective localization of membrane proteins to either the apical or basolateral domains forms the entire basis of their directional transport. What has emerged from these studies is that a direct pathway exists whereby the *trans*-Golgi network (TGN) is able to sort membrane proteins into distinct trafficking vesicles capable of targeted delivery to either the apical or basolateral membrane domains (Keller and Simons, 1997). A number of sorting signals that direct these membrane proteins to the distinct trafficking systems have been identified. Apical signals are located in membrane or luminal/extracellular domains, while basolateral targeting signals are found in cytoplasmic domains. Presumably, these molecular zip codes are utilized either as instructions to direct the segregation of proteins into vesicles preordained for transit to the proper destination, or as the primary determinants of polarized trafficking via direct interaction with the delivery machinery (molecular motors and their cytoskeletal tracks), respectively. While a major effort has been directed at identifying components of the sorting machinery that interact with these targeting signals, none have been identified to date.

Additional mechanisms that contribute to polarized protein localization exist at the plasma membrane (Yeaman et al., 1999). Specific components of the SNARE plasma membrane fusion machinery have a polarized expression in epithelial cells, suggesting that they play a role in selective fusion of properly targeted vesicles (Low et al., 1998). Other mechanisms operating at the level of the plasma membrane, such as selective retention of the protein within the initially targeted domain, or transcytosis of the protein to the alternative membrane domain, can also impact on polarity.

More recent studies reveal that many of these processes are fundamental to both neurons and epithelia (Bradke and Dotti, 1998). In most cases, the apical and the basolateral domains of epithelial cells are analogous to the axonal and somatodendritic domains of neurons, respectively. Sorting of newly synthesized membrane proteins occurs in the TGN located in the cell body of neurons (see figure). Previous studies have indicated that axonal sorting signals, like apical ones, are generally in membrane or luminal/extracellular domains of these proteins, while somatodendritic signals, like basolateral signals, are found in cytoplasmic domains. However, there are also contradictions to this "analogous membrane domain" hypothesis. Previous structure–function studies of determinants of polarized protein localization in central neurons have been limited to nonneuronal proteins expressed in cultured neurons (e.g., influenza hemagglutinin, vesicular stomatitis virus glycoprotein) or studies on individual neuronal proteins (e.g., APP, transferrin receptors, synaptobrevin). In the few cases where neuronal proteins have been expressed in neurons for structure–function analyses, no general axonal or somatodendritic targeting signals have emerged, perhaps due to the structural variety among the examined proteins.

The current paper by Stowell and Craig (1999 [this issue of *Neuron*]) is a new study to analyze a family of highly related neuronal plasma membrane proteins for targeting signals in neurons. This work focuses on metabotropic glutamate receptors (mGluRs), a family of at least six protein isoforms that act to couple excitatory glutamate neurotransmission and G protein–mediated intracellular signaling pathways. Depending on the mGluR subtype, glutamate-induced G protein activation can lead either to activation of phospholipase C from postsynaptic sites or to inhibition of adenylyl cyclase at both pre- and postsynaptic locations. The differential targeting of these receptors to pre- or postsynaptic membranes determines their role in either antegrade or retrograde synaptic signaling in the nervous system.

By using mGluRs as their model proteins, Stowell and Craig could take advantage of the wealth of information from previous structure–function studies on the huge family of G protein–coupled receptors and the high degree of structural similarity within mGluRs to guide rational design of mutants and chimeras. The initial observation was that different mGluR isoforms transiently expressed in cultured hippocampal neurons by infection with replication-defective viral vectors exhibited different subcellular distributions. Among these, mGluR2 was observed only in the somatodendritic domain, while mGluR7 was expressed throughout the neuron. A number of possibilities existed for these results. The first was that mGluR2 contained a specific positive somatodendritic targeting signal that was absent in mGluR7. Alternative explanations included the existence of a positive axonal signal on mGluR7 but not mGluR2 or an "axon exclusion" signal specific to mGluR2. Through a series of ingeniously designed truncation mutants and chimeric proteins, the existence of "axon exclusion" and "axon targeting" signals in the cytoplasmic C-terminal tails of mGluR2 and mGluR7, respectively, was proposed (see figure). The axon targeting signal of mGluR7 appeared to dominate, as appending this onto the end of full-length mGluR2 or onto the somatodendritic protein telencephalin led to expression throughout the somatodendritic domain and the axon.



Cartoon of a Prototypical Neuron Showing the Components of the Endomembrane Pathway for Membrane Protein Biosynthesis

Sorting of membrane proteins into distinct populations of transport vesicles destined for the axon (red) and for the somatodendritic (blue) compartments occurs in the TGN (purple). Sorting of mGluR isoforms is directed by signals in the cytoplasmic tail, as indicated in the cartoon.

One major question that arises from these studies relates to general principles of axonal versus somatodendritic protein sorting in neurons. As discussed by Stowell and Craig, the exclusive axonal targeting that is widespread in nature has been difficult to reproduce when recombinant proteins are expressed in cultured neurons. mGluR7 is found predominantly in axons in situ, for example. These authors point to the fact that generating and maintaining an exclusive axonal localization may involve more than selective axonal targeting, and propose that axonal membrane proteins may initially be uniformly distributed and only achieve polarity through localized differential turnover. As such, the short incubation times dictated by transient protein expression in cultured neurons may not be sufficient to generate the proper localization. Given this, one would expect that any endogenous membrane protein destined for axonal localization in cultured hippocampal neurons would initially be expressed uniformly, followed by enrichment in the axon through selective turnover. Future studies on the dynamics of the targeting of mGluR7 or other axonal membrane proteins may clarify these issues and solve the discrepancy between in situ and in vitro localization of axonal membrane proteins. However, identification of cellular proteins that exhibit differential interaction with the distinct targeting signals on the cytoplasmic domains of mGluRs characterized by Stowell and Craig may allow for the identification of components of the polarized protein trafficking machinery in neurons that have remained so elusive in epithelial cells.

It is surprising that both of the sorting signals identified by Stowell and Craig are found in the cytoplasmic tail of the mGluRs. Taken together with recent observations that synaptobrevin contains a cytoplasmic axonal targeting motif (West et al., 1997), these findings suggest that, unlike apical targeting in epithelial cells, the axonal targeting machinery of neurons can utilize cytoplasmic sorting signals. It is interesting to note that the cytoplasmic tail of G protein–coupled receptors such as the mGluRs also plays an important modulatory role and is the site for modification by phosphorylation and for interaction with arrestin and other cellular proteins. This raises the possibility that the targeting of mGluRs in neurons could be dynamically modulated via such modifications in this region critical for targeting, and may provide a mechanism to generate the observed cellular variability in mGluR localization.

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Trying versus Succeeding: Event-Related Designs Dissociate Memory Processes

We have all experienced the frustration of trying to remember a name or fact that feels as if it is at the tip of our tongue but remains inaccessible despite our best efforts to retrieve it. This common occurrence provides a heuristic demonstration that acts of remembering can be separated into two types of processes—one associated with the *effort* of retrieving and one associated with *success* in retrieving. In the instance of the "tip-of-the-tongue" phenomenon, effort is exerted but information is not successfully retrieved. While this exact experience is not the focus of the study by Ranganath and Paller in this issue of *Neuron* (1999), the phenomenon illustrates the issue that is explored; namely, understanding how and where the processes associated with retrieval effort and retrieval success occur in the brain. Ranganath and Paller have shed new light on the question of what brain regions are involved in effort and success during episodic memory (e.g., see Tulving, 1983) by mapping event-related potentials (ERPs).

Ranganath and Paller (1999) cleverly designed a behavioral procedure to encourage varied levels of retrieval effort, while simultaneously monitoring whether or not information was successfully retrieved. They employed a recognition memory task where subjects attempted to discriminate between studied and novel pictures (line drawings of common objects). In the "specific test" condition, subjects were only to say that an item was old if it appeared exactly as it did when studied. While some of the old test items were identical to those presented during the study period, others had been subtly changed in size and shape (the pictures were scaled, altering their height and width). In this manner, the specific test required considerable retrieval effort to be exerted; to remember accurately, subjects were required to develop a strategy that made use of very specific perceptual details. The second condition, the "general test," demanded considerably less effort. Subjects were required to endorse an item as old regardless of a change in exact perceptual details. ERPs were compared both within and across the different testing conditions. By isolating electrophysiological correlates of successfully retrieved items, in the context of varied retrieval demands, Ranganath and Paller were able to dissociate neural correlates of retrieval effort from those of success. They found that scalp potentials localized over left frontal cortex tracked the strategic demands of the retrieval task independent of whether information was successfully recognized. By contrast, differences between old and new items were found in scalp potentials over right frontal cortex, suggesting a complementary correlate of retrieval success.

To fully appreciate the significance of Ranganath and Paller's study, it is necessary to consider the origins of the debate over localization of retrieval effort versus retrieval success. The issue arises because of a controversy concerning the role of the prefrontal cortex in memory retrieval. A host of neuroimaging studies have revealed that certain areas of prefrontal cortex are involved in memory retrieval (reviewed by Buckner, 1996; Fletcher et al., 1997; Tulving et al., 1994). In particular, activity in areas of right anterior and dorsolateral prefrontal cortex have been consistently reported across a variety of memory tasks and materials (e.g., for recognition and cued recall, and for words and pictures). The question is: does this activity represent retrieval effort, retrieval success, or both?

The difficulty in resolving this debate is exemplified by comparing two studies employing positron emission tomography (PET) that were conceptually similar yet produced findings that led to opposing conclusions. In the first case, Nyberg et al. (1995) employed a simple recognition memory test in which subjects studied a list of words and were then asked to discriminate between old (studied) and new (unstudied) items. Brain activity was measured during the recognition test, with the key manipulation being a contrast between two experimental conditions in which all the test items were either old (attempt and success in retrieval) or new (attempt to retrieve but with no success), respectively. Relative to a control condition (silent reading), the right prefrontal cortex was found to be active for both memory conditions, with little difference between them. That is, the prefrontal cortex was active both when all test items were old and when all test items were new. Consequently, Nyberg et al. (1995) concluded that activity in the prefrontal lobes was related to the effort or attempt to retrieve, regardless of whether retrieval was successful.

In the second case, Rugg et al. (1996) also employed a recognition memory test and again contrasted activity in two experimental conditions. In this study, the key manipulation between the experimental conditions was to vary the proportion of old and new items in each test list, such that there was either a high (4:1) or low (1:4) ratio of old to new test items. Significantly, the critical test conditions were *embedded* within a much longer test list, in the hope that subjects would be unlikely to notice the different ratios of old to new test items and would therefore be unlikely to vary their retrieval effort. Thus, subjects were tested on a long list of items, but brain scans were only taken during the critical test conditions where the proportion of old to new items had been manipulated. Relative to a control condition (in which all items were new) significant prefrontal activity was found in both memory conditions, with greater activity occurring when more old items were present. Thus, Rugg et al. (1996) found that prefrontal activity varied as a function of the number of old items presented, suggesting that the prefrontal lobes are sensitive to whether retrieval is actually successful.

How can two studies both designed to answer the same question, using the same technique and similar experimental protocols, come to such radically different conclusions? The answer lies primarily in the technique itself, namely PET. An inherent feature of PET studies is the fact that they are limited to the use of blocked designs. Within a blocked design, brain activity is measured in distinct experimental conditions, periods of time during which a series of sequential experimental trials are presented. This produces a measure of brain activity that is averaged across the entire series of trials (or block) regardless of variation in the types of stimuli presented or subjects' responses to those stimuli. Consequently, experimental manipulations are limited to changes across different blocks of stimuli, for example old versus new test items in the Nyberg et al. (1995) study and varied proportions of old and new test items in the Rugg et al. (1996) study. While these manipulations were designed to encourage retrieval success in one condition and discourage it in the other, the blocked design of PET did not allow either study to compare activation specifically for trials in which retrieval was successful with those in which it was not: because of the averaging across experimental conditions in a blocked design, retrieval effort and retrieval success could not be clearly separated. By contrast, the ERP technique employed by Ranganath and Paller allows the use of event-related designs. In event-related studies, data can be separated post hoc according to the different classes of experimental stimuli that were presented *and* the different responses made by each subject (i.e., contingent upon subject's performance).

The experiment presented by Ranganath and Paller illustrates the advantage of event-related techniques nicely (see also Johnson et al., 1997; Schacter et al., 1997; Donaldson and Rugg, 1999; Duzel et al., 1999; and Wilding, 1999, for related manipulations). The strength of

the approach used by Ranganath and Paller is not simply in their use of separate blocked recognition conditions to encourage varied retrieval strategies. Rather, the analysis possible in their study relied both on a manipulation of retrieval demands across blocks of trials *and* the use of event-related procedures to isolate correlates of individual recognition events. By crossing the two levels of analysis—examining the effects concerned with the overall strategy (across blocks) and those concerned with individual items (within each block in an event-related manner)—they were able to distinguish between the neural correlates of retrieval effort and success.

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How Hallucinations Make Themselves Heard

Schizophrenia is a common disorder with a lifetime risk of about 1%. The age of onset is typically in the mid twenties and many sufferers never fully recover. The effects of the illness can be devastating for the sufferer and for his or her family. Although there is evidence of structural and functional brain abnormalities in schizophrenia, the causes of the disorder remain unknown (Straube and Oades, 1992). Auditory hallucinations are the most common symptom of this disorder, being reported by about 65% of patients with schizophrenia (David, 1994). The patient does not hear just sounds but fully formed verbal communications that appear to emanate from a particular speaker or group of speakers. These speakers often seem omniscient (they can read the patient's thoughts) and are usually hostile, as in the following example:

> Days later while in the Metropolis again, I was once more startled by these same pursuers, who had threatened me several days before. It was night-time. As before, I could catch part of their talk, but, in the theatre crowds, I could see them nowhere. I heard one of them, a woman, say: "You can't get away from us; we'll lay for you and get you after a while!" To add to the mystery, one of these "pursuers" repeated my thoughts aloud verbatim. I tried to elude these pursuers as before, but this time I tried to escape from them by means of subway trains, darting up and down subway exits and entrances, jumping on and off trains, until after midnight. But, at every station where I got off a train, I heard the voices of these pursuers as close as ever (L. Percy King, from a letter written in the 1940s protesting the writer's imprisonment in a mental hospital).

Hallucinations can cause considerable distress because the voices continually criticize the patient and may command the patient to act against his or her wishes:

> Only a short time before I was confined to my bed I began to hear voices, at first only close to my ear, afterwards in my head, or as if one was whispering in my ear—or in various parts of the room... These voices commanded me to do, and made me believe a number of false and terrible things (from John Percival, Esq., *A Narrative of the Treatment Experienced by a Gentleman, During a State of Mental Derangement* [1840]; examples quoted in Peterson, 1982).

How is it that, in the absence of any sensory input, the hallucinating patient can have an experience that is indistinguishable from a real, external voice? Discovering the physiological basis of hallucinations would provide a major insight into the neural basis of phenomenological consciousness as well as pointing toward possible physiological mechanisms underlying hallucinations. The new generation of brain imaging techniques provide the opportunity to localize any brain activity associated with the occurrence of hallucinations. Functional magnetic resonance imaging (fMRI) is particularly suited to this purpose; since its temporal resolution is relatively high (in the order of seconds), scanning can be carried on continuously and can be repeated over a number of sessions. It would seem at first sight an easy matter to scan patients while they indicated when hallucinations were occurring. In practice, suitable patients are difficult to find. Furthermore, the timing of the hallucinations is not under the control of the patient or the experimenter and the temporal sequence of the hallucinations that happen to occur must be appropriate to the scanning protocol. Additionally, many patients are unwilling to give detailed information about when the hallucinations are occurring.

In this issue of *Neuron*, Dierks and his colleagues (1999) report results from a series of three suitable patients in whom fMRI was used to identify brain activity during hallucinations. These patients were identified by screening all the patients with a history of hallucinations

who entered a university clinic over an 18 month period. These patients were able to indicate when hallucinations were occurring by pressing a button. Using a technique originally developed by Silbersweig et al. (1995) in a positron emission tomography (PET) study, brain regions were identified in which activity systematically increased at times when hallucinations were occurring. This technique depends on performing a cross-correlation between occurrence of hallucinations and brain activity. Using this method, it does not matter that the occurrence of the hallucinations is unpredictable as long as the durations of the hallucinations and the intervening hallucination-free periods are of the order of 10–100 s. Such periods are suitable for the scanning protocol with acquisition of a whole brain image every 4 s. The regions identified by this method were in the temporal lobe, in particular primary auditory cortex, the frontal operculum (part of Broca's area), and components of the so-called limbic system. Dierks and his colleagues confirmed the identity of primary auditory cortex by also scanning the patients while they heard real sounds, including speech. Previous imaging studies of auditory hallucinations using less sensitive techniques have not given very consistent results. However, some of these studies have also implicated temporal lobe structures (Liddle et al., 1992; Silbersweig et al., 1995), limbic regions (Silbersweig et al., 1995), and the frontal operculum (McGuire et al., 1993), but the observation of activity in primary auditory cortex is a new and important finding.

What do these results tell us about the origins of auditory hallucinations? A number of authors (e.g., David, 1994; Frith, 1996) have suggested that these experiences have their origin in the patient's own inner speech or thought. The observation of activity in the frontal operculum related to hallucinations is consistent with this idea, since overt speech, covert speech, and auditory imagery are associated with activation in this region (McGuire et al., 1996). If hallucinations are associated with inner speech then the abnormality does not lie in the occurrence of inner speech. This is an activity in which we all indulge. Rather it lies in the false perception that this speech is being generated externally and by some one else. Dierks and his colleagues point out that, in normal volunteers, inner speech is not associated with activity in primary auditory cortex (McGuire et al., 1996). They go on to make the very interesting suggestion that it might be the abnormal occurrence of activity in primary auditory cortex during inner speech that leads to a false attribution of the voice to an external source: it is the activity in primary auditory cortex that lends the experience the quality of a real, external sound rather than the quality of mental imagery. Along similar lines, it has been suggested that activity in primary visual cortex may be necessary (but not sufficient) to have the full experience associated with conscious vision (Weiskrantz, 1986).

There is, however, another possibility that needs to be considered. When interpreting the pattern of brain activity associated with hallucinations, we have to distinguish between primary effects, that is the activity in which the hallucinations have their origin, and secondary effects, that is activity reflecting the response to the experience of hearing a voice. It is possible that, in the presence of an auditory hallucination, patients direct their attention to the auditory modality and that it is this shift of attention that increases activity in primary auditory cortex. It is well established in normal volunteers that activity in primary auditory cortex can be enhanced by attention to auditory stimuli in the absence of a change in stimulation (Woldorff et al., 1993). The need for focused attention in the auditory modality is particularly great during MRI scanning since this technique is associated with loud background noise. Evidence against this interpretation comes from a study by David et al. (1996), in which it was observed that, in patients experiencing auditory hallucinations, primary auditory cortex was less responsive to external stimuli (David et al., 1996). However, further imaging studies of the effects of attention to sounds in primary auditory cortex and how these effects interact with auditory hallucinations will be needed to resolve this issue. In particular, it will be important to replicate the observations of Dierks and his colleagues and, in addition, to observe whether the response of primary auditory cortex to real speech is enhanced or attenuated by the simultaneous presence of auditory hallucinations.

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Harm's Way: Polyglutamine Repeats and the Activation of an Apoptotic Pathway

The process of apoptosis, in which cells undergo a tightly controlled and coordinated death, proceeds via the activation of caspase proteases that cleave key substrates. The activation or inactivation of these substrates is responsible for the "packaging" of the dying cell for clearance by phagocytes and is probably responsible for actually killing the cell in many cases. How the proteases become activated, then, is of central importance to our understanding of cell life and death in a variety of physiological and pathological situations. In the paper by Yuan and colleagues in this issue of *Neuron* (Sanchez et al., 1999), the authors propose a novel pathway for the activation of caspases by proteins with extended polyglutamine repeats of the sort associated with several neurodegenerative diseases, including Huntington's disease, spinocerebellar ataxias, dentatorubral–pallidolyusian atrophy, and others (Perutz, 1999). They suggest that polyglutamine repeat proteins assemble into insoluble aggregates containing one of the caspases, which becomes activated, leading to apoptosis. Although it is difficult to relate the in vitro studies to in vivo disease, they show that this caspase, which is normally soluble, partitions with an insoluble fraction in extracts from the brains of several patients with Huntington's disease. This raises the intriguing possibility that cytosolic aggregation of polyglutamine repeat proteins contributes to neurodegenerative disease via the recruitment, binding, and activation of caspases.

There are currently about a dozen human caspases identified, and only a subset of these are known to be involved in apoptosis. All caspases are expressed as single chain proenzymes with low proteolytic activity; upon cleavage, these autoassemble into tetramers and increase their enzymatic activity about 100-fold (Ashkenazi and Dixit, 1998) (see figure). This cleavage can occur in two ways. The first is via the action of another protease. Active caspases can cleave procaspases to yield the active subunits, and this forms the basis for caspase cascades that amplify the effects of an active caspase (see figure, panel D).

The second way in which procaspases are cleaved and activated is by bringing together two or more procaspase molecules such that the low proteolytic activity of each proform can act on its neighbor to cleave and activate it. This depends on the binding of adaptor molecules to protein interaction regions located within the prodomains of some of the procaspases (see figure) (Green, 1998). The oligomerization of the adaptor molecules therefore controls the activation of the caspase, which in turn can act on other caspases to activate them.

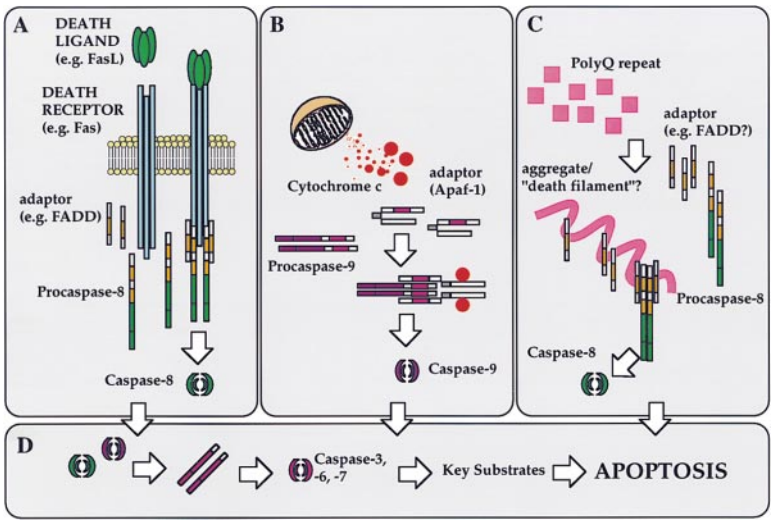
So far, only two types of functional adaptor molecules are known. The first type forms complexes with the intracellular domains of so-called "death receptors," apoptosis-inducing members of the tumor necrosis factor receptor family (Ashkenazi and Dixit, 1998). One of these adaptors is FADD/MORT1, which binds directly or indirectly to some of these death receptors and recruits caspase-8, leading to its activation (see figure, panel A).

The second type of adaptor molecule is Apaf-1, which aggregates upon binding to cytochrome c released from mitochondria during apoptosis (Zhou et al., 1999). The aggregated Apaf-1 recruits procaspase-9, leading to its transcleavage and activation (see figure, panel B). These two types of adaptor molecules help to define two types of death pathway, one involving death receptors and one involving the mitochondria (and cytochrome c release).

Now there may be another. Sanchez et al. (1999) examined the ability of polyglutamine repeats of 50 or more units to trigger caspase activation and apoptosis in cells transfected with the expression constructs. They found that the cell death was blocked by a dominant negative FADD (containing only the bit that normally binds to Fas) and by a dominant negative caspase-8. Both of these proteins effectively localized to the large polyglutamine aggregates in the cells, suggesting that procaspase-8 is similarly recruited. They showed that expression of a polyglutamine repeat led to endogenous caspase-8 appearing in an insoluble fraction in the cell, presumably with the insoluble polyglutamine aggregates. Thus, recruitment of procaspase-8 to the polyglutamine aggregates may play a role in polyglutamine-induced apoptosis. In support of this, they found that a cell line lacking caspase-8 was resistant to polyglutamine-induced apoptosis.

The model in panel C of the figure is consistent with their observations. Polyglutamine repeats form aggregates that bind to adaptor proteins such as FADD, which has itself been shown to form filamentous structures in cells upon overexpression (Perez and White, 1998). FADD (and perhaps other adaptor molecules) recruits procaspase-8, which transactivates. The ability of FADD DN to block binding and activation of procaspase-8 suggests that the binding of this adaptor (and any other relevant adaptor) is to a limited number of sites on the polyglutamine filament. A procaspase-8 with a mutated active site blocks caspase activation and apoptosis, presumably because it complexes with endogenous procaspase-8 but cannot participate in the transactivation event. Whether other procaspases are present in the complex is not known, but procaspase-8, at least, appears to be required for caspase activation and subsequent death, at least in the cell lines examined.

Recent studies have shown that the formation of large insoluble aggregates is not a requirement for apoptosis induced by polyglutamine repeat proteins (e.g., Saudou et al., 1998). This does not rule out, however, the possibility that smaller polyglutamine polymers, perhaps more closely resembling the "death filaments" formed by FADD and mentioned above, trigger apoptosis via recruitment of adaptor proteins and procaspases as described here. Further, another recent study has shown that while polyglutamine repeats can induce caspase activation, they also induce a caspase-independent cell death (Moulder et al., 1999). Interestingly, FADD has also recently been found to be capable of inducing a caspase-independent cell death (Kawahara et al., 1998). It will be interesting to determine whether these phenomena are related.



Pathways to Caspase Activation and Apoptosis

(A) Transactivation of procaspase-8 by association with adaptors such as FADD and ligated death receptors. Binding of ligand to a death receptor causes trimerization and assembly of an intracellular complex that includes adaptor proteins (e.g., FADD) and procaspases (e.g., procaspase-8). Bringing the procaspase-8 molecules into close proximity leads to their transactivation and processing into mature caspase-8, which can then act on other procaspases to activate them (arrows).

(B) Transactivation of procaspase-9 by association with aggregated Apaf-1, induced by cytochrome c. Cytochrome c released from mitochondria following a proapoptotic induction binds to Apaf-1, which causes it to change conformation, aggregate, and bind procaspase-9. Bringing the procaspase-9 molecules into close proximity leads to their transactivation and processing into mature caspase-9.

(C) Suggested aggregation and transactivation of procaspase-8 by association with polymerized polyglutamine repeat proteins. Polymerized polyglutamine repeat proteins bind adaptor molecules such as FADD, which in turn bind procaspase-8. Procaspase-8 then transactivates as in (A), leading to the generation of mature caspase-8.

(D) Active caspases produced by the processes in (A) through (C) cleave and activate other caspases, which in turn cleave key substrates to orchestrate the apoptotic phenotype and death of the cell.

The extended polyglutamine repeats associated with several neurodegenerative diseases can aggregate independently of other influences in vitro, but in vivo this may be facilitated through the action of "tissue" transglutaminase, an enzyme that links glutamine to the ϵ-amino groups of lysines on other proteins. In recent studies, the polyglutamine repeat protein huntingtin was shown to be an effective substrate for transglutaminase, and the rate constant of the reaction increased with the length of the polyglutamine repeat (Kahlem et al., 1998). Further, transglutaminase inhibitors blocked the formation of huntingtin polymers (Kahlem et al., 1998) and the formation of filamentous aggregates and apoptosis induced by expression of the dentatorubral–pallidoluysian atrophy protein (Igarashi et al., 1998).

But the possible role of transglutaminase in apoptosis may be more general. For example, ectopic expression of the tissue transglutaminase gene can trigger apoptosis in cells from normal individuals (i.e., without polyglutamine repeat disease), and expression of an antisense construct for this gene was observed to block spontaneous and retinoic acid–induced apoptosis in a neuroblastoma cell line (Melino et al., 1994). One could envision that even in the absence of pathogenic proteins with extensive polyglutamine repeats, the action of transglutaminase in some circumstances can create similar filamentous structures capable of recruiting adaptor proteins and procaspases (such as FADD and procaspase-8), leading to caspase activation and apoptosis. Therefore, there may be various ways to trigger the generation of "death filaments" that recruit procaspases and lead to apoptosis. If so, the studies of Yuan and colleagues (Sanchez et al., 1999) may have done more than suggest a mechanism for cell death in a pathologic setting, they may have identified a new physiological death pathway.

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