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37. The Jak1 (Fig. 2, A through D) and Jak2 antisera were as described (*21*); the Jak1 antiserum used for Fig. 2E was raised in rabbits against an $NH_2$-terminal peptide (*35*). Rabbit antiserum to Tyk2 was raised and affinity-purified against an engineered glutathione-S-transferase fusion product containing a portion of human Tyk2.
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# Targeting of $G\alpha_{i2}$ to the Golgi by Alternative Spliced Carboxyl-Terminal Region

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**Heterotrimeric guanosine triphosphate (GTP)–binding proteins (G proteins) may participate in membrane traffic events. A complementary DNA (cDNA) was isolated from a mouse pituitary cDNA library that corresponded to an alternatively spliced form of the gene encoding the G protein alpha subunit $G\alpha_{i2}$. The cDNA was identical to that encoding $G\alpha_{i2}$ except that the region encoding for the carboxyl-terminal 24 amino acids was replaced by a longer region encoding 35 amino acids that have no sequence similarity with $G\alpha_{i2}$ or other members of the G protein family. This alternative spliced product and the corresponding protein ($sG_{i2}$) were present in several tissues. Specific antibodies revealed that $sG_{i2}$ was localized in the Golgi apparatus, suggesting a role in membrane transport. Thus, alternative splicing may generate from a single gene two G protein alpha subunits with differential cellular localization and function.**

Proteins that bind GTP participate in the control of many cellular events (*1*). GTP-binding proteins are divided into two classes: the small Ras-related GTP-binding proteins and the heterotrimeric GTP-binding proteins (G proteins). Genetic and biochemical studies in yeast have shown that the first class participates in membrane trafficking (*2*). In mammalian cells this role has been assigned to members of the Arf and Rab families of small GTP-binding proteins (*2*). Heterotrimeric G proteins have classically been defined as the intracellular transducers of signals at the cell surface. However, they also appear to participate in the regulation of membrane trafficking events (*3–5*). A nonhydrolyzable analog of GTP, GTP-γ-S, stimulates GTP-binding proteins and inhibits membrane transport. This effect was thought to result from activation of monomeric GTP-binding proteins. However, $AlF_4^-$, a compound that activates heterotrimeric but not monomeric GTP-binding proteins, has the same effect as GTP-γ-S on membrane trafficking (*3*). These and other results suggest that heterotrimeric GTP-binding proteins can also influence transport in both secretory and endocytic pathways (*4–7*). Two G proteins, $G\alpha_{i3}$ (*8*, *9*) and $G\alpha_s$ (*10*), have been shown to be associated with the Golgi apparatus and their activation modifies the transport of proteins (*9*, *10*).

The data presented in this paper demonstrate the existence of a protein generated by alternative splicing of the gene encoding $G\alpha_{i2}$ ($G_{i2}$). The alternatively spliced transcript encodes a protein with a different COOH-terminus that confers localization in the Golgi apparatus. The alternative localization of this form of $G_{i2}$, which we term $sG_{i2}$, suggests that this protein may function in the control of membrane transport events.

We screened a mouse pituitary cDNA library (*11*) at low stringency (40% formamide, 42°C) with a $G_{i2}$ cDNA fragment including sequences conserved between the three inhibitory G protein α subunits (amino acids 200 to 320) (*12*) and isolated several cDNAs. Three isoforms of $G_{i2}$ were obtained. Two of the cDNAs incorporated an alternative 3′ untranslated region and corresponded to previously described cDNAs (Fig. 1) (*12*, *13*). The third isoform is identical to $G_{i2}$ through the coding region until amino acid position 331, where the remainder of the COOH-terminal coding region is substituted by a different sequence that encodes 35 amino acids, resulting in an open reading frame of 366 amino acids (Fig. 1). The nucleotide and amino acid sequences of this segment are not similar to those of other G proteins.

To determine the genomic structure of the 3′ region of the mouse $G_{i2}$ gene, a genomic library was screened with an Xba I–Xho I fragment (Fig. 1) containing the divergent COOH-terminal fragment of $sG_{i2}$. This fragment contains the sequences from the Xba I site of $sG_{i2}$, 3′ to the end of the cDNA. In the human genome the $G_{i2}$ gene is encoded by nine exons (*14*). The eighth and ninth exons contain the COOH-terminal portion of the coding region and the 3′ untranslated sequences, respectively (*14*). Accordingly, we isolated two phages containing the corresponding region from the mouse genome and determined the sequences of exons 8 and 9 and the boundaries of intron 8 (Fig. 1C). By comparison with the cDNA sequences, we established that the alternative 3′ region present in $sG_{i2}$ is derived from exon 9 and so results from alternative splicing. Both the predicted donor and acceptor splice sites that generate this isoform are noncanonical.

We confirmed the existence of $sG_{i2}$ by

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reverse transcriptase–polymerase chain reaction (RT-PCR) analysis (*15*). The COOH-terminal region of $G_{i2}$ was amplified from pituitary RNA (Fig. 1B). The analysis confirmed the presence of three fragments of different length, which correspond to the two previously described $G_{i2}$ cDNAs (*12*, *13*) and the $sG_{i2}$ form. We also used ribonuclease protection assays (*16*) for quantitative analysis of the distribution and expression of $sG_{i2}$ relative to $G_{i2}$. The $sG_{i2}$ transcript was present in all the tissues analyzed, and the amount of its expression was proportional to that of $G_{i2}$ (Fig. 2). We confirmed the open reading frame of the putative $sG_{i2}$ protein by translating the $sG_{i2}$ cDNA in vitro (*17*). The product migrated more slowly than $G_{i2}$ (Fig. 3A), confirming its predicted larger molecular size. Polyclonal antibodies were raised to a peptide corresponding to the first 15 COOH-terminal amino acids specific to $sG_{i2}$. With this antibody, anti-$sG_{i2}$ (Fig. 3B), we detected a band of ~42 kD in membrane extracts from brain and pituitary. This protein comigrated precisely with a band obtained from extracts of cultured cells transfected with an expression vector containing $sG_{i2}$ cDNA (Fig. 3B). The anti-$sG_{i2}$ is able to immunoprecipitate $sG_{i2}$ produced by in vitro translation (Fig. 3A). The anti-$sG_{i2}$ was blocked only by the specific peptide (Fig. 3C). The observation that $sG_{i2}$ is widely expressed suggests that it has a basic function. Overexpression of $sG_{i2}$ in different cell lines did not alter the intracellular concentration of adenosine 3′,5′-monophosphate (cAMP) or inositol phosphates (*18*). Also, $sG_{i2}$ lacks the site of adenosine diphosphate (ADP)–ribosylation by pertussis toxin (PTX), which is the hallmark of the inhibitory G proteins (*19*). Therefore, this G protein probably cannot be inactivated by PTX.

To study whether the alternative

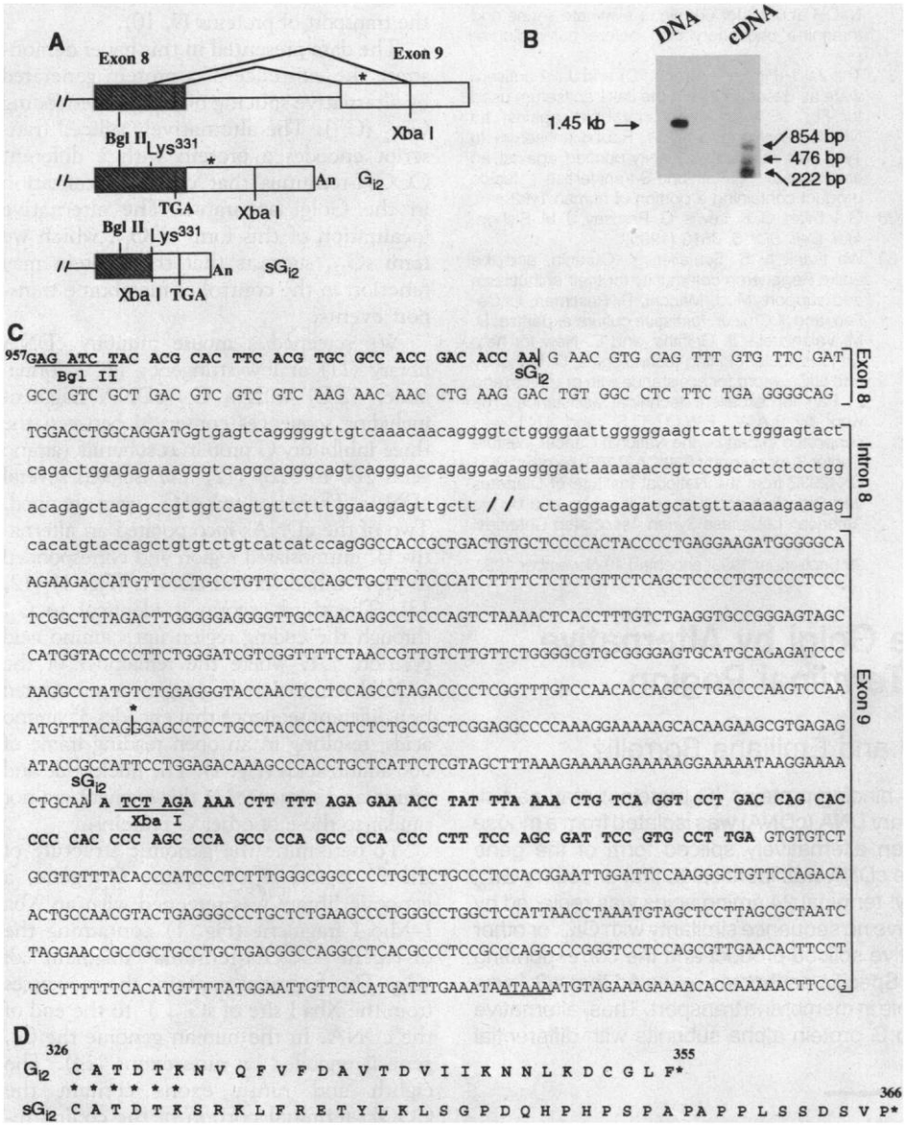


**Fig. 1.** Molecular structure of $sG_{i2}$. (**A**) Schematic representation of the 3′ end of the gene encoding $G_{i2}$ and $sG_{i2}$ (top) and the cDNAs (below). Exon 8 contains the coding sequence from nucleotides 877 to 1091. This region includes the TGA sequence and 23 nucleotides of the 3′ untranslated region. Exon 9 contains the remainder of the 3′ untranslated sequence of the gene. (**B**) PCR analysis of mouse genomic DNA and RNA from pituitary gland. The 854-bp fragment corresponds to $G_{i2}$ (*12*), whereas the 476-bp band corresponds to a 3′ alternative splicing variant generated by the use of an acceptor site labeled by an asterisk in (A) and (C); this splicing variant lacks the first 378 bp of exon 9 (*13*). The 222-bp fragment corresponds to $sG_{i2}$. (**C**) Nucleotide sequence of the 3′ end of the gene encoding $G_{i2}$ and $sG_{i2}$. The sequence displayed extends from the Bgl II site to the 3′ end of exon 8, the junction of intron 8, and the complete exon 9 sequence of the mouse $G_{i2}$ gene. Triplets in bold represent the $sG_{i2}$ coding region; lines indicate the donor and acceptor sites. The capital letters represent the $G_{i2}$ mRNA sequences. The asterisk refers to an additional splice variant acceptor site that deletes parts of the 3′ untranslated sequence of $G_{i2}$ (*13*). (**D**) Amino acid sequence of the COOH-terminus of $G_{i2}$ extending from amino acid 326 aligned with the COOH-terminus of $sG_{i2}$.

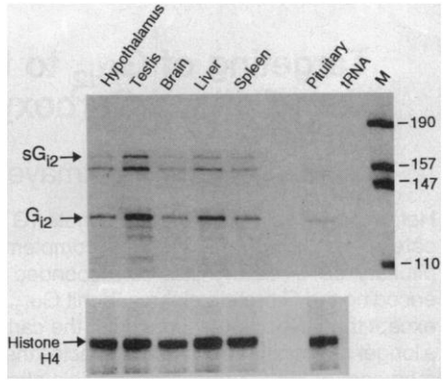


**Fig. 2.** Distribution of $sG_{i2}$ in various tissues. The tissues analyzed are indicated. (**A**) A cDNA fragment of $sG_{i2}$ extending from the Bgl II site to 20 bp downstream from the TGA was used as a probe. Ribonuclease protection reactions (*28*) included yeast tRNA (10 μg) as control or total RNA (10 μg) from various tissues. The fully protected fragment of $sG_{i2}$ was 164 nucleotides long. Two $G_{i2}$-specific fragments were obtained, a large fragment (127 nucleotides), which is protected from the Xba I site to the 3′ end, and a 35-nucleotide fragment, which corresponds to a protected fragment extending from the Bgl II site to the Xba I site, where splicing takes place. The smaller fragment is not resolved on this gel. The positions of four molecular size marker fragments are shown (M in base pairs). A riboprobe hybridizing to the histone H4mRNA was used as an internal control to standardize the amount of RNA used in each sample. Samples were analyzed on a 6% acrylamide denaturing gel.

COOH-terminal region of $sG_{i2}$ could affect the subcellular localization of the protein, we did immunocytochemical analysis of COS-7 cells and JEG-3 cells. JEG-3 cells do not express $G_{i2}$ (*20, 21*) and were used as negative controls, whereas COS-7 cells constitutively express the gene (*20*). Although no immunoreactivity was detected in JEG-3 cells (*18*), the analysis of COS-7 cells revealed a labeling of intracellular membrane compartments, most likely the Golgi apparatus (Fig. 4, B, D, and F). In contrast, immunostaining of COS-7 cells with the $G_{i2}$-specific antibody AS7 (anti-$G_{i2}$) (*22*) highlighted mainly the plasma membrane (Fig. 4A). The anti-$G_{i2}$ recognizes the COOH-terminal decapeptide of $G_{i2}$ and therefore does not cross-react with $sG_{i2}$.

To document the Golgi localization of $sG_{i2}$, we treated COS-7 cells with a fluorescent (Bodipy) ceramide analog that has been shown to stain the Golgi apparatus (*23*). The staining of the Golgi by this method was comparable to that obtained with the anti-$sG_{i2}$ (Fig. 4, C and D). In addition to the Golgi, some other vesicular structures were labeled with both ceramide and anti-$sG_{i2}$, although they were brighter with anti-$sG_{i2}$. A minor perinuclear staining was visible with anti-$sG_{i2}$, possibly due to the labeling of the endoplasmic reticulum or to extensions of the Golgi apparatus not evidentiated by ceramide. The localization of $sG_{i2}$ was further characterized by double staining experiments with monoclonal antibody to β-COP (anti–β-COP) and anti-$sG_{i2}$. The protein β-COP is associated with the Golgi apparatus and nonclathrin coated vesicles (*24*). Double staining experiments of fixed COS-7 cells with anti–β-COP and anti-$sG_{i2}$ demonstrated that the two antigens colocalize in the same cellular compartment (Fig. 4, E and F).

The finding that β-COP and $sG_{i2}$ colocalize in the same cellular compartment is of interest because it has been shown that β-COP needs the participation of a heterotrimeric G protein to initiate its association to the membrane (*5*). The COOH-terminal portion of $G_{i2}$, in common with that portion of other G proteins, mediates coupling of these proteins to membrane receptors, and this interaction is essential for G protein activation (*19, 25, 26*). Thus, because the conserved COOH-terminal region in $sG_{i2}$ has been replaced, the specificity of interaction with membrane receptors may be modified or abolished.

The COOH-terminal 24 amino acids of G proteins mediate coupling of certain G protein–coupled membrane receptors to the appropriate intracellular effector. Switching of the COOH-terminal amino acids of the G protein $G\alpha_q$ with the corresponding region of $G_{i2}$ confers on the dopamine D2 and adenosine 1 receptors, inhibitors of the

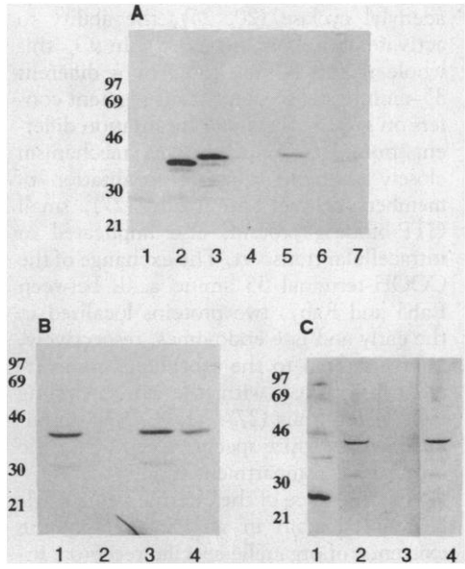


**Fig. 3.** Characterization of the $sG_{i2}$ protein. (**A**) In vitro translation of the vector alone (lane 1), $G_{i2}$ (lane 2), and $sG_{i2}$ (lane 3) RNA. The product of $sG_{i2}$ is larger than that obtained by the translation of $G_{i2}$. The predicted sizes are 41.5 kD for $sG_{i2}$ and 40.5 kD for $G_{i2}$. Immunoprecipitations of the in vitro–translated $G_{i2}$ and $sG_{i2}$ proteins with anti-$sG_{i2}$ (lanes 4 and 5) and anti-$G_{i2}$ (lanes 6 and 7). Each antibody immunoprecipitated only the specific protein. (**B**) Protein immunoblot analysis of transfected and endogenous $sG_{i2}$ protein. Membranes were prepared as described (*21*). JEG-3 cells transfected with the $sG_{i2}$ expression vector (lane 1) or untransfected (lane 2) and membranes from brain (lane 3) and pituitary (lane 4). A band of the expected size was detected in transfected JEG-3 cells, which do not endogenously express the $G_{i2}$ gene (*20, 21*), and in brain and pituitary. The immunoprecipitation and immunoblot were done with anti-$sG_{i2}$, the rabbit polyclonal antibody 293, at a dilution of 1:100. (**C**) The specificity of anti-$sG_{i2}$ was tested by peptide competition. Samples of membranes prepared from mouse brain were electrophoresed and blotted. Strips of the blot were then incubated with preimmune serum (lane 1), anti-$sG_{i2}$ (lane 2), anti-$sG_{i2}$ in the presence of the peptide used for the immunization (lane 3), and anti-$sG_{i2}$ in the presence of a nonspecific peptide (lane 4). Molecular sizes (in kilodaltons) are indicated.

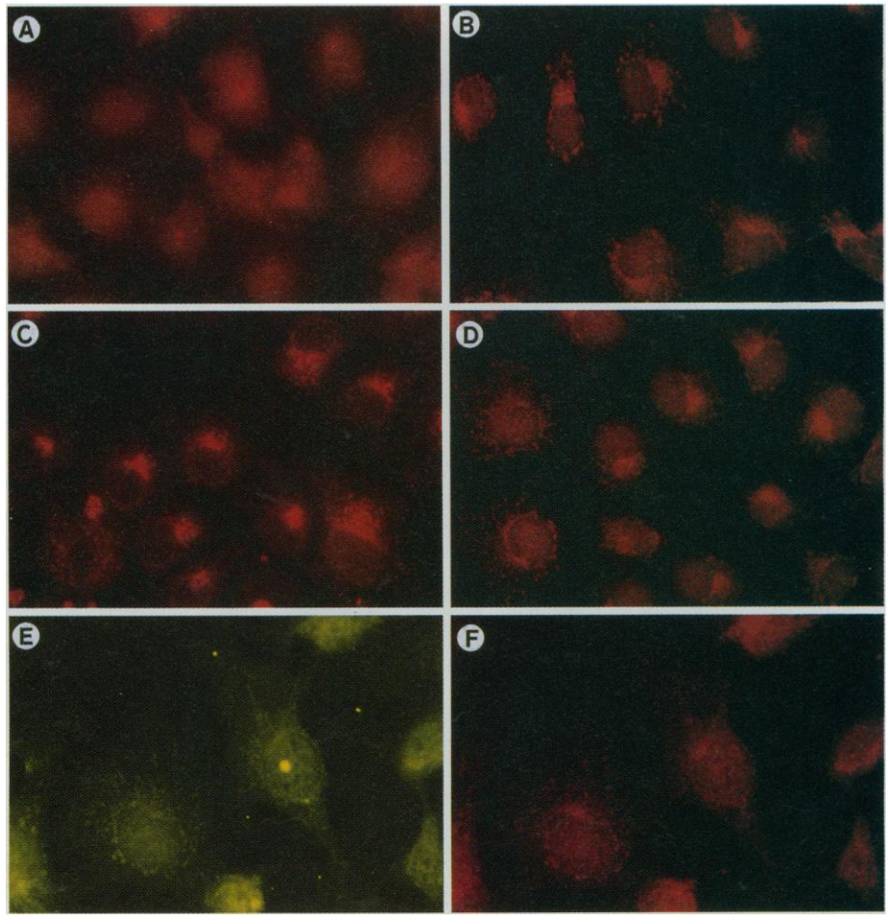


**Fig. 4.** Immunocytochemical localization of $sG_{i2}$. (**A**) Immunostaining of fixed COS-7 cells with anti-$G_{i2}$ (AS7) and anti-$sG_{i2}$ (**B**). (**C**) Bodipy ceramide staining of living COS-7 cells compared with anti-$sG_{i2}$ staining of fixed cells (**D**). Double staining of COS-7 cells with antibodies to β-COP (M3A5) (*24*) (**E**) or $sG_{i2}$ (**F**). Cells were processed as described (*29*).

adenylyl cyclase (*20*, *25*), the ability to activate phospholipase C (*25*). In $sG_{i2}$ this whole region is substituted by a different 35–amino acid segment. This segment confers on $sG_{i2}$ a subcellular localization different from that of $G_{i2}$. This mechanism closely resembles that for localization of members of the Rab family (*27*), small GTP-binding proteins also implicated in intracellular transport. The exchange of the COOH-terminal 35 amino acids between Rab5 and Rab7, two proteins localized in the early and late endosomes, respectively, retargets them to the subcellular compartment associated with the corresponding COOH-terminus (*27*). The COOH-terminus may recognize specific receptors in the appropriate compartment.

The presence of the alternative COOH-terminal domain in $sG_{i2}$ may imply the existence of organelle-specific receptors involved in its recognition. The function of this protein might be synergistic with that of other GTP-binding proteins in the regulation of membrane transport mechanisms.

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15. For PCR analysis cDNA from mouse pituitary RNA was prepared (*20*) with 2 μg of total RNA. A portion of the reverse transcribed RNA was then used for PCR reactions. In each reaction 5′ and 3′ oligonucleotide primers (1 μg of each) were used. The 5′ oligonucleotide sequence GCCAACAAG-TACGACGAGGCA corresponds to position 879 to 899 of the $G_{i2}$ coding sequence. The 3′ oligonucleotide corresponds to the last 18 nucleotides of the $sG_{i2}$ coding region illustrated in Fig. 1C. To amplify mouse genomic DNA, we used 500 ng of intact DNA. The DNA was previously denatured at 94°C for 10 min. PCR was done under the same conditions used to amplify the cDNA described elsewhere (*21*). PCR products were separated on agarose gels, blotted, and hybridized with a $^{32}P$-labeled oligonucleotide probe corresponding to the first 18 nucleotides of the $sG_{i2}$ translated sequence in exon 9 (Fig. 1C).
16. The $sG_{i2}$ cDNA fragment from the Bam HI site at position 634 to 20 base pairs (bp) 3′ from the TGA was inserted into the Hind II site of the pBluescript polylinker. The plasmid DNA was linearized at the Bgl II site, and a uniformly labeled antisense transcript was synthesized from the T3 RNA polymerase promoter, incorporating [$\alpha$-$^{32}P$]uridine triphosphate. RNA probes were obtained with a Riboprobe kit (Promega). The ribonuclease protection assays were done as described (*28*).
17. The cDNAs corresponding to $G_{i2}$ and $sG_{i2}$ were subcloned in the Not I site of the expression vector p513 (*11*), linearized, and transcribed in vitro with T7 RNA polymerase. Purified RNA was then translated and labeled in vitro with a rabbit reticulocyte lysate system (Promega) including [$^{35}S$]methionine (Amersham). The products were analyzed on 10% polyacrylamide gel. Immunoprecipitations were done as in J. R. Barber and I. Verma, [*Mol. Cell. Biol.* **7**, 2201 (1987)].
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29. COS-7 cells were fixed in acetone:methanol and incubated overnight with the specific antibody at the appropriate dilution. No specific staining was obtained when $sG_{i2}$ was preincubated with an excess amount of the peptide used to obtain the antibody. The antibody 293 was visualized with Texas red–labeled goat antibody to rabbit immunoglobulin G. The M3A5 mouse monoclonal antibody was revealed by fluorescein–labeled goat antibody to mouse immunoglobulin G. The secondary antibody used for $sG_{i2}$ was also used with AS7. The Bodipy ceramide (Molecular Probes, Oregon) staining of living COS-7 cells was done as in (*23*).


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# The Met Proto-Oncogene Mesenchymal to Epithelial Cell Conversion


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**Coexpression of the human Met receptor and its ligand, hepatocyte growth factor/scatter factor (HGF/SF), in NIH 3T3 fibroblasts causes the cells to become tumorigenic in nude mice. The resultant tumors display lumen-like morphology, contain carcinoma-like focal areas with intercellular junctions resembling desmosomes, and coexpress epithelial (cytokeratin) and mesenchymal (vimentin) cytoskeletal markers. The tumor cells also display enhanced expression of desmosomal and tight-junction proteins. The apparent mesenchymal to epithelial conversion of the tumor cells mimics the conversion that occurs during embryonic kidney development, suggesting that Met-HGF/SF signaling plays a role in this process as well as in tumors that express both epithelial and mesenchymal markers.**


The *met* proto-oncogene product (Met) is a member of the family of tyrosine kinase growth factor receptors (*1*, *2*), and its ligand is hepatocyte growth factor/scatter factor (HGF/SF) (*3*–*5*). HGF/SF mediates liver regeneration in vivo (*6*), induces differentiation of Madin-Darby canine kidney (MDCK) epithelial cells into branching tubules (*7*), and promotes epithelial cell motility and invasiveness in vitro (*5*, *8*). Two lines of evidence (*9*) suggest that Met is involved in the formation and maintenance of epithelial lumenal structures: (i) Met is expressed in epithelial cells bordering lumenal structures in a variety of tissues, including cells that border the mammary duct, and (ii) treatment of certain carcinoma cell lines with human HGF/SF ($HGF/SF^{hu}$) induces the formation of lumenal structures in vitro.

NIH 3T3 cells produce murine $HGF/SF^{mu}$


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