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RAB6C Is a Retrogene that Encodes a Centrosomal Protein Involved in Cell Cycle Progression

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Rab-GTPases are key regulators of membrane transport, and growing evidence indicates that their expression levels are altered in certain human malignancies, including cancer. Rab6C, a newly identified Rab6 subfamily member, has attracted recent attention because its reduced expression might confer a selective advantage to drug-resistant breast cancer cells. Here, we report that *RAB6C* is a primate-specific retrogene derived from a *RAB6A′* transcript. *RAB6C* is transcribed in a limited number of human tissues including brain, testis, prostate, and breast. Endogenous Rab6C is considerably less abundant and has a much shorter half-life than Rab6A′. Comparison of the GTP-binding motifs of Rab6C and Rab6A′, homology modeling, and GTP-blot overlay assays indicate that amino acid changes in Rab6C have greatly reduced its GTP-binding affinity. Instead, the noncanonical GTP-binding domain of Rab6C mediates localization of the protein to the centrosome. Overexpression of Rab6C results in G1 arrest, and its specific depletion generates tetraploid cells with supernumerary centrosomes, revealing a role of Rab6C in events related to the centrosome and cell cycle progression. Thus, *RAB6C* is a rare example of a recently emerged retrogene that has acquired the status of a new gene, encoding a functional protein with altered characteristics compared to Rab6A′.

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Introduction

Rab GTPases constitute the largest branch of the Ras superfamily and are involved in regulating intracellular protein trafficking pathways.[1] *Ypt6/RAB6* is one of five ancestral *RAB* genes conserved from yeast to humans. During evolution, *RAB6* expanded by gene duplication and alternative splicing, resulting in the expression of three *RAB6* isoforms in *Homo sapiens*. The primordial *RAB6A′/A* gene is encoded on human chromosome 11 and generates two spliced variants, *RAB6A′* and *RAB6A*, while *RAB6B* on chromosome 3 is a gene duplication of *RAB6A/A′* not subject to splicing.[2,3] Rab6A, 6A′, and 6B localize at the late Golgi cisternae and *trans*-Golgi network (TGN), as well as itinerant tubulovesicular carriers that move along microtubules mainly in the plus-end direction.[4–6] Rab6A′ appears to be the closest functional equivalent to yeast homolog Ypt6, and like in budding yeast, depletion or overexpression of mutant forms of Rab6A′ affects several trafficking routes that intersect the Golgi.[7] This includes early endosome-to-TGN retrieval pathways that carry proteins such as TGN46, mannose 6-phosphate receptors, or internalized glycolipid-bound toxins;[8–13] recycling of Golgi-resident enzymes to the endoplasmic reticulum;[14–17] and efficient delivery and exocytosis of cargo to the plasma membrane.[5] In each pathway, the function of Rab6A′ may be manifold, acting in conjunction with other molecular components to dock incoming vesicles,[18] organize subdomains for membrane budding/fission,[19] or recruit/activate molecular motors.[5,20,21]

The diverse and dynamic functions of the Rab6 isoforms are reflected in the identification of more than 15 interacting effector proteins that include motors, motor adapters, tethers, and structural

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Abbreviations used: GFP, green fluorescent protein; TGN, *trans*-Golgi network; ORF, open reading frame; OWM, Old World monkey; BrdU, bromodeoxyuridine; FRAP, fluorescence recovery after photobleaching; HA, hemagglutinin epitope; siRNA, short interfering RNA; GDI, guanine dissociation inhibitor; GST, glutathione *S*-transferase; UTR, untranslated region; HRP, horseradish peroxidase; PBS, phosphate-buffered saline; NCBI, National Center for Biotechnology Information.

proteins.[8,9,20,22–25] Some effort has been made to understand the specific roles of the structurally and functionally related Rab6 subfamily isoforms. Rab6A and Rab6A′ are the most ubiquitously and abundantly expressed members, differing in just three amino acids, and have similar GTP-binding properties.[2] How redundant or essential the individual activities of Rab6A and Rab6A′ are to the membrane-trafficking pathways mentioned above is still under investigation.[26] The particular function of the more divergent Rab6B isoform is easier to infer, it being selectively expressed in neuronal cells, and is presumed to have a novel spatiotemporal role necessary for the complex trafficking pathways that operate in specialized cell types.[3] Finally, in addition to promoting membrane trafficking during interphase, at least one of the Rab6 isoforms plays a role during mitosis. Thus, Rab6A′ is required for proper inactivation of the Mad2 spindle assembly checkpoint, and unexpectedly, this second function requires neither prenylation nor GTP hydrolysis by Rab6A′.[27]

Recently, two groups discovered a fourth Rab6 isoform denoted as *WTH3* or *RAB6C*. *WTH3* was isolated as a differentially methylated DNA fragment, and *RAB6C* was identified as a novel cDNA in the context of the LIFEdb project.[28,29] Rab6C shows highest identity to Rab6A′ (91%) but possesses an extra carboxy-terminal extension of 46 amino acids after the hypervariable region, suggestive of unique functional properties. In accordance with this idea, green fluorescent protein (GFP)-tagged Rab6C did not show subcellular localization at the Golgi or delay transport of the secretory cargo VSV-G to the plasma membrane like Rab6A′.[30] Instead, Rab6C was found to have a nucleo-cytoplasmic distribution.[31] The promoter region of *RAB6C* is associated with a CpG island that is partially methylated in primary cells isolated from effusions of breast cancer patients and hypermethylated in drug-resistant cell lines but not in paired drug-sensitive cell lines.[31,32] Hypermethylation of *RAB6C* results in downregulation of its mRNA levels, and addition of histone deacetylase and methyltransferase inhibitors reversed epigenetic silencing of *RAB6C*.[32] Stable re-expression of *RAB6C* in a multidrug-resistant uterine sarcoma cell line resulted in 8–35 times increased sensitivity to the chemotherapeutic agent doxorubicin.[29] Conversely, reduction of *RAB6C* expression in HEK293 cells using an RNAi strategy made them 3–7 times more tolerant to doxorubicin than wild-type HEK293 cells.[33] More recently, *RAB6C* was found to harbor an unusual but functional p53-binding motif within its promoter region. Overexpression of p53 increased levels of *RAB6C* mRNA in HeLa cells but not in drug-resistant MCF-7 cells, where promoter methylation abrogated transactivation by p53. Furthermore, overexpression of Rab6C in HEK293 cells stimulated apoptosis.[33,34] These results lead to the current hypothesis that *RAB6C* is a candidate tumor suppressor gene encoding a pro-apoptotic factor and necessarily downregulated during establishment of drug resistance in order to avoid cell death.

Here, we completely reassess *RAB6C* in light of our finding that it originated by retrotransposition, a mechanism whereby mRNA is reverse transcribed and randomly integrated into the genome.[35] Most retrotransposition events result in inactive pseudogenes and are a problematic source of experimental artifacts.[36] Rarely though, a new retrogene encoding a protein with modified or novel functions can emerge. Here, we demonstrate that retrogene *RAB6C* is such an example, encoding a biologically relevant protein that is, however, completely unconventional as a Rab. Instead, primate-restricted Rab6C is a centrosomal protein with a role in processes related to regulation of centrosome duplication and cell cycle progression.

Results

RAB6C is a hominoid-specific intronless gene derived from RAB6A′ mRNA

Within the human genome, *RAB6C* (GenBank accession 84084) maps to chromosome 2q21.1 (Supplementary Fig. S1a). Given its single-exon structure and that it shares highest identity to *RAB6A′* transcript (97%), *RAB6C* was likely generated by retrotransposition of a fully processed *RAB6A′* mRNA. A second *RAB6C-like* intronless gene (GenBank accession 150786) exists approximately 1.38 Mb downstream from *RAB6C* in an inverted orientation (Supplementary Fig. S1a). The sequences of *RAB6C* and *RAB6C-like* are almost identical over 21,000–24,000 bp of flanking region, indicating that one of the copies is the result of an intrachromosomal segmental duplication rather than a second retrotransposition event.

To determine the timing of the retrotransposition event, we searched for intronless *RAB6C* orthologues in other species. *RAB6C* was absent from bovine and rodent genomes, suggesting that it was acquired during anthropoid evolution. Further analysis revealed the occurrence of *RAB6C* in four apes (chimpanzee, gorilla, orangutan, and gibbon) but not in any of the available Old World monkey (OWM) genomes (Supplementary Table S1). To verify that *RAB6C* is indeed absent from OWM, we screened genomic DNA isolated from rhesus macaque and Vero and COS-7 cell lines originated from green monkey. A chimpanzee genomic sample served as a control for the existence of the *RAB6C* gene. Primers designed against conserved sequences of *RAB6C* only amplified a PCR product of the expected size of intronless *RAB6C* in chimpanzee (Supplementary Fig. S1b). We can thus estimate that the retrotransposition event giving rise to *RAB6C* occurred after the OWM–hominoid split and before separation of the lesser ape and other hominoid lineages, between 21 and 25 MYrs ago (Fig. 1a).[37] Inspection of the putative translation products corresponding to *RAB6C* and *RAB6C-like* revealed preservation of the open reading frame (ORF) in human, chimpanzee, gorilla, and orangutan. In contrast, multiple stop codons in the gibbon sequence prevent an elongated ORF,