protease (NS3-4A) and an RNA polymerase (NS5B) — are attractive drug targets. In the 2000s, inhibitors of these enzymes and of another non-enzymatic but essential HCV protein (NS5A), referred to as direct acting antivirals (DAAs), emerged as the lead targets for HCV drug development. In late 2011, two NS3-4A protease inhibitors were approved for human use in combination with PEGylated interferon and ribavirin, raising treatment success to more than 70% for patients with HCV genotype 1 (there are six highly divergent and variable genotypes of the virus).

However, euphoria over this advance was short-lived. Patients with advanced disease were treated but many others were not, owing to the additional, often severe, side effects of this drug combination and the emergence of viral resistance. In the meantime, and continuing into the present, dozens of new compounds were being tested in the clinic. In 2013, more-potent DAAs, in combination with PEGylated interferon and ribavirin, were approved, as was the first all-oral regimen, consisting of a NS5B-targeting DAA combined with ribavirin alone.

The recent clinical studies[5–11] present the next wave of interferon-free, all-oral, DAA-based regimens, which are likely to be approved in the near future for HCV treatment. Without delving into details and trade names, several key points about these trials emerge. First, they include multiple all-oral combinations that can achieve success rates of more than 95%. 'Success' for HCV treatment means no detectable virus 12 weeks after stopping treatment. Unlike drug treatments for hepatitis B and HIV, most HCV researchers believe that this endpoint represents a durable cure that lowers the risk of progressive liver disease. Second, these treatments are effective in patients who are in greatest need and are most difficult to treat — those with advanced fibrosis and cirrhosis, those who are co-infected with HIV, and even liver-transplant candidates and recipients. Also noteworthy is that the new drug combinations promise shorter treatment times (12 weeks and possibly even less) and minimal side effects; as a result, fewer people are expected to discontinue their treatment.

So from a mystery virus and a 5% treatment-success rate, we have come to an era of cure rates of more than 95% (Fig. 1). Game over, right? Not quite. What about viral resistance to the drugs? With nearly 200 million infected individuals, 6 diverse viral genotypes and around 1 trillion viral variants being generated per day per infected person, it is likely that HCV will have some tricks up its sleeve to develop resistance. However, some of the new DAAs, in particular sofosbuvir, which targets the active site of NS5B, have an extremely high barrier to resistance, and there have been only rare glimpses of resistant variants in clinical observations with multiple viral genotypes[13]. Combining potent DAAs, each with lower resistance barriers, can still be highly effective at avoiding the build-up of resistance. Nonetheless, resistance will undoubtedly occur and should be taken into account to guide treatment decisions. The current drugs are also less effective against genotype 3 HCV, which is common in South Asia, although pan-genotype drugs are in development.

Another barrier is identifying those infected. Most people are unaware of their HCV infection[14], and only a small minority has been treated[15]. Although some health agencies have recommended universal screening of high-risk groups, implementing such policies is challenging and time-consuming. And once infected individuals are identified, how will society pay for their treatment? The current price tag for cutting-edge HCV treatment in the United States is more than US$80,000 for a 12-week course. Competition among pharmaceutical companies may lower this price, but most people infected with HCV live in countries that cannot afford the new treatments. Fortunately, there is movement in the pharmaceutical industry to provide for low-cost drug production in certain countries, such as Egypt, where an estimated 10% of the population is infected. Finally, getting rid of the virus does not always erase the risk of future liver-related problems — patients still need to be monitored routinely for liver function and cancer, particularly those whose infection had led to cirrhosis.

With the new drugs that are in hand or on the horizon, we have the means to eradicate this virus, possibly without needing a vaccine. However, the challenge now is to extend these great medical advances on a national and global scale to those in need — something that has not been terribly effective in the past. We can hope that implementing these transformative HCV advances will help to create a model for success, for this and other widespread human diseases. ■

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NEUROLOGICAL DISORDERS

Quality-control pathway unlocked

A modified ubiquitin protein has been identified by three independent studies as the missing link in a cellular quality-control pathway that is implicated in Parkinson's disease. SEE LETTER P.162

ASA ABELIOVICH

Parkinson's disease, a progressive neurodegenerative disorder, has long been hypothesized to be caused by defects in organelles called mitochondria, which power mammalian cells through the production of ATP molecules. An accumulation of dysfunctional mitochondria may lead not only to a cellular energy crisis, but also to excessive production of toxic by-products. Two enzymes implicated in Parkinson's disease, PINK1 and parkin[1,2], are thought to be involved in the disposal of defective mitochondria, but how the two proteins interact has been unclear. A trio of studies (by Kane *et al.*[3], writing in the *Journal of Cell Biology*; by Kazlauskaite *et al.*[4], in the *Biochemical Journal*; and by Koyano *et al.*[5], on page 162 of this issue) now report that phosphorylated ubiquitin protein is the link between PINK1 and parkin, providing insights into a complex system of parkin regulation.

Kinase enzymes such as PINK1 alter the behaviour of target proteins through the addition of phosphate groups, a process called phosphorylation. PINK1 is imported to mitochondria and, in healthy cells, undergoes

rapid degradation[6]. However, if mitochondria are defective or damaged (for example by exposure to CCCP, a poison that blocks ATP production), PINK1 accumulates, becoming anchored to the outer mitochondrial membrane with its kinase domain exposed to the cytoplasm.

Damaged mitochondria also attract parkin, which is otherwise dispersed throughout the cytoplasm in healthy cells[7]. Parkin is a ubiquitin ligase, which adds ubiquitin proteins (either singly or in polyubiquitin chains) both to itself through autoubiquitination and to nearby target proteins. Ubiquitinated proteins can serve as a signal to the cell that a cellular compartment should be degraded, which in damaged mitochondria leads to their timely disposal[7], a process known as mitophagy.

Mutations in either *PINK1* or *PARKIN* that underlie rare familial forms of Parkinson's disease disrupt mitophagy, implicating this cellular pathway in Parkinson's disease[7]. Furthermore, *PINK1* mutations impede the recruitment of parkin to damaged mitochondria, suggesting that the proteins act in a linear pathway. Consistent with a PINK1–parkin quality-control pathway, mutations in *pink1* or *parkin* in fruit flies cause accumulation of defective mitochondria and cellular degeneration[8,9].

Initial models proposed that PINK1 phosphorylates and so activates parkin in damaged mitochondria. Although direct phosphorylation of parkin by PINK1 has been documented[10], this modification does not seem to be sufficient for full activation of parkin's ubiquitin-ligase activity[3–5,10]. In search of a functional connection between PINK1 and parkin, three groups undertook cell-wide protein analyses and biochemical studies, and found the missing link between the two — phosphorylated ubiquitin (phospho-ubiquitin).

Each study showed that, in cells in which PINK1 was activated by CCCP treatment, PINK1 phosphorylates ubiquitin at a serine amino-acid residue (serine 65). Strikingly, a corresponding serine-65 residue in a ubiquitin-like domain is the aforementioned target of PINK1 phosphorylation on parkin[10]. Subsequent analyses by all three groups demonstrated that modified ubiquitin, in turn, induces parkin activity (Fig. 1).

Koyano and co-workers found that modified ubiquitin alone could not fully activate parkin — complete activation required coincident modification of parkin's ubiquitin-like domain as well as of ubiquitin, each at their respective serine-65 residues. A unique aspect of this group's work is their use of a strain of yeast that harbours a mutant form of ubiquitin lacking the serine-65 residue, which cannot be phosphorylated by PINK1. When the authors added human PINK1 and parkin to these cells, they found that parkin was not activated, underscoring the idea of an ordered pathway for mitophagy.

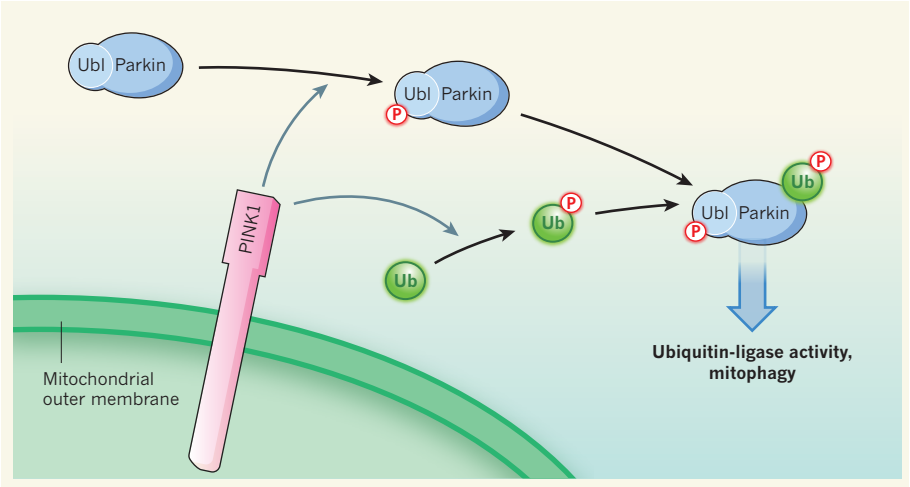

Figure 1 | PINK1 and parkin in mitochondrial quality control. Mitochondrial damage leads to anchoring of the PINK1 enzyme to the outer mitochondrial membrane, with its kinase domain facing the cytoplasm. PINK1 adds a phosphate group (P) to the ubiquitin-like domain (Ubl) of the ubiquitin-ligase enzyme parkin. Three studies[3–5] find that PINK1 also phosphorylates the ubiquitin (Ub) protein itself. Phosphorylated ubiquitin directly binds to and activates parkin. Activated parkin ligates ubiquitin and phospho-ubiquitin molecules to nearby target proteins, leading to disposal of the damaged mitochondria through mitophagy.

Whereas all three studies implicate phosphorylated ubiquitin as an intermediary in the PINK1–parkin pathway, the role of direct phosphorylation of parkin by PINK1 seems more complex. Koyano and colleagues report that modification of both ubiquitin and parkin at serine-65 is necessary for full activation of parkin in cells. But Kane and colleagues found evidence that modification of ubiquitin alone can activate parkin. This discrepancy is likely to relate to the distinct assays used in the studies, rather than to a biological difference.

Consistent with phospho-ubiquitin's activating role, Kane *et al.* and Koyano *et al.* found that it binds directly to parkin. Koyano and colleagues took the studies a step further, demonstrating that phospho-ubiquitin can still be used by parkin as a substrate for ubiquitination and autoubiquitination. But, surprisingly, the group found that parkin could be activated by phospho-ubiquitin that was mutated or modified such that it could not act directly as a substrate in ubiquitination. This implies that phospho-ubiquitin binds to and activates parkin separately from its role as a substrate.

Clues as to how this could be achieved might be gleaned from recent crystallographic analyses of parkin[11,12]. A phospho-peptide binding pocket has been proposed[11] to lie within an inhibitory domain in parkin that, when the protein is inactive, occludes access to its catalytic active site. Kazlauskaite *et al.* speculate that the active site of parkin could be exposed by conformational changes brought about by the binding of phospho-ubiquitin's phosphate group to this inhibitory domain.

Kane and co-workers' data point to another role for phospho-ubiquitin — recruiting parkin to the outer membrane of damaged mitochondria. A particularly interesting idea is that such recruitment may generate a positive feedback loop, in which recruited parkin would be predicted to ligate additional phospho-ubiquitin to nearby proteins, attracting yet more parkin.

A subset of known parkin substrates, including the proteins mitofusin 2 and Miro, regulate mitochondria[13,14], and their ubiquitination by parkin may be required for normal mitophagy. It will be important to determine whether activation by phospho-ubiquitin affects parkin's target selection, the fate of ubiquitinated target proteins, or the structure of polyubiquitin chains formed on targets. Finally, drugs that mimic the effects of phospho-ubiquitin may be candidate therapeutics for inherited and sporadic forms of Parkinson's disease. ■

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