STAT5B-mediated Growth Hormone Signaling Is Organized by Highly Dynamic Microtubules in Hepatic Cells*

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In the last decade, the notion that microtubules are critical to the spatial organization of signal transduction and contribute to the transmission of signals to downstream targets has been proposed. Because the STAT5B transduction and transcription factor is the major STAT protein activated by growth hormone stimulation in hepatocytes and is a crossroads between many signaling pathways, we studied the involvement of microtubules in STAT5B-mediated growth hormone signaling pathway in the highly differentiated and polarized WIF-B hepatic cell line. We showed that depolymerization of the microtubule network impaired STAT5B translocation to the nucleus upon growth hormone treatment. A significant amount of STAT5B binds to microtubules, while STAT5A and STAT3 are exclusively compartmentalized in the cytosol. Moreover, taxol-induced stabilization of microtubules released STAT5B from its binding, and we show that STAT5B binds specifically to the highly dynamic microtubules and is absent of the stable microtubule subpopulation. The specific involvement of dynamic microtubule subpopulation in growth hormone signaling pathway was confirmed by the inhibition of growth hormone-induced STAT5B nuclear translocation after stabilization of microtubules or specific disruption of highly dynamic microtubules. Upon growth hormone treatment, MT-bound STAT5B was rapidly released from microtubules by a dynein-dependent transport to the nucleus. Altogether, our findings indicate that the labile microtubule subpopulation specifically and dynamically organizes STAT5B-mediated growth hormone signaling in hepatic cells.

Once bound to specific receptors, a large number of cytokines, growth factors, and hormones act through a sequence of steps allowing the rapid transport of information from the plasma membrane to subcellular targets. This information often results in the phosphorylation of transcription factors that subsequently migrate to the nucleus where they initiate specific gene transcription. How transcription factors are organized as molecular targets downstream of receptor activation and how they move rapidly through the cytoplasm from their activation site to the nucleus often remains unclear. The notion that microtubules (MTs)1 might be involved in the cytoplasmic activation and/or trafficking of various transcription factors and protein kinases has emerged, as many signal transduction molecules, including transcription factors and protein kinases, have been shown to interact with MTs (1). For example, the glucocorticoid receptor was shown to colocalize with the MT network in mammalian cells (2, 3). The tumor suppressor protein p53 also uses MT tracks to migrate to the nucleus (4). Various protein kinases such as the MAP kinase kinase kinase MLK2 (5) or the MAP kinases ERK1 and ERK2 (6) were also shown to interact or to colocalize with MTs or with microtubule-associated proteins (7).

In this study, we investigated the putative involvement of the MT network in GH signaling in the highly differentiated WIF-B hepatic cell line (8, 9). After ligand binding, the activated GH receptor activates the JAK2 tyrosine kinase, which in turn may phosphorylate three proteins from the STAT family: STAT1, STAT3, and STAT5. This tyrosine phosphorylation is followed by STAT homo- and/or heterodimerization and translocation to the nucleus where the activated dimers bind to the γ interferon-activated sequence in the promoter of target genes. In hepatocytes, STAT5B is the major STAT protein activated in response to a physiological pulse of GH (10). In addition to the classic JAK-STAT pathway, GH binding to its receptor may also result in the JAK2-dependent activation of ERK/MAPK serine-threonine kinases (11), to modulate the activity of STAT5 isoforms (12). The involvement of MTs in GH signaling is suggested as GH treatment was proposed to stabilize the MT network (13) and to activate the MT-bound MAP kinases ERK1 and ERK2, which may also phosphorylate STAT5 on serine residues (14, 15). That MTs might be involved in organizing STAT-mediated signaling pathways remains controversial however. Indeed, EGF-induced STAT1 nuclear translocation was found to be inhibited upon MT depolymerization by colchicine (16) while interferon α- and γ-induced STAT1 translocation appeared not to depend on MTs (17). This prompted us to examine the possibility that MTs might be involved in the organization of STAT5B-mediated transduction in response to GH treatment in hepatic cells. Furthermore, as pointed out by the differential effects of TGF-β or lysophosphatidic acid signaling on MT subsets (18, 19), MT subpopulations might not be involved to the same extent in cell signaling. In eukaryotic cells, the differentiation of the MT network into

1 The abbreviations used are: MT, microtubule; MAP, mitogen-activated protein; ERK, extracellular signal-regulated kinase; STAT, signal transducer and activator of transcription; FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine isothiocyanate; PBS, phosphate-buffered saline; PIPES, 1,4-piperazine-dithanesulfonic acid; GH, growth hormone; GR, glucocorticoid receptor.
subpopulations mainly results from the selective stabilization of some MTs, which can be identified by their higher resistance to dilution- or nocodazole-induced depolymerization and which exhibit high concentrations of post-translationally modified tubulin (for review, see Ref. 20). As it seems likely that these highly dynamic and stable MT subpopulations differ in their molecular environment, we also examined the possibility that a specific MT subpopulation might be involved in the cytoplasmic organization of STAT5B-mediated signaling pathway.

**EXPERIMENTAL PROCEDURES**

**Antibodies and Chemicals—**Anti-STAT5B, directed against the C-terminal part of the molecule (C-17, anti-STAT5A (L-20), and anti-STAT3 (K-15) antibodies were bought from Santa Cruz Biotechnologies. The specificity of the anti-STAT5B and anti-STAT5A antibodies was verified in all the cell lines studied. Anti-α-tubulin (clone D6-11B1), anti-acetyl-tubulin (clone 6-11B1), anti-rabbit IgG FITC conjugates, anti-mouse TRITC conjugates, anti-rabbit, and anti-mouse horseradish peroxidase conjugates were purchased from Sigma. Anti-GM130 mouse antibodies were from Transduction Laboratories. Anti-PCNA antibodies were from Caltag laboratories (San Francisco, CA). Paclitaxel (Taxol®) and nocodazole were purchased from Sigma.

**Cell Culture and Treatments—**WIF-B cells were cultured in F12 (Coon's modified medium, Sigma) supplemented with 5% fetal calf serum, 1 mM dithiothreitol, pH 7.9), then resuspended in extraction solution (20 mM HEPES, 20 mM KCl, 30% sucrose, pH 7.9). After centrifugation (3000 × g, 4 °C, 5 min), the cell pellet was resuspended in lysis buffer (20 mM HEPES, pH 7.9, 20 mM KCl, 30% sucrose, 0.5% Nonidet P-40, 1 mM dithiothreitol) supplemented with protease- and phosphatase-inhibitor mixture (1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 20 μM leupeptin, 100 μM sodium orthovanadate, and 1 mM sodium fluoride) and incubated on ice for 1 min. After centrifugation (4000 × g, 4 °C, 10 min), the nuclear pellet was resuspended with washing buffer (20 mM HEPES, 20 mM KCl, 15% sucrose, 1 mM dithiothreitol, pH 7.9), then resuspended in extraction buffer (20 mM HEPES, 5% glycerol, 400 mM NaCl, 1 mM dithiothreitol, pH 7.9) supplemented with the above-described inhibitor mixture, and incubated on ice for 1 h. After centrifugation (25000 × g, 4 °C, 30 min), the supernatant was used as nuclear extract. Nuclear extract protein concentrations were measured using the Bradford assay (Bio-Rad). 

**MT Purification—**Cells were washed twice with PEM buffer (80 mM PIPES, 2 mM EGTA, 1 mM MgCl₂, pH 6.9), and soluble proteins were extracted in the same buffer supplemented with 0.05% Triton X-100 and the antiprotease and antiphosphatase mixture. Soluble protein extraction was followed by one rinse in Triton-free buffer. We verified that under such conditions, cellular MTs were preserved at the morphological level by immunofluorescence labeling of α-tubulin. MTs were then depolymerized by the combined action of calcium (5 mM CaCl₂) and cold (4 °C, 1 h) in PEM buffer (80 mM PIPES, 1 mM MgCl₂, pH 6.9) containing 1% Nonidet P-40 and the antiprotease and antiphosphatase mixture. The efficiency of MTs depolymerization was checked at the morphological level. Soluble and microtubular fractions were concentrated and dialyzed against PEM in Centricron 10 filtration devices (Millipore Corp., Bedford, MA), then cleared by centrifugation (25000 × g, 1 h, 4 °C). MTs were then depolymerized by centrifugation at 25000 × g for 30 min at 37 °C and washed once with PEM buffer supplemented with 1 mM GTP. MTs were then directly depolymerized in SDS-PAGE sample buffer while soluble proteins were supplemented with 4X concentrated SDS-PAGE sample buffer.

**Miscellaneous—**Cells were washed twice with PEM buffer, and soluble proteins were extracted in the same buffer supplemented with 0.05% Triton X-100 and the antiprotease and antiphosphatase mixture. Soluble protein extraction was followed by one rinse in Triton-free buffer. Incubation of extracted cells in a PEM buffer during 30 min at 37 °C allowed the dilution-induced depolymerization of the most labile MTs. To be sure that no dynamic MT remained in extracted cells, cells were further incubated for a prolonged period of time with PEM (3 h at 37 °C). Stable MTs that remained in permeabilized cells were subsequently depolymerized by the combined action of calcium (5 mM CaCl₂) and cold (4 °C, 1 h) in PEM buffer containing 0.1% Nonidet P-40 and the antiprotease and antiphosphatase mixture. The solutions containing MT proteins were dialyzed against PEM and concentrated using Centricron 10 filtration devices, then centrifuged (25000 × g, 1 h, 4 °C). MTs were then repolymerized by addition of 20 μM taxol and 1 mM GTP (45 min, 37 °C) and centrifuged (25000 × g, 30 min, 37 °C). MT pellets were rinsed once with polymerization buffer supplemented with 1 mM GTP. Dynamic and stable MTs were then directly depolymerized in SDS-PAGE sample buffer and subsequently analyzed by Western blot.

**Western Blot Analysis—**Proteins were subjected to SDS-PAGE in 9% polyacrylamide gel and transferred onto polyvinylidene difluoride membrane. Blots were probed with anti-α-tubulin or anti-acetyl-tubulin monoclonal antibody, anti-STAT5B, anti-STAT5A, or anti-STAT3 polyclonal antibodies. Primary antibodies were detected with anti-mouse or goat anti-rabbit immunoglobulins conjugated with horseradish peroxidase and an enhanced chemiluminescence assay (ECL, PerkinElmer Life Science Products Inc., Boston, MA) on Kodak Biomax MR films. Band intensities were quantified by densitometry.

**Immunofluorescence—**After appropriate treatment, WIF-B cells cultured on glass coverslips were rinsed twice with PEM buffer and permeabilized with the same buffer containing 0.05% Triton X-100 (37 °C, 3 min). After two rinses with Triton-free buffer and two rinses with PBS, cells were fixed with cold methanol (5min, −20 °C). Cells were incubated with primary antibodies diluted in PBS for 1 h at 37 °C, washed three times with PBS, and incubated (1 h, 37 °C) with FITC-conjugated or with a mixture of FITC- and TRITC-conjugated antibodies for single or double labeling experiments, respectively. After three washes with PBS, the coverslips were examined by epifluorescence using a Leica DMLB microscope with a ×100 objective or by confocal microscopy using a Zeiss LSM 510 microscope.

**Plasmid Construction and Mutagenesis—**Full-length STAT5B inserted in Myc-tagged pUHD vector (generous gift from M. Pallardy, INSERM U461, Chatenay-Malabry, France) was introduced into pEGFP-C1 vector (Clontech, Palo Alto, CA) on SmaI and SacII sites. STAT5B deletion mutants were generated by mutagenesis using the QuikChange™ Site mutagenesis kit (Stratagene) with oligonucleotide primers designed to intro a stop codon in position 779 (Gln779). Mutations were confirmed by direct sequence analysis. For C-ter STAT5B mutant (Cter), the sequence encoding the C-terminal tail of STAT5B (115 amino acids) was amplified by PCR using primers designed to also introduce a SstII site upstream of the PCR product. The final PCR product was digested with SstII and XbaI and purified before ligation into the pCDNA3 vector (Invitrogen).

**Transient Transfections—**HuH7 cells grown on glass coverslips in 6-well plates were transfected with 1 μg of DNA using 3 μl of FuGENE 6 reagent (Roche Applied Science) according to the manufacturer's protocol and used 24 h after transfection.

**MT Depolymerization Impairs GH-induced Nuclear Translocation of STAT5B but Not That of STAT3—**To determine whether MTs are actually involved in the STAT5B-mediated GH signaling pathway in hepatic cells, we performed experiments in which the MT network of WIF-B cells was depolymerized by a 4-h nocodazole treatment prior to GH induction. We have previously shown that, in these conditions, the MT network is fully depolymerized in WIF-B cells (22). At increasing times after GH treatment, nuclear extracts were prepared and

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2 A. Dreechou and T. Phung-Koskas, personal unpublished results.
analyzed by Western blot for the presence of STAT5B or STAT3 (Fig. 1A). PCNA, a resident nuclear protein, was revealed as loading control. Consistent with previous studies (24), GH treatment induced a rapid translocation of STAT5B into the nucleus, the maximum effect being reached after 5 min. By contrast, when the whole MT network was disrupted by nocodazole treatment, STAT5B translocation was dramatically slowed down and was about 2-fold less effective than that measured in control cells (Fig. 1A) whereas the overall (nuclear + cytoplasmic) STAT5B level was unaffected (Fig. 1B, lane 0). Comparatively, GH-induced nuclear translocation of STAT3 was not affected in the absence of MTs, for up to 45 min (Fig. 1A). The latter result suggests that, in the GH signaling pathway, the events which occur upstream of STAT activation do not depend on the integrity of the MT network. This also means that MTs would play a role in the latest cytoplasmic steps of
GH signaling, i.e. STAT5B activation and/or nuclear translocation. As expected by STAT5B phosphorylation on a tyrosine residue by the JAK2 kinase after GH stimulation (25, 26), a double band of STAT5B was early observed in the Western blot analysis of total cell lysates (Fig. 1B). The kinetics of STAT5B phosphorylation upon GH treatment was not impaired after MT depolymerization (Fig. 1B), indicating that the impairment of nuclear translocation we observed (Fig. 1A) did not result from an altered activation of STAT5B but from a downstream inhibition involving MTs.

These biochemical results were confirmed in immunofluorescence experiments in which cells were double-stained with antibodies to STAT5B and α-tubulin. After a 5-min GH treatment of WIF-B cells, STAT5B was mainly translocated in the nucleus. When cells were pretreated with nocodazole for 1 h to depolymerize most of the MT network (Fig. 1C, lower panel), nuclear translocation of STAT5B dramatically decreased compared with controls (Fig. 1C, upper panel). Altogether, these results clearly indicate that nuclear translocation of STAT5B induced by GH treatment depends upon the integrity of the MT network.

**STAT5B Specifically Binds to MTs Compared with STAT5A or STAT3**—To test whether STAT5B actually interacts with MTs, we analyzed separately cytosolic and MT fractions purified from WIF-B cells by Western blot. A significant fraction (25%) of cellular STAT5B co-purified with the MT fraction (Fig. 2A). No STAT5B was pelleted in the absence of MTs i.e. when this fraction was purified from cells in which the MT network was fully depolymerized by a 4 h nocodazole treatment (results not shown). To make sure that this interaction was not restricted to WIF-B cells, we further checked that STAT5B was present in the MT fractions obtained from other cell lines. Fig. 2A shows that a significant proportion of STAT5B interacts with MTs in all cell types we analyzed (MDCK, HuH7, and MDA-MB 231 cells).

Compared with STAT5B, STAT3 and STAT5A were found exclusively in the cytosolic fraction (Fig. 2B) as suggested by others (15), clearly indicating that STAT5B specifically associates with MTs. Interestingly, STAT5A and STAT5B are highly homologous and differ mainly by a short C-terminal tail (27), suggesting that this region could be directly involved in the differential binding of the two proteins on MTs. To evaluate the putative role of STAT5B C-terminal domain in MT binding, we performed transfection experiments in HuH7 hepatoma cells with a plasmid encoding GFP-tagged wild-type STAT5B or GFP-tagged STAT5B with deletion of its last 8 amino acids (Q779). As shown in Fig. 2C, wild-type construction bound to MTs in the same proportion than endogenous STAT5B (MT fraction versus cytosolic fraction). Moreover, C-terminally truncated STAT5B (Q779) still bound to MTs to the same extent as the wild-type construction, suggesting that this part of the molecule is not directly involved in MT binding. This result was confirmed by transfecting HuH7 cells with a plasmid encoding the C-terminal tail of STAT5B (115 amino acids), which appeared to localize strictly in the cytosolic fraction (Fig. 2D).

To confirm STAT5B-MT interaction, we performed double immunofluorescence labeling of α-tubulin and STAT5B in WIF-B cells, which were first permeabilized in PEM buffer to extract soluble cytosolic proteins. As shown in Fig. 3, STAT5B colocalized and/or was closely juxtaposed to MTs. Interestingly, STAT5B staining was not uniform along MTs, but occurred in discrete locations, suggesting that it might be compartmentalized into specific MT sites.

**STAT5B Specifically Interacts with Highly Dynamic MTs**—Dynamic and stable MT subpopulations might not be involved to the same extent in cell signaling, and alteration of their dynamic properties may play a role in modulating the activation or the nuclear translocation of MT-bound signaling effec-
FIG. 4. STAT5B specifically interacts with dynamic microtubules. A, WIF-B cells cultured for 24 h in serum-free medium were treated with or without 20 μM taxol for 2 h, then permeabilized in MT-stabilizing buffer containing 0.05% Triton X-100. The soluble cytosolic fraction was discarded. The MTs remaining in the permeabilized cells were depolymerized by the combined action of calcium and cold, then purified by assembly with GTP and taxol, and analyzed by Western blot with anti-STAT5B and α-tubulin. The STAT5B contents of each MT fraction was normalized relative to the tubulin contents after densitometry. B, WIF-B cells cultured on glass coverslips were detergent-extracted with 0.05% Triton X-100 in PEM buffer (3 min, 37 °C) and rinsed with Triton-free PEM buffer (panels a and b). Dynamic MTs were depolymerized by incubating extracted cells for 30 min at 37 °C with detergent-free buffer (panels c and d). Extracted cells were then incubated during 3 h with PEM buffer (panels e and f), and stable MTs were further depolymerized by the combined action of calcium and cold (panels g and h). All samples were fixed with methanol (~20 °C, 5 min) and processed for the double immunofluorescence labeling of α-tubulin (panels a, c, e, g) and acetylated-tubulin (panels b, d, f, h). Scale bar, 10 μm. C, WIF-B cells were treated as described in B. Dynamic and stable MT fractions were clarified and purified as described under “Experimental Procedures” and analyzed by Western blot for α-tubulin, acetylated-tubulin, and STAT5B.

MTs. Strikingly, STAT5B was detected very specifically in the dynamic MT fraction (there was no signal in the stable MT fraction) (Fig. 4C). Together with the results from taxol experiments, these data clearly indicate that MT-bound STAT5B is specifically enriched and compartmentalized in the dynamic MT subpopulation.

Stabilization of MTs or Selective Disruption of Dynamic MTs Impairs STAT5B Nuclear Translocation after GH Treatment—To further explore the functional specialization of dynamic and stable MTs in the GH signaling pathway, we performed experiments in which MTs were stabilized by treatment of WIF-B cells with taxol (20 μM, 2 h) prior to GH stimulation. In these conditions, the effectiveness of STAT5B nuclear translocation was decreased (Fig. 5A) indicating that the inhibition of MT dynamics impaired STAT5B targeting to the nucleus.

We also performed experiments in which highly dynamic MTs were specifically depolymerized using the 201-F compound. We have previously shown that 201-F compound selectively disassembles dynamic MTs without affecting stable MTs in WIF-B cells (22). As expected, in the presence of a normal MT network, GH treatment induced STAT5B and STAT5A translocation into the nucleus. In cells treated with 201-F, in which only the stable MT subset was preserved, STAT5B remained in the cytoplasm after GH induction, indicating that the dynamic MT subpopulation is required for STAT5B transport to the nucleus (Fig. 5B). As expected from our previous finding that STAT5A is only cytosolic, the GH-induced nuclear transport of STAT5A was not impaired in the absence of dynamic MTs (Fig. 5B). Altogether, these observations indicate that unaltered dynamic MTs are essential for the transport of STAT5B to the nucleus after GH stimulation.

MT-bound STAT5B Is Activated upon GH Stimulation—We further addressed the functional significance of STAT5B-MT binding by analyzing STAT5B-MT interaction upon GH stim-
FIG. 5. Alteration of MT dynamics or selective disruption of dynamic MTs impairs STAT5B nuclear translocation after GH treatment. 

A, WIF-B cells were serum-starved for 24 h before the experiments. After treatment with (●) or without (●) taxol (20 μM, 2 h), cells were stimulated with 50 nM GH as indicated. Nuclear extracts were prepared and analyzed by Western blots (10 μg of nuclear proteins were loaded for each sample) probed with anti-STAT5B antibody (upper panel) and anti-PCNA as loading control (lower panel). After quantification by densitometry, STAT5B nuclear fractions were expressed as the percentage of the maximal translocation.

B, after treatment without (vehicle) or with 201-F (25 μM, 1 h), 24 h serum-starved WIF-B cells were treated for 5 min with 50 nM GH. Cells were then fixed directly without prior extraction (methanol, −20 °C, 5 min) and processed for the double immunofluorescence labeling of STAT5B or STAT5A and α-tubulin (as indicated). Scale bar, 10 μm.
Such an organization consists in a compartmentalization between the cytosol and the dynamic MT network as we showed a specific interaction of the STAT5B isoform with MTs, while STAT5A and STAT3, which are also activated by GH treatment, remained exclusively cytosolic. While STAT5B mainly differs from STAT5A by its 8 last amino acids and a C-terminal deletion of 12 amino acids (27), we found that the C-terminal tail of STAT5B is not directly involved in the binding of STAT5B to MTs. Differences in amino acid composition scattered in the other domains of the molecule (Liu et al., 27) would then be responsible for the differential binding between STAT5A and STAT5B. Another difference between the two STAT5 isoforms is the capacity for STAT5A to be specifically phosphorylated on a serine residue by the ERK MAP kinases (15), such phosphorylation being required for its full activation. Upon GH stimulation, ERK2 actually mediates serine phosphorylation of STAT5A, but not that of STAT5B (12). As a subpopulation of ERKs has been shown to interact with the MT cytoskeleton (14) (6), our finding that STAT5A is not MT-associated, contrary to STAT5B, was surprising. Consistent with such an observation, GH-induced nuclear translocation of STAT5A was not impaired when either dynamic MTs were depolymerized or the whole MT network was disassembled in WIF-B cells and in fibroblasts (15). An exclusive cytosolic compartmentalization might however allow STAT5A to interact transiently with MT-bound activated ERKs (6) while this would not be the case if STAT5A were bound to MTs in another location. Despite the fact that no gene has been shown yet to be transactivated selectively by only one STAT5 isoform, it is likely that each isoform has specific functions (for review, see Ref. 31). For example it has recently been shown that the constitutive activation of STAT5B but not that of STAT5A contributes to squamous cell tumorigenesis in vivo (32). These specific functions are probably a reflection of their capacity to interact with different proteins required for target gene activation.

The cytoplasmic organization of STAT5B is quite complex; like STAT3 it was shown to be embedded in multimolecular structures termed statosomes (33). Our results also point out the high degree of STAT5B organization on the MT cytoskeleton. It not only binds specifically to dynamic MTs but its binding appears restricted to discrete domains that might be privileged sites where STAT5B might interact with other signaling partners. In this respect, STAT5B was already shown to interact directly with the glucocorticoid receptor (GR) in hepatic and mammary cells (34, 35). Interestingly, the GR is also MT-bound (3) and is associated with hsp90 (36, 37), the FKBPs immunophilin (38) and the dynein intermediate chain (39). Whether STAT5B would participate in such complexes and whether these would actually favor its interaction with other signaling molecules remains to be determined.

That STAT5B was preferentially bound to highly dynamic MTs and absent from the stable MT-associated proteome is to our knowledge the first evidence that MT subpopulations function specifically in organizing some signaling pathways. It is known for a decade that highly dynamic and stable MTs have specific functions during the interphase. Indeed, labile MTs are specifically involved in fibroblastic locomotion (40), in axonal growth (41) or in mediating Golgi-to-plasma membrane trafficking of secretory proteins in hepatic cells (22). Conversely, stable MTs are specialized in organizing vimentin intermediate filaments in fibroblasts (42, 43), in mediating ER-to-Golgi (44) and post-Golgi trafficking of integral proteins (22), in mediating the dispersal of the Golgi complex upon nocodazole treatment (45) or in the export from endocytic recycling compartment to cell surface (46). The proteome associated with

**DISCUSSION**

In this study, we investigated the involvement of MTs in the GH signaling pathway in the highly differentiated WIF-B hepatic cell line. WIF-B cells are highly responsive to GH induction in terms of activation and nuclear translocation of STAT factors, with kinetics similar to those observed in rat liver (10). We studied the interaction of STAT5B with the MT cytoskeleton as it is the major STAT protein activated in response to GH in hepatocytes (31).

We showed that cells exhibit a sophisticated cytoplasmic organization of the STAT5B-mediated GH signaling pathway:
each subpopulation would provide a molecular basis of functional specialization, including the organization of cell signaling as we show here for STAT5B.

The fact that MTs are not a passive scaffold for STAT5B but actively participate in GH signaling is also supported by our findings. That STAT5B nuclear translocation is inhibited after MT disruption is reminiscent of other reports in which an alteration of the MT network or of its dynamics affects various signaling pathways. For example, both MT depolymerization and stabilization activate the Jun N-terminal kinase pathway (47), MT depolymerization activates NF-B in HeLa cells (48), EGF-induced nuclear translocation of STAT1 is inhibited after MT depolymerization (16), or p53 nuclear accumulation is altered after MT depolymerization but is enhanced after suppression of MT dynamics (4, 49). The active participation of MTs in GH signaling is confirmed by the GH-dependent activation and drop in MT-bound STAT5B. Several mechanisms might have explained such a release from MTs. STAT5B might first be sequestered on MTs as shown for the Smad proteins in the TGF-β signaling pathway (50), or for cubitus interruptus included in complexes with Costal2 in the Hedgehog pathway (51). The release of STAT5B from MTs might then have occurred after a GH-induced conformational change resulting either from phosphorylation or from a transient change in MT dynamics. That STAT5B phosphorylation on tyrosine 699 would be directly responsible for its release from MTs is unlikely for two reasons: (i) the kinetics of release is relatively slow, allowing a fraction of activated STAT5B to bind to MTs for several minutes (ii) the mutation of STAT5B substituting tyrosine 699 with an aspartate to add a negative charge and mimic phosphorylation did not impair the binding of mutated STAT5B to MTs.2

MT dynamics is also a potential target for various signaling pathways: HGF stimulates MT dynamics in MDCK cells (52) whereas TGF-β and lysophosphatidic acid stabilize MT subsets in NIH-3T3 fibroblasts (18, 19). As GH was proposed to promote tubulin polymerization and MT stabilization in CHO cells transfected with the GH receptor (13) one could also consider a potential decrease of MT dynamics to be responsible for STAT5B release. However, in our hands, GH did not cause any major alteration in MT dynamics.3 Furthermore, although we found that a release of STAT5B from MTs also occurred upon taxol-induced MT stabilization, such a release did not favor nuclear translocation.

Alternatively to a sequestration and a direct release of activated STAT5B from MTs, we showed that dynein is involved in STAT5B nuclear translocation. The drop in STAT5B binding to MTs might thus result from its rapid trafficking toward MT minus ends near the nucleus prior to release. MTs may func-

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FIG. 7. Overexpression of p50/dynamitin impairs the nuclear accumulation of STAT5B after GH induction. WIF-B cells were transiently transfected with the plasmid encoding p50/dynamitin. Twenty-four hours after transfection, cells were treated (GH) or not (Control) with 50 nM GH for 1 min or 5 min and subjected without prior extraction to a double immunofluorescence labeling with antibodies to STAT5B and to GM130 as indicated. Scale bar, 10 μm.

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3 T. Phung-Koskas, unpublished data.
tion as tracks to target signaling factors using molecular motors. An increasing number of transcription or signaling factors is shown to interact with molecular motors. With regard to dynein, it was shown that IκB interacts with the dynein light chain (53). Heterocomplexes containing the GR and the hsp90 chaperone interact with the dynein intermediate chain (39). There is also recent functional evidence that dynein mediates the trafficking of MT-bound p53 toward the nucleus upon activation (4) and that hsp90-binding immunophilins link p53 to dynein during this transport (54). More surprisingly, interactions may also take place that involve kinases as the MLK2 kinase (5) or the tumor suppressor APC were shown to interact with the kinesin-like KIF3 (55). Cubitus interruptus is bound to the kinesin-related protein Costal 2 in the Hedgehog signaling pathway (56, 57), and JIP scaffolding proteins for the JNK signaling pathway were found to bind conventional kinesin with the kinesin-like KIF3 (55). That GR associates with the dynein intermediate chain (39). That STAT5B-mediated GH signaling pathway is highly organized (58). Regarding the case of STAT5B, dynein-mediated trafficking pathway were found to bind conventional kinesin (56, 57), and JIP scaffolding proteins for the JNK signaling pathway were found to bind conventional kinesin (58).

Altogether, our findings point out to which extent the STAT5B-mediated GH signaling pathway is highly organized at the cytoplasmic level. They also suggest that its organization would allow a very precise regulation of the interactions with other signaling pathways either in the cytosol or on the MTs.

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