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Tubby-tagged balancers for the Drosophila X and second chromosomes

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We generated *FM7a* and *CyO* balancer chromosomes bearing a *Tubby*^{1} (*Tb*^{1}) dominant transgene. Flies heterozygous for these *FM7a* and *CyO* derivatives exhibit a phenotype undistinguishable from that elicited by the *Tb*^{1} mutation associated with the *TM6B* balancer. We tested two of these *Tb*-bearing balancers (*FM7-TbA* and *CyO-TbA)* for more than 30 generations and found that the *Tb*^{1} transgene they carry is stable. Thus, these new *Tb*-tagged balancers are particularly useful for balancing lethal mutations and distinguish homozygous mutant larvae from their heterozygous siblings.

One of the great advantages of *Drosophila melanogaster* as model organism is the availability of balancer chromosomes. These chromosomes suppress recombination with their homologues, allowing the maintenance of lethal and sterile mutants as balanced heterozygotes. All balancers carry dominant markers that are visible in adult flies, but only a subset have markers that unambiguously distinguish homozygous mutant larvae from their heterozygous siblings. The latter balancers include those that express high levels of the GFP protein under the indirect control, via the UAS/GAL4 system, of either the *Kruppel (Kr)*,[1] or *hsp70*,[2] promoter. In addition, there are direct-drive balancers that express GFP under the control of the *actin A5c* promoter[3] or YFP under the control of *Deformed* (*Dfd* HZ2.7rev) or *glass* (GMR) enhancer elements.[4] Each of the GFP- or YFP-expressing balancers has specific advantages, but all share a common drawback: These balancers require the use of a dissecting microscope equipped with an UV light source, which for reliable fluorescence detection is preferably used in the dark.

The *TM6B* balancer[5] carries the *Tb*^{1} dominant mutation, which results in squat larvae and pupae. We have been using this balancer for many years to unambiguously distinguish homozygous mitotic mutants dying at late larval stages from their heterozygous siblings (reviewed in ref. 6). This balancer proved particularly useful when mutant larvae are rare and one has to examine several vials (or bottles) to find third instar larvae suitable for dissection and cytological analysis. To generate new tools for easy detection of larvae homozygous for lethal mutations on the X or the second chromosome, we decided to generate *Tb*-marked versions of *FM7a*[7] and *CyO*,[8] respectively.

We have previously demonstrated that the Tubby phenotype results from the deletion in the *Tb*^{1} chromosome of DNA encoding amino acids 167–190 of the TwdlA cuticle protein.[9] A genomic fragment spanning the *TwdlA*^{Tb1} transcription unit, as well as 1 kb of DNA upstream, was inserted into a modified polylinker sequence in the pCaSpeR vector. Using standard methods, we co-injected DNA with a Δ*2-3* transposase source into *w*^{118} embryos, selected for *w*$^{+}$ eye color, and generated balanced stocks. All such stocks (n = 10) exhibited the squat larval and pupal phenotype characteristic of the *Tb*^{1} mutation. One such stock, referred to hereafter as *P{Tb*^{1}*}/CyO*, was used as the starting point for mobilizing *Tb*^{1} onto X and 2nd chromosome balancers.

To generate *FM7a-P{Tb*^{1}*}* we crossed *FM7a; P{Tb*^{1}*}/CyO; TMS*/+ males to *w/w* females (*TMS* is a Δ*2-3*-bearing third chromosome balancer expressing the P-element transposase described in ref. 10); from approximately 1,000 progeny we recovered two *FM7* chromosomes that co-segregated with *Tb* (we propose to name these balancers *FM7-TbA* and *FM7-TbB).* To generate a second chromosome balancer with a *P{Tb*^{1}*}* insertion we used a *CyO* balancer bearing the additional markers *S* and *bw*^{1} (designated as *CyO-Sbw* in FlyBase). We crossed *w/w; P{Tb*^{1}*}/CyO-Sbw; TMS*/+ females to *w*; *CyO*/*Sco* males and recovered three *CyO-Sbw-P{Tb*^{1}*}* chromosomes from approximately 1,000 progeny (we propose to name these balancers *CyO-TbA, CyO-TbB* and *CyO-TbC)*. To assess the utility of these *FM7a* and *CyO Tb*-bearing balancers, we compared their *Tb* phenotype with the *Tb*^{1} mutant phenotype associated with *TM6B*.[5] To quantify the squat phenotype elicited by the balancers we measured the axial ratio[11] of larvae and pupae (AR, length/width) heterozygous for each balancer. It has been previously shown that the AR does not depend on larval and pupal size and provides a reliable measure of the *Tb* phenotype.[9] As shown in **Figure 1**, the ARs observed in *FM7-TbA*, *FM7-TbB, CyO-TbA, CyO-TbB*, *CyO-TbC* and *TM6B* heterozygotes are fully comparable and significantly different from those of wild-type or non-*Tb*-bearing larvae and pupae. We thus conclude that the *FM7a* and *CyO Tb*-tagged chromosomes are highly suitable to distinguish larvae and pupae

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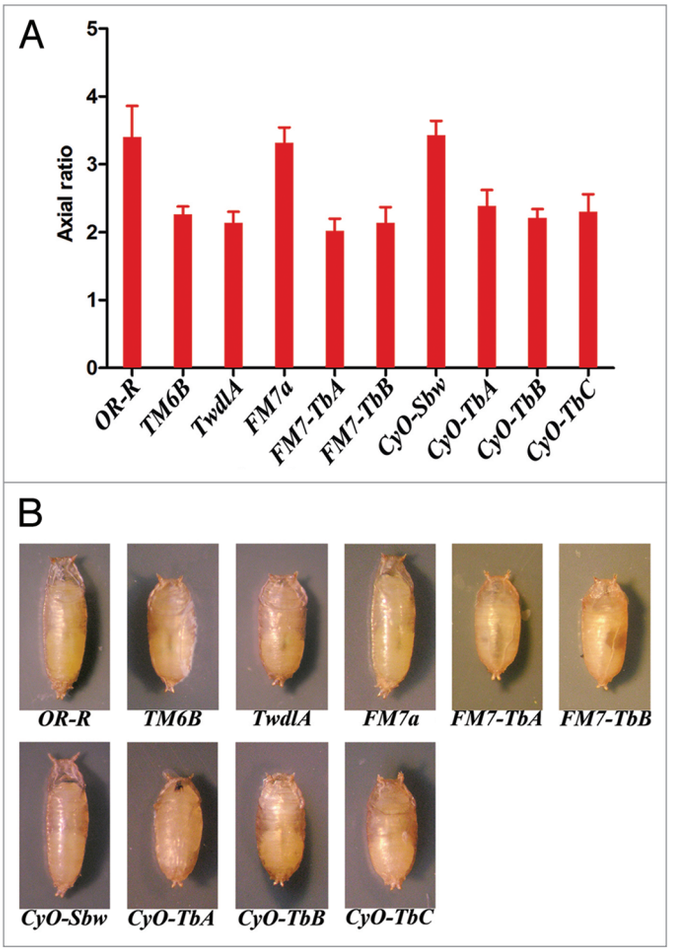

Figure 1. Quantitation of the squat phenotype of pupae heterozygous for different *Tb*-bearing chromosomes or balancers; *OR-R* is a wild-type Oregon R stock; *TwdlA* contains a single copy of the *TwdlA*^{Tb1} transgene; *FM7a* and *CyO-Sbw* are the balancer chromosomes used to construct the *Tb*-bearing derivatives indicated as *FM7-TbA, FM7-TbB, CyO-TbA, CyO-TbB* and *CyO-TbC*. (A) Axial ratios (ARs; length/width, ±standard deviation) determined from digital photographs of at least 40 pupae of each genotype (B) examples of pupae heterozygous for different *Tb* mutations and transgenes.

that bear the balancers from those that are homozygous for the balanced chromosome.

We generated the two *FM7a* and the three *CyO Tb*-bearing balancers in October 2009 and March 2010, respectively. Within two months from the time of their generation, one balancer of each type (*FM7-TbA* and *CyO-TbA*) was used to rebalance 40 chromosomes bearing different lethal mutations associated with either the X or the second chromosome. These stocks were reexamined in March 2011 (after more than 30 generations) and none of them had lost the *Tb* marker from the balancer. We thus conclude that the *Tb*^{1} transgene is very stable and that the *FM7-TbA* and *CyO-TbA* balancers are suitable for the establishment and long-term maintenance of balanced stocks.

Using inverse PCR (an EcoRI fragment was ligated and amplified using the 5'-CAGCTCCATAGTTATAGCCGC and CGTTAAGTGGATGTCTTCTTG-3' primers), we also determined the insertion sites of the *Tb*^{1} transgenes within the *FM7-TbA* and *CyO-TbA* balancers. The insertion in *FM7-TbA* mapped at 17C in the intergenic space between *CG15047* and *beadex*; accordingly, this balancer is homozygous-viable. The *Tb*^{1} transgene of *CyO-TbA* was inserted into the *Cytochrome P450 reductase (Cpr)* gene in region 26C3. We did not determine whether this insertion inactivates the *Cpr* gene.

In summary, we have generated *Tb*-marked versions of *FM7a* and *CyO*. These chromosomes carry a stable *Tb*^{1} transgene that has the same expressivity as the original *Tb*^{1} mutation. We believe that the use of the *FM7-TbA* and *CyO-TbA* balancers will facilitate a number of experimental strategies, allowing researchers to readily distinguish homozygous larvae and pupae from their heterozygous siblings simply by looking through the vial or the bottle used to grow the flies. In addition, the availability of balancers with different dominant larval markers such as *Tb* or GFP will allow construction of stocks carrying two lethal mutations balanced on *Tb*- and GFP-bearing balancers, respectively. This will be particularly helpful for selection and analysis of single and double mutants from the same culture. The *FM7-TbA* and *CyO-TbA* balancers can be obtained from the Bloomington Drosophila Stock Center at Indiana University (http://flystocks.bio.indiana.edu).

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