A continuous cell line derived from larval fat bodies of *Thysanoplusia intermixta* (Lepidoptera: Noctuidae)

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Abstract

A continuous cell line has been established from larval fat bodies of a lepidopteran insect, *Thysanoplusia intermixta*. These cells were cultured in MGM-450 medium. The cell line, designated as TI-1, was a suspension of cells with a doubling time of 36 h and consisted mostly of spindle-shaped cells, although other shapes, notably spherical cells, also existed. The chromosome number of the cell line varied considerably with the mode of 150–179. This cell line was found to be susceptible to *Autographa californica* NPV (AcNPV). Isozyme analysis showed that the cells had distinctive patterns from other insect cell lines.

Key words: Insect, cell culture, characterization, enzyme activity, karyotype

INTRODUCTION

As of 1989, more than 400 insect cell lines had been reported, most being derived from lepidopteran and dipteran species (Lynn, 1991). Insect cell lines have become increasingly important as a tool in the production of insect pathogenic viruses and recombinant proteins by the use of the baculovirus expression vector as well as in studies on physiology and developmental biology (Vaughn, 1981; Granados et al., 1987; Luckow, 1991; Hink et al., 1991; Lynn, 1992; Jarvis, 1993). This is especially true with cell lines from lepidopteran species: the cell lines from *Spodoptera frugiperda*, such as Sf-21 (Vaughn et al., 1977) and its clone Sf-9 (Summers and Smith, 1987), have been extensively used to produce recombinant proteins, and other cell lines such as Tn-368 (Hink, 1970) and Tn-5B1-4 (Granados et al., 1994) from *Trichoplusia ni* are also available for the same purpose (Wang et al., 1992; McKenna et al., 1998). It should be noted, however, that cell lines originating from different insect species tend to differ in their capacity to produce virus or express recombinant proteins (Hink et al., 1991). Therefore, there is a need to develop additional lepidopteran cell lines as substrates for recombinant baculoviruses and for baculovirus-expressed proteins (Gelernter and Federici, 1986; Maiorella et al., 1988).

In this paper, I describe a new cell line developed from the fat body tissues of *T. intermixta*, which is a polyphagous pest feeding on the leaves of a wide variety of vegetables and field crops including carrots.

MATERIALS AND METHODS

Establishment of continuous cell line. Larvae of *T. intermixta* were collected in Tokyo and reared on an artificial diet (Kawasaki et al., 1987) at 25°C under a 16 h light : 8 h dark photoperiodic regime. Adults were fed on a 10% sugar solution absorbed in cotton. Final stadium larvae were used as materials for cell cultures. The larvae were surface-sterilized with 70% ethanol, allowed to dry under a stream of clean air, and then the fat body tissues were dissected out. Small pieces of tissues were rinsed with the culture medium and subsequently transferred to 2 ml of MGM-450 medium (10% FBS) (Mitsuhashi and Inoue, 1988) in 35 mm plastic tissue culture Petri dishes (Falcon, Oxnard, CA). Cultures were sealed with Parafilm (American National Can, Greenwich, CT) and incubated at 25°C. The first subculture of the primary cultures was performed after 28 days. Thereafter, half of the medium was replaced at intervals of 14 days followed by 4 to 5 day intervals.
Culture media. MGM-450 medium supplemented with 10% FBS was used for both the primary cultures and the subcultures. For subcultures after the 200th passage, MGM-443 medium (10% FBS) (Mitsuhashi, 1982) and MGM-448 medium (10% FBS) (Mitsuhashi, 1984) were also used. The MGM-448 medium is a modification of MGM-443 medium with the addition of 0.05% PVP K-90, 1% bovine plasma albumin, 0.002% fetuin, 0.01% cytochrome C, and 0.02% inosine, and the MGM-450 medium is a further modification by supplementing with 0.3% tryptosephosphate broth.

Isozyme analysis. To confirm the identity of the TI-1 cell line, an extract of the cell line was subjected to enzyme analysis using the Corning Authenticitik with four enzymes as follows; phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), isocitrate dehydrogenase (ICD), and malic enzyme (ME). For comparison, the isozyme patterns of the following lepidopteran cell lines were also examined: a swallowtail, Papilio xuthus cell line (NIAS-PX-64) (Mitsuhashi, 1973), a cabbage armyworm, Mamestra brassicae, cell line (NIAS-MaBr-92) (Mitsuhashi and Shozawa, 1985), a Spodoptera litura cell line (TUAT-SpLi-221) (Mitsuhashi, 1995) and a Trichoplusia ni cell line (Tn-368) (Hink, 1970) were also analysed. The Trichoplusia ni cell line was supplied by Dr. M. Nagata (Univ. of Tokyo).

Karyotype analysis. A karyotype of the cells which continuously proliferated was examined by treatment with colchicine at a final concentration of $5 \times 10^{-6}$ M. Cells were incubated in colchicine containing medium for 3 h at 25°C to accumulate mitotic metaphase cells. After centrifugation at $150 \times g$, cells were then suspended in 0.6% potassium chloride for 15 min at room temperature. The cells were again spun down, and suspended in 1 ml of 0.6% potassium chloride solution. To this cell suspension 5 ml of Carnoy fixative was gently added and allowed to stand for 15 min, then the fluid was replaced with pure fixative by centrifugation. Fixation was continued for another 15 min. Finally, cells were suspended in an appropriate amount of fixative and placed on glass slides. The glass slides were air-dried. The cells were stained with 7% Giemsa solution. Chromosome number of T. intermixta 6th stadium larvae was determined by preparations from the testis of 5th stadium larvae, according to a modification of the method described by Imai et al. (1977).

Virus infection. An inoculum of Autographa californica nuclear polyhedrosis virus (AcNPV) was obtained from Dr. J. Mitsuhashi (Tokyo University of Agriculture). Cells in MGM-450 medium were inoculated by adding two drops of the virus solution and the cells were incubated at 25°C.

Fig. 1. Growth curves of the TI-1 cells adapted to (A) MGM-443 medium, (B) MGM-448 medium, and (C) MGM-450 medium (original cells), in various media (MGM-443, MGM-448 and MGM-450 media). Proliferation of the cells was measured at the 400 passage. Temperature: 25°C. ■: MGM-443 medium (10% FBS), ○: MGM-448 medium (10% FBS), ▲: MGM-450 (10% FBS).
RESULTS AND DISCUSSION

Primary cultures were made from the fat body tissues of the last stadium larvae of *T. intermixta*. Various types of cells migrated from the explanted fat body tissues. One of these consisted of large fat cells. They adhered to the bottom of the vessel, and spread their cyto-

plasm thinly. Epithelial-like cells were also observed and formed small cell sheets. Another type of cell was hemocyte-like cells which were mostly spherical in shape. However, their size varied considerably and sometimes were spindle-shaped. This type of cell usually did not adhere to the bottom of the vessel, but was suspended in the medium. The number of migrating cells increased with the advance of the culture. Only hemocyte-like cells proliferated. The first subculture was performed 28 days after the culture was initiated. Of three primary cultures, one culture gave continuously multiplying cells. This cell line has now been cultured for more than 600 passages and can be stored in 10% glycerol in MGM-450 (10% FBS) medium for at least 5 years at –100°C. Subcultures of the line were routinely performed at 4- to 6-day intervals using ratios of cell suspension to fresh MGM-450 (10% FBS) medium of 1:3 to 1:4. This cell line has been designated as TI-1.

During the first several passages, the cell growth was slow. The growth rate appeared to have stabilized after the 22nd passage. At the 200th passage, a portion of the TI-1 cells were transferred into MGM-443 (10% FBS) or MGM-448 (10% FBS) medium, and became adapted to the respective media. Growth curves of these cells in MGM-443, MGM-448 and MGM-450 media at the 300 passage are shown in Fig. 1. Growth was most rapid in the original cells cultured with MGM-450 medium and the population doubling time of the cells was about 36 h (Fig. 1C). In this culture, the highest cell density achieved was $3 \times 10^6$ cells/ml. Growth of cells adapted to MGM-443 (Fig. 1A) or MGM-448 medium (Fig. 1B) was inferior to that

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Fig. 2. Photomicrographs of the TI-1 cells. (A) Phase-contrast micrograph of the TI-1 cells. Bar represents 100 μm. (B) Metaphase chromosomes. Bar represents 10 μm. (C) Polyhedra of *Autographa californica* nuclear polyhedrosis virus formed in the nuclei of infected TI-1 cells. Bar represents 20 μm.

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Fig. 3. Chromosome numbers of the TI-1 cell line at the 200th passage.
Table 1. Electrophoretic mobility of isozymes from several insect cell lines
(mm from the origin)

<table>
<thead>
<tr>
<th>Cell linesa</th>
<th>Enzymesb</th>
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<tbody>
<tr>
<td>ICD  ME  PGI  PGM</td>
<td></td>
</tr>
<tr>
<td>TI-1  6  15  9  13</td>
<td></td>
</tr>
<tr>
<td>Tn368  4  12  9  13</td>
<td></td>
</tr>
<tr>
<td>PX-64  7  28  10  12</td>
<td></td>
</tr>
<tr>
<td>MaBr-92  9  11  11  20</td>
<td></td>
</tr>
<tr>
<td>SpLi-221B  14  (12)  14  18</td>
<td></td>
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<tr>
<td>19  14</td>
<td></td>
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<td>17</td>
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</tbody>
</table>

\[a\] PX-64, NIAS-PX-64 derived from pupal ovaries of *Papilio xuthus*; MaBr-92, NIAS-MaBr-92 derived from larval hemocytes of *Mamestra brassicae*; Tn368, derived from embryos of *Trichoplusia ni*; SpLi-221B, TUAT-SpLi-221B derived from ovaries of *Spodoptera litura*.

\[b\] ICD, isocitrate dehydrogenase; ME, malic enzyme; PGI, phosphoglucone isomerase; PGM, phosphoglcomutase.

of the original cells continuously cultured with MGM-450 medium (Fig. 1C, △). In all cultures, faster growth was achieved with both MGM-450 medium and the medium to which they adapted.

The morphologies of the cells in the established cell line are shown in Fig. 2A. During the early passages, the cell populations consisted of small spherical cells. Thereafter, cells with a spindle or spherical shape came to predominate. After subculturing, most of the cells adhered loosely to the bottom of the culture flask at first, and then gradually became detached from the flask, resulting in suspension in the medium. The attached cells could be easily dispersed by pipetting.

Chromosome analysis of the cells after 200 passages revealed that the cells had chromosomes typical of lepidopteran cell lines (Mitsuhashi and Shozawa, 1985). It is difficult to count chromosome number accurately, because a nucleus contained many microchromosomes (Fig. 2B). The mode of chromosome number of the cell line was from 150 to 169, however, the chromosome number varied to a great extent (Fig. 3). Heteroploidy and polyploidy have been commonly found among lepidopteran cell lines (Mitsuhashi, 1981; Hink and Hall, 1989; Hara et al., 1993). Since the chromosome number of spermatocytes of *T. intermixta* was 31 (data not shown), the TI-1 cell line appears to be mainly composed of pentaploid cells.

The TI-1 cells were susceptible to *Autographa california* nuclear polyhedrosis virus (AcNPV) (Fig. 2C). Polyhedra could be observed 3 days post-infection. The cell infection rate determined on the basis of the formation of polyhedra 4 days post-infection was approximately 15%. The TI-1 cell line was characterized by isozyme analysis using the Authentikit system. The four enzymes proposed by Tabachnick and Knudson (1980) were used. This analysis revealed distinctive patterns from all the other cell lines examined, including Tn-368 cell line of *T. ni*, a related species of *T. intermixta* (Table 1).

In the present study, I established a cell line derived from fat body tissues of *T. intermixta*. This cell line may be applicable to studies of insect pathogenic viruses and baculovirus-expression-vector systems.

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