Anhang A

Anhang

A.1 Übersicht viraler IFN-Antagonisten

Tab. A.1: Virale Antagonisten der IFN-Induktion.

Virus	**Gen/Protein**	**Mechanismus**	**Referenz**
BRSV	NS1 und NS2	Bindung an dsRNA und Inhibition der PKR	Bossert et al., 2003
Ebola	VP35		Basler et al., 2003; Basler et al., 2000
Influenza A	NS1		Lu et al., 1995; Talon et al., 2000
ReV	σ3		Jacobs und Langland, 1998
Porcines Rotavirus	NSP3		Langland et al., 1994
VV	E3L		Chang et al., 1992
AV	VAI-RNA	Bindung an RNA ohne PKR-Aktivierung	Robertson und Mathews, 1996
EBV	EBER-RNA		Sharp et al., 1993
HIV	TAR-RNA		Gunnery et al., 1990
HSV	ICP34.5	Inhibition von eIF-2α	Cassady et al., 1998
VV	K3L		Davies et al., 1992
ASFV	IκB-Homolog	Inhibition von NFκB	Powell et al., 1996
Influenza B	NS1-B	Inhibition von IRF-3	Dauber et al., 2004; Donelan et al., 2004
HPV-16	E6		Ronco et al., 1998
PV	unbekannt	PKR Abbau	Black et al., 1993

Tab. A.2: Virale Antagonisten des IFN-*Signalings*.

Virus	Gen/Protein	Mechanismus	Referenz
EBV	EBNA-2	Blockiert ISG-Transkription	Bejarano und Masucci, 1998
HHV-8	IRF- Homolog		Zimring et al., 1998
HPIV-3, SeV	unbekannt	Blockiert STAT-1-Phosphorylierung	Young et al., 2000
MPV	T-Antigen	Bindet Jak1	Weihua et al., 1998
HPV-16	E7	Bindet p48	Barnard und McMillan, 1999
AV	VAI-RNA	Inhibition ADAR	Lei et al., 1998
VV	E3L		Liu et al., 2001
HBV	Kapsid	Inhibition von Mx	Rosmorduc et al., 1999
VV	unbekannt	Inhibition von iNOS	Bellows et al., 2003
ReV	$\sigma 3$	Inhibition von OAS/RNase L	Smith et al., 2005
RoV	NSP3		Rios et al., 1995
VV	E3L		Beattie et al., 1995; Hornemann et al., 2003
HIV	unbekannt		Martinand et al., 1999; Roy et al., 1990
HSV	unbekannt		Cayley et al., 1984
AV	E1A	Reduktion von STAT-1 und p48	Leonard und Sen, 1996
hCMV	unbekannt	Reduktion von Jak1 und p48	Miller et al., 1998
SV5	V-Protein	STAT-1-Abbau	Didcock et al., 1999
mCMV	M27	STAT-2-Abbau	Khan et al., 2004
HPIV-2	unbekannt		Young et al., 2000

Tab. A.3: Andere Virale IFN-Antagonisten.

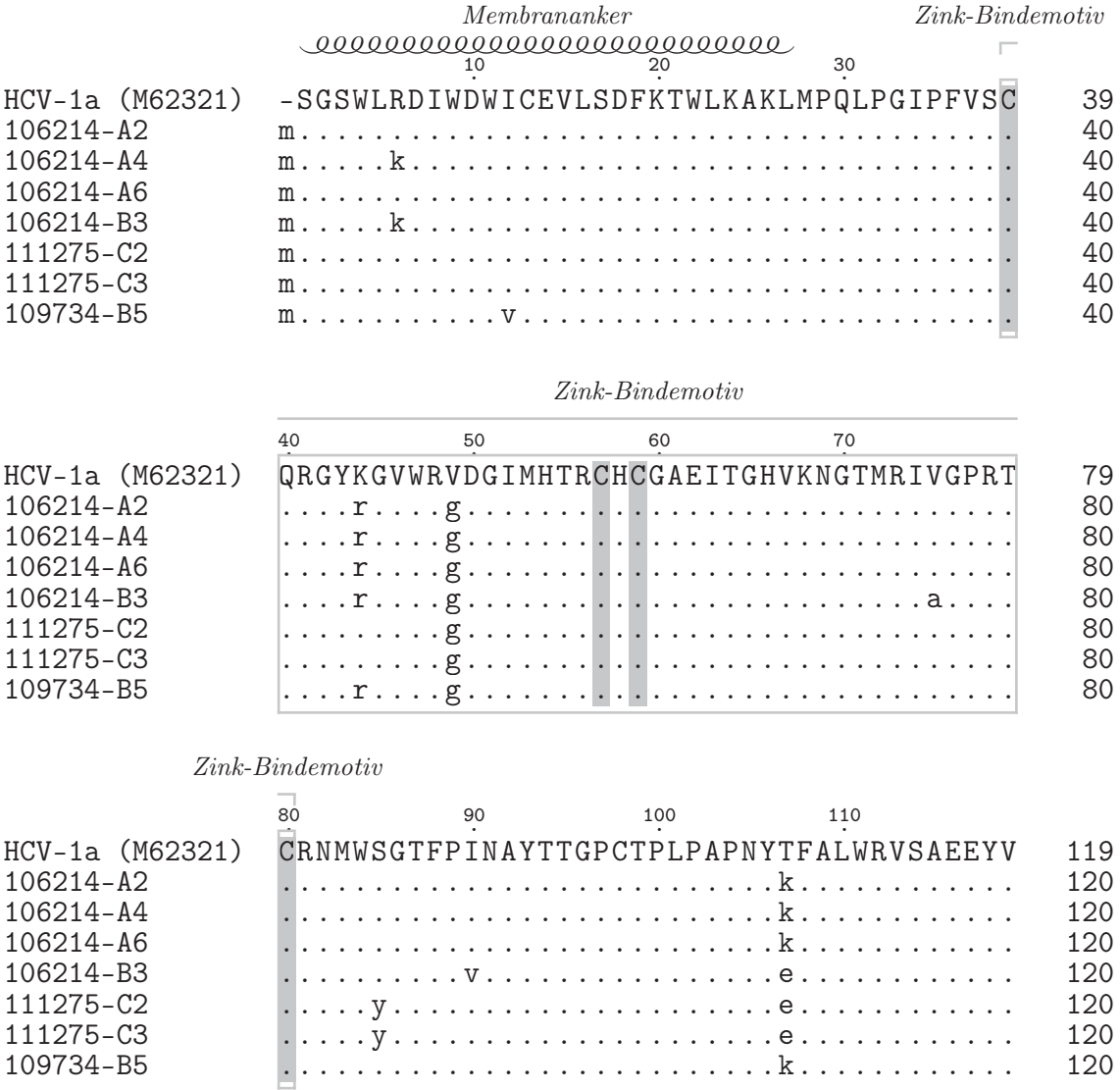
Virus	Gen/Protein	Mechanismus	Referenz
Pockenviren	Löslicher IFN-γ Rezeptor	Blockierung des Typ-2 IFN-*Signalings*	Alcami und Smith, 1995
Pockenviren	Löslicher IFN-α/β Rezeptor	Blockierung des Typ-1 IFN-*Signalings*	Symons et al., 1995

A.2 Sequenzdaten und Alignments

A.2.1 Aminosäuresequenzen der NS5A-Klone

Im Folgenden sind die Aminosäuresequenzen der verwendeten pCAGGS-NS5A-Klone dargestellt. Alle verwendeten Klone wurden im kodierenden Bereich komplett durchsequenziert. Der Übersicht halber wurden die Klone nach Genotyp sortiert und als Alignment mit den entsprechenden Referenzsequenzen dargestellt. Für den Genotyp 1a wurden die Klone mit der Sequenz des NS5A-ORF aus dem HCV-1-Genom [acc. M62321, Choo et al., 1991] verglichen, für den Genotyp 1b mit HCV-J [acc. D90208, Kato et al., 1990] und für den Genotyp 3a mit HCV-NZL1 [acc. D17763, Sakamoto et al., 1994]. Das Alignment wurde mittels ClustalW (Thompson et al., 1994) erstellt und über TEXshade(Beitz, 2000) grafisch dargestellt.

Genotyp 1a



```
                   120       130       140       150
HCV-1a (M62321)    EIRQVGDFHYVTGMTTDNLKCPCQVPSPEFFTELDGVRLH   159
106214-A2          ....l..y...........r....................   160
106214-A4          ....l..y................................   160
106214-A6          ....l..y...........r....................   160
106214-B3          ....l..y................................   160
111275-C2          ..kr....................................   160
111275-C3          ..kr.........i..........................   160
109734-B5          ......................................i.   160
```

```
                   160       170       180       190
HCV-1a (M62321)    RFAPPCKPLLREEVSFRVGLHEYPVGSQLPCEPEPDVAVL   199
106214-A2          ........................m...............   200
106214-A4          ........................................   200
106214-A6          ........................m...............   200
106214-B3          ........................................   200
111275-C2          .......s................................   200
111275-C3          .......s................................   200
109734-B5          ...........d....t....t.................v   200
                      |____|
                     NS4-Bind.
```

```
                   200       210       220       230
HCV-1a (M62321)    TSMLTDPSHITAEAAGRRLARGSPPSVASSSASQLSAPSL   239
106214-A2          ........................................   240
106214-A4          .......................................f   240
106214-A6          ........................................   240
106214-B3          ........................................   240
111275-C2          ...............r..........e.............   240
111275-C3          ...............r..........e.............   240
109734-B5          .........l................l.............   240
                                       |___________|   |__
                                  Hyperphosphorylierung  ISDR
                                                       |__
                                                    PKR-Bind.
```

```
                   240       250       260       270
HCV-1a (M62321)    KATCTANHDSPDAELIEANLLWRQEMGGNITRVESENKVV   279
106214-A2          ...............v........................   280
106214-A4          ...............v........k...............   280
106214-A6          ...............v........................   280
106214-B3          ...............v........................   280
111275-C2          ......................k.................   280
111275-C3          e.....................k.................   280
109734-B5          .....t............s.....................   280
                   _____________________________________|
                                  ISDR
                   ________________________________________
                                PKR-Bind.
```

```
                  280        290        300        310
HCV-1a (M62321)   ILDSFDPLVAEEDEREISVPAEILRKSRRFAQALPVWARP   319
106214-A2         ..............................tp........   320
106214-A4         ............................r....tp........   320
106214-A6         ..........................f...tp........   320
106214-B3         ..............................tp........   320
111275-C2         ..........................................   320
111275-C3         ..........................................   320
109734-B5         v....e.........m...........k.kp........   320
                  ____________________________
                       PKR-Bind.

                  320        330        340        350
HCV-1a (M62321)   DYNPPLVETWKKPDYEPPVVHGCPLPPPKSPPVPPPRKKR   359
106214-A2         e.....i...........e.......................   360
106214-A4         e.....i...........e.......................   360
106214-A6         e.....i...........e.......................   360
106214-B3         e.....i...r....q..e.......................   360
111275-C2         ......l......g.............qq............   360
111275-C3         ......i......g.............qq............   360
109734-B5         ............................q............   360
                                                  ________
                                                    NLS

                  360        370        380        390
HCV-1a (M62321)   TVVLTESTLSTALAELATRSFGSSSTSGITGDNTTTSSEP   399
106214-A2         ..................k.............d.......   400
106214-A4         ........v.........k.............dm......   400
106214-A6         ......................p........d.......   400
106214-B3         ..................k.............d.......   400
111275-C2         ..................k................s...a   400
111275-C3         ..i...............k................a...a   400
109734-B5         .......n..........qt.........aa.sa......   400
                  _____              ____________________
                   NLS                      V3-Region

                  400        410        420        430
HCV-1a (M62321)   APSGCPPDSDAESYSSMPPLEGEPGDPDLSDGSWSTVSSE   439
106214-A2         ..........................................g   440
106214-A4         ..........................................g   440
106214-A6         ..........................................g   440
106214-B3         ..........v...............................g   440
111275-C2         .....s....................................   440
111275-C3         .....s....................................   440
109734-B5         t.........n................................   440
                  ____________
                   V3-Region
```

```
                  440
HCV-1a (M62321)   ANAEDVVCCS   449
106214-A2         .dt.......   450
106214-A4         .dt.......   450
106214-A6         .dt.......   450
106214-B3         .dt.......   450
111275-C2         .gt.......   450
111275-C3         .gt.......   450
109734-B5         .gt.......   450
```

Genotyp 1b

Membrananker

```
                                  10        20        30
HCV-1b (D90208)   --SGSWLKDVWDWICTVLSDFKTWLQSKLLPRLPGLPFLS    38
108413-B6         mg..............a..............v....    40
108414-D2         mg.....r........a..............v....    40
110072-C1         mg.....r........t..............v..f.    40
110072-C2         mg.....r........t..............v..f.    40
107881-F2         mg..............t..............v..f.    40
```

Zink-Bindemotiv

```
                  40        50        60        70
HCV-1b (D90208)   CQRGYKGVWRGDGIMQTTCPCGAQITGHVKNGSMRIVGPK    78
108413-B6         ..................................... .    80
108414-D2         .....r...........s..................r    80
110072-C1         .......i.......h...s..................    80
110072-C2         .......i.......h...s..................    80
107881-F2         ..............r.....a............r    80
```

Zink-Bindemotiv

```
                  80        90        100       110
HCV-1b (D90208)   TCSNTWHGTFPINAYTTGPCTPSPAPNYSRALWRVAAEEY   118
108413-B6         ..........................k........d.    120
108414-D2         .......r.....................  .....    120
110072-C1         ..........................t.........s...   120
110072-C2         ...i......................t.........s...   120
107881-F2         ..  ..... ..... ..... ..... ..... .....    120

                  120       130       140       150
HCV-1b (D90208)   VEVTRVGDFHYVTGMTTDNVKCPCQVPAPEFFTEVDGVRL   158
108413-B6         ..................................l.....   160
108414-D2         m.....  ..... ..... ..... ..... .....    160
110072-C1         ..................i.....  ..... .....    160
110072-C2         ..................i.....  ..... .....    160
107881-F2         ..............t.....  ..... ..... .....   160
```

```
                    160         170         180         190
HCV-1b (D90208)  HRYAPVCKPLLREEVVFQVGLNQYLVGSQLPCEPEPDVAV  198
108413-B6        .....a.r....d..t.l......p........l....t.  200
108414-D2        .....a.........t.........................  200
110072-C1        .....a.........t.........................  200
110072-C2        .....a.........t.........................  200
107881-F2        .....a.........t.....................m.  200
```

NS4-Bind.

```
                    200         210         220         230
HCV-1b (D90208)  LTSMLTDPSHITAETAKRRLARGSPPSLASSSASQLSAPS  238
108413-B6        ..............a.........................  240
108414-D2        ..............a.r.......................  240
110072-C1        ........................................  240
110072-C2        ........................................  240
107881-F2        v............m.........................  240
```

Hyperphosphorylierung *ISDR*

PKR-Bind.

```
                    240         250         260         270
HCV-1b (D90208)  LKATCTTHHDSPDADLIEANLLWRQEMGGNITRVESENKV  278
108413-B6        .......r.....i..........................  280
108414-D2        ........................................  280
110072-C1        ........................................  280
110072-C2        p.......................................  280
107881-F2        ........................................  280
```

ISDR

PKR-Bind.

```
                    280         290         300         310
HCV-1b (D90208)  VILDSFDPIRAVEDEREISVPAEILRKPRKFPPALPIWAR  318
108413-B6        ........l..e.............rsk.......m...  320
108414-D2        ........l..e.....v.........s......m.v...  320
110072-C1        ........l..e.....v.........ts......m.....  320
110072-C2        ........l..e...w.v.........ts......m.....  320
107881-F2        ........l..e...g.vp.e......s............  320
```

PKR-Bind.

```
                    320          330          340          350
HCV-1b (D90208)  PDYNPPLLESWKDPDYVPPVVHGCPLPSTKAPPIPPPRRK   358
108413-B6        ..........................p...........   360
108414-D2        ..........................p...........   360
110072-C1        .......i..................p.....v......   360
110072-C2        .......i..................p.....v......   360
107881-F2        ..........................p.........k.   360
                                                  NLS
```

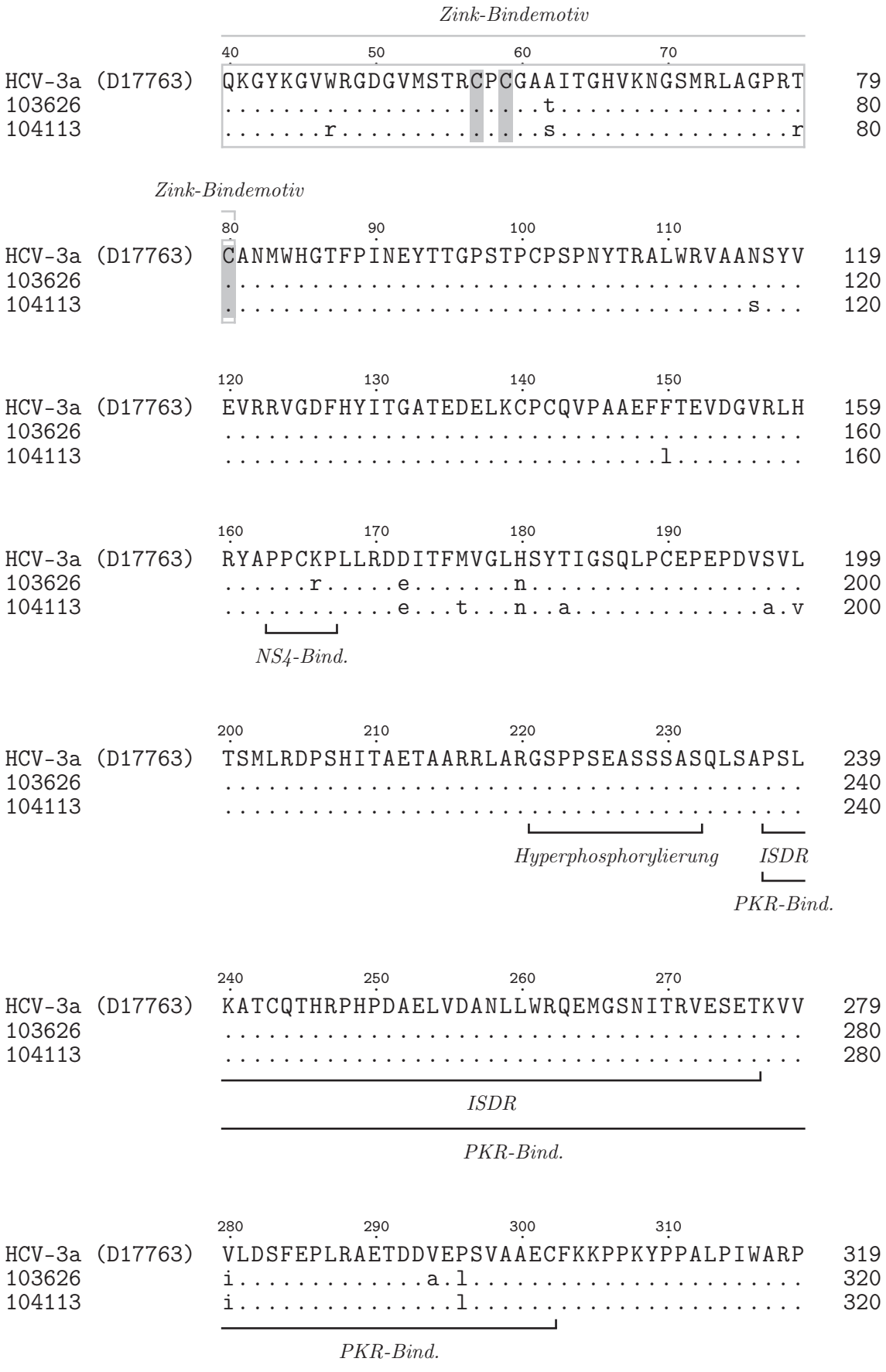
```
                    360          370          380          390
HCV-1b (D90208)  RTVVLTESTVSSALAELATKTFGSSGSSAVDSGTATGPPD   398
108413-B6        ......................g.e.........sa...   400
108414-D2        ......................e.........sa...   400
110072-C1        ......................d.....a.r....a..g   400
110072-C2        ......................d.....a.r....a..g   400
107881-F2        ........s.........a...s..e.........a...   400
                 NLS                   V3-Region
```

```
                    400          410          420          430
HCV-1b (D90208)  QASDDGDKGSDVESYSSMPPLEGEPGDPDLSDGSWSTVSG   438
108413-B6        .p.n...t..............................e   440
108414-D2        .p..n..t...a..........................e   440
110072-C1        .p.....t....g.........................e   440
110072-C2        .p....gt....g.........................e   440
107881-F2        ...g...a..............................e   440
                 V3-Region
```

```
                    440
HCV-1b (D90208)  EAGEDVVCCS   448
108413-B6        ..s.......   450
108414-D2        ..s.......   450
110072-C1        ..........   450
110072-C2        ..........   450
107881-F2        ..s.......   450
```

Genotyp 3a

```
                          Membrananker                  Zink-Bindemotiv
                          oooooooooooooooooooooooooooo
                               10          20          30
HCV-3a (D17763)  -SDDWLRTIWDWVCSVLADFKAWLSAKIMPALPGLPFISC    39
103626           m.g....i.........s...t.................    40
104113           m.g....d......t..s...t.................    40
```


Zink-Bindemotiv
40 50 60 70
HCV-3a (D17763) QKGYKGVWRGDGVMSTRCPCGAAITGHVKNGSMRLAGPRT 79
103626 .....t..... 80
104113 .....r.....s.....r 80
Zink-Bindemotiv
80 90 100 110
HCV-3a (D17763) CANMWHGTFPINEYTTGPSTPCPSPNYTRALWRVAANSYV 119
103626 120
104113 .....s... 120
120 130 140 150
HCV-3a (D17763) EVRRVGDFHYITGATEDELKCPCQVPAAEFFTEVDGVRLH 159
103626 160
104113 .....l..... 160
160 170 180 190
HCV-3a (D17763) RYAPPCKPLLRDDITFMVGLHSYTIGSQLPCEPEPDVSVL 199
103626 .....r.....e.....n..... 200
104113 .....e...t...n..a.....a.v 200
NS4-Bind.
200 210 220 230
HCV-3a (D17763) TSMLRDPSHITAETAARRLARGSPPSEASSSASQLSAPSL 239
103626 240
104113 240
Hyperphosphorylierung
ISDR
PKR-Bind.
240 250 260 270
HCV-3a (D17763) KATCQTHRPHPDAELVDANLLWRQEMGSNITRVESETKVV 279
103626 280
104113 280
ISDR
PKR-Bind.
280 290 300 310
HCV-3a (D17763) VLDSFEPLRAETDDVEPSVAAECFKKPPKYPPALPIWARP 319
103626 i.....a.l..... 320
104113 i.....l..... 320
PKR-Bind.

```
                      320       330       340       350
                        .         .         .         .
HCV-3a (D17763)       DYNPPLLDRWKAPDYVPPTVHGCALPPRGAPPVPPPRRKR   359
103626                ...........................q............   360
104113                ........................................   360
                                                          |___|
                                                           NLS
```

```
                      360       370       380       390
                        .         .         .         .
HCV-3a (D17763)       TIQLDGSNVSAALAALAEKSFPSSKPQEENSSSSGVDTQS   399
103626                .v......................................   400
104113                ........................................   400
                      |___|                |__________________|
                       NLS                      V3-Region
```

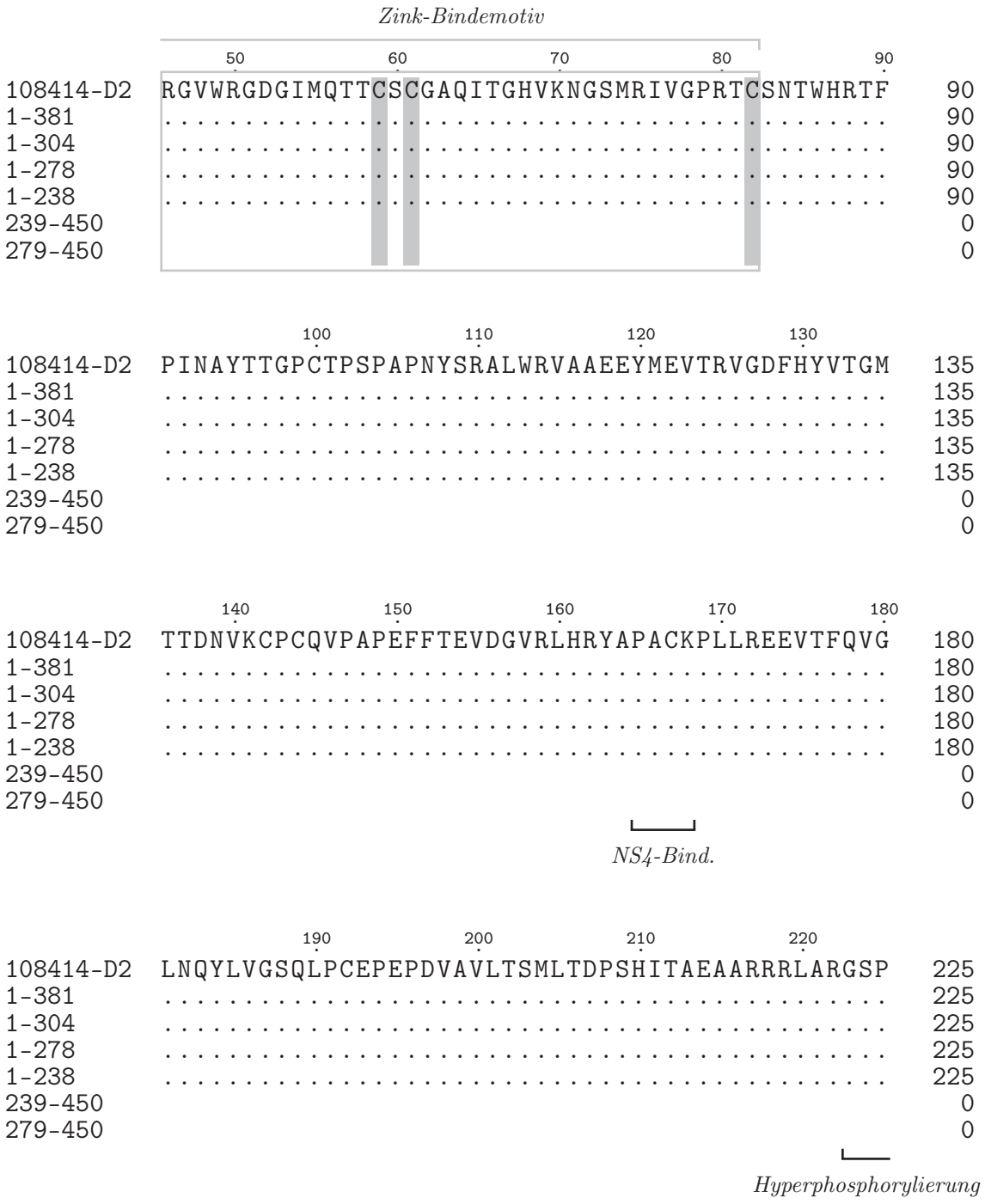
```
                      400       410       420       430
                        .         .         .         .
HCV-3a (D17763)       STTSKVPPSPGGESDSESCSSMPPLEGEPGDPDLSCDSWS   439
103626                ........................................   440
104113                ........................................   440
                      |__________|
                       V3-Region
```

```
                      440       450
                        .         .
HCV-3a (D17763)       TVSDSEEQSVVCC   452
103626                .............   453
104113                .............   453
```

A.2.2 Aminosäurealignment der NS5A-Deletionsmutanten

Im Folgenden sind die Aminosäuresequenzen der verwendeten pCAGGS-NS5A-Deletionsmutanten dargestellt. Sechs verschiedene Mutanten, vier C-terminal deletierte: NS5A(1–381, 1–304, 1–278 und 1–238), sowie zwei N-terminal deletierte: NS5A(239–451,279–451),wurden aus dem Klon pCAGGS-NS5A-108414-D2 generiert. Alle Mutanten wurden im kodierenden Bereich komplett durchsequenziert.

```
                          Membrananker                  Zink-Bindemotiv
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~                |----
                        10        20        30        40
                         .         .         .         .
108414-D2     MGSGSWLRDVWDWICTVLADFKTWLQSKLLPRLPGVPFLSCQRGY    45
1-381         .............................................    45
1-304         .............................................    45
1-278         .............................................    45
1-238         .............................................    45
239-450                                                         0
279-450                                                         0
```


Zink-Bindemotiv
50 60 70 80 90
108414-D2 RGVWRGDGIMQTTCSCGAQITGHVKNGSMRIVGPRTCSNTWHRTF 90
1-381 90
1-304 90
1-278 90
1-238 90
239-450 0
279-450 0
100 110 120 130
108414-D2 PINAYTTGPCTPSPAPNYSRALWRVAAEEYMEVTRVGDFHYVTGM 135
1-381 135
1-304 135
1-278 135
1-238 135
239-450 0
279-450 0
140 150 160 170 180
108414-D2 TTDNVKCPCQVPAPEFFTEVDGVRLHRYAPACKPLLREEVTFQVG 180
1-381 180
1-304 180
1-278 180
1-238 180
239-450 0
279-450 0
NS4-Bind.
190 200 210 220
108414-D2 LNQYLVGSQLPCEPEPDVAVLTSMLTDPSHITAEAARRRLARGSP 225
1-381 225
1-304 225
1-278 225
1-238 225
239-450 0
279-450 0
Hyperphosphorylierung

```
                230       240       250       260       270
108414-D2    PSLASSSASQLSAPSLKATCTTHHDSPDADLIEANLLWRQEMGGN   270
1-381        .............................................   270
1-304        .............................................   270
1-278        .............................................   270
1-238        .............                                   238
239-450                  m................................    33
279-450                                                        0
```

Hyperphosphorylierung *ISDR*

PKR-Bind.

```
                    280       290       300       310
108414-D2    ITRVESENKVVILDSFDPLRAEEDEREVSVPAEILRKSRKFPPAM   315
1-381        .............................................   315
1-304        ..................................              304
1-278        ........                                        278
1-238                                                        238
239-450      .............................................    78
279-450             m.....................................    38
```

ISDR

PKR-Bind.

```
                320       330       340       350       360
108414-D2    PVWARPDYNPPLLESWKDPDYVPPVVHGCPLPPTKAPPIPPPRRK   360
1-381        .............................................   360
1-304                                                        304
1-278                                                        278
1-238                                                        238
239-450      .............................................   123
279-450      .............................................    83
```

NLS

```
                    370       380       390       400
108414-D2    RTVVLTESTVSSALAELATKTFGSSESSAVDSGTASAPPDQPSDN   405
1-381        .....................                           381
1-304                                                        304
1-278                                                        278
1-238                                                        238
239-450      .............................................   168
279-450      .............................................   128
```

NLS *V3-Region*

```
                410         420         430         440         450
108414-D2  GDTGSDAESYSSMPPLEGEPGDPDLSDGSWSTVSEEASEDVVCCS   450
1-381                                                      381
1-304                                                      304
1-278                                                      278
1-238                                                      238
239-450    .............................................   213
279-450    .............................................   173
           ________
           V3-Region
```

A.2.3 Weitere Sequenzdaten

A.2.4 Sequenz des p48-cDNA-Klons

```
                10         20         30         40         50
p48  ATGGCATCAGGCAGGGCACGCTGCACCCGAAAACTCCGGAACTGGGTGGT    50
       M  A  S  G  R  A  R  C  T  R  K  L  R  N  W  V  V

                60         70         80         90        100
p48  GGAGCAAGTGGAGAGTGGGCAGTTTCCCGGAGTGTGCTGGGATGATACAG   100
       E  Q  V  E  S  G  Q  F  P  G  V  C  W  D  D  T

               110        120        130        140        150
p48  CTAAGACCATGTTCCGGATTCCCTGGAAACATGCAGGCAAGCAGGACTTC   150
       A  K  T  M  F  R  I  P  W  K  H  A  G  K  Q  D  F

               160        170        180        190        200
p48  CGGGAGGACCAGGATGCTGCCTTCTTCAAGGCCTGGGCAATATTTAAGGG   200
       R  E  D  Q  D  A  A  F  F  K  A  W  A  I  F  K  G

               210        220        230        240        250
p48  AAAGTATAAGGAGGGGGACACAGGAGGTCCAGCTGTCTGGAAGACTCGCC   250
       K  Y  K  E  G  D  T  G  G  P  A  V  W  K  T  R

               260        270        280        290        300
p48  TGCGCTGTGCACTCAACAAGAGTTCTGAATTTAAGGAGGTTCCTGAGAGG   300
       L  R  C  A  L  N  K  S  S  E  F  K  E  V  P  E  R
```

```
                310       320       330       340       350
p48  GGCCGCATGGATGTTGCTGAGCCCTACAAGGTGTATCAGTTGCTGCCACC   350
       G   R   M   D   V   A   E   P   Y   K   V   Y   Q   L   L   P   P

                360       370       380       390       400
p48  GGGAATCGTCTCTGGCCAGCCAGGGACTCAGAAAGTACCATCAAAGCGAC   400
       G   I   V   S   G   Q   P   G   T   Q   K   V   P   S   K   R

                410       420       430       440       450
p48  AGCACAGTTCTGTGTCCTCTGAGAGGAAGGAGGAAGAGGATGCCATGCAG   450
       Q   H   S   S   V   S   S   E   R   K   E   E   E   D   A   M   Q

                460       470       480       490       500
p48  AACTGCACACTCAGTCCCTCTGTGCTCCAGGACTCCCTCAATAATGAGGA   500
       N   C   T   L   S   P   S   V   L   Q   D   S   L   N   N   E   E

                510       520       530       540       550
p48  GGAGGGGGCCAGTGGGGGAGCAGTCCATTCAGACATTGGGAGCAGCAGCA   550
       E   G   A   S   G   G   A   V   H   S   D   I   G   S   S   S

                560       570       580       590       600
p48  GCAGCAGCAGCCCTGAGCCACAGGAAGTTACAGACACAACTGAGGCCCCC   600
       S   S   S   S   P   E   P   Q   E   V   T   D   T   T   E   A   P

                610       620       630       640       650
p48  TTTCAAGGGGATCAGAAGTCCCTGGAGTTTCTGCTTCCTCCAGAGCCAGA   650
       F   Q   G   D   Q   K   S   L   E   F   L   L   P   P   E   P   D

                660       670       680       690       700
p48  CTACTCACTGCTGCTCACCTTCATCTACAACGGGCGCGTGGTGGGCGAGG   700
       Y   S   L   L   L   T   F   I   Y   N   G   R   V   V   G   E

                710       720       730       740       750
p48  CCCAGGTGCAAAGCCTGGATTGCCGCCTTGTGGCTGAGCCCTCAGGCTCT   750
       A   Q   V   Q   S   L   D   C   R   L   V   A   E   P   S   G   S
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                760         770         780         790         800
p48  GAGAGCAGCATGGAGCAGGTGCTGTTCCCCAAGCCTGGCCCACTGGAGCC   800

       E   S   S   M   E   Q   V   L   F   P   K   P   G   P   L   E   P

                810         820         830         840         850
p48  CACGCAGCGCCTGCTGAGCCAGCTTGAGAGGGGCATCCTAGTGGCCAGCA   850

       T   Q   R   L   L   S   Q   L   E   R   G   I   L   V   A   S

                860         870         880         890         900
p48  ACCCCCGAGGCCTCTTCGTGCAGCGCCTTTGCCCCATCCCCATCTCCTGG   900

       N   P   R   G   L   F   V   Q   R   L   C   P   I   P   I   S   W

                910         920         930         940         950
p48  AATGCACCCCAGGCTCCACCTGGGCCAGGCCCGCATCTGCTGCCCAGCAA   950

       N   A   P   Q   A   P   P   G   P   G   P   H   L   L   P   S   N

                960         970         980         990        1000
p48  CGAGTGCGTGGAGCTCTTCAGAACCGCCTACTTCTGCAGAGACTTGGTCA  1000

       E   C   V   E   L   F   R   T   A   Y   F   C   R   D   L   V

               1010        1020        1030        1040        1050
p48  GGTACCTTCAGGGCCTGGGCCCCCCACCGAAGTTCCAGGTAACACTGAAT  1050

       R   Y   L   Q   G   L   G   P   P   P   K   F   Q   V   T   L   N

               1060        1070        1080        1090        1100
p48  TTCTGGGAAGAGAGCCATGGCTCCAGCCATACTCCACAGAATCTTATCAC  1100

       F   W   E   E   S   H   G   S   S   H   T   P   Q   N   L   I   T

               1110        1120        1130        1140        1150
p48  AGTGAAGATGGAGCAGGCCTTTGCCCGATACTTGCTGGAGCAGACTCCAG  1150

       V   K   M   E   Q   A   F   A   R   Y   L   L   E   Q   T   P

               1160        1170        1180
p48  AGCAGCAGGCAGCCATTCTGTCCCTGGTGTAG  1182

       E   Q   Q   A   A   I   L   S   L   V   .
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Zusammenfassung (englisch)

Chronic hepatitis C is currently treated with pegylated interferon (IFN)-α in combination with the nucleoside analog «ribavirin». Success of the combination therapy strongly depends on the viral genotype. Today approximately 40 percent of the genotype-1 and 80 % of the genotype-3 patients show a sustained virus clearance.

The mechanism of resistance of HCV against antiviral IFN-therapy is subject of this study. The basis of the molecular characterisation constitute sera of nine patients with genotypes 1a/b and 3a that have been characterised in clinical trials. Based on recent literature the emphasis of this work was put on the nonstructural protein 5A (NS5A).

Using functional reporter-assays the influence of 14 different NS5A-proteins was analysed. Isolates of the genotypes 1a/b and 3a from patients with different therapy-responses (non-response (NR), sustained virologic response (SVR) und breakthrough (BT) were characterised.

It was shown that NS5A functionally blocks IFN-signaling by inhibiting the transcription factor p48 (IRF-9). All 14 NS5A-proteins analysed were functionally capable of inhibiting IFN-stimulated response element (ISRE)-induced gene expression. Consequently this effect is independent of the therapy-response of the patient and independent of the viral genotype. Co-expression of p48 lead to a rescue of IFN-signaling in NS5A-transfected cells but not in cells transfected with the IFN-antagonist M27.

Mutagenesis studies have shown that the functional domain for this inhibition resides on the n-terminal 238 amino acids of NS5A and neither the ISDR, the PKR nor the V3 region are involved in this process. The n-terminally deleted mutants NS5A-239–450 and NS5A-279–451 show no influence on type-1-IFN induced signaling.

An effect on IFN-induction was also observed, although it did not compare to the known inhibitors of this pathway, such as the Influenza A protein «NS1» and the Ebola protein «VP35».

A biological relevance was shown using the recombinant Influenza A virus «delNS1», which is IFN-sensitive. NS5A was capable of rescuing viral growth in IFN-competent MDCK2-cells. Hence NS5A was capable of complementing the IFN-antagonist NS1.

This work adds to the observations that NS5A functionally inhibits IFN-signaling. It shows for the first time that NS5A can inhibit p48 (IRF-9) and blocks ISRE-dependent gene expression.