The Generation of Cloned *Drosophila melanogaster*

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Running title: The generation of Drosophila clones

Keywords: Cloning, Drosophila melanogaster, embryonic nuclear transfer

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ABSTRACT:

We report here the first successful use of embryonic nuclear transfer to create viable adult *Drosophila melanogaster* clones. Given the generation time, cost-effectiveness, and relative ease of embryonic nuclear transplant in *Drosophila*, this method can provide an opportunity to further study the constraints on development imposed by transplanting determined or differentiated nuclei.

NOTE:

Cloning at the organismal level refers to the creation of a genetically identical individual from an existing individual, generally through nuclear transfer. Embryonic and somatic nuclear transplantation has been successful to varying degrees in amphibians (GURDON and UEHLINGER 1966), arthropods (ILLMENSEE 1968), fish (LEE *et al.* 2002), and mammals (WILMUT *et al.* 2002). This technology can be exploited to create stem cells for use in therapeutic cloning and is being used to increase the production of transgenic mammals producing pharmacologically important compounds. In many cases, the technology is constrained by a lack of fundamental understanding of the nuclear reprogramming events that occur following transplantation, resulting in a high frequency of developmental defects in the cloned offspring (SHI *et al.* 2003). We have successfully used embryonic nuclear transfer to create viable adult
Drosophila clones. Embryos that hatch but fail to develop to adulthood exhibit characteristic developmental defects; hence we can potentially use this system to identify gene mutations or conditions which encourage complete nuclear reprogramming. The developmental programming of nuclei is a fundamental epigenetic process based, in part, on histone modification and packaging so the events involved in nuclear reprogramming in Drosophila are likely conserved across taxa. The method outlined herein provides a straightforward, cost-effective means of studying the effects of epigenetic interactions on nuclear transplants.

Host embryos laid by white-eyed w¹¹¹⁸ females were fertilized by homozygous ms(3)K81 males. These males generate sperm incapable of participating in pronuclear fusion and thus the resulting embryos are unable to complete embryogenesis under control of their own DNA (YASUDA et al. 1995). Embryos donating nuclei possessed green florescent protein-labeled histone 2AvD (H2AvDGFP) (CLARKSON and SAINT 1999) thus donor nuclei were easily distinguishable from those of the host. Less than 2 µL of cytoplasm was aspirated from preblastoderm stage embryos 70-100 minutes after egg laying. Nuclei were drawn laterally from the ventral face of the embryo and between five to fifteen nuclei were transplanted to the ventral area of a 10-30 minute old recipient embryo. Nuclei drawn from a single donor embryo were injected into between 1 and 6 recipients, potentially allowing for the generation of more than one clone from a single donor embryo. Recipient embryos were incubated at
18°C until the completion of embryogenesis at which point larvae were raised on standard *Drosophila* culture medium.

Of the 820 *w*1118 host embryos into which *H2AvDGFP* nuclei were injected, 61 (7.4%) expressed *H2AvDGFP* from the donor nuclei, 14 (1.7%) of those hatched as larvae, and 5 (0.6%) eclosed as fertile adults expressing fluorescence from the *H2AvDGFP* marker transgene (Figure 1). These individuals represent the first cloned adult *Drosophila*.

Evidence that these individuals represent animals derived from the injected embryonic nuclei stems from analysis of the mitochondrial and nuclear DNA of the clones. The *Drosophila* mitochondrial genome is highly variable in size (LEWIS *et al.* 1994), and the length of the A+T rich region differs between the *w*1118 and *H2AvDGFP* strains. This difference was detectable using PCR. Cloned animals possess nuclear DNA derived from the donor embryo but mitochondrial DNA from the host egg. Reciprocal transplantations (*w*1118 nuclei injected into *H2AvDGFP* host embryos) exhibited a reciprocal pattern of nuclear and mitochondrial DNA (Figure 2). DNA analysis on adult clones derived from *H2AvDGFP* donor nuclei yielded identical results.

The failure of the majority (98.3%) of the cloned embryos to develop normally is likely a consequence of multiple factors. In order to determine the percentage of embryos rendered inviable from mechanical damage intrinsic to the nuclear
transplant procedure, viable w^{1118} nuclei were injected into diploid w^{1118} host embryos. Of 202 embryos injected 21.8% (44) hatched, compared with 1.7% of cloned embryos, suggesting that approximately 80% of transplant recipient embryos die from mechanical damage. Of the remaining 20%, in some cases non-uniform concentrations of EGFP expression suggest the failure of donor nuclei to replicate and/or distribute themselves throughout the embryo. Characteristic defects in those expressing EGFP which die shortly after hatching, such as the absence of mouth hooks, defects in the tracheal system, and disorganized/absent spiracles, could potentially be due to epigenetic constraints on reprogramming of donor nuclei.

The first attempts to clone Drosophila by embryonic nuclear transplantation produced approximately 1% of embryos able to complete embryogenesis and only one developed as far as the third instar larval stage (ILLMENSEE 1968, ILLMENSEE 1972). The failure of these cloned Drosophila to survive to adulthood likely resulted from the failure to activate the unfertilized egg. The technique reported here allows for the generation of cloned adult Drosophila.

The rate at which developmental defects arise and the rate at which viable adult Drosophila clones are generated are comparable to that observed in mammals (WILMUT et al. 2002); approximately 10% of clones survive through embryogenesis, and approximately 1% develop into viable adults. Failure to properly reprogram mammalian embryonic and somatic nuclei in cloning
frequently manifests itself as placental abnormalities, fetal overgrowth, and premature death (SHI et al. 2003). Likewise, the abnormalities seen in inviable Drosophila clones could be due to incomplete reprogramming of donor nuclei. As the genetic regulation of early development is well characterized in Drosophila and there is a wealth of mutations affecting early development and maintenance of differentiated cell states, this method can be used to quickly and easily asses constraints on reprogramming of nuclei when cloning.

ACKNOWLEDGMENTS

We thank L. A. McEachern, N. Gorguy, M. Hart and V. Walker for discussion and comments on the manuscript. The H2AvDGFP stock was generously provided by S. Campbell, we thank the Bloomington Drosophila stock center for all other stocks. The work was supported by a Natural Sciences and Engineering Research Council (NSERC) discovery grant to V. K. L. and W. A. M. was supported by a fellowship from the Nova Scotia Health Research Foundation.

LITERATURE CITED:


**FIGURE LEGENDS**

Figure 1: Drosophila adult derived from embryonic nuclear transplant expressing H2AvDGFP.

Figure 2: PCR analysis of nuclear and mitochondrial DNA from cloned Drosophila.

(a) Analysis of nuclear DNA: Primers 5’ACTGTTTATTGCCCCCTC 3’ and 5’GTCGTCGAACAAAAGGTG 3’ amplified a 330bp fragment of white exon 6 present in both w1118 and the CaSpeR-4 P-element transformation vector used to create the H2AvDGFP strain3. Primers 5’ ACATCAAATTGTCTGCGG 3’ and 5’ CGCTCGTTGCAGAATAGT 3’ amplified a 569bp fragment of the CaSpeR-4 P-element transformation vector present in H2AvDGFP but absent in w1118 due to a deletion from approximately +2100 to +11000 relative to the w start codon. PCR allowed for the molecular detection of the CaSpeR-4 based transgene in nuclear transplant recipients.
Genomic multiplex PCR at the *white* locus (1.5% agarose gel). lane 1: *H2AvDGFP* genomic control DNA exhibits a 569bp band from the CaSper-4 derived *H2AvDGFP* P-element transformation vector and a 330bp band from *white* exon 6, lane 2: *w^1118* genomic control DNA exhibits the 330bp band but not the 569bp Casper-4 band, lane 3: *w^1118* nuclei injected in to *H2AvDGFP* host embryo. The 569bp CaSper-4 derived fragment is absent while the 330bp *w^1118* fragment is present indicating only *w^1118* nuclei in the cloned embryo, lane 4: *H2AvDGFP* nuclei injected in to *w^1118* host embryo. The 569bp CaSpeR-4 derived fragment and the 330bp *w^1118* fragment are present indicating *H2AvDGFP* nuclei in the cloned embryo, lane 5: DNA negative control, lane 6: 100bp ladder (MBI Fermentas). DNA was extracted from cloned late-stage embryos and adults using a technique modified from Hatton and O’Hare (http://www.bio.ic.ac.uk/research/ohare/t01816.htm).

(b) Analysis of mitochondrial DNA: Primers 5’AATAACAAATTTTTAAGCC 3’ and 5’ GAATAGGGGGAATAAATT 3’ amplified a variable region of the mitochondrial genome approximately 759bp in *w^1118* and 773bp in *H2AvDGFP*, distinguishing host from donor mitochondria.

PCR across variable region of mitochondrial genome (4% acrylamide gel). lane 1: *H2AvDGFP* control amplifies a 773bp fragment, lane 2: *w^1118* control amplifies a 759bp fragment, lane 3: *w^1118* nuclei injected in to *H2AvDGFP* host exhibits the
773bp fragment from the $H2AvDGFP$ host embryo mitochondria, lane 4: $H2AvDGFP$ nuclei injected in to $w^{1118}$ host exhibits the 759bp fragment from the $w^{1118}$ host embryo mitochondria, lane 5: DNA negative control, lane 6: 100bp ladder (MBI Fermentas).