Minireview

Prenylation of Rab GTPases: molecular mechanisms and involvement in genetic disease

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Abstract Small GTPases of the Rab family regulate membrane transport pathways. More than 50 mammalian Rab proteins are known, many with transport step-specific localisation. Rabs must associate with cellular membranes for activity and membrane attachment is mediated by prenyl (geranylgeranyl) post-translational modification. Mutations in genes encoding proteins essential for the geranylgeranylation reaction, Rab escort protein and Rab geranylgeranyl transferase, underlie genetic diseases. Choroideremia patients have loss of function mutations in REP1 and the murine Hermansky–Pudlak syndrome model *gunmetal* possesses a splice-site mutation in the α-subunit of RGGT. Here we discuss recent insights into Rab prenylation and advances towards our understanding of both diseases.

Key words: Rab; GTP-binding protein; Protein prenylation; Protein traffic; Choroideremia; Hermansky–Pudlak syndrome; Griscelli disease

1. Introduction

Rab proteins are small GTP-binding proteins which form the largest family within the Ras-like GTPase superfamily. Rabs are believed to regulate membrane transport pathways by acting as molecular switches that alternate between active (GTP-bound) and inactive (GDP-bound) conformations. In their active GTP-bound conformation, Rabs fulfil their role in vesicle formation, organelle movement and membrane tethering/fusion by recruiting a diverse group of effector proteins [1–4]. More than 50 Rab proteins have been identified in mammalian cells [5]. Each Rab is believed to be localised to a specific subcellular compartment and some show tissue-specific variation in expression level, reflecting the complexity and variety of trafficking events found in mammalian cells [1–4].

2. Prenylation of Rab proteins

Rab proteins are intrinsically soluble proteins, whose activity is dependent upon association with the cytoplasmic leaflet of cellular membranes. This association is mediated by a lipid modification of the C-terminus termed prenylation. Prenylation is a common mechanism for membrane association of cytoplasmic proteins, estimated to affect approximately 0.5% of all intracellular proteins [6]. Prenylation consists of the covalent attachment, via thioether linkage, of a C15 (farnesyl) or C20 (geranylgeranyl) isoprenoid group to a C-terminal cysteine residue in the context of a 'prenylation motif'.

Protein prenylation is catalysed by protein prenyl transferases of which three enzymes are known: protein farnesyl transferase (PFT), protein geranylgeranyl transferase-type I (PGGTI, also known as C*AAX*-GGT), and Rab geranylgeranyl transferase (RGGT, also known as PGGTII). All three enzymes are heterodimers consisting of an α- and β-subunit [7].

PFT and PGGTI, which share a common α-subunit, define the functional class of C*AAX* prenyl transferases. They recognise a C*AAX* tetrapeptide prenylation motif at the C-terminus of their substrates, where C represents a cysteine residue, *A* an aliphatic residue and *X* any amino acid. The identity of the residue at position *X* is sufficient to determine which enzyme will modify which protein. When *X* is a methionine or serine, as in Ras proteins, then the protein is farnesylated by PFT. However, when *X* is a leucine residue, as in Rho proteins, then the protein is geranylgeranylated by PGGTI [8,9]. C*AAX* prenylation motifs are also found in a variety of other proteins such as γ-subunits of heterotrimeric G-proteins, nuclear lamins, G-protein-coupled receptor kinases, fungal mating pheromones and retinal cyclic GMP phosphodiesterase [8,9].

RGGT defines a second class of prenyl transferases which differs in many ways from the C*AAX* prenyl transferases. RGGT was originally identified as the enzyme that modified Rab proteins, and so far no other substrates are known [10,11]. The structure of RGGT has been solved recently [12]. The β-subunit consists of an α–α barrel and forms the lipid-binding site as shown for PFT [11–13]. The α-subunit is larger than that found in the C*AAX* prenyl transferases, consisting of a helical domain similar to PFT plus two other domains of unknown function: a leucine-rich repeat and an immunoglobulin-like domain [12].

Rab proteins, unlike other small GTPases, exhibit a variety of prenylation motifs at their C-termini, containing either one or, more frequently, two cysteine residues, both of which are modified by geranylgeranyl groups [14]. Also, the prenylation motif in Rab proteins is insufficient by itself for substrate recognition by RGGT. Biochemical evidence indicates that

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unprenylated Rab must first bind to a 95 kDa protein termed Rab escort protein (REP, originally termed component A of RGGT) in order to be recognised as a substrate for RGGT [11,15]. The role of REP in prenylation is supported by the finding that deficiency of REP results in hypoprenylation of Rab proteins in vivo [16,17]. Together these observations suggest that the REP:Rab complex is the true substrate of RGGT [11,14].

Two REP proteins are found in mammals [18]. Both REP1 and REP2 are ubiquitously expressed and are structurally related to another Rab-binding protein, Rab GDP dissociation inhibitor (RabGDI), which is thought to catalyse the insertion and retrieval of prenylated Rab proteins into and out of cellular membranes [11,19]. Recent results suggest that REPs and GDIs interact with Rab proteins in a conserved way, using amino acid residues in the conserved 'Rab-binding platform' [20]. Conserved positions that distinguish Rab proteins from other small GTPases, RabF motifs, are likely involved in the binding of Rab to REPs and GDIs [5,21,22].

Rab prenylation occurs in several steps (Fig. 1). Newly synthesised Rabs bind to one of the two REP proteins and this complex then associates with RGGT. This enzyme contains one GGPP-binding site and recent studies indicate that GGPP-binding increases the affinity of RGGT for the REP:Rab complex suggesting that the enzyme may already be bound to a single GGPP group at this stage [23,24]. Geranylgeranylation of the most N-terminal cysteine follows [25]. In the case of some Rabs, e.g. Rab8 or Rab13, which contain a lone cysteine residue available for prenylation, dissociation of the enzyme occurs at this point. However, a second cysteine residue is available in most cases and RGGT catalyses another round of lipid-binding and transfer. In this case, it is unclear whether RGGT dissociates from the mono-prenylated REP:Rab-GG complex, due to the reduction in the affinity of RGGT for the mono-prenylated complex in the absence of GGPP, or whether it can recruit a second geranylgeranyl group while bound to the REP:Rab-GG complex. Finally, after the second GG group is transferred, binding of an additional lipid group to the β-subunit destabilises the complex and the enzyme dissociates from the di-prenylated REP:Rab complex. [24]. REP may then deliver the geranylgeranylated Rab to cellular membranes [26,27] where it can interact with specific effectors and perform its biological role.

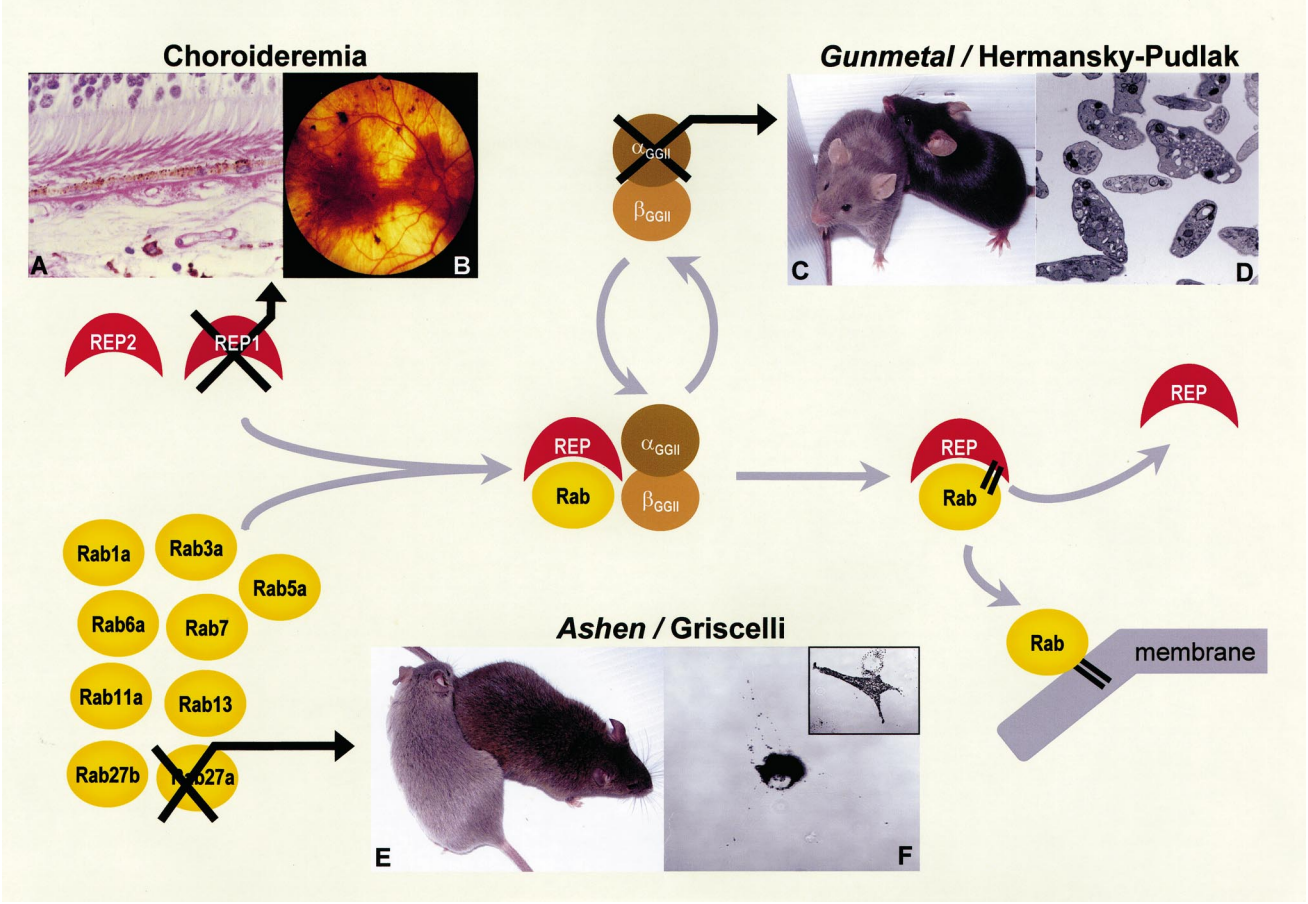

Fig. 1. Rab prenylation and disease of mice and man. Newly synthesised Rabs bind to one of two REP proteins. Mutations in REP1 cause CHM, an X-linked retinal degeneration. Histologic sections of retina reveal early pathological changes in a female carrier of the CHM mutation (A), and the fundus of the eye of an affected male shows marked peripheral retinal degeneration (B). The REP:Rab complex is the substrate for the enzyme RGGT which catalyses geranylgeranylation of the Rab protein. Mutations in the α-subunit of RGGT result in hypopigmentation as observed in the *gm* mouse model of HPS (C) and platelet dysfunction characterised by macrothrombocytopenia and reduced number of α- and dense granules (D). Mutations in one Rab protein, Rab27a, result in hypopigmentation as observed in the *ash* mouse model of GS (E), due to clustering of melanosomes in the perinuclear region of melanocytes (F). Inset in F shows normal distribution of melanosomes in wild-type melanocytes.

3. REP and choroideremia (CHM)

CHM is an X-linked chorioretinal degeneration characterised by progressive night blindness and loss of peripheral vision starting in the second or third decade of life which invariably results in complete blindness within two or three decades of the initial manifestation of symptoms [28,29]. The gene mutated in this disease was identified by Cremers and co-workers [30] and named CHM. All known CHM mutations are predicted to result in loss of CHM protein function due to the production of truncated, non-functional or unstable polypeptides [29].

A function of CHM became apparent when REP1 was purified and cloned, and observed to correspond to the CHM gene product [28,31]. This suggested that CHM was caused by improper Rab prenylation, but it remained unclear how loss of a function essential for every cell type resulted in an eye-specific phenotype. The observation that lymphoblasts from CHM patients still contained RGGT activity led to the identification of a second REP protein, REP2, and to the hypothesis that REP2 incompletely compensates the loss of REP1 in CHM patients thus leaving a subset of Rab proteins unprenylated [32]. Consistent with this hypothesis, one Rab protein was observed to be selectively unprenylated in CHM lymphoblasts [33]. This Rab was subsequently identified as Rab27a and shown to be highly expressed in the same layers of the rat eye that are observed to degenerate in CHM patients, the retinal pigment epithelium (RPE) and the choroid [33].

Rab27a, one of two isoforms of the Rab27 subfamily, was recently shown to localise to melanosomes, melanin containing granules present in melanocytes, and to be involved in the transport of these organelles to the periphery of melanocytes [34–36]. Therefore, one possibility is that retinal degeneration in CHM patients might be triggered by defects in transport of melanosomes in RPE and choroidal melanocytes. However, recent data call into question the relationship between Rab27a hypoprenylation and chorioretinal cell death. Firstly, Rab27a is highly expressed in a number of other tissues which are apparently unaffected in CHM patients [33,37,38]. Secondly, loss of function mutations in the human and murine *RAB27A* genes result in Griscelli syndrome (GS) and the *ashen* (*ash*) mutant mouse, respectively. GS is characterised by partial cutaneous albinism and immunodeficiency, neither of which is reported as affecting CHM patients [36,39]. In both GS patients and the *ash* mouse, the absence of Rab27a protein results in immunodeficiency due to failure of cytotoxic T lymphocytes to secrete the contents of their lytic granules [39–41], and albinism due to defects in melanosome transport [34–36].

In spite of these observations, the involvement of Rab27a in the pathogenesis of CHM cannot be discounted at present as the *ash* mutant examined in the above studies is maintained in the inbred laboratory mouse strain (C3H) which is homozygous for the *rd* mutation [42]. This mutation of the β-subunit of cyclic GMP phosphodiesterase causes complete degeneration of photoreceptors shortly after birth. The absence of observed retinal degeneration in GS is understandable as their mean life expectancy is 5 years in the absence of early bone marrow transplantation [43] and to our knowledge no reports of long term health of transplanted patients are published. In our view, it is still likely that dysfunction of Rab27a contributes to CHM pathogenesis perhaps in combination with other retinal Rabs similarly hypoprenylated and partially dysfunctional. Another possibility is that REP1 may have other functions yet undiscovered required for survival of retinal cells.

4. RGGT and Hermansky–Pudlak syndrome (HPS)

HPS is a group of recessively inherited disorders caused by mutation in multiple genes whose products are involved in the biogenesis and function of three related organelles: melanosomes in melanocytes, platelet dense granules and lysosomes. The disease manifests as hypopigmentation (partial albinism), prolonged bleeding and ceroid deposition in lysosomes [44,45].

The *gunmetal* (*gm*) mutant is one of 14 murine models of HPS. In addition to the above defects, the *gm* mutant exhibits other abnormalities relative to wild-type animals; they have fewer, larger platelets, reduced platelet α-granule content, and reduction in killing capacity of cytotoxic T cells [40,46]. Recently, the *gm* mutation was identified as a G to A substitution in a splice-acceptor site in the gene encoding the murine RGGT α-subunit [47]. As a consequence, the major RGGT transcript in the *gm* mutant lacks a start codon and is nonfunctional. However, the *gm* mutant contains a reduced level of RGGT activity due to the low frequency activation of a cryptic splice-acceptor site within RGGT-α RNA, which results in the production of a transcript containing a start codon.

This observation suggested that deficiency of Rab(s) prenylation results in the HPS phenotype, a hypothesis remarkably similar to the one proposed for CHM. Consistent with this possibility, a subset of Rabs accumulate unprenylated in *gm* cytosol, including Rab27a [40,46,47]. Interestingly, Rab27 isoforms are highly expressed in the tissues affected in the *gm* mouse suggesting that the *gm* phenotype is at least in part a result of reduced Rab27 function. However, a number of observations indicate that hypoprenylation-induced defects in the function of other unknown Rabs contribute significantly towards more general disruption of membrane transport which triggers the pathological changes observed in this mutant. The most obvious of these is that although both the *gm* and *ash* mutants exhibit hypopigmentation and immunodeficiency, the fundamental cell biological defects underlying these phenotypes differ. In *gm*, immunodeficiency appears to result from defects in T cell cytotoxic granule polarisation rather than in secretion of granule contents as observed in *ash* [40]. Our preliminary observations of melanosome distribution in melanocytes derived from *gm* neonates indicate that hypopigmentation in this mutant does not result from failure to transport melanosomes to the peripheral cytoplasm (E. Sviderskaya, A.N. Hume, D.C. Bennet and M.C. Seabra, unpublished observations) as is observed in *ash* [36]. Identification and characterisation of the other Rabs which accumulate unprenylated in the cytosol of *gm* mutant should provide further insight into Rab prenylation and function, the pathogenesis of HPS and other human lysosomal storage disorders.

5. Conclusions

In conclusion, deficiencies in the Rab prenylation machinery can lead to different diseases (Fig. 1). Hypoprenylation of one or more Rab proteins seems to be the cause of the

different diseases, although it is impossible to rule out at this point other molecular mechanisms. It is intriguing that while prenylation is required for the function of most Rab proteins, deficiencies in the prenylation machinery only affect certain tissues. A possible explanation is that these restricted pathologies result from members of the Rab family having a range of affinities for REP1, REP2 and/or RGGT, together with a differential tissue requirement.

Rab27 appears to be a common denominator in CHM and *gm*, suggesting that it is not a good substrate for prenylation. As we cannot detect significant differences in binding of REP1 and REP2 to Rab27 in vitro (B. Larijani and M.C. Seabra, unpublished observations), other yet undescribed factors may regulate Rab prenylation in vivo. If this is the case, elucidating the nature of these factors will bring further insight to the understanding of Rab prenylation and these pathologies.

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References

[1] Chavrier, P. and Goud, B. (1999) Curr. Opin. Cell Biol. 11, 466–475.
[2] Guo, W., Sacher, M., Barrowman, J., Ferro-Novick, S. and Novick, P. (2000) Trends Cell Biol. 10, 251–255.
[3] Zerial, M. and McBride, H. (2001) Nat. Rev. Mol. Cell Biol. 2, 107–119.
[4] Deacon, S.W. and Gelfand, V.I. (2001) J. Cell Biol. 152, F21–F24.
[5] Pereira-Leal, L. and Seabra, M.C. (2000) J. Mol. Biol. 301, 1077–1087.
[6] Epstein, W.W., Lever, D., Leining, L.M., Bruenger, E. and Rilling, H.C. (1991) Proc. Natl. Acad. Sci. USA 88, 9668–9670.
[7] Casey, P.J. and Seabra, M.C. (1996) J. Biol. Chem. 271, 5289–5292.
[8] Spence, R.A. anad Casey, P.J. (2000) in: The Enzymes (Tamanoi, F. and Sigman, D., Eds.), Vol. XXI, pp. 1–18, Academic Press, NY.
[9] Yokoyama, K. and Gelb, M.H. (2000) in: The Enzymes (Tamanoi, F. and Sigman, D., Eds.), Vol. XXI, pp. 105–130, Academic Press, NY.
[10] Seabra, M.C., Goldstein, J.L., Sudhof, T.C. and Brown, M.S. (1992) J. Biol. Chem. 267, 14497–14503.
[11] Seabra, M.C. (2000) in: The Enzymes (Tamanoi, F. and Sigman, D., Eds.), Vol. XXI, pp. 131–154, Academic Press, NY.
[12] Zhang, H., Seabra, M.C. and Deisenhofer, J. (2000) Struct. Fold. Des. 8, 241–251.
[13] Terry, K.L., Long, S.B. and Beese, L.S. (2000) in: The Enzymes (Tamanoi, F. and Sigman, D., Eds.), Vol. XXI, pp. 19–46, Academic Press, NY.
[14] Farnsworth, C.C., Seabra, M.C., Ericsson, L.H., Gelb, M.H. and Glomset, J.A. (1994) Proc. Natl. Acad. Sci. USA 91, 11963–11967.
[15] Anant, J.S., Desnoyers, L., Machius, M., Demeler, B., Hansen, J.C., Westover, K.D., Deisenhofer, J. and Seabra, M.C. (1998) Biochemistry 37, 12559–12568.
[16] Seabra, M.C., Ho, Y.K. and Anant, J.S. (1995) J. Biol. Chem. 270, 24420–24427.
[17] Bialek-Wyrzykowska, U., Bauer, B.E., Wagner, W., Kohlwein, S.D., Schweyen, R.J. and Ragnini, A. (2000) Mol. Microbiol. 35, 1295–1311.
[18] Desnoyers, L., Anant, J.S. and Seabra, M.C. (1996) Biochem. Soc. Trans. 24, 699–703.
[19] Wu, S.K., Zeng, K., Wilson, I.A. and Balch, W.E. (1996) Trends Biochem. Sci. 21, 472–476.
[20] Alory, C. and Balch, W.E. (2000) J. Cell Biol. 150, 89–103.
[21] Overmeyer, J.H., Wilson, A.L., Erdman, R.A. and Maltese, W.A. (1998) Mol. Biol. Cell 9, 223–235.
[22] Beranger, F., Cadwallader, K., Porfiri, E., Powers, S., Evans, T., de Gunzburg, J. and Hancock, J.F. (1994) J. Biol. Chem. 269, 13637–13643.
[23] Desnoyers, L. and Seabra, M.C. (1998) Proc. Natl. Acad. Sci. USA 95, 12266–12270.
[24] Thomä, N.H., Iakovenko, A., Kalinin, A., Waldmann, H., Goody, R.S. and Alexandrov, K. (2001) Biochemistry 40, 268–274.
[25] Shen, F. and Seabra, M.C. (1996) J. Biol. Chem. 271, 3692–3698.
[26] Alexandrov, K., Horiuchi, H., Steele-Mortimer, O., Seabra, M.C. and Zerial, M. (1994) EMBO J. 13, 5262–5273.
[27] Wilson, A.L., Erdman, A. and Maltese, W.A. (1996) J. Biol. Chem. 271, 10932–10940.
[28] Seabra, M.C. (1996) Ophthalmic Genet. 17, 43–46.
[29] van den Hurk, J.A., Schwartz, M., van Bokhoven, H., van de Pol, T.J., Bogerd, L., Pinckers, A.J., Bleeker-Wagemakers, E.M., Pawlowitzki, I.H., Ruther, K., Ropers, H.H. and Cremers, F.P. (1997) Hum. Mutat. 9, 110–117.
[30] Cremers, F.P., van de Pol, D.J., van Kerkhoff, L.P., Wieringa, B. and Ropers, H.H. (1990) Nature 347, 674–677.
[31] Seabra, M.C., Brown, M.S. and Goldstein, J.L. (1993) Science 259, 377–381.
[32] Cremers, F.P., Armstrong, S.A., Seabra, M.C., Brown, M.S. and Goldstein, J.L. (1994) J. Biol. Chem. 269, 2111–2117.
[33] Seabra, M.C., Ho, Y.K. and Anant, J.S. (1995) J. Biol. Chem. 270, 24420–24427.
[34] Hume, A.N., Collinson, L.M., Rapak, A., Gomes, A.Q., Hopkins, C.R. and Seabra, M.C. (2001) J. Cell Biol. 152, 795–808.
[35] Bahadoran, P., Aberdam, E., Mantoux, F., Bille, K., Yalman, N., de Saint-Basile, G., Casaroli, R., Ortonne, J.-P. and Ballotti, R. (2001) J. Cell Biol. 152, 843–850.
[36] Wilson, S.M., Yip, R., Swing, D.A., O'Sullivan, T.N., Zhang, Y., Novalk, E.K., Swank, R.T., Russell, L.B., Copeland, N.G. and Jenkins, N.A. (2000) Proc. Natl. Acad. Sci. USA 97, 7933–7938.
[37] Chen, D.J., Guo, T., Miki, M., Tachibana, M. and Gahl, W.A. (1997) Biochem. Mol. Med. 60, 27–37.
[38] Ramalho, J.S., Tolmachova, T., Hume, A.N., McGuigan, A., Gregory-Evans, C.Y., Huxley, C. and Seabra, M.C. (2001) BMC Genet. 2, 2.
[39] Menasche, G., Pastural, E., Feldmann, J., Certain, S., Ersoy, F., Dupuis, S., Wulffraat, N., Bianchi, D., Fischer, A., Le Deist, F. and de Saint-Basile, G. (2000) Nat. Genet. 25, 173–176.
[40] Stinchcombe, J.C., Barral, D.C., Mules, E.H., Booth, S., Hume, A.N., Machesky, L.M., Seabra, M.C. and Griffiths, G.M. (2001) J. Cell Biol. 152, 825–834.
[41] Haddad, E.K., Wu, X., Hammer, J.A. and Henkart, P.A. (2001) J. Cell Biol. 152, 835–842.
[42] Pittler, S.J. and Baehr, W. (1991) Proc. Natl. Acad. Sci. USA 88, 8322–8326.
[43] Klein, C., Philippe, N., Le Deist, F., Fraitag, S., Prost, C., Durandy, A., Fischer, A. and Griscelli, C. (1994) J. Pediatr. 125, 886–895.
[44] Swank, R.T., Novak, E.K., McGarry, M.P., Rusiniak, M.E. and Feng, L. (1998) Pigment Cell Res. 11, 60–80.
[45] Huizing, M., Anikster, Y. and Gahl, W.A. (2000) Traffic 1, 823–825.
[46] Swank, R.T., Jiang, S.Y., Reddington, M., Conway, J., Stephenson, D., McGarry, M.P. and Novak, E.K. (1993) Blood 81, 2626–2635.
[47] Detter, J.C., Zhang, Q., Mules, E.H., Novak, E.K., Mishra, V.S., Li, W., McMurtie, E.B., Tchernev, V.T., Wallace, M.R., Seabra, M.C., Swank, R.T. and Kingsmore, S.F. (2000) Proc. Natl. Acad. Sci. USA 97, 4144–4149.