Binding of matrix attachment regions to nuclear lamin is mediated by the rod domain and depends on the lamin polymerization state

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Abstract The nuclear matrix maintains specific interactions with genomic DNA at sites known as matrix attachment regions (M/SARs). M/SARs bind in vitro to lamin polymers. We show that the polymerized α-helical rod domain of lamin Dm_0 provides by itself the specific binding to the *ftz* M/SAR. In contrast, unpolymerized rod domain does not bind specifically to this M/SAR. Non-specific binding to DNA is also observed with Dm_0 containing a point mutation that impairs its ability to polymerize or with the isolated tail domain. These data suggests that the specific binding of lamins to M/SARs requires the rod domain and depends on the lamin polymerization state.

Key words: Nuclear lamina; MAR; SAR; *Drosophila melanogaster*

1. Introduction

Detailed cytogenetic studies of the three-dimensional folding of *Drosophila* polytene nuclei revealed close associations between the nuclear envelope and centromeres, telomeres and loci of intercalary heterochromatin. These studies also found that a significant part of the length of each salivary polytene chromosome abut the nuclear envelope [1,2]. Associations between the nuclear envelope and telomeres/centromeres were also recorded in *Drosophila* embryos [3]. In addition, a three-dimensional electron microscopy study revealed a close association between the nuclear envelope and chromatin [4].

The interactions between the nuclear envelope and chromatin are likely to be mediated by the nuclear lamina, which is a proteinaceous meshwork of intermediate filaments present under the inner membrane of the nuclear envelope (reviewed in [5–9]). The major proteins of the nuclear lamina are the lamins. Like all intermediate filaments, lamins are composed of a short N-terminal (head) domain, a central α-helical rod domain and a C-terminal (tail) domain which contains a nuclear localization signal and a CaaX box [10,11]. At the first level of polymerization, lamins form a parallel unstaggered dimer through coiled-coil formation of the rod domain. Lamin dimers can form filaments and paracrystals in vitro through a longtitudinal head-to-tail interaction and several types of lateral interactions [12–14]. The lamin tail domain contains several sequences that are highly conserved between all lamins, for which the function has yet to be explored.

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Rat liver lamins A, B and C and *Drosophila* lamin Dm_0 bind with high affinity to matrix-associated regions (M/SARs) [15,16]. These DNA sequences are held responsible for mediating the interaction between the nuclear matrix and chromatin (reviewed in [17,18]). M/SARs are several hundred base pairs long and contain stretches of AT-rich sequences which are likely to form an 'open' chromatin configuration. Indeed, the binding of M/SARs to lamin polymers involves single-stranded regions. In addition, this binding is saturable and requires the minor groove [16]. Lamin polymers also bind to the 120p1.4 *Drosophila* centromeric sequence [19] and to telomeric sequences [20].

In this report, we investigated which region in lamin Dm_0 is important for the specific interaction with M/SAR DNA sequences and whether this interaction depends on the formation of lamin polymers. We show that the α-helical rod domain of lamin Dm_0 is sufficient for the specific interaction with the M/SAR fragment of the *Drosophila fushi tarazu* (*ftz*) gene. For this specific interaction to occur, it is essential that the lamin is organized above the level of the dimer.

2. Experimental

2.1. Expression and purification of wild-type and mutant lamin Dm_0 proteins

All different lamin Dm_0 constructs were derived from previously described pT7Dm_0 [21]. For convenient expression, the *Nde*I-*Eco*RI fragment of pT7Dm_0 containing the entire reading frame was subcloned into the pET20b(+) vector (Novagen). All other Dm_0 constructs were designed by PCR [22] and inserted into pET20b(+), which resulted in the addition of the His-tag sequence AAAELHHHHHH (single-letter code of amino acids) at the C-terminus of these proteins. The R64 > H mutation was produced by overlap extension PCR [23]. The rod domain was designed to contain the region between Lys-55 to Thr-413 [24]. The tail domain was initially designed between Asn-411 and Met-622. Expression of this construct in *E. coli* resulted in a significant appearance of a slightly smaller polypeptide. N-terminal sequencing of the smaller peptide revealed that it is a degradation product of the N-terminus beginning at Ser-425. Therefore, a new construct was designed which contains the region between Ser-425 and Met-622. All other constructs were recovered without visible degradation products (not shown).

Lamin Dm_0 was expressed in *E. coli* and recovered from inclusion bodies by isoelectric focusing in 5 M urea as described previously [21]. The purified Dm_0 was dialyzed against buffer H (500 mM NaCl, 30 mM Tris-HCl pH 7.5, 1 mM DTT, 5 mM benzamidine, 0.5 mM PMSF, 1 μg/ml aprotinin and 1 μM pepstatin A). In contrast, all other constructs were recovered as soluble proteins. After induction with IPTG for 2–4 h at 30°C, the *E. coli* cells were broken by a French pressure-cell followed by two 150,000 × *g* centrifugations at 4°C in a Beckman TLA-100 rotor. The different Dm_0-derived polypeptides were purified to near-homogeneity from the supernatant by a one-step affinity chromatography on a His-bound resin column (Novagen). Following extraction of the protein from the His-bound column with 150–300 mM imidazole, the protein was concentrated and the imidazole was removed by dialysis.

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2.2. M/SAR-binding to aggregated lamin constructs

Plasmid pFKH2 [25,26] was digested with *Eco*RI into three fragments: 1.2-kb M/SAR fragment of the *Drosophila ftz* gene, 2.4-kb non-M/SAR fragment containing *ftz* sequences and 4.4-kb pAT153 vector fragment. The restriction fragments were endlabeled with [α-^{32}P]dATP (800 Ci/mMol, 10 mCi/ml, Rotem Industries, Israel) using the *E. coli* DNA polymerase-klenow fragment. Unincorporated nucleotides were removed by a QIAquick spin PCR purification kit (Qiagen). Aggregates of Dm_0 and rod domain were obtained by diluting 1.5 mg/ml of Dm_0 or 2 mg/ml rod domain incubations to a final concentration of 40 μg/ml in 50 mM sodium acetate pH 5.5, 25 mM $CaCl_2$ for 30 min at 20°C and then for 60 min at 4°C. Aggregates were centrifuged for 30 min/15,000 × *g*/4°C and resupended in buffer B (50 mM sodium acetate pH 5.5, 50 mM NaCl, 25 mM $CaCl_2$, 1 mM EDTA, 1 mM DTT, 5% glycerol, 5 mM benzamidine, 0.5 mM PMSF, 1 μg/ml aprotinin and 1 μM pepstatin A). Under these experimental conditions, all Dm_0 and rod domain molecules were recovered in the pellet fraction as determined by SDS-PAGE (not shown). Radioactively labeled restriction fragments of the pFKH2 (5 ng/20,000 cpm), 200 μg BSA (fraction V; Sigma) and sonicated *E. coli* competitor DNA (average size of about 3 kb) were added, resulting in a total volume of 50 μl, and incubation was for 12 h at 37°C. Following binding, Dm_0 or rod domain aggregates were centrifuged in a microfuge (30 min/15,000 × *g*). The separated supernatant and pellet fractions were treated for 30 min at 50°C, with 50 μg/ml Proteinase K, followed by phenol extraction and ethanol precipitation. DNA fragments were analysed by 1.2% agarose gel electrophoresis. The results of these experiments were visualized both by autoradiography and by phosphoimager (Fuji).

2.3. M/SAR-binding to non-aggregated lamin constructs

His-bound resin (Novagen) was preabsorbed with sonicated *E. coli* DNA (1 mg/ml) for 3 h at 20°C, followed by extensive washes with buffer S (50 mM Tris-HCl pH 7.5, 50 mM NaCl, 25 mM $CaCl_2$, 1 mM EDTA, 1 mM DTT, 5% glycerol, 5 mM benzamidine, 0.5 mM PMSF, 1 μg/ml aprotinin and 1 μM pepstatin A). Rod domain, tail domain and R64 > H proteins were dialyzed against buffer S. The purified proteins (2 μg) were incubated with radioactively labeled restriction fragments of the pFKH2 (5 ng/20,000 cpm), 200 μg BSA (fraction V; Sigma), sonicated *E. coli* competitor DNA and 10 μl His-bound resin in a total volume of 50 μl for 12 h at 37°C. The His-bound resin beads were precipitated, washed with 50 μl of buffer S and the wash was added to the supernatant fraction. Under these conditions, all lamin molecules were recovered with the beads as judged by SDS-PAGE analysis (not shown). The separated supernatant and pellet fractions were treated for 30 min at 50°C with 50 μg/ml Proteinase K, followed by phenol extraction and ethanol precipitation. Fragments were analysed by 1.2% agarose gel electrophoresis. The results of these experiments were visualized both by autoradiography and by phosphoimager (Fuji). In some of the binding experiments with the isolated tail domain, we used buffer B.

3. Results

3.1. Specific binding of ftz M/SAR DNA to Dm_0 and rod domain paracrystals

It was previously shown that A- and B-type vertebrate lamins as well as *Drosophila* lamin Dm_0 polymers bind specifically to different M/SARs [15,16]. However, these studies did not address the question of which lamin domain(s) are involved in binding. To address this question, we expressed and purified to near-homogeneity several Dm_0 constructs, including wild-type Dm_0, Dm_0 with a point mutation in Arg at position 64 (R64 > H) that inhibits polymerization (data not shown), isolated rod domain (aa 55–413) and isolated tail domain (aa 425–622). The purified proteins were analysed for their ability to bind specifically a M/SAR fragment of the *Drosophila ftz* gene [25,26]. When purified Dm_0 is dialyzed against buffer B (which has low ionic strength, a pH of 5.5 and calcium ions), it forms paracrystals (data not shown). Dm_0 paracrystals were incubated with endlabeled *Eco*RI fragments of pFKH2: a 4.4-kb fragment of pBR322 sequences, a 2.4-kb *ftz* non-MAR/SAR fragment and a 1.2 M/SAR-containing *ftz* fragment [25,26], in the presence of increasing amounts of *E. coli* competitor DNA. Bound fragments were separated from unbound fragments by centrifugation. The fragments were then subjected to agarose gel-electrophoresis and autoradiographed (Fig. 1A). At competitor DNA concentrations of 0–50 μg/ml, all three labeled fragments were recovered with the sedimented Dm_0 paracrystals (Fig. 1A). In contrast, when the concentration of *E. coli* DNA was above 100 μg/ml, only the 1.2-kb *ftz* M/SAR fragment remained associated with the paracrystals, whereas the other two non-M/SAR fragments were recovered in the supernatant fraction (Fig. 1A). Recovery of the *ftz* M/SAR fragment in the supernatamt required concentrations of above 1–2 mg/ml of the competitor DNA (data not shown). The finding that Dm_0 paracrystals bind the *ftz* M/SAR fragment suggests that Dm_0 recognizes this sequence in a similar way to other M/SARs [15,16].

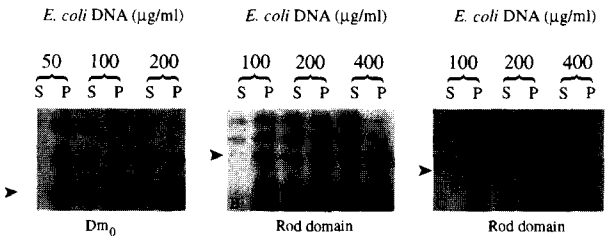

Fig. 1. Specific M/SAR-binding to Dm_0 and to α-helical rod domain aggregates. Dm_0 and α-helical rod domain were expressed in *E. coli* and purified. Each binding reaction (50 μl) contained 2 μg of lamin, 200 μg BSA, 5 ng (20,000 cpm) of ^{32}P-labeled *Eco*RI fragments of pFKH2 *ftz* plasmid and different concentrations of sonicated *E. coli* DNA. Incubation was for 12 h at 37°C in buffer B. Aggregates of Dm_0 (A) or α-helical rod domain (B,C) were pelleted and DNA fragments in the pellet (P) and supernatant (S) were purified and analysed by agarose gel electrophoresis and autoradiography. In panel C, the reaction was performed in the presence of His-bound resin.

When the pH is lowered to about 5.5, the α-helical rod domain of Dm_0 forms paracrystals (data not shown), similar to the rod domain of human lamins A and C [14,27]. Paracrystals of the Dm_0 rod domain were produced at pH 5.5 and incubated with endlabeled fragments of pFKH2 *ftz* plasmid in the presence of *E. coli* competitor DNA. The paracrystal-bound fragments were separated from the unbound fragments and analysed by gel-electrophoresis and autoradiography (Fig. 1B,C). The results of these experiments show that, while the non-M/SAR fragments were released by the competitor DNA, the M/SAR fragment remained associated with rod domain polymers. The concentrations of competitor DNA that were required to release the non-M/SAR fragments (Fig. 1A,B) or the M/SAR fragment (not shown) were similar for paracrystals of isolated rod domain and full-length lamin Dm_0 protein. The finding that paracrystals of the rod domain specifically bind M/SAR sequences indicates that a binding site for M/SARs is localized within the α-helical rod domain.

3.2. Unpolymerized Dm_0 does not bind to the ftz M/SAR DNA fragment

Luderus et al. [15,16] showed that aggregates of lamin Dm_0 as well as vertebrate A- and B-type lamins bind specifically to different M/SARs with high affinity. However, these studies did

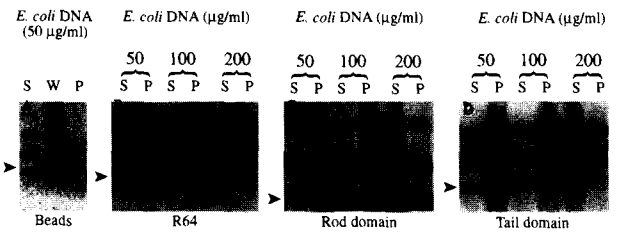

Fig. 2. Unpolymerized R64 > H, α-helical rod domain or tail domain of Dm_0 do not bind the *ftz* M/SAR fragment. R64 > H (B), α-helical rod domain (C) and tail domain (D) of Dm_0 were expressed in *E. coli* and purified to near-homogeneity. Reactions were performed in buffer S. Each binding reaction (50 μl) contained 2 μg of polypeptide, 5 ng (20,000 cpm) of ^{32}P-labeled *Eco*RI fragments of pFKH2 *ftz* plasmid, 200 μg BSA, His-bound resin and different concentrations of sonicated *E. coli* DNA. Incubation was for 12 h at 37°C. Beads were then precipitated and washed once with 50 μl of buffer S. DNA fragments were recovered from the beads (P) and from wash and supernatant (S) and analysed by agarose gel electrophoresis and autoradiography. Binding reaction in the absence of lamin polypeptides (A) demonstrates that all pFKH2 fragments are recovered in the supernatant (S) and wash (W).

not address directly the question of the role of the polymerization in binding. In order to address this question, we performed the binding reaction with several different unpolymerized Dm_0 constructs and used the His-tag, fused to the C-end of these polypeptides to capture and separate the DNA fragments which remained bound to these proteins. An important control was to show that His-bound resin does not interfere with the binding of the *ftz* M/SAR fragment to rod domain polymers. As shown in Fig. 1C, in the presence of beads, the *ftz* M/SAR fragment remained bound to paracrystals of the rod domain, whereas the non-M/SAR fragments dissociated from the paracrystals (Fig. 1, cf. B and C). In addition, the naked resin beads have no affinity to the restriction fragments of pFKH2. As shown in Fig. 2A, when binding was performed in the absence of lamin, a single wash of the resin beads with buffer S (Fig. 2A) or buffer B (not shown) removed all restriction fragments from the beads.

In order to inhibit paracrystal formation of lamin Dm_0, we introduced a point mutation changing Arg at position 64 to His (R64 > H). R64 > H molecules (2 μg) were incubated with the *Eco*RI-endlabeled fragments of pFKH2 in the presence of *E. coli* competitor DNA. Restriction fragments that remained associated with the R64 > H were separated from the unbound fragments by affinity purification using His-bound resin and analysed (Fig. 2B). The results of these experiments show that in the presence of competitor *E. coli* DNA both M/SAR fragment and the non-MA/SAR fragments were released from R64 > H molecules at the same concentration of competitor *E. coli* DNA (between 100–200 μg/ml).

As shown above, polymers of the α-helical rod domain of lamin Dm_0 bind specifically to the *ftz* M/SARs fragment. In order to study M/SAR interaction with the unpolymerized lamin rod domain, we performed the binding reaction at pH 7.5. The rod domain does not aggregate at this pH. Rod domain molecules (2 μg) were incubated at pH 7.5 with the *Eco*RI-endlabeled fragments of pFKH2, in the presence of *E. coli* competitor DNA. As shown in Fig. 2C, both the 1.2-kb M/SAR fragment and the 2.4- and 4.4-kb non-M/SAR fragments were released from the rod domain molecules at similar competitor DNA concentrations. Incubation of the restriction fragments with the tail domain of lamin Dm_0 gave similar results (Fig. 2D). These results demonstrate that, while Dm_0 shows a general low affinity to DNA, specific interaction with M/SARs requires its organization above the level of the dimer.

4. Discussion

In this report, we show that binding of the *ftz* M/SAR fragment to aggregates of the α-helical rod domain of lamin Dm_0 is specific and is similar to binding of the ftz M/SAR to full-length lamin Dm_0. Previous experiments demostrated that other coiled-coils, such as polymers of the cytoplasmic intermediate filament desmin and the nuclear matrix protein NuMA, also bind specifically M/SARs. However, these coiled-coil proteins bind with significant lower affinity than all tested lamins [16]. The high concentrations of competitor DNA that is required to displace the *ftz* M/SAR fragment from paracrystals of the lamin rod domain demonstrate that the site for the higher-affinity binding to M/SARs is localized within the rod domain of lamin. In view of the extensive sequence similarity between the rod domains of both A- and B-type lamins, it is likely that the binding of all lamins to MARs/SARs is mediated by the rod domain.

At pH 7.5, when the α-helical rod domain does not form paracrystals, the amount of competitor DNA that was required to release the *ftz* M/SAR fragment was similar to the concentration of competitor DNA required to displace the two other non-M/SAR fragments of pFKH2 and was comparable to that required to release the non-M/SAR fragments from paracrystals. Therefore, a second conclusion from these experiments is that specific binding of the *ftz* M/SAR fragment to the α-helical rod domain requires its polymerization beyond the level of the dimer. The lack of specific binding to the *ftz* M/SAR fragment was also observed with the tail domain and with the complete lamin Dm_0 protein containing a point mutation in the head domain (R64 > H), which impairs its ability to form paracrystals. There are several possible explanations why binding M/SARs requires polymerization. One explanation is that lamin dimers contain a M/SAR-binding site with an affinity too low to be detected in this study. Lamin polymerization causes clustering of these binding sites, resulting in high-avidity binding. If true, the difference in the apparent affinity for M/SARs and non-M/SARs, which is too small to be detected with unpolymerized molecules, is readily detectable with polymers. Another possible explanation is a difference in protein-folding between polymerized and unpolymerized lamin, which is important for the specific binding. For instance, the interface between lamin dimers in a paracrystal may create a M/SAR-binding site. We note, however, that aggregates formed by the R64 > C mutation (Arg-64 to Cys mutation in full-length lamin that can form unordered aggregates) bind to M/SAR sequences with the same specificity as full-length wild-type lamin Dm_0 (N.S. unpubl. obs.), suggesting that a specific ordering of lamin dimers in the polymerization product is not needed for specific binding. The lack of specific binding to the unpolymerized rod domain or to the R64 > H polypeptides also suggests that, if binding between M/SAR sequences and nuclear lamina occurs in vivo [15,19], it requires the formation of lamin polymers. In addition, since Dm_0 also shows lower-affinity binding to non-M/SARs, the possibility of additional lower-affinity binding of DNA se-

quences other than M/SARs by the nuclear lamina can not be ruled out.

Work by several groups [14,28–31] has shown that lamins also interact in vitro with chromosomal proteins. It was also demonstrated that one such binding site to chromosomal proteins is present within the α-helical rod domain of human lamins A and C [14] and another site is located in the lamin tail domain [31]. Therefore, the lamin rod domain is not only required for lamin polymerization but may have additional roles, such as binding to M/SARs sequences and chromosomal proteins. Direct functional studies will be required to assess the significance of rod domain's association with M/SARs as well as with chromatin.

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References

[1] Hochstrasser, M., Mathog, D., Gruenbaum, Y., Saumweber, H. and Sedat, J.W. (1986) J. Cell Biol. 102, 112–123.
[2] Hochstrasser, M. (1987) Chromosoma 95, 197–208.
[3] Hiraoka, Y., Dernburg, A.F., Parmelee, S.J., Rykowski, M.C., Agard, D.A. and Sedat, J.W. (1993) J. Cell Biol. 120, 591–600.
[4] Belmont, A.S., Zhai, Y. and Thilenius, A. (1993) J. Cell Biol. 123, 1671–1685
[5] Hutchison, C.J., Bridger, J.M., Cox, L.S. and Kill, I.R. (1994) J. Cell Sci. 107, 3259–3269
[6] Georgatos, S.D., Meier, J. and Simos, G. (1994) Curr. Opin. Cell Biol. 6, 347–353.
[7] Moir, R.D. and Goldman, R.D. (1993) Curr. Opin. Cell Biol. 5, 408–411.
[8] Nigg, E.A. (1992) Semin. Cell Biol. 3, 245–253.
[9] Paddy, M.R., Agard, D.A. and Sedat, J.W. (1992) Semin. Cell Biol. 3, 255–266.
[10] Hennekes, H. and Nigg, E.A. (1994) J. Cell Sci. 107, 1019–1029
[11] Firmbach, Kraft I. and Stick, R. (1995) J. Cell Biol. 129, 17–24.
[12] Heitlinger, E., Peter, M., Lustig, A., Villiger, W., Nigg, E.A. and Aebi, U. (1992) J. Struct. Biol. 108, 74–89.
[13] Heins, S. and Aebi, U. (1994) Curr. Opin. Cell Biol. 6, 25–33.
[14] Glass, C.A., Glass, J.R., Taniura, H., Hasel, K.W., Blevitt, J.M. and Gerace, L. (1993) EMBO J. 12, 4413–4424.
[15] Luderus, M.E., de Graaf, A., Mattia, E., den Blaauwen, J.L., Grande, M.A., de Jong, L. and van Driel, R. (1992) Cell 70, 949–959.
[16] Luderus, M.E., den Blaauwen J.L., de Smit, O.J., Compton, D.A. and van Driel, R. (1994) Mol. Cell Biol. 14, 6297–6305.
[17] Gasser, S.M., Amati, B.B., Cardenas, M.E. and Hofmann, J.F. (1989) Int. Rev. Cytol. 119, 57–96.
[18] Freeman, L.A. and Garrard, W.T. (1992) Crit. Rev. Eukaryot. Gene Expr. 2, 165–209.
[19] Baricheva, E.A., Berrrios, M., Bogachev, S.S., Borisevich, I.V., Lapik, E.R., Sharakhov, I.V., Stuurman, N. and Fisher, P.A. (in press) Gene.
[20] Shoeman, R.L. and Traub, P. (1990) J. Biol. Chem. 265, 9055–9061.
[21] Ulitzur, N., Harel, A., Feinstein, N. and Gruenbaum, Y. (1992) J. Cell Biol. 119, 17–25.
[22] Higuchi, R. (1989) Using PCR to engineer DNA. in PCR Technology Principles and Application (Erlich, H.A., Ed.), Stockton Press.
[23] Ho, S.N., Hunt, H.O., Morton, R.M., Pullen, J.K. and Pease, L.R. (1988) Gene 77, 51–59.
[24] Gruenbaum, Y., Landesman, Y., Drees, B., Bare, J.W., Saumweber, H., Paddy, M.R., Sedat, J.W., Smith, D.E., Benton, B.M. and Fisher, P.A. (1988) J. Cell Biol. 106, 585–596.
[25] Amati, B. and Gasser, S.M. (1990) Mol. Cell Biol. 10, 5442–5454.
[26] Amati, B., Pick, L., Laroche, T. and Gasser, S.M. (1990) EMBO J. 9, 4007–4016.
[27] Moir, R.D., Quinlan, R.A. and Stewart, M. (1990) FEBS Lett. 268, 301–305.
[28] Glass, J.R. and Gerace, L. (1990) J. Cell Biol. 111, 1047–1057.
[29] Hoger, T.H., Krohne, G. and Kleinschmidt, J.A. (1991) Exp. Cell Res. 197, 280–289.
[30] Yuan, J., Simos, G., Blobel, G. and Georgatos, S.D. (1991) J. Biol. Chem. 266, 9211–9215.
[31] Taniura, H., Glass, C. and Gerace, L. (1995) J. Cell Biol. 131, 33–44.