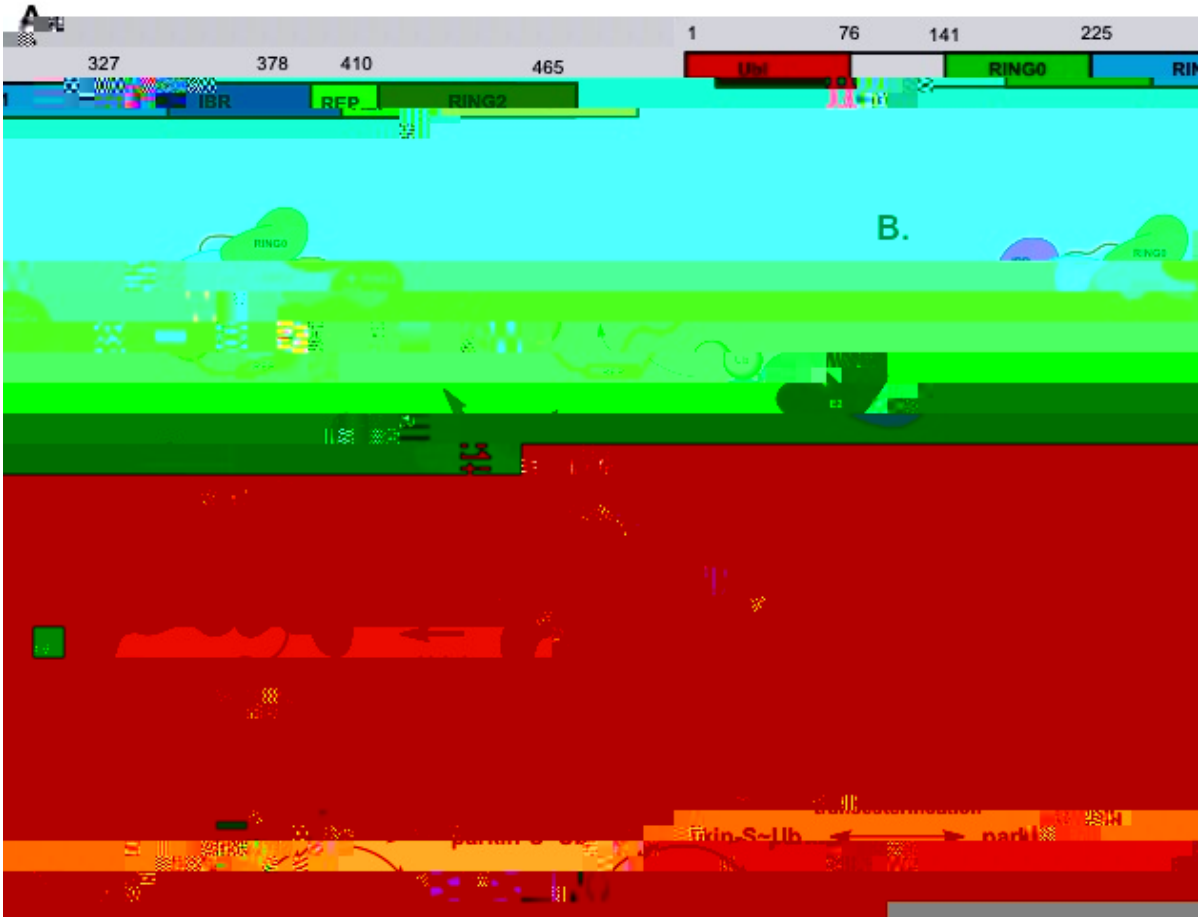
operate together in a pathway that maintains the health and normal function of mitochondria, with parkin likely acting downstream of PINK1[7,8]. Subsequent studies in mammalian cells demonstrated that parkin mediates the engulfment of impaired mitochondria by the autophagy pathway. This is one avenue in which the cell can quarantine and destroy hazardous material from within to protect itself from further damage. For impaired mitochondria, this process is specifically termed mitophagy, and the action of parkin here is dependent on PINK1[9]. Parkin responds to mitochondrial damage following PINK1 accumulation at the outer mitochondrial membrane when these organelles have lost their membrane potential[10]. In recent years, it has come to light that parkin manages a portfolio of mechanisms that act to maintain mitochondrial health, termed [5,11]. Through these mechanisms, parkin may play a major role in safeguarding neurons, raising the exciting possibility that parkin activity could be fine tuned as a therapeutic strategy in PD.

Wielding the recently discovered full-length crystal structure of parkin[12] as a framework, the goal of my project was to compare the function of mutant forms of the enzyme to the wild type form. Mutations were engineered to alter amino acids in parkin domains that we hypothesized would impact its ability to carry out crucial enzymatic functions. After expressing parkin in mammalian cancer cell lines, I examined the performance of mutant and wild type parkin at two major endpoints: (a) in mitochondrial translocation (recruitment) and (b) eventual clearance of the organelles (mitophagy), in response to a chemical trigger that simulates the effects of severe damage to mitochondria.

The ubiquitin ligase cycle of parkin proceeds like a game of “hot-potato,” where an E2 conjugating enzyme will first pass ubiquitin (the “potato”) to the active site of parkin (located at cysteine-431; see figure 1B). Following this, parkin will pass ubiquitin to a substrate protein[13]. Ubiquitin can then act to target a protein for degradation or promote mitophagy, amongst other pathways. There is evidence that in biochemical reactions carried out in a test tube ( ), the first step of this “hot potato” cycle, called transthiolation, is rate-limiting. On the other hand, the subsequent step (transesterification) likely occurs at a greater rate[12]. In my experiments in living cells, I found that PD-causing mutations which interfere with the initial step of E2-parkin binding (at threonine-240/T240), greatly impair parkin re2 (2 (t) 0.(f ( ) Tj ET Q q 0.24 0 0 0.24 440.1803 g(rki) 0.2Tf [(2

responding to mitochondrial damage with only a brief delay compared to wild type parkin. By contrast, single mutations that selectively impair the transesterification step of catalysis, for instance a mutation at histidine residue 433, do not detectably slow down parkin recruitment in cells.

Overall, these findings advance evidence that the transthiolation step in parkin catalysis is rate-limiting for the response of this enzyme to mitochondrial distress in cells. The results here suggest that if we are to upregulate parkin activity in PD brains as a neuroprotective strategy, for example with small molecule drugs, the transthiolation step would serve as a robust focus for manipulation. Further elucidation of parkin's role in mitochondrial quality control mechanisms may bring us closer to finding a tool to slow PD progression by helping vulnerable neurons survive. With our continued efforts, we aim to give these neurons the boost they need to halt neurodegeneration in its tracks.



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