# Subcellular distribution of farnesyl protein transferase in rat liver

Jacob Grünler, Ingela Parmryd*

*Department of Biochemistry, Arrhenius Laboratories for Natural Sciences, Stockholm University, S-106 91 Stockholm, Sweden*

Received 18 June 1999

**Abstract Farnesyl protein transferase (FPT) activity was measured in rat liver subcellular fractions by using an unspecific acceptor for the farnesyl groups. The highest specific activity was found in mitochondria and it exceeded that of the microsomes three-fold. Considerably lower specific activities were found in the nuclei and cytosol. Further subfractionation revealed that the mitochondrial FPT activity is located in the matrix. The β-subunit of the mitochondrial enzyme has an apparent molecular mass of 46 kDa, which is similar to its cytosolic counterpart. The results suggest that protein farnesylation can take place in a number of subcellular organelles.**



*Key words:* Isoprenylation; Prenylation; Farnesyl protein transferase

## 1. Introduction

Prenylated proteins have a broad distribution in organisms as diverse as yeast, higher plants and mammals [1–3]. Prenylation involves the binding of farnesol to a C-terminal cysteine residue or the binding of geranylgeraniol to one or two C-terminal cysteine residues. In both cases, the bond(s) formed is of the thioether type. Recently, pentaprenol and hexaprenol were found to be bound to proteins via thioether linkages [4,5]. Additional prenylating molecules such as isopentenyl adenine [6], dolichyl phosphate [7] and dolichol [8] have also been demonstrated to be bound to proteins but, in these cases, the type of binding remains to be characterised.

Many proteins with important cellular functions have been identified as having a farnesyl modification. These include lamin B [9], rhodopsin kinase [10], Ras proteins [11], ANJ 1 [12] and tyrosine phosphatases [13]. In order for these proteins to be correctly localised and exert their cellular functions they are dependent on farnesylation.

To date, three different prenyl protein transferases have been isolated and characterised from mammalian brain tissue and yeast: farnesyl protein transferase (FPT) [14] and geranylgeranyl protein transferases I [15] and II [16]. Rat brain FPT is a heterodimeric protein consisting of α- and β-subunits with molecular masses of 48 and 46 kDa, respectively. In yeast, the β-subunit has a lower molecular mass [17]. The β-subunit is responsible for binding both the polypeptide and farnesyl pyrophosphate (FPP) substrates in a catalytic cleft [18,19] and the catalysis is zinc dependent [20]. The enzyme recognises the C-terminal sequence -$CX_1X_2X_3$, where $X_3$ most often is serine or methionine.

In vivo labelling of spinach seedlings with [$^3$H]mevalonate demonstrated that extensive protein prenylation occurs in plants and prenylated polypeptides were present in all subcellular fractions studied [2]. A large proportion of the protein-bound radioactivity that could be released by methyl-iodide hydrolysis proved to be farnesol. To date, FPT has been considered to be a cytosolic enzyme, and the question of its subcellular distribution has not yet been fully addressed. However, using immunohistochemical procedures, it has been suggested that farnesylation of prelamin A takes place in the nucleus [21].

Multiple locations for various mammalian mevalonate pathway enzymes have been described. For instance several of the initial enzymes, originally thought to be exclusively cytosolic, are also present in peroxisomes [22,23] which, like microsomes, also contain the terminal enzymes for cholesterol and dolichol biosynthesis [24].

Recently, it has been demonstrated that mitochondrial mitoplasts contain several prenylated polypeptides [5], which prompted us to investigate the localisation of FPT in greater detail. In this study we have analysed FPT in isolated subcellular fractions from rat liver homogenate. The general distribution of this enzyme suggests that protein farnesylation occurs at cellular locations other than the cytosol and, in organelles such as mitochondria, presumably after import of newly synthesised proteins. The observed distribution emphasises the importance of prenylated proteins at the organelle function level.

## 2. Materials and methods

### 2.1. Materials

Male Sprague-Dawley rats weighing 150 g were used. Tritiated FPP (specific activity 0.21 Ci/mmol) was prepared as described earlier [25]. DL-Dithiothreitol (DTT) was of ultrapure grade and obtained from Sigma. Mammalian FPT standard, Ras proteins and FPT antiserum were the kind gift of Dr P.J. Casey, Durham, NC, USA. All chemicals were obtained from Sigma, and solvents were of reagent grade.

### 2.2. Cellular fractionation of rat liver

Nuclear, mitochondrial, microsomal and peroxisomal fractions were prepared according to previously published procedures [26–28]. For the preparation of cytosol, the supernatant remaining after preparation of microsomes was recentrifuged at 105 000×*g* for 2 h. The washed mitochondria were subfractionated according to Sottocasa et al. [29]. All steps were carried out on ice or at 4°C.

The contamination in isolated fractions was determined by analysis of marker enzymes. The microsomal marker employed was NADPH-cytochrome *c* reductase (0.032 μmol NADPH oxidised/min/mg protein), the mitochondrial marker was cytochrome *c* oxidase (1.42 μmol of cytochrome *c* oxidised/min/mg protein) and for peroxisomes, urate oxidase activity was measured (0.35 μmol oxidised/min/mg protein). Lactate dehydrogenase activity was measured to determine cytosolic contamination. After washing the organelles with 0.15 M Tris buffer,

*Corresponding author. Fax: (46) (8) 15 36 79.
E-mail: ingela@biokemi.su.se

*Abbreviations:* DTT, dithiothreitol; FPP, farnesyl pyrophosphate; FPT, farnesyl protein transferase; OGP, *n*-octyl β-D-glucopyranoside


PII: S0014-5793(99)00892-3

pH 8.0, no measurable lactate dehydrogenase activity could be detected.

The percentage contamination on a protein basis was calculated for the isolated fractions, by using the measured specific activities. The nuclear fraction contained 2%, 3% and 3% of mitochondrial, microsomal and peroxisomal protein, respectively. The mitochondrial fraction contained 3% microsomal and 7% peroxisomal protein. The contamination of peroxisomes with mitochondria and microsomes was 1% and 3%, respectively. The microsomal fraction contained 1% mitochondria and 2% peroxisomes.

*2.3. Comparison of homogenisation procedures*

Rat liver was homogenised in a Potter-Elvehjem homogeniser by three strokes at 345 rpm. The resulting homogenate was split into five aliquots of which four were subjected to further homogenisation procedures which were either 10 strokes in a Potter-Elvehjem homogeniser at 1450 rpm, treatment with a Turrax blender for 20 s at 12 000 rpm, treatment with a Turrax blender for 60 s at 24 000 rpm, or pulse sonication for 60 s. Cytosol was isolated from the various homogenates by centrifugation at $11\,500 \times g$ for 20 min followed by a second centrifugation step of the resulting supernatants at $105\,000 \times g$ for 90 min. All steps were carried out on ice or at 4°C.

*2.4. FPT assay*

FPT activity was assayed by measuring the amount of [$^3$H]farnesyl transferred from [$^3$H]FPP to DTT in polypropylene tubes [30]. Unless otherwise indicated, each reaction mixture contained the following components in a final volume of 50 μl: 50 mM 3-[*N*-morpholino]propanesulphonic acid (pH 7.5), 50 mM KF, 100 μM $ZnCl_2$, 50 μM [$^3$H]FPP, 0.05% *n*-octyl β-D-glucopyranoside (OGP) and 2 mM DTT. Reactions were started by the addition of 5–30 μg of protein. A fresh DTT solution was made up for each experiment. Appropriate controls, omitting DTT, were performed for each measured point and subtracted as background. Before measuring any activities in the nuclei, they were subjected to pulse sonication for 60 s. Protein content was estimated according to Lowry et al. [31].

In a subset of experiments, FPT activity was measured by quantitating the amount of $^3$H transferred from [$^3$H]FPP into H-Ras or K-Ras protein employing the filter assay method [14]. The reaction mixture contained the following components in a volume of 50 μl: 50 mM Tris-HCl, pH 7.7, 20 mM KCl, 5 mM $MgCl_2$, 50 μM $ZnCl_2$, 2 mM dithiothreitol, 0.05% OGP, 1 μM Ras protein, 5 μM [$^3$H]FPP (specific activity 3 Ci/mmol) and 50 μg of protein.

*2.5. Electrophoresis and Western blotting*

The samples to be analysed by SDS-PAGE were solubilised with 3% lithium dodecyl sulphate, 75 mM Tris-HCl (pH 8.0), 15% glycerol, 75 mM dithioerythritol by incubation at 90°C for 5 min. The protein profiles were resolved on 13% acrylamide gels [32]. Western blotting was performed as described by Towbin et al. [33]. The proteins were visualised by enhanced chemiluminescence.

## 3. Results

*3.1. Subcellular distribution of FPT*

In a previous study the activity of spinach seedling FPT was characterised, employing DTT as an unspecific acceptor for the farnesyl moiety [30]. A similar investigation has also been performed with rat liver FPT and the results obtained were similar to those of its spinach counterpart. Minor differences in the characteristics of the two enzymes in pH optimum and activation by addition of $Zn^{2+}$ were observed. These were taken into consideration when the activities were measured from the different organisms.

Table 1
Subcellular distribution of FPT activity in rat liver

| Fraction | Activity (pmol/mg protein/min) |
|---|---|
| Nuclei | 6.39 ± 1.0 |
| Mitochondria | 75.6 ± 17 |
| Peroxisomes | 2.16 ± 0.62 |
| Microsomes | 26.8 ± 5.5 |
| Cytosol | 1.92 ± 0.38 |

The various subcellular fractions were isolated from rat liver prior to incubation with DTT and [$^3$H]FPP in polypropylene tubes. The values shown are means ± S.D. of five experiments.

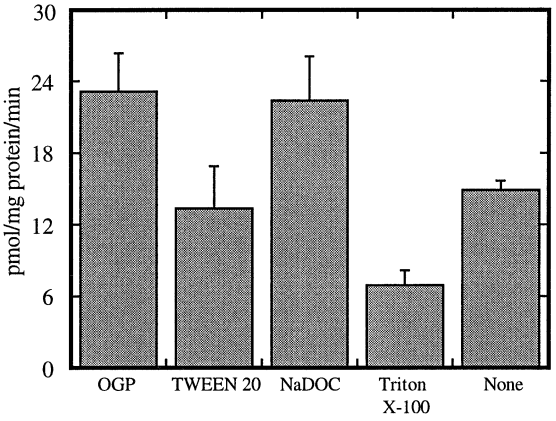


Fig. 1. Effect of detergentia on rat liver FPT activity. FPT activity was measured in the soluble fraction from rat liver, homogenised by pulse sonication by incubation with DTT and [$^3$H]FPP in polypropylene tubes. Detergentia were employed at a concentration of 0.05% in the incubation medium according to the figure. The values shown are means ± S.D. of four experiments.

The distribution of FPT activity was studied in various isolated subcellular fractions from rat liver. Since many of the enzymes involved in isoprenoid metabolism are present in non-membrane-bound forms in the luminal compartments of organelles, measurement of their activities requires detergentia to permeabilise the membrane to their substrates. For this reason, soluble FPT activity from pulse-sonicated rat liver homogenate was studied in the presence of various detergentia (Fig. 1). Both OGP and sodium deoxycholate were found to stimulate FPT activity, while Tween 20 and particularly Triton X-100 inhibited the same. All the following experiments were performed in the presence of OGP.

Interestingly, the specific activity of FPT in rat liver mitochondria by far exceeded that of the other organelle fractions (Table 1). The enzyme activity in microsomes was about 30% of that in mitochondria, and a lower activity was found in the nuclear fraction. Unexpectedly, only a very modest FPT activity was observed in the cytosolic fraction. No activity could

Table 2
Effect of homogenisation procedure on the amount of cytosolic rat liver FPT

| Homogenisation method | Activity in cytosol (% of homogenate) |
|---|---|
| 3 × 345 rpm | 4.42 |
| 10 × 1450 rpm | 13.3 |
| Turrax 12 000 rpm 20 s | 12.7 |
| Turrax 24 000 rpm 60 s | 26.6 |
| Pulse sonication 60 s | 54.6 |

Rat liver was homogenised in a Potter-Elvehjem homogeniser by three strokes at 345 rpm. The resulting homogenate was split into five aliquots, of which four were subjected to further homogenisation as described. Cytosol was isolated from the various homogenates by ultracentrifugation and FPT was assayed by the DTT method. The values shown have been calculated to compare activities on a per gram liver basis and are the means of three independent experiments. The S.D. did not exceed 13% for any measured point.

be detected in the nuclear fraction unless it was sonicated prior to enzyme measurements.

As a comparison, FPT activity was also measured employing the filter assay method using a Ras protein as the substrate for the reaction. The cytosol was the only fraction that contained an activity that could farnesylate both H-Ras and K-Ras (not shown).

### 3.2. Effect of homogenisation

In our fractionation procedures mild shearing forces were employed during homogenisation, which raised the question of the extent to which the preparation methods may effect the measured distribution of FPT. When the two homogenisation procedures, three strokes in a Potter-Elvehjem homogeniser at 345 rpm and 10 strokes at 1450 rpm were compared, the amount of FPT appearing in the cytosol increased three-fold when the more extreme forces were used (Table 2). In a similar approach, several Turrax blender homogenisation methods using different speeds and time periods were also compared. The measurable cytosolic FPT activity rose with increasing speed. Sonication is very efficient at rupturing membranes and this homogenisation method resulted in more than half of the total homogenate FPT activity appearing in the cytosol.

### 3.3. Mitochondrial FPT

Since the highest specific activity of FPT in rat liver was recovered in isolated mitochondria, subfractionation of this organelle was performed (Table 3). Removal of the outer membrane and the inter-membrane space resulted in 65% of the total mitochondrial activity being present in the mitoplasts. Further subfractionation of the latter fraction revealed that the majority of the FPT activity was present in the matrix. The relatively high level of the enzyme activity in the inner membrane and inter-membrane space is probably due to leakage of the enzyme from the matrix followed by adsorption during the subfractionation procedure.

The presence of FPT in the matrix was further established by Western blotting. The antisera employed, P121, was raised against purified mammalian FPT holoprotein and preferentially recognises its β-subunit, which has a molecular mass of 46 kDa (Fig. 2, lane 3). A single rat liver mitochondrial polypeptide of the same molecular mass reacted with the antisera (Fig. 2, lane 1). This polypeptide was enriched in the matrix when it was compared with whole mitochondria on a protein basis (Fig. 2, lane 2).

Table 3
Distribution of FPT activity in subfractions of rat liver mitochondria

| Subfraction | Activity (% of total mitochondrial) | Activity (% of mitoplastic) |
|---|---|---|
| Outer membrane | 3.85 ± 1.4 | |
| Inter-membrane space | 31.3 ± 4.0 | |
| Mitoplasts | 64.9 ± 7.3 | |
| Inner membrane | | 38.9 ± 4.8 |
| Matrix | | 61.0 ± 4.1 |

The various subfractions were isolated from rat liver mitochondria prior to incubation with DTT and [$^3$H]FPP in polypropylene tubes. The values shown are means ± S.D. of four experiments.

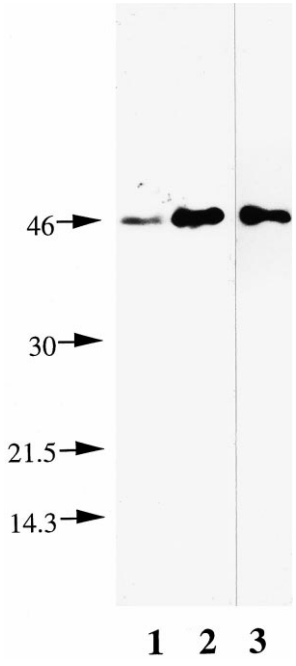


Fig. 2. Reaction of rat liver proteins with FPT antiserum. Western blot showing the reaction of rat liver proteins with P121 antiserum. The individual fractions were isolated as described. Lane 1: 90 μg of mitochondrial protein. Lane 2: 90 μg of matrix protein. Lane 3: 200 ng of mammalian FPT. The position of molecular mass standards is indicated on the left of the figure.

## 4. Discussion

According to the original concepts, the initial part of the mammalian mevalonate pathway that leads to FPP is present exclusively in the cytosol, while the terminal reactions which produce the various end products are associated with organelles. Recent investigations, however, have demonstrated a wide distribution of the enzymes involved in the biosynthetic processes of mevalonate pathway lipids. In liver, several of the initial reaction sequence enzyme activities have been found in peroxisomes [22], endoplasmic reticulum [34] and mitochondria [35]. Prenyl protein transferases have been studied almost exclusively in cytosolic fractions and their cellular distribution has not been thoroughly investigated. Upon analysis of the organelles of rat kidney and liver, a large number of prenylated polypeptides were discovered, particularly in mitochondria and microsomes, which raised the question as to whether prenylation occurs locally or before the targeting of the proteins to organelles [5].

Investigation of FPT revealed that the enzyme is present not only in the cytosol. In fact, its specific activity in the cytosolic fraction was considerably lower than in the various organelles. Subfractionation of liver mitochondria, which was the organelle fraction that had the highest specific FPT activity, demonstrated that the bulk of the enzyme is present in the matrix. Previous studies have established that FPP synthesis also takes place in the mitochondrial matrix [35], and the main function for this isoprenoid in mitochondria might be as a substrate for FPT.

The DTT assay should not be specific for enzymes recognising particular C-terminal sequences and might, therefore, be able to pick up farnesylating enzymes other than the classical FPT, provided that the bond formed is of a thioether

type. The discovery of new FPT isoenzymes could be hampered if peptides with identical sequences as those used to purify the classical FPT are utilised as substrates when searching. Other potential FPTs might then not be detected if their substrate specificities differ from that of the classical FPT. DTT, being an unspecific acceptor for the farnesyl group, provides a solution to this problem. The existence of multiple enzymes capable of transferring farnesyl groups to proteins is supported by findings in several organisms. Mammalian cells have been reported to contain two FPTs of different molecular masses that also differ in their divalent cation requirements [36]. Furthermore, yeast cells have been shown to possess two FPT activities that display different polypeptide specificities [37]. The results presented here suggest that several FPTs with different polypeptide specificities exist in rat liver, since only the cytosolic activity characterised here could utilise H-Ras as a substrate.

FPT appears to be a soluble protein within organelles. During various homogenisation procedures, mechanical forces are applied and it is well established that these forces cause organelle damage. Release of soluble enzymes to the cytosol can be observed to different extents, and this is generally used to estimate the intactness of the organelles. Applying different homogenisation procedures to rat liver, we could vary the level of FPT in the isolated cytosol. Consequently, the type of homogenisation applied to break the tissue will be one of the determining factors defining the FPT level present in a particular cytosolic fraction.

The multiple localisation of FPT activity naturally raises the question whether the enzyme distribution pattern is influenced to some extent by contamination of the individual subcellular fractions. The isolation procedures employed resulted in limited contamination as demonstrated by marker enzyme measurements.

Recently, it has been demonstrated that various farnesyl transferase inhibitors induce the release of cytochrome *c* from mitochondria and caspase 3 activation in transformed, but not in untransformed, cells [38]. They thereby stimulate apoptosis of cancer cells. The mechanism behind this is not clear, but it was suggested that (an) as yet unidentified farnesylated protein(s) is (are) involved in antagonising the release of cytochrome *c* in transformed cells. Our results showing that mitochondria contain both prenylated polypeptides [5] and FPT activity are in line with this hypothesis. However, the potential connection requires further investigation since the FPT activity in mitochondria does not have the same polypeptide specificity as the cytosolic FPT. It therefore seems unclear why it should be inhibited by peptidomimetics designed from cytosolic FPT substrates. Cytochrome *c* resides on the side of the inner membrane facing the inter-membrane space, so any farnesylated proteins present in its vicinity might become prenylated outside, as well as inside, the mitochondria. Both the inter-membrane space and the matrix were shown to contain FPT activity and it is possible that these activities represent different enzymes. We are presently trying to identify substrate proteins for the mitochondrial FPT(s).

Most proteins require co- and/or post-translational modifications for functional reasons such as activation, targeting and appropriate binding. Prenylation represents an important and irreversible modification mediating membrane anchoring and protein-protein interactions. Consequently, FPT, being present in various organelles, may have a basic role in maintaining a number of important cellular functions.

*Acknowledgements:* We would like to thank Dr E. Swiezewska for the synthesis of isoprenoids, Dr P.J. Casey for the Ras proteins, FPT standard and P121 antisera and Dr M. Runquist for valuable advice on fractionation procedures. This work was supported by grants from the Swedish Cancer Society and the Swedish Medical Research Council.

## References

[1] Clarke, S. (1992) Annu. Rev. Biochem. 61, 355–386.
[2] Shipton, C.A., Parmryd, I., Swiezewska, E., Andersson, B. and Dallner, G. (1995) J. Biol. Chem. 270, 566–572.
[3] Epstein, W.W., Lever, D., Leining, L.M., Bruenger, E. and Rilling, H.C. (1991) Proc. Natl. Acad. Sci. USA 88, 9668–9670.
[4] Parmryd, I. (1998) Protein Prenylation in Higher Eukaryotes, Ph.D. Thesis, Stockholm University, Stockholm.
[5] Parmryd, I. (1999) Arch. Biochem. Biophys. 364, 153–160.
[6] Faust, J.R. and Dice, J.F. (1991) J. Biol. Chem. 266, 9961–9970.
[7] Thelin, A., Löw, P., Chojnacki, T. and Dallner, G. (1991) Eur. J. Biochem. 195, 755–761.
[8] Hjertman, M., Wejde, J., Dricu, A., Carlberg, M., Griffiths, W.J., Sjövall, J. and Larsson, O. (1997) FEBS Lett. 416, 235–238.
[9] Wolda, S.L. and Glomset, J.A. (1988) J. Biol. Chem. 263, 5997–6000.
[10] Inglese, J., Glickman, J.F., Lorenz, W., Caron, M.G. and Lefkowitz, R.J. (1992) J. Biol. Chem. 267, 1422–1425.
[11] Hancock, J.F., Magee, A.I., Childs, J.E. and Marshall, C.J. (1989) Cell 57, 1167–1177.
[12] Zhu, J.-K., Bressan, R.A. and Hasegawa, P.M. (1993) Proc. Natl. Acad. Sci. USA 90, 8557–8561.
[13] Cates, C.A., Michael, R.L., Stayrook, K.R., Harvey, K.A., Burke, Y.D., Randall, S.K., Crowell, P.L. and Crowell, D.N. (1996) Cancer Lett. 110, 49–55.
[14] Reiss, Y., Goldstein, J.L., Seabra, M.C., Casey, P.J. and Brown, M.S. (1990) Cell 62, 81–88.
[15] Moores, S.L., Schaber, M.D., Mosser, S.D., Rands, E., O'Hara, M.B., Garsky, V.M., Marshall, M.S., Pompliano, D.L. and Gibbs, J.B. (1991) J. Biol. Chem. 266, 14603–14610.
[16] Seabra, M.C., Brown, M.S., Slaughter, C.A., Sudhof, T.C. and Goldstein, J.L. (1992) Cell 70, 1049–1057.
[17] Gomez, R., Goodman, L.E., Tripathy, S.K., O'Rourke, E., Manne, V. and Tamanoi, F. (1993) Biochem. J. 289, 25–31.
[18] Long, S.B., Casey, P.J. and Beese, L.S. (1998) Biochemistry 37, 9612–9618.
[19] Strickland, C.L., Windsor, W.T., Syto, R., Wang, L., Bond, R., Wu, Z., Schwartz, J., Le, H.V., Beese, L.S. and Weber, P.C. (1998) Biochemistry 37, 16601–16611.
[20] Huang, C.-C., Casey, P.J. and Fierke, C.A. (1997) J. Biol. Chem. 272, 20–23.
[21] Lutz, R.J., Trujillo, M.A., Denham, K.S., Wenger, L. and Sinensky, M. (1992) Proc. Natl. Acad. Sci. USA 89, 3000–3004.
[22] Krisans, S.K. (1992) Am. J. Respir. Cell Mol. Biol. 7, 358–364.
[23] Ericsson, J., Appelkvist, E.-L., Thelin, A., Chojnacki, T. and Dallner, G. (1992) J. Biol. Chem. 267, 18708–18714.
[24] Parmryd, I. and Dallner, G. (1996) Biochem. Soc. Trans. 24, 677–682.
[25] Keenan, R.W. and Kruczek, M.E. (1976) Biochemistry 15, 1586–1591.
[26] Tollbom, Ö., Valtersson, C., Chojnacki, T. and Dallner, G. (1987) J. Biol. Chem. 263, 1347–1352.
[27] Grünler, J., Olsson, J.M. and Dallner, G. (1995) FEBS Lett. 358, 230–232.
[28] Blobel, G. and Potter, R.V. (1966) Science 154, 1662–1665.
[29] Sottocasa, J.L., Kuylenstierna, B., Ernster, L. and Bergstrand, A. (1967) Methods Enzymol. 10, 448–463.
[30] Parmryd, I., Shipton, C.A., Swiezewska, E., Andersson, B. and Dallner, G. (1996) Eur. J. Biochem. 234, 723–731.
[31] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.

[32] Laemmli, U.K. (1970) Nature 227, 680–685.
[33] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[34] Ericsson, J., Thelin, A., Chojnacki, T. and Dallner, G. (1992) J. Biol. Chem. 267, 19730–19735.
[35] Runquist, M., Ericsson, J., Thelin, A., Chojnacki, T. and Dallner, G. (1994) J. Biol. Chem. 269, 5804–5809.
[36] Vogt, A., Sun, J., Qian, Y., Tan-Chiu, E., Hamilton, A.D. and Sebti, S.M. (1995) Biochemistry 34, 12398–12403.
[37] Danjoh, I. and Fujiyama, A. (1996) Eur. J. Biochem. 236, 847–851.
[38] Suzuki, N., Urano, J. and Tamanoi, F. (1998) Proc. Natl. Acad. Sci. USA 95, 15356–15361.