Structure-function analysis of the RNA helicase MLE

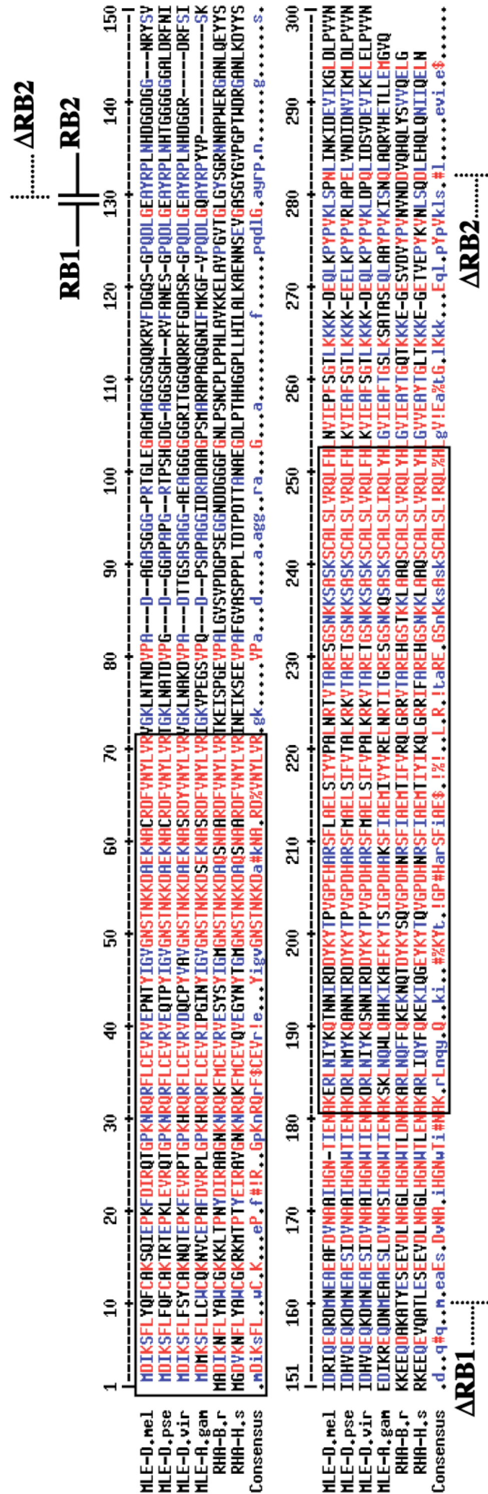
Supplemental Material

Annalisa Izzo[1], Catherine Regnard, Violette Morales[2], Elisabeth Kremmer[3] and Peter B. Becker*

Adolf-Butenandt-Institut, Molekularbiologie
80336 München, Germany

1. Supplemental Figures

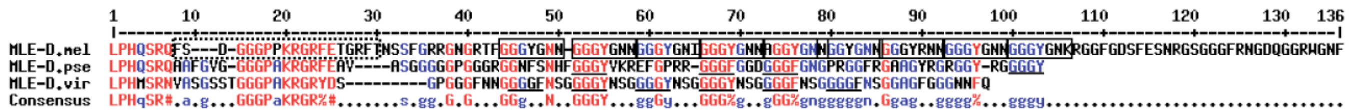
Supplemental Figure 1



Supplemental Figure 1.

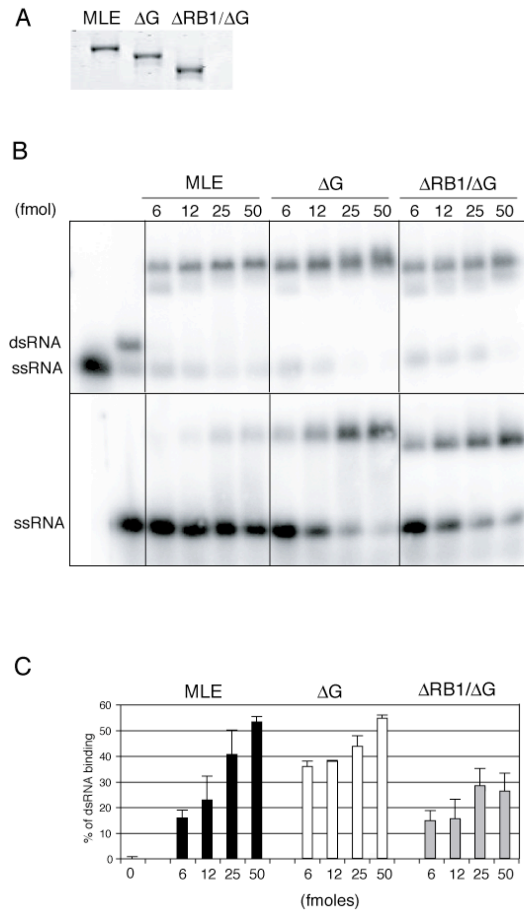
Multiple sequence alignment with hierarchical clustering (Corpet et al., NAR 16, 10881,1988) of the first 300 amino acids of MLE C-termini of MLE from *Drosophila melanogaster* (mel), *D. pseudoobscura* (pse) and *D. virilis* (vir) as well as human (H.s) and Zebrafish (B.r.) RHA. Conserved RB1 and RB2 domains are boxed. The extent of the expressed domains (RB1, RB2) and of the deletions (ΔRB1, ΔRB2) are indicated. The amino acids are displayed in their single letter code. The consensus sequence is annotated as follows: capital letters: full conservation (red colour); %: F or Y; #: N;D;Q;E. ! = I or V.

Supplemental Figure 2



Supplemental Figure 2. Multiple sequence alignment with hierarchical clustering (Corpet et al., NAR 16, 10881-10890,1988) of the glycine-rich C-termini of MLE from *Drosophila melanogaster* (mel), *D. pseudoobscura* (pse) and *D. virilis* (vir). The amino acids are displayed in their single letter code. The GGGYGNN heptad repeat (boxed in the melanogaster sequence) is not conserved in the other *Drosophilids*. However, a GGGY motif is present in all of them (underlined). The consensus sequence is annotated as follows: capital letters: full conservation (red colour); %: F or Y; #: N;D;Q;E. A two out of three conservation is indicated in blue. The dotted box marks the presumed NLS of *D. melanogaster* MLE. For details, see main text.

Supplemental Figure 3



Supplemental Figure 3. Contributions of the RB1 and G domains to MLE RNA binding. A. Coomassie stained gel of MLE and derivatives. B) EMSA and C) Filter binding assay.

2. Supplemental Materials and Methods

Plasmid construction. Details can be found in the Supplemental Material online.

pHis$_6$MLE: a 3897 bp DNA fragment, containing the MLE sequence (aa: 1 to 1289) was inserted into the pFastBacHTB Sf9 expression vector (Invitrogen) after digestion with NcoI/SacI.

pHis$_6$RB1: a 3547 bp DNA fragment was removed by StuI/NotI digestion from pHis$_6$MLE and the remaining linear plasmid (MLE aa: 1 to 121) was relegated after blunt end reaction.

pHis$_6$RB2: a 614 bp DNA fragment (MLE aa: 122 to 340) was cut out from pHis$_6$MLE by digestion with StuI/SphI and inserted into the pFastBacHTC Sf9 expression vector (Invitrogen) digested with the same enzymes.

pHis$_6$RB1-RB2: a 2931 bp DNA fragment was removed by SphI digestion from pHis$_6$MLE and the remaining linear plasmid (MLE aa: 1 to 340) was relegated after blunt end reaction.

pMLE-flag: the MLE sequence (aa: 1 to 1289) was PCR amplified from the *MLE* cDNA using the primers MLE-BamHI.rv (5´-CGCGGGATCCATGGATATAAAATC-3´) and MLE-SacI.fw (5´-TATGCAGAGCTCTTTCGATTTCGAACCC-3´), containing BamHI and SacI sites, respectively. The product was digested BamHI/SacI and inserted into the pPACK-BACK-flag expression vector (Invitrogen). The correct sequence was confirmed by sequencing.

pFastBac-MLE-flag: The BamHI/XbaI DNA fragment containing the *MLE-flag* sequence was removed from pPACK-BACK-MLE-flag and inserted into the pFastBac1 Sf9 cells expression vector (Invitrogen).

pFastBac-MLE^{GET}-flag: the EcoRI/StuI DNA fragment in pMLE-flag was replaced with the corresponding fragment containing the GKT-GET mutation from the pHis-MLE^{GET} (a gift from C.G. Lee, {Lee, 1997 #1355}).

pFastBac-MLE$^{\Delta RB1}$-flag: pFastBac1-MLE-flag was digested with BamHI and StuI, followed by blunt end reaction and religation.

pFastBac-MLE$^{\Delta RB2}$-flag: a 369 bp BamHI/StuI fragment containing the first dsRNA binding domain (dsRB1) of MLE was isolated from pFastBac-MLE-flag and cloned into pFastBac1, cut with the same enzymes, to obtain pFastBac-MLE^{RB1}. In a second step, pFastBac-MLE-flag was digested with HindIII, followed by blunt-ending with Klenow

Polymerase and XbaI digestion. The resulting HindIII/XbaI 3210 bp fragment was inserted into pFastBac-MLE^{RB1} digested with StuI and XbaI.

pFastBac-MLE$^{\Delta Gbox}$-flag was obtained in a multistep procedure: a BamHI/EcoRI fragment was isolated from pFastBac-MLE-flag and inserted into the pFastBac1 vector to obtain pFastBac-MLE$^{\Delta Gbox}$. Subsequently, the pFastBac-MLE$^{\Delta Gbox}$ vector was digested BamHI/ SacI and the corresponding 3543 bp fragment was inserted into pFastBac-MLE-flag, lacking the BamHI/SacI fragment, to obtain pFastBac-MLE$^{\Delta G}$-flag.

pFastBacMLE$^{G\text{-}box}$-flag: a 400 bp DNA fragment (MLE aa: 1163 to 1289) was PCR amplified from the p*MLE-flag* using the primers G-box-BamHI.rv and MLE-SacI.fw (5´-TATGCAGAGCTCTTTCGATTTCGAACCC-3´), containing BamHI and SacI sites, respectively. The product was digested BamHI/SacI and inserted into the pMLE-flag lacking the BamHI/SacI fragment. The correct sequence was confirmed by sequencing.

pFastBacMLE$^{\Delta RB1 \Delta G}$-flag: pFastBacMLE$^{\Delta G}$ was digested with BamHI and StuI and religated.

pMLE-GFP: the *MLE* sequence (aa: 1 to 1289) was amplified by PCR using the primers MLE-XmnI forward (5´-CGCGAACCCCTTCACCCCCATGGATATAAAA-3´) and the MLE-AgeI reverse (5´-TATAACCGGTCGGATCCAAGCTAATCG-3´). The PCR product was cloned into pEGFP-1 plasmid (Clontech) digested with XmnI and AgeI.

pMLE^{GET}-GFP: the 3870 bp BstEII/PstI DNA fragment in MLE-GFP was replaced with the corresponding fragment containing the K413E mutation, from pFastBac-MLE^{GET}-flag.

pMLE$^{\Delta RB1}$-GFP: the 3504 bp StuI/AgeI DNA fragment from pMLE-GFP was inserted into pEGFP-1 plasmid digested with XmnI/AgeI.

pMLE$^{\Delta RB2}$-GFP: a HindIII/BglII DNA fragment, lacking both RB1 and RB2 sequences, was cut out from the pMLE-GFP plasmid and inserted into the pMLE-GFP plasmid digested with StuI/BglII and containing only the RB1 sequence.

pMLE$^{\Delta G}$-GFP: plasmid pMLE-GFP was digested with ScaI/AgeI, followed by blunt ending and religation.

pMLE$^{\Delta G}$-GFP-NLS was obtained by inserting an artificial NLS behind the GFP tag of the pMLE$^{\Delta G}$-GFP. The fragment containing the NLS sequence from the SV40 large T-antigen {Lanford, 1986 #349}, was obtained by annealing the two complementary oligonucleotides NLS-NotI.fw (5´-GTACAAGGATCCAAAAAGAAGAGAAAGGTATAAGC-3´) and NLS-BsRGI.rv (5´-GGCCGCTTATACCTTTCTCTTCTTTTTGGATCCTT-3´), containing NotI and BsRGI sites, respectively.

pMLE$^{G\text{-}box}$*-GFP*: the MLE fragment containing the G-box, was PCR amplified with primers MLE-XmnI-G-box.fw (5´-CGGAATACCTTCCCATGGTGCCGCACCAAT-3´) and MLE-AgeI.rv primers using pMLE-GFP as a template. The PCR product was digested XmnI/AgeI and inserted into the pEGFP.