

Cloning of a Rab3 isoform 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 adipocytes. It is increased during differentiation of 3T3-L1 cells into

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 with 0.5 ml of oligo(dT)-cellulose (Collaborative Research)

proteins. DNA from the subtracted library (40 ng) and 1 μ M following temperature cycle for 29 cycles: 94°C for 2 min.

antisense primer in 100 μ l of reaction mix (GeneAmp PCR core 50°C for 1 min. and 72°C for 20 sec.

reagents, Perkin-Elmer/Cetus) containing 1.5 mM MgCl₂

were subjected to the following temperature cycles: 1 cycle of

after addition of 1 μ M 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%

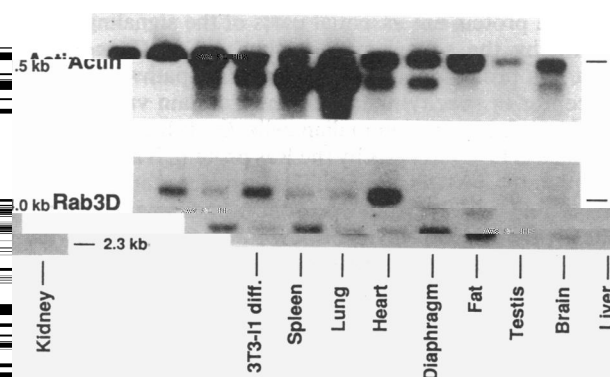
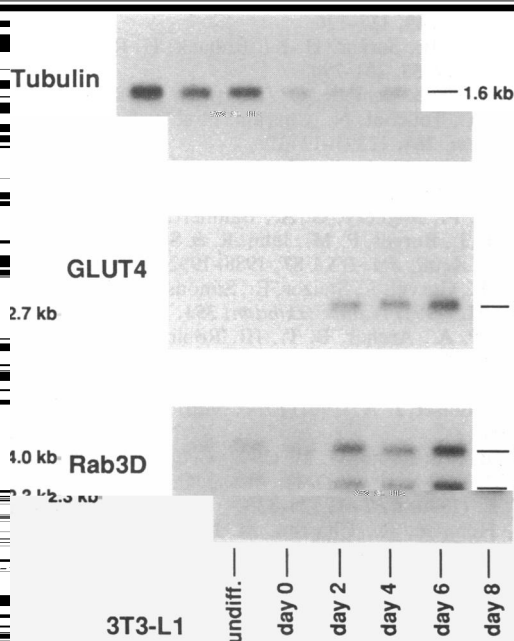
cDNA libraries were constructed from 3T3-L1 preadipocyte fibroblasts and differentiated adipocytes in the vector

pcDNA-1 (18). After subtraction of the adipocyte library with the preadipocyte one, the enrichment for GLUT4 cDNA was

formamide in the hybridization solution) with the ³²P-labeled

>100-fold (not shown).

Rat	3A	¹ MAS	ATDSRYGQKES	DQNFDMFKILIIGNSSVGKTSFLFRYADDSFTPAFVSTV
Bovine	3A	MAS	ATDARYGQKES	DQNFDMFKILIIGNSSVGKTSFLFRYADDSFTPAFVSTV



DISCUSSION

Here we describe the cloning and properties of an additional

We thank Brian Seed for advice and help in making the subtractive library and Bernard Thorens for helpful discussion. This research