

See page 303

## A Peptide Fusion a Day Keeps the Aggregates Away

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Many neurodegenerative diseases are associated with the formation of insoluble structures composed primarily of protein aggregates. Examples include Alzheimer disease (neurofibrillary tangles and amyloid plaques), Parkinson disease (Lewy bodies), and a class of diseases provoked by extension of the length of polyglutamine (polyQ) sequences in particular proteins. The extended polyQ sequences result from abnormal expansion of CAG repeats in the genes encoding the affected proteins, and they are thus inherited. In this issue, Popiel *et al.*<sup>1</sup> tested a new therapeutic strategy to treat a *Drosophila* model of polyQ disease that involves feeding to the flies a fusion peptide designed to disrupt polyQ aggregates.

The major polyQ diseases are spinocerebellar ataxias, dentatorubral-pallidoluysian atrophy, spinobulbar muscular atrophy, and Huntington disease.<sup>2</sup> The role of the polyQ aggregates in disease pathology is not clear. Some investigators have proposed that aggregates are beneficial because they provide “hot spots” for the accumulation of potentially deleterious factors, including the mutated protein itself (polyQ may confer adverse properties to the soluble proteins), so as to prevent it from intermingling with and affecting the activity of other proteins.<sup>3</sup>

However, a majority of investigators consider aggregates detrimental, in particular because they trap several factors indispensable for proper cell metabolism or function. The concept of aggregate toxicity forms the basis for the search for molecules capable of

disrupting the aggregates or blocking their formation.<sup>4</sup> For example, some investigators have proposed the development of “ $\beta$ -breakers,” because it often happens (as in the case of prions) that insolubility is a consequence of conformational alteration of  $\alpha$ -helices into  $\beta$ -sheets.<sup>5</sup>

Nagai and colleagues<sup>6,7</sup> recently adopted a similar strategy, using phage display to identify six short polyQ binding peptides (PQBP1–PQBP6) that could inhibit polyQ polymerization *in vitro*. One of their peptides, PQBP1, could antagonize polyQ-induced formation of inclusion bodies, neuronal degeneration, and premature death when expressed as a dimer, (PQBP1)<sub>2</sub>, in a *Drosophila* model of polyQ disease. These studies suggested the possibility of using gene therapy to express the inhibitory peptide from a viral vector to treat these diseases. However, the group decided to explore an alternative strategy based on the use of transduction peptides.

Transduction peptides constitute a class of short peptides that can gain access to the cytoplasm and nucleus after internalization by living cells.<sup>8,9</sup> Such peptides can be fused to hydrophilic cargoes that can then be delivered into cells both *in vitro* and *in vivo*. Common examples include Penetratin and Tat. Penetratin is also called Antennapedia (Antp), because it corresponds to a 16 amino acid long sequence that is present in the *Drosophila* transcription factor, Antennapedia. Tat is an arginine-rich peptide found in the sequence of the HIV transcription factor, TAT. Many other peptides have been found that share vector properties similar to those of Antp and Tat, including poly-Arg and Transportan.<sup>10,11</sup>

Importantly, transduction peptides are not internalized through identical routes. For example, internalization of Tat re-

quires endocytosis and is energy dependent, whereas Antp internalization takes place at 4 °C. Regardless of the mechanism of entry, all such peptides must cross the lipid bilayer, either at the level of the plasma membrane, as is the case for Antp, or after endocytosis, as for Tat.<sup>8,9</sup> It must be underscored that attaching a cargo to a transduction peptide can modify the properties of the cargo and/or the vector. Each chimeric cargo-vector (or vector-cargo) construct must therefore be viewed as a unique entity that must be tested for entry and for activity.

In the new work, Popiel and colleagues used Antp and Tat to internalize PQBP1. They first demonstrated that the fusion peptides prevented polyQ-induced polymerization *in vitro*, establishing that the biological activity of PQBP1 was not impeded by the vector. They then tested the internalization of Tat-PQBP1 and Antp-PQBP1 by COS cells and found that both fusions were active and exhibited little toxicity, and that their internalization decreased the formation of inclusion bodies after expression of polyQ constructs.

Because Antp seemed more efficient and less toxic than Tat in their model, the authors selected the Antp fusion for *in vivo* experiments that made use of the same model used to demonstrate that genetic co-expression of polyQ and (PQBP1)<sub>2</sub> could antagonize the formation of inclusion bodies, block neuronal death, and prevent death of the flies. However, instead of expressing PQBP1 genetically, they fed the flies *Drosophila* food containing 0.2 mM Antp-PQBP1. The authors again observed decreased inclusion body formation, decreased neuronal death and an extension of life.

This is not the first study to show that a transduction peptide can be used to deliver a cargo with physiological activity *in vivo*. Outside of the central nervous system (CNS), both Antp and Tat have been shown to exhibit internalization activity when injected intraperitoneally or into the circulation. However, in the CNS, most experiments thus far have involved injection of Tat fusions into the brain parenchyma or in peritoneum of the rodent. Injection into the peritoneum resulted in

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strong physiological effects, suggesting that the peptide-cargoes could cross the blood-brain barrier.<sup>12</sup>

The article by Popiel and colleagues presents the first case of oral ingestion of a transduction peptide with an activity in the nervous system. Although these results are of great interest, an important caveat is that it is quite difficult to make the jump from flies to mammals with such technology. Even if passage across the intestinal epithelium and into the brain could be reproduced in rodents, many points would have to be resolved before transduction peptides could be used as pharmacological tools. There have been no studies of the possible toxic or mutagenic activities of these peptides. In addition, one must also be able to target this new class of pharmacological agents to the right cells and, once in the cells, to the right subcellular compartment.

There is thus plenty of work ahead. However, considering the results gathered in only a few years by a small number of research groups, it is obvious that this new technology is very promising. A main advantage of transduction peptides is that they provide access to intracellular targets, as viral vectors do, but that they remain very classical pharmacological agents. If proteins such as interferon or insulin can be used therapeutically, there is no reason to doubt the interest of approaches using transduction peptides or nonpeptidic compounds modeled after these peptides.

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See page 295

# Armed Interference: Oncolytic Viruses Engineered to Carry Antitumor shRNAs

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A diverse array of oncolytic viruses are being developed for the treatment of cancer.<sup>1,2</sup> These therapeutic agents are naturally and/or genetically targeted to replicate selectively in cancer cells. The resulting “oncolysis” is a novel mechanism of action (MOA) for cancer treatment and seems in many cases to be effective against apoptosis-resistant cells. In addition to this primary MOA, oncolytic viruses can demonstrate secondary MOAs such as induction of tumor-specific cytotoxic T lymphocytes,<sup>3</sup> anti-angiogenic cytokines,<sup>4</sup> and chemosensitization.<sup>5</sup>

The next generation of oncolytic viruses have additional MOAs through therapeutic transgene “arming.”<sup>6</sup> These therapeutic payloads are expressed selectively in cancer cells during replication, resulting in complementary MOAs. Examples include JX-594 (targeted vaccinia expressing granulocyte-macrophage colony-stimulating factor (hGM-CSF), Jennerex Biotherapeutics, San Francisco, CA),<sup>7</sup> OncoVex (herpes simplex virus (HSV) expressing hGM-CSF, Biovex, Woburn, MA),<sup>8</sup> and MV-NIS (measles virus expressing the sodium iodide symporter gene, Mayo Clinic, Rochester, MN).<sup>9</sup> In addition, anti-angiogenic and antivascular gene products (*e.g.*, soluble vascular endothelial growth factor receptor (VEGF-R))

have been expressed in the context of a targeted oncolytic virus.<sup>10</sup> Therefore, these armed oncolytic viruses are designed to wage a multipronged attack against cancer.

In this issue, Yun and colleagues<sup>11</sup> report proof-of-concept studies on the expression of a small inhibitory RNA (siRNA) from an oncolytic virus. They expressed a small hairpin (sh) RNA against VEGF from an *E1A-CR2* gene region-deleted adenovirus (Ad). The authors compared this virus to important controls, including the same oncolytic Ad lacking the shRNA expression cassette and a replication-incompetent Ad expressing the anti-VEGF shRNA. They demonstrated that shRNA expression and anti-VEGF effects were greater and more prolonged in the context of the oncolytic vector as compared with the replication-deficient vector. In addition, the shRNA-armed virus demonstrated superior efficacy over the same virus without shRNA arming. An anti-angiogenic MOA was shown both *in vitro* and *in vivo*. Interestingly, the Ad *E1A* protein also showed anti-VEGF and anti-angiogenic effects.

siRNA technologies hold promise for the treatment of cancer. However, thus far this approach has had only limited success *in vivo* because of several hurdles.<sup>12</sup> These include difficulties in achieving high-level expression selectively in cancers, particularly after intravenous administration. The application of shRNA technology in the context of systemically deliverable oncolytic viruses such as Ads or vaccinia viruses may be particularly effective at overcoming such

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