

Benchmarks

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Ethylene as a Gaseous Reporter Molecule for Monitoring Gene Expression in Cultured Cells

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Reporters or signaling molecules are indispensable tools in current studies of gene activities, protein distribution, gene transfer and biosensing (2). A reporter molecule that is suitable for *en masse* measurement of gene activities in transgenic animals in a noninvasive manner is yet to be identified. We therefore intended to develop the plant hormone ethylene as a novel, noninvasive reporter molecule to profile spatial and temporal gene expression in animal cells. Here, we report the establishment of the plant ethylene biosynthetic pathway in an insect cell line IPLB-Sf21 of the fall armyworm *Spodoptera frugiperda* (3) as a step toward that goal.

Production of 1-aminocyclopropane-1-carboxylic acid (ACC)-dependent ethylene in higher plants is through the precursor S-adenosyl-L-methionine (SAM). SAM is first converted to ACC by ACC synthase and then to ethylene by ACC oxidase (8). An artificial dual functional enzyme, ACSO, has been created by in-frame fusion of ACC synthase and ACC oxidase (5). This fusion enzyme juxtaposes the active sites of ACC synthase with ACC oxidase in close proximity and catalyzes the two sequential steps of the ACC-dependent ethylene biosynthesis in an *in vitro* assay system (5), and it has been shown to produce ACC-dependent ethylene in the yeast *Saccharomyces cerevisiae* (7).

The insect cell line IPLB-Sf21 was purchased from Clontech Laboratories (Palo Alto, CA, USA). Production of the recombinant ACC oxidase fusion enzyme in the insect cells was carried out as reported (4). A 2.35 kb recombinant gene encoding the dual functional fusion enzyme ACSO (5) was inserted into the viral expression vector pBac-PAK8, and the resultant recombinant clone was transfected into the insect cell line IPLB-Sf21. Ethylene and ACC productions by the transfected insect cells were determined according to the

following method. After removing the spent medium, 5 mL 27°C TNM-FH medium (Grace's medium with 3.3 g/L of lactalbumin hydrolysate and 3.3 g/L of yeastolate) were added to the culture flask, and the insect cells were detached from the flask wall by pipetting the medium up and down five or six times. Cells, at the density of 3×10^6 cells per 25 mL flask determined by using a hemocytometer, were incubated at 27°C for 3 h. The medium was then removed, and 1.5 mL TNM-FH medium and 0.3 mL recombinant viruses ($\text{moi} = 10$) were added to the cells. After incubation at 27°C for 1.5 h, an additional 3.5 mL TNM-FH medium were added, and the culture was incubated at 27°C. Two days later, iron (II) sulfate-7-hydrate and sodium ascorbate were added to the transfected cells to a final concentration of 100 μM and 30 mM, respectively. The flask containing the transfected cell culture was sealed with a rubber septum and incubated at 27°C for 20 h before ethylene and ACC were quantitated. A gas sample of 2 mL in the headspace of the flask was removed for gas chromatography (1). Five hundred microliters of the cell culture were centrifuged at $2000 \times g$ for 2 min, and 450 μL supernatant were assayed for ACC content as previously described (6).

Table 1 shows that greater amounts of ACC and ethylene were produced by the insect cells transfected with the ACC oxidase recombinant virus. Around sixfold more ACC was produced by the ACSO cells. It was noted that the ACC concentration is a bit higher in the absence of ACC oxidase cofactors iron (II) and ascorbic acid. This correlates with increasing ethylene production under the same conditions. Much lower levels of ACC were observed in the control cells. The detected levels of ACC are not likely the result of authentic ACC molecules because the detection method we used, the Lizada and Yang assay, is known to generate background levels of artifacts (6). Ethylene production by insect cells in the presence of the ACC oxidase cofactors iron (II) and ascorbic acid was nearly sevenfold higher than ethylene production without these cofactors, which indicates that a functional ACC oxidase is present in the ACSO cells and that ethylene is able to diffuse

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Table 1. ACC and Ethylene Production in the Transfected Insect Cells

	ACC Concentration in Culture Media (mM)	Ethylene Concentration in Flask Headspace (nL/1 × 10 ⁸ cells/h)
ACSO ^a	125.60 ± 0.82	11.87 ± 1.35
ACSO + Fe ²⁺ , Vc ^b	98.65 ± 10.88	69.32 ± 9.41
Control	22.83 ± 1.85	3.14 ± 0.13
Control + Fe ²⁺ , Vc	3.83 ± 0.06	9.35 ± 0.25

Each value represents the average and standard deviation of a triplicate measurement.

^aACSO = ACC oxidase
^bVc = Ascorbic acid

through the membranes of these cells. The highest ethylene production rate reached 69.32 nL/1 × 10⁸ cells/h in the ACC oxidase-containing cells. The basal level of ethylene detected may be the contribution of lipid oxidation of the dead insect cells.

The above data demonstrated that a plant ethylene biosynthetic pathway can be reconstituted in non-plant cells by simply acquiring ACC synthase and ACC oxidase, and the two-carbon olefin gas ethylene is diffusible across these cell membranes. Our results show ethylene to be a quite useful reporter molecule for the study of gene activities in transgenic animals as (i) the method for measuring gaseous ethylene is simple and sensitive—a concentration as little as 30 ppb can be detected by gas chromatography; and (ii) the method avoids invasive procedures of sample collection and therefore is uniquely useful for those applications where continuously monitoring gene activities of the same organisms is required.

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