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Cell lines ☆

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ABSTRACT

We review the properties and uses of cell lines in *Drosophila* research, emphasizing the variety of lines, the large body of genomic and transcriptional data available for many of the lines, and the variety of ways the lines have been used to provide tools for and insights into the developmental, molecular, and cell biology of *Drosophila* and mammals.

1. Introduction

In this paper we describe the range of *Drosophila* cell lines that are currently available, and the ways in which they are useful for research. Most lines are distributed by the *Drosophila* Genomics Resource Center (DGRC), whose staff includes the authors of this paper. The DGRC website (https://dgrc.cgb.indiana.edu) publishes regularly updated descriptions of each of the lines, along with specific, individualized culture requirements and we will not reproduce that material here. Instead, we offer general guidelines and suggestions for the use of the lines. For a different, though dated, perspective, we recommend Echalier's monograph on *Drosophila* cell lines [1].

2. The uses of cell lines in *Drosophila* developmental biology

Drosophila cell lines have been available for almost 50 years, and have progressively become more integral to the toolkit for *Drosophila* research. Permanent cell lines are, without question, not normal cells; one must use common sense in choosing the experiments for which they are suitable and validate the results *in vivo*. Substantial genomic rearrangements have occurred in all cell lines [2,3], and they do not behave identically to the cells from which they are derived. But thoughtful use of cell lines rarely gives misleading results, and often provides invaluable insights. Table 1 lists a series of examples of the successful use of *Drosophila* cell lines to support this claim. Several points are worth emphasizing here:

(1) Cell line work complements genetic and developmental experiments in flies. The following are just three of many possible examples: Dominant-negative versions of the ecdysone receptor subunit EcR that were identified by transient expression experiments in Kc cells [4] have become standard tools for genetic studies of the ecdysone pathways in flies [5–10]. Interactions of S2 cells expressing Notch and its ligands [11] led to an extended study of the Notch pathway with complementary experiments in S2 cells and flies [12–15]. The recent identification in S2 cells of a receptor for the secreted morphogen Fog led to the verification of that receptor as a critical component of Fog function in gastrulation [16].
(2) Because cell lines provide large amounts of homogeneous tissue which can easily be manipulated, they make possible molecular and biochemical work that is otherwise extremely difficult in an organism as small and complex as *Drosophila*. A recent modENCODE meta-analysis [17] illustrates this point forcefully. Predictive models could be built only for cell lines because it was only in the cell lines that histone marks, polymerase localization, origins of replication, and transcription could be aligned in a single cell type. Until we can perform single-cell analyses of transcription and chromatin immunoprecipitation, we must rely on cell lines as an alternative. Table 1 cites several additional studies making use of the same approach.

Abbreviations: DGRC, *Drosophila* Genomics Resource Center; modENCODE, Model Organism Encyclopedia of DNA Elements.

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Table 1
Examples of uses of cell lines.

Type of application	Examples
Source of large quantity of homogeneous material for preparation of extract or protein purification	Isolation of non-muscle myosin from S2, S3, and Kc [89] Isolation and characterization of basement membrane components from S2, Kc, and DM [35,90,91] Preparation of cell-free transcription system from Kc [92,93]
Studies of basic cellular mechanisms	Processing and function of siRNAs , miRNAs, piRNAs [94,95,44] Alignment of chromosomes at the metaphase plate [96] Somatic cell chromosome pairing [97] Validation of computationally predicted splice enhancers [98] Studies of cytoskeletal mechanics using fluorescent- or epitope-tagged markers [99–107,34,108]
Isolation and characterization of purified transgene products following transformation	Atlantic salmon serum C-type lectin [109] Human clotting factor IX [110]
In vivo test tubes for detecting interaction and properties of transgenes and their products	"promoter-bashing" studies of (1) miRNA genes [111], *nkd* [112], chicken GAS41 [113], mammalian erythroid promoters [114] (all in S2) Examination of surveillance systems for splicing errors using the human β-globin gene [115] Characterization of a *Drosophila* muscarinic acetylcholine receptor [116] Description of an inhibitory factor for transcription of mammalian NGFI-A [117] Examination of interactions between Notch and its ligands Delta and Serrate [14,12] Identification of ecdysone response elements in *hsp27* [118,119] and *Eip71CD* [120] and functional elements in the ecdysone receptor [4]
Dissection of pathways by RNAi screening in a suitable cell line	(see Mohr [82] for details)
Study of pathogens, using *Drosophila* cells as a heterologous host	*Yersinia enterocolitica* [121] *Plasmodium* [122] Rabies virus [123]
Studies of distinct properties of individual lines	*mbn*2 and S2 as models for studies of the fly immune response [124–127] The long axon-like processes of the CNS line BG2-c2 for studies of mitochondrial transport [128] ML-DmD8 as a model for myogenesis [129] ML-DmD17-c3 as a model for cell migration [130]
Genomic analysis, using modENCODE data as baseline	Demonstration that band, interband, and band/interband boundary properties are conserved between polytene and diploid cells [131–133] Demonstration that HSF binds preferentially in regions of open chromatin structure [134] Demonstration that X-chromosome binding of the dosage compensation complex MSL is targeted to open chromatin structures, using the male lines S2-DRSC and ML-DmBG3-c2 and the female line Kc167 [135]
Secretion of labile signaling proteins for use *in vitro*	Wg [136] Fog [16]

(3) Cell lines are frequently used in studies of human genes or pathogens, offering an alternate environment which complements observations in the normal host. Several examples of such studies are listed in the Table 1.

The utility of cell lines has increased enormously in the past decade for two reasons: The *Drosophila* Genomics Resource Center (DGRC), established in 2003, has made over 150 diverse cell lines available to the community; and the NIH-sponsored project Model Organism Encyclopedia of DNA Elements (modENCODE, 2007–2012) produced an enormous amount of high quality, publicly available genomic data on *Drosophila* cell lines, including chromatin marks [18–20], insulators [21], replication origins and timing [22,23], and histone variants [24] for 4 "core" cell lines (the embryonic lines Kc167 and S2-DRSC, the wing disc line CME Cl.8+, and the CNS line ML-DmBG3-c2), plus transcription data for these 4 and 23 additional cell lines [25–28].

3. Available cell lines

Drosophila cell lines were originally made from embryos at various stages of development [29–32]; this continues to be the most common starting material for generating new lines [33,34]. Several of these lines were observed to have properties characteristic of hemocytes [35,36], but global transcriptome analysis shows a remarkable diversity among the embryonic lines (see below). Cell lines have also been made from embryos of about a dozen non-*melanogaster* species of *Drosophila* [37,38]. During the 1980s, cell lines were established from two tissues of late third-instar larvae: imaginal discs [39,40] and the central nervous system [41]. A cell line was also established from tumorous blood cells of larvae mutant for *l*(2)*mbn* [42]. The cell line fGS/OSS was made from a mixture of germ cells and somatic sheath cells of adult ovaries mutant for *bam* [43]; two lines containing only the sheath cells were then derived from fGS/OSS [43,44]. While *Drosophila* cell lines have traditionally been made without benefit of the malignant transformation that underlies the establishment of most mammalian cell lines, the Simcox lab has recently shown that the over-expression of specific oncogenes or null mutations in specific tumor-suppressor genes in fly embryos greatly enhances the efficiency of the establishment of new cell lines [33,45].

We will not attempt here to describe techniques for the production of new cell lines from flies. For help on this subject, we refer the reader to a Miyake lab protocol available on the DGRC website [46], and to the laboratories of Amanda Simcox and Alain Debec, where new cell lines continue to be established.

Some lines, particularly the embryonic line S2, have been stably transformed to produce large numbers of variants suitable for a variety of specialized purposes. Some of these transformants are listed in Table 1, and a few examples of transformed lines expressing fluorescent markers are illustrated in Fig. 1. In addition, embryonic lines have been made from mutant flies to serve a variety of purposes; a recent example is a series of lines made from embryos homozygous for both a gene-trap transposon that tags the tubulin-binding protein Jupiter with GFP and a null mutation in the