# Colony formation of *Drosophila* cells in semisolid medium containing agarose

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*Summary.* Four *D. melanogaster* established cell lines were tested for their ability to form clones. A plating efficiency of about 0.5% was achieved with a medium containing agarose, reaching 1% with agarose supplemented with a small amount of conditioned medium. Each clone, examined cytologically, displayed a specific and stable karyotype.

Established *Drosophila* cell lines generally consist of highly heterogeneous populations of cell types and karyotypes[2-5]. This heterogeneity may be an important factor in the establishment and evolution of a cell line. However, homogeneous cell populations are indispensable for certain genetic or biochemical investigations. Cloning of *Drosophila* cells from established cell lines has been attempted by various methods and with different results[6-10] (for a review, see also Schneider and Blumenthal[11]). This report describes the cloning of 4 *Drosophila* cell lines in soft agarose medium, without feeder-layer. It includes a cytological characterization of some of the clones obtained.

*Materials and methods.* Cell cultures: Four *Drosophila melanogaster* established cell lines of embryonic origin were employed in the present study: 1XII, 3.38, 0.57, 1.56. (For the karyological characterization of these lines, see below under 'Karyotype analysis'). All these lines were obtained in our laboratory from the wild stock Varese of *D. melanogaster*[12] according to the method of growing cells in vitro described by Echalier and Ohanessian[13]. These lines were maintained in D225 medium[13]supplemented with 15% foetal calf serum. Tests for detection of mycoplasmas were made[14,15]. The results were negative for all lines.

Cell cloning: A method similar to that described by Macpherson[17] for mammalian cells was employed. A base layer of 2 ml of D225 medium containing 0.5% Bacto-agar (Difco) was set in Petri dishes (35 × 10 mm) and allowed to solidify. Then a 2nd layer with various amounts of cells ($10^3$–$5 \times 10^4$) in 0.6 ml of D225 medium containing 0.3% agar was added. After the solidification of the upper layer, plates were sealed with parafilm and incubated at 25 °C. 20 Petri dishes were set up for each cell concentration. The dishes were examined weekly under an inverted microscope. Colony counts were made on unstained cultures on the 20th day after plating. Only colonies consisting of more than 50 cells were considered. A 2nd series of experiments was carried out using agarose (BDH) instead of Bacto-agar. A 3rd set of experiments was made by using agarose and conditioned medium (4MC) instead of fresh medium. Conditioned medium was prepared following the procedure described by Nakajima and Miyake[8]. In short, the cells were grown in large quantities in Jena glass bottles; on the 4th day, the medium was replaced with fresh medium which was harvested 1 day later and centrifuged. In a 4th set of experiments, conditioned medium was used only for the cell suspension, fresh medium being used for the base layer.

Clone subculturing: Colonies were removed from the agar medium with a Pasteur pipette. The agar drops containing the colonies were placed in the wells of a microtiter tissue culture plate (Falcon Plastics) with fresh medium. One week later, the cells were detached from the well bottom by vigorous pipetting and seeded in 9 $cm^2$ culture flasks, then transferred to larger (30 $cm^2$) glass flasks.

Karyotype analysis: Karyological analysis of 8 clones (2 from each line) was performed. Air-dried preparations were stained with acetic orcein (Fluka) or with quinacrine dihydrochloride dye (K&K Laboratories). Slides were examined through a Zeiss photomicroscope with phase contrast or with UV excitation for fluorescence observation. The karyological analysis of line 1.56 and of line 3.38 was performed in 1979[4], while the analysis of line 1XII and line 0.57 was carried out just before cloning.

Line 1.56: this line was characterized by cells having an X and a fragment of the Y, the major autosomes were normal, chromosomes of the IV pair were not noticed (78.6%). Tetraploid cells were present (21.4%).

Line 3.38: cells showed the presence of 1 chromosome X and a fragment of chromosome Y, the II pair was normal, an interchange between the homologues of the III pair was noticed, no chromosomes of the IV pair were observed (92%). A low percentage of tetraploid cells was present (8%).

Line 1XII: this line showed a chromosome X and a fragment of chromosome Y, the major autosomes were normal, only 1 chromosome of the IV pair was observed (53.9%). The percentage of tetraploid cells was high (46.1%).

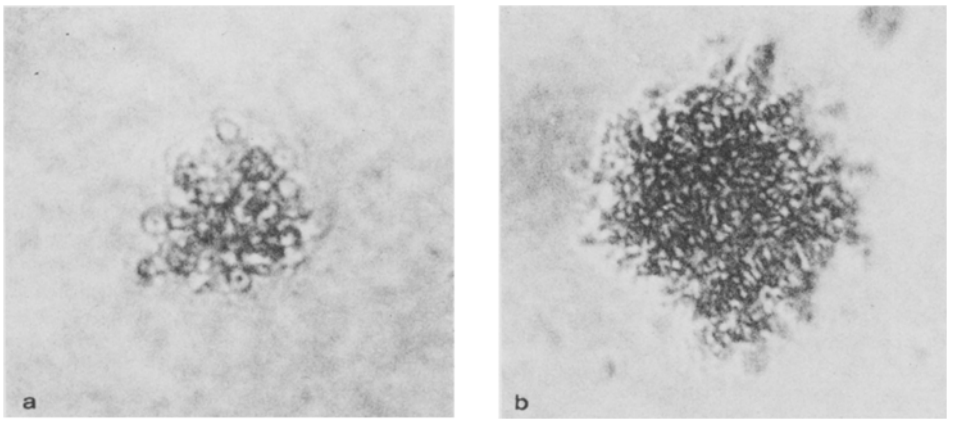
Line 0.57: cells were characterized by having an X and a fragment of the Y, only 1 chromosome of the IV pair was observed, in 11.3% of the metaphases an interchange between a chromosome of the II pair and a chromosome of the III pair was observed, while in 67.5% of the metaphases the major autosomes were normal. Tetraploid cells were present (21.2%).

*Results.* As can be seen in table 1, there was growth of colonies in D225 medium containing Bacto-agar, but the plating efficiency was very low (from 0.05% for line 1.56 to

Table 1. Colony formation in agar, agarose, agarose + 4MC medium

| Medium | No. of cells per 35 mm dish | Cell lines 1XII No. of colonies[a] ± SE | %PE[b] | 0.57 No. of colonies[a] ± SE | %PE[b] | 3.38 No. of colonies[a] ± SE | %PE[b] | 1.56 No. of colonies[a] ± SE | %PE[b] |
|---|---|---|---|---|---|---|---|---|---|
| D225 + agar | $10^3$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | $10^4$ | 7 ± 0.46 | 0.07 | 11 ± 0.43 | 0.11 | 15.9 ± 0.81 | 0.16 | 5.3 ± 0.45 | 0.05 |
| | $5 \times 10^4$ | 69.4 ± 2.17 | 0.14 | 81.8 ± 0.56 | 0.16 | 94.8 ± 0.67 | 0.19 | 55.7 ± 0.74 | 0.11 |
| D225 + agarose | $10^4$ | 50.8 ± 0.86 | 0.51 | 62.9 ± 0.81 | 0.63 | 73.7 ± 0.78 | 0.74 | 44.4 ± 0.77 | 0.44 |
| | $5 \times 10^4$ | 266 ± 0.91 | 0.53 | 281.9 ± 0.76 | 0.56 | 358.2 ± 1.19 | 0.71 | 239 ± 1.19 | 0.47 |
| D225 + agarose + 4MC medium | $10^3$ | 9 ± 0.36 | 0.99 | 10.1 ± 0.53 | 1 | 13.5 ± 0.60 | 1.35 | 6 ± 0.39 | 0.60 |
| | $5 \times 10^3$ | 52.2 ± 0.69 | 1.04 | 59.8 ± 0.63 | 1.20 | 64.9 ± 0.91 | 1.30 | 47.7 ± 0.79 | 0.95 |
| | $10^4$ | 101.3 ± 0.62 | 1.01 | 107.6 ± 0.68 | 1.07 | 114 ± 0.93 | 1.14 | 92.8 ± 0.83 | 0.93 |

[a]The number of colonies in 20 dishes was averaged in every experiment. [b]Plating efficiency.



*a* A colony of *D. melanogaster* line 3.38 in agarose plus 4MC medium at 7 days. *b* The same colony at 14 days. ×450.

0.19% for line 3.38) and clones took nearly 2 months to become visible to the naked eye. In the other series of experiments, in which agar was replaced by agarose, cells multiplied faster than in agarized medium and colonies were formed more effectively. The plating efficiency ranged from 0.44% (line 1.56) to 0.74% (line 3.38). In the 3rd set of experiments, on which fresh medium was completely replaced by conditioned medium, cells underwent 1-3 cell divisions, then stopped growing. In the 4th set of experiments, in which conditioned medium was used only for cell suspension, a very active cell multiplication occurred. Clones took only 1 month to become visible to the naked eye (fig. a and b). Plating efficiency reached a mean value of 1%, starting from a relatively small number of cells per surface unit (520 cells/cm$^2$ i.e. $5\times10^3$ cells/35 mm plate). The karyological analysis (table 2) revealed that each clone had a rather homogeneous karyotype while the parental line showed karyological heterogeneity. All the cells of clone 1 from line 1XII examined showed a karyotype with 1 X and a fragment of Y, while in clone 2 the cells were tetraploid. Clone 1 (tetraploid) and clone 2 (diploid) from line 3.38 had cells with an X plus a fragment of Y and an interchange between the homologues of the III pair. In clone 1 from line 0.57 an X and a fragment of Y were observed in all cells, while in clone 2 all the cells were characterized by a karyotype with the X plus a fragment of Y and an interchange between 1 chromosome of the II pair and 1 chromosome of the III pair. Cells of the 2 clones from line 1.56 revealed the same karyotype, with an X and a fragment of Y.

*Discussion.* Four cell lines were used in this experiment. They showed similar plating efficiencies (about 1%) in agarose medium supplemented with a small amount of conditioned medium after plating at low density (520 cells/cm$^2$). This cloning method may therefore be reliably applied to different cell lines.

The absence of mycoplasmas in these lines may explain the low plating efficiency. It is well known that mycoplasmas can mediate colony formation[17] and their presence may be responsible for high values of plating efficiency. In fact, Nakajima and Miyake[8] and Wyss[10] have reported high values of plating efficiency for lines $GM_1$ and $GM_2$ and for line KcHP respectively; however, their reports do not give the results of mycoplasma testing.

The method described is useful because it does not require a feeder-layer and the formation of clones from irradiated cells is excluded. In fact, our experience showed that in *Drosophila melanogaster* cell lines some cells started to multiply even 2 months after irradiation with 60.000 rad.

Table 2. Karyotypes of the 4 parental lines and of the respective clones

| Lines and clones | 2n X+fr.Y[a] | X+fr.Y[a] T (2;3) | X+fr.Y[a] T (3;3) | 4n | Total |
|---|---|---|---|---|---|
| 1XII | 196 53.9% | – | – | 168 46.1% | 364 |
| Clone 1 | 91 100% | – | – | – | 91 |
| Clone 2 | – | – | – | 101 100% | 101 |
| 3.38 | – | – | 209 92% | 18 8% | 227 |
| Clone 1 | – | – | – | 99 100% | 99 |
| Clone 2 | – | – | 108 100% | – | 108 |
| 0.57 | 137 67.5% | 23 11.3% | – | 43 21.2% | 203 |
| Clone 1 | 103 100% | – | – | – | 103 |
| Clone 2 | – | 154 100% | – | – | 154 |
| 1.56 | 275 78.6% | – | – | 75 21.4% | 350 |
| Clone 1 | 125 100% | – | – | – | 125 |
| Clone 2 | 104 100% | – | – | – | 104 |

[a]An X chromosome and a fragment of Y.

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