

Gene Transfer and Cloning of the Amino-Acid Transport System L from Human Cells

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Neutral amino acids are transported into Chinese hamster ovary cells by three transport systems.¹ System A and ASC are Na⁺-dependent and serve for low-molecular-weight amino acids. System L is Na⁺-independent and serves for branched-chain amino acids and aromatic amino acids. Transport activity of System L can be derepressed by severe starvation for leucine. It can also be derepressed in a temperature-sensitive leucyl-tRNA synthetase mutant cell line (CHO-025C1).² Fusion of the CHO-025C1 mutant cell line with human leukocytes resulted in complementation.

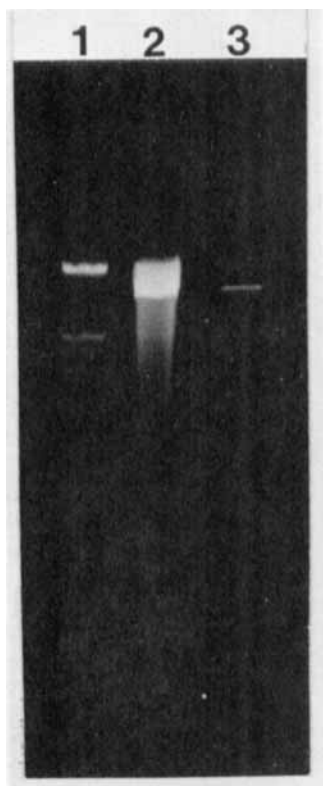


FIGURE 1. Human cosmid library (108K) DNA digested with *EcoRI* (lane 2). Lane 1 and lane 3 represent DNA size standards (lambda DNA digested with *EcoRI* and *HindIII*) and the cosmid vector, pCV108, digested with *EcoRI*, respectively.

With this approach, it was shown that human chromosome 5 encodes leucyl-tRNA synthetase³ and human chromosome 20 encodes amino-acid transport System L.² The details of these experiments are reported in another article.⁴ We are presently attempting to clone and isolate human gene sequences that code for amino-acid System L. For this approach, we are using the CHO-025C1 cell line, which requires high levels of leucine (0.4 mM) to survive at 37°C or above. Human gene sequences contained in a cosmid library (108K) obtained from Lau and Kan⁵ were transformed into the CHO cells and transformants were selected. The cosmid vector, pCV108, codes for G418 resistance when expressed in mammalian cells and codes for both kanamycin and ampicillin resistance when present in *E. coli*. DNA prepared from this library when

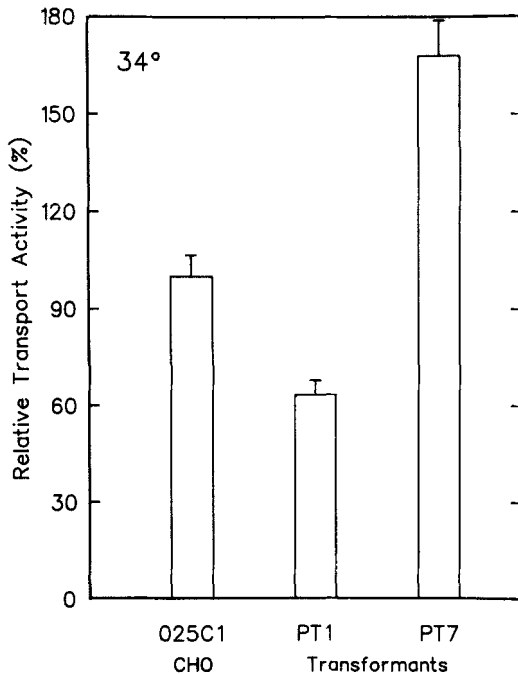


FIGURE 2. Leucine transport activity assay. Cell line PT7 is representative of a class of transformants that showed elevated levels of transport activity over the recipient cell line (025C1). PT1 cell line represents a second class of transformants that have survived the screening processes, but does not have increased transport activity.

restricted with *EcoRI* gave one clear band representing the cosmid vector pCV108 and a smear of human DNA sequences of various lengths indicating a good representation of the complexity of the human genome (FIG. 1). First, transformants that were resistant to G418 were selected. In a second selection, survivors resistant to higher temperatures in the presence of low levels of leucine (0.1 mM) were isolated. Some of these transformants have high levels of System L transport activity (FIG. 2) suggesting that human genes for leucine transport have been integrated into chromosomes of the transformed CHO cells. Another class of transformants that survived the selection procedures but did not have increased transport may represent transformants that have acquired the human leucyl-tRNA synthetase gene. Currently attempts are being made to rescue these human sequences by using a lambda *in vitro* packaging system.

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