Fibroblast growth factors are a family of intercellular signaling molecules with multiple and varied roles in animal development. Most are exported from cells by means of a classical amino-terminal signal sequence that is cleaved from the mature protein during its passage through the secretory pathway. Fibroblast growth factor-9 (Fgf-9) does not contain a recognizable signal sequence, although it is efficiently secreted. In this study, we show that Fgf-9 enters the endoplasmic reticulum and traverses the Golgi complex in a similar manner to other constitutively secreted proteins. Deletion and point mutation analysis has revealed an atypical non-cleaved signal sequence within the amino-terminal region of Fgf-9. Moreover, the first 28 amino acids of Fgf-9 can function as an efficient non-cleaved signal peptide when appended to the amino terminus of green fluorescent protein.

Fibroblast growth factors (Fgfs) are a large family of secreted polypeptides that display pleiotropic properties in vitro (1, 2). In addition to acting as mitogens for a wide spectrum of cell types, they can also act to promote cell migration, induce or inhibit cell differentiation. In vivo, Fgf signaling has been implicated in many aspects of animal development, from myoblast migration in Caenorhabditis elegans to a range of inductive and patterning roles in mammals (3, 4). In humans, genetic linkage analysis has implicated three members of the Fgf receptor gene family as the underlying cause of several skeletal dysplasias and autosomal dominant craniosynostosis syndromes (5). In adult mammals, Fgfs are involved in wound healing and can act as potent factors in angiogenesis, whereas their inappropriate expression has been associated with proliferative disorders such as cancer.

Many members of the Fgf family possess a classical amino-terminal signal sequence for export from cells by the constitutive secretory pathway that includes the endoplasmic reticulum (ER) and Golgi complex/trans-Golgi network. However, two notable exceptions are Fgf-1 and Fgf-2 that are located both in the cytoplasm and nucleus (6–8). The cytoplasmic forms of these Fgfs appear to be secreted by novel mechanisms (9–11).

Fgf-3 is also unusual in that it is not only secreted but also an extended uncleaved form is localized in the cell nucleus (12). In this example the dual subcellular localization appears to be the result of competition between a weak classical signal peptide and an adjacent nuclear localization signal (13). Other exceptions are Fgf-11 to Fgf-14 (also known as FGF homologous factors 1 to 4) that represent a less closely conserved group found predominantly in fetal and adult brain, where they reside primarily as nuclear proteins (14). Fgf-9 was discovered as a secreted factor from a glioma cell line and originally named glia-activating factor (15). Expression of Fgf-9 in cell culture demonstrated that it was glycosylated and efficiently secreted, although sequencing of the major secreted isoform showed it had only lost the amino-terminal methionine from the primary translation product (15, 16). Despite its secretion and glycosylation, the amino-terminal domain of Fgf-9 is mildly hydrophobic and does not demonstrate the expected characteristics of a signal peptide (Fig. 1A). More recently Fgf-16 has been identified and shown to be closely related to Fgf-9 on the basis of its primary sequence, and like Fgf-9 it too is secreted although lacking a classical signal sequence (17). Thus it would seem that this family of highly related proteins has evolved or acquired a diverse set of signals for their export from cells. In this report we have analyzed the sequences involved in the secretion of Fgf-9, and we show there is a non-cleaved signal sequence in the amino-terminal domain that directs protein into the constitutive secretory pathway.

EXPERIMENTAL PROCEDURES

mFGF9 Cloning and Constructs—The mouse Fgf-9 coding sequence was generated by reverse transcriptase-PCR from adult mouse brain RNA. The primers were chosen from the rat Fgf-9 sequence (18). The forward primer was GGATCCCATGCTCCCTTATCCGCTTAGAAGT including a BanHI site (underlined), and the reverse primer was GGAAATCTTCAGCTTTGCTTAGAATATCC including an EcoRI site (underlined). The PCR was performed with 4 cycles at 94 °C for 30 s, 53 °C for 1 min, and 72 °C for 1 min followed by 25 cycles with an annealing temperature of 58 °C. The PCR fragment that was identical to the sequence previously published (18) was cloned into pcDNA3 vector (Invitrogen), as were all subsequent modified mFGF9 cDNAs.

Amino-terminal truncations, internal deletions, and point mutations of the mFGF9 cDNA sequence were generated by PCR (Pfu polymerase, Stratagene), and the resulting mutations were confirmed by sequencing. The amino-terminal deletions were constructed using the same 3’ oligonucleotide primer, CCGAATTCCTCGACATGGCTCCCTTAGGTGAAGT including a BanHI site (underlined) and containing a unique BamHI site (underlined) to facilitate subsequent manipulation. The 5’ primers were as follows: mFGF9Δ6, CGGGATCCGGCATGGGAGCTATTTCCGGGTGCTG; mFGF9Δ12, CGCGGATCCCTCGACATGGCTCCCTTAGGTGAAGT; mFGF9Δ19, CGCGGATCCCTCGACATGGCTCCCTTAGGTGAAGT; mFGF9Δ26, CGCGGATCCCTCGACATGGCTCCCTTAGGTGAAGT; mFGF9Δ34, CGCGGATCCCTCGACATGGCTCCCTTAGGTGAAGT; mFGF9Δ42, CGCGGATCCCTCGACATGGCTCCCTTAGGTGAAGT; mFGF9Δ50, CGCGGATCCCTCGACATGGCTCCCTTAGGTGAAGT; mFGF9Δ57, CGCGGATCCCTCGACATGGCTCCCTTAGGTGAAGT; mFGF9Δ63, CGCGGATCCCTCGACATGGCTCCCTTAGGTGAAGT. The sequences of the mutants are schematically depicted in Fig. 3. For the internal deletion mutants, mFGF9Δ32-50 and mFGF9Δ32-52, a two-step cloning strategy was used. The 3’ portion of both mutants encompassed residues 52 to the carboxyl terminus (forward primer, GCAGTCACGGAAATCATGATTTA/reverse primer, GCAGTCACGGAAATCATGATTTA).
1 ml of the same medium but containing 200 uM FGF9 (Prepotech) overnight at 4 °C. Beads were washed once in buffer A (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM EDTA, pH 8.0, 1% Triton X-100) and the reverse primers containing an XbaI site (underlined) adjacent to the translation stop codon (bold). The EcoRI/XbaI-digested PCR product was cloned into the mFGF9-GFP chimera a two-step strategy was used. The GFP from the vector pEGFP-N1 (CLONTECH) was amplified by PCR using the following oligonucleotides: forward primer AAAG-GAGAGAATTCCTCAACATGCTGAGGGAATTCTTCACTGGA/reverse primer GCTCTAGAGCGTTCACCAAGATCTGGCAGTTTGCCGAGTTAC; mFGF9G20K/V22K, ATGATCGAATTCCACCGGCAACCCGTTTGGCTTAGAATATCCTTAT). An EcoRI site made the conservative amino acid changes from DL to EF.

For the point mutants mFGF9G20K, mFGF9V22K, and mFGF9G20K/V22K, the same 5'-oligonucleotides contain. CCGGATCC-TCCACCAGGAAATTCCTCAACATGCTGAGGGAATTCTTCACTGGA, and the reverse primers containing an XbaI site (underlined) adjacent to the translation stop codon (bold). The EcoRI/XbaI-digested PCR product was cloned into the mFGF9:GFP construct (9 residues 52 to the carboxyl terminus) had been removed. The resulting construct (mFGF9:GFP) contains a cDNA encoding the first 28 amino acids of FGF9 fused in-frame to enhanced GFP. A vector (GFP) with the enhanced GFP under the control of the cytomegalovirus promoter was used as a control.

Immunoblotting Analysis—Control COS-1 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% newborn calf serum. The cells were transiently transfected with 4 μg of different plasmid DNA constructs using LipofectAMINE according to the manufacturer’s instructions (Life Technologies, Inc.). In some experiments, tunicamycin was used at 10 μg/ml (Calbiochem) and was added on day 1 after transfection. Cell extracts were prepared by lysis in RIPA buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% deoxycholate, 0.1% SDS, 1% Nonidet P-40) at 4 °C for 30 min, passing the cells through a 23-gauge needle followed by one cycle of freezing/thawing. Cell extracts and cell supernatants obtained by centrifugation were incubated with heparin-Sepharose beads overnight at 4 °C. The beads were washed twice with phosphate-buffered saline (PBS), twice with buffer IV (see under Mitogen Assays) in COS-1 cells. Immunoblotting revealed a major secreted band in COS-1 cell supernatants corresponding to the predicted size of 30 kDa with a smaller, less intense 27-kDa band. By comparison only very small amounts of similarly sized proteins were detected in the cell extracts (Fig. 1B). In the presence of tunicamycin, the secreted and cell-associated FGF9 protein migrated on SDS-PAGE as a single band of 27 kDa (Fig. 1B), consistent with the inhibition of N-linked glycosylation on the single predicted consensus site (Fig. 1A). To establish directly the subcellular location of FGF-9, transfected COS-1 cells were analyzed for FGF-9 and the Golgi 58K protein by immunofluorescence. Faint reticular staining over the cytoplasm and strong perinuclear staining was observed for FGF-9, whereas the Golgi 58K protein gave the expected peri-nuclear staining characteristic of the Golgi complex (Fig. 2, A and B). Combination of the two optical sections and the phase contrast image clearly demonstrates a predominant co-localization of the two proteins and confirms that FGF-9 is associated with the Golgi complex and therefore enters the constitutive secretory pathway (Fig. 2D).

Identification of Candidate Sequences Involved in Secretion—Analysis of the primary protein sequence of FGF-9 using a computer-based program failed to identify a candidate signal peptide region (Ref. 20 and data not shown). In the aminoterminal 60 amino acid residues there are two peaks of weak hydrophobic character within the first 35 residues followed by a weak hydrophilic region (Fig. 1A). In contrast, a typical signal sequence usually shows a strong hydrophobic region at the amino terminus. The remaining portion of FGF-9 essentially constitutes a highly conserved region shared by other FGFs. To determine whether the amino terminus was necessary for secretion, we generated a series of deletions by progressively removing sequences from the 5′ end of the FGF-9 cDNA, while retaining a 5′-methionine codon to allow translation initia-
Fig. 1. Fgf-9 is efficiently secreted and N-glycosylated. A, the derived amino acid sequence for mouse Fgf-9. The single consensus site for asparagine-linked glycosylation is marked in bold letters, and the highly conserved region shared by all Fgfs is indicated by arrows. Below the sequence is a hydrophobicity profile demonstrating the weak hydrophobic nature of the amino terminus. B, immunoblot analysis of secreted (SN) and cell-associated (CP) Fgf-9. Equivalent amounts of extracts were analyzed to afford a direct assessment of secretion efficiency. Cells were grown in the absence (-) or presence (+) of 10 μg/ml tunicamycin.

Fig. 2. Subcellular localization of Fgf-9 by immunofluorescence microscopy. A, COS-1 cells transfected with FGF9wt stained with antiserum to FGF9; B, antiserum to Golgi 58K protein. C, cells visualized by phase contrast microscopy. By using confocal microscopy a composite of A–C, with the yellow color indicating co-localization of Fgf-9 and Golgi 58K protein (D).

after transfection of COS-1 cells with these deletion mutants, the amount of Fgf-9 proteins in the cells and supernatants was determined by immunoblotting (Fig. 3B). The degree of N-linked glycosylation was also assessed by comparison with similar extracts prepared from cells grown in the presence of tunicamycin. As N-linked glycosylation only occurs in the ER, this provides evidence for export of the mutant proteins through the secretory pathway. Deletion of the first 6 amino acids caused a profound reduction in the overall level of Fgf-9 synthesis, as can be seen by comparing expression from the full-length cDNA to other deletion mutants (Fig. 3B). However, despite an overall reduction in yield, a significant proportion of Fgf-9 protein was glycosylated and exported. A similar result was also obtained for FGF9Δ12 expression. Removal of 19 amino acids (FGF9Δ19) also resulted in a secreted and glycosylated Fgf-9, but most of the protein remained cell-associated and non-glycosylated. After removal of 22 amino acids (FGF9Δ22) only a small amount of the truncated Fgf-9 was secreted, and most of this was not glycosylated. Deletions of 26 and 37 amino acids from the amino terminus resulted in a single non-secreted and non-glycosylated product.

To gain further insight into the processing of the various amino-terminal deletion mutants, appropriately transfected COS-1 cells were analyzed by immunofluorescent microscopy. As already shown above (Fig. 2), wild-type Fgf-9 was almost entirely associated with the Golgi complex (Fig. 4, A–D). The 12 amino acid deletion mutant (FGF9Δ12) also showed clear staining of the Golgi as well as staining of the cytoplasm (Fig. 4, E–H). For the mutant FGF9Δ19, there was still staining of the Golgi but the cytoplasmic staining predominated, whereas the FGF9Δ37 showed no co-localization with the Golgi marker and relatively even staining throughout the cell (Fig. 4, I–L and M–P, respectively). Hence, the increasing amounts of non-glycosylated protein were associated with the progressive amino-terminal deletion of Fgf-9 and were paralleled by the presence of increasing amounts of cytoplasmic protein (compare Figs. 3 and 4).

Kinetics of Fgf-9 Intracellular Processing—To examine the efficiency of export and stability of Fgf-9, a moderately secreted mutant (FGF9Δ19) and a non-secreted mutant (FGF9Δ37) were studied along with the full-length protein. Cultures of appropriately transfected COS-1 cells were pulsed with radiolabeled methionine and chased up to 6 h (Fig. 5). After 30 min of chase, approximately 50% of the labeled wild-type protein was detected in the supernatant, and nearly all the cell-associated protein was glycosylated, indicating that most had entered the secretory pathway. Subsequently, the proportion of extracellular Fgf-9 increased at the expense of the cell-associated protein, and by 6 h more than 80% of the labeled protein had been secreted. By comparison, the truncated FGF9Δ19 protein although glycosylated was secreted less efficiently (Fig. 5), and immunofluorescent staining (Fig. 3) showed that some protein remained in the cytoplasm and was probably never exported. This would also explain the low level of cell-associa-
ated glycosylated Fgf-9Δ19 throughout the chase period. The non-secreted mutant protein (FGF9Δ37) showed a small amount of non-glycosylated protein in the medium probably arising from a small amount of cell lysis. From these experiments it would seem that native Fgf-9 is very efficiently secreted. Moreover, the proportion of FGF9Δ19 protein that enters the secretory pathway is also rapidly exported since there is no intracellular accumulation of its glycosylated form.

Deleted Forms of Fgf-9 Retain Biological Activity—It was possible that the more extensive truncations of Fgf-9 were not secreted because of incorrect folding of the mutant proteins that may have compromised the function of the signal sequence or caused the protein to be re-routed to the lysosomes. As an indirect assessment of correct folding, we used a mitogenicity assay as a sensitive assessment of biological activity. Recombinant proteins from cell-free transcription/translation reactions were tested directly in a thymidine incorporation assay to measure their ability to induce DNA synthesis. All the mutant proteins, including those that were not exported from cells, were biologically active, indicating that even severely truncated proteins are functional and therefore retain their tertiary structure (Fig. 6).

Redundancy of the Fgf-9 Signal Sequence—To define more precisely the sequences responsible for Fgf-9 secretion, internal deletion mutants of the amino-terminal region were tested for their ability to be N-glycosylated and exported. As proteins with deletions of up to 19 amino acids from the amino terminus were still glycosylated and secreted, whereas those with deletions of more than 26 amino acids were intracellular and cytoplasmic, it was anticipated that the core of the cryptic signal peptide would not reside in the first 19 amino acids. To investigate whether sequences downstream of amino acid 19 would function as a signal sequence, internal deletions of amino acids 28–52 and 20–52 were generated as shown (Fig. 7A). The smaller deletion (FGF9Δ28–52) was secreted as efficiently as wild-type Fgf-9, more surprisingly the larger deletion (FGF9Δ20–52) was also secreted, although it was less efficiently synthesized and the product poorly glycosylated. Nevertheless, considerably more Fgf-9 mutant protein accumulated in the supernatant than was found in the cell (Fig. 7B). This would suggest that the amino acid sequences between 28 and 52 are not necessary for secretion, whereas those upstream of position 28 are required for efficient secretion. However, proteins with deletions up to amino acid 19 can still enter the secretory pathway, albeit very inefficiently, suggesting that the long moderately hydrophobic sequence covering the amino-terminal region of the protein must be redundant in its ability to act as a signal peptide. To determine whether it was important for secretion that the region located toward the center of the domain was hydrophobic, the glycine at residue 20 and the valine at residue 22 were mutated to lysine as single mutations and as the double mutant on a FGF9Δ28–52 background. The
two point mutations resulted in proteins that were efficiently secreted, whereas the double point mutation was secreted, but to a lower efficiency, suggesting that neither position was critical, but a significant decrease in hydrophobicity diminished the efficiency of secretion.

The Amino-terminal Region Acts as a Non-cleavable Signal Peptide—To determine whether the amino-terminal sequences of Fgf-9 are sufficient to act as an efficient signal sequence, they were joined to a heterologous protein that normally resides in the cytoplasm and nucleus. DNA encoding the amino-terminal 28 amino acids of Fgf-9 was joined to a cDNA encoding a GFP open reading frame. The GFP provided a marker that could be directly and easily detected in cells. In initial experiments, appropriately transfected COS-1 cells were labeled overnight with [35S]methionine, and it was observed that virtually all the labeled FGF9-GFP hybrid protein was exported from the cell (Fig. 8A). In control cultures, GFP was distributed between the cell extract and supernatant. The appearance of GFP in the cell supernatant was expected as expression of GFP in these cells caused a significant amount of cell death as indicated by numerous floating cells at the time of processing. The toxicity associated with expressing GFP in cell culture has been reported by others (21). Interestingly, no toxicity was observed for FGF9GFP expressing COS-1 cells, perhaps reflecting a change in the intracellular trafficking route. To determine whether the hybrid protein was secreted without cleavage of the signal sequence, its size was compared with the primary translation product synthesized in vitro. The two proteins from in vivo and in vitro sources were indistinguishable by SDS-PAGE, indicating that the signal sequence was not cleaved and behaves in a similar fashion when joined to GFP as in its normal context (Fig. 8B). To determine directly whether the efficient export of FGF9GFP was due to its redirection into the secretory pathway, we utilized the autofluorescence of GFP to examine the subcellular distribution of the control and hybrid protein. Fluorescence microscopy showed that GFP resided throughout the cell in a pattern that demonstrated both a cytoplasmic and nuclear distribution (Fig. 9, A–D). In contrast FGF9GFP showed a clear perinuclear staining that was coincident with Golgi staining (Fig. 9, E–H). The low intensity of staining reflects its efficient export (Fig. 8A). Taken together, these findings show that the first 28 amino acids of Fgf-9 can act as an efficient non-cleaved signal sequence for the export of a heterologous protein.

DISCUSSION

Fgfs are a highly related family of proteins that show a strong conservation of amino acid residues over the central core region but with quite divergent sequences at their amino and carboxyl termini. Although many members of the Fgf family
contain a classical amino-terminal signal sequence, Fgf-1 and Fgf-2 do not and appear to be primarily located in the cytoplasm and cell nucleus. However, they are also exported by novel mechanisms that bypass the constitutive secretory pathway (6–11). Another anomaly concerns Fgf-3 that not only encodes a signal sequence but also a nuclear import signal (12). These act in competition with each other giving rise to dual intracellular location of the proteins (13). More recently Fgf-9 and Fgf-16, which share a high degree of sequence conservation (73% homology), were shown to be secreted efficiently despite the lack of a signal sequence for secretion (15, 17). Moreover, the secreted form of Fgf-9 retains its amino-terminal region, apart from the initiating methionine. In the study reported here, we show that the weakly hydrophobic sequences contained in the first 28 residues of Fgf-9 are sufficient when fused to GFP to function as a non-cleaved signal peptide. However, a deletion analysis of the amino-terminal region of Fgf-9 indicates that the amino acids further downstream can functionally compensate for loss of the immediate amino-terminal residues, suggesting that both hydrophobic domains in the amino-terminal domain can form part of a signal sequence. This is suggested by the finding that deletion of up to 22 amino-terminal residues still gives rise to a secreted protein, although the efficiency of export gets progressively worse with increasing size of the deletion (Figs. 3 and 4). Moreover, an internal deletion removing amino acids 20–52 reduces but does not abolish glycosylation and secretion of the protein (Fig. 7). However, the residues at the immediate amino terminus of Fgf-9 are required for its very efficient secretion, and an internal deletion of residues 28–52 had no significant effect on its secretion efficiency. Together these results suggest that the first 28 amino acids encompass the *bona fide* signal sequence, a finding supported by the efficient export of FGF9GFP. The identification of a weakly hydrophobic sequence that confers secretory properties upon a protein is unusual but not unique (22). Two other known examples are ovalbumin and plasminogen-activator inhibitor type 2 (PAI-2) which are both members of the serpin family. Although ovalbumin is an efficiently secreted protein, there is disagreement about the location of the amino acid residues important for its secretion (23, 24). In one study amino acids 231–279 from ovalbumin were shown to compete with authentic signal sequences for entry

**FIG. 5. Intracellular processing of Fgf-9 and Fgf-9 mutants.** A, autoradiograph from ³⁵S-methionine-labeled COS-1 cells transfected with FGF9wt, FGF9Δ19, or FGF9Δ37. Labeled (0.5 h) cells were chased for the hours indicated at the top of the panel. Tracks represent immunoprecipitates of cell supernatants (SN) and cell extracts (CP). Positions of bands representing glycosylated and non-glycosylated protein (upper and lower or single arrow, respectively) are indicated. The positions of relevant molecular mass marker proteins are indicated on the right of the panels. B, relative amounts of ³⁵S-labeled Fgf-9 protein in supernatant (SN) and cell extract (CP) after pulse/chasing for the times indicated in A. Quantitation was by phosphorimaging of data shown in A, and band intensities are expressed as the percentage of protein distributed between supernatant and cell extract at each time point.

**FIG. 6. Biological activity of Fgf-9 amino-terminal deletion mutants.** [³H]Thymidine incorporation (cpm, vertical axis) by C57MG cells. Quiescent cells were treated with newborn calf serum (NCS), fetal calf serum (FCS), recombinant FGF9 (rFGF9), or in vitro translated protein (hatched bars) as shown along the horizontal axis. Bars represent the S.E. with n = 4.
into microsomes in vitro, whereas another study implicated amino acids 22–41 as sufficient for translocating a heterologous protein across the ER membrane of *Xenopus* oocytes. The protease inhibitor PAI-2 has a non-glycosylated cytoplasmic form and a secreted form that is glycosylated (25, 26). The signal sequence of PAI-2 is composed of two hydrophobic domains located near the amino terminus (27). It would appear that both hydrophobic domains are necessary for ER import, a process that can be improved by increasing the hydrophobicity of either domain. However, although the overall characteristics of the Fgf-9 signal sequence are similar to that of PAI-2, its export appears to be much more efficient.

At present it is not clear if the non-cleaved signal sequence of Fgf-9 is part of an in vivo regulatory mechanism that determines whether and when the protein is secreted from cells. For example, PAI-2 is not secreted efficiently from U-937 cells until they are stimulated with phorbol ester, suggesting that the atypical signal sequence may serve some regulatory role (28). Alternatively, the amino-terminal residues may contribute to the biological properties of Fgf-9, which requires their retention in the mature protein.

During the preparation of this manuscript another study on the secretion of Fgf-9 reported that the internal hydrophobic region, located around amino acid 100 (see Fig. 1A), was crucial for Fgf-9 secretion and acted as an internal signal sequence (29). In this study the main observation was that deletion of 90 amino acids from the start of Fgf-9, which leaves its central hydrophobic domain at the amino terminus, yielded a polypeptide that was translocated into microsomes and that deletion or point mutation of the central hydrophobic region abolished Fgf-9 secretion. However, the subcellular location of these mutant proteins was not determined. The conclusions from their work are that the central hydrophobic region is crucial for translocation but that the amino-terminal sequences also make a contribution. The different conclusions deduced from our study and that of Miyakawa *et al.* (29) probably reflect both differences in methodology and interpretation. In our study we show that the amino-terminal 28 amino acids of Fgf-9 can act

**Fig. 7.** Identification of regions within the amino-terminal sequence affecting Fgf-9 secretion. A, schematic depiction of the amino-terminal sequence of Fgf-9 and the internal deletion and specific point mutations of Fgf-9 generated in this region. B, immunoblot analysis of equivalent amounts of cell supernatants (SN) and cell extracts (CP) from COS-1 cells transfected with the different Fgf-9 mutants shown in A.

**Fig. 8.** Amino acids 1–28 of Fgf-9 confer secretion properties to GFP. A, autoradiograph of 35S-labeled proteins immunoprecipitated with an antiserum to GFP from the cell supernatants (SN) and cell extracts (CP) of COS-1 cells transfected with GFP or FGF9GFP. Positions of the predicted band sizes of GFP and the FGF9GFP (27 and 29 kDa, respectively) are indicated by arrows. B, autoradiograph of 35S-labeled FGF9GFP. Tracks show protein immunoprecipitated from the supernatant of transfected COS-1 cells (SN FGF9GFP) or produced by in vitro translation (IVT FGF9GFP). A control (no DNA) for the in vitro translation is indicated.
as an efficient non-cleaved signal sequence in its natural context, as well as when joined to the amino terminus of a heterologous protein.

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FIG. 9. Subcellular localization of GFP and FGF9GFP by fluorescence microscopy. COS-1 cells transfected with GFP (A–D) or FGF9GFP (E–H) were stained with antibody to Golgi 58K protein (B and F) or examined for native fluorescence (C and G). A and E, cells visualized by phase contrast microscopy. Composite images of A–C and E–G (D and H), respectively. Yellow color indicates co-localization of GFP and Golgi 58K proteins.
Fibroblast Growth Factor 9 Secretion Is Mediated by a Non-cleaved Amino-terminal Signal Sequence
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