Parkinson Disease: From Cellular and Animal Models to Genomics

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Abstract: Parkinson disease is one of the most common human neurodegenerative diseases. Its importance has led to a large number of studies focused on the development of cellular and animal models for the disease. We first discuss the potentials and limitations of the available mammalian models for PD. The results obtained so far in some alternative models, such as yeasts or invertebrates (*Drosophila*, *Caenorhabditis*), that may be used to develop rapid genetic or pharmacological screenings, are also summarized. Finally, we briefly discuss the results derived from novel approaches, such as the analysis of expression profiles using microarrays and proteomic analyses of cellular and animal models of Parkinson disease. Integration of the data derived from all those approaches emerges as a significant problem to be solved in the next few years.

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INTRODUCTION

Parkinson disease (PD) is one of the most prevalent neurodegenerative diseases, affecting more than 1% of people older than 60 years. The main symptoms of the disease are resting tremor, bradykinesia, rigidity and postural instability. These symptoms are caused by the death of dopaminergic neurons in several regions of the brain, and most prominently in the substantia nigra, pars compacta. Therefore, the disease is chronic and progressive (reviewed in [1, 2]). About 90% of PD cases are said to be sporadic or idiopathic, meaning that there are not obvious causes that elicit the disease. Environmental factors may significantly contribute to the development of sporadic PD. However, it is known that genetic factors play also a significant role. On one hand, about 10% of PD cases are familial, being associated to particular mutations in certain genes (Table **1**. See recent reviews: [3-6]). On the other hand, global studies have shown that sporadic PD also has a significant genetic influence [7], and evidence for an involvement of some of the genes that generate familial PD in sporadic PD is growing [5,8]. Particularly, mutations in *LRRK2* may explain about 1-2% of the sporadic cases of PD [9]. Genetic analyses may thus shed light on the underlying causes that cause both sporadic and familial PD.

The cellular mechanisms associated to dopaminergic neuron loss are still poorly understood [4,8]. The main cytological hallmark is the presence in those neurons of proteinaceous cytoplasmic inclusions known as Lewy bodies (LBs), mainly formed by two proteins, ubiquitin and α–synuclein. The composition of LBs suggested that dopaminergic cell death may be in some way related to the mechanisms of protein degradation and turnover, perhaps through the ubiquitin-proteasome system. The study of familial PD cases indirectly supports this hypothesis. Two of the genes for which mutations have been shown to cause PD are obviously related to ubiquitin metabolism. One of them, called *parkin* (or *PARK2*) encodes a ubiquitin ligase, and recessive mutations in this gene cause early- or even juvenile-onset PD. Another gene, called *UCHL1* (*Ubiquitin C-terminal hydrolase L1*, also known as *PARK5*), encodes a ubiquitin hydrolase, that may also behave as a ubiquitin ligase in some circumstances [10]. A rare missense mutation in *UCHL1* has been shown to generate autosomal dominant forms of PD and a particular polymorphism in this gene seems to confer protection against sporadic PD [11] (see also [5] for a review). A third clue pointing towards protein control mechanism is the finding that rare dominant mutations as well as duplications and triplications in the gene *PARK1*, that encodes α–synuclein, may also cause familial PD (reviewed in [12]). The cellular function of α–synuclein is unknown. However, as we mentioned above, α–synuclein is one of the main constituents of LBs, suggesting it is involved in the pathologic process that leads to dopaminergic neuronal death. That parkin and α–synuclein are functionally related is shown by the fact that a glycosilated form of α–synuclein is a substrate of parkin [13] and that parkin and α–synuclein may contribute to generate Lys-63 polyubiquitin chains [14]. UCHL1 seems to act also on α–synuclein [10]. α–synuclein monomers tend to aggregate to form amyloid fibrils, which are found in LBs. Moreover, they may also form nonfibrilary oligomers that have been called protofibrils. It is unclear which one of these two types of aggregation is more dangerous to the cell. The conspicuous LBs might be part of the pathologic process or even the proximal cause of death of the cell, but they might alternatively be an extreme response by a cell under such level of stress that is compromising its function. Actually, the fact that the mutant forms of α–synuclein that generate PD promote the formation of protofibrils suggests that these oligomeric forms may be the main cause of α–synuclein-associated neurotoxicity. In any case, it seems clear that anomalies in α–synuclein protein metabolism, due to abnormal forms of the protein or simply an excess of it, may contribute to familiar PD (see reviews: [4,15]).

In parallel to the evidence that suggest an involvement of the mechanisms of protein control in PD, a second line of

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Table 1. Parkinson Disease-Related Genes

GENE	CHROMOSOME LOCATION	TYPE OF HEREDITY	PHENOTYPIC FEATURES	LEWY BODIES
α-synuclein (PARK1)	4q21	Autosomal dominant	Early onset, dementia	Yes
Parkin (PARK2)	6q25	Autosomal recessive	Juvenile Slow progression	Rarely
PARK3	2p13	Autosomal dominant	Late onset, Dementia Rapid progression	Yes
UCHL1 (PARK5)	4p14	Autosomal dominant? Susceptibility to sporadic PD	Late onset	Yes
PINK1 (PARK6)	1p35-36	Autosomal recessive	Early onset Slow progression	?
DJ1 (PARK7)	1p36	Autosomal recessive	Early onset Slow progresion	?
LRRK2 (PARK8)	12q12	Autosomal dominant	Late onset (Sporadic?)	Yes/No
PARK9	1p36	Autosomal recessive	Kufor-Rakeb syndrome	?
PARK10	1p32	Autosomal recessive (?)	Late onset	?
PARK11	2q36-37	Autosomal dominant (?)	?	?
NR4A2 (Nurr1)	2q22-23	Autosomal dominant	Late onset	?
MAPT (Tau)	17q21	Susceptibility to sporadic PD		

research strongly supports that other mechanisms may also significantly contribute to the genesis of the disease. Several chemical agents have been shown to specifically damage dopaminergic neurons. The analyses of the mechanisms of action of the two best known, 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), has firmly established a link between dopaminergic neuron death and mitochondrial dysfunction leading to oxidative stress (reviewed in [16]). Other products, that in some cases have been shown to contribute to the sporadic disease (e. g. pesticides such as rotenone, maneb or paraquat), may similarly damage the cells. Moreover, there is good evidence showing that one of the genes whose mutations cause familial PD, named *DJ-1* or *PARK7*, is part of a mechanism of defence to cope with oxidative stress ([17,18]; other roles have however been attributed to this protein, see [19]). Recent results by Shendelman *et al.* [20] suggest that one of DJ-1 main roles is to act as a chaperone that is activated by oxidative stress. Evidence for a protective role in mitochondria has been also described [21]. Similarly, PINK1 is a mitochondrial protein that has been shown to protect cells against mitochondrial dysfunction [22].

In summary, there are at least two well-founded work hypotheses regarding the types of stress that may lead to dopaminergic neuron death (Fig. (**1**)). It is reasonable to expect both hypotheses to be reflecting related aspects of PD. This is most clearly shown by Shendelman *et al.* [20] demonstration that the oxidative-stress activated chaperone function of DJ-1 is used to avoid the generation of protein aggregates. Particularly, α–synuclein is one of the targets of DJ-1, which suppresses the generation of α–synuclein protofibrils [20]. Moreover, in cell lines, both abnormal forms of DJ-1 and wild-type DJ-1 protein have been shown to interact with parkin, although the wild-type DJ-1 and parkin proteins only interact under oxidative stress conditions [23]. In summary, oxidative damage and protein aggregation are linked and the products encoded by genes involved in familial PD are probably part of a single pathway (Fig. (**1**)). It is obvious, however, that we still lack a sufficient understanding of the molecular and cellular mechanisms leading to PD. For example, we do not have a clear view of the relative contributions of protein metabolism-associated stress and oxidative stress in sporadic PD or of all the precise cellular connections between those two types of stress. Therefore, the development of cellular or animal models of PD to understand the multiple cell functions that are susceptible to be damaged along PD progression has become a priority.

MAMMALIAN MODELS: MANY HINTS AND A BIT OF DISAPPOINTMENT

The obvious animal models for PD are mammalian species, which have a dopaminergic system very similar to the human one. Rodent species, and most especially the mouse, would seem to be the best experimental choice. The possi-

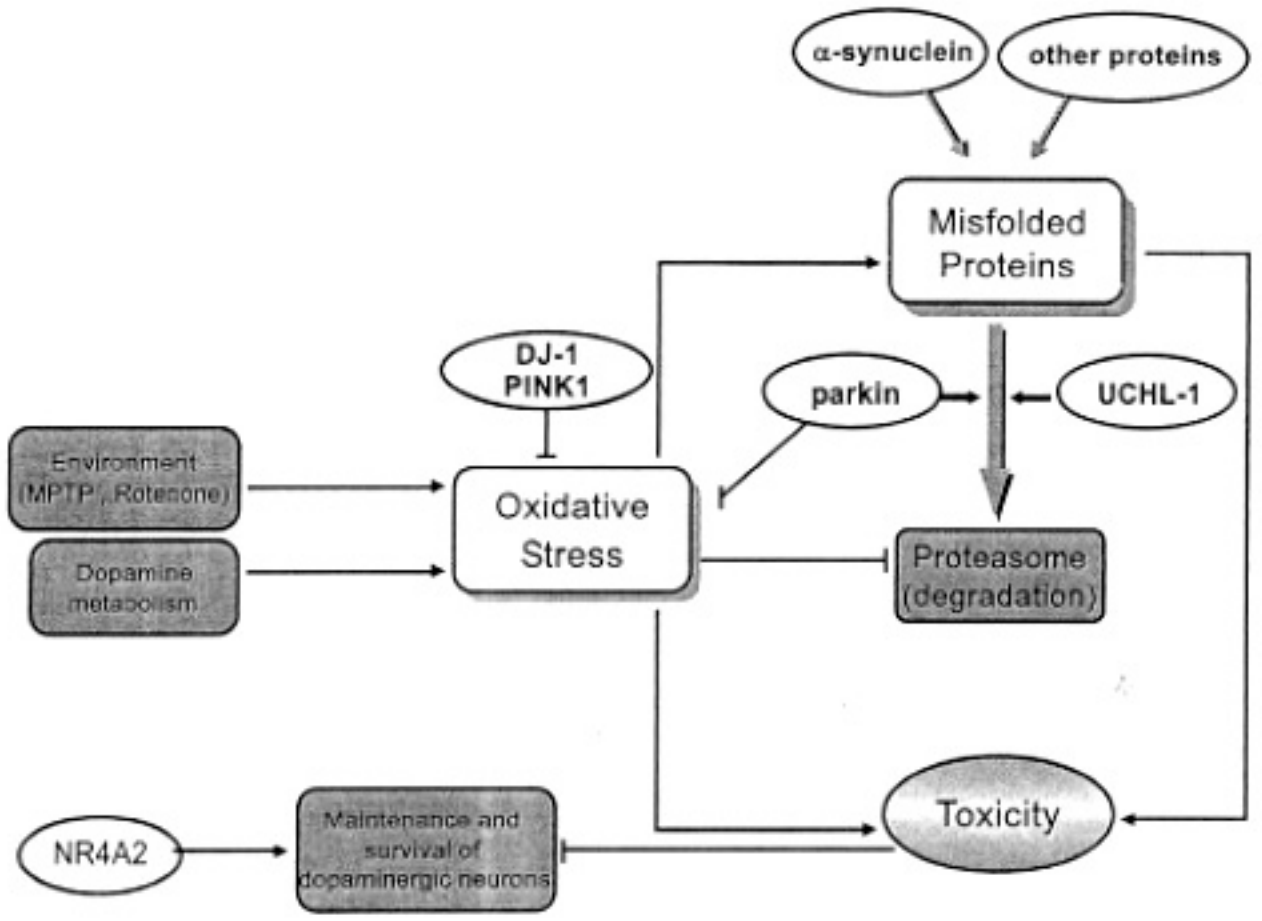

Fig. (1). A schematic view of the main processes involved in the genesis of PD and the possible impact of PD-related genes on those processes.

bility of generating both knockout and transgenic animals in mouse opens venues for the precise study of PD-related genes *in vivo*. Also, the toxics known to generate loss of dopaminergic neurons in humans may be similarly administered to rodents to observe their precise molecular and cellular effects.

The truth is, however, that, despite intense efforts, there is still no mouse genetic model that fully recapitulates the characteristics of the human disease [8,24,25]. For example, in the case of α–synuclein, it was reasonable to expect that overexpression of the protein, or simply expression of the mutant forms that contribute to the human disease, would generate a PD-related syndrome in mice. Thus, multiple transgenic mice that express both wild type and mutant forms of α–synuclein have been generated (reviewed in [26]). The phenotypes of those transgenic models are varied, including in some cases α–synuclein inclusions and massive neuronal pathology. However, none of them shows signs of dopaminergic cell death. These results strongly suggest that mice dopaminergic neurons are less susceptible to α–synuclein anomalies that human ones. α–synuclein knockout mice have only mild phenotypes, with subtle changes in the dopamine system that may be related to alterations in the mobilization of synaptic vesicles and differential dopamine recruitment [27-29]. Interestingly, null *α–synuclein* mice are less susceptible to MPTP than wild-type mice [30-34], a result that suggest that α–synuclein is a vulnerable point when cell metabolism confronts oxidative damage.

As we already indicated, loss of function of *parkin* is associated to PD in humans. However, none of four putative knockout mice for the *parkin* locus has substantia nigra cell loss. The first two of them [35,36] showed subtle biochemical and behavioural differences respect to wild type mice. A second report by one of those groups [37] suggested an involvement of *parkin* in mitochondrial function and in the control of oxidative damage. A third study found no differences between mutant and wild-type mice [38]. However, *parkin* has a complex alternative splicing pattern and the elimination of its second or third exons, performed to generate those three *parkin* mutants, may still allow the generation of some types of parkin protein. The elimination of the seventh exon of *parkin* -- that supposedly must cause a full knockout of the gene, a fact confirmed by Western blot analyses that failed to detect any form of parkin protein -- has stronger phenotypes, especially loss of locus coeruleus neurons that leads to low norepinephrine concentrations in the olfactory bulb and spinal cord of the mutants [39]. This last result may be optimistically valued, as several studies have shown that the locus coeruleus is affected in PD patients in presymptomatic stages, before any susbstantia nigra cell death (reviewed in [40]). Therefore, it is possible that *parkin* knockout mice are recapitulating the first stages of PD.

Knockout mice for *DJ-1* were also envisaged as promising models. However, they do not show any signs of dopaminergic neuron loss, but only altered dopamine metabolism and behavioural deficits, in addition to increased sensitivity to MPTP [41-43]. Finally, spontaneous knockout mice for *UCHL1* are also known as *gad* (gracile axonal dystrophy) mice [44], due to the fact that the mutation causes a characteristic early sensory ataxia. They suffer from axonal degeneration and also have spermatogenesis defects [45,46]. So far, no transgenic *UCHL1* mice expressing the human abnormal proteins have been generated. Therefore, no obvious model for the dominant form of the disease that seems to be associated to missense mutations in *UCHL1* has been yet obtained. Similarly, *PINK1* or *LRRK2* mice models are not yet available.

All these results suggest that the generation of a genetic model for PD in mice based on our current knowledge of the functioning of familial PD genes is a very difficult task. The single exception may be *NR4A2* mutant mice, for which some promissory results have been very recently obtained. Null mutants for that transcription factor lack nigral dopaminergic neurons and die shortly after birth [47]. However, heterozygotes for a loss of function *NR4A2* mutation survive to adulthood and show some most relevant phenotypes, as increased sensitivity to MPTP and, especially, progressive loss of dopaminergic neurons with age that leads to motor deficits [48,49]. In human substantia nigra, NR4A2 also decreases with age, in a way that correlates with dopaminergic neuron loss [50]. In summary, heterozygote *NR4A2* mice may be the only genetic model of this species in which at least part of the parkinsonian phenotypes are present. Mutants for other transcription factors required for the maintenance of dopaminergic neurons (*engrailed* genes, *Pitx3*) are also being explored to determine their possible connections with PD [51-53]

A second important research venue has been to explore whether mammals respond similarly to humans to drugs that are known to cause dopaminergic neuron death in order to create toxic models of PD. Several of those drugs, such as MPTP, 6-OHDA, rotenone, etc. have been used to mimic the disease, both in rodents and primates (reviewed in [8,16,24,54]). The MPTP models are the most widely used. Both acute and chronic treatments with MPTP lead to nigral cell death in susceptible mammals, generating a syndrome that is similar in many ways to PD [8]. Although these models are promising, they have some significant shortcomings. For example, monkeys treated with MPTP lose nigral neurons, but not neurons of other nuclei typically affected in PD, such as the locus coeruleus. Typical Lewy bodies are also lacking. Finally, some animals mildly affected by the treatment spontaneously recover [8,54].

In summary, mammalian models are contributing to our understanding of PD, but the disease still has not been fully reproduced in any of those models. We can predict that, in spite of its inherent problems, mammalian models will certainly yield new insights in the future. For example, the combination of toxic and genetic models has begun to be explored only recently. As we already mentioned, MPTP or other drugs may be administered to transgenic or knockout PD model mice in order to test increase or decrease of sensitivity to the toxin. These combined approaches are certainly worth exploring. There are however some aspects of PD that may well be difficult to examine in model mammals. On one hand, biochemical and cellular experiments to understand the wild-type function of PD-related molecules are difficult in these organisms. On the other hand, simpler organisms may provide advantages for genetic screening that may lead to the discovery of novel genes conferring susceptibility to the initiation or progression of the disease. These considerations naturally lead to the idea of generating cellular and invertebrate animal models of PD.

YEASTS AND OTHER CELLULAR MODELS OF PD

Different cell types have been used to investigate diverse cellular aspects of PD pathogenesis, such as the molecular mechanisms that lead to cell death by toxins such as MPTP or rotenone, by overexpression of α-synuclein, etc. Many experiments have been performed on commonly used lines, such as the chromaffin-derived rat PC12 line, while other authors have opted for using cell lines with features that make them more similar to dopaminergic neurons (e. g. several human neuroblastoma lines, mouse MN9D, rat MES23.5, etc.). It is however unclear how the metabolism of these lines precisely relates to that of true dopaminergic cells. These have been used only sparsely, due to the intrinsic limitations of experimenting with primary cell lines. Neural stem cells are also interesting systems. They have been used to study differentiation to dopaminergic neurons and their potential in cell therapy in PD is well known [55], so we can expect a large number of studies focused on them in the near future (see e. g. [20]).

A radically different idea has been to use yeast cells to study the cellular and molecular mechanisms impinged by PD-related molecules. It is obvious that the immense time since mammalian and fungi diverged makes difficult to believe that yeast cells may be a good general model for those studies. However, the goal is to use the simplicity of yeast cell analyses to perform the types of experiments that are precluded or much more difficult in other systems [56]. Thus, Willigham *et al.* [57] generated a screen in *Saccharomyces cerevisiae* for genes that, when mutated, cause lethality in conjunction with expression of human α–synuclein. In this way, it is possible to infer which protein products are able to interact, directly or indirectly, with α–synuclein *in vivo*. They discovered 86 interactions, which were interpreted as corresponding mainly to conserved genes (i. e. with human orthologs) and which products were found to be involved, more often than expected by chance, in lipid metabolism and vesicle-mediated transport [57]. These potential effects of α–synuclein on lipid and vesicle function were demonstrated to actually occur *in vivo* in yeast cells [58,59]. Interestingly, some mutations that may increase oxidative stress, such as those that affect the superoxide dismutase *sod2* gene, or an increase of intracellular iron, that also leads to oxidative stress, are lethal in combination with α–synuclein in yeasts [57,60,61]. Toxicity is also increased when human α–synuclein is expressed together with human tau [61]. In summary, analyses in yeast cells may yield rapid insights on the potential functions of poorly understood proteins such as α–synuclein.

INVERTEBRATE MODELS OF PARKINSON DISEASE

If we are planning to perform genetic analyses, two obvious invertebrate models are the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*. These organisms have dopaminergic neurons that have been carefully located and can be studied with conventional techniques [62-65]. *Drosophila* has been extensively studied as a model for neurodegenerative diseases [66-68]. The interest of using *Drosophila* for PD research derives from the striking work by Feany and Bender [69] that suggested that the fly might provide an animal model more similar to humans than the available mouse models. Feany and Bender [69] showed that normal and mutant forms of human α–synuclein, when expressed in dopaminergic neurons lead

to age-dependent cell loss, inclusions similar to Lewy bodies and behavioural deficits, also age-dependent. Moreover, expression of α–synuclein in the eye led to retinal degeneration, allowing for easy genetic or pharmacological screenings for modifiers of that phenotype. These results are even more notable considering that invertebrates lack synuclein genes, and therefore it was unexpected that *Drosophila* could react to α–synuclein expression in such a specific way. This precursor work has been followed by several significant studies that demonstrated that expression of the chaperone Hsp70 was able to suppress α–synuclein-dependent dopaminergic cell death [70] and that geldanamycin, a drug that activates stress response pathways, leading among other effects to an increase of Hsp70 protein levels, was also protective [71,72]. It has been also shown that the dopamine precursor L-DOPA, used to ameliorate PD symptoms, as well as dopamine receptor agonists, improve α-synuclein transgenic flies results in behavioral tests [73]. The similarity between the action of α–synuclein in *Drosophila* and humans was even more strikingly showed by the demonstration that, as occurs in Lewy bodies in human brains affected by PD and other synucleopathies [74], human α–synuclein is phosphorylated at position Ser129 in *Drosophila* transgenic lines [75]. This modification affects the ability of α–synuclein to generate fibrils [74], and a very recent report demonstrates that this phosphorylation is critical for neurotoxicity and aggregation formation in *Drosophila* [76]. All these results demonstrate that *Drosophila* may be an excellent model to understand the so far obscure molecular and cellular actions of α–synuclein, both in normal and pathological conditions. A caveat may be that the phenotypes generated by α–synuclein expression may be not fully penetrant in all conditions, with some transgenic flies being essentially normal [77].

Drosophila does have orthologs of other familial PD-related genes, such as *parkin*, *DJ-1* or *UCHL1*. Of them, only *parkin* mutants have been extensively analyzed. Null mutants often survive until adulthood, but they are short-lived. They lack any dopaminergic cell death, presenting instead muscle apoptosis and mitochondrial anomalies that lead, among other effects, to male sterility. They are also more sensitive to oxidative stress induced by paraquat than wild-type flies [78,79]. A first screening for suppressors or enhancers of parkin-induced partial lethality has shown that loss of function of oxidative stress genes enhance the lethal phenotype [80]. Appropiate expression of human or *Drosophila parkin* is able to suppress the neurotoxicity generated by α–synuclein or by another substrate of parkin in humans, known as Pael receptor, that also tends to produce protein aggregates [81,82]. *Drosophila* is also sensitive to drugs, such as rotenone, that lead to dopaminergic cell loss [83].

By contrast with *Drosophila*, the usage of the eight-cell dopaminergic system of *C. elegans* as a PD model may be said to be still in its infancy. Lakso *et al.* [84] demonstrated that expression of human wild type or mutant α–synucleins in *C. elegans* leads to dopaminergic neuron loss and motor deficits. *C. elegans* dopaminergic neurons are also sensitive to 6-OHDA [85] and expression of the human chaperone torsin A or its *C. elegans* counterpart TOR-2 protects against 6-OHDA-induced damage and also against the damage caused by α–synuclein expression [86].

We can summarize that the invertebrate models, most especially *Drosophila*, have already been shown to be very interesting, recapitulating several of the typical features of human parkinsonian syndromes. The near future will certainly provide new insights into PD based on the study of such simple animals.

GENOMIC AND PROTEOMIC ANALYSES OF PD MODELS

There are several fields in which genomic and proteomic data may provide useful information when applied to human diseases. On one hand, comparative genomics provides a background to estimate the potential values of the different cellular or animal models when compared with humans. On the other hand, functional genomics, and most especially the analysis of genome-wide expression profiles, may provide useful insights into the coordinated regulation of the genes involved in the pathological process. Finally, proteomics may offer direct information of the cellular processes that occur with disease progression. All these approaches are fully complementary, and we can expect that the integration of the data they generate will give us novel views of the molecular and cellular processes associated to a disease. It is therefore very interesting to compare the results provided by genomic and proteomic data with those obtained by more conventional approaches.

Comparative genomics work on PD genes is in progress by our group and others [87-89]. Although still relatively underdeveloped, some significant results are however available. For example, a comparative view casts some doubts about the yeast models: most of the key genes known to be involved in PD do not have orthologs in yeasts. For example, *parkin* belongs to a family of genes known as RBR. Genes of the RBR family exist in yeast, but they belong to subfamilies unrelated to the one that includes *parkin*. Parkin protein presents also a characteristic combination of domains that is absent in yeast RBRs. In summary, paralogs of *parkin*, but not true orthologs, exist in yeasts [87,89]. Work in progress in our group has established that similar results are obtained for the other familial PD-related genes. These results suggest that it is very doubtful that the cellular processes that characterize the disease in humans may be reproduced, at least fully, in yeasts. As we already indicated, invertebrates contain most of those genes (the exception being α–synuclein), suggesting that the invertebrate models might indeed replicate the processes found in our species.

It is obvious that genome-wide approaches may yield significant functional insights in such a complex syndrome as PD. Several groups have generated significant data using microarray analyses (or in one case SAGE analysis) of PD-affected individuals or models of PD (Table **2**). A few studies not included in that table have explored the effects of methamphetamine in mice, which some authors consider to be also a model for PD [90-92]. Data are still too fragmentary to arrive to solid conclusions based on those studies. Both the models and methods used have been very heterogeneous (see Table **2**). Moreover, much important information is still missing: human data is restricted to a single study and most genetic models of PD have not been analyzed yet. In any case, some interesting hints pointing towards some well-

Table 2. Genome-Wide Profile Expression Experiments of PD Models

Model	Tissue analyzed	Comparison	Main groups of up-regulated genes	Main groups of down-regulated genes	Refs.
Human	Substantia nigra	Individuals affected by PD vs. non-affected individuals	Cell adhesion/cytoskeleton; Extracellular matrix; Cell cycle; Protein modification/phosphorylation; Protein metabolism; Transcription; Inflammation/stress	Signal transduction; Protein degradation; Dopaminergic transmission/ metabolism; Ion transport; Protein modification/phosphorylation; Energy pathways/glycolisis	[95]
Mouse	Substantia nigra, part of striatum, some hippocampus	Chronic MPTP-treated vs. non-treated	Receptors; Growth factors; Interleukins	Cell cycle regulators; stress response proteins	[96]
Mouse	Substantia nigra, Ventral tegmental area	Acute MPTP-treated vs. non-treated	Only mitochondrial genes analyzed: 5 genes up regulated	Four mitochondrial genes down-regulated	[97]
Mouse	Whole brain	*UCHL1* mutants vs. wild-type	RNA metabolism; Transport; Cytoskeleton; Channels; Signal transduction;	Protein degradation; Transcription regulation	[98]
Mouse	Substantia nigra	Chronic MPTP treated vs. non-treated	Apoptosis	Axonal transport; vesicular transport and docking	[99]
Rat	Striatum	Injected with 6-OHDA vs. non-treated	None, varied genes	Signal transduction; cyclins; transcription factors; receptors	[100]
Drosophila	Heads	Presymptomatic 1-day-old transgenic α-synuclein vs. wild type	Few, varied genes	Mitochondrial; Lipid metabolism	[101]
Drosophila	Heads	Symptomatic 10-days-old or 30-days-old transgenic α–synuclein vs. wild type	Energetic metabolism; Detoxification response	Very few genes	[101]
Drosophila	Whole pupae	*Parkin* mutants vs. wild-type	Immune system, Oxidative damage; electron transport	Few, varied genes	[102]
Human neuro-blastoma-derived SH-SY5Y cells	-	Treated with MPP^+ (oxidation product of MPTP) vs. non-treated	Few genes, stress response.	Signal transduction; Mitochondrial; receptors	[103]
Mouse mesen-cephalon-derived MN9D cells	-	Treated with 6-OHDA vs. non-treated	Cellular response to endoplasmic reticulum stress	Not shown	[104]
Mouse mesen-cephalon-derived MN9D cells	-	Treated with MPTP vs. non-treated	Cellular response to endoplasmic reticulum stress	Not shown	[104]
Mouse SN4741 cells, derived from embryonic substantia nigra	-	Treated with H_2O_2 (to generate oxidative stress) vs. non-treated	Oxidoreductase; mitochondrial proteins; endoplasmic reticulum	Nuclear/transcriptional	[105]
Rat chromaffin-derived PC12 cells	-	Treated with 6-OHDA vs. non-treated	Cellular response to endoplasmic reticulum stress	Not shown	[106] [SAGE analysis]

known cellular aspects of PD (oxidative stress and mitochondria; protein degradation) have been found in almost all analyses. However, we must be sceptical, to avoid a tendency to over-interpreting the data in agreement with the expectations of what we know of PD. The truth is that the logic of most significant results, i. e. why particular genes are affected, remains inscrutable. In fact, the available information has not yet been deeply meta-analyzed to conclude whether significant trends exist. However, these results, preliminary as they are, harbour the great promise of allowing the establishment of models in which to rapidly test neuroprotective strategies based on reversion of the effects of damaging agents on gene expression (see review [93]). It is reasonable that microarray experiments may contribute also to indicate which ones are the best models of PD and to establish the relationships at the cellular levels among the effects of drugs or mutations related to the disease.

Finally, the future of proteomic analyses relevant for PD is best shown by a significant study in which more than 250 α–synuclein interacting proteins have been characterized by ICAT/mass spectrometry analysis of cell culture lysates [94]. In spite of the fact that α–synuclein-interacting proteins and proteins in the Lewy bodies in which α–synuclein is found had been extensively studied, these authors detected more than 200 novel putative α–synuclein-interacting proteins. Many of them were part of signal transduction pathways, involved in the ubiquitin-proteasome system or in responses to oxidative stress. Cell cultures to which rotenone was added showed significant differences in the complexes in which α–synuclein was found, with 51 proteins being significantly more or less represented in rotenone-treated vs. non-treated cultures [94]. These results show the power of novel proteomic approaches to unravel the cellular context of protein function. Again, integration of this kind of data with those generated by conventional approaches is a desirable goal for the immediate future.

We can conclude that the recognition of the important genetic component of PD and the development of cellular and animal models of the disease has largely contributed to our current knowledge of its molecular causes, although there are still many weak points in our understanding. The combination of genomic- and proteomic-scale analyses with conventional genetic and biochemical analyses will provide a novel framework and we may expect will dramatically increase our knowledge. However, the standardization of the methods and the integration of the results emerge as the most important problems to be solved in the next few years.

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