Biochemical and Biophysical Research Communications 392 (2010) 53–57

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The mitogaligin protein is addressed to the nucleus via a non-classical localization signal

Pauline Robinet, Lucile Mollet, Patrick Gonzalez [1], Thierry Normand, Stéphane Charpentier, Fabienne Brulé, Martine Dubois, Alain Legrand *

Centre de Biophysique Moléculaire (Affiliated with the University of Orléans), CNRS UPR4301, Rue Charles Sadron, 45071 Orléans Cedex 2, France

ARTICLE INFO

Article history:
Received 29 December 2009
Available online 7 January 2010

Keywords:
Unconventional nuclear localization signal
Mitogaligin
Dual localization

ABSTRACT

Mitogaligin, a protein encoded by *galig*, an internal cytotoxic gene of the galectin-3 locus, is mostly a mitochondrial protein. Mitochondrial targeting is due to an already identified mitochondrial localization signal. Interaction of mitogaligin with mitochondria leads to cytochrome *c* cytosolic leakage and ultimately to cell death. We have previously pointed out that mitogaligin can also be directed to the nucleus when the mitochondrial addressing signal is inactivated, indicating a possible dual intracellular localization of the protein. When expressed in the nucleus, mitogaligin exhibits also apoptotic properties leading to cell death. In this report, we show that nuclear addressing of mitogaligin depends on a sequence differing from classical signals containing basic, lysine or proline-tyrosine rich residues. The signal consists of a long sequence of amino acids residues based on a series of a short repetitive degenerated sequence.

Introduction

We have previously identified a cell death inducer gene, *galig*, located within the human galectin-3 locus [1,2]. The gene produces two unrelated proteins encoded from two overlapping open reading frames. Expression of this gene causes cell shrinkage, cytoplasm vacuolization, nuclei condensation, and eventually cell death. These alterations are associated with extramitochondrial release of cytochrome *c* and aggregation of mitochondria [2]. Mitogaligin, one of the two proteins produced by *galig*, is a 96 amino acid protein highly cationic and exceptionally rich in tryptophan residues [1]. This protein is mainly localized in mitochondria, destabilizing its membrane by an unknown process which involves interaction with cardiolipin [3], a specific phospholipid of mitochondria [4]. The mitochondrial addressing of mitogaligin relies on the already described internal sequence (residues 31–47) [2,3].

Fluorescent microscopy observations regularly indicate that a minor fraction of mitogaligin is specifically targeted to the nucleus [5]. This uneven distribution, or eclipsed distribution, is commonly associated with proteins targeted to different cellular compartments [6]. In addition, we have shown that neutralization of the mitochondrial localization signal, unmasks specific addressing to nucleus. Interestingly, nuclear mitogaligin also induced cell death through a pathway exhibiting typical properties of apoptosis at the morphological level (cell shrinkage, cytoplasm vacuolization, and nuclei condensation) and at the molecular level (caspase activation, DNA fragmentation, translocation of Bax from cytosol to mitochondria) [5]. These observations indicated that mitogaligin might express cytotoxic properties not only when addressed to mitochondria but also when targeted to the nucleus.

These results suggested that mitogaligin contains two localization signals, a major mitochondrial one and a minor nuclear one, actives when the mitochondrial localization signal is invalidated. This is not unusual because mitochondrial signals are generally recognized during translation and before appearance of other targeting peptides [7]. Thus, moving a mitochondrial targeting sequence away from the N-terminus frequently weakens its recognition [8,9]. This phenomenon has also been observed for mitogaligin, and we have shown that the mitochondrial targeting sequence of the peptide, i.e. amino acid (31–47), is invalidated when EGFP is fused to the N-terminal part of mitogaligin. In these conditions, the nuclear signal predominates and mitogaligin accumulates in the nucleus [5].

In this paper, we have undertaken to delineate such a signal sequence using truncated forms of mitogaligin. Our data point out that mitogaligin targeting to the nucleus results from a non-conventional signal, unrelated to already known nuclear localization signal (NLS) and associated to a repetitive domain of mitogaligin.

Materials and methods

Cell line and transfections. HeLa cells (ATCC, CCL-2) were cultured in E-MEM medium (Invitrogen) plus 10% fetal calf serum (Invitro-

* Corresponding author. Fax: +33 238255540.
E-mail address: alain.legrand@cnrs-orleans.fr (A. Legrand).
[1] Present address: Children's Hospital, Forshungslabor, Eythstrasse 24, Haus 16, 89075 ULM, Germany.

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.12.162

gen). Transfections were performed using DNA-polyethylenimine complexes [10], as described [1]. Twenty-four or 48 h after transfection, HeLa cells were fixed in 4% paraformaldehyde and analyzed by confocal fluorescence microscopy (LSM510, Carl Zeiss S.A.S.) using fluorescein filters (excitation at 488 nm and detection at 520 nm).

Expression vectors. Construction of the plasmid pEGFP-MG, used as a reference in this work, has previously been described [5]. This vector produces EGFP fused to the N-terminus of mitogaligin (EGFP-mitogaligin). pEGFP-MG was used as a template for PCRs to generate plasmids encoding truncated from of EGFP-mitogaligin. The resulting plasmids were designed according to the remaining residues of mitogaligin. For example pEGFP-MG(18–60) indicates that the plasmid produces the chimeric protein containing EGFP and residues 18–60 of mitogaligin.

Results

The repetitive domain of mitogaligin is a non-conventional NLS

Conventional NLS sequences consist of short sequences rich in positive charges and the signals may have mono- or bipartite organizations [11–16]. Analysis of the mitogaligin primary structure with different software dedicated to prediction of cellular localization revealed no apparent classical NLS. Although mitogaligin is highly positively charged due to 12 arginine residues, these charges are not clustered but rather regularly distributed along the protein (Fig. 1A). Mitogaligin is encoded by the third exon of the galectin-3 gene, in an alternative reading frame of galectin-3. This exon is essentially composed of repetitive DNA which generates a repetitive domain made of 8 U containing nine amino acid residues [17–19]. In mitogaligin, this domain corresponds to residues 13–86 (Fig. 1A). All units contain one or two positive charges thus explaining why they are somewhat regularly spaced.

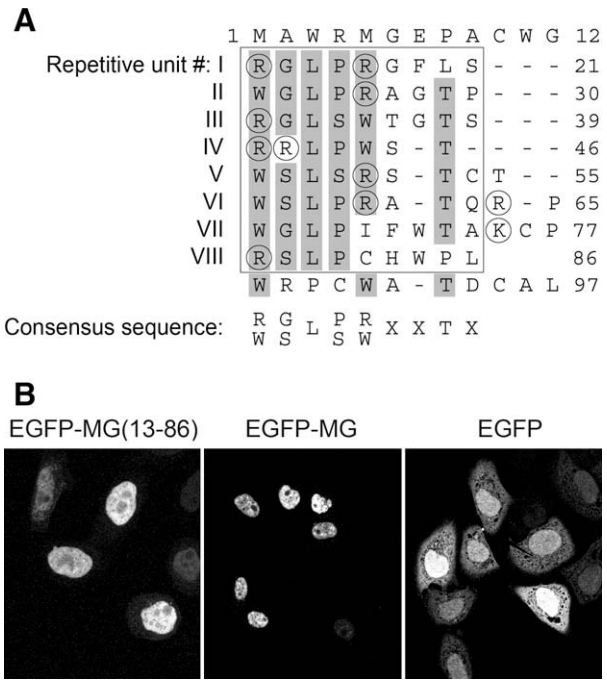

Fig. 1. Repetitive domains in mitogaligin sequence and subcellular localization of full mitogaligin. (A) Eight repetitive units (numbered I–VIII) are evidenced in the mitogaligin amino acid sequence. DNA repetitive domain encodes amino acid sequence from R13 to L86. The eight repetitive units are arranged according to the best alignment. Highlighted letters represent conserved amino acid residues. Circles surround positively charged amino acids. The consensus sequence deduced from this repetitive domain is: $R_{50\%}/W_{50\%}$-$G_{50\%}/S_{37,5\%}$-$L_{100\%}$-$P_{75\%}S_{25\%}$-$R_{50\%}/W_{25\%}$-X-X-$T_{75\%}$-X. (B) HeLa cells were transfected with plasmids encoding EGFP-MG(13–86), EGFP-MG and EGFP and analyzed under confocal microscopy.

We wondered whether the nuclear accumulation of EGFP-mitogaligin was related to this particular primary structure of mitogaligin. To test this hypothesis, the plasmid pEGFP-mitogaligin-(13–86) encoding EGFP fused to the repetitive domain of mitogaligin, was constructed. This recombinant protein does not contain the 12 N-terminus and 11 C-terminus amino acid residues of mitogaligin. Upon transfection of HeLa cells, green fluorescence was restricted to the nucleus as observed for the parental protein (Fig. 1B). The nuclear fluorescence was not due to an artifact such as EGFP cleavage because the dual labeling obtained using an anti-mitogaligin antibody, tagged the nucleus as well (data not shown). This observation firstly indicates that the nuclear targeting of mitogaligin does not involve the 12 first and 11 last amino acid residues and secondly that the repetitive domain of the protein is sufficient to function as a nuclear transportation signal.

Serial deletions of C-terminal and N-terminal residues of mitogaligin

To further localize the boundaries of the nuclear targeting sequence, we applied the conventional strategy which consists in serial deletions starting from the C-terminal or the N-terminal ends of mitogaligin. Both categories of deletions were generated from the plasmid pEGFP-MG encoding the chimeric EGFP-mitogaligin (Fig. 1B). These plasmids were transfected into HeLa cells and observed under fluorescence microscopy. No difference of localization between mutants and the full protein was noted when the first 60 residues of mitogaligin were conserved (Fig. 2A, EGFP-MG(01–74), EGFP-MG(01–60)). In contrast, nuclear accumulation was abolished with plasmids encoding shorter forms of mitogaligin (Fig. 2A, EGFP-MG(01–46), EGFP-MG(01–38), EGFP-MG(01–30)).

In parallel, serial deletions from the N-terminus of mitogaligin were also generated from the same parental plasmid. Nuclear localization was not affected upon withdrawal of the 30 first residues of mitogaligin (Fig. 2B, EGFP-MG(31–97)). Shorter forms of mitogaligin were clearly delocalized into cytosol (Fig. 2B, EGFP-MG(38–97, EGFP-MG(42–97), EGFP-MG(48–97), EGFP-MG(74–97)). These results do not help to delineate very precisely the mitogaligin nuclear addressing sequence. However, they suggest that it lies within the core region of mitogaligin, between residues 31 and 60.

The central region of mitogaligin is not sufficient for nuclear accumulation

Considering these results, it was expected that transfection of a plasmid encoding a chimeric protein containing EGFP fused to residues (31–60) of mitogaligin would produce a nuclear protein. Sequence was not enough to function as such as a nuclear location signal.

These astonishing results suggested that additional sequences were necessary to get a functional addressing signal. Therefore, we have performed extensions of the central region (31–60) of mitogaligin downstream and upstream (Fig. 3B and C). Extension of the (31–60) sequence to (31–79) restored nuclear targeting. Likewise, nuclear targeting was also observed when the sequence (31–60) was extended from its N-terminal end. While sequence (18–60) was not sufficient for nuclear localization, sequence (13–60) induced a net recovering of nuclear targeting (Fig. 3C). In summary, only one of these two additional sequences located on each sides of (31–60) is necessary to restore nuclear addressing in conjunction with (31–60).

At this stage, the central region of mitogaligin seemed imperative for nuclear localization, and it was expected that deletion of the (31–46) region of mitogaligin in EGFP-mitogaligin would generate a cytosolic protein. Surprisingly, such a protein was clearly localized into the nucleus (Fig. 4A). Consequently, the structure

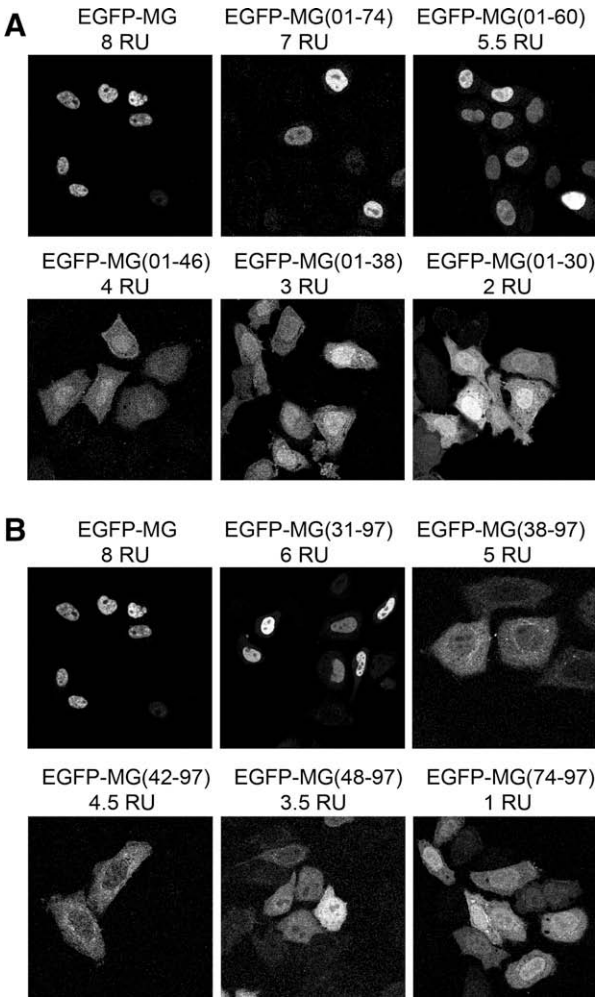

Fig. 2. Subcellular localization of EGFP-mitogaligin mutants. Plasmids encoding truncated forms of EGFP-mitogaligin were constructed and transfected in HeLa cells. Twenty-four hours after transfection, cells were fixed and observed under confocal microscopy. (A) Deletions from the C-terminal end of mitogaligin in EGFP-mitogaligin. Plasmids encode full mitogaligin (MG), MG(01–74), MG(01–60), MG(01–46), MG(01–38) or MG(01–30), all fused to EGFP. (B) Deletions from the N-terminal part of mitogaligin in EGFP-mitogaligin. Plasmids encode MG(31–97), MG(38–97), MG(42–97), MG(48–97) or MG(74–97). For each construct, the number of repetitive units (RU) is indicated.

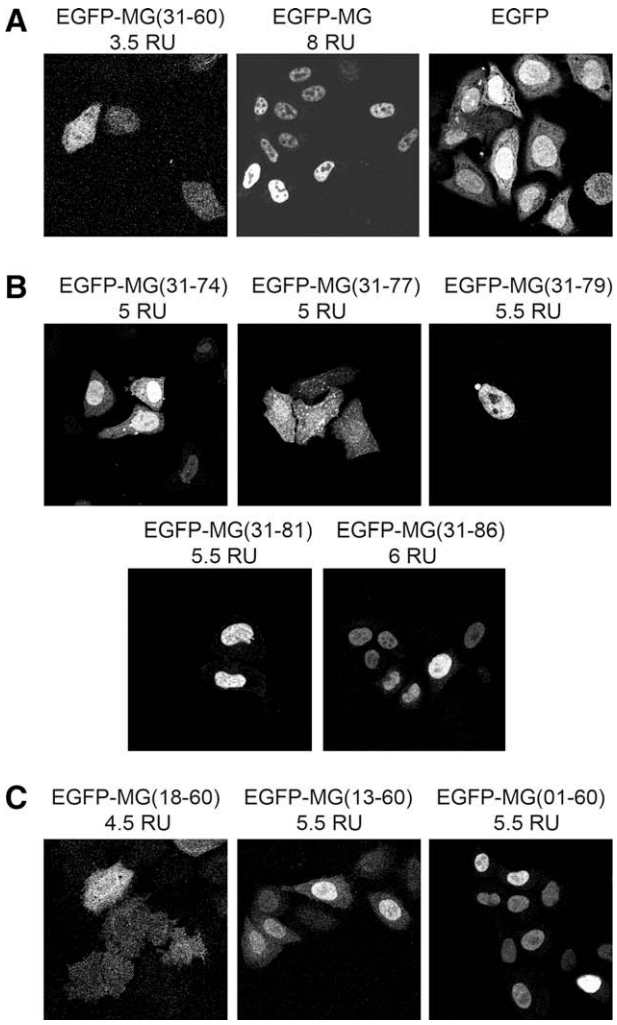

Fig. 3. The nuclear localization of mitogaligin is not related to a single short NLS. Plasmids encoding modified forms of EGFP-mitogaligin were transfected in HeLa cells. Expression was analyzed under confocal microscopy. (A) Transfection of a plasmid encoding the core region of mitogaligin (31–60) is not sufficient for nuclear localization. (B) Extension of the C-terminus of sequence (31–60) restores nuclear targeting. Transfected plasmids encode MG(31–74), MG(31–77), MG(31–79), MG(31–81), and MG(31–86) fused to EGFP. (C) Extension of the N-terminus of sequence (31–60) also restores nuclear targeting of mitogaligin. Plasmids encode MG(01–60), MG(13–60), and MG(18–60). For each construct, the number of repetitive units (RU) is indicated.

of the nuclear signal of mitogaligin is more complex that can be anticipated for a conventional NLS [13].

The nuclear localization of mitogaligin is related to the number of repetitive units

The data generated above displayed apparent contradictory results. From deletion experiments in Fig. 2, the sequence (31–60) appeared important for nuclear targeting function. However, the sequence by itself was not sufficient for accurate addressing and one additional sequence belonging to mitogaligin-(01–30) or mitogaligin-(60–79) needed to be present for nuclear localization. Furthermore, deletion of the sole sequence (31–46) of mitogaligin in EGFP-mitogaligin did not prevent nuclear distribution. To resolve these apparent contradictions, we reasoned that each repetitive unit displays a weak nuclear targeting signal not active enough by itself for nuclear addressing. As mitogaligin is essentially translated from repetitive DNA, the signal would be the result of the accumulation of the corresponding repetitive units that combinatorially contributes to overall nuclear localization. Table 1 reports the repeated unit composition of mitogaligin in all the vectors used above. Indeed, the significant difference between these vectors was the number of repetitive units. In fact, all truncated forms of mitogaligin with five complete repetitive units or less exhibited cytosolic distribution while mutated mitogaligins containing more than five integral units had a nuclear localization.

To test whether the number of repetitive units was indeed more critical than the actual sequence for the nuclear addressing function, a vector containing a duplication of the sequence (31–60) has been constructed. This plasmid produces a recombinant protein with seven full repetitive units versus 3.5 for the vector containing a single copy of the sequence (31–60) and eight for the plasmid expressing the whole nuclear mitogaligin. Transfection experiments indicated that the protein constituted by the two linked sequences (31–60) was nuclear (Fig. 4B, right) while the vector with no duplication produced a cytosolic protein (Fig. 4B, left). Thus, increasing the number of repetitive units is sufficient to enhance the nuclear targeting function.

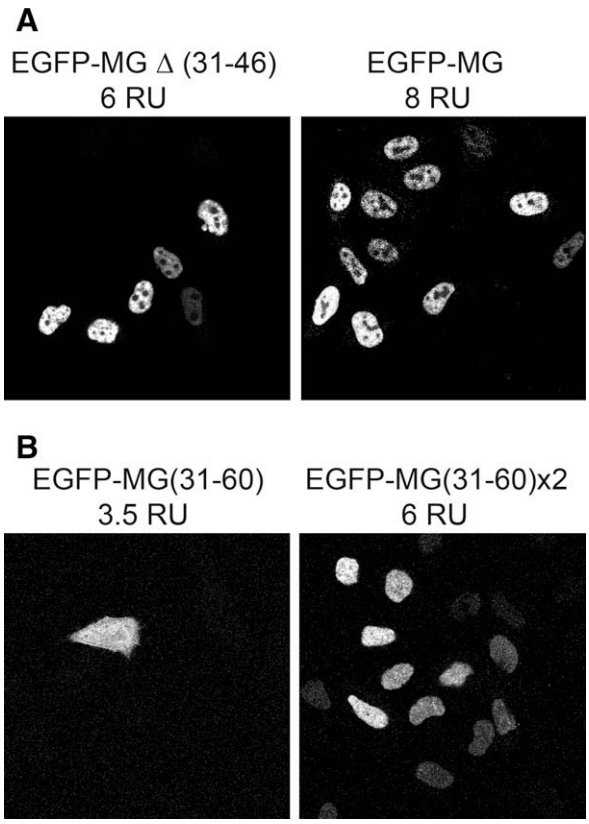

Fig. 4. (A) Deletion of the region (31–46) of mitogaligin in the plasmid pEGFP-MG does not modify nuclear localization. Cells were transfected with pEGFP-MG Δ(31–46), a vector encoding mitogaligin devoid of the sequence 31–46 (left panel) or pEGFP-MG, a vector encoding EGFP-mitogaligin (right panel). Twenty-four hours after transfection, cells were observed under confocal microscopy. Numbers of repetitive units (RU) are indicated. (B) Nuclear localization of mitogaligin depends on the number of repetitive units. Left panel: plasmid pEGFP-MG(31–60) encodes sequence 31–60 of mitogaligin fused to EGFP and contains about 3.5 U of the repetitive sequence (see text). Right panel: plasmid pEGFP-MG(31–60) × 2 contains a duplication of the same sequence also fused to EGFP and includes seven complete repetitive units. Twenty-four hours after transfection, cells were analyzed with confocal microscopy.

Table 1
Numbers of repetitive units (RU) and cellular localization of the modified mitogaligins. All mutant mitogaligins are in fusion with EGFP at their N-terminus. N/C stands for nucleus/cytosol. Localizations were determined under confocal microscopy. MG (first lane) is the complete mitogaligin. The repetitive units (RU) were numbered according to Fig. 1A nomenclature.

Proteins	Fig.	Numbers of complete RU	Additional residues from incomplete RU	Localization
MG	1–4A	8 (I–VIII)	0	N
MG(13–86)	1B	8 (I–VIII)	0	N
MG(01–74)	2A	7 (I–VII)	0	N
MG(31–60) × 2	4B	2 × 3 (III–V)	2 × 5 (VI)	N
MG(31–97)	2B	6 (III–VIII)	0	N
MG(31–86)	3B	6 (III–VIII)	0	N
MG Δ(31–46)	4A	6 (I–II, V–VIII)	0	N
MG(01–60)	2A, 3C	5 (I–V)	5 (VI)	N
MG(13–60)	3C	5 (I–V)	5 (VI)	N
MG(31–81)	3B	5 (III–VII)	4 (VIII)	N
MG(31–79)	3B	5 (III–VII)	2 (VIII)	N
MG(38–97)	2B	5 (IV–VIII)	1 (III)	C
MG(31–74)	3B	5 (III–VII)	0	C
MG(31–77)	3B	5 (III–VII)	0	C
MG(18–60)	3C	4 (II–V)	3 (I) + 5 (VI)	C
MG(42–97)	2B	4 (V–VIII)	5 (IV)	C
MG(01–46)	2A	4 (I–IV)	0	C
MG(48–97)	2B	3 (VI–VIII)	7 (V)	C
MG(31–60)	3A, 4B	3 (III–V)	5 (VI)	C
MG(01–38)	2A	2 (I–II)	8 (III)	C
MG(01–30)	2A	2	0	C
MG(74–97)	2B	1 (VIII)	0	C

Discussion

The experiments presented in this report lead to the conclusion that the nuclear targeting sequence of mitogaligin constitutes a new class of nuclear addressing signal. This result is really not surprising considering, as mentioned above, that the primary structure of mitogaligin reveals no predictable NLS. Although the primary structure of mitogaligin contains an abundance of basic residues, these residues are not clustered as it has been described for a numerous of mono-, bi- or tripartite signals [20].

We used the classical experimental approach to delineate the targeting signal, based on transfection of plasmids encoding truncated forms of mitogaligin fused to the C-terminal end of EGFP. A first set of experiments indicated that the mitogaligin nuclear addressing sequence was related to the (13–86) repetitive domain of the protein (Fig. 1). This made a first difference with other known NLS which do not exhibit such a repetitive structure even for non-conventional sequences [21].

Serial deletions, used to delineate more precisely the mitogaligin addressing sequence boundaries, generated unexpected and apparent contradictory results. Fig. 2 revealed the importance of residues between 31 and 60. The role of this sequence in the nuclear targeting of mitogaligin was reinforced by the fact that replacement of an arginine doublet ($R_{40}R_{41}$) with glutamate residues in mitogaligin-(01–60) or mitogaligin-(31–97) diminishes considerably the nuclear localization (data not shown). However, peptide (31–60) was clearly insufficient to independently function as a nuclear targeting signal (Fig. 3A). Recovering of the nuclear addressing function required an extension of sequences located upstream or downstream to the (31–60) peptide (Fig. 3B and C). Surprisingly, removal of mitogaligin region (31–46) did not prevent nuclear addressing indicating that this sequence was not essential (Fig. 4A).

The simplest interpretation for these contradictory observations is to consider the mitogaligin nuclear transportation sequence as a modular structure: each repetitive unit in mitogaligin constitutes a weak functional addressing signal and the efficient nuclear localization results from the cooperation between these repetitive units. Nuclear localization is always observed when the recombinant mitogaligin contains more than five complete units of the repetitive sequence while a cytosolic localization is constantly detected when five or less repetitive units are present (Table 1). In other words, in mitogaligin, each repetitive unit independently exhibits a low affinity for its carrier and synergy, achieved by more than 5 U, generates sufficient avidity to constitute a functional targeting signal. Consequently, in order to get efficient addressing of mitogaligin to nucleus, the number of repetitive units is more critical that the sequence *per se*. This can explain the fact that the mitogaligin nuclear addressing signal is exceptionally long when compared to a classical NLS [20]. The mutant EGFP-mitogaligin-(31–60) illustrates this modular structure. This mutant contains about 3.5 repetitive units and appears diffused in the cytoplasm confirming that this sequence is not sufficient to display addressing function. However, duplication of the same sequence, (31–60), induces nuclear targeting (Fig. 4B).

The following degenerate consensus sequence of the repetitive unit can be established: [W/R]-[G/S]-L-[P/S]-[R/W]-X-X-T-X. The first five residues of the repetitive units are much more conserved than the C-terminal end for each unit. As compared to the different

classes of classical NLS which harbor clusters of basic amino acid residues, the repetitive units contain one or two positively charged residues. This constitutes another major difference. Accumulation of more or less regularly spaced basic residues, due to addition of the repetitive units could be the key to generate an efficient signal.

The sequence of mitogaligin required for nuclear targeting is particularly long as compared to classical NLS. However, this is not a unique case. An increasing number of cellular or viral proteins have been found to utilize non-classical NLS for nuclear localization [22–28]. For example, the nuclear localization sequence of VP22, a viral protein from bovine herpesvirus 1, is a 103 amino acid sequence [28]. However, a major difference with mitogaligin is that no repetitive domain is found within the sequence. Interestingly, like mitogaligin, VP22 also contains a mitochondrial addressing signal which overlaps the nuclear localization signal [28]. It is thought that these long NLS are more related to their structure rather than to their linear sequence. The deletion experiments support the hypothesis that it can be the same for mitogaligin. The structure of mitogaligin has yet to be characterized. Because mitogaligin does not present structural homology with other known proteins, such a structure will have to be elucidated through experimental procedures.

In summary, we have characterized a new type of nuclear importation signal. This sequence does not match a minimal consensus for other known classical NLS. The mitogaligin nuclear addressing signal presents a modular organization based on addition of eight repetitive units of nine amino acid residues indicating that the sequence is much longer than classical NLS. However, the mitogaligin targeting sequence can be shortened but should contain at least five complete units to be functional. This structure can explain the fact that the mitochondrial localization signal (MLS) is dominant over the nuclear signal. It is known that MLS are recognized during translation [7]. Consequently, the mitogaligin MLS could be recognized before translation of the whole long nuclear addressing domain occurred. The mechanism of importation of this new type of NLS remains to be elucidated.

Acknowledgments

This work was supported by grants from the Comités Départementaux du Cher et de l'Indre de la Ligue Nationale Contre le Cancer. P.R. is supported by a fellowship from the Ministère de l'Enseignement Supérieur et de la Recherche.

References

[1] M. Guittaut, S. Charpentier, T. Normand, M. Dubois, J. Raimond, A. Legrand, Identification of an internal gene to the human Galectin-3 gene with two different overlapping reading frames that do not encode Galectin-3, J. Biol. Chem. 276 (2001) 2652–2657.
[2] M. Duneau, M. Boyer-Guittaut, P. Gonzalez, S. Charpentier, T. Normand, M. Dubois, J. Raimond, A. Legrand, Galig, a novel cell death gene that encodes a mitochondrial protein promoting cytochrome *c* release, Exp. Cell Res. 302 (2005) 194–205.
[3] P. Gonzalez, M. Duneau, S. Charpentier, T. Normand, L. Mollet, M. Dubois, A. Legrand, Destabilization of membranes containing cardiolipin or its precursors by peptides derived from mitogaligin, a cell death protein, Biochemistry 46 (2007) 7374–7382.
[4] D. Ardail, J.P. Privat, M. Egret-Charlier, C. Levrat, F. Lerme, P. Louisot, Mitochondrial contact sites. Lipid composition and dynamics, J. Biol. Chem. 265 (1990) 18797–18802.
[5] P. Gonzalez, P. Robinet, S. Charpentier, L. Mollet, T. Normand, M. Dubois, A. Legrand, Apoptotic activity of a nuclear form of mitogaligin, a cell death protein, Biochem. Biophys. Res. Commun. 378 (2009) 816–820.
[6] N. Regev-Rudzki, O. Pines, Eclipsed distribution: a phenomenon of dual targeting of protein and its significance, Bioessays 29 (2007) 772–782.
[7] J.C. Mueller, C. Andreoli, H. Prokisch, T. Meitinger, Mechanisms for multiple intracellular localization of human mitochondrial proteins, Mitochondrion 3 (2004) 315–325.
[8] K.N. Truscott, N. Pfanner, W. Voos, Transport of proteins into mitochondria, Rev. Physiol. Biochem. Pharmacol. 143 (2001) 81–136.
[9] W. Neupert, J.M. Herrmann, Translocation of proteins into mitochondria, Annu. Rev. Biochem. 76 (2007) 723–749.
[10] O. Boussif, F. Lezoualc'h, M.A. Zanta, M.D. Mergny, D. Scherman, B. Demeneix, J.P. Behr, A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine, Proc. Natl. Acad. Sci. USA 92 (1995) 7297–7301.
[11] D. Kalderon, B.L. Roberts, W.D. Richardson, A.E. Smith, A short amino acid sequence able to specify nuclear location, Cell 39 (1984) 499–509.
[12] C.V. Dang, W.M. Lee, Identification of the human c-myc protein nuclear translocation signal, Mol. Cell. Biol. 8 (1988) 4048–4054.
[13] C. Dingwall, R.A. Laskey, Nuclear targeting sequences – a consensus?, Trends Biochem Sci. 16 (1991) 478–481.
[14] J. Robbins, S.M. Dilworth, R.A. Laskey, C. Dingwall, Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence, Cell 64 (1991) 615–623.
[15] D.L. Sherman, P.J. Brophy, A tripartite nuclear localization signal in the PDZ-domain protein L-periaxin, J. Biol. Chem. 275 (2000) 4537–4540.
[16] S.C. Hsu, M.C. Hung, Characterization of a novel tripartite nuclear localization sequence in the EGFR family, J. Biol. Chem. 282 (2007) 10432–10440.
[17] B.J. Cherayil, S. Chaitovitz, C. Wong, S. Pillai, Molecular cloning of a human macrophage lectin specific for galactose, Proc. Natl. Acad. Sci. USA 87 (1990) 7324–7328.
[18] Y. Oda, H. Leffler, Y. Sakakura, K. Kasai, S.H. Barondes, Human breast carcinoma cDNA encoding a galactoside-binding lectin homologous to mouse Mac-2 antigen, Gene 99 (1991) 279–283.
[19] A. Raz, P. Carmi, T. Raz, V. Hogan, A. Mohamed, S.R. Wolman, Molecular cloning and chromosomal mapping of a human galactoside-binding protein, Cancer Res. 51 (1991) 2173–2178.
[20] A. Lange, R.E. Mills, C.J. Lange, M. Stewart, S.E. Devine, A.H. Corbett, Classical nuclear localization signals: definition, function, and interaction with importin alpha, J. Biol. Chem. 282 (2007) 5101–5105.
[21] H. Siomi, G. Dreyfuss, A nuclear localization domain in the hnRNP A1 protein, J. Cell Biol. 129 (1995) 551–560.
[22] T. Wolff, G. Unterstab, G. Heins, J.A. Richt, M. Kann, Characterization of an unusual importin alpha binding motif in the borna disease virus p10 protein that directs nuclear import, J. Biol. Chem. 277 (2002) 12151–12157.
[23] A. Kretsovali, C. Spilianakis, A. Dimakopoulos, T. Makatounakis, J. Papamatheakis, Self-association of class II transactivator correlates with its intracellular localization and transactivation, J. Biol. Chem. 276 (2001) 32191–32197.
[24] P. Lischka, G. Sorg, M. Kann, M. Winkler, T. Stamminger, A nonconventional nuclear localization signal within the UL84 protein of human cytomegalovirus mediates nuclear import via the importin alpha/beta pathway, J. Virol. 77 (2003) 3734–3748.
[25] E. Lombardo, J.C. Ramirez, J. Garcia, J.M. Almendral, Complementary roles of multiple nuclear targeting signals in the capsid proteins of the parvovirus minute virus of mice during assembly and onset of infection, J. Virol. 76 (2002) 7049–7059.
[26] C. Lufei, X. Cao, Nuclear import of Pin1 is mediated by a novel sequence in the PPIase domain, FEBS Lett. 583 (2009) 271–276.
[27] P. Wang, P. Palese, R.E. O'Neill, The NPI-1/NPI-3 (karyopherin alpha) binding site on the influenza a virus nucleoprotein NP is a nonconventional nuclear localization signal, J. Virol. 71 (1997) 1850–1856.
[28] J. Zhu, Z. Qiu, C. Wiese, Y. Ishii, J. Friedrichsen, G. Rajashekara, G.A. Splitter, Nuclear and mitochondrial localization signals overlap within bovine herpesvirus 1 tegument protein VP22, J. Biol. Chem. 280 (2005) 16038–16044.