A BACwards glance at neurodegeneration: molecular insights into disease from LRRK2, SNCA and MAPT BAC-transgenic mice

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Abstract
BAC (bacterial artificial chromosome)-transgenic mice expressing a transgene from an entire genomic locus under the control of the native promoter offer the opportunity to generate more accurate genetic models of human disease. The present review discusses results of recent studies investigating PD (Parkinson’s disease) and tauopathies using BAC-transgenic mice carrying either the LRRK2 (leucine-rich repeat kinase 2), α-synuclein (SNCA) or MAPT (microtubule-associated protein tau) genes. In all lines, expression of the WT (wild-type) gene resulted in physiologically relevant protein expression. The effect of expressing the mutant form of a gene varied depending on the mouse strain or the particular disease mutation used, although it was common to see either neurochemical or behavioural differences in these animals. Overall, BAC technology offers an exciting opportunity to generate a wide range of new animal models of human-disease states.

Introduction
Traditionally, transgenic mice are generated by the injection of a transgene expression cassette containing the gene of interest, driven by a heterologous promoter, directly into the fertilized mouse oocytes [1]. Although useful, this has its limitations. Expression of the gene is under the control of a heterologous promoter and therefore may not be temporally or anatomically appropriate. In addition to this, it does not allow for the investigation of multiple isoforms of a protein that are the result of alternative splicing. Maybe most importantly, the size of the DNA transgene to be introduced is restricted by the cloning capacity of the plasmid being used.

In the last decade, these limitations have been addressed by the use of BACS (bacterial artificial chromosomes) as vectors for introducing DNA into the mouse genome. BACS have the capacity to hold up to 100–300 kb of foreign DNA, meaning that they are capable of holding the entire genomic locus of most mammalian genes. Since the gene of interest is controlled by its native promoter and flanked by endogenous regulatory elements, expression occurs in a physiologically, spatially and temporally relevant manner (Figure 1). In the field of neurodegeneration, this provides a unique tool for comparing the effect of expressing a WT (wild-type) gene with that of a gene harbouring a mutation known to be associated with disease. This review discusses recent results obtained through the use of BAC-transgenic mice.

LRRK2 (leucine-rich repeat kinase 2)
Mutations in the LRRK2 gene are the most common genetic cause of PD (Parkinson’s disease; for recent reviews see [2–4]). Although many functions have been proposed for LRRK2, the exact pathological role of the protein, in the pathways leading to PD, are unknown. In human tissue, in situ hybridization and RT (reverse transcription)–PCR studies have shown that LRRK2 mRNA expression can be found throughout the brain [5,6]. Immunohistochemistry in healthy human brain confirmed LRRK2 protein expression in various regions, including the hippocampus, the temporal and parietal cortices, caudate putamen and dopaminergic neurons of the substantia nigra [6–8]. In tissue from a PD brain, LRRK2 immunoreactivity was also seen in numerous regions including the hippocampus, locus coeruleus, striatum and raphe nucleus [7,8]. However, its presence in the pathological hallmark of PD and LBs (Lewy bodies) remains controversial [6–8]. In an attempt to better understand the function of LRRK2, mutations in BAC-transgenic mice carrying the human LRRK2 gene have been generated by several groups [9–12].

Protein distribution was investigated in BAC-WT LRRK2 transgenic mice using Western blotting and immunohistochemistry, and LRRK2 was found to be ubiquitously expressed in most brain regions [9,10] providing evidence that location of expression in the mouse model appeared to mimic that seen in the human brain. In these animals, expression of human LRRK2 is much higher than that of the...
endogenous murine protein [10–12]. The phenotypic effect of WT LRRK2 transgene expression remains unresolved, with some groups reporting no behavioural or neurochemical phenotype [11] and others reporting hyperactivity and increased striatal dopamine content and release [10]. Direct comparison between studies can be hampered, however, when differing batteries of phenotypic tests and methods of assay quantification are used [10,11].

In addition to WT LRRK2 transgenic mice, BAC-transgenic mice harbouring the LRRK2R1441G missense mutation have been generated [11]. Transgene expression was found to be 5–10-fold higher than endogenous mouse Lrrk2, but similar to WT LRRK2 transgene expression [11]. At the age of 3 months, the LRRK2R1441G mice were phenotypically normal; however, mutant transgenic animals went on to develop age-related progressive motor deficits. By 10–12 months, the LRRK2R1441G mice were showing visually apparent immobility, which was ameliorated after treatment with the dopamine precursor L-dopa. These effects were absent from WT LRRK2 mice, indicating that the deficits seen in the LRRK2R1441G mice are the results of the mutation and not overexpression of the LRRK2 protein [11]. In addition to the motor deficits, the LRRK2R1441G mice also exhibited axonal pathology similar to that observed in PD patients as well as impaired dopamine release as measured by intrastriatal microdialysis in freely moving mice. Immunohistochemistry for TH (tyrosine hydroxylase; an enzyme critical for dopamine production) in 9–10-month-old LRRK2R1441G mice revealed that the number of dopamine neurons in the SNpc (substantia nigra pars compacta) was unchanged; however, the average cell body size and number of TH-positive dendrites were reduced. Abnormal TH staining was observed in the striatum and piriform cortex, with axons appearing fragmented and showing signs of beading.

In 2010, a study on BAC mice carrying the LRRK2G2019S mutation was published. The study reported that the
mutation resulted not only in an age-related decrease in evoked dopamine release as measured by fast-scan cyclic voltammetry [10], which is consistent with the reported effect of the LRRK2\textsuperscript{R1441G} mutation [11], but also in a decrease in dopamine tissue content. In contrast, unlike LRRK2\textsuperscript{R1441G}, expression of LRRK2\textsuperscript{G2019S} did not result in any motor deficits despite having reduced dopamine content and release [10]. This discrepancy could potentially be explained by the milder nature of the LRRK2\textsuperscript{G2019S} mutation compared with LRRK2\textsuperscript{R1441G} or by the different mouse strains used. The G2019S mutation was bred on to a C57BL/6j background, whereas the R1441G mutation was bred on to an FVB background. This complication is not unusual in comparing mouse models of disease.

Histological examination of the LRRK2\textsuperscript{G2019S} mouse brain found no evidence of typical PD neurodegeneration. In the WT LRRK2 mice, there was a reduction in phospho-tau accumulation that the authors interpreted as showing a protective role for normal LRRK2 in the healthy brain [10]. Expression of the G2019S mutation did not have this protective effect [10]. In the second study [11], expression of WT LRRK2 did not appear to offer protection as non-transgenic and WT-LRRK2 mice had the same level of phospho-tau. In the LRRK2\textsuperscript{R1441G} mice, there was a significant increase in tau pathology and phospho-tau detection, accompanied by axonal pathology [11].

These lines of mice provide a genetic model of PD that between them represent the motor, neurochemical and pathological features of the disease. However, these mice do not represent the only models of PD as it is known that genetic variation in the genes encoding \(\alpha\)-synuclein (SNCA) and microtubule-associated protein tau (\(\alpha\)-MAPT), are associated with neurodegenerative disease, including PD [13,14].

\(\alpha\)-Synuclein

The \(\alpha\)-synuclein protein encoded by the SNCA gene locus is found aggregated in LBs, the neuropathological hallmark of PD. Although \(\alpha\)-synuclein has been implicated in a number of neuronal processes, including dopamine homeostasis, synaptic vesicle release, regulation of fatty acid metabolism and molecular chaperoning (for reviews, see [15–17]), its exact role in the pathway to disease pathology is unknown.

In 2010, a study of SNCA mutations expressed from BAC transgenes was published [18]. Nussbaum and co-workers generated mice expressing WT or mutant (A30P or A53T) \(\alpha\)-synuclein from SNCA BACs and crossed the transgenics with \(\alpha\)-synuclein null animals [18]. Generating transgenic mice expressing the transgene on a null background allowed analysis of the effects of the mutations without endogenous murine \(\alpha\)-synuclein confounding the results. The human WT SNCA transgenic mice acted as a control to show that any changes were not due to expression of the human gene rather than the mutation being expressed.

Transgenic mice expressing the WT or mutant human SNCA gene expressed the transgene at twice the endogenous levels on the knockout null background. Motor function of the mice was assessed using the accelerating rotorod and open-field tests. The only line to show differences from control animals was the A53T line. These 6-month-old mice showed a significant impairment on the rotorod that got progressively worse with age. Motor deficits in the A53T mice were further confirmed in the open-field test where, at 6 months, the total distance travelled was significantly less compared with controls. In comparison, no motor abnormalities were seen in the A30P mice using either test [18].

Despite the A53T mice showing impairment in motor function at a young age, no PD-related pathology was found in the brain of any of the lines until the age of 22 months. Additionally, there was no alteration in dopamine or its metabolite, DOPAC (3,4-dihydroxyphenylacetic acid), in striatal tissue from any of the mice [18].

In addition to degeneration of the nigro-striatal pathway, other non-motor symptoms of PD include loss of the sense of smell (hyposmia) and gastrointestinal dysfunction [19,20]. These symptoms were also investigated using the synuclein BAC mice. Time spent exploring a novel odour and latency to dig for food beneath bedding were used to test the animals’ olfaction. Neither test detected changes in the animals’ capability to smell; furthermore, no degeneration of the olfactory bulb was observed [18].

Investigation of gastrointestinal function found that both the A30P and A53T mice produced a reduction in faecal mass accompanied by a reduction in water content of the stools, indicative of reduced gut motility. This was confirmed when whole gut transit time was measured and found to be reduced in the mutants compared with controls [18]. It was interesting to note that although the A30P mice showed no motor impairment or pathology there was gastrointestinal dysfunction at a young age in these animals and the A53T mutants.

This is one of the first reports of using BAC-transgenic animals to investigate mutations in the SNCA gene. These animals, especially the A53T line, seem to recapitulate many symptoms of the early stages of PD and may provide a useful and convenient mammalian model for future studies. However, as a note of caution, both lines of SNCA mutant mice in this study were bred to homozygosity for the randomly inserted transgene to elevate transgene expression and generate a phenotype. It is therefore possible that any changes observed in these mice may be due to disruption of the genome where the transgene was inserted. Most transgenic animal models are maintained as heterozygous for the transgene so that if the expression of an endogenous gene is disrupted by transgene insertion one copy remains intact in a heterozygous animal. For studies of homozygous transgenics multiple lines of mice carrying the same mutation, but derived independently, are required to confirm that the observed changes are due to the mutation and not site of insertion.

In an attempt to better understand the genetic regulation of SNCA expression, recent work used BAC-transgenic mice to investigate the polymorphic microsatellite site, NACP (non-amyloid component of plaques) Rep1, found in the promoter.
Table 1 | Overview summary of the main findings from studies into neurodegenerative disease using BAC-transgenic mice carrying the LRRK2, SNCA or MAPT gene loci

<table>
<thead>
<tr>
<th>Model</th>
<th>Age of onset</th>
<th>Neurochemical phenotype</th>
<th>Protein deposition</th>
<th>Neuronal morphology</th>
<th>Behavioural phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRRK2 (WTOX)</td>
<td>N/A</td>
<td>Not reported</td>
<td>None</td>
<td>None</td>
<td>None reported</td>
<td>[11]</td>
</tr>
<tr>
<td>LRRK2R1441G</td>
<td>10 months</td>
<td>Decreased dopamine release</td>
<td>None</td>
<td>Phospho-tau</td>
<td>SNpc: decreased cell body size, TH-positive dendrites; Striatum: beaded and fragmented axons</td>
<td>Hypokinesia</td>
</tr>
<tr>
<td>LRRK2 WT</td>
<td>12 months</td>
<td>Increased dopamine release</td>
<td>Decreased phospho-tau</td>
<td>None</td>
<td>Hyperactivity and improved motor performance</td>
<td>[10]</td>
</tr>
</tbody>
</table>
| LRRK2G2019S   | 12 months    | Decreased dopamine content and release | None | None | None | No details given on whether the mice developed a PD-like phenotype. It would be interesting to see whether the presence of the Rep1 allele in question is sufficient to produce motor impairments.

region of SNCA. Recent GWAS (genome-wide association studies) have shown association of the SNCA locus with sporadic PD and earlier work indicated that certain alleles of Rep1 are associated with an increased risk of idiopathic PD [21]. To better understand the role of Rep1 in disease, mice carrying Rep1 allelic variants were generated [22]. It was reported that mice carrying the risk allele generated 70% more SNCA-mRNA and 1.25 times more α-synuclein protein than the protective allele. Although this study has been useful for investigating the importance of the ‘at risk’ allele on α-synuclein levels in the brain, no details were given on whether the mice developed a PD-like phenotype. It would be interesting to see whether the presence of the Rep1 allele in question is sufficient to produce motor impairments.

**MAPT**

BAC technology has also been used to investigate the function and dysfunction of MAPT or tau, a protein that self-aggregates in a number of different neurodegenerative disorders known as tauopathies, such as AD (Alzheimer’s disease), FTD (frontotemporal dementia) and PSP (progressive supranuclear palsy). Although PD is not a tauopathy, MAPT genetic variation is also associated with PD [23], placing MAPT firmly at the centre of a range of neurodegenerative processes. Tau is normally associated with microtubules; however, in tauopathies, the protein becomes hyperphosphorylated, resulting in self-aggregation (for a review, see [24]). These tau deposits can be seen throughout the brain of patients suffering from tauopathies [25]. In the human brain, MAPT is alternatively spliced to give rise to six different isoforms of the protein [26]. Transgenic mice based on cDNA models investigate only a single isoform under the control of a heterologous promoter. However, in BAC-transgenic mice, all six isoforms can be expressed and gene transcription is under the control of the native promoter [27,28].

The first BAC-transgenic mouse used to study tau was reported in 2000. The presence of all six isoforms in the brain of transgenic mice was confirmed by RT–PCR and Western blotting [27]. Immunohistochemistry found that the protein was localized to the axons, as is seen in the normal human brain. However, since these mice also expressed endogenous tau it is not an ideal model to examine human tau. In a follow-up study, the mice generated by Duff and co-workers were crossed with tau-knockout mice [28]. When human WT tau was expressed in these animals, age-dependent changes in tau localization were observed [28]. In 6-week-old animals, tau was localized as normal to axons, but by 3 months, tau protein had started to accumulate in cell bodies and by 9 months somato-dendritic accumulation started to resemble that seen in the early AD human brain. This staining was not seen in age-matched normal non-transgenic mice expressing only murine tau [28]. It was also reported that paired-helical filament tau could be isolated from the brains of tau BAC-transgenic animals, but not from controls [28]. By 15 months of age, tau deposition was accompanied by significant cell loss, as was evident from increased ventricle size and
cell loss is associated with the expression of WT tau. However, since tau protein levels in these mice are approximately 4-fold higher than in WT mice, tau accumulation might be the result of expressing human tau in a mouse, or the result of tau overexpression.

Although these early studies provide a promising tool for investigating tau protein, to date there are no reports in the literature of BAC-transgenic animals being used to investigate mutations in MAPT that are known to cause tauopathies. These animals would not only provide a model for these hereditary diseases, but also be a useful tool for examining the mechanisms responsible for tau accumulation.

Summary and perspectives

Despite being a relatively new advance in the field of transgenics, BAC technology is already being widely used to more accurately generate a range of neurodegenerative disease models (Table 1). In parallel work, studies underway at Rockefeller University have further developed BAC technologies to generate an atlas of the mouse brain that maps gene expression. The GENSAT project collects data from EGFP (enhanced green fluorescent protein) BAC-transgenic mice (for reviews, see [30,31]). The coding sequence of EGFP is expressed under the control of the gene locus, accurately mapping protein expression. BAC technology is also being applied to generate rat transgenics. The use of rats has a number of advantages over mice; however, owing to difficulties in genome manipulation rats have been previously overlooked for the study of genetic mutations. Taken together, BAC-transgenic animals will provide a much more physiologically relevant model of neurodegenerative disease and are likely to form the basis of many new and exciting investigations in the future.

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References

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