Medizinische Fakultät der Charité – Universitätsmedizin Berlin
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aus dem Institut für Virologie
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Dissertation

Die Rolle des Helicase-Primase-Komplexes von Herpes-simplex-Virus Typ 1 bei der DNA-Replikation des adenoassoziierten Virus

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INHALTSVERZEICHNIS

Abkürzungsverzeichnis

Nicht aufgenommen sind die Abkürzungen für chemische Elemente und internationale Standardeinheiten (SI-Einheiten).

A	Alanin
AAV, rAAV	(rekombinantes) adenoassoziiertes Virus
Abb.	Abbildung
bidest.	zweifach destilliertes Wasser
bp, kbp	Basenpaar, Kilobasenpaare
bzw.	beziehungsweise
Ci	Curie
CMV	Zytomegalievirus
D	Asparaginsäure
Da	Dalton
d.h.	das heißt
DNA, ssDNA	(einzelsträngige) Desoxyribonukleinsäure
EDTA	Ethylendiamintetraessigsäure
FCS	fetales Kälberserum
FITC	Fluoresceinthionylcarbonat
G	Glycin
GTP	Guanosin-5´-triphosphat
h	Stunde
HSV, rHSV	(rekombinantes) Herpes-simplex-Virus
ICP	*infected cell protein*
ITR	*inverted terminal repeat*
K	Lysin
mAk	monoklonaler Antikörper
min	Minuten
MOI	Multiplizität der Infektion
ND10	*nuclear domain 10, promyelotic leukemia nuclear body*
NTP, dNTP	(Desoxy)nukleosidtriphosphat

ORF	offenes Leseraster
ori	*origin of replication*
Page	Polyacrylamidgelelektrophorese
PBS, PBS-T	phosphatgepufferte Salzlösung, mit Tween®
pCM	Plasmid unter CMV-Promotorkontrolle
PCR	Polymerase-Kettenreaktion
pfu	*plaque forming units*
PML	*promyelocytic leukemia protein*
Q	Glutamin
R	Arginin
RBE	*rep-binding element*
RF1, RF2	Replikationsform 1, Replikationsform 2 von AAV
RNA, mRNA	(*messenger*-)Ribonukleinsäure
s.	siehe
SDS	Natriumdodekylsulfat
Tab.	Tabelle
TNF	*tumor necrosis factor*
Tris	Tris(hydroxymethyl)-Aminomethan
TRITC	Tetramethylrhodaminisothiocyanat
trs	*terminal resolution site*
u.a.	unter anderem
U_L, UL	*unique long* von HSV
V	Valin
v.a.	vor allem
vgl.	vergleiche
% [v/v]	Volumenprozent
% [w/v]	Gewichtsprozent
wt	Wildtyp
z.B.	zum Beispiel

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7 ZUSAMMENFASSUNG

Das adenoassoziierte Virus (AAV) ist ein helferabhängiges Virus, das für seine produktive Vermehrung die Koinfektion mit einem Helfervirus benötigt, z.B. dem Herpes-simplex-Virus (HSV). Als HSV-Helferproteine dienen vier HSV-Replikationsproteine, die zusammen mit dem AAV-Replikationsprotein Rep den Minimalkomplex für die Replikation von AAV bilden. Die vier benötigten HSV-Helferproteine sind das *single-strand DNA-binding protein* ICP8 und ein trimerer Protein-Komplex mit Helicase- und Primase-Aktivität. Nach Koinfektion von AAV und HSV wird das einzelsträngige AAV-DNA-Genom in subnukleäre HSV-Replikationskompartimente transloziert, in denen das AAV-Genom repliziert werden kann. Hierbei kolokalisieren HSV-ICP8 und AAV-Rep in Gegenwart des einzelsträngigen (ssDNA) AAV-Genoms.

In der vorliegenden Arbeit konnte gezeigt werden, dass für die ssDNA-abhängige Rekrutierung von AAV-Rep in nukleäre Replikationskompartimente das Zusammenwirken der vier HSV-Helferproteine hinreichend und notwendig ist. Für die Bildung der nukleären Replikationskomplexe mussten weder die HSV-Helicase noch die HSV-Primase enzymatisch aktiv sein. Dies wurde gezeigt durch Mutanten der HSV-Helicase bzw. -Primase, bei denen gezielt einzelne Aminosäure-Austausche in die katalytischen Zentren eingeführt wurden. Alle Mutanten hatten ihre enzymatische Aktivität vollständig verloren unter Erhalt der Interaktionsfähigkeit als Proteinkomponenten des trimeren Helicase-Primase-Komplexes.

Um zu testen, ob während des weiteren Verlaufs der AAV-DNA-Replikation die enzymatische Aktivität von HSV-Primase bzw. -Helicase benötigt wird, wurden AAV-DNA-Replikationsanalysen nach Transfektion der vier HSV-Helfergenkonstrukte durchgeführt. Auch hierbei wurden sowohl Helicase als auch Primase vor allem als strukturelle Komponenten der Replikationskomplexe benötigt. Dies entsprach den Erwartungen, da das AAV-ssDNA-Genom an den Enden über partiell doppelsträngige palindromische Strukturen verfügt, die durch komplementäre Rückfaltung die Funktion des Primers für die DNA-Polymerase übernehmen. Bei Präsenz der HSV-Polymerase nach HSV-Infektion zeigten die AAV-Replikationsuntersuchungen, dass bei enzymatisch aktiver HSV-Primase AAV effizienter repliziert wird, möglicherweise durch Rekrutierung des HSV-Polymerase-komplexes zur Replikation des AAV-Genoms.

Die HSV-Helicase besitzt wie AAV-Rep ATPase- und Helicase-Aktivität. Deshalb überraschte der weitere Befund, dass die enzymatisch aktive HSV-Helicase bei Transfektion des minmalen HSV-Replikationskomplexes eine nachweisbare, wenn auch geringe Steigerung der AAV-DNA-Replikationsrate zeigte. Als Erklärung kommt eine Stimulation der homologen Rekombination zur Auflösung hochmolekularer AAV-DNA-Replikations-intermediate in Frage. Aufgrund der entgegengesetzten Polarität der HSV-Helicase könnte diese auch die Rep-Helicase funktionell komplementieren. Die Quantifizierung der AAV-Replikationsintermediate zeigte zudem, dass zusätzlich bislang noch unbekannte, weitere HSV- oder zelluläre Faktoren existieren müssen, die die AAV-Replikation weiter stimulieren.

Aufbauende Untersuchungen werden nötig sein, um die strukturellen und funktionellen Interaktionen des Multiprotein-Komplexes aus AAV-Rep, HSV-*single-strand DNA-binding protein*, dem trimeren HSV-Helicase-Primase-Komplex und der AAV-ssDNA zu entschlüsseln. Neben dieser grundlegenden Frage sind die Untersuchungen auch für die Weiterentwicklung der AAV-Vektortechnologie von Bedeutung. Für die weitere Optimierung effizienter Verpackungssysteme für die AAV-Vektorproduktion im biotechnologisch großen Maßstab sind HSV-basierte Systeme äußerst vielversprechend. Deren Weiterentwicklung hängt wesentlich von einem guten Verständnis der Interaktionen zwischen AAV, HSV und zellulären Faktoren während der einzelnen Schritte der AAV-Replikation ab.

8 ANHANG

8.1 MONOCLONAL ANTIBODIES AGAINST UL5

The HSV helicase-primase complex (UL5-UL8-UL52 complex) was purified from *Spodoptera frugiperda* cells infected with a recombinant baculovirus that expresses all three proteins (AcUL5-UL8-UL52, essentially as described (Dodson *et al*., 1989)). The purified protein was used to immunize female BALB/c mice, and hybridoma lines secreting monoclonal antibodies (mAbs) were generated as described previously (McLean *et al.*, 1994). Mabs reactive with the complex were initially identified by an ELISA assay, and subsequently assessed for their ability to interact with the individual components by Western Blotting using extracts of *Spodoptera frugiperda* cells infected with baculovirus expressing UL5, UL8 or UL52 (Stow *et al.*, 1992).

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8.2 VERÖFFENTLICHUNGEN

A) Artikel in Zeitschriften:

Hattermann, K., Maerz, A., Slanina, H., Schmitt, C., and Mankertz, A. (2004). Assessing the risk potential of porcine circoviruses for xenotransplantation: consensus primer-PCR-based search for a human circovirus. *Xenotransplantation* **11,** 547-550.

Slanina, H., Weger, S., Stow, N. D., Kuhrs, A., and Heilbronn, R. (2006). Role of the Herpes Simplex Virus Helicase-Primase Complex during Adeno-Associated Virus DNA Replication. *J. Virol.* **80,** 5241-5250.

B) Vorträge:

Slanina, H., Weger, S., Kuhrs, A., Stow, N. D., Weller, S. K., and Heilbronn, R. (2003). Functional Analysis of the HSV Helicase-Primase-Complex for the Replication of Adeno-Associated Virus. *Jahrestagung der Gesellschaft für Virologie*, Berlin.

Slanina, H., Weger, S., Stow, N. D., Kuhrs, A., and Heilbronn, R. (2006). Role of the Herpes Simplex Virus Helicase-Primase Complex during Adeno-Associated Virus DNA Replication. *IXth Parvovirus Workshop*, Les Diablerets, Schweiz.

C) Poster:

Slanina, H., Weger, S., Kuhrs, A., Stow, N. D., and Heilbronn, R. (2005). Functional Analysis of the HSV Helicase-Primase-Complex for AAV DNA replication. *Jahrestagung der Gesellschaft für Virologie*, Hannover.

Slanina, H., Weger, S., Stow, N. D., Kuhrs, A., and Heilbronn, R. (2006). Role of the Herpes Simplex Virus Helicase-Primase Complex during Adeno-Associated Virus DNA Replication. *Jahrestagung der Gesellschaft für Virologie*, München.

8.3 SELBSTSTÄNDIGKEITSERKLÄRUNG

„Ich, Heiko Slanina, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema „Die Rolle des Helicase-Primase-Komplexes von Herpes-simplex-Virus Typ 1 bei der DNA-Replikation des adenoassoziierten Virus“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

Berlin, 26.09.2006

Heiko Slanina

8.4 DANKSAGUNGEN

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8.5 LEBENSLAUF

Mein Lebenslauf wird aus Datenschutzgründen in der elektronischen Version meiner Arbeit nicht mit veröffentlicht.