

DEVELOPMENT

Insulin Staining of ES Cell Progeny from Insulin Uptake

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Recent reports describe derivation of insulin-containing cells from embryonic stem (ES) cells (1–5) and putative adult stem cells (6–8). Of particular note is the report that mouse ES cells efficiently form islet-like structures in vitro (1). Using this protocol (1) on five ES cell lines, both murine and human, we reproduced the finding that 10 to 30% of cells stain with antibodies to insulin. Fifty-micrometer clusters of insulin-staining cells were produced as described (1) (Fig. S1).

Despite antibody staining, we did not detect insulin 1 mRNA by reverse transcription–polymerase chain reaction (RT-PCR) and insulin 2 mRNA detection was weak. Multiple primers used during all five stages of the protocol (1) confirmed these results. RT-PCR controls detected insulin transcripts from a single pancreatic β cell among 1 million non- β cells. Insulin gene expression was also assessed in ES cells with lacZ insertions downstream of the endogenous insulin or pdx1 promoters. Only about 1/100,000 cells was X-gal–positive despite insulin antibody staining in 10 to 30% of cells. Similarly, differentiated human ES cells expressing green fluorescent protein from an insulin promoter did not show fluorescence above background (9). Moreover, the insulin-positive cells did not stain with an antibody for C-peptide, a byproduct of de novo insulin synthesis. Nuclei of insulin-staining cells were small, condensed, and TUNEL⁺, suggesting apoptosis (Fig. 1, A to D). Electron microscopy of differentiated cells failed to demonstrate the granules characteristic of β cells.

Differentiated insulin-positive cells were reported to contain 1 μ g of insulin per mg of total protein (1). This is less than 0.02% of the insulin found in the media to which these cells are exposed, raising the possibility that insulin is

concentrated from the medium. ES cells differentiated in media without exogenous insulin did not stain for insulin, and differentiated ES cells

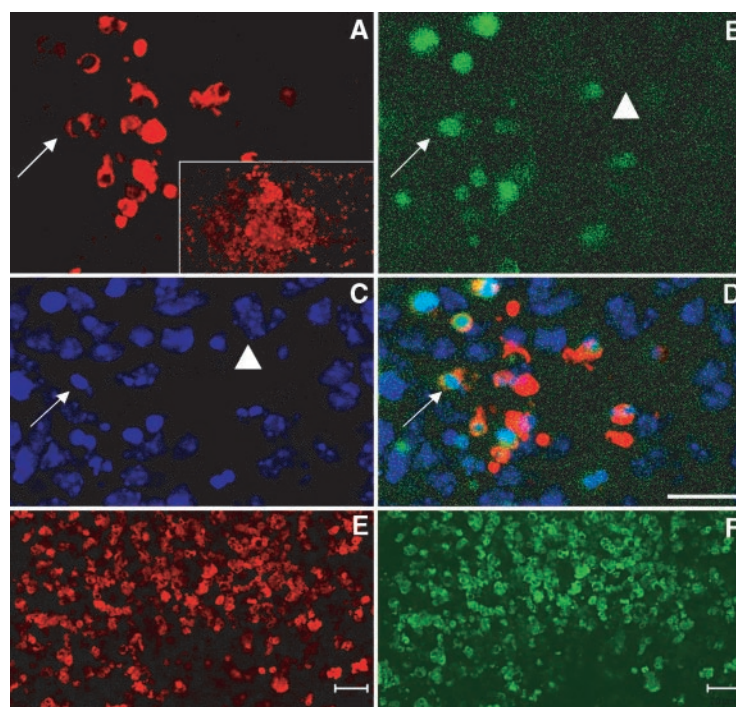


Fig. 1. Confocal images of insulin staining in mouse ES cell progeny. (A) Insulin staining (red). The inset demonstrates a typical aggregate of insulin-staining cells. (B) TUNEL⁺ nuclei (green). (C) Nuclear staining with DAPI (purple). (D) Composite image of (A) to (C). Cells that stain for insulin have condensed nuclei and are TUNEL⁺ (arrows). Cells with normal nuclei are TUNEL[−] and do not stain for insulin (arrowheads). (E and F) Insulin-staining cells (red) (E) are identical to cells that have taken up FITC-conjugated insulin (green) (F). Controls showed no leakage of the FITC or rhodamine signals. Scale bars, 20 μ m.

subsequently cultured in insulin-deficient media lost insulin staining. (This release of absorbed insulin may mimic genuine secretion.) Some absorbed insulin is retained for more than 3 weeks in insulin-deficient media. Therefore, the mere persistence of insulin immunoreactivity in a transplant of ES cell progeny is insufficient evidence of β cell differentiation or function.

ES cells differentiated in the presence of fluorescein isothiocyanate (FITC)–conjugated insulin concentrate FITC-insulin in the cells that stain with antibody to insulin (Fig. 1, E and F). When medium is supplemented with FITC-conjugated albumin, a distinct cell population con-

centrates this protein. Cells unrelated to β cells also concentrate insulin: Murine embryonic fibroblasts grown in the N2-based media used in the five-stage protocol (1) or in medium used to culture human embryoid bodies (2) produce cells with TUNEL⁺ nuclei that are insulin immunoreactive.

We cannot exclude the possibility that the paucity of β cell differentiation in these cultures is due to cell line variability or suboptimal culture conditions. We do conclude that insulin staining alone can overestimate genuine β cell differentiation when exogenous insulin is present. Furthermore, RT-PCR cannot quantify the number of cells within a population that produce insulin transcripts. Several methods should be combined for reliable analysis of insulin-expression including C-peptide staining, electron microscopy, Northern analysis, in situ hybridization, metabolic labeling, demonstration of biphasic insulin secretion, and transplantation assays for β cell function that demonstrate rescue of the diabetic phenotype for more than a month.

References and Notes

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10. We thank N. Lumelsky and R. McKay for help in reproducing their culture regimen (7) and for comments on the manuscript. We thank R. Perak, N. Benvenisty, and members of the Melton lab. The following individuals kindly provided reagents: C. Wright (R1 Pdx1-lacZ ES cells), D. Bucchini (Ins2-lacZ ES cells), A. McMahon (AV3 ES cells), R. McKay (nestin antibody, E14.1 ES cells), and J. Itskovitz-Eldor (H9 human ES cells). A detailed protocol is available upon request.

Supporting Online Material

www.sciencemag.org/cgi/content/full/299/5605/363/DC1
Methods and Materials
Fig. S1

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