Overview of MARCM-Related Technologies in *Drosophila* Neurobiological Research

Tsai-Chi Hsu,[1,2] Kai-Yuan Ku,[1,2] Hung-Chang Shen,[1] and Hung-Hsiang Yu[1,3]

[1]Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan
[2]These authors contributed equally to this work; K.Y.K., T.C.H., H.C.S., and H.H.Y. composed the manuscript
[3]Corresponding author: *samhhyu@gate.sinica.edu.tw*

Mosaic analysis with a repressible cell marker (MARCM)–related technologies are positive genetic mosaic labeling systems that have been widely applied in studies of *Drosophila* brain development and neural circuit formation to identify diverse neuronal types, reconstruct neural lineages, and investigate the function of genes and molecules. Two types of MARCM-related technologies have been developed: single-colored and twin-colored. Single-colored MARCM technologies label one of two twin daughter cells in otherwise unmarked background tissues through site-specific recombination of homologous chromosomes during mitosis of progenitors. On the other hand, twin-colored genetic mosaic technologies label both twin daughter cells with two distinct colors, enabling the retrieval of useful information from both progenitor-derived cells and their subsequent clones. In this overview, we describe the principles and usage guidelines for MARCM-related technologies in order to help researchers employ these powerful genetic mosaic systems in their investigations of intricate neurobiological topics. © 2020 by John Wiley & Sons, Inc.

Keywords: cell lineage analysis • genetic mosaic systems • MARCM • twin-spot MARCM

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INTRODUCTION

Myriad and diverse cell types must be systematically assembled during the development of multi-tissue organisms. A great challenge for biologists is to reveal how genetic and molecular programs control where, when, and what various types of cells are generated and organized into tissues during crucial developmental processes, e.g., growth, proliferation, fate determination, and differentiation. Analysis of phenotypes in animals with mutated genes of interest represents an elegant approach to elucidate the function of genes and molecules in these processes. However, it is impossible to conduct such phenotypic analyses on essential genes because the homozygous mutant organisms are not viable. Thanks to the invention of genetic mosaic systems that permit creation of cells with different genotypes in the same organism, biologists can now investigate the function of such essential genes of interest (and other genes) in complicated biological processes [a timeline of genetic mosaic systems is provided by Lee (2014)].

Two tools—FLP (Flippase recombinase)/FRT (FLP recognition target), which mediates site-specific DNA recombination, and GAL4/UAS (upstream activation sequence), which permits binary-controlled gene expression—were combined to make genetic mosaic systems more efficient and controllable for studying intricate biological questions (Brand

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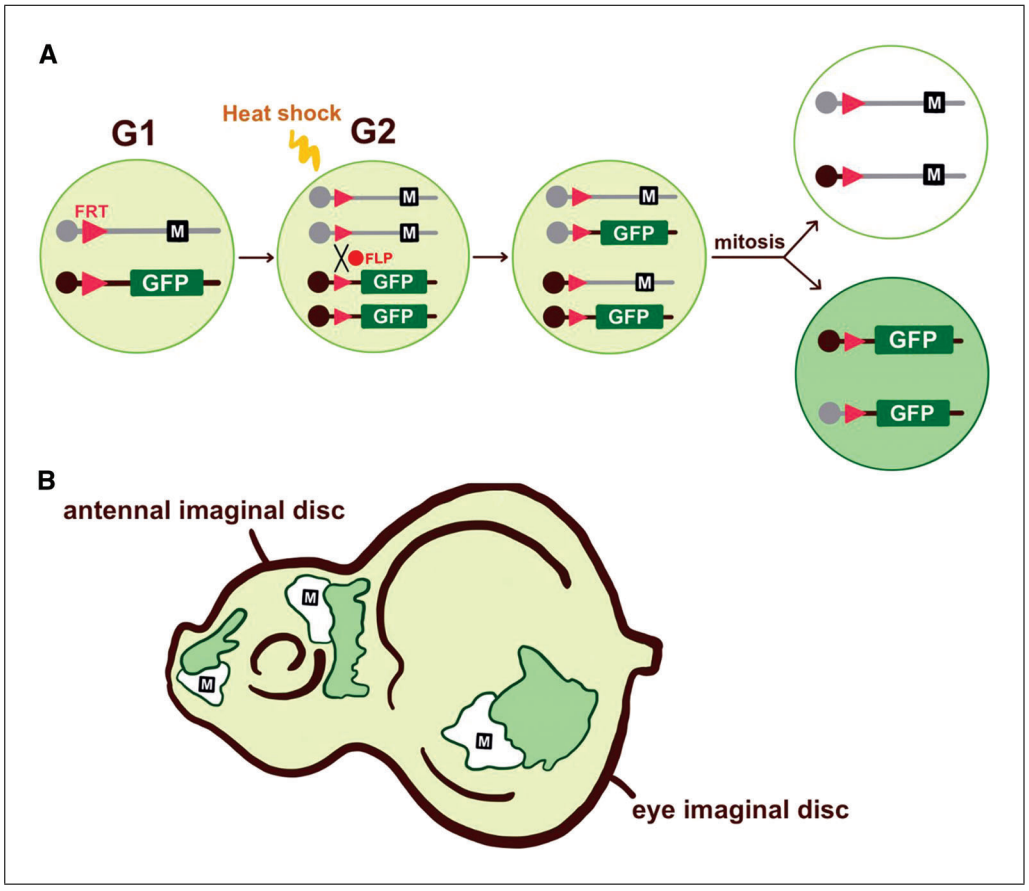

Figure 1 The conventional FLP/FRT-based genetic mosaic system. (**A**) In the conventional FLP/FRT-based genetic mosaic system, a mutation of the gene of interest (indicated by M) and a reporter (e.g., GFP) are placed distal to FRT sites, which are inserted at *trans* positions of homologous chromosomes in progenitor cells. Upon heat-shock induction of FLP to induce FRT-site-specific DNA recombination, the recombinant chromosomes are segregated into two progeny cells such that one cell contains homozygous mutated alleles (an invisible cell) and the other cell is brightly labeled (carries two alleles of GFP). The two progeny cells can subsequently develop into twin clones after multiple rounds of proliferation, as shown in B. (**B**) Schematic of eye-antennal imaginal discs illustrates clonal patterns observed in the conventional FLP/FRT-based genetic mosaic system.

& Perrimon, 1993; Xu & Rubin, 1993). The standard arrangement of conventional FLP/FRT-based genetic mosaic systems is two FRT sites inserted at *trans* positions of homologous chromosomes, with a mutated gene of interest and a reporter placed distal to the FRT sites (Fig. 1A). After FLP/FRT-mediated site-specific DNA recombination occurs, the recombinant chromosomes are segregated into twin cells (or clones), such that one cell (or clone) contains homozygous mutant alleles and the other carries two reporter alleles in a background tissue, the remaining cells of which have one mutant allele and one reporter allele (Fig. 1A). Since the generation of mosaic clones requires cellular propagation, this type of genetic mosaic system is an ideal tool for cell-lineage analyses to trace derivatives of different cell types from common progenitors. Using genetic mosaic systems to conduct cell lineage and phenotypic analyses is therefore a powerful approach to tease apart how, what, where, and when convoluted biological processes may be orchestrated during development by different genetic and molecular programs.

In conventional FLP/FRT-based genetic mosaic systems, the mutant clone is invisible (negative labeling) while its twin and the rest of the background tissue are labeled with the reporter (Fig. 1B). This type of system works very well for cells that are arranged in regular positions and possess identifiable morphologies, e.g., cells in imaginal discs (Lee & Luo, 1999; Fig. 1B). However, negative-labeling genetic mosaic systems are extremely difficult to use in neurobiological studies due to the highly variable cell-body positions and

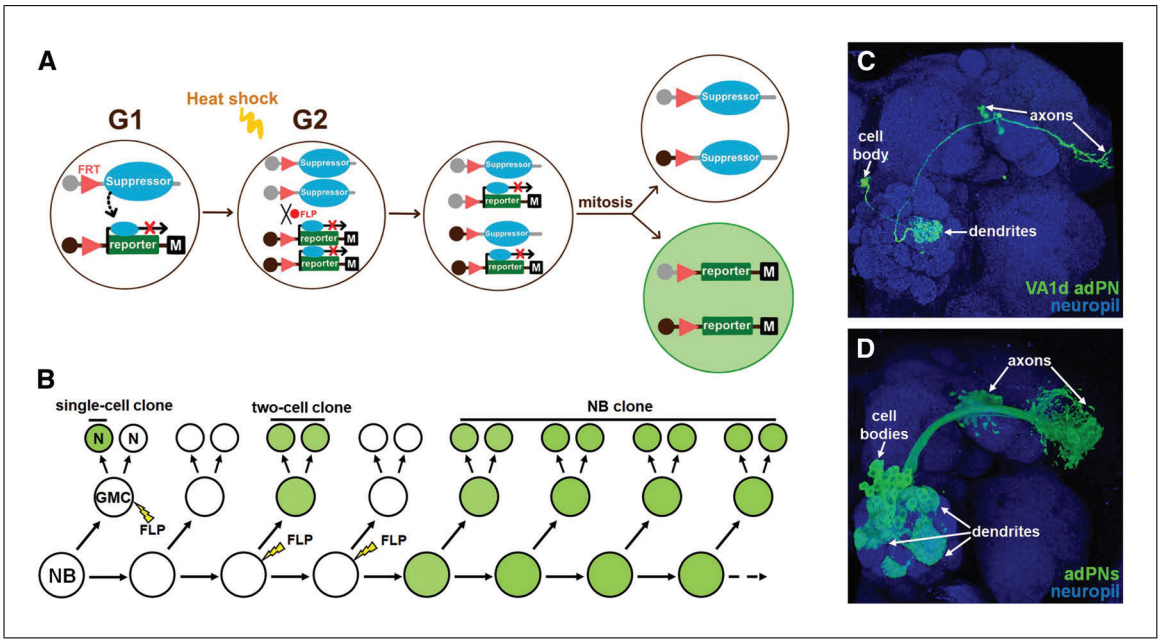

Figure 2 MARCM systems and their clonal patterns. (**A**) In MARCM systems, a suppressor (e.g., GAL80, QS or GFP RNAi) is placed in *trans* with the mutated gene of interest (indicated by M) and a reporter (e.g., mCD8::GFP), which initially makes neural progenitors invisible. Upon heat-shock induction of FLP to induce FRT-site-specific DNA recombination, the recombinant chromosomes are segregated into two progeny cells, such that one cell contains homozygous mutated alleles (mCD8::GFP-positive neuron) and the other cell remains invisible (carries two suppressor alleles). (**B**) Schematic shows neurogenesis in most neural lineages of the *Drosophila* central brain: neuroblasts (NBs) undergo asymmetric division to generate self-renewing NBs and ganglion mother cells (GMCs), and GMCs subsequently divide to produce two daughter neurons (Ns). Three clonal patterns—single-cell, two-cell and NB clones—are predicted and often observed in MARCM experiments when FLP is induced in GMCs or NBs. (**C-D**) Two examples of MARCM clones reveal morphological patterns of a single VA1d anterodorsal projection neuron [adPN; single-cell clone (C), and overall adPNs (NB clone; D) derived from the ALad1 neural lineage.

entangled neurite morphologies of neurons. To solve this issue, MARCM (mosaic analysis with a repressible cell marker) and related technologies were invented to positively label neurons of interest (Lee & Luo, 1999; Potter, Tasic, Russler, Liang, & Luo, 2010; Yu, Chen, Shi, Huang, & Lee, 2009); these renovated FLP/FRT-based genetic mosaic systems have made a significant impact in *Drosophila* neurobiological research, as they have been widely applied in important studies on brain development and neural circuit formation, including those investigating neuronal type and neural lineage, neurogenesis, cell fate specification, neuronal morphogenesis, and differentiation (Chiang et al., 2011; Lee, Lee, & Luo, 1999; Lee, Marticke, Sung, Robinow, & Luo, 2000; Marin, Jefferis, Komiyama, Zhu, & Luo, 2002; Yu et al., 2013; Zhu et al., 2006). Step-by-step protocols for MARCM-related genetic mosaic technologies (e.g., MARCM and twin-spot MARCM) are detailed elsewhere (Shen, Hsu, Chung, & Yu, 2017; Wu & Luo, 2006). In this overview, we summarize the principles and usage guidelines for MARCM and related genetic mosaic technologies in the study of *Drosophila* neurobiology.

MARCM TECHNOLOGIES

As a renovation of conventional FLP/FRT-based genetic mosaic systems, MARCM technologies incorporate a new component, the suppressor, which makes the background tissue invisible. The mutated gene of interest is then placed in *trans* with the suppressor, and after recombination and mitosis, this arrangement permits positive labeling of cells derived from one of two progeny cells (suppressor-negative) in otherwise unmarked tissues (cells derived from the other twin cell and the background tissue all remain suppressor-positive; Lee & Luo, 1999; Fig. 2A). With regard to neurogenesis in the *Drosophila* brain, a limited number of neural stem cells, called neuroblasts (NBs), undergo asymmetric division to generate self-renewing NBs and ganglion mother cells (GMCs; Goodman & Doe, 1993; Fig. 2B). The GMCs then undergo another round of division to produce

two daughter cells, which differentiate into neurons (Goodman & Doe, 1993; Fig. 2B). Therefore, three clonal patterns are predicted and usually observed in MARCM experiments, including single-cell, two-cell, and NB clones (Lee & Luo, 1999; Fig. 2B). The single-cell and two-cell clones portray neuronal morphology at single-cell resolution, whereas NB clones reveal morphological patterns of the entirety of neurons derived from a common NB (Lee & Luo, 1999; Fig. 2C-D). In this section, we describe the principles and highlight important points regarding the usage of three MARCM systems.

GAL80-Based MARCM

GAL80-based MARCM is the prototypical and still most often used MARCM technology in *Drosophila* neurobiological research. In this system, ubiquitously expressed GAL80 (a repressor of GAL4-driven expression) is driven by the tubulin promoter (tubP-GAL80); GAL80 blocks the expression of a reporter under the control of GAL4 drivers (e.g., UAS-mCD8::GFP), such that labeling is limited to cells where GAL4 is expressed and GAL80 is absent (Lee & Luo, 1999; Fig. 2A). In this system, FLP expression is usually controlled by a heat-shock promoter (hs-FLP), which permits generation of stochastic MARCM clones at any developmental time of interest (Lee & Luo, 1999). Upon the expression of FLP in NBs or GMCs, FRT-mediated chromosome recombination is activated, permitting the segregation of GAL80 into two daughter cells. This segregation event is followed by mitosis, which generates single-cell, two-cell, and NB clones, with GAL80-negative cells able to be visualized as mCD8::GFP-positive (Lee & Luo, 1999; Fig. 2B-D). The mCD8::GFP signal usually appears around 24 to 48 hr after the induction of MARCM clones due to the persistence of GAL80. Since heat-shock-induced FLP expression is stochastic, occasional mixtures of single-cell clones and two-cell or NB clones are unavoidable. Mild heat-shock induction may help to minimize the occurrence of unwanted clonal mixtures. However, strong heat-shock induction to produce a high level of FLP is necessary to generate NB clones at the mid-larval stage (due to the dilution of FLP levels in quickly dividing NBs). Despite the potential occurrence of clonal contamination, GAL80-based MARCM allows researchers to easily investigate the function of genes of interest in neurons with homozygous mutations, even if the whole animal mutation is lethal to the organism, so long as the mutation is not lethal to the cells of interest. More importantly, GAL80-based MARCM allows researchers to express target genes under the control of GAL4 in neurons of interest for ectopic overexpression experiments and in mutant neurons for rescue experiments, facilitating the exploration of gene function in specific neuronal types and demonstrating the power of MARCM in neurobiological research. Besides its application in studies of gene function, GAL80-based MARCM has also been utilized to identify distinct types of mushroom body (MB) neurons and olfactory projection neurons (PNs) in the olfactory system; it has also been used to label a substantial number of neurons and neural lineages in efforts to reconstruct neural circuits in the *Drosophila* central brain (Chiang et al., 2011; Jefferis, Marin, Stocker, & Luo, 2001; Lee et al., 1999; Marin et al., 2002; Yu et al., 2013).

QS-Based MARCM

QS-based MARCM is a genetic mosaic system analogous to GAL80-based MARCM, which is constructed by replacing all GAL80-, GAL4-, and UAS-related components with the equivalent constructs from the Q binary–controlled gene expression system, i.e., QS (suppressor), QF (transcription factor that binds and activates QUAS promoter), and QUAS (promoter equivalent to UAS; Potter et al., 2010; Fig. 2A). Theoretically, QS-based MARCM can do everything that the GAL80-based MARCM can do, and it possesses all the same pros and cons. However, we note that QF has been changed to QF2 to reduce toxicity, and the tools available in the QF/QUAS system are generally not as compatible and sophisticated as those used in the GAL4/UAS system (Riabinina et al., 2015).

RNAi-Based MARCM

Since the primary goal of MARCM technologies is to positively label cells (or clones) of interest and leave the rest of the cells unmarked, it is also possible to generate MARCM clones by utilizing RNA interference (RNAi) as a suppressor to specifically silence mCD8::GFP expression. In RNAi-based MARCM, the same GAL4 drivers are used to control expression of both GFP RNAi and mCD8::GFP (Yu et al., 2009; Fig. 2A). Besides the clonal contamination issue shared by all MARCM technologies, RNAi seems to persist longer than GAL80 when expressed by GAL4 drivers in progenitors (i.e., NBs and GMCs; see the twin-spot MARCM section

below for further discussion of this issue). Therefore, strong drivers that post-mitotically express GAL4 are preferred in RNAi-based MARCM to avoid problems associated with RNAi longevity (Yu et al., 2009). In addition, conducting ectopic overexpression and rescue experiments in RNAi-based MARCM is not as straightforward as it is in GAL80- and QS-based MARCM systems. In RNAi-based MARCM systems, overexpression and rescue transgenes should be engineered with the RNAi target sequences in the 5′ or 3′ untranslated regions to work effectively. Despite these complications, functional versions of RNAi-based MARCM are available in the GAL4/UAS system and other LexA/lexAop binary-controlled gene expression systems (Awasaki et al., 2014; Yu et al., 2009).

TWIN-SPOT MARCM AND SIMILAR TECHNOLOGIES

Because MARCM technologies only label one of the twin daughter cells and leave the rest of cells invisible, potentially useful information from the unmarked twin is lost and comparisons of both twins are not feasible. Therefore, MARCM systems described in the previous section are not suitable for high-resolution analyses of neural lineages that quickly switch cell fates or for precision analyses of gene functions in identical neurons of different animals (Yu et al., 2009, 2010). These limitations were overcome by the development of new genetic mosaic systems, including twin-spot MARCM, coupled MARCM, and twin-spot generator, all of which permit the labeling of twin cells (and their derived clones) with two distinct colors in otherwise unmarked background tissues (Griffin et al., 2009; Potter et al., 2010; Yu et al., 2009). In this section, we highlight the principles and important points regarding the usage of these twin-spot/colored genetic mosaic systems.

Twin-Spot MARCM

In the twin-spot MARCM system, two RNAi-based suppressors, GFP-RNAi and rCD2-RNAi, are placed at *trans* sites of homologous chromosomes to independently inhibit the expression of their respective reporters, mCD8::GFP and rCD2::RFP (Yu et al., 2009; Fig. 3A). Following FLP/FRT-mediated site-specific mitotic recombination, GFP-RNAi and rCD2-RNAi are segregated into the twin daughter cells, respectively, de-repressing the expression of rCD2::RFP and mCD8::GFP in individual cells (Yu et al., 2009; Fig. 3A). Two clonal patterns are predicted and typically seen in twin-spot MARCM experiments: paired single-cell clones and two-cell associated with NB clones (Yu et al., 2009; Fig. 3B). Since the information derived from one side of the twin cells can be utilized as a reference for the other side, twin-spot MARCM enables high-resolution neural lineage analyses for birth-dating neurons derived from common NBs and improves phenotypic analyses of identical neurons in different animals for precise investigations into gene function (Yu et al., 2009, 2010; Fig. 3B-C). The power of twin-spot MARCM in high-resolution neural lineage analyses was demonstrated by the birth-dating of 40 neuronal subtypes among 80 anterodorsal projection neurons (adPNs) and 39 GMC-derived pairs of lateral projection neurons and local interneurons in the ALad1 and ALl1 neural lineages, respectively (Lin, Kao, Yu, Huang, & Lee, 2012; Yu et al., 2010; Fig. 3D). In addition to allowing high-resolution neural-lineage analysis, twin-spot MARCM also permits improved phenotypic analyses to accurately disclose gene functions in neurons. As such, twin-spot MARCM was used to detect a single temporal fate change that requires *chinmo* (a BTB-zinc finger nuclear protein) in one of six GAL4-OK107-positive central complex neurons; this phenotype would likely have been misclassified as an axonal guidance and misprojection defect if investigators had used single-colored MARCM technologies (Yu et al., 2009).

Just like the obstacles faced with RNAi-based MARCM, the efficiency and persistence of RNAi suppressors and reporters determine whether twin-spot MARCM experiments can be successfully conducted in neurobiological studies. Because the same GAL4 drivers are used to express RNAi suppressors and reporters in twin-spot MARCM, drivers that strongly express GAL4 in differentiated neurons but not in NBs and GMCs are preferred; this reduces the lifetime of RNAi suppressors and facilitates high expression of reporters in post-mitotic neurons for improved twin-spot MARCM results (Yu et al., 2009). For example, when using a strong MB driver (GAL4-OK107) to express GAL4 in MB progenitors and their derivatives (i.e., MB γ, α′/β′, and α/β neurons) in twin-spot MARCM experiments, MB γ neurons were unfaithfully labeled, since only single GFP- or RFP-positive neurons (instead of pairs of GFP and RFP neurons) were observed in paired single-cell clones (Yu et al., 2009). Intriguingly, this issue was no longer observed when

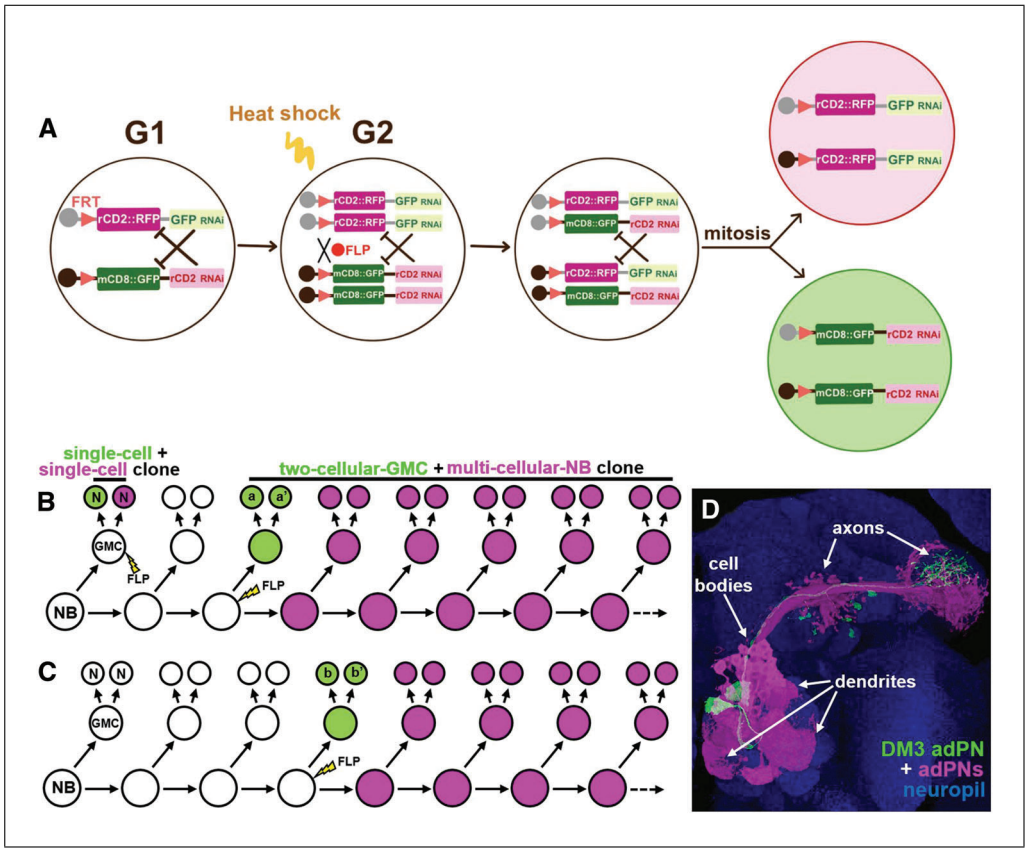

Figure 3 The twin-spot MARCM system. (**A**) Two RNAi suppressors, GFP RNAi and rCD2 RNAi, are used to independently inhibit the expression of two respective reporters, rCD2::RFP and mCD8::GFP, in the twin-spot MARCM system. After activation of FLP/FRT-mediated site-specific DNA recombination, the recombinant chromosomes with GFP RNAi and rCD2 RNAi are segregated into two progeny cells, which allows expression of rCD2::RFP and mCD8::GFP, respectively. A mutation of the gene of interest can be associated with either mCD8::GFP or rCD2::RFP for the phenotypic analysis. (**B-C**) Two clonal patterns (paired single-cell clones, and two-cell associated with NB clones) are predicted and often observed in twin-spot MARCM experiments when FLP is induced in GMCs or NBs, respectively. Twin-spot MARCM permits high-resolution neural-lineage analyses for birth-dating neurons derived from common NBs. For instance, the birth of a and a′ neurons (the pair of green neurons shown in panel B) occurs one cell cycle prior to the birth of b and b′ neurons (the pair of green neurons shown in panel C). The birth timing can be determined by counting the cell numbers in the magenta multi-cellular (derived from NB) sides of different twin-spot MARCM clones in panels B and C. (**D**) Two-cell clones associated with NB in twin-spot MARCM experiments are sometimes observed as a single neuron (e.g., single green DM3 adPN) associated with a group of neurons (e.g., magenta adPNs), following the death of one of two semi-lineages in the neural lineage of interest (e.g., ALad1 neural lineage).

MB247-GAL4 was used instead; MB247-GAL4 is an MB driver that specifically expresses GAL4 in differentiated MB neurons (Yu et al., 2009). Besides the problems of efficiency and persistence of RNAi suppressors and reporters, the twin-spot MARCM system has similar issues as the RNAi-based MARCM system for overexpression and rescue experiments. Therefore, the design of transgenes for such experiments should follow the principles outlined in the RNAi-based MARCM section. Also, like RNAi-based MARCM, twin-spot MARCM is available in both GAL4/UAS and LexA/lexAop systems (Awasaki et al., 2014; Yu et al., 2009).

Coupled MARCM

In contrast to twin-spot MARCM, coupled MARCM uses two independent suppressors, GAL80 from the GAL4/UAS system and QS from the Q system (Potter et al., 2010). In coupled MARCM experiments, tubP-GAL80 and tubP-QS are placed distal to FRT sites in *trans* positions of two homologous chromosomes, such that two independent reporters driven by the GAL4/UAS and Q systems are

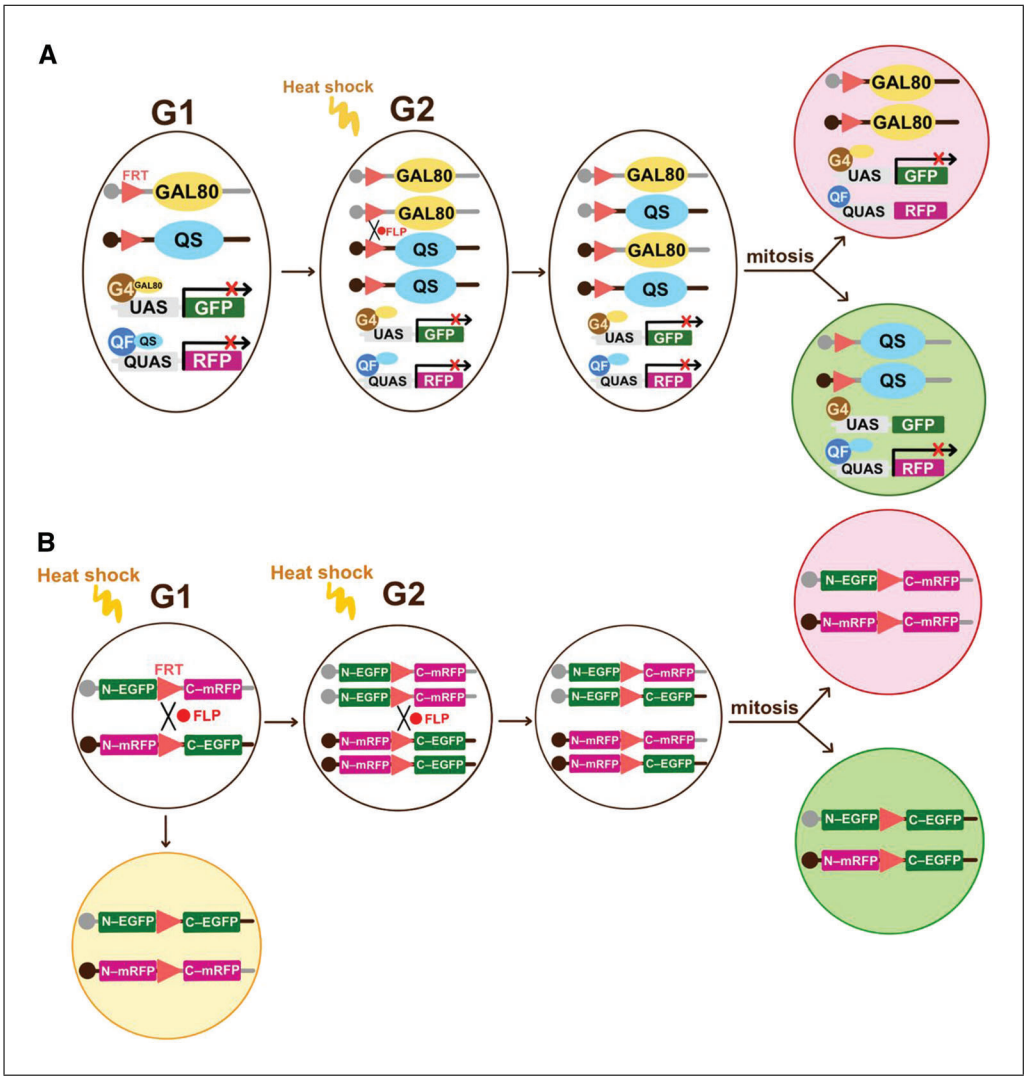

Figure 4 The coupled MARCM and twin-spot generator systems. (**A**) GAL80 and QS suppressors are used to independently control the expression of two distinct reporters [e.g., GFP and RFP driven by GAL4 (indicated by G4) and QF, respectively] in twin cells after FLP/FRT-mediated mitotic recombination in the coupled MARCM system. (**B**) In the twin-spot generator system, two reporters, enhanced GFP (EGFP) and monomeric RFP (mRFP), are initially non-functional, and upon FLP/FRT-mediated site-specific DNA recombination, EGFP and mRFP are reconstructed and expressed as two functional reporters. Because the FLP/FRT-mediated recombination event also occurs at G1 phase, yellow cells that express both reporters are often seen in twin-spot generator experiments.

expressed in the twin cells (or clones; Fig. 4A; Potter et al., 2010). Because the coupled MARCM system requires two independent drivers to control the expression of the different reporters, researchers should carefully select GAL4 and QF drivers that can faithfully label the same neurons in the neural lineages of interest to avoid confusing results (Lee, 2014).

Twin-Spot Generator

Technically, the twin-spot generator is not a suppressor-based genetic mosaic system. In this system, DNA fragments encoding two reporters, enhanced GFP (EGFP) and monomeric RFP (mRFP), are initially designed as non-functional genes (Griffin et al., 2009): the N-termini of EGFP and mRFP are out of frame with the C termini of mRFP and EGFP, respectively (Fig. 4B). Upon the induction of FLP to activate FRT-mediated site-specific DNA recombination, DNA fragments encoding EGFP and mRFP are reconstructed to express two in-frame functional reporters (Griffin et al., 2009; Fig. 4B). The advantage of the twin-spot generator system is that it requires fewer transgenic components than twin-spot and coupled MARCM

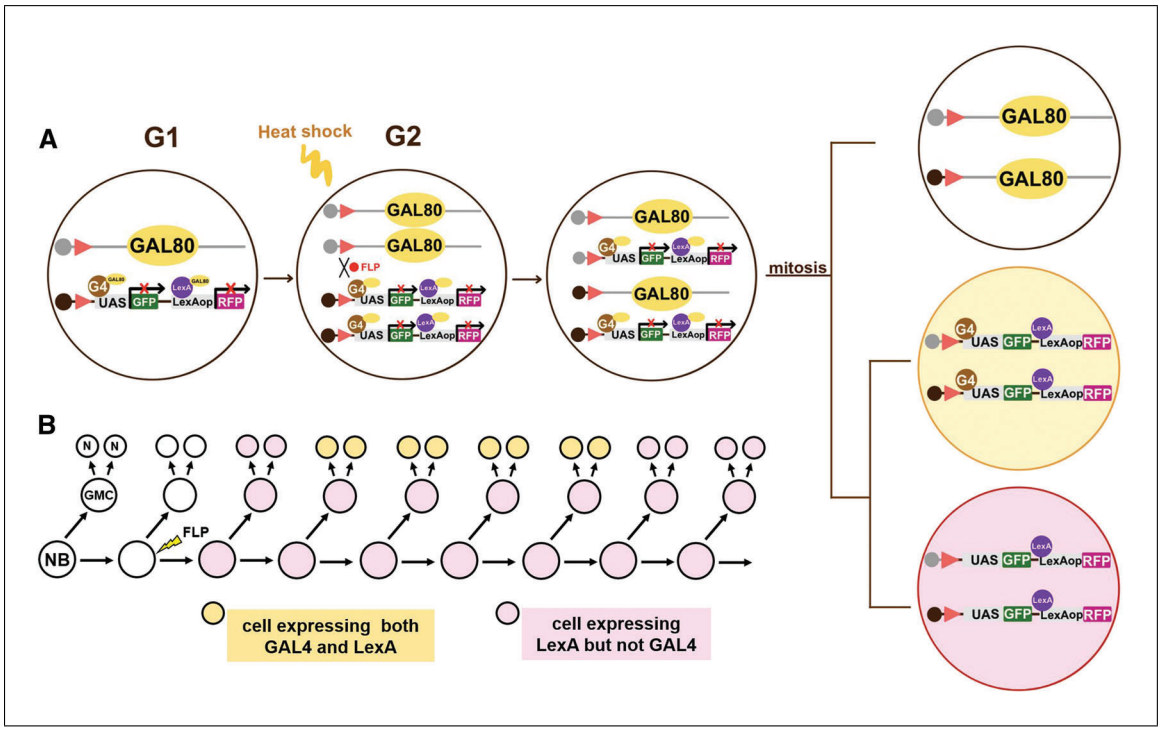

Figure 5 The dual-expression-control MARCM system. (**A**) In the dual-expression-control MARCM system, GAL80 can be used to inhibit two reporters (e.g., GFP and RFP) driven by GAL4 drivers of interest and a pan-cell LexA driver, tubP-LexA::GAD (indicated by LexA). As with the GAL80-based MARCM system, one of the twin cells is labeled (GAL80-negative) and the other is invisible (GAL80-positive) in the dual-expression-control MARCM system. However, the labeled GAL80-negative cells may express either FRP alone or both reporters, depending on the absence/presence of GAL4, which allows researchers to determine the coverage of neurons of interest for GAL4 drivers. The same principles can be applied in the QS-based MARCM system by replacing UAS, GAL4, LexA::GAD, and GAL80 components with QUAS, QF, LexA::QFAD, and QS components, respectively. (**B**) The schematic illustrates a hypothetical neural pattern observed in a GAL80-based dual-expression-control MARCM system. Only the progeny of four GMCs in mid-lineage express GAL4, while earlier and later GMCs express LexA::GAD but lack GAL4.

systems. However, since FLP/FRT-mediated site-specific DNA recombination also occurs in non-mitotic cells, EGFP and mRFP may be co-expressed in the same cells in the twin-spot generator system (Fig. 4B), making visualization of true twin-spot clones in the study of the brain development a challenging task (Lee, 2014).

OTHER MARCM-RELATED TECHNOLOGIES

Dual-Expression-Control MARCM

Not all drivers are suitable for conducting gene function studies and cell lineage analyses in genetic mosaic systems (e.g., MARCM, twin-spot MARCM and coupled MARCM), as the drivers might not be expressed in the neurons of interest. Therefore, prior to conducting experiments, the coverage of drivers in neurons of interest should be validated in the dual-expression-control MARCM system, which is a regulatory system featuring independently controlled expression of genes using the GAL4/UAS (or QUAS/QF) and LexA/lexAop systems (Lai & Lee, 2006; Riabinina et al., 2015; Fig. 5A). Since a pan-cell driver (e.g., tubP-LexA::GAD) is used in the LexA/lexAop system, and the expression of reporters (e.g., lexAop-RFP) driven by LexA::GAD can be inhibited by GAL80, the coverage of neurons (e.g., labeled by GFP) using GAL4 drivers can be determined in dual-expression-control MARCM experiments (Fig. 5A-B). Despite the fact that the dual-expression-control MARCM system was initially designed with GAL80-based MARCM, the same principles can be applied in the QS-based MARCM system by replacing UAS, GAL4, LexA::GAD, and GAL80 components with QUAS, QF, LexA::QFAD, and QS components, respectively. Using this approach, researchers found that one of the most frequently used drivers for studying adPNs, GAL4-GH146, only labels about 45 early-born adPNs, while missing 32 late-born adPNs in the ALad1 neural lineage (Lai & Lee, 2006; Lai, Awasaki, Ito, & Lee, 2008;

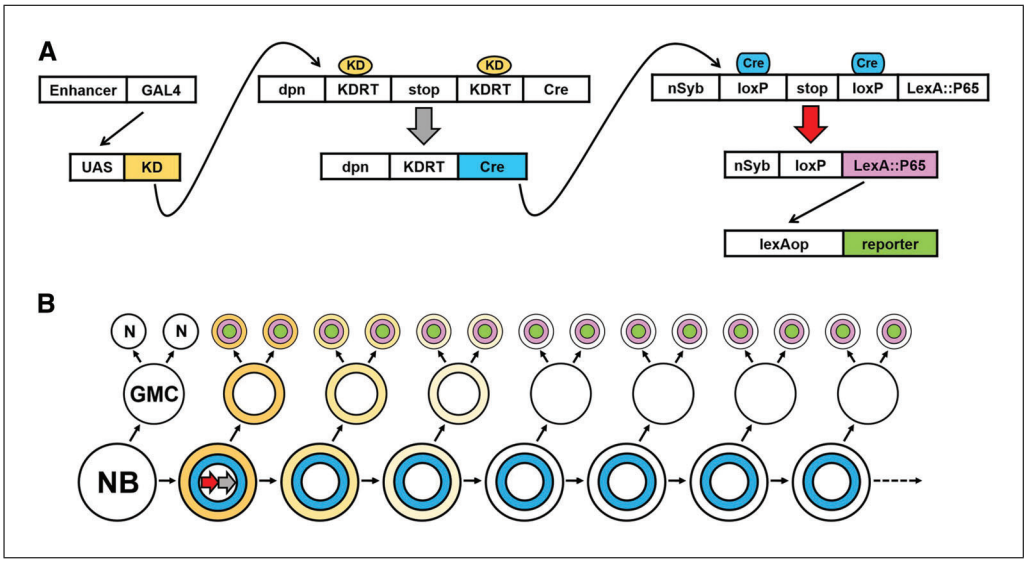

Figure 6 A strategy to convert patterned NB activities into neural lineage-restricted drivers. (**A-B**) Converting patterned NB activities into neural lineage-restricted drivers can be achieved by the following three steps. First, transient GAL4 activities expressed by drivers (e.g., "Enhancer-GAL4") permit the expression of KD recombinase (yellow) in NBs and non-NB cells; in sequentially derived cells, KD recombinase is gradually reduced by dilution (lighter yellow). Second, the KD recombinase kicks out the KDRT stop cassette (gray arrow) in a transgene of "KDRT-stop-KDRT-Cre" driven by a pan-NB Deadpan (dpn) promoter to limit expression of Cre recombinase to the NBs of interest (blue). Finally, the Cre recombinase removes the other stop cassette in a "loxP-stop-loxP-LexA::P65" transgene only in the NBs of interest (red arrow). Since LexA::P65 (magenta) is now under the control of a pan-neural nSyb promoter, LexA::P65 then drives reporter expression (green) only in differentiated neurons, but not in NBs and GMCs, of the specific neural lineages.

Yu et al., 2010). Thus, dual-expression-control MARCM revealed a major limitation of using this driver in genetic mosaic systems for investigations of adPNs within the ALad1 neural lineage.

Neural Lineage-Restricted Driver-Based MARCM-Related Technologies

A key to successfully performing genetic mosaic analyses in *Drosophila* neurobiological research is the use of appropriate drivers in MARCM-related systems. If drivers are not available, patterned NB activities may be converted into neural lineage-restricted drivers to identify appropriate drivers for the study (Awasaki et al., 2014; Fig. 6A-B). In this strategy, GAL4 drivers or direct promoters are used to express KD recombinase in NBs of interest (Awasaki et al., 2014). The KD recombinase then kicks out a stop cassette to limit the expression of Cre recombinase in NBs by intersecting with a pan-NB Deadpan (dpn) promoter in specific neural lineage patterns (Awasaki et al., 2014; Fig. 6A-B). The Cre recombinase is subsequently used to remove the other stop cassette and permit LexA::P65 to drive reporter expression under the control of a pan-neural synaptobrevin (nSyb) promoter only in differentiated neurons, but not in NBs and GMCs, of the specific neural lineages (Awasaki et al., 2014; Fig. 6A-B). By coupling such neural lineage–restricted drivers with MARCM-related technologies, researchers can broaden their choice of neurons of interest to solve complicated biological problems. The advantage of this neural lineage-restricted driver approach is that it irreversibly converts GAL4 activity in NBs into LexA drivers that function in all neurons subsequently derived from the NBs. These drivers can then serve as ideal tools for comprehensive cell-lineage analyses using twin-spot MARCM. For instance, R13C01-GAL4 may be converted into drivers for comprehensive investigation of AL11, VLPl1, VLPl2, and VLP14 neural lineages in cell-lineage analyses (Awasaki et al., 2014). However, the downside of this neural lineage–restricted driver-based approach is that it requires many transgenic components, and it is therefore not a user-friendly technology for neurobiologists.

CONCLUSION

The MARCM-related positive genetic mosaic labeling technologies described in this overview were invented to visualize

and study neurons of interest in *Drosophila*. In mouse, a related genetic mosaic system called mosaic analysis with double markers (MADM; the design of twin-spot generator was adapted from MADM) was also developed to investigate neurons of interest (Zong, Espinosa, Su, Muzumdar, & Luo, 2005). Using these technologies, researchers may gather information about neuronal morphologies and neural lineages, both of which are essential in investigations of brain development and neural circuit formation. Similar to other technologies, MARCM-related approaches carry distinct advantages and limitations. Researchers should be aware of these characteristics prior to application of the technologies in gene-function studies and cell-lineage analyses. In this overview, we summarize the principles and usage guidelines of MARCM-related technologies. Armed with this information, researchers should be able to employ these powerful genetic mosaic systems to explore uncharted territory in important neurobiological topics.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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