A strategy for generation and balancing of autosome: Y chromosome translocations

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We describe a method for generation and maintenance of translocations that move large autosomal segments onto the Y chromosome. Using this strategy we produced (2;Y) translocations that relocate between 1.5 and 4.8 Mb of the 2nd chromosome. All translocations were easily balanced over a male-specific lethal 1 (msl-1) mutant chromosome. Both halves of the translocation carry visible markers, as well as P-element ends that enable molecular confirmation. Halves of these translocations can be separated to produce offspring with duplications and with lethal second chromosome deficiencies. Such large deficiencies are otherwise tedious to generate and maintain.

Introduction

Translocations are traditionally generated by randomly induced chromosome breakage. Use of the site-specific FLIP recombinase (FLP), which recombines FLP recombination target (FRT) sites, was pioneered by Golic and Lindquist, and has been widely used for generation of deficiencies with defined end points. This method also enables recombination between non-homologous chromosomes, but at a lower efficiency. Translocations that move autosomal material onto the sex chromosomes allow predictable transmission of material now linked to the X or Y chromosome, making these particularly useful. We obtained insertions of FRT in G9a, situated at 1A1 on the tip of the X chromosome, for the purpose of generating X:A translocations. However, one of these insertions segregates with the Y chromosome, and in fact is inserted in a duplication of the X on the Y chromosome (Dp(1;Y)y+). The duplicated region comprises 1A1–1B, and includes y`. Using this Y-linked FRT site as one endpoint, we generated large translocations between the Y and 2nd chromosomes with an efficiency similar to that for production of autosomal to X translocations. Both halves of these translocations carry visible markers, enabling maintenance by selection. Full translocations were easily balanced over the male-specific lethal 1 (msl-1) mutation for maintenance without selection.

Results and Discussion

We obtained insertions of specialized P-elements, P(RS3) and P(RS5), for the purpose of generating translocations as described in Golic and Golic (see Table 1 for stock descriptions). These elements have FRT sites flanking the 5’ exon or 3’ exon, respectively, of the mini-white gene. Flies carrying intact elements have dark red eyes. FLP-mediated recombination between the flanking FRT sites deletes one exon to generate reduced P(RS3r) and P(RS5r) elements, which retain a single FRT site and the 3’ (RS3r) or the 5’ (RS5r) exons of the mini-white gene. Flies with reduced elements have white eyes.

We used P(RS3) elements with insertion sites at cytological positions 22A2 and 25B1 on 2L, and 56C6 and 57B1 on 2R from the Drosophila Genetic Resource Center (DGRC, Kyoto, Japan). Two P(RS5) elements inserted in G9a at cytological position 1A1 on the tip of the X chromosome were also obtained. Although these elements were believed to be close to one another, the mini-white marker of one segregated with the Y chromosome (DGRC Stock # 125639). PCR confirmed that this stock had a P- element in G9a, as well as a copy of G9a lacking the insertion. This is consistent with insertion on Dp(1;Y)y`, the Y chromosome present in the strain used to generate the original insertions, which carries 1A1–1B on the Y chromosome (personal communication, Cook K). This insertion has been renamed Dp(1;Y)y`, P[w[+mW.Scer\ FRT.hj] = RS5/G9a[5-HA-2731] y` w[+118] and is now available from the Bloomington Drosophila Stock Center (BDSC # 3649).

We performed matings to determine whether the Y-linked FRT site could recombine with autosomal sites. The Golic laboratory has demonstrated that the recovery of recombinants between non-homologous chromosomes is dramatically enhanced by a third FRT site. In accord with these findings, we were unable to obtain 2;Y translocations with 2 FRT sites (approximately 348 vials screened). We then generated flies carrying the Y-linked P(RS5r) element and 2 second chromosome P(RS3r) elements,
one on 2L and one on 2R. Both P{RS3r} elements were oriented to enable production of 2;Y translocations (Fig. 1). Cytological and molecular analysis was then used to identify the 2nd chromosome break point. The number of vials scored for each set of endpoints and the translocations recovered are presented in Table 2.

Table 2. Recovery of translocations in this study

| 2nd chromosome FRT sites | # Vials scored | Translocations recovered (#) | Rate of recovery of translocations |
|-------------------------|--------------|------------------------------|-----------------------------------|
| 22A2 and 56C6           | 140          | 22A2 (3)                     | 2.14%                             |
| 22A2 and 57B1           | 107          | 0                            | -                                 |
| 25B1 and 56B6           | 105          | 0                            | -                                 |
| 25B1 and 57B1           | 238          | 57B1 (1), 25B1 (1)           | 0.84%                             |
| All                     | 590          | 5                            | 0.85%                             |

All dysgenic males carried a Y-linked FRT site and the indicated FRT sites on 2L and 2R. The rate of recovery of translocations was (# independently recovered translocations/ # vials scored) × 100.

Figure 1. Mating scheme used to generate T(2;Y) translocations. The 3rd chromosome FLP source, [w’ ovo-FLP], was balanced over TM3-Ser1 to enable translocations to be positively identified. In practice, the eye color of translocations is sufficiently dark as to be readily distinguished in the [w’ ovo-FLP] background.

Table 1. Full genotype and stock numbers of Drosophila strains used in this study

| Description | Stock #, supplier | Genotype | Flybase Id # |
|-------------|-------------------|-----------|--------------|
| P{RS5} on Y | 43649 (BDSC)      | Dp(1;Y)y[+], P[w[+mW.Scer/FRT.hs] = RS5 G9a[5-HA-2731] y[+]/w[1118] | FBab0010476; FBti0059095 |
| P{RS3} at 22A2 | 124255 (DGRC) | w[1118]; P{RS3}CG18131[CB-6892–3] | FBti0059228 |
| P{RS3} at 25B1 | 124291 (DGRC) | w[1118]; P{RS3}CG3036[UM-8026–3] | FBti0029115 |
| P{RS3} at 56C6 | 124216 (DGRC) | w[1118]; P{RS3}CG1837–3 | FBti0058933 |
| P{RS3} at 57B1 | 123609 (DGRC) | w[1118]; P{RS3}CG-5407–3 | FBti0028557 |
| 3rd chromosome FLP | 8705 (BDSC) | w[*]; wg(Sp-1)/CyO; P[w[+mC] = ovo-FLP.R1F1A | FBti0072900 |
| X-linked FLP | 8727 (BDSC) | P[w[+mC] = ovo-FLP.R1M1A, w[*] | FBti0072898 |
| msl-1       |                  | y w; msl1l/CyO       |             |
even in flies retaining the $FLP$ chromosome. Use of an $X$-linked $FLP$ source (BDSC # 8727) would obviate the need to distinguish male offspring with light and dark eyes. The $Y$-linked half of the translocation can be followed by the $y^+$ marker, which continues to segregate with the $Y$ chromosome. Both halves of the translocation are thus marked, making it possible to follow them independently in a $yw$ background. Furthermore, each half of the translocation can be molecularly confirmed by amplification with genomic primers flanking the insertion sites and outward facing primers in a $yw$ background. Furthermore, each half of the translocation are thus marked, making it possible to follow them independently $Y$ segregate with the $Y$ chromosome insertion on $Y$ of translocation between the used to confirm the second chromosome break point. A schematic of translocation between the $Y$-linked 1A1 insertion and the 22A2 insertion on 2L is depicted in Figure 2. Flies with both ends of the translocation were $w^+$, and, when used as template for PCR amplification, produced amplicons from proximal and distal $P$-element ends for the 1A1 and 22A2 insertions (Fig. 2B). Males with 2 wild type second chromosomes, and thus carrying half of the translocation as a duplication on the $Y$ chromosome, were $w^+$.

As expected, these males lacked PCR products from the distal end of the insertion in $G9a$ and the proximal end of the second chromosome insertion (Fig. 2B). No adult $w^+$ females were recovered from these stocks, indicating that these deficiencies are completely lethal.

We recovered 5 independent (2; $Y$) translocations with endpoints at 22A2 (3), 25B1 (1), and 57B1 (1). Translocations were named by the 2nd chromosome break point, for example, $T(2;Y)25B1$. The rate of recovery of translocations was 0.85% (# independently recovered translocations/ # vials scored) × 100. This is comparable to translocation rates previously reported (0.33 to 0.97%).$^4$

Both halves of translocations are clearly visible on polytene squashes (Fig. 3A and B). All 3 translocations can be separated to create viable ($Dp(2;Y)22A2$) or subviable ($Dp(2;Y)25B1$ and $Dp(2;Y)57B1$) duplications. Duplications can sometimes be observed pairing with the wild type 2nd chromosomes (Fig. 3C). Translocation moves an $X$ telomere from the $Y$ chromosome to the tip of the truncated 2nd chromosome. This can be seen in a polytene preparation from the $T(2;Y)57B1$ translocation (Fig. 3D). Importantly, both translocation and duplication males retain all fertility factors on the $Y$ chromosome, and are thus fertile.

The $w^+$ and $y^+$ markers associated with each half of these translocations make it possible to maintain them by selection in a $yw$ background. However, $Dp(2;Y)22A2$ is viable, indicating that smaller translocations will be at a risk for loss without selection. Translocations were initially balanced by mating exceptional $XXY$ females to a $CyO$ ($In(2LR)O$) second chromosome. While possible to create stocks with both sexes carrying balanced translocations, these are plagued by low fertility. To enable easier maintenance, translocations were balanced over an $msl-1$ mutation (Fig. 4). Females homozygous for $msl-1$ are fully viable and fertile, however $msl-1$ is male lethal.$^6$ The $msl-1$ gene, situated at 36F on 2L, is proximal to or on the opposite arm from translocation break points, and thus is present on the translocated second chromosome but absent from separated duplications. Stocks balanced by this method are vigorous and easily maintained. To control the risk of recombination posed by rare $XXY$ females, duplicate stocks are maintained and periodically inspected. The location of other male-specific lethal genes, such as $mle$ (42A6) and $msl-3$ (65E4), suggests that all (2; $Y$) translocations and (3; $Y$) translocations on 3R, or 3L distal to 65E4, could be balanced by a similar strategy.

**Materials and Methods**

Flies were maintained at 25 °C on standard cornmeal agar food. All fly strains used in this study are presented in Table 1. Matings to generate translocations were set with 5 males and approximately 20 $yw$ females per vial. A generalized overview
The primers used for the molecular characterization of translocations are:

- **Proximal 1A1:** 1A1(+) – CCAAGGCTGC ACCCAAGGCT CTGCTCCCAC and 1A1_R1 - CGTTCTGCCGGCA GCGAAGTCGA CTG;
- **Distal 1A1:** Pry4(+) – TAATCAACAA TCATATCGCT GTCTCAC TCAG and 1A1_F1 - GAAGTGCGTG CGTGTGCGTG CGTC;
- **Proximal 2L at 22A2:** Pry4(+) – TAATCAACAA TCATATCGCT GTCTCAC TCAG and 22A2_R1 – GTGCTGCCCA TCATCCTTAT GCC and Distal 2L at 22A2: Plac1(+) – CCAAGGCTGC ACCCAAGGCT CTGCTCCCAC and 22A2_F1 – CCGTCGCCTG CTGTGTTACGC.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
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