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Cloning of a Rab3 isotype predominately expressed in adipocytes

GIULIA BALDINI*, TOBIAS HOHL*, HERBERT Y. LIN*†, AND HARVEY F. LODISH*†

*Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142; and †Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

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ABSTRACT **We have isolated the cDNA for Rab3D, an additional member of the small molecular weight GTP-binding protein family. Rab3D message is abundant in mouse adipo-**

homogenized for 60 sec with a Polytron homogenizer and incubated at 37°C for 1 hr. The samples were then adjusted to 0.4 M NaCl and incubated at room temperature for 1 hr

proteins. DNA from the subtracted library (40 ng) and 1 μM antisense primer in 100 μl of reaction mix (GeneAmp PCR core reagents, Perkin–Elmer/Cetus) containing 1.5 mM $MgCl_2$, were subjected to the following temperature cycles: 1 cycle of after addition of 1 μM sense primer by 29 cycles of 94°C for 2 min, 55°C for 1 min, and 72°C for 20 sec. The subtracted library was transferred to Biotrans nylon membranes (ICN) which were pretreated and then hybridized at high stringency (50% formamide in the hybridization solution) with the ^{32}P-labeled following temperature cycle for 29 cycles: 94°C for 2 min, 50°C for 1 min, and 72°C for 20 sec.

RESULTS

cDNA libraries were constructed from 3T3-L1 preadipocyte fibroblasts and differentiated adipocytes in the vector pcDNA-1 (18). After subtraction of the adipocyte library with the preadipocyte one, the enrichment for GLUT4 cDNA was >100-fold (not shown).

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Rat     3A    1 MAS ATDSRYGQKESS DQNFDYMFKILIIGNSSVGKTSFLFRYADDSFTPAFVSTV
Bovine  3A      MAS ATDARYGQKESS DQNFDYMFKILIIGNSSVGKTSFLFRYADDSFTPAFVSTV
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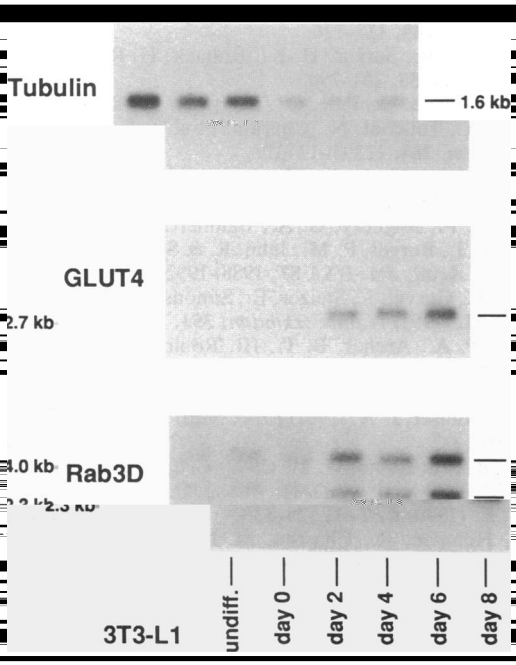

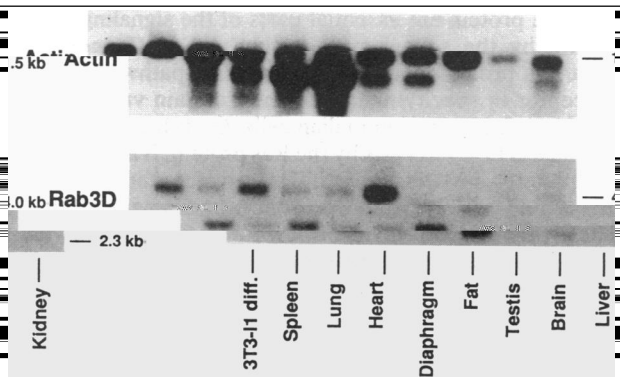

Here we describe the cloning and properties of an additional

library and Bernard Thorens for helpful discussion. This research