

2 MATERIAL

2.1 Cell lines

<u>BL60-2</u> :	human Burkitt's lymphoma cell line
<u>HeLa</u> :	human cervix carcinoma cell line
<u>H9</u> :	human T cell lymphoma line
<u>MEF</u> :	murine embryonal fibroblasts

2.2 Bacterial strains

<i>E. coli XLI-blue</i>	Stratagene, La Jolla, CA, USA
<i>E. coli BL21(DE3)pLysE</i>	Invitrogen, Karlsruhe, Germany

2.3 Chemicals and reagents

Acridine orange	Sigma-Aldrich, St. Louis, MO, USA
Acrylamide	Roth, Karlsruhe, Germany
Agarose	BMA, Rockland, ME, USA
Ammoniumpersulfate	Sigma-Aldrich, St. Louis, MO, USA
Amplify solution	Amersham, Buckinghamshire, UK
Aprotinin	Sigma-Aldrich, St. Louis, MO, USA
β-thioglycerol	Sigma-Aldrich, St. Louis, MO, USA
ATP	Sigma-Aldrich, St. Louis, MO, USA
Bacto-agar	Difco/BD, Sparks, MD, USA
Bacto-tryptone	Difco/BD, Sparks, MD, USA
Bacto-yeast extract	Difco/BD, Sparks, MD, USA
Bathocuproine disulfonic acid	Sigma-Aldrich, St. Louis, MO, USA
BCA protein assay reagents	Pierce, Rockford, IL, USA
Big Dye terminator ready reaction mix	Applied Biosystems, Foster City, CA
Biotinylated UTP and CTP	Enzo Diagnostics, Farmingdale, NY, USA
Bovine albumine, fraction V	Roth, Karlsruhe, Germany
Calcium ionophore A 23187	Roche, Basel, Switzerland
Complete protease inhibitor cocktail	Roche, Basel, Switzerland
Coomassie brilliant blue R-250	Serva, Heidelberg, Germany

Custom made RPA probe sets	Pharmingen, San Diego, CA, USA
DAPI (4.6-diamidino-2-phenylindole)	Sigma-Aldrich, St. Louis, MO, USA
dNTP mix (10 mM each)	Clontech, Palo Alto, CA, USA
Dulbecco's MEM	Biochrom, Berlin, Germany
Ethidium bromide	Sigma-Aldrich, St. Louis, MO, USA
ExpressHyb solution	Clontech, Palo Alto, CA, USA
Fetal calf serum (FCS)	Biochrom, Berlin, Germany
Glutamax	Gibco/Invitrogen, Karlsruhe, Germany
Hepes	Roth, Karlsruhe, Germany
hOrc RPA template set	Pharmingen, San Diego, CA, USA
IPTG	Sigma-Aldrich, St. Louis, MO, USA
Metafectene	Biontex, Munich, Germany
Microscint 20	Packard BioScience, Groningen, Netherlands
Mowiol 4-88	Calbiochem, La Jolla, CA, USA
Non-fat dry milk powder ("Sucofin")	TSI, Zeven, Germany
NuPAGE LDS sample buffer	Invitrogen, Karlsruhe, Germany
Paraformaldehyde	Merck, Whitehouse Station, NJ, USA
PMSF (phenyl-methyl-sulfonylfluoride)	Sigma-Aldrich, St. Louis, MO, USA
RPMI-1640	PAA, Cölbe, Germany
Sodium pyruvate	Gibco/Invitrogen, Karlsruhe, Germany
Staurosporine	Roche, Basel, Switzerland
Streptavidine alexa fluor 568 conjugate	Molecular Probes, Eugene, OR, USA
Template suppression reagent	Applied Biosystems, Foster City, CA
Triton X-100	Sigma-Aldrich, St. Louis, MO, USA
Trypsin/EDTA Solution (10x)	Biochrom, Berlin, Germany
Tween 20	Sigma-Aldrich, St. Louis, MO, USA

All chemicals and reagents not listed above were purchased from Roth, Karlsruhe, Germany. Chemicals were normally used in p.a. grade.

2.4 Radiochemicals

[α - ³³ P]dATP (3000 Ci/mmol, 10 mCi/ml)	NEN, Boston, MA, USA
[α - ³² P]dCTP (3000 Ci/mmol, 10 mCi/ml)	NEN, Boston, MA, USA
[α - ³² P]UTP (3000 Ci/mmol, 10 mCi/ml)	NEN, Boston, MA, USA
[6- ³ H]-thymidine (10 Ci/mmol, 1 mCi/ml)	NEN, Boston, MA, USA
L-[4,5- ³ H]leucine (161 Ci/mmol, 1 mCi/ml)	Amersham, Buckinghamshire, UK

2.5 Enzymes and peptides

CaspACE FITC-VAD-fmk	Promega, Madison, WI, USA
DNase I, RNase-free	Roche, Basel, Switzerland
Recombinant human caspase-3	Pharmingen, San Diego, CA, USA
Recombinant human caspase-6	Pharmingen, San Diego, CA, USA
Recombinant human caspase-7	Pharmingen, San Diego, CA, USA
Recombinant human caspase-8	Pharmingen, San Diego, CA, USA
Recombinant human caspase-10	Calbiochem, La Jolla, CA, USA
Restriction Enzymes	MBI Fermentas, St.Leon-Rot, Germany
Taq polymerase	Eppendorff, Hamburg, Germany
T4-DNA-Ligase	Promega, Madison, WI, USA
z-DEVD-fmk	Calbiochem, La Jolla, CA, USA
z-VAD-fmk	Calbiochem, La Jolla, CA, USA

2.6 Antibodies

Biotinylated mouse anti-FLAG M5	Sigma-Aldrich, St. Louis, MO, USA
Biotinylated rabbit anti-mouse Ig F(ab') ₂	Dako, Glostrup, Denmark
Goat anti-human IgM F(ab) ₂	Dianova, Hamburg, Germany
Goat anti-mouse IgG F(ab) ₂	Dianova, Hamburg, Germany
Goat anti-mouse IgG-HRP	Promega, Madison, WI, USA
Goat anti-rabbit IgG-HRP sc-2004	Santa Cruz Biotechnology, Santa Cruz, CA, USA
Mouse anti-CD95	Pharmingen, San Diego, CA, USA
Mouse anti-Cdc6 Ab-1 (DCS-180)	NeoMarkers, Fremont, CA, USA
Mouse anti-FLAG M5	Sigma-Aldrich, St. Louis, MO, USA
Mouse anti-V5	Invitrogen, Karlsruhe, Germany
Rabbit anti-Caspase-3	Pharmingen, San Diego, CA, USA
Rabbit anti-DNA-PK p350	Pharmingen, San Diego, CA, USA
Rabbit anti-His tag ab 9108	Abcam, Cambridge, UK
Rabbit anti-Mcm2	Pharmingen, San Diego, CA, USA
Rabbit anti-Mcm3	Pharmingen, San Diego, CA, USA
Rabbit anti-Mcm4	Pharmingen, San Diego, CA, USA
Rabbit anti-Mcm5	Pharmingen, San Diego, CA, USA
Rabbit anti-Mcm6	Pharmingen, San Diego, CA, USA
Rabbit anti-Mcm7	Pharmingen, San Diego, CA, USA

2.7 Kits

Advantage-HF2 PCR Kit	Clontech, Palo Alto, CA, USA
Atlas human cDNA expression array I	Clontech, Palo Alto, CA, USA
Enhanced Chemiluminescence System	Amersham, Buckinghamshire, UK
HuGeneFL Array	Affymetrix, Santa Clara, CA, USA
InViSorb Apoptosis Detection Kit II	Invitex, Berlin, Germany
HexaLabel Kit	MBI Fermentas, St.Leon-Rot, Germany
Lig'nscribe Kit	Ambion, Austin, TX, USA
Magnetic Dynabeads M-280 streptavidine	Dynal Biotech, Oslo, Norway
MAXIscript	Ambion, Austin, TX, USA
Nucleobond Midi and Maxi Kits	Clontech, Palo Alto, CA, USA
pGEMT cloning Kit	Promega, Madison, WI, USA
QIAEXII Agarose Gel Extraction Kit	Qiagen, Hilden, Germany
QIAprep Spin Miniprep Kit	Qiagen, Hilden, Germany
QuikChange Site-Directed Mutagenesis Kit	Stratagene, La Jolla, CA, USA
RiboQuant multi-probe RPA system	Pharmingen, San Diego, CA, USA
RNeasy Mini Kit	Qiagen, Hilden, Germany
RPA III kit	Ambion, Austin, TX, USA
SuperScript 2 RT-PCR Kit	Invitrogen, Karlsruhe, Germany
TNT coupled reticulocyte lysate system	Promega, Madison, WI, USA
QIAexpressionist Kit	Qiagen, Hilden, Germany

2.8 Plasmids

pCDNA3.1 vector	Invitrogen, Karlsruhe, Germany
pCDNA6/V5-HisA vector	Invitrogen, Karlsruhe, Germany
pEGFP-N3	Clontech, Palo Alto, CA, USA
pGEMT Easy	Promega, Madison, WI, USA
pRSET B T7 expression vector	Invitrogen, Karlsruhe, Germany

2.9 Oligonucleotides

Gene specific oligonucleotides were ordered from Biotex, Berlin, Germany.

2.9.1 Oligonucleotides for Mcm3

MCM3-FLAG+:	GAA TTC TGA TGG CGG GTA CCG TGG
MCM3-FLAG-:	CTC GAG TCA GAT GAG GAA GAT GAT GAT GC

FLAG-MCM3.1+: GGA ATT CTG GCG GGT ACC GTG GTG C
 MCM3.1-: GCA TGC TTA CAT CTC CTG GAT GG
 FLAG-MCM3.2+: GGA ATT CTG GAG CTG ATC AGT GAC AAC C
 MCM3.2-: GCA TGC TTA GCC ATT TTC TAG GTC TC
 FLAG-MCM3.3+: GGA ATT CTG GCC AAG TCA TTG GCC CC
 MCM3.3-: GCA TGC TTA GCT ATC CTG GCT GCG
 FLAG-MCM3.4+: GGA ATT CTG GCT GGC ATC CAT GCT C
 MCM3.4-: GCA TGC TTA CTG GCG AGT CTT CC
 FLAG-MCM3.5+: GGA ATT CTG GCA GAG GAA GCT GTG G
 MCM3.5-: GCA TGC TCA GAT GAG GAA GAT GAT GC
 MCM3.5MA+: GCC AGC CAG CTG CCA AAG ATG GG
 MCM3.5MA-: CCC ATC TTT GGC AGC TGG CTG GC
 MCM3.5MB+: CCA AAG ATG GGG CTT CAT ACG ACC
 MCM3.5MB-: GGT CGT ATG AAG CCC CAT CTT TGG
 MCM3.5MC+: GGG ATT CAT ACG CCC CCT ATG AC
 MCM3.5MC-: GTC ATA GGG GGC GTA TGA ATC CC
 MCM3.5MD+: GAC TTC AGT GCC ACA GAG GAG G
 MCM3.5MD-: CCT CCT CTG TGG CAC TGA AGT C
 T7-FLAG+: GGA GGT CTA TAT AAG CAG AGC
 ECO-FLAG-: CAG AAT TCC AGC ACA CTG GCG GCC
 MCM3.5MA-doppel+: GCC AGC CAG CTG CCA AAG CTG GG
 MCM3.5MA-doppel-: CCC AGC TTT GGC AGC TGG CTG GC
 MCM3-Nterm-: CTC GAG TCA TTT GGC ATC TGG CTG GC
 MCM3-Cterm+: GAA TTC TGG GGG ATT CAT ACG ACC CC

2.9.2 Oligonucleotides for Cdc6

CDC6-FLAG+: GGA TCC ATG CCT CAA ACC CGA TCC
 CDC6-FLAG-: CTC GAG TTA AGG CAA TCC AGT AGC
 FLAG-CDC6.1+: GGA TCC GGC AAG AAA GAG AAT GG
 CDC6N1-: GCA TGC TTA GAG ATC CAG GGT ATT AGC
 FLAG-CDC6N2+: GGA TCC GGC CAG GAT GTA TTG TAC
 CDC6N2-: GCA TGC TTA GCA AAC CAA GAT CTT CTG
 T7-FLAG+: GGA GGT CTA TAT AAG CAG AGC
 BAM-FLAG-: GGA TCC CTT GTC ATC ATC ATC C
 CDC6-SEVD+: CAG AAG TTG CTG GTA ACA GGA TGA CC
 CDC6-SEVD-: GGT CAT CCT GTT ACC AGC AAC TTC TG

CDC6-SEVD-Nterm-: CTC GAG TTA AAC TTC TGA GAT GAT GAC TTG G
 CDC6-SEVD-Cterm+: GGA TCC GGT AAC AGG ATG ACC TTG AGC
 CDC6-MUT540+: GGG AAA GGG AGA TGG CTG TCA TCA GG
 CDC6-MUT540-: CCT GAT GAC AGC CAT CTC CCT TTC CC
 CDC6-MUT860+: GGT ATT GGA CGA GAT GGC TCA ACT GG
 CDC6-MUT860-: CCA GTT GAG CCA TCT CGT CCA ATA CC
 CDC6WT-V5+: GGA TTG CCT TAT CTC GAG CAT GCA TCT AGA GG
 CDC6WT-V5-: CCT CTA GAT GCA TGC TCG AGA TAA GGC AAT CC
 CDC6N-V5+: CTC AGA AGT TTA TCT CGA GCA TGC ATC TAG AGG
 CDC6N-V5-: CCT CTA GAT GCA TGC TCG AGA TAA ACT TCT GAG

2.9.3 Oligonucleotides for RPA II

ID2 132+: GGT CCG TTA GGA AAA ACA GCC
 ID2 471-: GGA ATT CAG AAG CCT GCA AGG
 NF-ATC 2212+: CCA AGA CCA TAC TAC AGC CAG C
 NF-ATC 2354-: GGA GAA AGG TCG TGG AGC TTG G
 EGR-1 1509+: GCA TAC CAA GAT CCA CTT GCG
 EGR-1 1857-: CGA AAG CCC TGT GGA GGC
 EGR-2 723+: CCA GAC TAT CCT GGA TTC TTT CC
 EGR-2 1025-: GCG AGG CCT CAG AAT GGG C
 TAFII30 289+: CCA TAT CTA ACG GGG TTT ACG
 TAFII30 424-: CCA GTC ACT GCA TCT GGG
 DUSP5 873+: CCT ACA CTA CAA ATG GAT CCC
 DUSP5 1050-: GGT CTT CAT AAG GTA AGC C
 TTP 536+: GCC ACC CTC CTG TGC TTC G
 TTP 795-: CCA GAC GCT GAT AGG AGT GG
 RGS1 267+: CCA AGG ATG TAC TTT CTG CTG C
 RGS1 496-: GCA GCA TCT GAA TGC ACA AAT GC
 NEFA 912+: GGT ATG GGA AGA GAC TGA TGG
 NEFA 1132-: CCT CAT TCA TTA CAT GTT CCC
 CD70 412+: GGA CCA GAG CTG GAC AAG G
 CD70 702-: GGT CTC ATC AGT GTT TCG GG
 1D-myosin 989+: CCA GAA GAT GAT CGA GGT GG
 1D-myosin 1368-: CGA TCA TCC ACA CTT TGG CC
 KIAA0053 778+: GCA GGA CAA CCT GGT GAA GC
 KIAA0053 1034-: GGA GAC TAT AGT TGT CAC G

Scya4 120+:	CGT GAC TGT CCT GTC TCT CC
Scya4 376-:	CCA GGT CAT ACA CGT ACT CC
EV12A 316+:	GCT AAC AGT ACT TCT TCC TGG
EV12A 608-:	GCC ATG TTG TTG TTG TTT TCC GC
EMP3 288+:	CCT TCA TCC TGT TCA TGT TCC
EMP3 474-:	GCC AGG GCG AAG CAG TAT CC
GAPDH 884+:	GCT ACA CTG AGC ACC AGG TGG
GAPDH 976-:	GGT CGT TGA GGG CAA TGC C

2.10 Media and buffers

<u>Caspase buffer:</u>	20 mM PIPES, 100 mM NaCl, 10 mM DTT, 1 mM EDTA, 0.1% CHAPS, 10% sucrose, pH 7.2
<u>CSK buffer:</u>	10 mM PIPES pH7.0, 100 mM NaCl, 300 mM sucrose, 3 mM MgCl ₂ , 0.5% Triton X-100, 1 mM ATP and 1 tablet/10 ml Complete protease inhibitor cocktail (115)
<u>Coomassie solution:</u>	0.25% coomassie brilliant blue R-250, 10% glacial acetic acid, 45% ethanol
<u>Destaining solution:</u>	7.5% glacial acetic acid, 25% ethanol
<u>Immunoblot transfer buffer:</u>	25 mM Tris-base, 192 mM glycine, 20% methanol, pH 8.3
<u>2 x Laemmli sample buffer:</u>	100 mM Tris-base, 20% glycerol, 8% SDS, 10% β-mercaptoethanol, 0.01% bromphenolblue, pH 6.8
<u>LB medium:</u>	10 g bacto-tryptone, 5 g yeast extract, 5 g NaCl ad 1 l H ₂ O
<u>10 x MOPS:</u>	0.2 M MOPS, 0.05 M Sodiumacetate, 0.01 M EDTA, pH 7.0
<u>Mowiol solution:</u>	4.2 ml 99.5% glycerine, 2.4 g Mowiol 4-88, 12 ml 0.2 M Tris-HCl pH8.5
<u>PBS:</u>	0.8 mM Na ₂ HPO ₄ , 0.2 mM NaH ₂ PO ₄ , 14 mM NaCl
<u>PBS-T:</u>	PBS + 0.05% Tween 20
<u>SDS lysis buffer:</u>	1 % SDS, 2 mM EDTA, 10 mM Tris-HCl, pH7.5
<u>20 x SSC:</u>	3 M NaCl, 0.3 M Tri-sodium citrate
<u>TBE:</u>	0.1 M Tris-base, 0.1 M boric acid, 2.5 mM EDTA
<u>TBS-T:</u>	0.5 M NaCl, 20 mM Tris, 0.1% Tween-20

2.11 Antibiotics

Ampicillin (100 µg/ml)	Sigma-Aldrich, St. Louis, MO, USA
Carbennicillin (50 µg/ml)	Roth, Karlsruhe, Germany
Chloramphenicol (34 µg/ml)	Fluka/Sigma-Aldrich, St. Louis, MO, USA
Gentamicin (25 µg/ml)	Gibco/Invitrogen, Karlsruhe, Germany
Kanamycin (30 µg/ml)	Sigma-Aldrich, St. Louis, MO, USA

Penicillin (100 units/ml)	PAN Biotech, Aidenbach, Germany
Streptomycin (100 µg/ml)	PAN Biotech, Aidenbach, Germany

2.12 Consumables

Immobilon-P PVDF membrane	Millipore, Bedford, MA, USA
Hybond-N membrane	Amersham, Buckinghamshire, UK
4-12% NuPAGE Bis-Tris gels	Invitrogen, Karlsruhe, Germany
Quick spin columns	Roche, Basel, Switzerland
Unifilter plate	Packard BioScience, Groningen, Netherlands
10-well slides	Roth, Karlsruhe, Germany
X-omat LS films	Kodak, Rochester, NY, USA

2.13 Special appliances

ABI PRISM 310 Genetic Analyzer	Applied Biosystems, Foster City, CA
Confocal laser scanner	Hewlett-Packard, Palo Alto, CA, USA
PT-200 thermal cycler	MJ Research, Waltham, MA, USA
Leica DC500 digital camera	Leica, Solms, Germany
FACSCalibur flow cytometer	BD Biosciences, San Jose, CA, USA
Leica DMLB microscope	Leica, Solms, Germany
TopCount scintillation counter	Packard BioScience, Groningen, Netherlands
UV Stratalinker	Stratagene, La Jolla, CA, USA

2.14 Special software

CellQuest 3.3	BD Biosciences, San Jose, CA, USA
Factura 2.2.2	Applied Biosystems, Foster City, CA
GeneWorks 2.3.1	Accelrys, San Diego, CA, USA
Microarray Suite 4.0	Affymetrix, Santa Clara, CA, USA
TINA 2.08e	Raytest, Straubenhardt, Germany
Leica IM1000 1.20	Leica, Solms, Germany