Structural requirements for nuclear localization of GCMa/Gcm-1

Said Hashemolhosseini[a,*], Karin Kilian[a], Elena Kardash[a], Peter Lischka[b], Thomas Stamminger[b], Michael Wegner[a,*]

[a]*Institut für Biochemie, Universität Erlangen-Nürnberg, Fahrstrasse 17, D-91054 Erlangen, Germany*
[b]*Institut für Klinische und Molekulare Virologie, Universität Erlangen-Nürnberg, D-91054 Erlangen, Germany*

Received 29 July 2003; revised 1 September 2003; accepted 9 September 2003

First published online 24 September 2003

Edited by Takashi Gojobori

Abstract GCM proteins constitute a small transcription factor family. Nuclear localization of *Drosophila* GCM is mediated by a typical bipartite nuclear localization sequence (NLS) close to the DNA-binding GCM domain. Here, we have analyzed nuclear localization of the mammalian GCM proteins. Whereas GCMb/Gcm-2 contained a classical bipartite NLS, nuclear localization of GCMa/Gcm-1 was mediated by two regions without resemblance to known NLS, one corresponding to the amino-terminal part of the GCM domain, the second defined as a tyrosine-and-proline-rich carboxy-terminal region. Nuclear import was counteracted by an amino-terminal nuclear export activity. This complex regulation of subcellular localization has important implications for GCMa/Gcm-1 function.

Key words: Nuclear localization sequence; Nuclear export signal; Nuclear import; Nucleocytoplasmic shuttling; Glial cells missing

1. Introduction

GCM (glial cells missing) proteins constitute a separate family of transcription factors [1]. The hallmark of all GCM proteins is the GCM domain [2], a zinc-coordinating, β-pleated sheet containing DNA-binding domain with a novel fold and mode of DNA recognition [3,4]. GCM proteins are present in vertebrates and some invertebrates, but appear to be absent in plants and fungi. Compared to other transcription factor families, the number of GCM proteins in a given species is low [4]. Thus, there are two GCM proteins in *Drosophila melanogaster* [5], and two GCM proteins in mammals [1].

All GCM proteins show highly similar DNA-binding characteristics and recognize a 5′-ATGCGGGT-3′ consensus motif or minor variations thereof [2,6,7]. However, GCM proteins fail to show significant sequence similarities outside the GCM domain, including functionally important regions such as the respective transactivation domains [7–9]. In addition to this low amount of sequence conservation, there also seems to be a lack of functional conservation [1]. The two *Drosophila* GCM proteins play essential and partially redundant roles in gliogenesis of the developing nervous system and in hemocyte development [10–14], whereas the two mammalian homologs GCMa/Gcm-1 and GCMb/Gcm-2 are important for placental and parathyroid development, respectively [15–17]. GCMa/Gcm-1 (henceforth called GCMa) is additionally expressed in thymus and kidney [18].

Being transcription factors, GCM proteins are expected to be primarily nuclear, and should contain nuclear localization signals (NLS). In transcription factors, NLS are frequently located within the DNA-binding domain or its immediate vicinity [19]. Sometimes, two functionally independent NLS have been mapped in a single transcription factor [20]. NLS are recognized by importin α/β heterodimers. Complexes between importins and cargo proteins are then targeted to the nuclear pore complex through which translocation of the importin-bound protein occurs in a manner dependent on the guanine nucleotide binding protein Ran and several Ran-interacting factors [21,22].

In some proteins, nuclear import is counteracted by a nuclear export mechanism that often involves a small, hydrophobic, leucine-rich nuclear export signal (NES) in the cargo protein [23,24], and the NES-interacting factor CRM1 [25,26]. Proteins containing both NLS and NES have the capacity to continuously shuttle between the cytoplasm and the nucleus.

We have previously mapped the NLS of *Drosophila* GCM to a short stretch of amino acids (KR-X_{10}-KRRR) between position 218 and 233 of the protein [8]. This NLS conforms to the consensus for bipartite NLS in which two clusters of basic amino acids are separated by a stretch of 10–12 amino acids [27]. It closely follows the DNA-binding GCM domain (amino acids 34–186), and thus occupies a position typical for an NLS in transcription factors. Analogously, the mammalian GCMb/Gcm-2 contains a putative NLS consisting of two basic clusters separated by nine amino acids which is situated immediately behind the GCM domain [28], but still has to be verified experimentally.

Sequence inspection of GCMa, in contrast, did not reveal any obvious candidate for an NLS with any of the commonly used prediction programs. We therefore set out to experimentally define the requirements for nuclear import of GCMa.

2. Materials and methods

2.1. Plasmid constructs

Full-length mouse GCMa, a GCMa variant with an internal deletion of amino acids 312–337 and several carboxy-terminally truncated versions (GCMa1–400; GCMa1–176) were fused to an amino-terminal T7 epitope and inserted between *Bam*HI and *Sal*I sites of pCMV5. In addition to wild-type GCMa, non-DNA-binding mutants (K74M and C76A, see [4,6]) were used. An expression plasmid for a green

*Corresponding author. Fax: (49)-9131-85 22484.
E-mail addresses: sh@biochem.uni-erlangen.de (S. Hashemolhosseini), m.wegner@biochem.uni-erlangen.de (M. Wegner).

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)01037-8

fluorescent protein (GFP)-GCMa fusion protein was generated by inserting the wild-type GCMa coding region between *Xho*I and *Eco*RI sites of pEGFP-N1 (Clontech Laboratories, Palo Alto, CA, USA). All β-galactosidase expression plasmids were based on the previously described pCMVlacZ [29], so that various fragments of mouse GCMa (see Figs. 2–4) or the region encompassing amino acids 176–189 of mouse GCMb/Gcm-2 (KRQMASFYQPQKRR) could be added to the 5′ end of the *lacZ* gene. A *lacZ* fusion carrying the NLS from SV40 T-antigen was used as positive control [29]. Fragments corresponding to amino acids 1–72 or amino acids 68–120 of mouse GCMa were also placed between the *Bam*HI and *Nhe*I sites of pGEX-GFP so that GCMa sequences were inserted in frame between glutathione *S*-transferase (GST) and GFP [30].

2.2. Tissue culture, transfection, immunocytochemistry and X-Gal staining

HeLa and NIH 3T3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum. HeLa cells were transfected in 35 mm plates using Superfect reagent (Qiagen). Forty-eight hours post transfection, cells were fixed using 1% paraformaldehyde and either stained for β-galactosidase activity in phosphate-buffered saline containing 1 mg/ml X-Gal or processed for immunocytochemistry [18] using a mouse monoclonal antibody directed against the T7 epitope (Novagen) and Cy3-coupled goat anti-mouse antibodies (Dianova), both diluted 1:200.

2.3. Purification and microinjection of GST-GFP fusion proteins

GST-GFP hybrid proteins were expressed in *Escherichia coli* BL21, purified on glutathione Sepharose 4B (Pharmacia Biotech, Germany), concentrated by ultrafiltration with a Nanosep filter (Pall-Filtron, USA) and microinjected into nuclei or cytoplasm of HeLa cells at 1.5 mg/ml in combination with rabbit IgG (1.0 mg/ml) using a CompiC INJECT computer-assisted injection system (Cellbiology Trading, Hamburg, Germany) as described [30]. Cells were fixed 1.5 h post injection with 3% paraformaldehyde, and the injected proteins were analyzed by immunofluorescence analysis.

2.4. Heterokaryon assays and immunofluorescence studies

Nucleocytoplasmic shuttling of GCMa was analyzed in a heterokaryon assay [31]. Briefly, transfected HeLa cells were seeded on glass coverslips together with an equal number of NIH 3T3 cells. Cells were incubated with 50 μg/ml cycloheximide 30 min prior to the fusion and throughout the experiment. Heterokaryons were formed by incubating the cells for 2 min in 50% polyethylene glycol 8000 in DMEM. After 2 h incubation at 37°C in the presence of cycloheximide, cells were fixed, processed for indirect immunofluorescence analysis, counterstained with 5 μg/ml of Hoechst 33258 (Sigma, Taufkirchen, Germany) and analyzed as described [30].

3. Results

3.1. Subcellular localization of mouse GCMa

To directly visualize the subcellular localization of GCMa, we transfected HeLa cells with an epitope-tagged version of mouse GCMa and performed immunocytochemistry on the transfected cells using an antibody directed against the amino-terminal T7 epitope. Most of the GCMa protein was localized to the nuclei of transfected cells as expected (Fig. 1a). Even GCMa mutants that were unable to bind to DNA due to exchange of an essential residue in the GCM domain were still predominantly nuclear (Fig. 1b) indicating that DNA binding and nuclear localization are separable functions. Moreover, shortening the protein by 36 amino acids from the carboxy-terminal end did not alter the subcellular localization arguing that GCMa does not need to be intact to correctly localize in cells (Fig. 1c). However, further shortening to a version that essentially corresponds to the GCM domain (amino acids 1–176) altered subcellular distribution. The GCM domain was only partially localized to the nucleus; a significant portion remained cytoplasmic (Fig. 1d). The relative amount of nuclear GCM domain was even further reduced when DNA binding was prevented by substitution of an essential amino acid (Fig. 1e). We conclude from the different subcellular distribution of full-length GCMa and the GCM domain that an important determinant for nuclear localization must be localized outside the GCM domain. Furthermore, if we assume active transport of the GCM domain into the nucleus, it must contain a signal for nuclear import. This nuclear import signal either functions inefficiently or is counteracted by a simultaneously present export function. The fact that a higher fraction of the GCM domain is nuclear in the presence of intact DNA binding could argue in favor of an additional export function, as retention of the GCM domain on DNA would decrease the rate of nuclear export and alter the relative subcellular distribution at steady state.

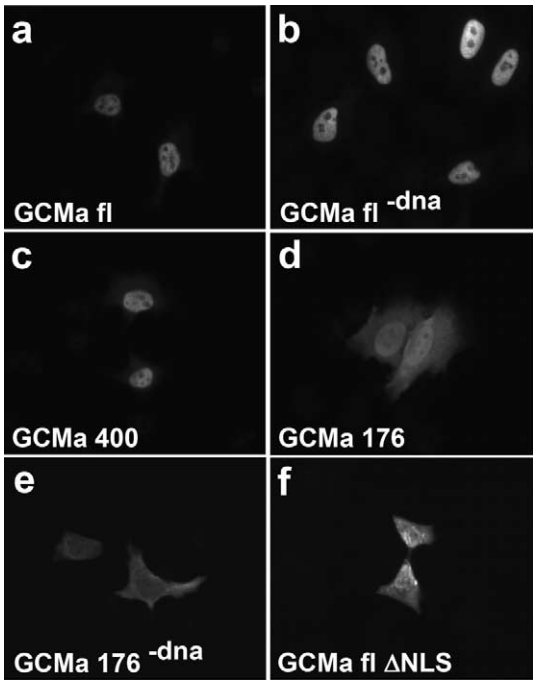

Fig. 1. Subcellular localization of wild-type and mutant mGCMa. Indirect immunofluorescence studies of HeLa cells transfected with the following T7 epitope-tagged GCMa proteins: (a) full-length wild-type GCMa, (b) full-length non-DNA-binding GCMa (C76A mutant), (c) carboxy-terminal GCMa deletion mutant (residues 1–400), (d) wild-type GCM domain (residues 1–176), (e) non-DNA-binding GCM domain (K74M mutant), (e) internal GCMa deletion mutant lacking amino acids 312–337 (GCMaΔNLS). Antibodies directed against the amino-terminal T7 epitope were used for detection.

3.2. Mapping the NLS outside the GCM domain

Commonly used prediction programs failed to detect typical nuclear localization signals within GCMa. For NLS identification we divided GCMa into five, slightly overlapping fragments (F1–F5, Fig. 2a) and fused them in frame to β-galactosidase. The subcellular distribution of these β-galactosidase fusions in transfected HeLa cells was determined by X-Gal staining. Unfused β-galactosidase was predominantly cytoplasmic and served as a control (β-gal in Fig. 2b). GCMa fragments F2, F3 and F5 exhibited cytoplasmic localizations (Fig. 2b). Fragment F1, in contrast, was both nuclear and cytoplasmic, indicating that the determinants for subcellular localization which might be present within the GCM domain

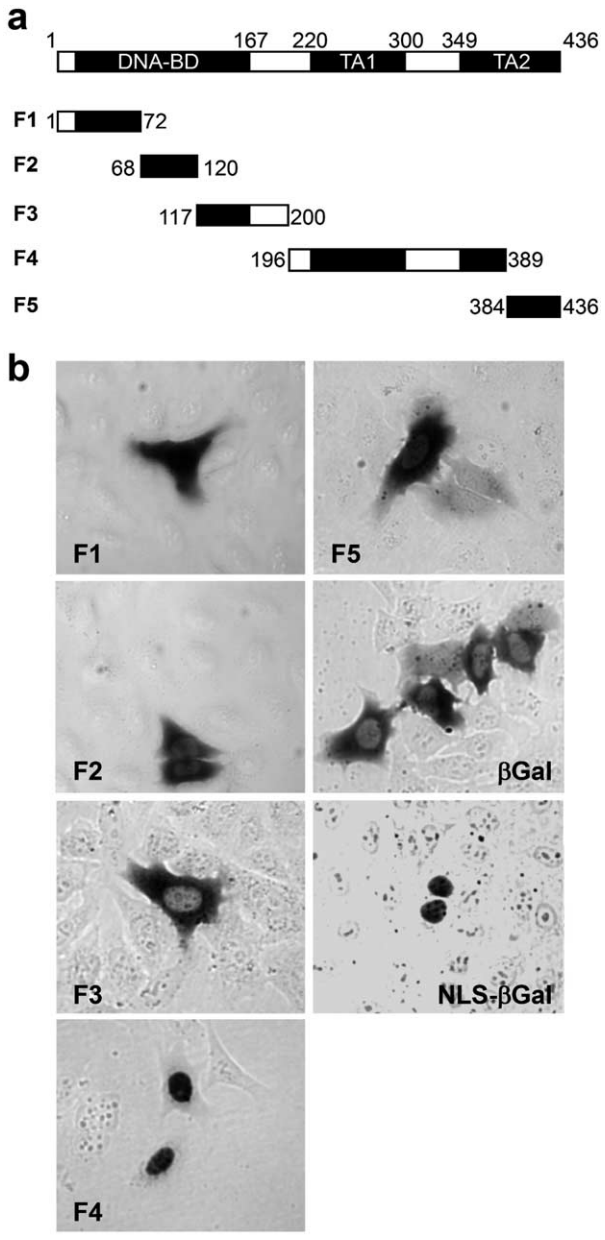

Fig. 2. NLS-carrying regions of GCMa. a: Domain structure of mouse GCMa with DNA-binding GCM domain (DNA-BD) and two transactivation domains (TA1 and TA2) as well as schematic representation of the GCMa fragments F1–F5 that were fused to the amino-terminus of β-galactosidase. Numbers indicate first and last residues present in protein domains or GCMa fragments. b: X-Gal staining of HeLa cells transfected with β-galactosidase fusions carrying the GCMa fragments depicted in panel a. βGal, β-galactosidase control for cytoplasmic localization. NLS-βGal, β-galactosidase carrying the NLS of SV40 T-antigen at its amino-terminus, control for nuclear localization.

(see Fig. 1d) should be localized to the first 72 amino acids. Only fragment F4, which encompassed amino acids 196–389, was able to drive the overwhelming amount of β-galactosidase fusion into the nucleus in a manner comparable to the NLS of SV40 T-antigen (compare F4 to NLS-βGal in Fig. 2b). Therefore, we conclude that an NLS is present in the GCMa region represented by fragment F4.

To define this NLS in more detail, we subdivided fragment F4 further (Fig. 3a). The first series of subfragments that were fused to β-galactosidase consisted of three fragments spanning amino acids 196–270 (F4/1), amino acids 271–311 (F4/2), and amino acids 312–389 (F4/3). Again, only one of the resulting β-galactosidase fusions (i.e. the one containing fragment F4/3) exhibited predominantly nuclear localization (Fig. 3b).

A further set of β-galactosidase fusions with different parts of the region between amino acids 312 and 389 of GCMa was generated and analyzed in transfected HeLa cells for their subcellular localization (fragments F4/3a–F4/3e in Fig. 4a). Only fragments F4/3b and F4/3e which contained the region immediately following amino acid residue 312 exhibited nuclear localization, the shortest of which was fragment F4/3e with a length of 25 amino acids spanning positions 312–337 (Fig. 4b). Fusions between β-galactosidase and GCMa fragments starting at later amino acid positions such as residue 331 (F4/3c), residue 337 (F4/3d) or residue 354 (F4/3a) stayed predominantly cytoplasmic. Thus, we have experimentally defined the 25 amino acids contained within F4/3e as the NLS of GCMa. Basic amino acids were completely missing. Instead, the NLS was rich in tyrosine, proline, serine and leucine residues (Fig. 4a).

Nuclear localization of β-galactosidase was also achieved when the putative NLS of GCMb/Gcm-2 was fused to the amino-terminus of *lacZ* instead of GCMa sequences (GCMb NLS in Fig. 4b). In direct comparison, the NLS of GCMb/Gcm-2 appeared to be somewhat more efficient in translocating β-galactosidase to the nucleus than the minimal

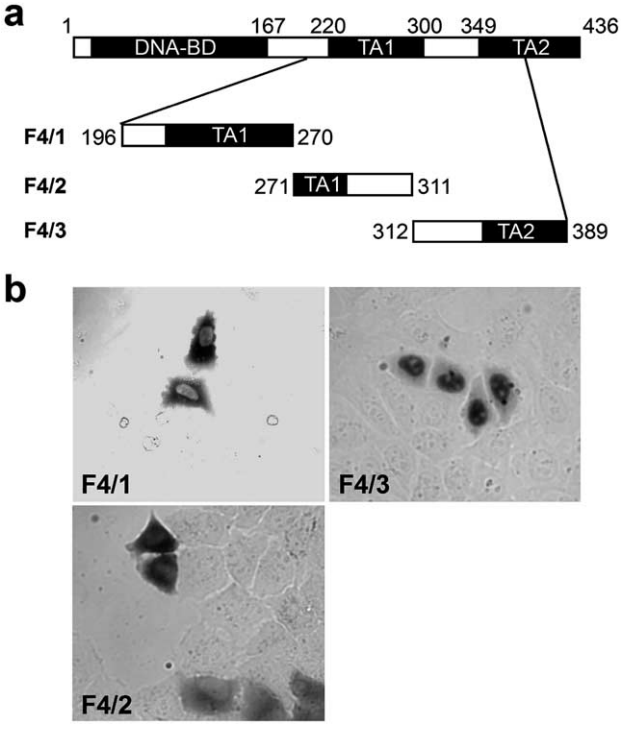

Fig. 3. Mapping the carboxy-terminal NLS of GCMa. a: Domain structure of mouse GCMa with DNA-binding GCM domain (DNA-BD) and two transactivation domains (TA1 and TA2) as well as schematic representation of the GCMa fragments F4/1–F4/3 that were fused to the amino-terminus of β-galactosidase. Numbers indicate first and last residues present in protein domains or GCMa fragments. b: X-Gal staining of HeLa cells transfected with β-galactosidase fusions carrying the GCMa fragments depicted in panel a. Not shown are the βGal and NLS-βGal controls which were performed in parallel.

NLS from GCMa as evident from the fact that residual cytoplasmic β-galactosidase staining was still visible with the GCMa NLS, but not with the GCMb NLS.

To confirm the importance of amino acids 312–337 for nuclear localization of GCMa, we generated a GCMa mutant in which this region was selectively deleted. This GCMaΔNLS mutant exhibited cytoplasmic as well as nuclear localization contrasting with the predominantly nuclear localization of wild-type GCMa (Fig. 1f). This altered subcellular distribution of the GCMaΔNLS mutant is unlikely to result from a severely disturbed overall conformation because it is still able to activate a GCM-dependent reporter plasmid in a manner very similar to the wild-type protein (data not shown). Its behavior clearly corroborates the importance of the NLS at residues 312–337 of GCMa for nuclear localization. However, it also argues for a second NLS within GCMa. Taking all experiments (see Figs. 1 and 2) into consideration, this component is most likely localized in the first 72 amino acids of GCMa.

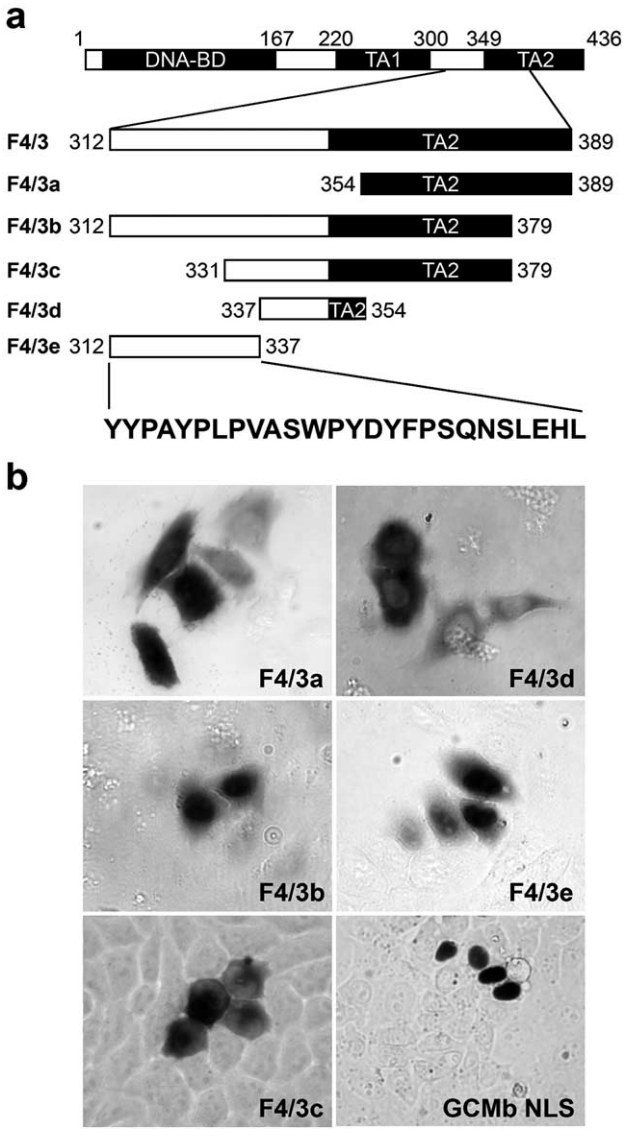

Fig. 4. Delineating the carboxy-terminal NLS of GCMa. a: Schematic representation of the GCMa fragments F4/3a–F4/3e that were fused to the amino-terminus of β-galactosidase. Numbers indicate first and last residues present in GCMa fragments. The amino acid sequence of the NLS of GCMa (corresponding to residues 312–337) is shown. b: X-Gal staining of HeLa cells transfected with β-galactosidase fusions carrying the GCMa fragments depicted in panel a or amino acids 176–189 of GCMb/Gcm-2 (GCMb NLS). Not shown are the βGal and NLS-βGal controls which were performed in parallel.

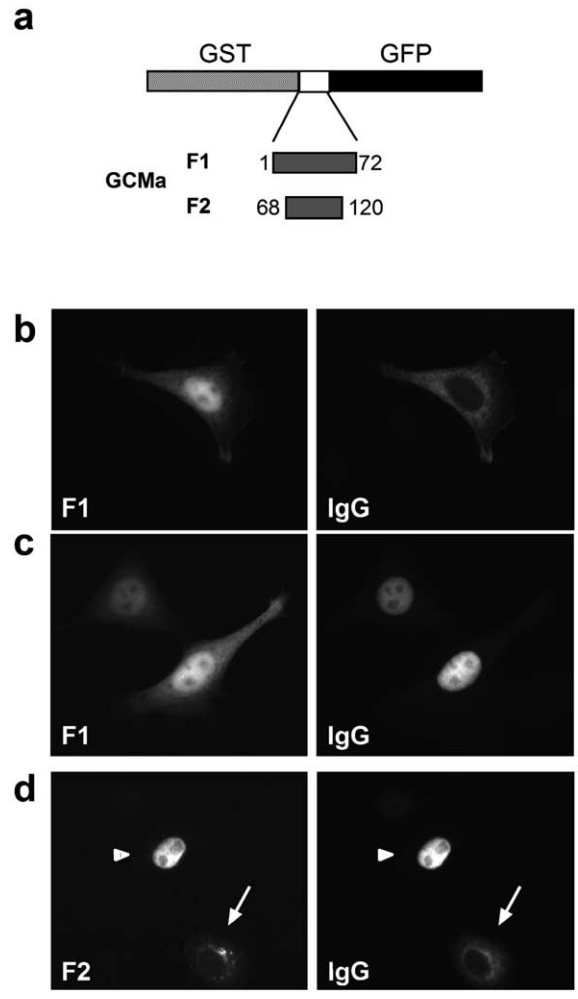

Fig. 5. Nuclear import and export functions of the amino-terminal GCMa region. a: Schematic representation of the GST-GFP fusion proteins carrying residues 1–72 (GST-F1-GFP) or residues 68–120 (GST-F2-GFP) of GCMa. b–d: Indirect immunofluorescence analysis of HeLa cells 1.5 h after microinjection with GST-GFP fusion proteins depicted in panel a and IgG. Left panels shown the GST-GFP fusions, right panels the co-injected IgG. b: Cytoplasmic microinjection, GST-F1-GFP. c: Nuclear microinjection, GST-F1-GFP. d: Cytoplasmic (arrow) and nuclear (arrowhead) microinjection, GST-F2-GFP.

3.3. *Analyzing the role of the GCM domain in subcellular localization of GCMa*

To directly address this question we performed microinjection experiments with a bacterially expressed fusion protein carrying amino acids 1–72 of GCMa sandwiched between a GST and a GFP portion (GST-F1-GFP in Fig. 5a). Injection sites were marked by co-injection of IgG (right panels in Fig. 5b–d). When injected into the cytoplasm, the fusion protein efficiently translocated into the nucleus confirming the presence of an NLS in this GCMa fragment (Fig. 5b, left panel). After nuclear injection, on the other hand, a significant frac-

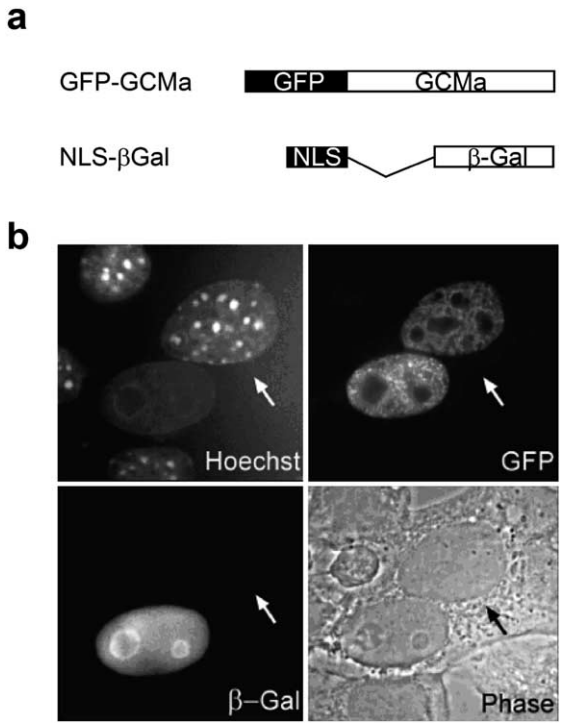

Fig. 6. Nucleocytoplasmic shuttling of GCMa. a: Schematic representation of GFP-GCMa and NLS-βGal proteins. b: Heterokaryon assay between HeLa cells cotransfected with expression plasmids for the proteins shown in panel a and 3T3 cells. Two hours later, cells were fixed, stained for βGal and counterstained with Hoechst 33258 dye (upper left panel). Murine nuclei are marked by an arrow. In interspecies heterokaryons that coexpressed GFP-GCMa and NLS-βGal, only GFP-GCMa was found in the murine nuclei (upper right panel), while NLS-βGal was restricted exclusively to the human nuclei (lower left panel). Heterokaryon in phase contrast (lower right panel).

tion of the fusion protein found its way into the cytoplasm arguing that amino acids 1–72 of GCMa also contain an export signal (Fig. 5c, left panel). As a control, we injected an analogous fusion protein carrying amino acids 68–120 of GCMa instead of amino acids 1–72. Similar to the co-injected IgG, this fusion protein remained in the subcellular compartment where it was injected independent of whether it was injected into the cytoplasm (arrow in Fig. 5d, left panel) or into the nucleus (arrowhead in Fig. 5d, left panel).

If GCMa contains NLS and NES sequences it should be able to relocate between cytoplasm and nucleus. To analyze this question, an interspecies heterokaryon analysis was performed. HeLa cells were cotransfected with a GFP-GCMa expression plasmid and an NLS-β-galactosidase control plasmid (Fig. 6a) that encodes a nucleus-restricted β-galactosidase (see Fig. 2b). Transfected cells were allowed to synthesize the ectopic proteins. Prior to and following heterokaryon formation (lower right panel in Fig. 6b) de novo protein synthesis was inhibited. In these heterokarya, human nuclei can be differentiated from murine nuclei by staining with Hoechst 33258 dye which yields a characteristic punctate pattern in murine nuclei and a diffuse stain in human nuclei (upper left panel in Fig. 6b). Two hours after heterokaryon formation, only GFP-GCMa was found to be present in both human and murine nuclei (upper right panel in Fig. 6b), whereas NLS-β-galactosidase was detected exclusively in the human nuclei (lower left panel in Fig. 6b). GFP-GCMa was thus selectively transported from the transfected HeLa cell nucleus into the cytoplasm and subsequently into the murine nucleus. We conclude that GCMa is able to leave and enter the nucleus and therefore constitutes a bona fide nucleocytoplasmic shuttling protein.

4. Discussion

From the fact that GCMa as a transcription factor has to function in the nucleus, it is expected to possess an NLS. Because of their characteristic signature, NLS are quite effectively predicted by computer algorithms as was the case for *Drosophila* GCM and GCMb/Gcm-2. In both proteins, the NLS is in the immediate vicinity of the DNA-binding GCM domain. Such proximity between NLS and DNA-binding domain is often observed in transcription factors and can be explained in evolutionary terms as ensuring co-segregation of two intimately linked characteristics.

In the case of GCMa, prediction programs failed to detect an NLS, thus making experimental definition necessary. In this study, we mapped two NLS within GCMa. One of these NLS is localized in the first 72 amino acids of the protein and thus within the GCM domain. The first 50 of these 72 amino acids either appear disordered in the crystal structure or are localized on the solvent-exposed surface of the GCM domain [4]. Therefore, they should be easily accessible for the nuclear import and export machinery. The second NLS was found closer to the carboxy-terminus between two regions that had previously been shown to function as transactivation domains [9]. No other function has previously been ascribed to this carboxy-terminal NLS-containing region.

Identification of the amino-terminal NLS was confounded by the simultaneous presence of an NES in the same fragment. While the nature and sequence of the amino-terminal NLS as well as its degree of conservation in other GCM proteins are difficult to predict at the moment, there is a good candidate for an NES in the same region. Residues 14–23 (**LSWDINDVKL**) conform roughly to the **L-**X_{2-3}**-L-**X_{2-3}**-L-X-L/I** consensus for small, hydrophobic, leucine-rich NES [32]. Comparative sequence analysis between GCMa and other GCM proteins indicates that this putative NES is not conserved in other GCM proteins.

Our data indicate that GCMa is a nucleocytoplasmic shuttling protein whose overall subcellular distribution depends on the activity of two NLS and one NES. Accordingly, deletion of one of the NLS allocates a greater proportion of mGCMa to the cytoplasm. The ability to shuttle might also be important for its function, as it might render GCMa responsive to cytoplasmic signaling events. Nucleocytoplasmic shuttling offers a unique opportunity for additional modulation of GCMa activity as well as for protein sequestration. Previous detection of a minor fraction of the endogenous GCMa protein in the cytoplasm of placental trophoblasts [33] might be taken as evidence that nucleocytoplasmic shuttling is indeed relevant for GCMa function in vivo. Interestingly, *Drosophila* GCM has also been reported to be nuclear in glioblasts of the fly embryo, but cytoplasmic in neuroglioblasts [34] arguing that nucleocytoplasmic shuttling might be a conserved feature within this family of transcription factors.

Astonishing is the absence of any similarity between known NLS and either NLS of GCMa. The NLS of GCMa thus

constitute highly unusual and novel nuclear import signals. Instead of basic residues, the most prominent amino acids within the carboxy-terminal NLS were tyrosines, prolines, serines and leucines. The overrepresentation of tyrosines and serines in the carboxy-terminal NLS of GCMa might indicate that this region undergoes phosphorylation in vivo which could influence its function as an NLS and thereby alter the overall distribution of GCMa in the cell. Several NLS have indeed been shown in the past to be modulated in their efficacy by phosphorylation sites within the NLS or in its immediate vicinity [35–37].

Many features indicate that GCM transcription factors arose late during evolution and have not expanded as much as most other transcription factor families [1]. The unusual NLS of GCMa might be yet another indicator of a relative young evolutionary age, as GCMa structure might not have had the time to be streamlined to a typical transcription factor. Alternatively, the particular features of NLS and NES may directly result from functional restraints imposed on GCMa. This will only be unravelled once a better understanding has been achieved of the molecular function of GCMa.

Acknowledgements: Supported by grants from the DFG to M.W. and T.S. (SFB 473).

References

[1] Wegner, M. and Riethmacher, D. (2001) Trends Genet. 17, 286–290.
[2] Akiyama, Y., Hosoya, T., Poole, A.M. and Hotta, Y. (1996) Proc. Natl. Acad. Sci. USA 93, 14912–14916.
[3] Cohen, S.X., Moulin, M., Schilling, O., Meyer-Klaucke, W., Schreiber, J., Wegner, M. and Muller, C.W. (2002) FEBS Lett. 528, 95–100.
[4] Cohen, S.X., Moulin, M., Hashemolhosseini, S., Kilian, K., Wegner, M. and Müller, C.W. (2003) EMBO J. 22, 1835–1845.
[5] Van De Bor, V. and Giangrande, A. (2002) Curr. Opin. Genet. Dev. 12, 465–472.
[6] Schreiber, J., Enderich, J. and Wegner, M. (1998) Nucleic Acids Res. 26, 2337–2343.
[7] Tuerk, E.E., Schreiber, J. and Wegner, M. (2000) J. Biol. Chem. 275, 4774–4782.
[8] Schreiber, J., Sock, E. and Wegner, M. (1997) Proc. Natl. Acad. Sci. USA 94, 4739–4744.
[9] Reifegerste, R., Schreiber, J., Gulland, S., Lüdemann, A. and Wegner, M. (1999) Mech. Dev. 82, 141–150.
[10] Hosoya, T., Takizawa, K., Nitta, K. and Hotta, Y. (1995) Cell 82, 1025–1036.
[11] Jones, B.W., Fetter, R.D., Tear, G. and Goodman, C.S. (1995) Cell 82, 1013–1023.
[12] Vincent, S., Vonesch, J.-L. and Giangrande, A. (1996) Development 122, 131–139.
[13] Kammerer, M. and Giangrande, A. (2001) EMBO J. 20, 4664–4673.
[14] Lebestky, T., Chang, T., Hartenstein, V. and Banerjee, U. (2000) Science 288, 146–149.
[15] Anson-Cartwright, L., Dawson, K., Holmyard, D., Fisher, S.J., Lazzarini, R.A. and Cross, J.C. (2000) Nat. Genet. 25, 311–314.
[16] Gunther, T. et al. (2000) Nature 406, 199–203.
[17] Schreiber, J., Riethmacher-Sonnenberg, E., Riethmacher, D., Tuerk, E.E., Enderich, J., Bösl, M.R. and Wegner, M. (2000) Mol. Cell. Biol. 20, 2466–2474.
[18] Hashemolhosseini, S., Hadjihannas, M., Stolt, C.C., Haas, C.S., Amann, K. and Wegner, M. (2002) Mech. Dev. 118, 175–178.
[19] Boulikas, T. (1994) J. Cell Biochem. 55, 32–58.
[20] Südbeck, P. and Scherer, G. (1997) J. Biol. Chem. 272, 27848–27852.
[21] Jans, D.A., Xiao, C.Y. and Lam, M.H. (2000) BioEssays 22, 532–544.
[22] Kuersten, S., Ohno, M. and Mattaj, I.W. (2001) Trends Cell Biol. 11, 497–503.
[23] Fischer, U., Huber, J., Boelens, W.C., Mattaj, I.W. and Lührmann, R. (1995) Cell 82, 475–483.
[24] Wen, W., Meinkoth, J.L., Tsien, R.Y. and Taylor, S.S. (1995) Cell 82, 463–473.
[25] Fornerod, M., Ohno, M., Yoshida, M. and Mattaj, I.W. (1997) Cell 90, 1051–1060.
[26] Stade, K., Ford, C.S., Guthrie, C. and Weis, K. (1997) Cell 90, 1041–1050.
[27] Dingwall, C. and Laskey, R.A. (1991) Trends Biochem. Sci. 16, 478–481.
[28] Kammerer, M., Pirola, B., Giglio, S. and Giangrande, A. (1999) Cytogenet. Cell Genet. 84, 43–47.
[29] Sock, E., Enderich, J., Rosenfeld, M.G. and Wegner, M. (1996) J. Biol. Chem. 271, 17512–17518.
[30] Rehberg, S., Lischka, P., Glaser, G., Stamminger, S., Wegner, M. and Rosorius, O. (2002) Mol. Cell. Biol. 22, 5836–5844.
[31] Pinol-Roma, S. and Dreyfuss, G. (1992) Nature 355, 730–732.
[32] Bogerd, H.P., Fridell, R.A., Benson, R.E., Hua, J. and Cullen, B.R. (1996) Mol. Cell. Biol. 16, 4207–4214.
[33] Nait-Oumesmar, B., Copperman, A.B. and Lazzarini, R.A. (2000) J. Histochem. Cytochem. 48, 915–922.
[34] Bernardoni, R., Kammerer, M., Vonesch, J.L. and Giangrande, A. (1999) Dev. Biol. 216, 265–275.
[35] Komeili, A. and O'Shea, E.K. (1999) Science 284, 977–980.
[36] Hübner, S., Xiao, C.Y. and Jans, D.A. (1997) J. Biol. Chem. 272, 17191–17195.
[37] Briggs, L.J., Stein, D., Goltz, J., Corrigan, V.C., Efthymiadis, A., Hubner, S. and Jans, D.A. (1998) J. Biol. Chem. 273, 22745–22752.