Kaji, S. and Y. Ushioda. Konan University, Kobe, Japan. The duration of cell cycle in the development of the Bar eye disc cells in vitro.

The experiments were carried out to determine the cell growth cycle of Bar and Oregon-R eye disc cells in the different larval stages under in vitro culture.

Eye discs from 70, 85 and 95 hr larvae after hatching were dissected in the modified Schneider's culture medium. Then, they were placed in EDTA-trypsin-collagenase solution (80 mg NaCl, 3 mg KCl, 3 mg $NaHCO_3$, 1 mg NaH_2PO_4, 5 mg glucose, 2 mg EDTA, 5 mg trypsin, 25 mg collagenase per 10 ml distilled water) for 30 minutes at room temperature. Dissociated cells were rinsed in culture medium, and then treated with colcemid (0.06 μg/ml) for 90 minutes. Colcemid is used in mitotic inhibitors for synchronization of the cultivated cells. After this treatment, the cell suspensions were carefully rinsed with culture medium, and then exposed with ^{3}H-thymidine (sp. act. 5.0 Ci/mM, 10 μCi/ml) for 40 minutes. After incubation with the tracer the cells were rinsed and further incubated for 1, 2, 4, 8, 12, 24 and 48 hrs in culture medium. Thereafter, cells were examined by means of autoradiography as described previously (Kaji and Ushioda 1979).

Table 1. ^{3}H-thymidine incorporation into the 70 h Oregon-R and Bar eye disc cells after exposed to colcemid in vitro.

		Duration of culture (hr)							
		0	1	2	4	8	12	24	48
No.grains per cell	Oregon-R	6.02	7.21	7.35	7.66	4.02	2.30	1.16	0.33
	Bar	5.24	6.49	6.78	6.65	3.15	1.89	0.92	0.41

Table 2. ^{3}H-thymidine incorporation into the 85 hr Oregon-R and Bar eye disc cells after exposed to colcemid in vitro.

		Duration of culture (hr)							
		0	1	2	4	8	12	24	48
No.grains per cell	Oregon-R	5.48	6.10	7.18	6.52	3.76	2.50	1.28	0.68
	Bar	4.22	5.14	5.13	5.06	3.71	2.28	1.01	0.62

Table 3. ^{3}H-thymidine incorporation into the 95 hr Oregon-R and Bar eye disc cells after exposed to colcemid in vitro.

		Duration of culture (hr)							
		0	1	2	4	8	12	24	48
No.grains per cell	Oregon-R	2.90	3.78	4.22	3.17	2.90	2.79	1.38	1.16
	Bar	3.12	3.52	4.40	3.36	3.17	2.51	1.63	1.49

Table 1 shows the rate of grain number in the 70 hr Oregon-R and Bar eye disc cells after incorporation of ^{3}H-thymidine. The number of grains in the cells decreased to about 50% for 8 hr, 25% for 12 to 24 hr, 12.5% for 24 to 48 hr culture. These results suggest that the duration of cell cycle in the 70 hr Oregon-R and Bar cells has an interval of about 8 hrs under in vitro conditions.

In the 85 hr cells, the incorporation rate decreased to about 50% for 12 hrs after tracer exposure (Table 2). It can be assumed that the 85 hr cell growth cycle was a longer interval than that of the 70 hr cells.

In the 95 hr mature larval eye disc cells, incorporation was about half the amount of the 70 hr cells. The grain number decreased to 50% for 24 hr culture. Thereafter, no change was seen until the 48 hour post-labeling time (Table 3). These results suggest that eye disc cells have almost no division in the mature larval stage.

Results from these in vitro cell culture experiments indicate that in the Bar eye disc cells, dividing ability and duration of cell growth cycle of the photoreceptor cells is the same as that of the wild type.

References: Kaji, S. and Y. Ushioda 1979, Annot. Zool. Japon. 52:1.