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The Carboxyl-terminal *CXXX* Sequence of $G_i\alpha$, but Not Rab5 or Rab11, Supports Ras Processing and Transforming Activity*

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Although the heterotrimeric $G_i\alpha$ subunit terminates in an apparent *CXXX* prenylation signal (CGLF), it is not modified by isoprenylation. To determine if the $G_i\alpha$ *CXXX* sequence can signal prenylation when placed at the carboxyl termini of normally prenylated proteins, we have characterized the processing and biological activity of chimeric oncogenic Ras proteins that terminate in the $G_i\alpha$ *CXXX* sequence (Ras/$G_i\alpha$). Surprisingly, these chimeras were prenylated both *in vivo* and *in vitro*, demonstrated significant membrane association, exhibited transforming activity, and induced transcriptional transactivation from Ras-responsive elements. We then extended these studies to determine if, unlike the CC or *CXC* carboxyl-terminal sequences of other Rab proteins, the carboxyl-terminal *CXXX* sequences of the Ras-related Rab5 and Rab11 proteins represent conventional *CXXX* prenylation signals that can support Ras processing and transforming activity. Unexpectedly, these Ras/Rab chimeras were nonprenylated, were cytosolic, and lacked detectable transforming or transcriptional transactivation activity. Taken together, these results suggest that the context within which a *CXXX* sequence occurs may also critically control the modification of a protein by prenylation, and that the Rab5 and Rab11 carboxyl termini do not possess conventional *CXXX* sequences. Instead, their CC*XX* and CC*XXX* motifs may represent additional classes of protein prenylation signals.

Recent studies have established the identity of many proteins that undergo post-translational modification by prenylation, the addition of an isoprenoid group, to one or more cysteines at or near their carboxyl termini (1–3). These include proteins involved in a wide variety of cellular processes such as signal transduction, intracellular vesicular transport, cytoskeletal organization, cell growth control and polarity, viral replication, and protein folding/assembly (reviewed in Ref. 4). Protein prenylation is currently known to be signaled by at least three distinct carboxyl-terminal motifs, designated *CXXX*,[1] CC, or *CXC* (where C is cysteine and *X* is any amino acid). Proteins terminating in *CXXX* sequences are specifically modified by the addition of either farnesyl or geranylgeranyl groups, which are attached by farnesyltransferase (FTase) or geranylgeranyltransferase I (GGTase I), respectively. In contrast, CC and *CXC* motifs signal modification only by geranylgeranyl groups, which are attached by a third enzyme, geranylgeranyltransferase II (GGTase II).

The *CXXX* prenylation sequence of Ras proteins has been the best studied and the most extensively characterized (4, 5). The modifications signaled by this sequence include farnesyl addition to the cysteine residue of the *CXXX* motif, followed by two closely linked modifications, proteolytic cleavage of the terminal *XXX* residues and carboxyl methylation of the now-terminal cysteine. Of the three *CXXX*-signaled modifications, the farnesylation step alone is both necessary and sufficient to trigger Ras membrane association and transforming activity (6). Nonfarnesylated mutant Ras proteins are no longer membrane associated and are defective in transforming activity (7, 8). The functions of other prenylated proteins have also been shown to be critically dependent on their prenylation (4).

Results from *in vitro* and *in vivo* prenylation studies strongly suggest that the *CXXX* tetrapeptide sequence alone is sufficient to signal isoprenylation of Ras proteins. First, synthetic tetrapeptides corresponding to the *CXXX* sequences of Ras proteins function both as efficient inhibitors of and as substrates for FTase activity *in vitro* (9–11). Second, the addition of a Ras *CXXX* sequence to normally nonprenylated proteins such as Protein A is sufficient to trigger their modification by isoprenoids (7). Furthermore, since substitution of the terminal residue of the Ras *CXXX* sequence with leucine results in modification of Ras by geranylgeranylation rather than by farnesylation (12–17), this suggests that GGTase I also possesses a simple *CXXX* tetrapeptide recognition sequence. These observations are in sharp contrast to those seen with the CC or *CXC* prenylation signal sequences characteristic of most Rab/YPT proteins (18–20), whose modification by GGTase II shows an absolute requirement both for sequence information in addition to the CC/*CXC* motifs and for protein conformation (11, 21–23).

We and others have utilized chimeric Ras proteins that terminate in heterologous sequences to determine the sequence requirements for protein prenylation. For example, it has been shown that *CXXX* sequences that signal geranyl-

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[1] The abbreviations used are: *CXXX*/CC/*CXC*, where *C* is cysteine and *X* is any amino acid; FTase, farnesyltransferase; GGTase I, geranylgeranyltransferase I; GGTase II, geranylgeranyltransferase II; MVA, mevalonic acid; PAGE, polyacrylamide gel electrophoresis; CAT, chloramphenicol acetyltransferase.

geranylation, rather than farnesylation (12, 14, 16, 17, 24), can support Ras prenylation and transforming activity (17, 24). In contrast, Ras proteins terminating in either the CC or CXC sequences characteristic of Rab/YPT proteins do not undergo isoprenylation and lack transforming activity (11, 23). Therefore, in the present study we have utilized chimeric Ras proteins to determine the following: (*a*) the basis for the lack of prenylation of the heterotrimeric $G_i\alpha$ protein, despite the presence on it of a carboxyl-terminal *CXXX* sequence, and (*b*) if the Rab5 and Rab11 carboxyl-terminal *CXXX* sequences, in contrast to the rab CC/CXC sequences, can support Ras processing and transforming activity. Unexpectedly, we observed that the $G_i\alpha$ *CXXX* sequence, but not the Rab5 or Rab11 *CXXX* sequences, could signal the prenylation, membrane association, and transforming activity of oncogenic Ras proteins. These results demonstrate that the sequences upstream of at least some *CXXX* sequences do critically influence protein prenylation, indicating the importance of considering the prenylation status of a given tetrapeptide *CXXX* sequence in the context of its intact protein, and they further suggest that the Rab CC*XX* and CC*XXX* sequences may represent additional classes of carboxyl-terminal motifs that are functionally distinct from the *CXXX* motif and are instead more related to the CC and CXC motifs of other Rab proteins.

EXPERIMENTAL PROCEDURES

Ras CXXX Mutagenesis and Molecular Constructs—Oncogenic [Leu[61]]H-*ras* or [Val[12]]K-*ras*4B mutant sequences that encode carboxyl-terminal *CXXX* mutants (Table I) were generated by oligonucleotide-directed mutagenesis using *Taq* polymerase chain reaction DNA amplification as described previously (20). All mutated sequences were verified by dideoxy sequencing. The resulting fragments were introduced into the pAT-*ras*H bacterial expression vector for expression and purification of recombinant Ras proteins and into the pZIP-NeoSV(X)1 retrovirus vector for expression in mammalian cells (25).

Cell Culture and Transformation Assays—NIH 3T3 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum, and DNA transfections by the calcium phosphate precipitation technique were done as described previously (25), using 10 ng/dish pZIP-*ras*H DNA encoding each mutant or authentic Ras protein. Transfected cells were maintained in growth medium, and transformed foci were quantitated after 14 days. Transfected NIH 3T3 cells were also selected in growth medium containing G418 (Geneticin, GIBCO/BRL) (400 μg/ml) to establish cell lines expressing each mutant Ras protein.

Subcellular Localization and Analysis of Post-translational Processing—G418-selected NIH 3T3 cells expressing each mutant protein were labeled overnight in growth medium supplemented with either [^{35}S]methionine/cysteine (Tran^{35}S-label, ICN) (200 μCi/ml) or [5-^{3}H]mevalonolactone (Du Pont-New England Nuclear) (200 μCi/ml), which is converted *in vivo* to [^{3}H]mevalonic acid (MVA). Metabolic labeling was done in the presence or absence of 50 μM compactin (provided by J. L. Goldstein, University of Texas Southwestern Medical Center, Dallas, TX). For fractionation analysis, labeled cells were separated into crude membrane (P100)- and cytosolic (S100)-containing fractions by centrifugation (100,000 × *g*, 30 min) as described previously (26). Ras proteins were immunoprecipitated from each fraction using mouse monoclonal antibodies 146-3E4 (specific for H-Ras) or 142-4E5 (recognizing a shared Ras determinant) (both from Quality Biotech, Camden, NJ), resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and subjected to fluorographic analysis.

Transcriptional Transactivation Assays—NIH 3T3 cells were transiently transfected with 100 ng of pZIP-*ras* DNA encoding authentic or mutant Ras proteins, which was calcium phosphate-precipitated along with 10 μg of carrier DNA and 1 μg of a chloramphenicol acetyltransferase (CAT) reporter gene (pBX4-CAT; kindly provided by B. Wasylyk) driven by a β-actin promoter containing four tandem copies of the Ras-responsive element from the polyomavirus enhancer element (27). Forty-eight h after transfection, cell lysates were prepared, and the CAT activity induced by each pZIP-*ras* construct was assayed as described previously (28).

RESULTS AND DISCUSSION

The $G_i\alpha$ CXXX Sequence Can Signal Ras Prenylation, Membrane Association, and Transforming Activity—To determine if the $G_i\alpha$ carboxyl-terminal *CXXX* sequence (CGLF) can signal prenylation when placed at the carboxyl termini of normally prenylated proteins, we generated constructs encoding chimeric oncogenic Ras proteins (designated H-Ras/$G_i\alpha$ and K-Ras/$G_i\alpha$) terminating in the *CXXX* sequence (CGLF) of the nonprenylated α1 subunit of the heterotrimeric G_i protein (Table I). The resulting mutant Ras sequences were then transfected into NIH 3T3 cells for characterization of their biological activity. Unexpectedly, both Ras/$G_i\alpha$ chimeras exhibited potent focus-forming activity (Table I), and cells stably expressing each of these proteins exhibited the same transformed morphology as those expressing authentic [Leu[61]]H-Ras and [Val[12]]K-Ras4B (Fig. 1, *panels b* and *c*). Since the ability of Ras proteins to induce transcriptional activation from promoters containing Ras-responsive elements correlates directly with transforming potential (28), we evaluated the Ras/$G_i\alpha$ chimeric proteins for this property. Both chimeras exhibited the ability to activate transcription via a Ras-responsive element (Fig. 2, *panel A*, *d*, and *panel B*, *d*). This activity was comparable to the strong induction observed with authentic [Leu[61]]H-Ras and [Val[12]]K-Ras (Fig. 2, *panel A*, *b*, and *panel B*, *a*), and contrasts sharply with the complete lack of ability of a nonprenylated cytosolic mutant ([Leu[61],Ser[186]]H-Ras) to induce transactivation (Fig. 2*A*, *c*). Taken together, these results suggest that the $G_i\alpha$ carboxyl-terminal sequence CGLF can be a functional prenylation signal sequence when placed downstream of Ras carboxyl-terminal sequences and can support Ras-transforming activity.

To confirm that the biologically active chimeric proteins

TABLE I

Carboxyl-terminal sequences and properties of chimeric Ras CXXX proteins

Protein	Carboxyl-terminal sequence	FFU[a]	MVA[b]
[Leu[61]]H-Ras	DESGPGCMSCKCVLS	1.00	+
[Val[12]]K-Ras4B	GKKKKKKSKTKCVIM	1.00	+
$G_i\alpha$	TDVIIKNNLKDCGLF	ND[c]	–
Rab5	LTEPTQPTRNQCCSN	0.00	+
Rab11	PTTENKPKVQCCQNI	0.00	+
H-Ras/$G_i\alpha$	DESGPGCMSCKCGLF	0.95	+
K-Ras/$G_i\alpha$	GKKKKKKSKTKCGLF	1.02	+
[Phe[189]]H-Ras	DESGPGCMSCKCVLF	0.94	+
[Phe[188]]K-Ras	GKKKKKKSKTKCVIF	0.82	+
[Gly[186]]K-Ras	GKKKKKKSKTKCGIM	1.11	+
H-Ras/Rab5	DESGPGCMSCKCCSN	0.00	–
H-Ras/Rab11	DESGPGCMSCKCQNI	0.00	–
[Cys[186]]K-Ras4B	GKKKKKKSKTKCCIM	1.00	+
[Asn[188]]K-Ras4B	GKKKKKKSKTKCVIN	1.00	+
[Ile[189]]H-Ras	DESGPGCMSCKCVLI	0.95	+

[a] Relative focus-forming activity in NIH 3T3 cells. Five × 10^6 cells were plated in quadruplicate 60-mm dishes and transfected with 10 ng of each pZIP-*ras* plasmid DNA construct. Foci were quantitated after 14 days, and the activity of each mutant was normalized to that of the authentic [Leu[61]]H-Ras or [Val[12]]K-Ras (1–4 × 10^3 foci/μg of DNA). Focus-forming units (FFU) are reported above as the average of at least three independent transfections into quadruplicate dishes. Mutant *ras* DNAs negative for focus-forming activity at 10 ng/dish were transfected at up to 5 μg/dish.

[b] Incorporation of [^{3}H]MVA into Ras proteins was determined following metabolic labeling of stably transfected cells expressing each *CXXX* variant as described under "Experimental Procedures." Labeling experiments were performed at least twice.

[c] Not determined.

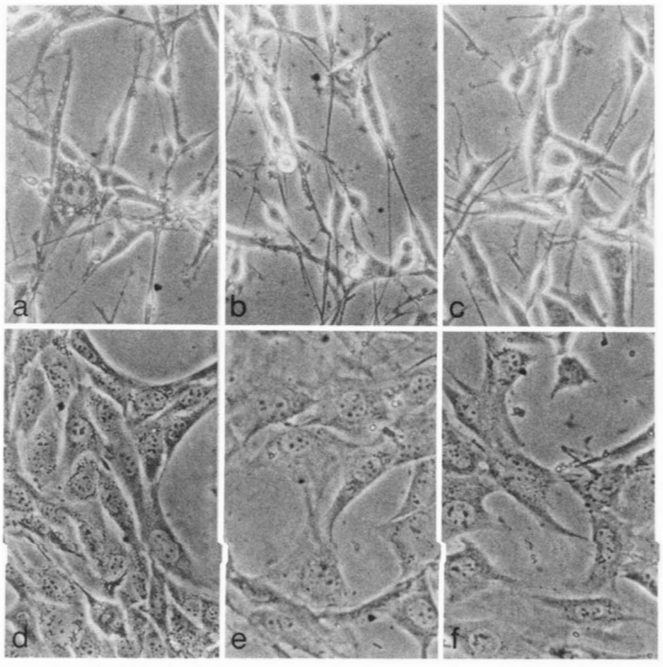

FIG. 1. **Morphology of NIH 3T3 cells expressing mutant Ras proteins with heterologous *CXXX* sequences.** Cells were transfected with 10 ng of each pZIP-*ras* (neo^r) construct and transfectants were selected in G418-containing medium. *a*, [Leu^{61}]H-Ras; *b*, H-Ras/G$_i\alpha$; *c*, K-Ras/G$_i\alpha$; *d*, control, untransformed cells; *e*, H-Ras/Rab5; *f*, H-Ras/Rab11.

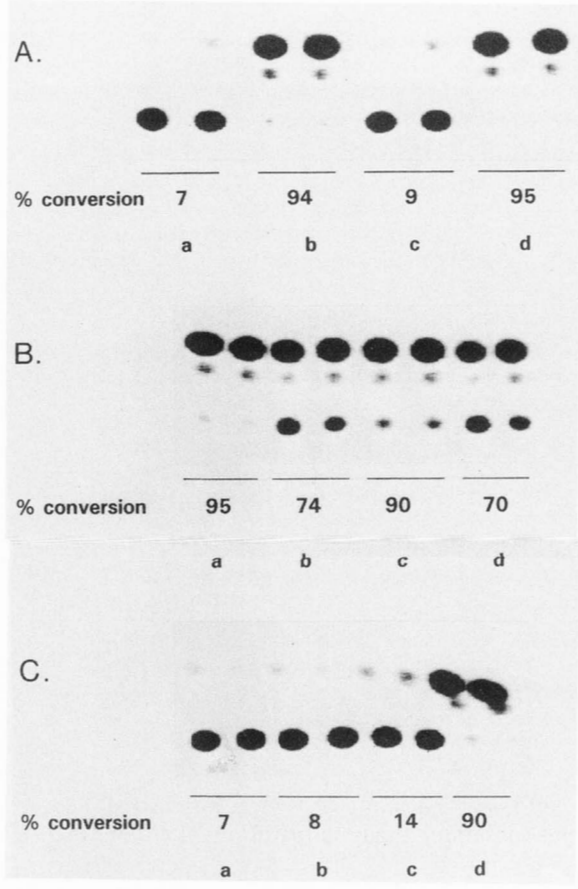

FIG. 2. **TLC analysis of transcriptional transactivation by Ras proteins terminating in different *CXXX* motifs.** NIH 3T3 cells were transiently co-transfected with a CAT reporter gene and the pZIP-*ras* plasmid DNAs as described under "Experimental Procedures." CAT activity present in cell lysates was determined by quantitating the conversion of unacetylated to acetylated chloramphenicol. Thin layer chromatograms of each sample were subjected to AMBIS β scanning for quantitation of the percentage of acetylated chloramphenicol. The numerical percent conversion shown is the average of the duplicate determinations shown; all experiments were performed in duplicate and repeated at least three times. *Panel A*, H-Ras/G$_i\alpha$: *a*, empty pZIP vector; *b*, [Leu^{61}]H-Ras; *c*, [Leu^{61},Ser^{186}]H-Ras; *d*, H-Ras/G$_i\alpha$. *Panel B*, K-Ras/G$_i\alpha$: *a*, [Val^{12}]K-Ras; *b*, [Val^{12},Phe^{188}]K-Ras; *c*, [Val^{12},Gly^{186}]K-Ras; *d*, K-Ras/G$_i\alpha$. *Panel C*, Ras/Rab: *a*, H-Ras/Rab5; *b*, H-Ras/Rab11, *c*, [Cys^{185}]H-Ras/Rab11; *d*, [Leu^{61},Ile^{189}]H-Ras.

were prenylated and membrane-associated, NIH 3T3 cells expressing H-Ras/G$_i\alpha$ were labeled metabolically with [^{35}S] methionine/cysteine. Fractionation analysis and subsequent immunoprecipitation with the H-Ras specific monoclonal antibody 146-3E4 revealed that the electrophoretic mobility of the membrane-associated ("P") form differs from that of the unprocessed, cytosolic ("S") form (Fig. 3*A*, Ras/G$_i\alpha$ *P*− and *S*−, respectively) and is consistent with farnesylated Ras protein. This mobility difference, although distinct from that of the fully processed (farnesylated, proteolytically clipped and carboxymethylated) authentic [Leu^{61}]H-Ras (Fig. 3*A*, compare *P*− forms of Ras and Ras/G$_i\alpha$), could be prevented by treatment with compactin, an inhibitor of mevalonate biosynthesis, suggesting that prenylation of H-Ras/G$_i\alpha$ had occurred *in vivo* (Fig. 3*A*, compare *S*/*P*− and *S*/*P*+). This was confirmed by the detection of incorporation of a product of [^{3}H]MVA into H-Ras/G$_i\alpha$ (data not shown).

Interestingly, the different mobilities of the membrane-associated forms of the Ras/G$_i\alpha$ chimeras compared to authentic Ras suggest that they are incompletely processed. The mobility at which the Ras/G$_i\alpha$ chimeras migrate is comparable to that of the partially processed, transforming Ras mutant [Tyr^{187}]K-ras, which we have shown previously (6) to be farnesylated, but not carboxymethylated. Furthermore, as assessed by laser densitometric scanning of the autoradiogram, only 26% of the chimeric protein partitioned into the crude membrane fraction (Fig. 3*A*, *Ras/G$_i\alpha$ P*−), compared to essentially all of the authentic [Leu^{61}]H-Ras (Fig. 3*A*, *Ras P*−), suggesting that a lower percentage of the chimeric protein becomes prenylated. Similar results were obtained upon fractionation and immunoprecipitation of K-Ras/G$_i\alpha$ (data not shown). Thus, when placed within the context of the Ras protein, the CGLF sequence does signal prenylation, although inefficiently compared to the Ras *CXXX* sequences. The lower amount of processed, functional Ras protein is nevertheless sufficient for full morphologic transformation of NIH

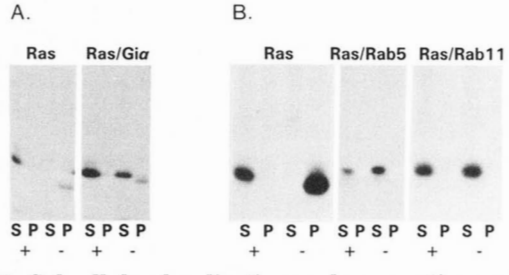

FIG. 3. **Subcellular localization and compactin sensitivity of H-Ras/G$_i\alpha$ and Ras/Rab chimeric proteins expressed in NIH 3T3 cells.** Cells were labeled metabolically with [^{35}S]methionine/cysteine in the presence (+) or absence (−) of compactin, an inhibitor of mevalonate biosynthesis. Following fractionation into crude soluble (*S*) and particulate (*P*) fractions at 100,000 × *g*, labeled chimeric proteins were visualized by immunoprecipitation with H-Ras-specific antibody 146-3E4 followed by SDS-PAGE and fluorography, as described under "Experimental Procedures."

3T3 cells (Fig. 1, *panels b* and c, and Table I).

Our observation that the Ras/$G_i\alpha$ mutants were biologically active and prenylated was unexpected in light of recent studies demonstrating that $G_i\alpha$ is not prenylated (29–33). Although it is possible that we detected prenylation of Ras/$G_i\alpha$ due to overexpression, we consider this unlikely, particularly since two of the studies demonstrating the failure of authentic $G_i\alpha$ to become prenylated were performed in COS cell transient overexpression systems (30, 32).

On the other hand, this observation is not altogether surprising, considering that the $G_i\alpha$ and H-Ras *CXXX* sequences differ by only 2 residues (CGLF *versus* CVLS), neither of which individually perturbs Ras prenylation or transforming activity to a significant extent (6). As determined in an *in vitro* reticulocyte lysate prenylation assay, changing only the final residue of H-Ras to phenylalanine ([Phe^{189}]H-Ras) produces a different specificity of prenylation, from farnesylation to geranylgeranylation, as expected when X_3 = Phe, without altering the efficiency of prenylation (data not shown). When expressed in NIH 3T3 cells, the [Phe^{189}]H-Ras protein is fully processed, and equally membrane-associated (data not shown) and transforming as the authentic [Leu^{61}]H-Ras protein (Table I). Similar prenylation and biological activity were observed with [Phe^{188}]K-Ras (Table I and Fig. 2*B*, *b*). Although a glycine residue at the X_1 position partially perturbs processing of an oncogenic [Val^{12},Gly^{186}]K-Ras protein (6), this mutant is nevertheless highly transforming (Table I and Ref. 6) and transcriptionally active (Fig. 2*B*, *c*). The small degree of impairment in prenylation seen in the mutant Ras proteins containing single substitutions at either the X_1 or X_3 position contrasts with the incomplete (~26%) prenylation observed with the doubly substituted CGLF sequence; taken together, these results suggest that the combination of glycine at X_1 and phenylalanine at X_3 compromises the signaling efficiency of the CGLF sequence, even when attached to a normally prenylated protein.

The results presented here indicate that sequences upstream of at least some *CXXX* sequences do critically influence protein prenylation, indicating the importance of considering the prenylation status of a given tetrapeptide *CXXX* sequence in the context of its intact protein. It has been demonstrated previously that the addition of the Ras *CXXX* sequence CVLS to the carboxyl terminus of $G_i\alpha$1 results in the prenylation of the $G_i\alpha$ polypeptide (32). Since the sequence CGLF terminates in phenylalanine, it should signal modification by GGTase I (11, 13). Our results therefore suggest that GGTase I may be influenced more critically by upstream sequences than is the FTase enzyme that prenylates authentic Ras proteins.

The Rab5 and Rab11 CXXX Sequences Cannot Signal Prenylation of Ras Proteins—Unlike the majority of Rab proteins, which terminate in CC or *CXC* motifs, both Rab5 and Rab11 terminate in apparent *CXXX* motifs (34). Therefore, it was expected that these Rab proteins, like non-Rab proteins terminating in conventional *CXXX* motifs, would be substrates for a *CXXX* prenyltransferase (*e.g.* FTase or GGTase I), rather than for the GGTase II enzyme, which prenylates Rab proteins terminating in CC or *CXC* motifs (22, 23). Furthermore, since oncogenic Ras-transforming activity can be supported by either farnesyl or geranylgeranyl addition (17, 24), it was expected that chimeric Ras proteins terminating in Rab5 or Rab11 *CXXX* sequences would not only be fully prenylated, but also membrane-associated, and transforming.

To address directly whether the Rab5 CCSN and Rab11 CQNI carboxyl-terminal sequences function as conventional *CXXX* prenylation signal sequences, constructs encoding chimeric oncogenic [Leu^{61}]H-Ras proteins terminating in either CCSN (Ras/Rab5) or CQNI (Ras/Rab11) were generated and transfected into NIH 3T3 cells. Unexpectedly, neither Ras/Rab chimeric protein was processed and both were biologically inactive. Both chimeras lacked any detectable focus-forming activity (Table I), and cells expressing these mutant proteins displayed the nontransformed morphology of normal NIH 3T3 cells (Fig. 1, *panels e* and *f*). Additionally, neither of these mutants was active in CAT assays (Fig. 2*C*, *a* and *b*). Fractionation analysis revealed that both proteins were completely cytosolic (Fig. 3*B*, *– lanes*) and migrated with unprocessed H-Ras protein (compare to *Ras S+*). Furthermore, compactin treatment did not affect their electrophoretic mobility (Fig. 3*B*, *+ lanes*), nor did these proteins incorporate [^{3}H]MVA in *in vitro* or *in vivo* prenylation analyses (data not shown).

The inability of the Rab5 sequence, CCSN, to signal Ras processing was particularly unexpected, since each of its *CXXX* residues, when introduced individually into the Ras *CXXX* sequence, can support Ras prenylation (Table I and Refs. 6, 11, and 35). Nor was the inability of the CQNI sequence of Rab 11 to signal prenylation due to its unique terminal residue, since a mutant H-Ras protein in which only the final residue was changed to isoleucine ([Ile^{189}]H-Ras) was still an excellent substrate for prenylation *in vitro* (data not shown) and *in vivo* (Table I), and was both transforming (Table I) and transcriptionally active (Fig. 2*C*, *d*). Therefore, while these Rab proteins possess apparent *CXXX* motifs, the requirement for additional upstream Rab sequences to complement the *CXXX* sequence suggests that the prenylation signals of Rab5 and Rab11 are not conventional *CXXX* motifs, but are instead more similar to those of Rab proteins terminating in CC or *CXC* sequences. Thus, the particular Rab5 *CCXX* and Rab11 *CCXXX* sequences may define two additional classes of prenylation signals.

The results presented here complement recent observations that Rab5 prenylation was not blocked by Rab5 carboxyl-terminal peptides and that Rab5 is not prenylated by GGTase I (36). We and others have recently shown that the Rab proteins terminating in CC and *CXC* motifs are modified by a common enzyme, GGTase II (22, 23), and competition studies suggest that Rab5 is also modified by GGTase II.[2] Whether the *CCXXX* motif of Rab11 is also recognized by GGTase II and whether the Rab5 and Rab11 proteins undergo a combination of modifications distinct from those signaled by the CC or *CXC* motifs remain to be determined.

The many members of the Rab protein family are believed to cycle between distinct intracellular compartments of the endocytic and exocytic secretory pathway to facilitate the accurate and unidirectional flow of vesicular transport (37). We speculate that the apparent complexity of Rab carboxyl-terminal motifs, which include CC, *CXC*, *CXXX*, *CCXX*, *CCXXX*, and possibly *CCX* sequences (reviewed in Ref. 34), may reflect a similar complexity of modifications at the carboxyl termini required to support their diverse intracellular locations and complex functions (37). Therefore, we anticipate that understanding the modifications signaled by each Rab carboxyl-terminal motif, defining the enzymes that catalyze these modifications, and determining whether there is a unique function for each motif will be critical to understanding the mechanism(s) by which Rab proteins regulate the complex processes of endocytic and exocytic vesicular transport. For example, we have recently demonstrated the involvement of Rab1a, Rab1b, and Rab2 in regulating vesicular transport between the endoplasmic reticulum and the Golgi

[2] R. Khosravi-Far, M. Sinensky, and C. Der, unpublished data.

complex (38, 39). These three Rab proteins terminate in CC sequences, and it will be important to determine if their ability to regulate transport can also be facilitated by Rab mutants that terminate instead in C*XC*, CC*XX*, or CC*XXX* sequences.

In summary, the addition of a C*XXX* sequence (CGLF) from a normally nonprenylated protein ($G_i\alpha$) to Ras upstream sequences results in the prenylation of Ras, whereas the addition of C*XXX* sequences (CCSN, CQNI) from two normally prenylated proteins (Rab5, Rab11) does not result in Ras prenylation. These results demonstrate that the presence on a given protein of a functional C*XXX* sequence is suggestive, but not conclusive, evidence, that the protein is modified by prenylation. Instead, the ability of a particular C*XXX* sequence to successfully signal isoprenylation must be evaluated within the context of the intact protein. Therefore, while the use of synthetic C*XXX* tetrapeptides has allowed detailed characterization of the substrate specificity of both FTase and GGTase I, the final determination of the physiological and pharmacological substrates for these enzymes may require detailed analyses of full-length proteins. Furthermore, these results indicate that the CC*XX* and CC*XXX* motifs of Rab5 and Rab11 do not appear to function as conventional C*XXX* motifs, but instead may represent new classes of prenylation signal sequences.

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