Title Page

Title: GFP transgenic animals in biomedical research: a review of potential disadvantages

Nándor Lipták*, Zsuzsanna Bősze, László Hiripi

Short title: GFP transgenic animals

NARIC-Agricultural Biotechnology Institute, Animal Biotechnology Department, Gödöllő, Hungary

*Corresponding author: Nándor Lipták, NARIC-Agricultural Biotechnology Institute, Animal Biotechnology Department, H-2100 Gödöllő, Szent-Györgyi A st 4. Hungary. E-mail: liptak.nandor@abc.naik.hu

Phone: +36-28-526-253

Fax: +36-28-526-151

Summary

Green Fluorescent protein (GFP) transgenic animals are accepted tools for studying various physiological processes, including organ development and cell migration. However, several *in vivo* studies claimed that GFP may impair transgenic animals' health. Glomerulosclerosis was observed in transgenic mice and rabbits with ubiquitous reporter protein expression. Heart-specific GFP expression evoked dilated cardiomyopathy and altered cardiac function in transgenic mouse and zebrafish lines, respectively. Moreover, growth retardation and increased axon swelling were observed in GFP and yellow fluorescent protein (YFP) transgenic mice, respectively. This review will focus on the potential drawbacks of the applications of GFP animals in biomedical research.

Keywords

GFP, transgenic animals, glomerulosclerosis, dilated cardiomyopathy

GFP as a fluorescent marker in transgenic animals

GFP, which was originally discovered in jellyfish *Aequorea victoria*, absorbs blue light and emits green fluorescence without any exogenous substrates (Shimomura *et al.*, 1962). Several variants of GFP with modified fluorescence spectra were designed in the last two decades, including enhanced GFP (EGFP) (Cormack *et al.*, 1996), YFP (Wachter *et al.*, 1998), Venus (Nagai *et al.*, 2002) etc. EGFP had increased extinction and more efficient folding, whilst Venus showed decreased sensitivity to both acidosis and Cl^- compared to wild-type GFP; for more details, see review (Zimmer, 2009). The development of the expression vector composed of the chicken β-actin promoter and cytomegalovirus enhancer (CAGGS), offered a chance for the researchers to create transgenic animals with ubiquitous reporter protein expression (Niwa *et al.*, 1991). After this innovation, GFP or its derivatives were successfully integrated into the genome of laboratory and livestock animals, e.g. mouse (Okabe *et al.*, 1997), pig (Garrels *et al.*, 2011; Park *et al.*, 2001), rabbit (Katter *et al.*, 2013; Takahashi *et al.*, 2007), etc. Recently, EGFP transgenic animals have been used for modelling human diseases (Garcia Diaz *et al.*, 2016; Shetty *et al.*, 2019), tissue engineering (Hirakata *et al.*, 2016) and helped the researchers to reveal the platelet biogenesis in lungs (Lefrancais *et al.*, 2017). Moreover, utilizing of GFP infected chondrocytes was valuable for studying developmental dysplasia and dislocation of the hip in rats (Ning *et al.*, 2019). Initially, most of the studies suggested that GFP is biologically inert. After the creation of GFP transgenic mice, the non-toxic nature of GFP in living cells was questioned by certain researchers (Liu *et al.*, 1999).

Immunogenicity of GFP

Intravenous administration of wild type pre-leukemia cells cause mortality in Balb/c mice while EGFP transformed leukemia cells did not cause systemic leukemia (Stripecke *et al.*, 1999). Further studies suggested that EGFP is highly immunogenic in Balb/c but only moderately immunogenic in other strains like C57BL/6. Not only the strain but also the route of administration of cells influence the level of immune response against EGFP expressing cells (Skelton *et al.*, 2001).

GFP-evoked dilated cardiomyopathy and altered cardiac function

Dilated cardiomyopathy was detected in transgenic mice with heart-specific GFP overexpression (Huang *et al.*, 2000). In four independent transgenic mouse lines, cardiac-specific GFP expression was driven by the α-myosin heavy chain gene (α-MyHC) promoter. Significantly increased heart mass/body mass ratio, four-chamber dilation, relatively thin myocardium was observed in two out of four transgenic FVB/N strains compared to non-transgenic controls. More than half of the 5-weeks old male mice died due to congestive heart failure from one of the two transgenic lines, while mice from the other line developed systolic dysfunction at 4 months of age. The severity of dilated cardiomyopathy positively correlated to the GFP expression level in the heart, suggesting a dose-dependent adverse effect of GFP (Huang *et al.*, 2000). Age-dependent abnormalities in cardiac morphology and function were published using transgenic zebrafish lines with heart-specific GFP expression. Decreased heart rate, stroke volume, and cardiac output (Ho *et al.*, 2007) and altered cardiac function (Avey *et al.*, 2018) were measured in 8-days old and adult myosin light chain 2 gene (cmlc2)-GFP transgenic zebrafishes, respectively; but GFP exerts no detrimental

actions on 3-days old zebrafish hearts (Huang *et al.*, 2011). Several attempts were made to discover how GFP overexpression affects cardiac physiology and induces dilated cardiomyopathy. In vitro studies reported that EGFP expression impairs actin-myosin interactions and contractile function of muscle cells (Agbulut *et al.*, 2006; Agbulut *et al.*, 2007), but other research groups did not confirm these findings (Resnicow *et al.*, 2008). In another study, heart-specific co-expression of EGFP and a calmodulin II kinase (CaMKII) inhibitory peptide, auto-camtide-3-inhibitor successfully counteracted the EGFP-evoked left ventricular dilatation and dysfunction; thus, EGFP may cause increased CaMKII activity in cardiomyocytes which led to dilated cardiomyopathy (Khoo *et al.*, 2008), see details in **Table 1**. Nevertheless, mild hypertrophy and cardiomyopathy was observed in α-MyHC tetracycline transactivator transgenic mice (McCloskey *et al.*, 2005), indicating that overexpression of any protein in the heart under the control of α-MyHC promoter may cause cardiomyopathy.

GFP-evoked glomerulosclerosis

In most cases, ubiquitous expression EGFP or its variants under the control of the CAGGS promoter did not affect fecundity (Garrels *et al.*, 2012; Hoffmann *et al.*, 2016), life span and had no measurable detrimental effect (Chou *et al.*, 2014; Garrels *et al.*, 2011; Yum *et al.*, 2018).

Nowadays, CAGGS-EGFP transgenic mice (C57BL/6-Tg(CAG-EGFP)1Osb/J, Jackson Laboratories) with ubiquitous reporter protein expression (Okabe *et al.*, 1997) are one of the most popular transgenic animals in biomedical research. However, 5-weeks old CAGGS-EGFP transgenic mice developed mild glomerulosclerosis and proteinuria (Guo *et al.*, 2007). The

pathological progression was slow, age-dependent and did not affect the life-span of the mice. Only CAGGS-EGFP transgenic mice with the highest EGFP expression in their glomeruli developed glomerulosclerosis. Three other transgenic strains with lower glomerular GFP expression showed normal renal histology, suggesting that high EGFP levels may have harmful effects on the glomeruli (Guo *et al.*, 2007). Supporting these findings, more severe proteinuria and glomerulosclerosis were observed in an EGFP- γ -Aminobutyric acid A receptor-associated protein (GABARAP) transgenic mouse strain after a single doxorubicin injection compared with non-transgenic mice (Takagi-Akiba *et al.*, 2012). Mild proteinuria, focal segmental glomerulosclerosis (FSGS) and glomerulomegaly were also detected in heterozygote CAGGS-Venus transgenic rabbits, while homozygote Venus transgenic rabbits developed FGSS, glomerulomegaly and microscopic hematuria, but not proteinuria (Liptak *et al.*, 2018). Similarly to CAGGS-EGFP transgenic mice, the mild glomerulosclerosis did not affect the life span. The Venus transgene was integrated at chromosome 8, at 61471914 bp (Katter *et al.*, 2013) in the intron 12–13 of the diaphanous related formin 3 gene (DIAPH3, Ensembl gene ID: ENSOCUG00000001719), thus, it is unlikely that the integration site of the transgene caused the FSGS. These data suggested that glomerulosclerosis in transgenic animals overexpressing the reporter GFP or its variants (EGFP, Venus) is not limited to mice. Underlying mechanisms of GFP-associated glomerulosclerosis are not clear; however, defective polyubiquitination (Baens *et al.*, 2006) and oxidative stress (Ganini *et al.*, 2017; Goto *et al.*, 2003) may have a role in the development of glomerular lesions. EGFP produces H_2O_2 and superoxide anion during its maturation in the presence of NADH, but these findings were restricted to *E.col*i and HeLA cell lines to date (Ganini *et al.*, 2017), see details in **Table 1**.

GFP-induced neuropathology

EGFP and β-galactosidase co-expression in the forebrain caused growth retardation, weakness and premature death in 25-30 days old transgenic mice from two independent lines (Krestel *et al.*, 2004). GFP alone did not have any of the abovementioned neuropathological effects in a third transgenic mouse line. The cytoplasmic aggregation of EGFP and β-galactosidase could be responsible for the premature death in the two transgenic lines. Doxycycline and granulocyte colony-stimulating factor (GCSF) treatment were effective in the prevention of early death of transgenic mice co-expressing EGFP and β-galactosidase, but did not prevent them from growth retardation (Krestel *et al.*, 2004). Doxycycline exerted its effect via downregulation of the EGFP expression, while GCSF had neuroprotective effect which was published earlier (Schabitz *et al.*, 2003).

In YFP transgenic mice, more severe axon swelling was observed in gracile tract, gracile nucleus and dorsal roots compared to non-transgenic mice at 8 and 12 months of age (Bridge *et al.*, 2009). YFP expression was driven by the neuron-specific Thy-1 promoter in the transgenic mice. Unfortunately, the exact genomic position of the YFP coding transgene is not available. Increased axon swelling was attributed by the excessive accumulation of the YFP in the central nervous system and not the position effect of the transgene (Bridge *et al.*, 2009). These observations were underlined by an independent research group later (Gatto *et al.*, 2015), see details in **Table 1**.

Conclusions

In the majority of GFP transgenic animal lines, GFP did not have any detectable harmful effect on animals' health, but there were a few exceptions. Heart-specific GFP overexpression caused dilated cardiomyopathy in mice and altered cardiac function in zebrafish. Transgenic mouse and rabbit strains with CAGGS promoter-driven ubiquitous EGFP or Venus expression developed mild glomerulosclerosis and proteinuria. Neuron-specific expression of YFP and co-expression of EGFP and β-galactosidase caused pathologic symptoms in mouse strains created by different research groups. These findings should be considered when *in vivo* studies using GFP transgenic animals are designed.

Acknowledgments

This work was supported by the National Research, Development and Innovation Office (NKFIH) grant no. 108921, NVKP_16-1-2016-0039 to Z.B. and 120870 to N.L.

Conflict of interest

The authors have no conflict of interest to declare.

References

AGBULUT, O, COIRAULT, C, NIEDERLANDER, N, HUET, A, VICART, P, HAGEGE, A, PUCEAT, M, and MENASCHE, P: GFP expression in muscle cells impairs actin-myosin interactions: implications for cell therapy. *Nat Methods* **3**, 331, 2006.

AGBULUT, O, HUET, A, NIEDERLANDER, N, PUCEAT, M, MENASCHE, P, and COIRAULT, C: Green fluorescent protein impairs actin-myosin interactions by binding to the actin-binding site of myosin. *J Biol Chem* **282**, 10465-71, 2007.

AVEY, SR, OJEHOMON, M, DAWSON, JF, and GILLIS, TE: How the expression of green fluorescent protein and human cardiac actin in the heart influences cardiac function and aerobic performance in zebrafish Danio rerio. *Journal of Fish Biology* **92**, 177-189, 2018.

BAENS, M, NOELS, H, BROECKX, V, HAGENS, S, FEVERY, S, BILLIAU, AD, VANKELECOM, H, and MARYNEN, P: The dark side of EGFP: defective polyubiquitination. *PLoS One* **1**, e54, 2006.

BRIDGE, KE, BERG, N, ADALBERT, R, BABETTO, E, DIAS, T, SPILLANTINI, MG, RIBCHESTER, RR, and COLEMAN, MP: Late onset distal axonal swelling in YFP-H transgenic mice. *Neurobiol Aging* **30**, 309-21, 2009.

CHOU, CJ, PENG, SY, WU, MH, YANG, CC, LIN, YS, CHENG, WT, WU, SC, and LIN, YP: Generation and characterization of a transgenic pig carrying a DsRed-monomer reporter gene. *PLoS One* **9**, e106864, 2014.

CORMACK, BP, VALDIVIA, RH, and FALKOW, S: FACS-optimized mutants of the green fluorescent protein (GFP). *Gene* **173**, 33-8, 1996.

GANINI, D, LEINISCH, F, KUMAR, A, JIANG, JJ, TOKAR, EJ, MALONE, CC, PETROVICH, RM, and MASON, RP: Fluorescent proteins such as eGFP lead to catalytic oxidative stress in cells. *Redox Biology* **12**, 462-468, 2017.

GARCIA DIAZ, AI, MOYON, B, COAN, PM, ALFAZEMA, N, VENDA, L, WOOLLARD, K, and AITMAN, T: New Wistar Kyoto and spontaneously hypertensive rat transgenic models with ubiquitous expression of green fluorescent protein. *Dis Model Mech* **9**, 463-71, 2016.

GARRELS, W, HOLLER, S, CLEVE, N, NIEMANN, H, IVICS, Z, and KUES, WA: Assessment of fecundity and germ line transmission in two transgenic pig lines produced by sleeping beauty transposition. *Genes (Basel)* **3**, 615-33, 2012.

GARRELS, W, MATES, L, HOLLER, S, DALDA, A, TAYLOR, U, PETERSEN, B, NIEMANN, H, IZSVAK, Z, IVICS, Z, and KUES, WA: Germline Transgenic Pigs by Sleeping Beauty Transposition in Porcine Zygotes and Targeted Integration in the Pig Genome. *PLoS One* **6**, 2011.

GATTO, RG, CHU, Y, YE, AQ, PRICE, SD, TAVASSOLI, E, BUENAVENTURA, A, BRADY, ST, MAGIN, RL, KORDOWER, JH, and MORFINI, GA: Analysis of YFP(J16)-R6/2 reporter mice and postmortem brains reveals early pathology and increased vulnerability of callosal axons in Huntington's disease. *Hum Mol Genet* **24**, 5285-98, 2015.

GOTO, H, YANG, B, PETERSEN, D, PEPPER, KA, ALFARO, PA, KOHN, DB, and REYNOLDS, CP: Transduction of green fluorescent protein increased oxidative stress and enhanced sensitivity to cytotoxic drugs in neuroblastoma cell lines. *Mol Cancer Ther* **2**, 911-7, 2003.

GUO, JK, CHENG, EC, WANG, L, SWENSON, ES, ARDITO, TA, KASHGARIAN, M, CANTLEY, LG, and KRAUSE, DS: The commonly used beta-actin-GFP transgenic mouse strain develops a distinct type of glomerulosclerosis. *Transgenic Research* **16**, 829-834, 2007.

HIRAKATA, E, TOMITA, N, TAMADA, Y, SUGURO, T, NAKAJIMA, M, KAMBE, Y, YAMADA, K, YAMAMOTO, K, KAWAKAMI, M, OTAKA, A, OKUMURA, H, and SUZUKI, S: Early tissue formation on whole-area osteochondral defect of rabbit patella by covering with fibroin sponge. *J Biomed Mater Res B Appl Biomater* **104**, 1474-82, 2016.

HO, YL, LIN, YH, TSAI, IJ, HSIEH, FJ, and TSAI, HJ: In vivo assessment of cardiac morphology and function in heart-specific green fluorescent zebrafish. *J Formos Med Assoc* **106**, 181-6, 2007.

HOFFMANN, OI, KEREKES, A, LIPTAK, N, HIRIPI, L, BODO, S, SZALOKI, G, KLEIN, S, IVICS, Z, KUES, WA, and BOSZE, Z: Transposon-Based Reporter Marking Provides Functional Evidence for Intercellular Bridges in the Male Germline of Rabbits. *PLoS One* **11**, e0154489, 2016.

HUANG, W, DENG, Y, DONG, W, YUAN, W, WAN, Y, MO, X, LI, Y, WANG, Z, WANG, Y, OCORR, K, ZHANG, B, LIN, S, and WU, X: The effect of excess expression of GFP in a novel heart-specific green fluorescence zebrafish regulated by nppa enhancer at early embryonic development. *Mol Biol Rep* **38**, 793-9, 2011.

HUANG, WY, ARAMBURU, J, DOUGLAS, PS, and IZUMO, S: Transgenic expression of green fluorescence protein can cause dilated cardiomyopathy. *Nature Medicine* **6**, 482-483, 2000.

KATTER, K, GEURTS, AM, HOFFMANN, O, MATES, L, LANDA, V, HIRIPI, L, MORENO, C, LAZAR, J, BASHIR, S, ZIDEK, V, POPOVA, E, JERCHOW, B, BECKER, K, DEVARAJ, A, WALTER, I, GRZYBOWKSI, M, CORBETT, M, RANGEL, A, HODGES, MR, BADER, M, IVICS, Z, JACOB, HJ, PRAVENEC, M, BOSZE, Z,

RULICKE, T, and IZSVAK, Z: Transposon-mediated transgenesis, transgenic rescue, and tissue-specific gene expression in rodents and rabbits. *Faseb Journal* **27**, 930-941, 2013.

KHOO, MS, GRUETER, CE, EREN, M, YANG, J, ZHANG, R, BASS, MA, LWIN, ST, MENDES, LA, VAUGHAN, DE, COLBRAN, RJ, and ANDERSON, ME: Calmodulin kinase II inhibition disrupts cardiomyopathic effects of enhanced green fluorescent protein. *J Mol Cell Cardiol* **44**, 405-10, 2008.

KRESTEL, HE, MIHALJEVIC, AL, HOFFMAN, DA, and SCHNEIDER, A: Neuronal co-expression of EGFP and beta-galactosidase in mice causes neuropathology and premature death. *Neurobiol Dis* **17**, 310-8, 2004.

LEFRANCAIS, E, ORTIZ-MUNOZ, G, CAUDRILLIER, A, MALLAVIA, B, LIU, FC, SAYAH, DM, THORNTON, EE, HEADLEY, MB, DAVID, T, COUGHLIN, SR, KRUMMEL, MF, LEAVITT, AD, PASSEGUE, E, and LOONEY, MR: The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. *Nature* **544**, 105-+, 2017.

LIPTAK, N, HOFFMANN, OI, SKODA, G, GOCZA, E, KEREKES, A, BOSZE, Z, and HIRIPI, L: Glomerulosclerosis in Transgenic Rabbits with Ubiquitous Venus Protein Expression. *Acta Veterinaria Hungarica* **66**, 281-293, 2018.

LIU, HS, JAN, MS, CHOU, CK, CHEN, PH, and KE, NJ: Is green fluorescent protein toxic to the living cells? *Biochemical and Biophysical Research Communications* **260**, 712-717, 1999.

MCCLOSKEY, DT, TURNBULL, L, SWIGART, PM, ZAMBON, AC, TURCATO, S, JOHO, S, GROSSMAN, W, CONKLIN, BR, SIMPSON, PC, and BAKER, AJ: Cardiac transgenesis with the tetracycline transactivator changes myocardial function and gene expression. *Physiol Genomics* **22**, 118-26, 2005.

NAGAI, T, IBATA, K, PARK, ES, KUBOTA, M, MIKOSHIBA, K, and MIYAWAKI, A: A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nat Biotechnol* **20**, 87-90, 2002.

NING, B, JIN, R, WANG, D, and SUN, J: The H19/let-7 feedback loop contributes to developmental dysplasia and dislocation of the hip. *Physiol Res* **68**, 275-284, 2019.

NIWA, H, YAMAMURA, K, and MIYAZAKI, J: Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* **108**, 193-9, 1991.

OKABE, M, IKAWA, M, KOMINAMI, K, NAKANISHI, T, and NISHIMUNE, Y: 'Green mice' as a source of ubiquitous green cells. *FEBS Lett* **407**, 313-9, 1997.

PARK, KW, CHEONG, HT, LAI, LX, IM, GS, KUHHOLZER, B, BONK, A, SAMUEL, M, RIEKE, A, DAY, BN, MURPHY, CN, CARTER, DB, and PRATHER, RS: Production of nuclear transfer-derived swine that express the enhanced green fluorescent protein. *Animal Biotechnology* **12**, 173-+, 2001.

RESNICOW, DI, HOOFT, AM, HARRISON, BC, BAKER, JE, and LEINWAND, LA: GFP fails to inhibit actin-myosin interactions in vitro. *Nature Methods* **5**, 212-213, 2008.

SCHABITZ, WR, KOLLMAR, R, SCHWANINGER, M, JUETTLER, E, BARDUTZKY, J, SCHOLZKE, MN, SOMMER, C, and SCHWAB, S: Neuroprotective effect of granulocyte colony-stimulating factor after focal cerebral ischemia. *Stroke* **34**, 745-51, 2003.

SHETTY, G, WU, Z, LAM, TNA, PHAN, TT, ORWIG, KE, and MEISTRICH, ML: Effect of hormone modulations on donor-derived spermatogenesis or colonization after syngeneic and xenotransplantation in mice. *Andrology* **7**, 257-265, 2019.

SHIMOMURA, O, JOHNSON, FH, and SAIGA, Y: Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. *J Cell Comp Physiol* **59**, 223-39, 1962.

SKELTON, D, SATAKE, N, and KOHN, DB: The enhanced green fluorescent protein (eGFP) is minimally immunogenic in C57BL/6 mice. *Gene Therapy* **8**, 1813-1814, 2001.

STRIPECKE, R, VILLACRES, MD, SKELTON, DC, SATAKE, N, HALENE, S, and KOHN, DB: Immune response to green fluorescent protein: implications for gene therapy. *Gene Therapy* **6**, 1305-1312, 1999.

TAKAGI-AKIBA, M, ASANUMA, K, TANIDA, I, TADA, N, OLIVA TREJO, JA, NONAKA, K, ASANUMA, E, KOMINAMI, E, UENO, T, and TOMINO, Y: Doxorubicin-induced glomerulosclerosis with proteinuria in GFP-GABARAP transgenic mice. *Am J Physiol Renal Physiol* **302**, F380-9, 2012.

TAKAHASHI, R, KURAMOCHI, T, AOYAGI, K, HASHIMOTO, S, MIYOSHI, I, KASAI, N, HAKAMATA, Y, KOBAYASHI, E, and UEDA, M: Establishment and characterization of CAG/EGFP transgenic rabbit line. *Transgenic Research* **16**, 115-20, 2007.

WACHTER, RM, ELSLIGER, MA, KALLIO, K, HANSON, GT, and REMINGTON, SJ: Structural basis of spectral shifts in the yellow-emission variants of green fluorescent protein. *Structure* **6**, 1267-77, 1998.

YUM, SY, LEE, SJ, PARK, SG, SHIN, IG, HAHN, SE, CHOI, WJ, KIM, HS, KIM, HJ, BAE, SH, LEE, JH, MOON, JY, LEE, WS, LEE, JH, LEE, CI, KIM, SJ, and JANG, G: Long-term health and germline transmission in transgenic cattle following transposon-mediated gene transfer. *BMC Genomics* **19**, 387, 2018.

ZIMMER, M: GFP: from jellyfish to the Nobel prize and beyond. *Chem Soc Rev* **38**, 2823-32, 2009.

Table 1

The collection of the *in vivo* data of GFP-induced pathology in transgenic animals

Affected	Transgenic strains	Symptoms	Possible explanations

organ systems			
Cardiovascular system	α-MyHC-EGFP mice	Dilated cardiomyopathy, premature death (Huang *et al.*, 2000).	Impaired actin-myosin interactions (Agbulut *et al.*, 2006), increased CaMKII activity (Khoo *et al.*, 2008).
	cmlc2-EGFP zebrafish	Altered cardiac function (Avey *et al.*, 2018; Ho *et al.*, 2007; Huang *et al.*, 2011).	
Urinary tract	CAGGS-EGFP mice	Glomerulosclerosis, proteinuria, tubulo-interstitial injury (Guo *et al.*, 2007).	Defective polyubiquitination (Baens *et al.*, 2006), oxidative stress (Ganini *et al.*, 2017**).**
	GABARAP-EGFP mice	Glomerulosclerosis, proteinuria (Takagi-Akiba *et al.*, 2012), (Liptak *et al.*, 2018).	
	CAGGS-Venus rabbits		
Central nervous system	lacZ-GFP mice	Growth retardation, premature death (Krestel *et al.*, 2004).	**G**FP/YFP excessive aggregation/accumulation.
	Thy-1 YFP mice	Increased axon swelling (Bridge *et al.*, 2009).	

Abbreviations: α-MyHC: α-myosin heavy chain gene, cmcl2: myosin light chain 2 gene, CamKII: calmodulin II kinase, GABARAP: γ -Aminobutyric acid A receptor-associated protein.