Ui-Tei, Kumiko, and Yuhei Miyata. 2000. Cell lines derived from *Drosophila* larval central nervous system and their practical applications. *Dros. Inf. Serv.* 83: 188-189.

Cell lines derived from *Drosophila* larval central nervous system and their practical applications.

Ui-Tei, Kumiko, and Yuhei Miyata. Department of Pharmacology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan.

The central nervous system (CNS) is composed of a large heterogeneous population of cells. This complexity makes it difficult to dissect neuronal functions at a cellular level. One way to resolve this problem is to use cell lines consisting of homogenous cells originated from the nervous system. To this end, we have succeeded in establishing cell lines from the CNS of *Drosophila* 3rd instar larva: 8 continuous cell lines, designated as ML-DmBG1~8, and clonal lines from 3 of them (Table 1; Ui *et al.*, 1994a). Here, we introduce these cell lines and some of the practical applications.

All parental cell lines (ML-DmBG1~8) and 10 colonial clones originating from ML-DmBG2 (ML-DmBG2-c1~10) reacted to the antibody to horseradish peroxidase (HRP) (Ui *et al.*, 1994a). HRP is a neuronal marker in insects. Thus, it is likely that these clonal cells are neuronal. Therefore, we analyzed neurotransmitters or their candidates in 10 clonal cell lines from ML-DmBG2. Acetylcholine (ACh), a major neurotransmitter in *Drosophila*, was found in 7 out of 10 clones, and substance P and proctolin were detected in 7 and 8 out of 10, respectively (Ui-Tei *et al.*, 1994b, 1995). Although catecholamine(CA)s, their metabolites, other amines (serotonin, octopamine), GABA and taurine were not detected in any clones, L-DOPA, a precursor of CAs, and somatostatin were expressed in all clones. Thus, these cell lines are also neuron-like with respect to chemical phenotypes. Thus, we assumed to be and used as neuronal model cells. We describe some biochemical studies using these cell lines below.

ML-DmBG2-c2 cells, which contains the highest amount of ACh among the 10 clonal lines, were used for investigating the mechanism of apoptosis (Ui-Tei, *et al.*, 1996; Nagano, *et al.*, 1998; Ui-Tei, *et al.*, 2000). Apoptosis is defined by its morphological feature and internucleosomal DNA fragmentation in vertebrates. However, a similar type of cell death was not reported biochemically in *Drosophila*. We showed for the first time that cell death characteristic for apoptosis also occurs in *Drosophila* cells (Ui-Tei, *et al.*, 1996). The cell death was induced in the clonal cells by the treatment with a calcium ionophore, and the characteristic morphological changes, such as nucleus condensation and membrane-bound apoptotic bodies, were observed. In this cell death, DNA fragmentation of nucleosomal size was clearly detected. Therefore, a typical apoptosis is shown to occur also in *Drosophila* cells. Furthermore, apoptosis could be induced by other chemicals in the cells. Based on these results, we concluded that the apoptotic pathway is not unique in the clonal cells (Ui-Tei *et al.*, 2000).

Although many of the established *Drosophila* cell lines lack adhesive properties, some of the CNS-derived cell lines such as ML-DmBG1 and 2 have cell-cell and cell-substratum adhesive properties. Using ML-DmBG1 cells, Hirano *et al.* (1991) revealed the first evidence that *Drosophila* integrins can function as receptors for cell-substratum adhesion recognizing vitronectin. ML-DmBG2-c6 among 20 lines screened showed the strongest adhesion activity when purified *Drosophila* laminin was used as substrate (Takagi *et al.*, 1998). Using this line, Takagi *et al.* showed that laminin-mediated cell spreading caused integrin clustering which colocalized with intracellular signaling molecules such

Table.1 List of cell lines derived from *Drosophila* larval CNS

Cell lines	Ploidy				Doubling time (h)	Reference
	n	2n	4n	other		
ML-DmBG1						Hirano et al., 1991
ML-DmBG1-c1	0	100	0	0	39	
ML-DmBG2	7	91	0	0		
ML-DmBG2-c1	0	83	13	4	53	
ML-DmBG2-c2	0	87	6	7	28	Ui-Tei et al., 1996 Santaren, 1996 Nagano et al., 1998 Ui-Tei et al., 2000
ML-DmBG2-c3	0	77	20	3	27	Santaren, 1996
ML-DmBG2-c4	0	9	88	3	31	Santaren, 1996
ML-DmBG2-c5	0	81	19	0	43	
ML-DmBG2-c6	0	89	11	0	42	Takagi et al., 1998 Takagi et al., 1999 Takagi et al., 2000
ML-DmBG2-c7		86			67	
ML-DmBG2-c8		90			54	
ML-DmBG2-c9		1	66		50	
ML-DmBG2-c10		97			34	
ML-DmBG3	0	90	10	0		
ML-DmBG3-c1	0	94	0	6	43	
ML-DmBG3-c2	0	95	0	5	37	
ML-DmBG3-c3						
ML-DmBG3-c4						
ML-DmBG3-c5						
ML-DmBG4	5	85	5	5		
ML-DmBG5						
ML-DmBG6						
ML-DmBG7						
ML-DmBG8						

as p21-activated protein kinase (PAK) and Enabled (Ena: a substrate for Abelson tyrosine kinase) (Takagi *et al.*, 1999). Furthermore, laminin is shown to trigger tyrosine - phosphoryla-tion of Ena and other unidentified proteins (Takagi *et al.*, 2000).

Protein synthe-sis patterns of three clones were analyzed using high resolution two-dimensional gel electrophoresis analysis by Santaren *et al.* (1996).

Drosophila is a valuable insect for studying biological mechanism because of the extensive knowledge of its genetics. However, its small body size makes biochemical and physiological approaches at the cellular level relatively difficult. Thus, the cell lines which offer large amounts for biochemical study are useful.

References: Hirano, S., K. Ui, T. Miyake, T. Uemura, and M. Takeichi 1991, Development 113:1007-1016; Nagano, M., H. Suzuki, K. Ui-Tei, S. Sato, T. Miyake, and Y. Miyata 1998, Neurosci. Res.31:113-121; Santaren, J. F., 1996, In Vitro Cell. Dev. Biol.Anim. 32: 434-440; Takagi, Y., K. Ui-Tei, T. Miyake, and S. Hirohashi 1998, Neurosci. Lett. 244: 149-152; Takagi, Y., K. Ui-Tei, and S. Hirohashi 1999, In Vitro Cell. Dev. Biol. Anim. 35: 549-552; Takagi, Y., K. Ui-Tei, and S. Hirohashi 2000, Biochem. Biophysic. Res. Commun. 270: 482-487; Ui, K., S. Nishihara, M. Sakuma, S. Togashi, R. Ueda, Y. Miyata, and T. Miyake 1994a, In Vitro Cell. Dev. Biol. 30A: 209-216; Ui-Tei, K., S. Nishihara, M. Sakuma, K. Matsuda, T. Miyake, and Y. Miyata 1994b, Neurosci. Lett. 174: 85-88; Ui-Tei, K., M. Sakuma, Y. Watanabe, T. Miyake, and Y. Miyata 1995, Neurosci. Lett. 195: 187-190; Ui-Tei, K., S. Sato, T. Miyake, and Y. Miyata 1996, Neurosci. Lett. 203: 191-194; Ui-Tei, K., M. Nagano, S. Sato, and Y. Miyata 2000, Apoptosis 5: 133-140.