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Evolutionary history of the *Rh* blood group-related genes in vertebrates

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Abstract *Rh* and its homologous Rh50 gene products are considered to form heterotetramers on erythrocyte membranes. Rh protein has Rh blood group antigen sites, while Rh50 protein does not, and is more conserved than Rh protein. We previously determined both *Rh* and *Rh50* gene cDNA coding regions from mouse and rat, and carried out phylogenetic analyses. In this study, we determined *Rh50* gene cDNA coding regions from African clawed frog and Japanese medaka fish, and examined the long-term evolution of the *Rh* blood group and related genes. We constructed the phylogenetic tree from amino acid sequences. *Rh50* genes of African clawed frog and Japanese medaka fish formed a cluster with mammalian *Rh50* genes. The gene duplication time between *Rh* and *Rh50* genes was estimated to be about 510 million years ago based on this tree. This period roughly corresponds to the Cambrian, before the divergence between jawless fish and jawed vertebrates. We also BLAST-searched an amino acid sequence database, and the *Rh* blood group and related genes were found to have homology with ammonium transporter genes of many organisms. Ammonium transporter genes can be classified into two major groups (amt α and amt β). Both groups contain genes from three domains (bacteria, archaea, and eukaryota). The *Rh* blood group and related genes are separated from both amt α and β groups.

Key words *Rh* blood group genes · Evolutionary history · Gene duplication

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Introduction

The human Rh blood group plays important roles in transfusion and clinical medicine, being involved in hemolytic diseases of newborns, autoimmune diseases, and mild hemolytic anemia. Rh polypeptides were observed to be phosphorylated M_r 30–32,000 membrane proteins using SDS-PAGE and immunoprecipitation (Gahmberg 1982; Moore et al. 1982), and the Rh blood group system is composed of two closely linked *D* and *CE* loci (Mouro et al. 1993). A protein was obtained together with the Rh gene product on immunoprecipitation with anti-Rh antibodies from human, and named the 50kD glycoprotein (Moore and Green 1987). This glycoprotein was considered to form a heterotetramer with *Rh* blood group gene products on erythrocyte membranes (Eyers et al. 1994). The nucleotide sequence of the human 50kD glycoprotein (Rh50) was determined, and its amino acid sequence was homologous with that of the human *Rh* gene (Ridgwell et al. 1992). That protein was also predicted to have the 12 transmembrane domains which are similar to those of the Rh blood group gene product (Avent et al. 1990). There are several names for this gene, such as *RHAG*, but we call this gene *Rh50* and the Rh blood group gene *Rh* hereafter for simplicity.

Nucleotide sequences of *Rh* genes in nonhuman primates have been reported (Mouro et al. 1994; Salvignol et al. 1994, 1995). We have also determined the nucleotide sequences of *Rh* and *Rh50* genes in mouse and rat and that of the *Rh50* gene for the crab-eating macaque, and considered their evolutionary relationships (Kitano et al. 1998; see also Matassi et al. 1999).

In this paper, we examined the long-term evolution of the *Rh* blood group and related genes through newly determined cDNA sequences for Japanese medaka teleost fish and African clawed frog and

analyzed the gene duplication time of *Rh* and *Rh50* genes.

Materials and methods

Animals and RNA preparation

Total RNAs were extracted from femora of African clawed frog (*Xenopus laevis*) and from whole body of Japanese medaka fish (*Oryzias latipes*), using ISOGEN (Nippon Gene). Reverse transcription was performed using SuperScript II reverse transcriptase and oligo dT-adaptor primer (Gibco-BRL).

Methods for degenerate PCR and rapid amplification of cDNA ends

We first performed degenerate PCR for the *Rh50* genes. Degenerate primers DP0065 (5′-TIGGIYTIGGCTTCCT-3′) and DP0077 (5′-GCYACIGCYARICCAAAGTA-3′) were used for the *Xenopus Rh50* gene, and degenerate primers DP0065 (5′-TIGGIYTIGGCTTCCT-3′) and DP0081 (5′-CCACIGCAACWCCTCCTGCIA-3′) were used for the Japanese medaka *Rh50* gene. We then performed 5′ rapid amplification of cDNA ends (5′RACE) using the 5′RACE System for Rapid Amplification of cDNA Ends version 2.0 (Gibco-BRL). 3′RACE was also carried out. To amplify the complete cDNA sequence, PCR was performed using gene-specific primers. These primers were designed on both noncoding regions determined by 5′ and 3′RACE methods. Primers DP0114 (5′-TCAGTCCCCTGCAGGTCTGAGAT-3′) and DP0112 (5′-TGCAGTTGTTGAAATGCCATTTAC-3′) were used for the *Xenopus Rh50* gene, and DP0107 (5′-CAAGAGTGATC AAGCATCGTACC-3′) and DP0109 (5′-GGTCTCCCCAGTG CAGCTTTAG-3′) were used for the Japanese medaka *Rh50* gene. PCR was performed in a 20-μl reaction containing 0.5–1 μl of first-strand cDNA, 1×Gene *Taq* Universal Buffer (Mg^{2+} free) (Nippon Gene), 1.5 mm $MgCl_2$, 0.2 mm dNTP, 10 pmol of each primer (designed on sites of 5′ and 3′ ends), and 1 unit of Ampli*Taq* Gold (PE Biosystems). Amplification was carried out in a DNA GeneAmp PCR System 9700 (PE Biosystems). PCR products were purified using MicroSpin Columns S-300 HR (Amersham Pharmacia Biotech). DNA sequencing was performed on PCR products using the BigDye Terminator Cycle Sequencing Kit and ABI PRISM 377 DNA sequencer (PE Biosystems). A progressive sequencing strategy was carried out with further primers to complete the sequence for the coding region of both strands of the cDNA.

Sequence analyses

CLUSTAL W version 1.6 (Thompson et al. 1994) was used for multiple alignment. Numbers of amino acid substitutions were estimated using Kimura's (1983) method. The dista program of the ODEN package (Ina 1994) was used to estimate the number of amino acid substitutions. The PredictProtein server (EMBL) was used for analyses of transmembrane helix location. The program protml of MOLPHY version 2.2 (Adachi and Hasegawa 1994) was used for the maximum-likelihood analyses.

Results and discussion

Sequence comparison of Rh, Rh50, and related genes

We sequenced *Rh50*-like genes in *Xenopus* and Japanese medaka (DDBJ/EMBL/GenBank International Nucleotide Sequence Database accession numbers AB036510 and AB036511, respectively). Nucleotide sequence lengths of these genes are 1275 bp for *Xenopus* and 1467 bp for Japanese medaka. GC contents of those genes for *Xenopus* and Japanese medaka are 45.9 and 48.9%, respectively. These values are similar to other *Rh50* genes (Kitano et al. 1998; Matassi et al. 1999).

Figure 1 shows the multiple alignment of amino acid sequences of *Rh*, *Rh50*, and related genes. Amino acid sequences of *Rh* genes for human (Avent et al. 1990; Cherif-Zahar et al. 1990; their amino acid sequences are identical), crab-eating macaque (Salvignol et al. 1994), mouse and rat (Kitano et al. 1998), and those of *Rh50* genes for human (Ridgwell et al. 1992), crab-eating macaque, mouse, and rat (Kitano et al. 1998) were used. We also included the *Drosophila Rh*-like gene (AF172639, AF181624, and AF193812; their amino acid sequences are identical), two *Rh*-like genes for *Caenorhabditis elegans* (Wilson et al. 1994), and a sponge *Rh*-like gene (Seack et al. 1997).

The 12 predicted hydrophobic membrane-spanning regions are surrounded by boxes (Fig. 1). These membrane-spanning regions did not include gaps and are relatively conserved.

The phylogenetic tree of Rh, Rh50, and related genes

Because membrane-spanning regions did not include gaps and are relatively conserved, we used only the 222 amino acid sites for membrane-spanning regions (Fig. 1) for tree construction. Figure 2 shows the maximum-likelihood tree for *Rh*, *Rh50*, and related genes using the JTT (Jones et al. 1992) model (the program protml of MOLPHY version 2.2 was used). The root was located by assuming the *Rh*-like gene of sponge as an outgroup. There are three clusters in this tree: (1) *Rh50* genes of mammals and *Rh50*-like genes of *Xenopus* and Japanese medaka, (2) *Rh* genes of mammals, and (3) genes of *Drosophila* and *C. elegans*. This tree suggests that *Xenopus* and Japanese medaka *Rh50*-like genes are orthologues of other *Rh50* genes and duplication of *Rh* and *Rh50* genes occurred before the speciation of teleosts and other vertebrates.

We also considered three possible tree topologies, using the maximum-likelihood method (Fig. 3A–C). Tree A shows the same topology as Fig. 2. In this case, we have to assume that frog *Rh* and fish *Rh* genes were lost (or the genes have not yet been identified). In tree B, duplication of *Rh* and *Rh50* genes occurred after speciation of fish and other vertebrates, and we have to assume that the *Xenopus Rh* gene was lost (or the gene has not yet been identified). In tree C, duplication of *Rh* and *Rh50* genes occurred after speciation of amphibians and other vertebrates.

We tried to obtain *Rh* orthologous genes for *Xenopus* and Japanese medaka using several degenerate primers, but could not obtain these cDNA sequences.

Fig. 1 Multiple alignment of amino acid sequences of *Rh* blood group and related genes. Amino acid sequences of *Rh* genes for human (M34015 and X54534; their amino acid sequences are identical), crab-eating macaque (L37054), mouse (AB015189), and rat (AB015191), and of *Rh50* genes for human (X64594), crab-eating macaque (AB015467), mouse (AB015192), and rat (AB015194) are shown. Amino acid sequences of *Rh*-like genes for *Drosophila* (AF172639, AF181624, and AF193812; their amino acid sequences are identical), *Caenorhabditis elegans* 1 (Z74026-B0240.1), *C. elegans* 2 (U64847-F08F3.3), and sponge (Y12397) are also depicted. Twelve membrane-spanning regions are *boxed*

Among the three possible trees in Fig. 3, tree A had the highest likelihood value. Interestingly, the human *Rh* gene locus is located on Chromosome (Chr) 1p34–p36 (Cherif-Zahar et al. 1991; Ruddle et al. 1972) and the human *Rh50* locus is located on Chr 6p11–21.1 (Cherif-Zahar et al. 1996; Ridgwell et al. 1992). These locations are close to two paralogous regions involving the major histocompatibility complex in the human genome (Kasahara 1999; Kasahara et al. 1996). Therefore, duplication between *Rh* and *Rh50* genes plausibly occurred by chromosomal or genome duplication in an ancestral vertebrate.

The branch lengths of *Rh* genes are much greater than those of *Rh50* genes, indicating a higher evolutionary rate in the *Rh* than in the *Rh50* gene. This pattern is consistent with the d_N result (Kitano et al. 1998). The ratios of *Rh*-d_N and *Rh50*-d_N were 2.0, 1.7, and 2.0 for human-macaque, mouse-rat, and primate-rodent comparisons, respectively. It is interesting that after the duplication which produced *Rh* and *Rh50* genes, the *Rh* gene lineage started to evolve more rapidly than the *Rh50* lineage. Kitano and Saitou (1999) have suggested that some kind of positive selection occurred on the primate lineage of *Rh* genes.

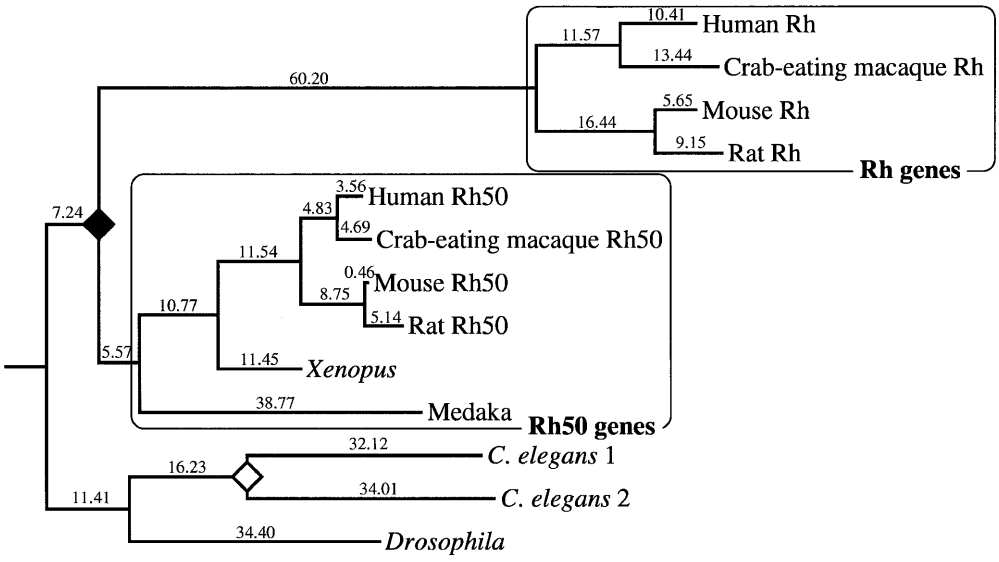


Fig. 2 The maximum-likelihood tree of *Rh* blood group and homologous genes generated using the 12 membrane-spanning regions. The *closed diamond* denotes the duplication event producing *Rh* and *Rh50* genes. The *open diamond* denotes another gene duplication in the nematode lineage. The root was located by assuming the Rh-like protein of sponge as an outgroup

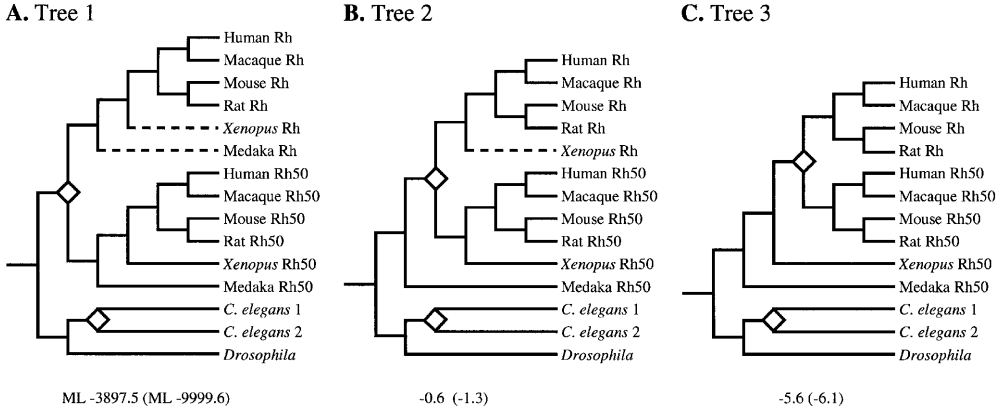


Fig. 3A–C Three possible tree topologies of *Rh* blood group and homologous genes. A likelihood difference from the maximum likelihood (*ML*) by 12 membrane-spanning regions is shown for each tree. Values of the maximum-likelihood analysis using all amino acid sites are shown in *parentheses*. The program protml of MOLPHY version 2.2 (Adachi and Hasegawa 1994) was used

Comparison of the number of amino acid substitutions

We estimated the number of amino acid substitutions (Table 1). The phylogenetic tree of Fig. 2 was used and number of amino acid substitutions of a single lineage was estimated applying Ishida and co-workers' (1995) method. We then estimated the evolutionary rates of *Rh* and *Rh50* genes using regression through the origin. Because the branch length of the *Xenopus Rh50* gene is much shorter than that of other genes, we did not use it for calibration.

Divergence times between human and macaque, between mouse and rat, between primates and rodents, and between tetrapods and fishes were assumed to be 23.3 (Kumar and Hedges 1998), 30 (Kitano et al., 1999), 112, and 450 (Kumar and Hedges 1998) million years ago, respectively, and they were used for calibration of the molecular clock. Numbers of amino acid substitutions were thus obtained as 83.87 and 41.24 for *Rh* and *Rh50* genes, respectively (Table 1). If we use these rates, the time of duplication (node indicated by the closed diamond in Fig. 2) producing *Rh* and *Rh50* genes was estimated to be about 372 or 514 million years ago from the data for *Rh* or *Rh50*, respectively. The estimate (372 million years ago) from the data for *Rh* is lower than the divergence time between tetrapods and fishes (450 million years ago). This lower estimate suggests that the evolutionary rate of *Rh* genes accelerated after

Table 1 Number of amino acid substitutions and divergence times. *Node*: *H-M* between human and macaque, *M-R* between mouse and rat, *P-R* between primates and rodents, *T-F* between tetrapods and fishes, *Rh/Rh50* between *Rh* and *Rh50* genes

	Node				
	H-M	M-R	P-R	T-F	Rh/Rh50
Rh[a]	11.93	7.40	23.67	–	83.87
Rh50[a]	4.13	2.80	10.25	35.67	41.24
Million years ago	23.3	30	112	450	514[b]

[a] Amino acid substitutions of a single lineage based on the phylogenetic tree in Fig. 2

[b] Estimated from numbers of amino acid substitutions of *Rh50* genes

the gene duplication. Therefore, a more plausible time of duplication is 514 million years ago based on the *Rh50* gene data. This period roughly corresponds to the Cambrian, before the divergence between jawless fish and jawed vertebrates lineages.

At least two genome duplications are suggested to have taken place in a common ancestor of jawed vertebrates (Kasahara 1999; Kasahara et al. 1996). The first genome duplication might have taken place after the divergence between jawless fish and other vertebrates. The gene duplication of *Rh* (1p34–p36) and *Rh50* (6p11–21.1) genes almost corresponds to this period. If true, another two duplicated *Rh*/*Rh50*-like genes exist (or existed) in jawed vertebrates.

The relationship to ammonium transporter proteins

Because products of *Rh* and *Rh50* genes are predicted to have 12 transmembrane domains, Rh and Rh50 proteins have been suggested to be related to ammonium transporter proteins (e.g., Marini et al. 1997b). We BLAST-searched (Altschul et al. 1990) the DDBJ amino acid sequence database (DAD) for homologous proteins. Two protein sequences of the human *Rh50* gene and the *Rh*-like gene of sponge were used to query sequences. Table 2 shows the list of protein sequences obtained. Seventeen ammonium transporter proteins were found. No ammonium transporter gene was found examining the complete genomic sequences of *Haemophilus influenzae* and *Mycobacterium genitalium*, whose natural environment is human tissue (Marini et al. 1997a). Ammonium transporter proteins carry the ammonium ion (NH_4^+) into cells (Marini et al. 1994). The ammonium ion is a nitrogen source supporting optimal growth for primitive organisms such as yeast.

Figure 4 shows the multiple alignment of transmembrane domains for ammonium transporter protein sequences compared with Rh, Rh50, and related protein sequences. Because the 1st, 2nd, 3rd, and 12th predicted membrane regions did not align well, these regions were eliminated.

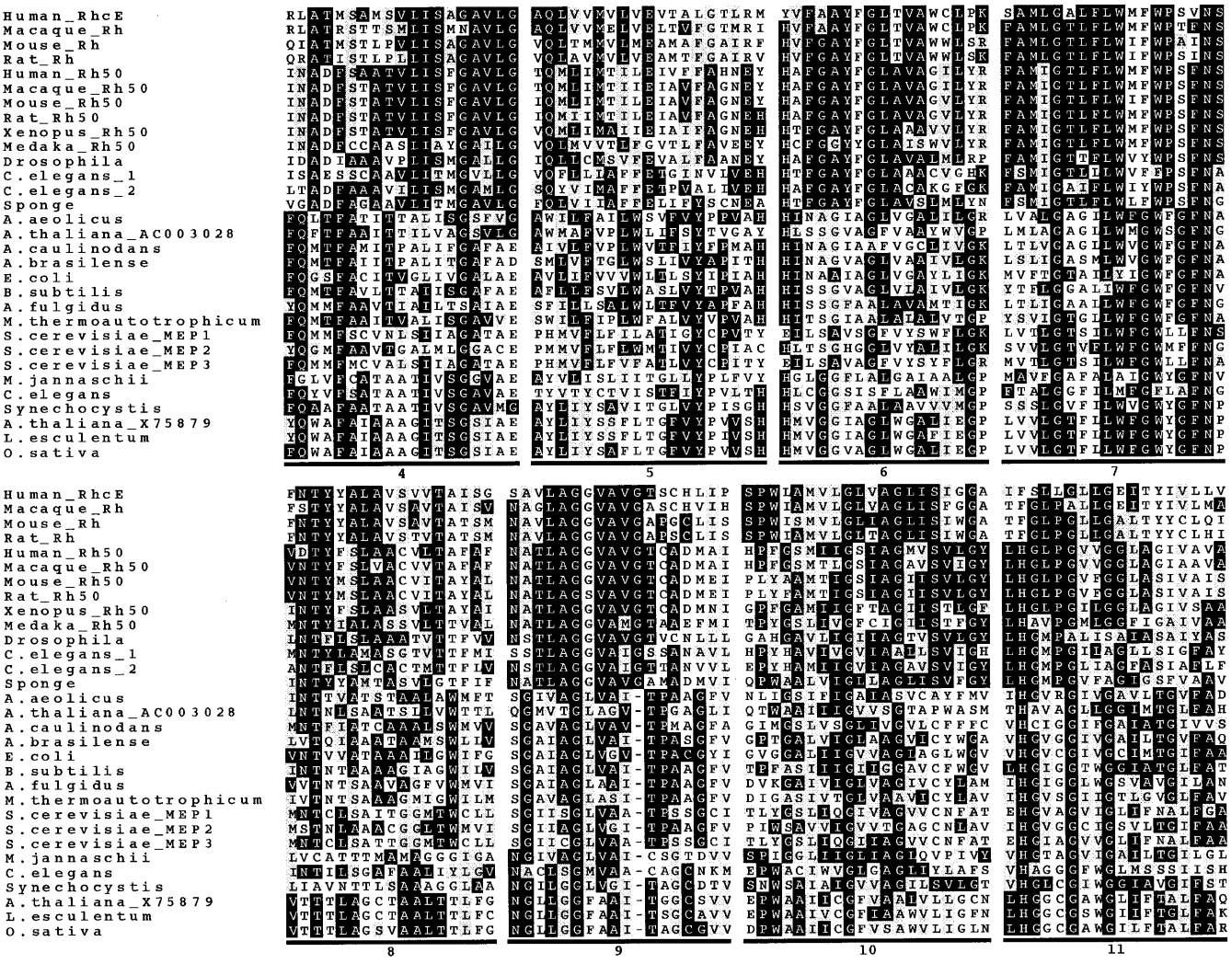
Figure 5 shows the neighbor-joining tree of the *Rh* blood group-related genes and ammonium transporter genes based on the multiple alignment of Fig. 4. Bootstrap values greater than 90% are shown. There are roughly three clusters in this tree; the α group of ammonium transporter genes (amt α group), the β group of ammonium transporter genes (amt β group), and the *Rh* blood group and related genes (*Rh* genes group). Both α and β groups of the ammonium transporter cluster include three kingdoms of life (bacteria, archaea, and eukaryota). However, the relationships of the three kingdoms in both the α and β groups are unclear. Because most bootstrap probabilities on branches in both α and β groups are not so high, statistical errors may cause these unclear relationships for the three kingdoms in both α and β groups. Although the root point of the *Rh* gene group is not appropriate (see Fig. 2), members of the *Rh* gene group form a cluster.

Marini and co-workers (1997a) and Van Dommelen and co-workers (1998) created phylogenetic trees for ammonium transporter proteins, and those trees also indicated two major groups, but they were not named. Therefore, we propose to call these two groups of ammonium transporter genes the α and β groups. Because most of the ammonium transporter genes are predicted from genome sequences, however, functional differences between gene products from the α and β groups are not known.

The universal ancestor of all the organisms might already have had an ammonium transporter gene, in which case the gene duplication occurred before the divergences of the three kingdoms. Therefore, most of the organisms may have amt α and/or amt β groups. For example, *Arabidopsis thaliana* has genes for both amt α and amt β, while *Saccharomyces cerevisiae* has only genes for the amt α group. Because we cannot define the root point of this tree, defining the divergence pathway of the *Rh* blood group-related genes is

Table 2 Ammonium transporter proteins

Accession No.	Organism	Taxon
AE000674	*Aquifex aeolicus*	Bacteria
ACJ225126	*Azorhizobium caulinodans*	Bacteria
AF005275	*Azospirillum brasilense*	Bacteria
L03216	*Bacillus subtilis*	Bacteria
U82664	*Escherichia coli*	Bacteria
D90901	*Synechocystis* sp.	Bacteria
AE001036	*Archaeoglobus fulgidus*	Archaea
AE000846	*Methanobacterium thermoautotrophicum*	Archaea
U67574	*Methanococcus jannaschii*	Archaea
AC003028	*Arabidopsis thaliana*	Eukaryota
X75879	*Arabidopsis thaliana*	Eukaryota
U53338 (C05E11.5)	*Caenorhabditis elegans*	Eukaryota
X95098	*Lycopersicon esculentum*	Eukaryota
AF001505	*Oryza sativa*	Eukaryota
Z72906	*Saccharomyces cerevisiae* (MEP1)	Eukaryota
Z71418	*Saccharomyces cerevisiae* (MEP2)	Eukaryota
U40829	*Saccharomyces cerevisiae* (MEP3)	Eukaryota



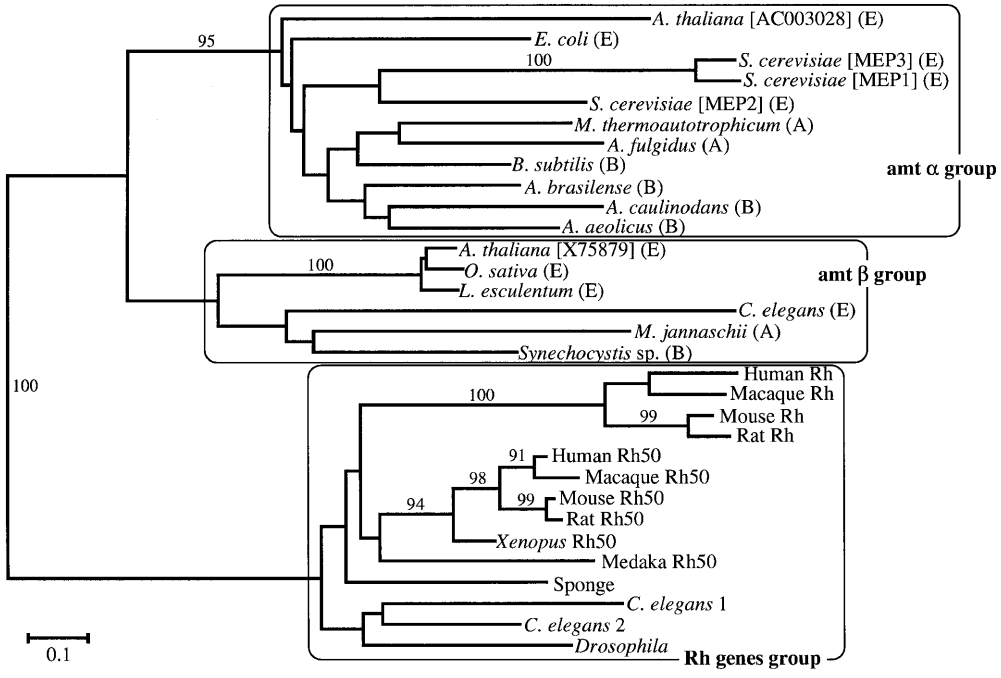


Fig. 5 The phylogenetic tree of ammonium transporter genes and the *Rh* blood group-related genes. Midpoint rooting was used. Bootstrap values greater than 90% are shown on each branch. *B*, *A*, and *E* in *parentheses* indicate taxa for bacteria, archaea, and eukaryota, respectively

Fig. 4 Multiple alignment of transmembrane domains for ammonium transporter proteins compared with Rh, Rh50, and their related proteins. This figure was made using BOXSHADE 3.21 (http://www.ch.embnet.org/software/BOX_form.html). *Bars with numbers* designate transmembrane domains

difficult. However, the *Rh* blood group and related genes appear to exist as essential membrane proteins in animals and, probably, other eukaryotes. Expression of *Rh* and *Rh50* genes is restricted to the erythrocyte in humans and, probably, in other vertebrates, and erythrocytes evolved in the vertebrate lineage. Therefore, the location of expression must have changed from some other cell type(s) to the erythrocyte membrane before duplication of the *Rh* and *Rh50* genes.

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References

Adachi J, Hasegawa M (1994) MOLPHY: programs for molecular phylogenetics, version 2.2. Institute of Statistical Mathematics, Tokyo

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Avent ND, Ridgwell K, Tanner MJ, Anstee DJ (1990) cDNA cloning of a 30 kDa erythrocyte membrane protein associated with Rh (Rhesus)-blood-group-antigen expression. Biochem J 271:821–825

Cherif-Zahar B, Bloy C, Le Van Kim C, Blanchard D, Bailly P, Hermand P, Salmon C, Cartron J-P, Colin Y (1990) Molecular cloning and protein structure of a human blood group Rh polypeptide. Proc Natl Acad Sci USA 87:6243–6247

Cherif-Zahar B, Mattei MG, Le Van Kim C, Bailly P, Cartron J-P, Colin Y (1991) Localization of the human Rh blood group gene structure to chromosome region 1p34.3–1p36.1 by in situ hybridization. Hum Genet 86:398–400

Cherif-Zahar B, Raynal V, Gane P, Mattei MG, Bailly P, Gibbs B, Colin Y, Cartron J-P (1996) Candidate gene acting as a suppressor of the RH locus in most cases of Rh-deficiency. Nat Genet 12:168–173

Eyers SAC, Ridgwell K, Mawby WJ, Tanner MJA (1994) Topology and organization of human Rh (rhesus) blood group-related polypeptides. J Biol Chem 269:6417–6423

Gahmberg CG (1982) Molecular identification of the human Rho (D) antigen. FEBS Lett 140:93–97

Ina Y (1994) ODEN: a program package for molecular evolutionary analysis and database search of DNA and amino acid sequences. Comput Appl Biosci 10:11–12

Ishida N, Oyunsuren T, Mashima S, Mukoyama H, Saitou N (1995) Mitochondrial DNA sequences of various species of the genus *Equus* with a special reference to the phylogenetic relationship between Przewalskii's wild horse and domestic horse. J Mol Evol 41:180–188

Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. Comput Appl Biosci 8:275–282

Kasahara M (1999) The chromosomal duplication model of the major histocompatibility complex. Immunol Rev 167:17–32

Kasahara M, Hayashi M, Tanaka K, Inoko H, Sugaya K, Ikemura T, Ishibashi T (1996) Chromosomal localization of the proteasome Z subunit gene reveals an ancient chromosomal duplication involving the major histocompatibility complex. Proc Natl Acad Sci USA 93:9096–9101

Kimura M (1983) The neutral theory of molecular evolution. Cambridge University Press, Cambridge, UK

Kitano T, Saitou N (1999) Evolution of Rh blood group genes have experienced gene conversions and positive selection. J Mol Evol 49:615–626

Kitano T, Sumiyama K, Shiroishi T, Saitou N (1998) Conserved evolution of the Rh50 gene compared to its homologous Rh blood group gene. Biochem Biophys Res Commun 249:78–85

Kitano T, Oota S, Saitou N (1999) Molecular evolutionary analyses of the Rh blood group genes and Rh50 genes in mammals. Zool Stud 38:379–386

Kumar S, Hedges SB (1998) A molecular timescale for vertebrate evolution. Nature 392:917–920

Marini AM, Vissers S, Urrestarazu A, Andre B (1994) Cloning and expression of the *MEP1* gene encoding an ammonium transporter in *Saccharomyces cerevisiae*. EMBO J 13:3456–3463

Marini AM, Soussi-Boudekou S, Vissers S, Andre B (1997a) A family of ammonium transporters in *Saccharomyces cerevisiae*. Mol Cell Biol 17:4282–4293

Marini AM, Urrestarazu A, Beauwens R, Andre B (1997b) The Rh (rhesus) blood group polypeptides are related to NH_4^+ transporters. Trends Biochem Sci 22:460–461

Matassi G, Cherif-Zahar B, Pesole G, Raynal V, Cartron J-P (1999) The members of the RH gene family (RH50 and RH30) underwent different evolutionary pathways. J Mol Evol 48:151–159

Moore S, Green C (1987) The identification of specific Rhesus-polypeptide-blood-group-ABH-active-glycoprotein complexes in the human red-cell membrane. Biochem J 244:735–741

Moore S, Woodrow CF, McClelland DB (1982) Isolation of membrane components associated with human red cell antigens Rh(D), (c), (E) and Fy. Nature 295:529–531

Mouro I, Colin Y, Cherif-Zahar B, Cartron J-P, Le Van Kim C (1993) Molecular genetic basis of the human Rhesus blood group system. Nat Genet 5:62–65

Mouro I, Le Van Kim C, Cherif-Zahar B, Salvignol I, Blancher A, Cartron J-P, Colin Y (1994) Molecular characterization of the Rh-like locus and gene transcripts from the rhesus monkey (*Macaca mulatta*). J Mol Evol 38:169–176

Ridgwell K, Spurr NK, Laguda B, MacGeoch C, Avent ND, Tanner MJ (1992) Isolation of cDNA clones for a 50 kDa glycoprotein of the human erythrocyte membrane associated with Rh (rhesus) blood-group antigen expression. Biochem J 287:223–228

Ruddle F, Ricciuti F, McMorris FA, Tischfield J, Creagan R, Darlington G, Chen T (1972) Somatic cell genetic assignment of peptidase C and the Rh linkage group to chromosome A-1 in man. Science 176:1429–1431

Salvignol I, Blancher A, Calvas P, Clayton J, Socha WW, Colin Y, Ruffie J (1994) Molecular genetics of chimpanzee Rh-related genes: their relationship with the R-C-E-F blood group system, the chimpanzee counterpart of the human rhesus system. Biochem Genet 32:201–221

Salvignol I, Calvas P, Socha WW, Colin Y, Le Van Kim C, Bailly P, Ruffie J, Cartron J-P, Blancher A (1995) Structural analysis of the RH-like blood group gene products in non-human primates. Immunogenetics 41:271–281

Seack J, Pancer Z, Mueller IM, Mueller WE (1997) Molecular cloning and primary structure of a Rhesus (Rh)-like protein from the marine sponge *Geodia cydonium*. Immunogenetics 47:493–498

Thompson JD, Gibson TJ, Higgins DG (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Van Dommelen A, Keijers V, Vanderleyden J, Zamaroczy M de (1998) (Methyl) ammonium transport in the nitrogen-fixing bacterium *Azospirillum brasilense*. J Bacteriol 180:2652–2659

Wilson R, Ainscough R, Anderson K et al. (1994) 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. Nature 368:32–38