expend a great deal of effort generating human Tsg101 knock-out clones by homologous recombination, as has been done to prove the involvement of cyclophilin A in HIV-1 replication[10].

None of this is necessary, however, as Sundquist and colleagues[6] have already done the job an easier way. They used small, double-stranded, interfering RNAs to shut off Tsg101 expression and show that L-domain function does indeed depend on Tsg101. This is a powerful technology, the technical details of which have only recently been worked out in mammalian cells[11], and it will surely transform how molecular biologists think about the follow-up investigation of two-hybrid hits.

We now know that the Pro-Thr-Ala-Pro motif of HIV-1 and Ebola recruits Tsg101. But what about the L-domains of other viruses? The Tyr-X-X-Leu motif of equine infectious anemia virus interacts with the AP-2 clathrin-associated adaptor protein complex[12], another set of proteins in the same membrane-sorting pathway. Pro-Pro-X-Tyr proteins bind to Nedd4-like family of E3 ubiquitin protein ligases[13], and there is clear evidence that mono-ubiquitination regulates protein sorting in the multivesicular body pathway[8]. Other evidence that ubiquitin ligases function in the same pathway as Tsg101 comes from the finding that the L-domain function of a Pro-Pro-X-Tyr-bearing virus, Moloney murine leukemia virus, is inhibited by the dominant negative Vps4 mutant[6]. Although different L-domains interact with different cellular factors, it nonetheless appears that all of these factors are components of the same endosomal/multivesicular-body pathway.

There is still much to find out, of course. Tsg101 normally functions as a complex with other cellular proteins[8]. Are all of the factors in this complex required for virion budding? How are they normally regulated? Do cellular antiviral responses regulate any of them? Does binding of viral structural proteins to the factors contribute to host cell killing? And, does such binding block the host cells' ability to present viral antigens to the immune system?

Finally, by working out the details of Tsg101 function, virus-specific targets might be identified that will steer development of new anti-viral inhibitors. In the same way that a given broad-spectrum antibiotic cures infection with several bacterial species, such drugs might rescue victims from the clutches of viruses as disparate as HIV-1 and Ebola.

1. Martin-Serrano, J., Zang, T. & Bieniasz, P.D. HIV -1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. *Nature Med.* **7**,1313–1319 (2001).
2. Göttlinger, H.G., Dorfman, T., Sodroski, J.G. & Haseltine, W.A. Effect of mutations affecting the p6 gag protein on human immunodeficiency virus particle release. *Proc. Natl. Acad. Sci. USA* **88**, 3195–3199 (1991).
3. Huang, M., Orenstein, J.M., Martin, M.A. & Freed, E.O. p6Gag is required for particle production from full-length human immunodeficiency virus type 1 molecular clones expressing protease. *J. Virol.* **69**, 6810–6818 (1995).
4. Wills, J.W. *et al.* An assembly domain of the Rous sarcoma virus Gag protein required late in budding. *J. Virol.* **68**, 6605–6618 (1994).
5. Parent, L.J. *et al.* Positionally independent and exchangeable late budding functions of the Rous sarcoma virus and human immunodeficiency virus Gag proteins. *J. Virol.* **69**, 5455–5460 (1995).
6. Garrus, J.E. *et al.* Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell* **107**, 55–65 (2001).
7. VerPlank, L. *et al.* Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55(Gag). *Proc. Natl. Acad. Sci. USA* **98**, 7724–7729 (2001).
8. Katzmann, D.J., Babst, M. & Emr, S.D. Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell* **106**, 145–155 (2001).
9. Vogt, V.M. Ubiquitin in retrovirus assembly: actor or bystander? *Proc. Natl. Acad. Sci. USA* **97**, 12945–12947 (2000).
10. Braaten, D. & Luban, J. Cyclophilin A regulates HIV-1 infectivity, as demonstrated by gene targeting in human T cells. *EMBO J.* **20**, 1300–1309 (2001).
11. Elbashir, S.M. *et al.* Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* **411**, 494–498 (2001).
12. Puffer, B.A., Watkins, S.C. & Montelaro, R.C. Equine infectious anemia virus Gag polyprotein late domain specifically recruits cellular AP-2 adapter protein complexes during virion assembly. *J. Virol.* **72**, 10218–10221 (1998).
13. Kikonyogo, A. *et al.* Proteins related to the Nedd4 family of ubiquitin protein ligases interact with the L domain of Rous sarcoma virus and are required for gag budding from cells. *Proc. Natl. Acad. Sci. USA* **98**, 11199–11204 (2001).

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α-synuclein and cytosolic dopamine: Stabilizing a bad situation

The intriguing finding that α-synuclein—a protein recently found to be mutated in some familial cases of Parkinson disease—and cytosolic dopamine interact to form adducts that stabilize a presumably toxic intermediate of fibril formation provides clues into the mechanism of neurodegeneration.

DAVID SULZER

Parkinson disease (PD) is a neurodegenerative disorder characterized by resting tremor, rigidity, and difficulty in initiating voluntary movement. These deficits are primarily due to the progressive death of neuromelanin-expressing dopamine (DA) neurons of the substantia nigra. The pathological hallmarks of PD are Lewy bodies; large non-membrane-bound inclusions composed of ubiquitinated and aggregated protein fibrils, which are absent in normal substantia nigra DA neurons.

Most PD cases are 'idiopathic'; that is, with no known genetic or environmental cause. However, mutations in at least three proteins underlie rare instances of autosomal PD. The first mutation to be discovered in PD patients was in a presynaptic protein, α-synuclein (α-SYN)[1]. In the 9 November issue of *Science*[2], Lansbury and colleagues suggest that DA and α-SYN might interact to initiate the specific neurodegeneration of substantia nigra neurons in familial and idiopathic PD. The interaction appears to produce a DA–α-SYN adduct that stabilizes a presumably toxic intermediate, the so-called protofibril, by inhibiting its conversion to fibrils.

α-SYN is a natively unfolded protein, but contains an A2 apolipoprotein α-helix-like region—a region that is suspected to reversibly bind vesicular membranes[3]. The findings that α-SYN knockout mice show an enhanced rate of recovery of DA synaptic transmission[4], whereas inhibition of α-SYN expression in culture reduces the population of synaptic vesicles in the 'reserve pool'[5], suggests that α-SYN functionally interacts with synaptic vesicles, perhaps by modulating vesicle trafficking. It is not yet clear how these apparently normal actions of α-SYN might relate to PD etiology.

A clue that mutant α-SYN may provide a toxic gain of function came from the

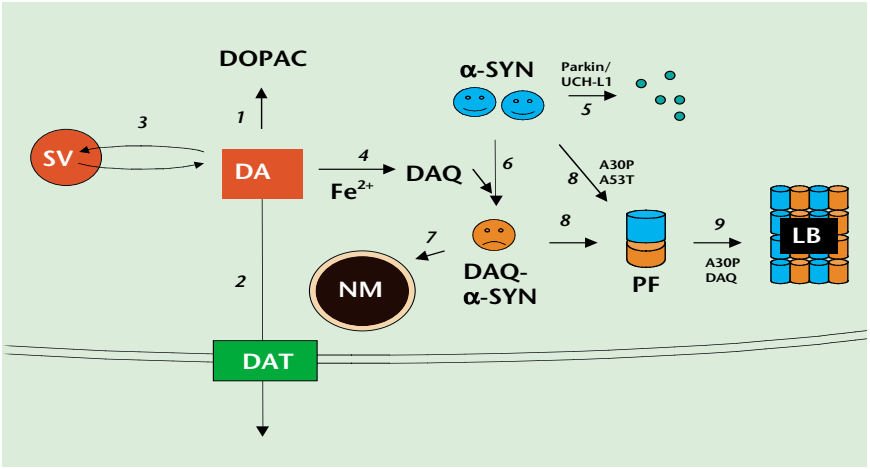

Fig. 1 Model for pathogenic interactions between dopamine and α-SYN in neuronal cytosol. Substantia nigra neurons possess multiple mechanisms to protect the cytosol from α-SYN/DA–α–SYN protofibrils. DA levels in the neuronal cytosol can be maintained at safe levels by (1) conversion to DOPAC by monoamine oxidase (2) release from the cell by reverse transport across the DA uptake transporter (DAT), and (3) accumulation by the vesicular monoamine transporter into synaptic vesicles (SV). However, 'excess' cytosolic DA is converted to a quinone (DAQ) in the presence of iron (4). 'Excess' or damaged α-SYN may be degraded to small peptides by the ubiquitin–proteasome pathway (5); parkin has been implicated in the recognition of the protein for ubiquitin-tagging, and UCH-L1 might also regulate this pathway. If DAQ is not sequestered by cytosolic antioxidants such as glutathione (not shown), it can react covalently with proteins including α-SYN (6). One way to sequester DA–α-SYN adducts from the cytosol may be by autophagy and formation of neuromelanin (NM) granules (7). The combination of native or mutant α-SYN and DA–α-SYN exacerbates the formation of pathogenic protofibrils (PF) (8). The cell attempts to safely sequester protofibrils by further oligomerization to fibrils and formation of Lewy bodies (LB)(9); the A30P α-SYN mutation and DA–α-SYN inhibit this step, thereby causing further toxic build up of protofibrils.

discovery that α-SYN is a major component of the insoluble fibrils of Lewy bodies in PD, and possibly in amyloid plaques in Alzheimer disease[6]. The transformation of α-SYN from a soluble state to mass aggregated fibrils involves an increase in β-sheet content and progressive oligomerization. Transient small units of oligomers of β-folded proteins are known as protofibrils. The two known α-SYN mutations that underlie autosomal PD enhance the rate of protofibril formation[7], whereas oligomerization of α-SYN with the closely related protein β-SYN inhibits fibrillization[8].

In an effort to identify drugs that interfere with α-SYN fibrillization, Conway *et al.*[2] tested many different commercially available compounds. Notably, the compounds found to block fibril formation were catecholamines, including DA and its precursor, L-DOPA. The block occurred during the process of oligomerization, by stabilizing the protofibril at the expense of fibril formation. Catecholamines are readily oxidized in the presence of iron to highly reactive metabolites, such as DA-quinone (DAQ), that covalently bind proteins. Because antioxidants that maintain DA in a reduced state also block its inhibition of fibril formation, it is likely that the DAQ metabolite plays a role in protofibril stabilization.

Conway *et al.* then reacted α-SYN with DA and isolated DAQ–α-SYN adducts. The native α-SYN and DAQ–α-SYN adduct were mixed at varying ratios to compare the rate of fibrillization. A low fraction of DAQ–α-SYN inhibited conversion of protofibrils to fibrils—in other words, DAQ may actually inhibit Lewy body formation by binding α-SYN.

A number of cellular mechanisms keep DA from associating with α-SYN and causing neurotoxicity. Several of these involve keeping cellular levels of DAQ below a certain threshold. Neurotoxicity due to elevated cytosolic DA and resulting DAQ formation has long been implicated in neurodegeneration from models of methamphetamine toxicity[9]. When cytosolic DA exceeds the native antioxidative buffering of the neuronal cytosol, DAQ and associated oxyradicals are produced. Cytosolic DA is normally maintained at a safe level via uptake into synaptic vesicles, release from the cytoplasm by reversal of the DA uptake transporter, and breakdown by monoamine oxidase. If DAQ is still produced, it can be sequestered by glutathione.

If, despite these protective mechanisms, DAQ remains and succeeds in modifying soluble cytosolic proteins such as α-SYN, the ubiquitin–proteasome pathway can degrade the damaged proteins. Parkin, a protein that in mutant form underlies rare cases of autosomal PD, may be required for α-SYN degradation by the ubiquitin–proteasome pathway[10]. Another protective mechanism could be by autophagy of cytosolic DAQ modified proteins, which results in accumulation of neuromelanin in substantia nigra during normal aging.

Perhaps these pathways are sufficient to inhibit PD in most individuals. However, for those for whom it is not, fibrillation and Lewy body formation may be a last resort for removing DAQ damaged α-SYN (Fig. 1). If this is true, the Lewy body will be thought of in a new light: as a desperate attempt by the neuron to save itself by sequestering protofibrils.

It is striking that substantia nigra neurons can display enormous Lewy bodies that occupy most of the perikariya, in addition to neuromelanin granules that occupy much of the remaining cytosol. If Lewy bodies were protective, the finding would be consistent with recent reports in other neurodegenerative disorders, including Huntington disease and spinocerebellar ataxia, where ubiquitinated fibrillar aggregates appear to be protective. In this light, it is interesting that the autosomal recessive PD linked to *parkin* mutations lacks Lewy bodies—an observation consistent with protofibrils, and not fibrils, being the toxic agents.

A requirement for cytosolic catecholamines in protofibril stabilization would provide an explanation of why most PD cell death occurs in substantia nigra and neuromelanin-expressing norepinephrinergic neurons of the locus coeruleus. Similarly, if α-SYN inhibits protofibril formation, a α-SYN deficiency may explain why Lewy bodies are formed in non-catecholaminergic neurons in a related disorder, Diffuse Lewy Body disease[8].

A caveat in these models is that experiments on α-SYN protofibril formation have been conducted in cell-free systems. Clearly, additional cytosolic factors such as glutathione, ubiquitination and autophagy will modify these pathways in the substantia nigra. It will be interesting to see if mice with mutant α-SYN exposed

to chemical models of PD neurodegeneration such as 6-hydroxy-dopamine, which readily produces DAQ, or MPTP, show results consistent with this model. It is also imperative to establish why protofibrils are neurotoxic. Given the possible functional association of α-SYN with synaptic vesicles, perhaps protofibrils perturb intracellular membranes; indeed the Lansbury group has noted a disruption of synthetic vescicles by protofibrils[11]. If DA synaptic vesicle membrane is damaged, protofibrils could further increase cytosolic DA levels.

As promising as these newly identified mechanisms are, they do not solve the central riddle of why some individuals develop idiopathic PD. However, the unification of two previously separate areas of investigation, cytosolic DA and α-SYN pathogenic mechanisms, has provided some intriguing clues that may help unravel this enigma.

1. Polymeropoulos, M. H. *et al.* Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science* **276**, 2045–2047 (1997).
2. Conway, K.A., Rochet, J.-C., Bieganski, R.M. & Lansbury, P.T. Kinetic stabilization of the α-synuclein protofibril by a dopamine-α-synuclein adduct. *Science* **294**, 1346–1349 (2001).
3. Clayton, D. F., & George J. M. The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. *Trends Neurosci.* **21**, 249–254 (1998).
4. Abeliovich, A. *et al.* Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron* **25**, 239–252 (2000).
5. Murphy, D.D., Rueter, S.M., Trojanowski, J.Q. & Lee, V.M. Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. *J. Neurosci.* **20**, 3214–3220 (2000).
6. Spillantini M.G., *et al.* α–synuclein in Lewy bodies. **388**, 839–840 (1997).
7. Conway, K.A. *et al.* Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. *Proc. Natl. Acad. Sci. USA* **97**, 571–576 (2001).
8. Hashimoto, M., Rockenstein, D., Mante, M., Mallory, M. & Masliah, E. β-synuclein inhibits α-synuclein aggregation: a possible role as an anti-parkinsonian factor. *Neuron* **32**, 213–223 (2001).
9. Cubells, J.F., Rayport, S., Rajendran, G. & Sulzer, D. Methamphetamine neurotoxicity involves vacuolation of endocytic organelles and dopamine-dependent intracellular oxidative stress. *J. Neurosci.* **14**, 2260–2271 (1994).
10. Shimura, H. *et al.* Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. *Science* **293**, 263–269 (2001).
11. Volles, M.J. *et al.* Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. *Biochemistry* **40**, 7812–7819 (2001).

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A new feature on the cholesterol-lowering landscape

Reduction in blood levels of low-density lipoprotein cholesterol reduces the risk of coronary heart disease. The identification of a new class of compounds that upregulate the low-density lipoprotein receptor may lead to new therapeutic advances. (pages 1332–1338)

DANIEL J. RADER

Increased low-density lipoprotein (LDL) cholesterol is an established risk factor for atherosclerosis, the underlying cause of coronary heart disease (CHD) and most strokes[1]. The most effective drugs for reducing blood levels of LDL cholesterol are the statins, which have been shown to significantly reduce the risk of coronary events and stroke in clinical trials[1]. These data have led to recent guidelines that expand the use of LDL-lowering drug therapy to more patients[2]; an estimated 36 million people in the United States alone are now candidates for drug therapy.

Despite the success with statins, there is still a need for new therapies to reduce LDL cholesterol. Some patients are unable to tolerate statins (due primarily to musculoskeletal symptoms) and the recent withdrawal of cerivastatin from the market as a result of several cases of rhabdomyolysis has heightened concerns about the safety and tolerability of statins. More importantly, many patients do not achieve the LDL cholesterol goal with statin therapy alone. In this issue, Grand-Perret *et al.*[3] describe the identification and characterization of a new class of compounds that reduce blood levels of cholesterol in an animal model. While the molecular mechanism of action of the compounds has not been completely elucidated, preliminary data suggest that they upregulate the LDL receptor (LDLR) through a different mechanism than the statins.

The molecular regulation of cellular sterol metabolism has been elucidated by Brown and Goldstein and their colleagues[4] (Fig. 1*a*). The LDLR gene promoter contains a sterol response element (SRE) that is required for regulating transcription of the gene encoding LDLR in response to cellular sterol content. Two SRE-binding proteins (SREBP-1 and -2) have been purified and cloned; they contain two transmembrane domains and are localized to the endoplasmic reticulum (ER). Another protein, termed SREBP-cleavage activating protein (SCAP), acts as a chaperone protein that transports the precursor SREBPs from the ER to the Golgi, where two proteases, site 1 and site 2 protease (S1P and S2P), sequentially cleave the SREBPs. The second cleavage liberates the mature SREBP proteins from the membrane, allowing them to enter the nucleus, bind to the SREs of target genes and, along with additional transcription factors, activate gene transcription. Responsiveness of the system to cellular sterol content is accomplished through a 'sterol-sensing domain' in SCAP. Under cholesterol-replete conditions, the SCAP–SREBP complex remains in an inactive form in the ER through active repression by sterols; in contrast, under cholesterol-depleted conditions, SCAP buds from the ER membrane and transports the SREBPs to the Golgi, where they become activated through proteolysis.

The statins inhibit HMGCoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, reducing cellular sterol content and thereby de-repressing the transport of the SCAP–SREBP complex and resulting in the upregulation of LDLR, which in turn transports blood LDL into the liver, reducing blood levels of LDL (Fig. 1*b*). In contrast, the compounds identified by Grand-Perret *et al.*[3] appear to act directly on the SCAP–SREBP complex, possibly by interacting directly with the sterol-sensing domain in SCAP.

A number of observations suggest that the newly identified compounds might be effective in lowering plasma LDL cholesterol levels. When administered to cells loaded with cholesterol (in which LDLR expression is downregulated) the compounds increase LDLR expression in a dose-dependent fashion that requires the presence of the SRE in the promoter. When administered to hamsters fed a high-fat diet, one of the compounds