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Note

The Generation of Cloned *Drosophila melanogaster*

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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.

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 that 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 w^{1118} 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). Embyros donating nuclei possessed green-fluorescent-protein-labeled histone 2AvD (H2AvDGFP; Clarkson and Saint 1999) so donor nuclei were easily distinguishable from those of the host. Less than 2 μl of cytoplasm was aspirated from preblastoderm stage embryos 70–100 min after egg laying. Nuclei were drawn laterally from the ventral face of the embryo and 5–15 nuclei were transplanted to the ventral area of a 10- to 30-min-old recipient embryo. Nuclei drawn from a single donor embryo were injected into one to six recipients, potentially allowing for the generation of more than one clone from a single donor embryo. In the trial reported here, two of the five adult clones, both females, originated from adjacent embryos, suggesting that they may have been derived from one donor. Recipient embryos were incubated at 18° 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

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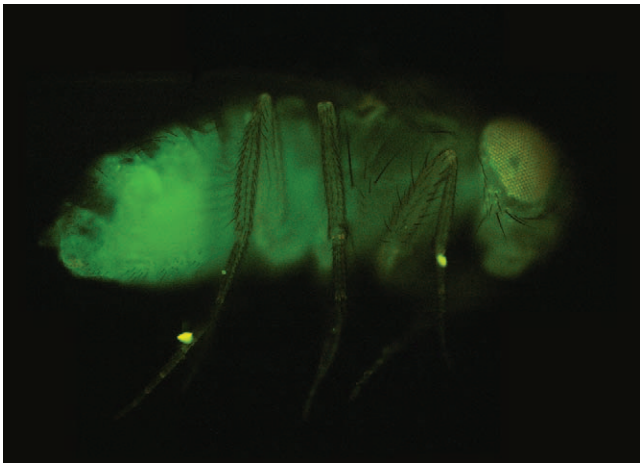

Figure 1.—Drosophila adult derived from embryonic nuclear transplant expressing *H2AvDGFP*.

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 of 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. 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 ~80% of transplant-recipient embryos die from mechanical damage. Of the remaining 20%, in some cases nonuniform concentrations of GFP expression suggest the failure of donor nuclei to replicate and/or distribute themselves throughout the embryo. Characteristic defects in those expressing GFP, which die shortly after hatching, such as the absence of mouth hooks, defects in the tracheal system, and disorganized or 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 ~1% of embryos able to complete embryogenesis and only one developed as far as the third instar larval stage (Illmensee 1968, 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

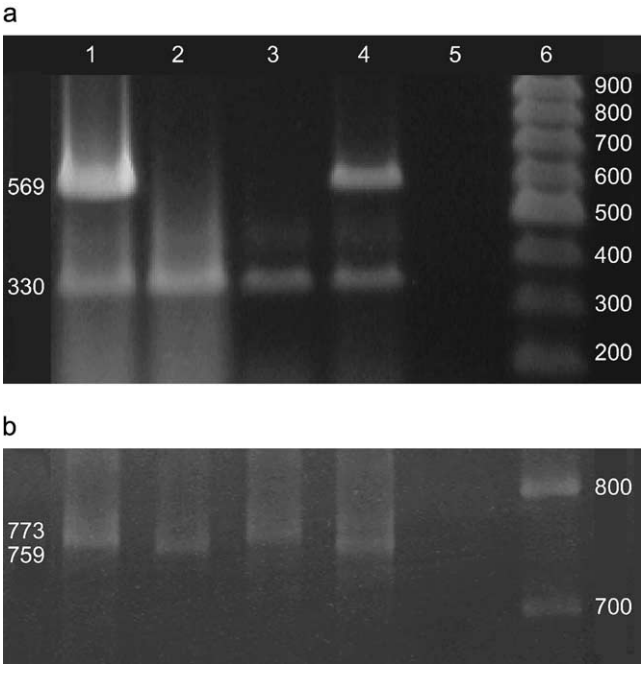

Figure 2.—PCR analysis of nuclear and mitochondrial DNA from cloned Drosophila. (a) Analysis of nuclear DNA: primers 5′-ACTGTTTATTGCCCCCTC-3′ and 5′-GTCGTCGAACAAA AGGTG-3′ amplified a 330-bp fragment of *white* exon 6 present in both w^{1118} and the CaSpeR-4 *P*-element transformation vector used to create the *H2AvDGFP* strain. Primers 5′-ACAT CAAATTGTCTGCGG-3′ and 5′-CGCTCGTTGCAGAATAGT-3′ amplified a 569-bp fragment of the CaSpeR-4 *P*-element transformation vector present in *H2AvDGFP* but absent in w^{1118} due to a deletion from ~+2100 to +11,000 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) is shown: lane 1, *H2AvDGFP* genomic control DNA exhibits a 569-bp band from the CaSper-4-derived *H2AvDGFP P*-element transformation vector and a 330-bp band from *white* exon 6; lane 2, w^{1118} genomic control DNA exhibits the 330-bp band but not the 569-bp Casper-4 band; lane 3, w^{1118} nuclei injected into the *H2AvDGFP* host embryo. The 569-bp CaSper-4-derived fragment is absent while the 330-bp w^{1118} fragment is present, indicating only w^{1118} nuclei in the cloned embryo; lane 4, *H2AvDGFP* nuclei injected into the w^{1118} host embryo. The 569-bp CaSpeR-4-derived fragment and the 330-bp w^{1118} fragment are present, indicating *H2AvDGFP* nuclei in the cloned embryo; lane 5, DNA negative control; lane 6, 100-bp 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′-GAATAGGGGGAA-TAAATT-3′ amplified a variable region of the mitochondrial genome ~759 bp in w^{1118} and 773 bp in *H2AvDGFP*, distinguishing host from donor mitochondria. PCR was performed across a variable region of the mitochondrial genome (4% acrylamide gel): lane 1, the *H2AvDGFP* control amplifies a 773-bp fragment; lane 2, the w^{1118} control amplifies a 759-bp fragment; lane 3, w^{1118} nuclei injected into the *H2AvDGFP* host exhibits the 773-bp fragment from the *H2AvDGFP* host embryo mitochondria; lane 4, *H2AvDGFP* nuclei injected into the w^{1118} host exhibits the 759-bp fragment from the w^{1118} host embryo mitochondria; lane 5, DNA negative control; lane 6, 100-bp ladder (MBI Fermentas).

the rate at which viable adult Drosophila clones are generated are comparable to that observed in mammals (Wilmut *et al.* 2002); ~10% of clones survive through embryogenesis, and ~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 assess constraints on reprogramming of nuclei when cloning.

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