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Expression analysis of mammalian mitochondrial ribosomal protein genes

Agnes Cheong[#], **Ranjana Lingutla**[#], **Jesse Mager**[*]

Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA, USA

Abstract

Mitochondrial ribosomal proteins (MRPs) are essential components for the structural and functional integrity of the mitoribosome complex. Throughout evolution, the mammalian mitoribosome has acquired new *Mrp* genes to compensate for loss of ribosomal RNA. More than 80 MRPs have been identified in mammals. Here we document expression pattern of 79 *Mrp* genes during mouse development and adult tissues and find that these genes are consistently expressed throughout early embryogenesis with little stage or tissue specificity. Further investigation of the amino acid sequence reveals that this group of proteins has little to no protein similarity. Recent work has shown that the majority of *Mrp* genes are essential resulting in early embryonic lethality, suggesting no functional redundancy among the group. Taken together, these results indicate that the *Mrp* genes are not a gene family descended from a single ancestral gene, and that each MRP has unique and essential role in the mitoribosome complex. The lack of functional redundancy is surprising given the importance of the mitoribosome for cellular and organismal viability. Further, these data suggest that genomic variants in *Mrp* genes may be causative for early pregnancy loss and should be evaluated as clinically.

Keywords

MRP; mitochondria; mitoribosome complex; *Mrpl1*; *Mrpl2*; *Mrpl3*; *Mrpl4*; *Mrpl9*; *Mrpl10*; *Mrpl11*; *Mrpl12*; *Mrpl13*; *Mrpl14*; *Mrpl15*; *Mrpl16*; *Mrpl17*; *Mrpl18*; *Mrpl19*; *Mrpl20*; *Mrpl21*; *Mrpl22*; *Mrpl23*; *Mrpl24*; *Mrpl27*; *Mrpl28*; *Mrpl30*; *Mrpl32*; *Mrpl33*; *Mrpl34*; *Mrpl35*; *Mrpl36*; *Mrpl37*; *Mrpl38*; *Mrpl39*; *Mrpl40*; *Mrpl41*; *Mrpl42*; *Mrpl43*; *Mrpl44*; *Mrpl45*; *Mrpl46*; *Mrpl47*; *Mrpl48*; *Mrpl49*; *Mrpl50*; *Mrpl51*; *Mrpl52*; *Mrpl53*; *Mrpl54*; *Mrpl55*; *Mrpl56*; *Mrpl57*; *Mrpl58*; *Mrpl59*; *Mrps2*; *Mrps5*; *Mrps6*; *Mrps7*; *Mrps9*; *Mrps10*; *Mrps11*; *Mrps12*; *Mrps14*; *Mrps15*; *Mrps16*; *Mrps17*; *Mrps18a*; *Mrps18b*; *Mrps18c*; *Mrps21*; *Mrps22*; *Mrps23*; *Mrps24*; *Mrps25*; *Mrps26*; *Mrps27*; *Mrps28*; *Mrps29*; *Mrps30*; *Mrps31*; *Mrps33*; *Mrps34*; *Mrps35*; *Mrps36*; *Mrps37*; *Mrps38*; *Mrps39*

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[*]Corresponding Author: Amherst, 661 North Pleasant Street, Amherst, Massachusetts 01003, USA; jmager@vasci.umass.edu.
[#]These authors contributed equally to this work.

Author statement:
RL initiated the project with help designing by JM. AC trained and supervised RL. RL performed the majority of RT-PCR. AC collected the samples, extracted RNA and repeated RT-PCR as needed. RL, AC and JM all wrote and edited the manuscript. All authors have read and approve the manuscript for submission.

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Introduction

Mitochondria are evolutionarily conserved organelles that mammals acquired from alphaproteobacteria through the process of endosymbiosis (1). One remarkable feature of these highly dynamic organelles is the presence of their own circular genome, the nucleoid. Phylogenetic analyses have suggested that most of the mitochondrial related genes found in the eukaryotic nucleus originated from the mitochondrial genome and integrated into the nuclear genome through ancient evolutionary events (1, 2). Though varied in size among species, the mitochondrial genome is quite small when compared to the nuclear genome (3). Over the course of evolution, the mammalian mitochondrial genome has retained the coding sequence for 37 genes which encode for two ribosomal RNAs (rRNA), 22 transfer RNAs (tRNA) and 13 proteins all of which are essential components of the electron transport chain (ETC), the central components of the oxidative phosphorylation (OXPHOS) system (3). The remaining mitochondrial related proteins are encoded by genes in the nuclear genome and translated by the cytosolic translation machinery. This includes all components of the mitochondrial transcription and translation system. The majority of these proteins contain a transit peptide sequence at the N-terminus which allows them to be recognized by the translocase of the outer membrane (TOM) complex and imported into the mitochondria.

Mitochondria are generally termed the “powerhouse” of the cell given their ability to generate vast amounts of energy. Depending on physiological conditions, mitochondria will respond through fusion or fission to achieve adequate energy demands (4–8). In addition to being the major cellular energy source, mitochondria also produce reactive oxygen species (ROS), an essential second messenger, and also have crucial roles in multiple metabolic and biogenetic pathways (9, 10). Hence, dysfunctional mitochondria can have myriad detrimental effects. Indeed, numerous clinical studies show that mutations in mitochondrial related genes are associated with multisystem disorders in humans and early embryonic lethality in mice (11–13). In particular, dysfunctional mitochondrial translation most often results in pre-gastrulation developmental arrest in mouse embryos due to insufficient ATP production and interruption in cell cycle (12).

The mitochondrial translation machinery is composed of tRNAs and the 55S mitoribosome complex which consists of two rRNAs (16S and 12S) and nuclear-encoded mitochondrial ribosomal proteins (MRP) (14). Similar to the cytosolic ribosome complex, the mitoribosome complex contains the P-site (the peptidyl site), A-site (the acceptor site) and E-site (the exit site) (14). The mitoribosome complex has undergone dynamic change during the course of evolution. The metazoan mitoribosome complex has lost some bacterial originated MRPs and regions of the rRNA and acquired new eukaryotic specific MRPs (15, 16). Distinct from the bacterial mitoribosome complex, the mammalian complex has higher protein content (14). In mammals, more than 80 *Mrp* genes have been identified and this group of genes is classified in two main categories: *Mrpl*, components of the large subunit, and *Mrps*, components of the small subunit. The naming of these mammalian *Mrp* genes is inherited from their prokaryotic homologs for consistency across organisms. Recently, additional proteins have been identified as MRPs based on their cellular localization in the

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mitochondria and association with other mitoribosome components (17). Currently, the total number of *Mrp* genes in mice has increased to 84 with 51 *Mrpl* and 33 *Mrps*.

Our group has recently characterized knock out phenotypes of embryos lacking the *Mrpl3*, -*l22*, -*l44*, -*s18c* and -*s22* genes along with description of 16 other mitochondrial related gene knockouts (12)(https://blogs.umass.edu/jmager/). Loss of any one of these essential mitochondrial related genes, results in one of two specific phenotypes - null embryos either fail to implant after the blastocyst stage or fail to initiate gastrulation, each with nearly identical mutant phenotype. The surprisingly consistent phenotypes and apparent lack of functional redundancy among the *Mrp* genes led us to investigate expression patterns and similarities among this large group of genes.

Results and Discussion

Mrp genes are widely expressed in various mouse tissues:

We evaluated the expression pattern of 79 *Mrp* genes (50 *Mrpl* and 29 *Mrps*) in oocytes, pre-implantation (zygotes and blastocysts) and gastrulation stage (E6.5-E8.5) embryos as well as several adult tissues (kidney, liver, heart, brain and testes). We used intron-spanning RT-PCR primers intentionally designed to amplify all known isoforms of each specific gene.

Forty-six of 50 *Mrpl* and 20 of 29 *Mrps* are ubiquitously expressed in all tissues tested (fig. 1). This includes *Mrpl1*, -*l3*, -*l4*, -*l9*, -*l10*, -*l11*, -*l13*, -*l14*, -*l15*, -*l16*, -*l17*, -*l18*, -*l19*, -*l21*, -*l22*, -*l23*, -*l24*, -*l28*, -*l30*, -*l32*, -*l33*, -*l34*, -*l35*, -*l36*, -*l37*, -*l38*, -*l39*, -*l40*, -*l41*, -*l42*, -*l43*, -*l44*, -*l45*, -*l46*, -*l47*, -*l48*, -*l49*, -*l50*, -*l51*, -*l52*, -*l53*, -*l54*, -*l55*, -*l56*, -*l57*, -*l58*, -*s5*, -*s6*, -*s9*, -*s10*, -*s11*, -*s14*, -*s15*, -*s16*, -*s17*, -*s18a*, -*s18b*, -*s18c*, -*s21*, -*s22*, -*s23*, -*s25*, -*s31*, -*s33*, -*s35*, and -*s36*. Details of those that do show temporal or tissue specificity are listed here (fig. 1): *Mrpl2* is absent in E8.5 yolk sac; *Mrpl12* is absent in E7.5 extraembryonic tissue; *Mrpl20* is absent in oocytes, zygotes and blastocysts; *Mrpl27* is absent in oocytes; *Mrps2* is absent in E8.5 embryonic tissue; *Mrps7* is absent in E7.5 extraembryonic tissue; *Mrps12* is absent in zygotes; *Mrps24* is absent in oocytes and zygotes; *Mrps26* is absent in oocytes and zygotes; *Mrps27* is absent in zygotes and blastocysts; *Mrps28* is absent in oocytes and zygotes; *Mrps30* is absent in zygotes; and *Mrps34* is absent in oocytes and zygotes. The only notable pattern across all of the *Mrp* genes is that there is varied expression during the pre-implantation stages, but nearly all *Mrp* are robustly expressed by E7.5.

Gastrulation is the first major differentiation and specification of the primary germ layers, a dynamic process involving drastic cell movement which requires energy. Given the role of MRPs in mitochondrial translation system, it is perhaps not surprising that they are all robustly expressed at this time. Three non-mutually exclusive explanations for the varied expression during preimplantation are plausible. The first is that preimplantation development is a series of reduction-divisions resulting in increasingly smaller blastomere size. Additionally, no active movement of cells has been documented in the embryo prior to implantation, suggesting a relatively low cellular energy requirement. The second contributing factor could be the large pool of functional maternal mitoribosomes present in the embryos, mitigating the need for new mitoribosome synthesis. A third possibility is that there may be distinct mitoribosome complexes required at different stages of development,

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allowing tissue specific expression. If this were true then one would predict tissue and/or temporal specific phenotypes for some *Mrp* knock-out alleles. However, this appears not to be the case for many *Mrp* genes.

It is important to note that there are a few discrepancies between our data and older microarray studies. Different from our RT-PCR panel, Zeng et al found that all *Mrp* are present during all cleavage stage and blastocyst stage embryos (18). We suspect that the microarray probes recognize distinct *Mrp* splice variants (whereas our primers amplified all variants), which may be of interest to investigate in the future.

Mrp genes are each essential for early embryo development

Our group has previously performed in-depth characterization of many different *Mrp* knockout phenotypes (12). Our study showed that the absence of each single *Mrp* gene results in pre-gastrulation arrest. We tabulated these data together with the embryonic phenotyping data collected by the International Mouse Phenotyping Consortium (IMPC) and other studies (19, 20). 22 of the 84 *Mrp* genes have been knocked out in the mouse, and all but 1 are lethal (95%). Null mouse pups were not recovered at weaning age in 5 of the strains and are identified as preweaning lethal: *Mrpl23*, *-l58*, *-s21*, *-s37* and *-s39*, and the analysis of early stages was not performed leading us to suspect that they are also early lethal phenotypes. 17 of the 22 are identified as embryonic lethal: *Mrpl3*, *-l12*, *-l18*, *-l22*, *-l33*, *-l44*, *-l47*, *-l51*, *-l59*, *-s5*, *-s12*, *-s18c*, *-s22*, *-s25*, *-s37*, *-s38*, and *-s39* (fig. 2). Among the embryonic lethal strains, *Mrpl3*, *-l22*, *-l44*, *-l59*, *-s18c*, *-s22*, and *-s25* null embryos were developmentally arrested at the pre-gastrulation stage (E6.5); *Mrpl12*, *-l47*, *-l51*, *-s5*, *-s12*, and *-s38* null embryos are likely to be arrested at the pre-gastrulation stage as these null embryos were not found at E9.5; and *Mrpl18*, *-l33* and *-s29* null embryos are not found at E12.5. Given the involvement of mitochondria in cell movement and proliferation and the large percentage of early embryonic lethal *Mrp* strains, we speculate that the great majority of *Mrp* knockouts will likewise undergo early developmental arrest. It is important to point out that we observe no correlation between temporal expression patterns of specific genes and their null phenotype. In other words, the phenotype/stage of lethality cannot be predicted or informed by a specific gene's expression.

Mrpl56 is the only knockout with homozygous viable mice (21). This gene encodes for the enzyme lactamase beta (LACTB) and *in vitro* study using mammalian cell line has shown that this enzyme has the ability to alter the mitochondrial lipid metabolism (22). Interestingly, when assessing possible protein-protein interaction in this MRP group based on the STRING database version 11.0 (23), MRPL56 was predicted to be the only member that does not show interaction with any other MRP members rising doubts regarding its involvement in the mitoribosome. This leads us to speculate that although MRPL56 is localized in the mitochondria, it may not be part of the mitoribosome complex. Supporting this idea, previous *in vitro* study utilizing mammalian cell line suggested MRPL56/ LACTB to be an endoribonuclease, having a similar role to MRPP3 and its family (24).

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MRPs share little/no amino acid similarity

To further explore the properties of MRP proteins, we compared the 79 amino acid sequences within this large group to identify possible conserved motifs. We performed T-Coffee multiple sequence alignment of all isoforms encoded by the *Mrp* genes and each pairwise alignment was scored with BLOSUM62 substitution matrices. The BLOSUM62 scores are alignment scores that indicate how closely related two amino acid sequences are to one another (considering alignment gaps, sequence lengths, and mutation rates). Thus, the greater the alignment score, the greater the sequence similarity between two MRP proteins. Interestingly, other than the 3 MRPS18 homologues (-S18A, -S18B and -S18C) (25), none of the other MRPs share any signature domains. Additionally, our analyses show that all alignment scores greater than 500 are pairwise alignments of MRP gene specific-isoforms and that all of the remaining pair-wise alignments result in very low scores (all <260, not shown), indicating little/no protein homology across all of the MRPs. Therefore we conclude that this group of genes is not a gene family, but rather a similarly named group of proteins which function in the mitoribosome. However, utilizing evolutionary trace analysis, it appears that each MRP does contain conserved amino acid residues across different eukaryotic lineages suggesting that the unique non-redundant role of each *Mrp* genes is not limited to particular species.

PROSITE analysis (26) indicates that only 15 of the 84 mammalian MRPs contain domains that are identified in other proteins. 9 of these MRPs share distinct signature domains with their orthologs in other species: MRPL3, -L13, -L24, -S2, -S5, -S9, -S11, -S12, and -S18 and the remaining 6 MRP members contain protein domains that are also found in proteins outside of the MRP group. MRPL3 contains an L3 signature domain that is also shared with its orthologs in other organisms. MRPL39 contains a TGS domain which is named after the threonyl-tRNA synthetase (ThrRS), GTPase, and guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase (SpoT) (27). MRPL42 contains a prokaryotic membrane lipoprotein lipid attachment site which is usually cleaved by specific lipoprotein peptidase in prokaryotes (28). Since this site overlaps with the transit peptide sequence in MRPL42, this domain may not have retained its function in mammalian mitoribosome. MRPL44 and MRPS5 contain dsRNA binding domains suggesting a potential interaction with the 16S/12S rRNA. MRPS18 contains the aminoacyl-tRNA binding domain inferring the importance of this protein in the A-site of the mitoribosome. MRPS27 and MRPS39 both contain the PPR domain which is commonly found in proteins that are involved in RNA processing (29). Lastly, MRPS37 contains a CHCH domain which signals the protein to be imported into the mitochondrial intermembrane space and these proteins are usually involved in mitochondrial biogenesis, maintenance, and translation (26, 30).

The central feature shared among this large group of proteins is their involvement in assembly and/or components of the mitoribosome complex. Based on the unique domains found in some MRPs, it is not surprising that each of these MRPs has a unique role in the mitoribosome complex that cannot be compensated by other MRPs. If MRPs have unique non-redundant functions, then it is not surprising that each gene is robustly expressed in all tissues and stages as we observe. Unique and essential roles within the mitoribosome would

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also explain the nearly identical knockout phenotype that we observe for many *Mrp* null embryos (12).

Conclusion

Clinical studies have shown that mitochondrial disorders such as cardiomyopathy, sensorineural deafness, diabetes, lactic acidosis, Leigh syndrome, and combined oxidative phosphorylation deficiency (COXPD) are all associated with mutations in *Mrp* genes (11, 31–40). Aside from mitochondrial diseases, *Mrp* genes have also been associated with carcinoma, breast and gastric cancers through their involvement in cell proliferation and apoptosis (41–45).

Although there are over 80 *Mrp* genes present in the mammalian genome, this group of genes shares little similarity and early lethality of *Mrp* null embryos suggests no functional redundancy. The ubiquitous expression of most *Mrp* genes further suggests the indispensable role of each *Mrp* member in diverse tissues and cell types. Throughout evolution, mitochondria continue to adjust to diverse energy demand and cell-physiological conditions. Understanding the relationship between these conserved and disease-associated genes and their specific roles in the mitochondrial translation machinery may benefit therapeutic approaches to mitochondrial diseases.

Materials and Methods

Mouse oocytes and embryo retrieval

All embryos and tissues were collected/dissected from C57BL/6NJ mice throughout this study. MII oocytes were retrieved from the infundibulum after superovulation with standard course of 5IU PMSG followed by 5IU HCG 48 hours later. To collect mouse embryos, wildtype male and female mice were housed together, and the morning of the copulation plug was defined as E0.5. Pre-implantation stage embryos were collected on E0.5 (for zygotic stage) and E3.5 (for blastocyst stage). Gastrulation stage embryos were collected on days E6.5, E7.5 and E8.5. Use of animals was approved by the University of Massachusetts IACUC protocol #2018–0003.

Reverse transcription polymerase chain reaction (RT-PCR) analysis

RNA was isolated from mouse tissues using either Qiagen RNeasy Plus Micro Kit (Qiagen 74034) or Roche High Pure RNA Isolation Kit (Roche 11828665001) and converted to cDNA using BioRad iScript cDNA Synthesis (BioRad 1708890). Subsequently, RT-PCR was performed for 38 cycles of 30s at 60°C, 72°C and 95°C with the primers listed in table 1 (see appendix). RT-PCR amplicon was electrophoresed in a 1.5% agarose containing 0.2 ug/ml ethidium bromide (Invitrogen 15585011). Gels were run at 120V in 1X TAE for 30min and subsequently visualized and photographed with Syngene inGeninus Bio Imaging system. Pooled C57BL/6NJ oocytes and embryonic tissues collected at the respective stages were used in this study and the RT-PCR analysis was repeated with a minimum of two times.

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Amino Acid Sequence comparison analysis

The amino acid sequences of 79 mitochondrial ribosomal proteins were obtained through the UniProt protein sequence database (46). A multiple sequence alignment of the Mrp protein sequences was performed using the Tree-based Consistency Objective Function for Alignment Evaluation program (T-Coffee) (47) and the maximum likelihood tree was visualized using Interactive Tree of Life (iTOL) (48).

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Appendix

Table 1.

Intron-spanning primers for RT-PCR analysis

Gene	Product size (bp)	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
Mrpl1	406	CTGCTGCTGCTGCTACAAAG	GCAGCTCCATTTTCTTCTGC
Mrpl2	420	GAGCAATGTCCTCCTTCAGC	GCCATCCTGCCTATGTGATT
Mrpl3	414	TGTTAGAAATCTCCACAGCAAGA	ATCTGTTTTGGTGGCAGTCC
Mrpl4	427	ATTTCAGAGCCGCGAGATAG	GCACTTTCATGGGCAACAT
Mrpl9	413	CGCGTGTATAAGTTGGTGGA	CGAGTGATAGGCTCCTCTGG
Mrpl10	424	ATGGCTCCAAGGCTGTGAC	TTTAAGACCCGCACCATTTC
Mrpl11	301	CAGTTCTGCAAAGAGTTCAACG	CAGAGCCAATGATGGAACG
Mrpl12	400	CAGATGCCCAGCGTCTGT	GGTTGATGCCCTGGACGTAG
Mrpl13	338	CACTTTTGCTCGAATGTGGT	GCCTTTGCATCATTGTCCTT
Mrpl14	400	CCATGTCAGCAGAGCATTCA	CACAAAGTTCTGAGCGATGG
Mrpl15	214	AGAAAGGCAGAGAGGAACCC	ACCTCTCCCATTCACAAGCT
Mrpl16	333	CCTTTCAAGCCCATCACTCG	CTGCGTCAAGTCATATGGGC
Mrpl17	414	CCATCTGCTGCGGAACTTG	CTTGCGTCCTGGTTATGGTG
Mrpl18	264	GAAACTGGAGTGTGGGTTCG	ACAACAACCTGGCCGTTAAG
Mrpl19	233	ACCCCTATGCCAGTGGAAAA	AAAGGTGCTGTACTCGGGAA
Mrpl20	218	CTCAGCATTTTCGGGGAAGG	TTTCCTGTTGAGCTCCACCT
Mrpl21	243	TCCTAAGACCTCCCTGAGCT	TTCTCCAGCCGAATCCTCTC
Mrpl22	215	TTTACCCACCTCAACTGCCT	CACTGCCATGTCTTGTGCTT
Mrpl23	217	GAAGATACCGTGCAGTTCCG	CTGGGAAAGTGAAGGTCTGC
Mrpl24	403	GCATCCAAAGCCACTTCCTC	CACGTTCTCCTGCCTCAGTA
Mrpl27	416	GGCGCTTACGCTGAGGAC	TGAAGGTTCCCTCAGGTTTG
Mrpl28	451	CCCCGGTACACTACAAACCT	TCATAGATGGCTGCTCTCCG
Mrpl30	406	AGGTGCAGAATCTCTCATTGG	CTGCTCCACAGGCTTCAGAT
Mrpl32	424	AGAGCTGGAGTCGCTTTGTC	ATCCTCTTGCCCTGATCCTT
Mrpl33	122	CTGCTCTCCGCTGTCTCTTT	CTCAGCTTCTCTCGGAGTCG
Mrpl34	231	GCCCTATTGAGTGGCAGGTA	TCAGTGGCTCAGGGACTTG

Gene	Product size (bp)	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
Mrpl35	400	CCCTTGAATGTCTTGGCATC	TGCTGCAGAACACAAATTCC
Mrpl36	218	TCTTCTCCTCGTTCCTGCTG	TTGTCGCTGTTTATGCTTGG
Mrpl37	400	TCTGGAGCCCATTACCTACG	CAGCTGTATGAGGCTGTCCA
Mrpl38	443	TACTGGAGCGGAAGCACTTT	CTCCTGTCCTTCAGCCACTC
Mrpl39	405	AGTTGTGCCATGCATCTGAG	TCTTGCATCAACGTCCAGAG
Mrpl40	420	ACACACCCACCAGAGAGCTT	AGGCTGGTATCCCGCTTTAT
Mrpl41	401	GTTTCCTGACTGCCGTGACT	GCGTGGGAAATTCTTAGGATA
Mrpl42	408	TGGGCGATATCAAACAGAACT	TCTGTCTCTGGGAGGATTCAG
Mrpl43	436	TGACCAGCGTCCTCCATAAT	ATCGAAGCTGGAGCAGAATC
Mrpl44	463	GGGGAGTGAAGAAGGGATTC	TCTGCACTGAGGGTCAACTG
Mrpl45	428	TTGGAGTATGCCAGGAAAGC	CAACGGACATGAACCACTTG
Mrpl46	425	CTCGGGAAACAGAAGCTGAC	GAGAAATCGCCTGACCTGAG
Mrpl47	447	ACCACGTTGTCAAGGAAAGG	CACATAAGGCATTGCGAAGA
Mrpl48	412	AGCAAGGCTTTTCCCTCCTA	ACTCGCTCATGGGTGGTAAG
Mrpl49	414	GACGGAAGCTGAGCCAGA	GCCAGGCTTTAAGTTGCTCA
Mrpl50	353	TGATCGTGTCTCGGTTAGCA	CATTTGGTGAAGCCTGGAGT
Mrpl51	302	CGGCAAGTAGGAGACTGTGG	ATGCAGGTCCTCAATGAACA
Mrpl52	329	GTGTCCGGAGATTGCACTG	CGGAAGTGGGCTTCTCAGTA
Mrpl53	240	GGTCAAGCTGGTTCGAGTTC	GAGGCCAAGGCACTTAACAT
Mrpl54	311	TCCTCAGAAGACCTGGAGGAT	GTGGCGCCAGATATTCTGTT
Mrpl55	333	CCTTTGGCCATTCTGCTTAG	TTCTTCCCTCTTCTGCTGGA
Mrpl56	313	TCCGGCACTACCGAGTTATC	CTGCTTCCCACAGTTTAGCC
Mrpl57	202	TCTTTCCAAGCGAAGGAGAG	GATGGTCTAGCTGGTCTGCAA
Mrpl58	409	GTTGACCTGAGCACAGCAAA	TCAGTTGGCTTCTCCTGCTT
Mrps2	489	GCCATTACAGCATTCGGATT	TTGCAGTTGGTGTCCACAAT
Mrps5	440	TCCCTGCATTGTGTAGCTTG	CTGGACGACCCCACTTTTTA
Mrps6	217	CGCTACGAGTTGGCTTTGAT	CAGCACTTGTCGGAGCATAA
Mrps7	400	AACCTTCCAGGGCTCACAC	ACCCAATCACAGGCTCACA
Mrps9	480	AAGACCCGGAAACTTTTGCT	CGGGTTCAATCAGTTGCTTT
Mrps10	419	CGCTCAGTGCCAGTATGAAG	CTGCGTCTTTGTCACTTCCA
Mrps11	413	TTAAGAAACTCCGGGTCGTG	ACTCCCTTTCCTGTGGCTTT
Mrps12	382	CCTTCTTTATGGCCTCACCA	GCAGTCATACTTGCCCCTCA
Mrps14	310	AGTTCCTCCATCAGCGTCAG	TAGCCCGTGGTCAGCTAAGT
Mrps15	437	GTAGCGCCAGTCTGCTCTCT	GGTCTGACGGAGGATTTTGA
Mrps16	387	GGTCCAGCTCACAACCATTT	TTGGCCTCTGACTCTGCTTT
Mrps17	324	ATAGTCCGTTCATCCGTCCA	GAGGGGGCTCTCTAGGTAGG
Mrps18a	444	CTGGTTATGGCTTCCAGCTC	ATACCCACCTTGTTCCAACG
Mrps18b	516	ATATGAGAGCGAGCCTTGGA	CCTTGATAGAGTCGGCGAAG
Mrps18c	418	GGTTGCTCTGTGCAGTGGTA	TTCTCGGTATCTGATGTTACAAACTC

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Gene	Product size (bp)	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
Mrps21	250	ATGGCTAAACATCTGAAGTTCATT	ACGGGTCTGCACGGTTCT
Mrps22	533	TACAACCACTGAAGCCACCA	AGCCATTCCACCAAAGTGTC
Mrps23	406	CCTTTCCACCCCTGAGAGAG	GTTTCTGGCTCCTGCTTCAC
Mrps24	566	CGGTCTGCGCCAAGAATC	TGTACTTGTACACGACCTTGGA
Mrps25	455	GGGACGTGGTATTCAAGGAG	ACTGGCTTTCAGAGCAGCTT
Mrps26	420	CGGAATTCTTCGTGTTGACC	CGGCCCAGTTGTAACTCTTC
Mrps27	444	TCACGGTTTGTGGACAACAT	GCCCACTGTGTTCCTTTGTT
Mrps28	510	AGAGCGCTTGTCTTCTCCAA	TTACTTGCTGGGTTGCTCCT
Mrps30	756	ACGGATTCTCACCAAGATGC	GCTCCAGTCCAAGCAAAAAG
Mrps31	407	GTTGACCTGAGCACAGCAAA	TCAGTTGGCTTCTCCTGCTT
Mrps33	301	GTCTCCGCTTTCGGAGTATG	TCTCTTCCCTTCTCCTTTCCTC
Mrps34	578	AAAGTAAGACCCCGGCTGAT	CGAGTCCTCTCCAGGTTCAG
Mrps35	434	TCTGCATTTGACTCCTGTGG	TCTTTGCCCACACATACTCG
Mrps36	263	GTGCAGGTAGTGAAGCCACA	CCACGCTGGATAAATTCCAT

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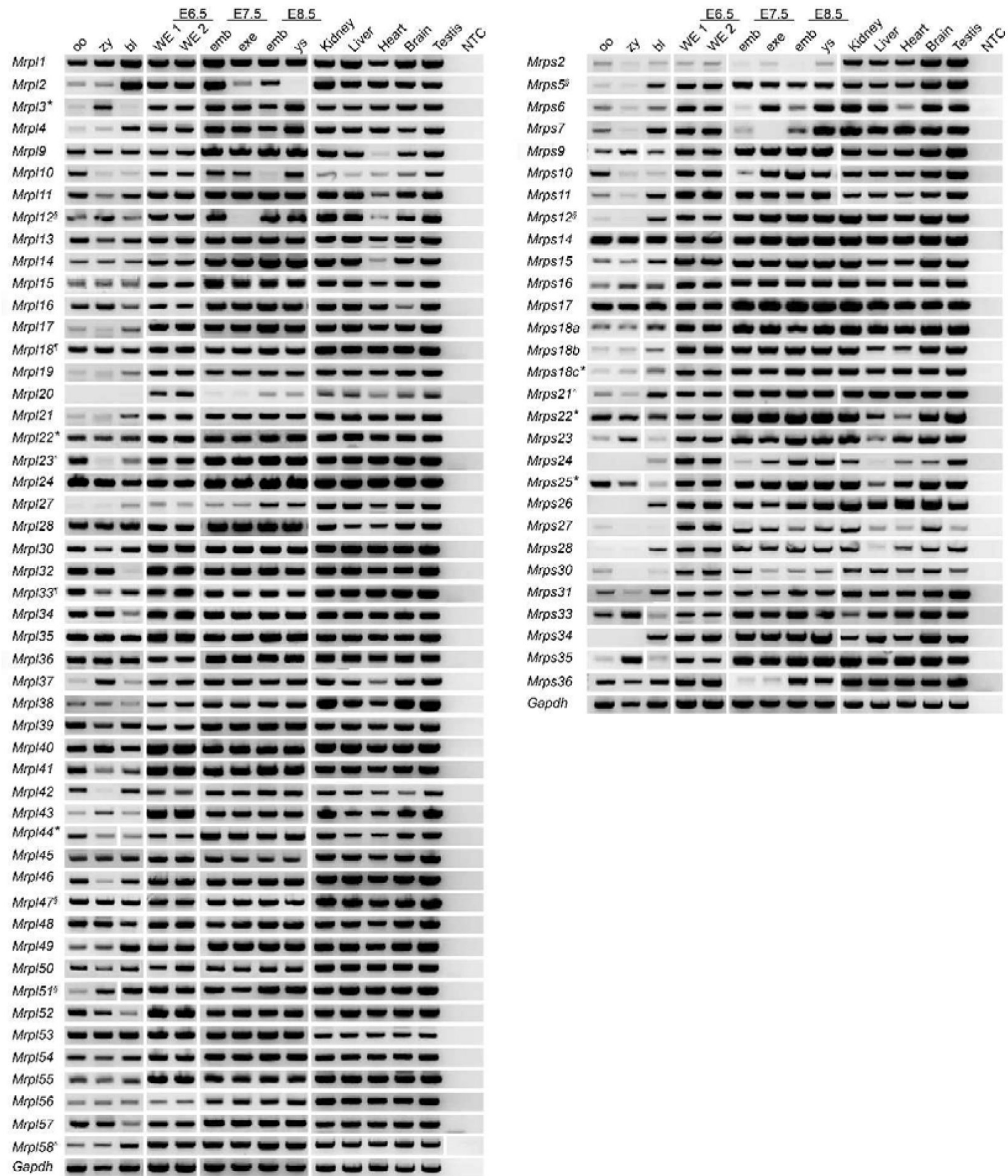

Figure 1.

79 Mrp genes are widely expressed throughout early embryo development and in adult mouse tissues. (A-B) RT-PCR gene expression patterns of the 50 *Mrpl* (A) and 29 *Mrps* (B) in MII oocytes, E0.5 zygotes, blastocysts and gastrulation stage embryos (E6.5-E8.5), as well as kidney, liver, heart, brain and testes. *Gapdh* was used as control. “oo” denotes oocytes, “zy” denotes zygotes, “bl” denotes blastocysts, “WE” denotes whole embryos, “emb” denotes embryonic portion, “exe” denotes extraembryonic portion, and “ys” denotes yolk sac. “NTC” denotes no template control. “*” denotes null embryos that are arrested at the pre-gastrulation stage (E6.5); “§” denotes null embryos that are likely to result null embryos that are arrested at E6.5 (they cannot be found at E9.5); “¶” denotes null embryos

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are not found at E12.5; and “^” denotes mouse knockout strains that failed to produce viable null mouse pups at weaning age but were not examined earlier.

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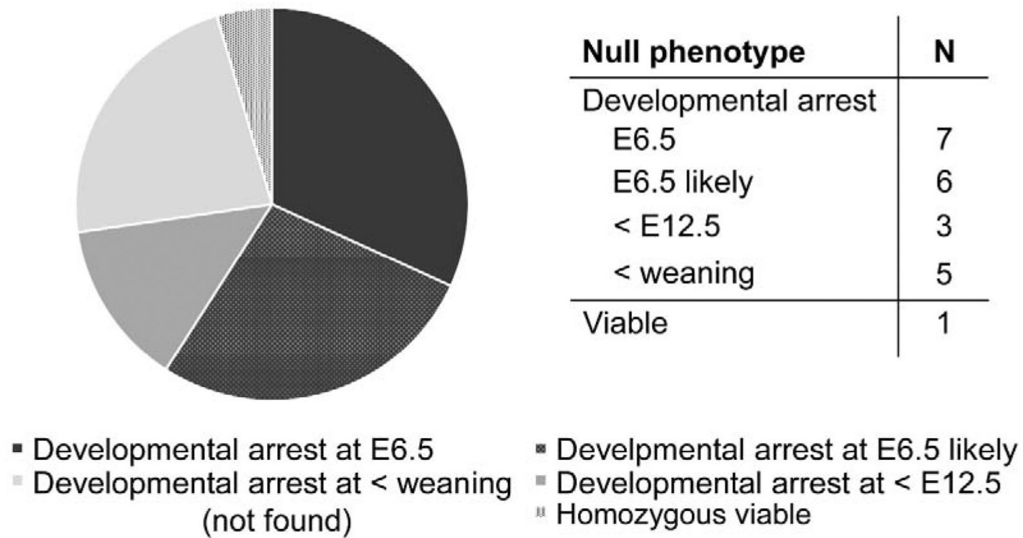

Figure 2.

95% lethality in Mrp knockouts. 22 *Mrp* genes have been deleted thus far in the mouse. 12 *Mrpl* (*Mrpl3*, *-l12*, *-l18*, *-l22*, *-l23*, *-l33*, *-l44*, *-l47*, *-l51*, *-l56*, *-l58*, and *-l59*) and 10 *Mrps* (*Mrps5*, *-s12*, *-s18c*, *-s21*, *-s22*, *-s25*, *-s29*, *-s37*, *-s38*, and *-s39*). Among these *Mrp* alleles, seven (*Mrpl3*, *-l22*, *-l44*, *-l59*, *-s18c*, *-s22*, and *-s25*) result in null embryos that are arrested at the pre-gastrulation stage (E6.5); six more (*Mrpl12*, *-l47*, *-l51*, *-s5*, *-s12*, and *-s38*) are likely to result null embryos that are arrested at E6.5 (they cannot be found at E9.5); *Mrpl18*, *-l33* and *-s29* null embryos are not found at E12.5; and five strains (*Mrpl23*, *-l58*, *-s21*, *-s37*, and *-s39*) failed to produce viable null mouse pups at weaning age but were not examined earlier. *Mrpl56* is the only strain that generated viable homozygous knockout animals. Data extracted from IMPC and other studies.