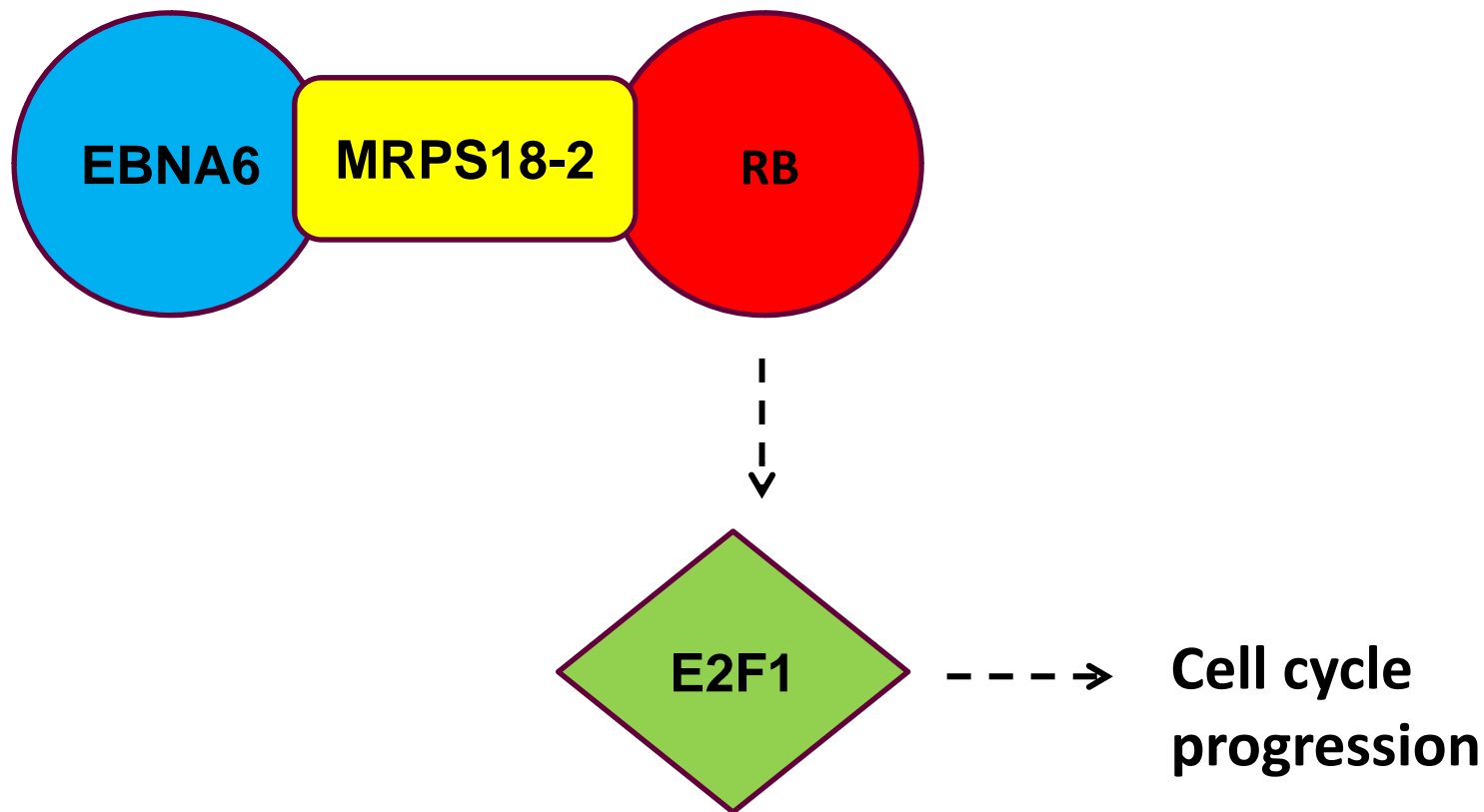


Evolutionary history of mitochondrial ribosomal proteins of S18 family and Gly132 polymorphism in colon cancer

Muhammad Mushtaq

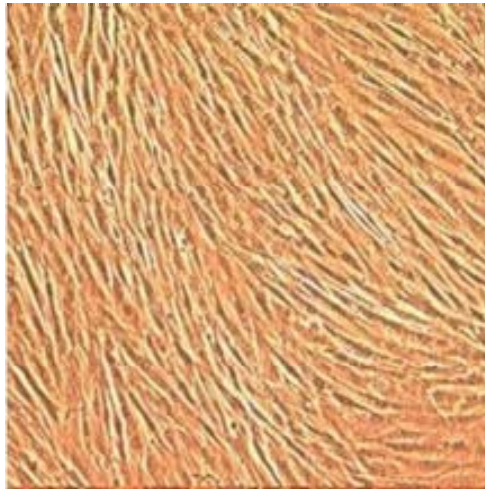
Department of Microbiology, Tumor and Cell Biology (MTC)

MRPS18-2 act as bridge between EBV encoded EBNA 6 and RB



Overexpression of MRPS18-2 protein resulted in REF immortalization

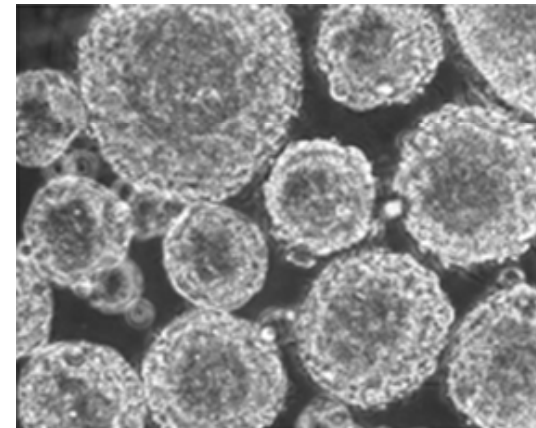
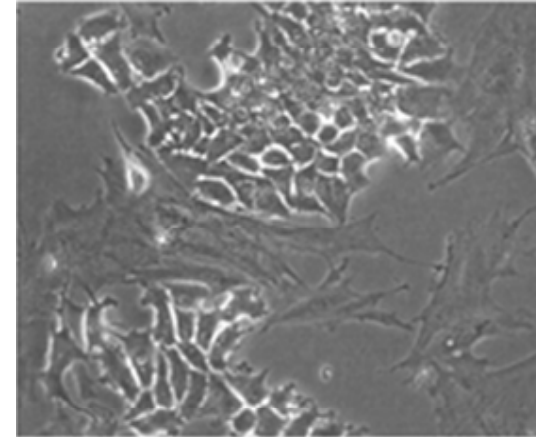
Rat embryonic
fibroblasts



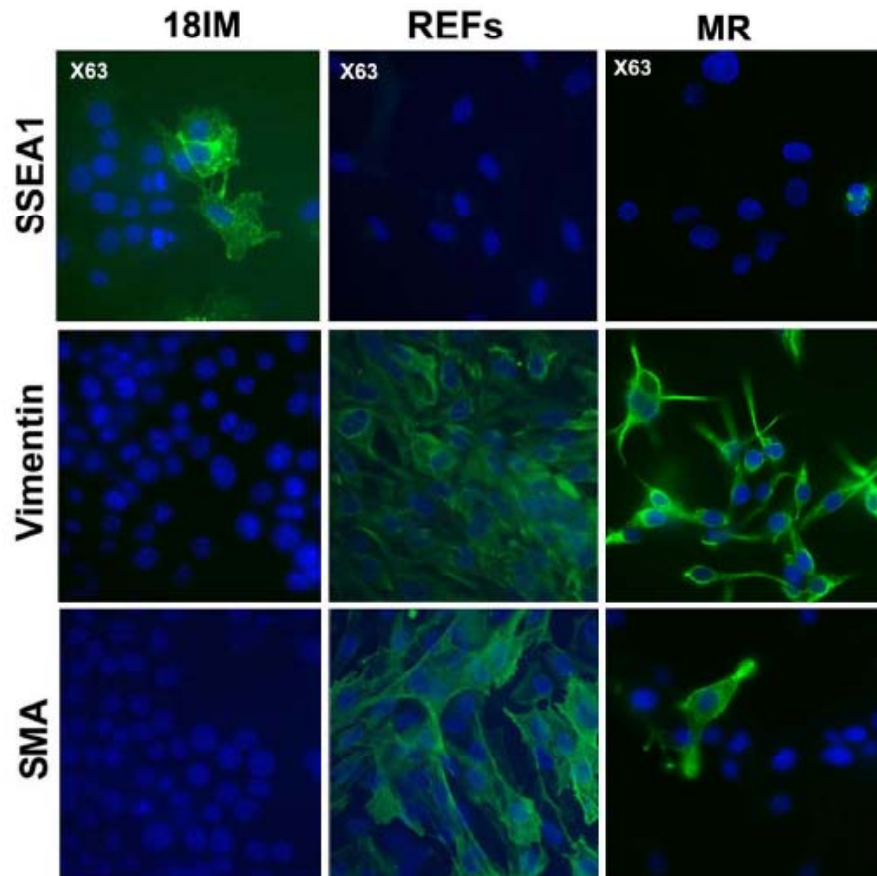
MRPS18-2



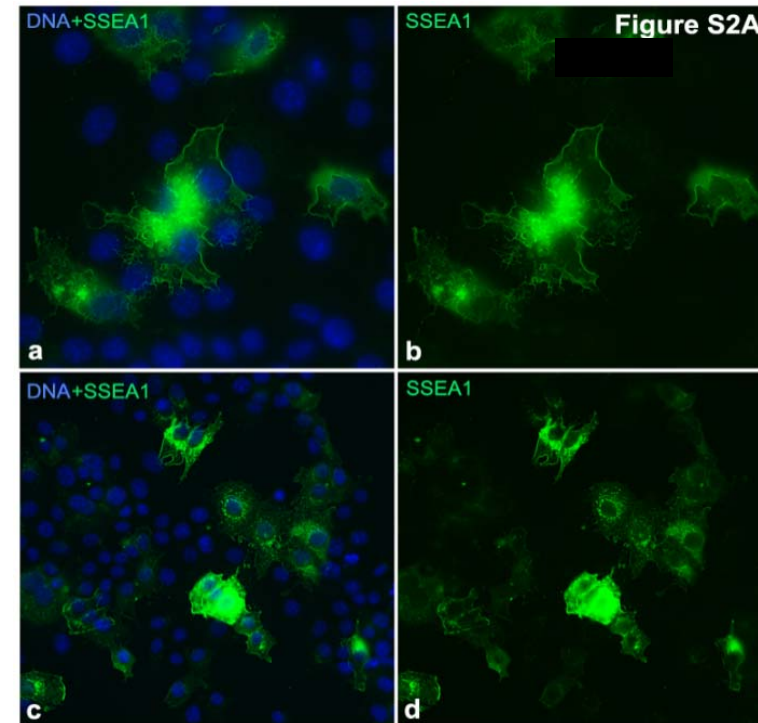
Immortalized cells (18IM)



Immortalized 18IM cells are de-differentiated



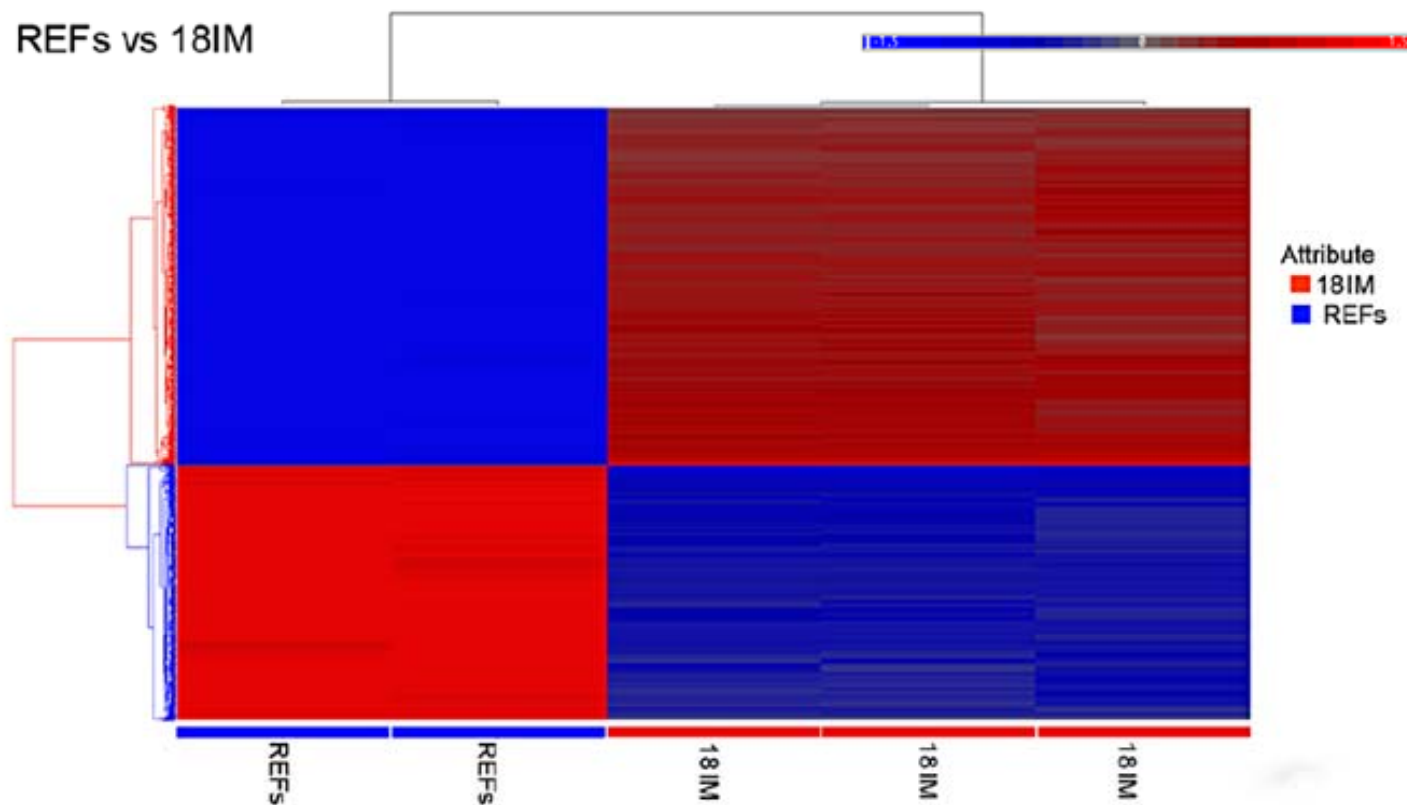
Growth for 2 years



Still 30% of cells express
SSEA-1

Microarray study confirmed stem cell phenotype of 18IM

Comparison of primary REFs and immortalized 18IM cells showed that 4209 genes and 19 pathways were changed



CLUSTAL W (1.82) multiple sequence alignment



```

S18_3 -----MAAVVAVCGGLGRKKLT 17
S18_1 -----MAALKALVSGCGRLLRGLLAGPAAT 25
S18_2 MAASVLNTVLRRLPMLSLFRGSHRVQVPLQTLCTKAPSEEDSLSSVPISPYKDEPWKYLE 60











S18_3 HLVTAAVSLTHPGTHTVLWRRGCSQQVSSNEDLPISMENPYKEPLKKCILCGKHVDYKNV 77
S18_1 SWSRLPARGFREVVETQEGKTTIEGRITATPKESPNNPSGQCPICRWNLKHKYNYYDD 85
S18_2 SEEYQERYGSRPVWADYRRNHKGGVPPQRTRKTCIRRNVVGNPCPICRDHKLHVDFRNV 120
          : . * * :













S18_3 QLLSQFVSPFTGCIYGRHITGLCGKKQKEITKAIKRAQIMGFMPVITYKDPAYLKDPKVCN 137
S18_1 VLLLSQFIRPHGGMLPRKITGLCQEEHRKIEECVKMAHRAGLLPNHRPRLPEGVVPKSKP 145
S18_2 KLLEQFVCAHTGIIFYAPYTGVCVKQHKRLTQAIQKARDHGLLIYHIPQVEPRDLDFSTS 180
          ** . . * : **:* ::::: :.: : * : *.:

S18_3 IRYRE----- 142
S18_1 QLNRYLTRWAPGSVKPIYKKGPRWNRVRMPVGSPLLRDNVCYSRTPWKLYH----- 196
S18_2 HGAVSATPPAPTLVSGDPWYPWYNWKQPPERELSRLRRLYQGHLEESGPPPESEMPKMPP 240

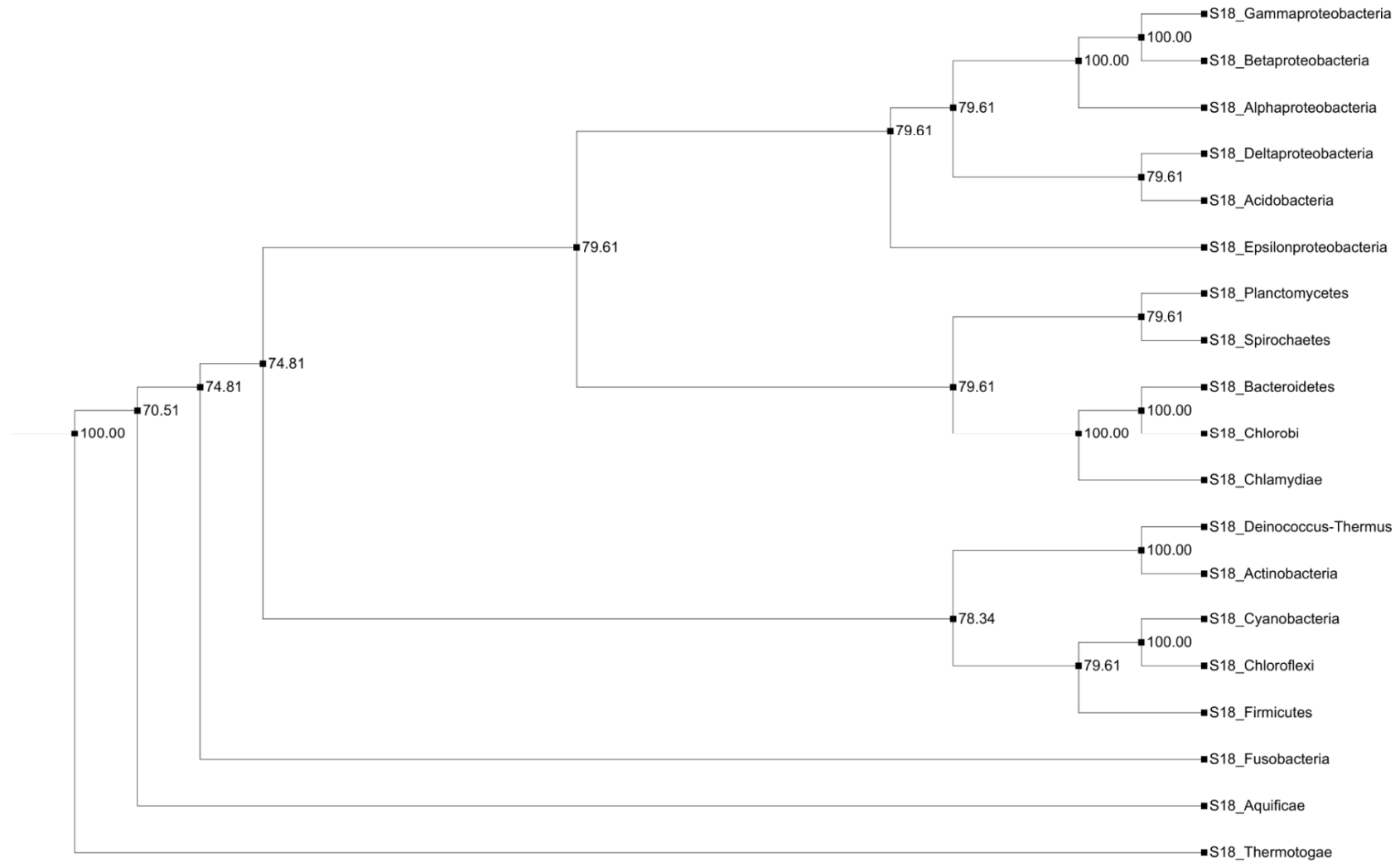
S18_3 -----
S18_1 -----
S18_2 RTPAEASSTGQTGPQSAL 258
    
```

Domain architecture of S18 proteins

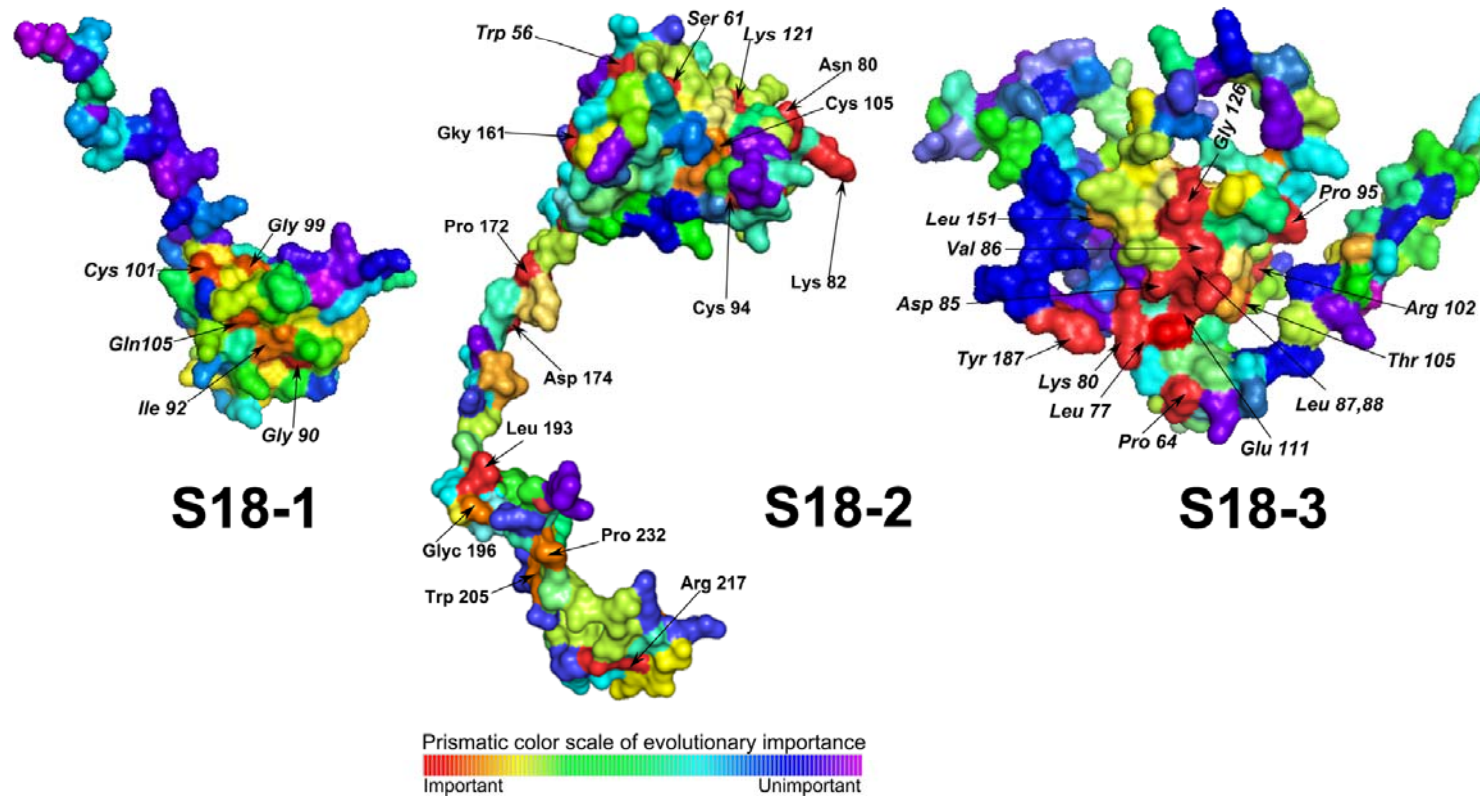
General Name	Domain architecture	Taxonomy span (nr seq)
30S Ribosomal protein S18		Cellular organisms (7582)
28S Ribosomal protein S18a, mitochondrial		Catarrhines (8)
protein S18 multi-domain protein		Bacteria (8)
Translation initiation factor IF-2		Bacteroidetes (8)
Actin cortical patch component		Eurotiomycetes (7)
transglycosylase		Bacteria (6)
Hypothetical protein VIC_003911		Vibrio (6)
ATPase		Cellular organisms (4)
Phosphogluconate dehydratase		Proteobacteria (3)
Hypothetical protein PAXINDRAFT_141109		Paxillaceae (3)

 Ribosomal_S18	 SPOR (Sporulation related)	 GAT_1 (Type 1 glutamine amidotransferase-like)
 IF2_N	 bS6 (bacterial ribosomal S6)	 Translation_Factor_II_like
 lysozyme_like	 F1-ATPase_like	 IF-2 (Translation-initiation factor 2)
 WD40	 PKc_like (Protein Kinases, catalytic)	 P-loop_NTPase (P-loop containing Nucleoside Triphosphate Hydrolases)

Consensus tree of bacterial S18 protein

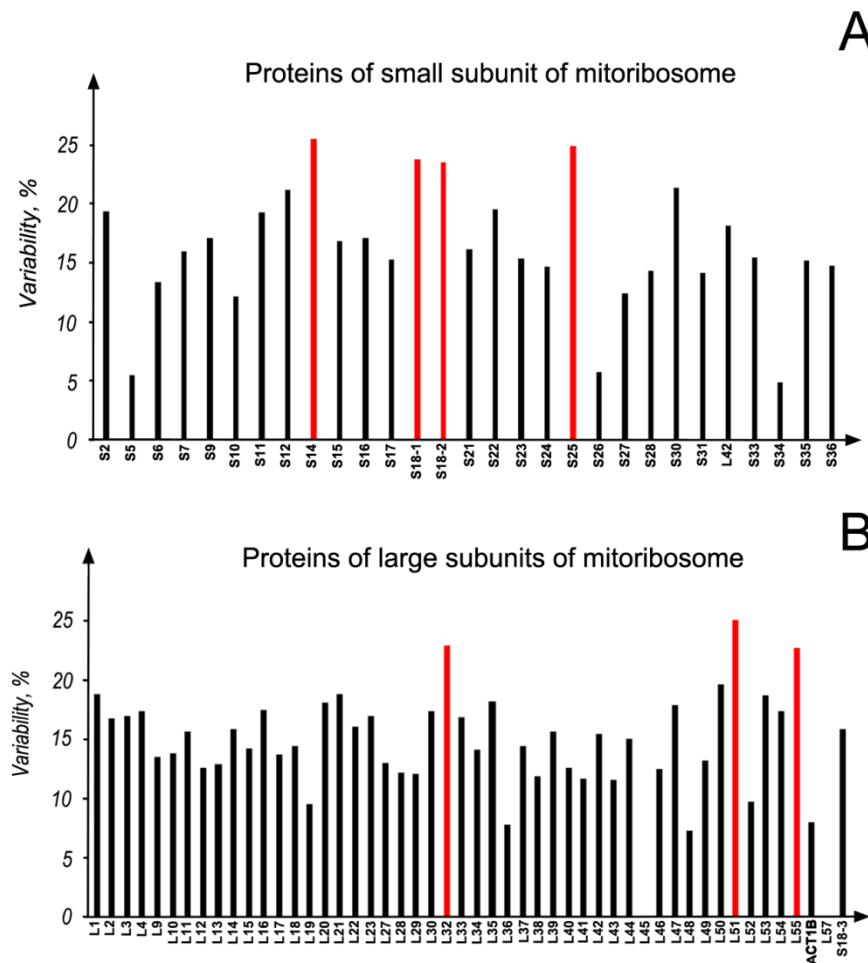


Evolutionary Trace Analysis (ETA)



Mutational analysis of all the mitoribosomal whole proteome in different cancers

Data derived from COSMIC database



Direct DNA sequencing of PCR products



Tumor DNA-Mutant Primer ↓

```

Query 1      TTTGTCTGCGCCACACG↓GTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC 60
|
|
|
Sbjct 23095  TTTGTCTGCGCCACACGGGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC 23154
|
Query 61     CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTTGTTTATG 105
|
|
Sbjct 23155  CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTTGTTTATG 23199
    
```

Tumor DNA-Wild Primer ↓

```

Query 1      TTTGTCTGCGCCACACGGGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC 60
|
|
|
Sbjct 23095  TTTGTCTGCGCCACACGGGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC 23154
|
Query 61     CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTTGTTTATG 105
|
|
Sbjct 23155  CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTTGTTTATG 23199
    
```

Normal DNA-Mutant Primer ↓

```

Query 3      TTTGTCTGCGCCACACG↓GTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC 62
|
|
|
Sbjct 23095  TTTGTCTGCGCCACACGGGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC 23154
|
Query 63     CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTT 100
|
|
Sbjct 23155  CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTT 23192
    
```

Normal DNA-Wild Primer ↓

```

Query 8      TTTGTCTGCGCCACACGGGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC 67
|
|
|
Sbjct 23095  TTTGTCTGCGCCACACGGGTATCATCTTCTATGCTCCATACACAGGTTAGCCCATCATC 23154
|
Query 68     CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTTGTTT 109
|
|
Sbjct 23155  CCTGCACCACCAGAGAGCTTTTCCTTGTGGCATGCCTTGTTT 23196
    
```

Conclusions

- S18 family of proteins are evolutionarily important proteins and the current study helps to know the evolutionarily history and pattern of duplications of S18 proteins during evolution.
- The Gly132 polymorphism might be an important biomarker for colon adenocarcinoma; therefore, it must be further investigated.
- The amino acids identified in ETA should be further investigated to identify the physiological role of these proteins which is majorily unknown.

Acknowledgements

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- George Klein

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