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Rescue of the Activity of HNH Nuclease Mutants – Towards Controlled Enzymes for Gene Therapy

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Abstract: Artificial nucleases are designed for *in vivo* gene engineering, as the DNA cleavage performed at a specific target site enhances the effectiveness of the cell's DNA repair machinery. The therapeutic potential of the above phenomenon stems from the knowledge that (i) the shifted reading frame can be restored by non-homologous end-joining, or (ii) a DNA of erroneous sequence – causing a genetic disease – can be corrected by homologous recombination in the presence of a suitable DNA template. Besides the advantageous properties of the nowadays applied zinc finger nucleases, TALE nucleases and the CRISPR/Cas9 system, they possess a residual citotoxicity. This is related to off-target cleavages, which could be prevented by the strict regulation of the enzymes. The studies on enzymes acting naturally in a controlled manner are beneficial to get better insight into their mechanism. Such enzymes or their appropriate domains may be the most promising alternatives to the presently applied ones. As an example, the DNA cleavage of the inactive HNH nuclease mutants is inducible in a multiple way. This property may be used for establishing a control mechanism and thus, in combination with specific DNA-binding domains they are good candidates for the catalytic site of artificial nucleases. Here we collect the results on the properties of the HNH nucleases that allow for their redesign into enzymes with possible therapeutic applications.

Keywords: Allosteric control, Artificial nuclease, CRISPR/Cas, HNH motif, TALE, zinc finger.

1. INTRODUCTION

Artificial nucleases are designed for specific gene engineering, which would otherwise be impossible with the available arsenal of the native enzymes. One of the most promising application of these agents is gene therapy [1-5], but they are also used for gene modifications in cells to create animal models [6,7], to improve properties of plants (e.g. targeted addition of a herbicide-tolerance gene [8]) [9,10], and to broaden our knowledge on genetic phenomena [11]. The basis of these experiments is that the double-strand cleavage of DNA induces the cells' repair machinery to correct the damaged molecule. By the help of the subsequent processes (i) the shifted reading frame can be restored or a random mutation can be induced for gene knockout purpose by non-homologous end-joining, or (ii) a DNA of erroneous sequence – causing a genetic disease – can be corrected, or a gene can be inserted in a targeted way by homologous recombination in the presence of a suitable DNA template [12-21]. Because of this large scientific potential, there is a high demand of constructing specific artificial nucleases, as it is demonstrated by the continuously increasing number of publications on this hot topic.

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2. CURRENT ARTIFICIAL NUCLEASES

One large group of artificial nucleases are the synthetic catalytic agents, mostly metal complexes, with all their advantages and drawbacks discussed in detail elsewhere [5,22-25]. The other class of the designed enzymes includes modified versions of native proteins constructed either by introducing mutations through molecular design or directed evolution methods or by creating chimera from independent catalytic and DNA binding domains. The main strategies applied in these experiments on artificial meganucleases constructed from homing endonucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR) with associated nucleases (Cas) were discussed in several recent reviews [26-33].

The success of gene editing with artificial nucleases quickly led to a human clinical trial with zinc finger/FokI nucleases [34]. Although the infusion of the autologous CD4 T-cells in which the CCR5 gene was inactivated by ZFNs proved to be safe within the limit of the mentioned study, we must be aware of possible side-effects of the artificial nucleases, which may arise from the off-target binding of the applied DNA recognition domains and unwanted DNA cleavage [32,33,35,36]. Since the first successful construction of a modular zinc finger nuclease [37] the most widely applied protein chimera utilize the functional FokI nuclease domain as their cleavage agent [38], which can only function upon dimerization on DNA. The relatively short recognition se-

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quence (9-12 base pairs) of each DNA binding domains may cause cleavages at off-target sites by weak interactions of one of the monomers with a non-specific site, or by DNA looping [39]. The off-target DNA binding may be further enhanced by any damage of the DNA recognition domain within the cell. It has to be mentioned that the same phenomena are excluded in the native FokI enzyme, as it acts in a controlled manner, the nuclease domain being under the allosteric inhibition of the DNA binding domain until the specific target sequence is recognized [40,41]. This advantageous property is, however, lost when this nuclease domain is fused to different DNA-binding domains.

Efforts have been put to improve the specificity and cytotoxic properties of the artificial enzymes. The redesign of the dimerization interface [42], construction of single chain quasi dimer nucleases [43] and context dependent assembly of the recognition motifs [44] represent examples of these attempts. The introduction of TALEs and later CRISPRs as DNA recognition domains led to the decrease of the the side-effects [32,33,45]. Engineering nicking enzymes in all kinds of the chimeric artificial nucleases also resulted in a substantial progress of this field [46-52]. Alternative nuclease domains have been applied in few cases in mainly monomeric chimera [53-58] and non-FokI-based zinc finger nucleases have also been developed by introducing mutations or small modifications into the zinc finger array, such as the exchange of the metal ion-binding cysteines to histidines [59-61]. Trials to establish controlled artificial nucleases through light-activation [62,63], DNA binding [64], cold-shock technique [65], protein-protein interaction [66], DNA modification [67] or metal ion dependent DNA binding [68] were also executed. Bioinformatics was utilized to predict ZFN and TALEN off-target cleavage [69].

3. HNH NUCLEASES

It is obvious that the previously described experiments are still in progress yet without clear reassuring outcome concerning the application in human therapy. This fact inspired a search for specific class of the nuclease domains with the potential to turn these agents into engineered protein chimera, combining them with DNA binding domains so that they can act in controlled manner. The latter property is indispensable for their eventual future therapeutic application, as it was shown above. Possible candidates could be among the nucleases with catalytic domains, which are dependent on allosteric features in their catalytic mechanism.

According to the topography of their catalytic sites superfamilies have been identified among the nucleases, such as the PD-(D/E)XK, HNH, LAGLIDADG and GIY-YIG groups of the enzymes [70-73]. While the catalytic residues vary largely among the members of most of the families, their identity and position within the HNH superfamily [70,71,74,75] is well conserved. This superfamily includes a number of specific (e.g. the I-HmuI [76] and I-PpoI [74] group I. homing endonucleases or Eco31I [77] HphI [78], KpnI [79,80], MnlI [81], Hpy99I [82], PacI [83] restriction endonucleases) the RNA guided Cas9 nuclease [84,85] and nonspecific enzymes (e.g. Nuclease A [86], Serratia nuclease [74], Vvn [87], CAD - caspase-activated DNase [88] and bacterial toxins – colicins [89] and pyocins [90]). Their sequences can be found in the Pfam [72] and HNH [91] databases, the latter dividing the proteins into 10 subgroups.

3.1. Structure of the HNH Motif

The HNH motif consists of two β-strands and an α-helix connected with flexible loops. It is structurally resembling to the well-known zinc-finger motif (also possessing a ββα-metal binding structure [92]) but its function is different. The classical active site of the HNH nucleases is characterized by a $HX_{10\text{-}17}NX_{6\text{-}11}$(H/N) motif, where X stands for any amino acid with the exception of those with side-chains for strong metal ion binding or proline. The first histidine (H) among the highly conserved residues behaves as a general base in DNA hydrolysis generating the nucleophile that attacks the scissile phosphodiester bond [70,84,93]. The following asparagine (N) plays a structural role, stabilizing the structure of the motif and positioning the catalytic histidine through extensive hydrogen-bonding network [70,94,95].

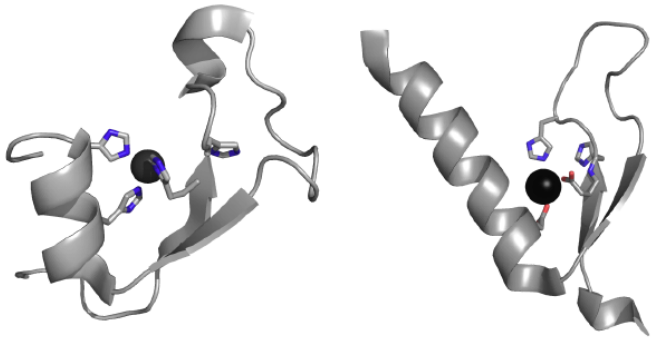

Fig. (1). The structure of the HNH (left: PDB ID 1M08) and HNN (right: PDB ID 2QNC) motifs.

The last conserved amino acid, which can either be a histidine or asparagines (H/N), serves for the metal ion binding. Mg^{2+} or Zn^{2+} is suggested in most of the cases to be the catalytically active cofactor. The nucleases such as e.g. the I-HmuI HNH homing endonuclease [76], PacI restriction endonuclease [83], T4 endonuclease VII [93], Cas9 nuclease [84,85] contain Mg^{2+} in the active site bound to the conserved second asparagine residue from HNN motif (Fig. **1**). On the other hand HNH nucleases offering histidines for metal ion binding prefer transition metal ions, such as Zn^{2+} in their active centre. Examples are provided by e.g. the bacteriophage P1 nuclease [66] and colicin E7 [96,97].

The I-PpoI His-Cys box and the I-CreII GIY-YIG homing endonucleases have the specific feature to possess an HNH motif beside their motifs characteristic for different superfamilies. The former contains two Zn^{2+}-ions [98,99] within the His-Cys box motifs, but its HNH-like active centre can be activated by Mg^{2+}, Ca^{2+}, Co^{2+} or Zn^{2+} [70]. The cleavage pattern of I-CreII is characteristic for the GIY-YIG enzymes, but the enzyme with mutated GIY-YIG residues proved to be still active in the presence of Zn^{2+} or Mg^{2+}, unlike upon the mutation at the conserved histidine residues of the HNH motif [100].

3.2. Nuclease Colicins

Nuclease colicins are plasmid encoded bacterial toxins produced by Escherichia coli to provide protection from re-

lated bacteria or bacteriophages by degradation of their chromosomal DNA [89, 101,102]. E-type colicins share the BtuB receptor to enter the target cell [103,104]. The C-terminal nuclease domains of the Colicin E2, E7, E8 and E9 (NColEs) serve as non-specific metallonucleases. Based on the topography of their active centre, these enzymes belong to the HNH superfamily of nucleases, showing high sequence similarity (Scheme **1**) with the $HHX_{14}NX_8HX_3H$ general sequence near their C-termini [105]. These nuclease colicins are the most widely investigated HNH enzymes.

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KRNKPGKATGKGKPVNNKWLNNAGKDLGSPVPDRIANKLRDKEFKSFDDFRKKFWEEVSKDPELSKQF
KRNKPGKATGKGKPVGDKWLDDAGKDSGAPIPDRIADKLRDKEFKSFDDFRKKFWEEVSKDPELSKQF
KRNKPGKATGKGKPVGDKWLDDAGKDSGAPIPDRIADKLRDKEFKNFDDFRKKFWEEVSKDPDLSKQF
GRDLPGKVTGTGTDVEGSWLAGAGEGLGAPVPTRIADRLRDREFSSFDAFRRSFWQEVAADPELAGQF

SRNNNDRMKVGKAPKTRTQDVSGKRTSFELHHEKPISQNGGVYDMDNISVVTPKRHIDIHRGK-576
KDSNKTNIQKGKAPFARKKDQVGGRERFELHHDKPISQDGGVYDMDNIRVTTPKRHIDIHRGK-581
KGSNKTNIQKGKAPFARKKDQVGGRERFELHHDKPISQDGGVYDMNNIRVTTPKRHIDIHRGK-582
KKGNQGRMKKGLAPRVRELEQAGKRHSTELHHVDLISDGGEVYNIDNIHVVTPKQHVEIHSGK-617
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Scheme 1. Multiple alignment of the sequences of nuclease domains of selected bacterial toxins (line 1: colicin E7, line 2: colicin E2, line 3: colicin E9 and line 4: S-type pyocin) is shown for identification of the conserved amino acids. The C-terminal sequence encompassing the catalytic HNH motif is underlined.

DNase colicins establish strong nonspecific interaction with the DNA within the major groove. At the same time, the catalytic HNH motif is located in the minor groove of the substrate [89,96,106-109]. The HNH motif formed by the C-terminal ~ 45 residues coordinates a single metal ion by the imidazole side-chains of three histidines (e.g. by H544, H569 and H573 in NColE7) in a distorted tetrahedral coordination sphere. This arrangement allows for the binding of a water molecule that can be exchanged to the phosphodiester group of DNA. Thus, the metal ion within the active site becomes close to the scissile phosphodiester group suggesting that it directly participates in the catalytic process. Indeed, the presence of the metal ions is essential for the catalytic activity [97]. For NColE7 Zn^{2+} [96,105] was suggested to be the physiological metal ion, but there is still a debate about the quality of metal ions in colicin nucleases [89,96,109-117]. The apo NColE7 e.g. could be reactivated to a different extent by Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Ca^{2+} and Sr^{2+} [97,105]. The metal ion having a free coordination site may have essential multiple roles in DNA-cleavage: it binds to the scissile phosphodiester, polarizes the P-O bond for nucleophylic attack and stabilizes the phosphoanion transition state and/or the leaving group. It is also common among hydrolases that the metal ion activates a water molecule either for nucleophilic attack, or for the protonation of the laving group. The former function in HNH nucleases is supposed to be generated by the most conserved His residue - not coordinating to the metal ion - as already mentioned above.

Colicins are simultaneously expressed with their immunity proteins to protect the host cell. These proteins inhibit the interaction of colicin nuclease domains with DNA by establishing charge complementarity interactions with the nuclease [105, 118-124]. Thus, most of the investigated colicin nuclease domains have been prepared together with their immunity proteins independently of whether they were active and inactive. The consequence of this is multiple: (i) The solution of the nuclease/immunity protein complex must be acidified in order to separate the proteins. Under such conditions the metal ion is lost from the nuclease. This raises the question of the quality of the bound metal ion. (ii) The immunity protein, as well as, the DNA substrate has been demonstrated to induce the proper folding of the mutated nuclease domain [125,126]. Thus, even if the mutation would affect the structure of the nuclease domain itself, this can not be detected in the presence of the immunity protein or the substrate.

Crystal structures of selected HNH nucleases showed a common feature of having a positively charged amino acid side-chain outside the HNH sequence, but spatially close to the active site. Examples are, e.g. the Vvn endonuclease (PDB ID 1OUP) [87], Sm endonuclease (PDB ID 1G8T) [127], Nuclease A (PDB ID 1ZM8) [86] NColE7 (PDB ID 3FBD) [108]. R57 has been identified by a mutational analysis in the Serratia nuclease to be essential for catalysis [74, 128]. Previous studies also demonstrated that the catalytic activity of NColE7 drops to a low level or is completely cancelled upon modification or deletion of the positively charged N-terminal amino acids [116,129,130]. The arginine residue being distal in the amino acid sequence, but becoming spatially close to the active site is a conserved amino acid, a common feature of the HNH nucleases. The role of this amino acid may be multiple. It has been suggested to bind and stabilize the cleaved phosphate product [131]. As the presence of this residue or any positively charged residue potentially replacing proved to be essential for the catalytic activity of the HNH motif [129,130,132] it can also be considered to participate directly in the catalytic mechanism. Being a flexible residue, it may facilitate the proton transfer from the histidine general base towards the leaving alcoholate [133]. Such a role has been suggested recently in other hydrolytic enzymes [134,135].

Not only the positive charges at the N-terminus, but also the amino acids with hydrophobic side-chains, such as the W tryptophan in NColE7 has a high impact on the catalysis by stabilizing the structure of the enzyme (and also the catalytic centre) through its interactions with a hydrophobic pocket, also involving residues from the HNH motif. It has been demonstrated, that the absence of this residue abolishes the metal ion binding within the active centre [126]. On the other hand, the protein folding and so the metal ion affinity is rescued by the interactions with the immunity protein or the DNA molecule [125,126]. It also has to be mentioned that the hydrolytic reaction is furthermore facilitated by the bending of the DNA [96] due to the protein-binding, to which the positively charged residues may contribute, as well.

Recently it has been demonstrated that the mutation of the histidine residue behaving as the general base in the catalytic reaction of the HNH nucleases led to the inactive enzyme, which however, become active in the presence of synthetic imidazole [136]. Similarly, activation of the truncated N-terminal NColE7 inactive mutant was achieved to a various extent in the presence of arginine or the KRNK tetrapeptide mimicking the deleted sequence (unpublished results).

3. CONCLUSION

The intriguing feature of certain HNH nucleases, such as the nuclease domains of colicins is that the residues deriving from the opposite end of the amino acid sequence to the ac-

tive centre influence the catalytic process to a large extent. Individual inactive nuclease domains can be defined, which can be activated by the presence of appropriate protein segments. The necessity of, e.g. the N-terminal amino acids for the enzyme function provides a good opportunity to develop NColE7 into an artificial nuclease. The designed positive allosteric control is expected to greatly enhance the specificity of the new enzyme [137]. In an enzyme of this kind the activation of the hydrolysis occurs only when the DNA recognizing domain binds to its specific target, precisely positioning the controlling sequence into the catalytic site (Fig. **2**). Any off-target cleavage arising from the non-specific binding of the zinc finger unit – even in the case of the eventual partial degradation within the cell – is expected to be precluded in such a nuclease, because the latter events result in loss of allosteric activation.

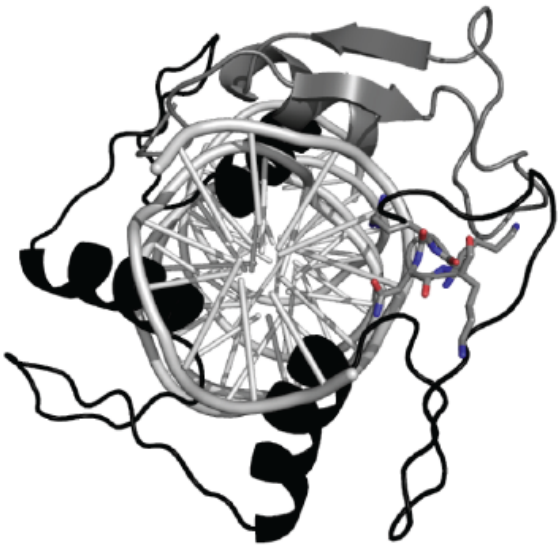

Fig. (2). The schematic view of a designed allosteric control in an HNH-based artificial nuclease. The zinc finger protein (black) positions the HNH motif and the regulatory positively charged sequence (both in grey – the latter highlighted by sticks) into a functional complex.

Furthermore, the fact that the missing essential residues or metal ions may be substituted by externally supplemented chemical agents, increases the chance to use the HNH nuclease domains as strictly controlled agents in gene engineering.

CONFLICT OF INTEREST

The author confirms that this article content has no conflicts of interest.

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REFERENCES

[1] Pauwels, K.; Podevin, N.; Breyer, D.; Carroll, D.; Herman, P. Engineering nucleases for gene targeting: safety and regulatory considerations. *New Biotechnol.*, **2014**, *31*, 18-27.

[2] Carroll, D. Genome engineering with targetable nucleases. *Annu. Rev. Biochem.*, **2014**, *83*, 409-439.

[3] Wood, A.J.; Lo, T.W.; Zeitler, B.; Pickle, C.S.; Ralston, E.J.; Lee, A.H.; Amora, R.; Miller, J.C.; Leung, E.; Meng, X.; Zhang, L.; Rebar, E.J.; Gregory, P.D.; Urnov, F.D.; Meyer, B.J. Targeted genome editing across species using ZFNs and TALENs. *Science*, **2011**, *333*, 307.

[4] Ochiai, H.; Miyamoto, T.; Kanai, A.; Hosoba, K.; Sakuma, T.; Kudo, Y.; Asami, K.; Ogawa, A.; Watanabe, A.; Kajii, T.; Yamamoto, T.; Matsuura, S. TALEN-mediated single-base-pair editing identification of an intergenic mutation upstream of BUB1B as causative of PCS (MVA) syndrome. *Proc. Natl. Acad. Sci. USA*, **2014**, *111*, 1461-1466.

[5] Gyurcsik, B.; Czene, A. *Future Med. Chem.*, **2011**, *3*, 1935-1966.

[6] Kaneko, T.; Mashimo, T. Creating knockout and knockin rodents using engineered endonucleases via direct embryo injection. *Methods Mol. Biol.*, **2015**, *1239*, 307-315.

[7] Bedell, V.M.; Ekker S.C. Using engineered endonucleases to create knockout and knockin zebrafish models. *Methods Mol. Biol.*, **2015**, *1239*, 291-305.

[8] Cai, CQ.; Doyon, Y.; Ainley, W.M.; Miller, J.C.; Dekelver, R.C.; Moehle, E.A.; Rock, J.M.; Lee, Y.L.; Garrison, R.; Schulenberg, L.; Blue, R.; Worden, A.; Baker, L.; Faraji, F.; Zhang, L.; Holmes, M.C.; Rebar, E.J.; Collingwood, T.N.; Rubin-Wilson, B.; Gregory, P.D.; Urnov, F.D.; Petolino, J.F. Targeted transgene integration in plant cells using designed zinc finger nucleases. *Plant Mol. Biol.*, **2009**, *69*, 699-709.

[9] Porteus, M.H. Plant biotechnology: zinc fingers on target. *Nature*, **2009**, *459*, 337-338.

[10] Peer, R.; Rivlin, G.; Golobovitch, S.; Lapidot, M.; Gal-On, A.; Vainstein, A.; Tzfira, T.; Flaishman, M.A. Targeted mutagenesis using zinc-finger nucleases in perennial fruit trees. *Planta*, **2014**,

[11] Urnov, F.D. Biological techniques: Edit the genome to understand it. *Nature*, **2014**, *513*, 40-41.

[12] Lieber, M.R. The mechanism of human nonhomologous DNA end joining. *J. Biol. Chem.*, **2008**, *283*, 1-5.

[13] Deriano, L.; Roth, D.B. Modernizing the nonhomologous end-joining repertoire: alternative and classical NHEJ share the stage. *Annu. Rev. Genet.*, **2013**, *47*, 433-455.

[14] Ousterout, D.G.; Perez-Pinera, P.; Thakore, P.I.; Kabadi, A.M.; Brown, M.T.; Qin, X.; Fedrigo, O.; Mouly, V.; Tremblay, J.P.; Gersbach, C.A. Reading frame correction by targeted genome editing restores dystrophin expression in cells from Duchenne muscular dystrophy patients. *Mol. Ther.*, **2013**, *21*, 1718-1726.

[15] Durai, S.; Mani, M.; Kandavelou, K.; Wu, J.; Porteus, M.H.; Chandrasegaran, S. Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells. *Nucl. Acids Res.*, **2005**, *33*, 5978-5990.

[16] Yan, Z.; Sun, X.; Engelhardt, J.F. Progress and prospects: techniques for site-directed mutagenesis in animal models. *Gene Ther.*, **2009**, *16*, 581-588.

[17] Stoddard, B.L. Homing endonucleases: from microbial genetic invaders to reagents for targeted DNA modification. *Structure*, **2011**, *19*, 7-15.

[18] Silva, G.; Poirot, L.; Galetto, R.; Smith, J.; Montoya, G.; Duchateau, P.; Pâques, F. Meganucleases and other tools for targeted genome engineering: perspectives and challenges for gene therapy. *Curr. Gene Ther.*, **2011**, *11*, 11-27.

[19] Lieberman-Lazarovich, M.; Levy, A.A. Homologous recombination in plants: an antireview. *Methods Mol. Biol.*, **2011**, *701*, 51-65.

[20] Kong, X.; Ball, A.R. Jr.; Pham, H.X.; Zeng, W.; Chen, H.Y.; Schmiesing, J.A.; Kim, J.S.; Berns, M.; Yokomori, K. Distinct functions of human cohesin-SA1 and cohesin-SA2 in double-strand break repair. *Mol. Cell Biol.*, **2014**, *34*, 685-698.

[21] Metzger, M.J.; Stoddard, B.L.; Monnat, R.J. Jr. PARP-mediated repair, homologous recombination, and back-up non-homologous end joining-like repair of single-strand nicks. *DNA Repair*, **2013**, *12*, 529-34.

[22] Harris, K.L.; Lim, S.; Franklin, S.J. Of folding and function: understanding active-site context through metalloenzyme design. *Inorg. Chem.*, **2006**, *45*, 10002-10012.

[23] Cowan, J.A. Chemical nucleases, *Curr. Opin. Chem. Biol.*, **2001**, *5*, 634-642.

[24] Franklin, S.J. Lanthanide-mediated DNA hydrolysis, *Curr. Opin. Chem. Biol.*, **2001**, *5*, 201-208.

[25] Wang, J.T.; Xia, Q.; Zheng, X.H.; Chen, H.Y.; Chao, H.; Mao, Z.W.; Ji, L.N. An effective approach to artificial nucleases using copper(II) complexes bearing nucleobases. *Dalton Trans.*, **2010**, *39*, 2128-2136.

[26] Stoddard, B.L., Homing endonucleases from mobile group I introns: discovery to genome engineering, *Mobile DNA*, **2014**, 5:7

[27] Jabalameli, H.R.; Zahednasab, H.; Karimi-Moghaddam, A.; Jabalameli, M.R. Zinc finger nuclease technology: advances and obstacles in modelling and treating genetic disorders. *Gene*, **2015**, *558*, 1-5.

[28] Chou, S.T.; Leng, Q.; Mixson, A.J. Zinc finger nucleases: Tailor-made for gene therapy. *Drugs Future.*, **2012**, *37*, 183-196.

[29] Doyle, E.L.; Stoddard, B.L.; Voytas, D.F.; Bogdanove, A.J. TAL effectors: highly adaptable phytobacterial virulence factors and readily engineered DNA-targeting proteins. *Trends Cell Biol.*, **2013**, *23*, 390-398.

[30] Sanjana, N.E.; Cong, L.; Zhou, Y.; Cunniff, M.M.; Feng, G.; Zhang, F. A transcription activator-like effector toolbox for genome engineering. *Nat. Protoc.*, **2012**, *7*, 171-192.

[31] van der Oost, J.; Westra, E.R.; Jackson, R.N.; Wiedenheft, B. Unravelling the structural and mechanistic basis of CRISPR-Cas systems. *Nat. Rev. Microbiol.*, **2014**, *12*, 479-492.

[32] Gaj, T.; Gersbach, C.A.; Barbas, C.F. 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol.*, **2013**, *31*, 397-405.

[33] Kim, H.; Kim, J.S. A guide to genome engineering with programmable nucleases. *Nat. Rev. Genet.*, **2014**, *15*, 321-334.

[34] Tebas, P.; Stein, D.; Tang. W.W.; Frank, I.; Wang, S.Q.; Lee, G.; Spratt, S.K.; Surosky, R.T; Giedlin, M.A.; Nichol, G.; Holmes, M.C.; Gregory, P.D.; Ando, D.G.; Kalos, M.; Collman, R.G.; Binder-Scholl, G.; Plesa, G.; Hwang, W.T.; Levine, B.L.; June, C.H. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. *N. Engl. J. Med.*, **2014**, *370*, 901-910.

[35] Händel, E.M.; Cathomen, T. Zinc-finger nuclease based genome surgery: it's all about specificity. *Curr. Gene Ther.*, **2011**, *11*, 28-37.

[36] Mussolino, C.; Cathomen, T. On target? Tracing zinc-finger-nuclease specificity. Nat. Methods., 2011, 8, 725-726.

[37] Kim, Y.G.; Cha, J.; Chandrasegaran, S. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. *Proc. Natl Acad. Sci. USA*, **1996**, *93*, 1156-1160.

[38] Li, L.; Wu, L.P.; Chandrasegaran, S. Functional domains in FokI restriction endonuclease. *Proc. Natl. Acad. Sci. USA,* **1992**, *89*, 4275-4279.

[39] Catto, L.E.; Ganguly, S.; Milsom, S.E.; Welsh, A.J.; Halford, S.E. Protein assembly and DNA looping by the FokI restriction endonuclease. *Nucl. Acids Res.*, **2006**, *34*, 1711-1720.

[40] Wah, D.A.; Hirsch, J.A.; Dorner, L.F.; Schildkraut, I.; Aggarwal, A.K. Structure of the multimodular endonuclease FokI bound to DNA. *Nature*, **1997**, *388*, 97-100.

[41] Wah, D.A.; Bitinaite, J.; Schildkraut, I.; Aggarwal, A.K. Structure of FokI has implications for DNA cleavage. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 10564-10569.

[42] Szczepek, M,; Brondani, V.; Buchel, J.; Serrano, L.; Segal, D.J.; Cathomen, T. Structure-based redesign of the dimerization interface reduces the toxicity of zinc-finger nucleases. *Nature Biotechnol.*, **2007**, *25*, 786-793.

[43] Minczuk, M.; Papworth, M.A.; Miller, J.C.; Murphy, M.P.; Klug, A. Development of a single-chain, quasi-dimeric zinc-finger nuclease for the selective degradation of mutated human mitochondrial DNA. *Nucl. Acids Res.*, **2008**, *36*, 3926-3938.

[44] Segal, D.J. Zinc-finger nucleases transition to the CoDA. *Nature Methods*, **2011**, *8*, 53-55.

[45] Mussolino, C.; Alzubi, J.; Fine, E.J.; Morbitzer, R.; Cradick, T.J.; Lahaye, T.; Bao, G.; Cathomen, T. TALENs facilitate targeted genome editing in human cells with high specificity and low cytotoxicity. *Nucl. Acids Res.*, **2014**, *42*, 6762-6773.

[46] Chan, S.H.; Stoddard, B.L.; Xu, S.Y. Natural and engineered nicking endonucleases--from cleavage mechanism to engineering of strand-specificity. *Nucl. Acids Res.*, **2011**, 39, 1-18.

[47] Ramirez, C.L.; Certo, M.T.; Mussolino, C.; Goodwin, M.J.; Cradick, T.J.; McCaffrey, A.P.; Cathomen, T.; Scharenberg, A.M.; Joung, J.K. Engineered zinc finger nickases induce homology-directed repair with reduced mutagenic effects. *Nucl. Acids Res.*, **2012**, *40*, 5560-5568.

[48] Kim, E.; Kim, S.; Kim, D.H.; Choi, B.S.; Choi, I.Y.; Kim, J.S. Precision genome engineering with programmable DNA-nicking enzymes. *Genome Res.*, **2012**, *22*, 1327-1333.

[49] Wu, Y.; Gao, T.; Wang, X.; Hu, Y.; Hu, X.; Hu, Z.; Pang, J.; Li, Z.; Xue, J.; Feng, M.; Wu, L.; Liang, D. TALE nickase mediates high efficient targeted transgene integration at the human multi-copy ribosomal DNA locus. *Biochem. Biophys. Res. Commun.*, **2014**, *446*, 261-266.

[50] Cho, S.W.; Kim, S.; Kim, Y.; Kweon, J.; Kim, H.S.; Bae, S.; Kim, J.S. Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. *Genome Res.*, **2014**, *24*, 132-141.

[51] Shen, B.; Zhang, W.; Zhang, J.; Zhou, J.; Wang, J.; Chen, L.; Wang, L.; Hodgkins, A.; Iyer, V.; Huang, X.; Skarnes, W.C. Efficient genome modification by CRISPR-Cas9 nickase with minimal off-target effects. *Nat. Methods*, **2014**, *11*, 399-402.

[52] Davis, L.; Maizels, N. Homology-directed repair of DNA nicks via pathways distinct from canonical double-strand break repair. *Proc. Natl. Acad Sci. USA*, **2014**, *111*, E924-932.

[53] Lin, J.; Chen, H.; Luo, L.; Lai, Y.; Xie, W.; Kee, K. Creating a monomeric endonuclease TALE-I-SceI with high specificity and low genotoxicity in human cells. *Nucl. Acids Res.*, **2015**, *43*, 1112-1122.

[54] Kleinstiver, B.P.; Wang, L.; Wolfs, J.M.; Kolaczyk, T.; McDowell, B.; Wang, X.; Schild-Poulter, C.; Bogdanove, A.J.; Edgell, D.R. The I-TevI nuclease and linker domains contribute to the specificity of monomeric TALENs. *G3 (Bethesda)*, **2014**, *4*, 1155-1165.

[55] Yanik, M.; Alzubi, J.; Lahaye, T.; Cathomen, T.; Pingoud, A.; Wende, W. TALE-PvuII fusion proteins-novel tools for gene targeting. *PLoS One.*, **2013**, *8*, e82539.

[56] Gabsalilow, L.; Schierling, B.; Friedhoff, P.; Pingoud, A.; Wende, W. Site- and strand-specific nicking of DNA by fusion proteins derived from MutH and I-SceI or TALE repeats. *Nucl. Acids Res.*, **2013**, *41*, e83

[57] Chan, S.H.; Bao, Y.; Ciszak, E.; Laget, S.; Xu, S.Y. Catalytic domain of restriction endonuclease BmrI as a cleavage module for engineering endonucleases with novel substrate specificities. *Nucl. Acids Res.*, **2007**, *35*, 6238-6248.

[58] Xu, S.Y.; Gupta, Y.K. Natural zinc ribbon HNH endonucleases and engineered zinc finger nicking endonuclease. *Nucl. Acids Res.*, **2013**, *41*, 378-390.

[59] Imanishi, M.; Negi, S.; Sugiura, Y. Non-FokI-based zinc finger nucleases. *Methods Mol. Biol.*, **2010**, *649*, 337-349.

[60] Negi, S.; Umeda, Y.; Masuyama, S.; Kano, K.; Sugiura, Y. Novel zinc finger nuclease created by combining the Cys(2)His(2)- and His(4)-type zinc finger domains. *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 2789-2791.

[61] Dhanasekaran, M.; Negi, S.; Sugiura, Y. Designer zinc finger proteins: tools for creating artificial DNA-binding functional proteins. *Acc. Chem. Res.*, **2006**, *39*, 45-52.

[62] Zaremba, M.; Siksnys, V. Molecular scissors under light control. *Proc. Natl. Acad. Sci. USA*, **2010**, *107*, 1259-1260.

[63] Yang, W.Y.; Breiner, B.; Kovalenko, S.V. C-Lysine conjugates: pH-controlled light-activated reagents for efficient double-stranded DNA cleavage with implications for cancer therapy. *J. Am. Chem. Soc.*, **2009**, *131*, 11458-11470.

[64] Strickland, D.; Moffat, K.; Sosnick, T.R. Light-activated DNA binding in a designed allosteric protein. *Proc. Natl. Acad. Sci. USA*, **2008**, *105*, 10709-10714.

[65] Doyon, Y.; Choi, V.M.; Xia, D.F.; Thuy, D.; Gregory, P.D.; Holmes, M.C. Transient cold shock enhances zinc-finger nuclease–mediated gene disruption. *Nature Methods*, **2010**, *7*, 459-460.

[66] Gruenig, M.C.; Lu, D.; Won, S.J.; Dulberger, C.L.; Manlick, A.J.; Keck, J.L.; Cox, M.M. Creating directed double-strand breaks with the Ref protein: a novel RecA-dependent nuclease from bacteriophage P1. *J. Biol. Chem.*, **2011**, *286*, 8240-8251.

[67] Xu, S.Y.; Corvaglia, A.R.; Chan, S.H.; Zheng, Y.; Linder, P. A type IV modification-dependent restriction enzyme SauUSI from Staphylococcus aureus subsp. aureus USA300. *Nucl. Acids Res.*, **2011**, *39*, 5597-5610.

[68] Lambert, A.R.; Sussman, D.; Shen, B.; Maunus, R.; Nix, J.; Samuelson, J.; Xu, S.Y.; Stoddard, B.L. Structures of the rare-cutting restriction endonuclease NotI reveal a unique metal binding fold involved in DNA binding. *Structure*, **2008**, *16*, 558-569.

[69] Fine, E.J.; Cradick, T.J.; Zhao, C.L.; Lin, Y.; Bao, G. An online bioinformatics tool predicts zinc finger and TALE nuclease off-target cleavage. *Nucl. Acids Res.*, **2014**, *42*, e42.

[70] Eastberg, J.H.; Eklund, J.; Monnat, R.; Stoddard, B.L. Mutability of an HNH nuclease imidazole general base and exchange of a deprotonation mechanism. *Biochemistry*, **2007**, *46*, 7215-7225.

[71] Orlowski, J.; Bujnicki, J.M. Structural and evolutionary classification of Type II restriction enzymes based on theoretical and experimental analyses. *Nucl. Acids Res.*, **2008**, *36*, 3552-3569.

[72] Finn, R.D.; Tate, J.; Mistry, J.; Coggill, P.C.; Sammut, S.J.; Hotz, H.R.; Ceric, G.; Forslund, K.; Eddy, S.R.; Sonnhammer, E.L.L.; Bateman, A. The Pfam protein families database. *Nucl. Acids Res.*, **2008**, *36*, D281-D288

[73] Finn, R.D.; Bateman, A.; Clements, J.; Coggill, P.; Eberhardt, R.Y.; Eddy, S.R.; Heger, A.; Hetherington, K.; Holm, L.; Mistry, J.; Sonnhammer, E.L.; Tate, J.; Punta, M. Pfam: the protein families database. *Nucl. Acids Res.*, **2014**, *42*, D222-230.

[74] Friedhoff, P.; Franke, I.; Meiss, G.; Wende, W.; Krause, K.L.; Pingoud, A. A similar active site for non-specific and specific endonucleases. *Nat. Struct. Biol.*, **1999**, *6*, 112-113.

[75] Mehta, P.; Katta, K.; Krishnaswamy, S. HNH family subclassification leads to identification of commonality in the His-Me endonuclease superfamily. *Protein Sci.*, **2004**, *13*, 295-300.

[76] Shen, B.W.; Landthaler, M.; Shub, D.A.; Stoddard, B.L. DNA binding and cleavage by the HNH homing endonuclease I-HmuI. *J. Mol. Biol.*, **2004**, *42*, 43-56.

[77] Jakubauskas, A.; Giedriene, J.; Bujnicki, J.M.; Janulaitis, A. Identification of a single HNH active site in type IIS restriction endonuclease Eco31I. *J. Mol. Biol.*, **2007**, *370*, 157-169.

[78] Cymerman, I.A.; Obarska, A.; Skowronek, K.J.; Lubys, A.; Bujnicki, M.J.M. Identification of a new subfamily of HNH nucleases and experimental characterization of a representative member, HphI restriction endonuclease. *Proteins*, **2006**, *65*, 867-876.

[79] Saravanan, M.; Bujnicki, J.M.; Cymerman, I.A.; Rao, D.N.; Nagaraja, V. Type II restriction endonuclease R.KpnI is a member of the HNH nuclease superfamily. *Nucl. Acids Res.*, **2004**, *32*, 6129-6135.

[80] Saravanan, M.; Vasu, K.; Ghosh, S.; Nagaraja, V. Dual role for Zn2+ in maintaining structural integrity and inducing DNA sequence specificity in a promiscuous endonuclease. *J. Biol. Chem.*, **2007**, *282*, 32320-32326.

[81] Kriukiene, E.; Lubiene, J.; Lagunavicius, A.; Lubys, A. MnlI--The member of H-N-H subtype of Type IIS restriction endonucleases. *Biochim. Biophys. Acta*, **2005**, *1751*, 194-204.

[82] Sokolowska, M.; Czapinska, H.; Bochtler, M. Crystal structure of the beta beta alpha-Me type II restriction endonuclease Hpy99I with target DNA. *Nucl. Acids Res.*, **2009**, *37*, 3799-3810.

[83] Shen, B.W.; Heiter, D.F.; Chan, S.H.; Wang, H.; Xu, S.Y.; Morgan, R.D.; Wilson, G.G.; Stoddard, B.L. Unusual target site disruption by the rare-cutting HNH restriction endonuclease PacI. *Structure*, **2010**, *18*, 734-743.

[84] Nishimasu, H.; Ran, F.A.; Hsu, P.D.; Konermann, S.; Shehata, S.I.; Dohmae, N.; Ishitani, R.; Zhang, F.; Nureki, O. Crystal structure of Cas9 in complex with guide RNA and target DNA. *Cell*, **2014**, *156*, 935-949.

[85] Anders, C.; Niewoehner, O.; Duerst, A.; Jinek, M. Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. *Nature*, **2014**, *513*, 569-573.

[86] Ghosh, M.; Meiss, G.; Pingoud, A.; London, R.E.; Pedersen, L.C. Structural insights into the mechanism of nuclease A, a beta beta alpha metal nuclease from Anabaena. *J. Biol. Chem.*, **2005**, *280*, 27990-27997.

[87] Li, C-L.; Hor, L-I.; Chang, Z-F.; Tsai, L-C.; Yang, W-Z.; Yuan, H.S. DNA binding and cleavage by the periplasmic nuclease Vvn: a novel structure with a known active site. *EMBO J.*, **2003**, *22*, 4014-4025.

[88] Scholz, S.R.; Korn, C.; Bujnicki, J.M.; Gimadutdinow, O.; Pingoud, A.; Meiss, G. Experimental evidence for a beta beta alpha-Me-finger nuclease motif to represent the active site of the caspase-activated DNase. *Biochemistry*, **2003**, *42*, 9288-9294.

[89] Papadakos, G.; Wojdyla, J.A.; Kleanthous, C. Nuclease colicins and their immunity proteins. *Q. Rev. Biophys.*, **2012**, *45*, 57-103.

[90] Ghequire, M.G.; De Mot, R. Ribosomally encoded antibacterial proteins and peptides from Pseudomonas. *FEMS Microbiol. Rev.*, **2014**, *38*, 523-568.

[91] Veluchamy, A.; Mary, S.; Acharya, V.; Mehta, P.; Deva, T.; Krishnaswamy, S. HNHDb: a database on pattern based classification of HNH domains reveals functional relevance of sequence patterns and domain associations. *Bioinformation*, **2009**, *6*, 80-83.

[92] Jantz, D.; Amann, B.T.; Gatto, G.J.; Berg J.M. The design of functional DNA-binding proteins based on zinc finger domains. *Chem. Rev.*, **2004**, *104*, 789-799.

[93] Biertümpfel, C.; Yang, W.; Suck, D. Crystal structure of T4 endonuclease VII resolving a Holliday junction. *Nature*, **2007**, *449*, 616-620.

[94] Huang, H.; Yuan, H.S. The conserved asparagine in the HNH motif serves an important structural role in metal finger endonucleases. *J. Mol. Biol.*, **2007**, *368*, 812-821.

[95] Kühlmann, U.C.; Moore, G.R.; James, R.; Kleanthous, C.; Hemmings, A.M. Structural parsimony in endonuclease active sites: should the number of homing endonuclease families be redefined? *FEBS Lett.*, **1999**, *463*, 1-2.

[96] Doudeva, L.G.; Huang, H.; Hsia, K-C.; Shi, Z.; Li, C-L.; Shen, Y.; Cheng, C-L.; Yuan, H.S. Crystal structural analysis and metal-dependent stability and activity studies of the ColE7 endonuclease domain in complex with DNA/Zn2+ or inhibitor/Ni2+. *Protein Sci.*, **2006**, *15*, 269-280.

[97] Ku, W.; Liu, Y.; Hsu, Y.; Liao, C.; Liang, P.; Yuan, H.; Chak, K. The zinc ion in the HNH motif of the endonuclease domain of colicin E7 is not required for DNA binding but is essential for DNA hydrolysis. *Nucl. Acids Res.*, **2002**, *30*, 1670-1678.

[98] Flick, K.E.; Jurica, M.A.; Monnat, R.J.; Stoddard, B.L. DNA binding and cleavage by the nuclear intron-encoded homing endonuclease I-PpoI. *Nature*, **1998**, *394*, 96-101.

[99] Galburt, E.A.; Jurica, M.S. The His-Cys box homing endonuclease family. In: *Homing Endonucleases and Inteins*; Belfort, M.; Derbyshire, V.; Stoddard, B.; Wood, D. Ed.; Springer-Verlag, Berlin, **2005**, pp. 85-102.

[100] Corina, L.E.; Qiu, W.; Desai, A.; David, L.; Herrin, D.L. Biochemical and mutagenic analysis of I-CreII reveals distinct but important roles for both the HNH and GIY-YIG motifs. *Nucl. Acids Res.*, **2009**, *37*, 5810-5821.

[101] Lin, Y-H.; Liao, C-C.; Liang, P-H.; Yuan, H.S.; Chak, K-F. Involvement of colicin in the limited protection of the colicin producing cells against bacteriophage. *Biochem. Biophys. Res. Commun.*, **2004**, *318*, 81-87.

[102] Chak, K-F.; Kuo, W-S.; Lu, F-M.; James, R. *J. Gen. Microbiol.*, **1991**, *137*, 91-100.

[103] Housden, N.G.; Kleanthous, C. Colicin translocation across the Escherichia coli outer membrane. *Biochem. Soc. Trans.*, **2012**, *40*, 1475-1479.

[104] Mora, L.; de Zamaroczy, M. *In vivo* processing of DNase colicins E2 and E7 is required for their import into the cytoplasm of target cells. *PLoS ONE*, **2014**, *9*, e96549.

[105] Sui, M-J.; Tsai, L-C.; Hsia, K-C.; Doudeva, L.G.; Ku, W-Y.; Han, G.W.; Yuan, H.S. Metal ions and phosphate binding in the H-N-H motif: Crystal structures of the nuclease domain of ColE7/Im7 in complex with a phosphate ion and different divalent metal ions. *Protein Sci.*, **2002**, *11*, 2947-2957.

[106] Hsia, K-C.; Chak, K-F.; Liang, P-H.; Cheng, Y-S.; Ku, W-Y.; Yuan, H.S. DNA binding and degradation by the HNH protein ColE7. *Structure*, **2004**, *12*, 205-214.

[107] Wang, Y-T.; Yang, W-J.; Li, C-L.; Doudeva, L.G.; Yuan, H.S. *Nucl. Acids Res.*, Structural basis for sequence-dependent DNA cleavage by nonspecific endonucleases. **2007**, *35*, 584-594.

[108] Wang, Y-T.; Wright, J.D.; Doudeva, L.G.; Jhang, H-C.; Lim, C.; Yuan, H.S. Redesign of high-affinity nonspecific nucleases with altered sequence preference. *J. Am. Chem. Soc.*, **2009**, *131*, 17345-17353.

[109] Mate, M.J.; Kleanthous, C. Structure-based analysis of the metal-dependent mechanism of H-N-H endonucleases. *J. Biol. Chem.*, **2004**, *279*, 34763-34769

[110] Pommer, A.J.; Kuhlmann, U.C.; Cooper, A.; Hemmings, A.M.; Moore, G.R.; James, R.; Kleanthous, C. Homing in on the role of transition metals in the HNH motif of colicin endonucleases. *J. Biol. Chem.*, **1999**, *274*, 27153-27160.

[111] Hannan, J.P.; Whittaker, S.B.M.; Hemmings, A.M.; James, R.; Kleanthous, C.; Moore, G.R. NMR studies of metal ion binding to the Zn-finger-like HNH motif of colicin E9 *J. Inorg. Biochem.*, **2000**, *79*, 365-370.

[112] Keeble, A.H.; Hemmings, A.M.; James, R.; Moore, G.R.; Kleanthous, C. Multistep binding of transition metals to the H-N-H

endonuclease toxin colicin E9. *Biochemistry*, **2002**, *41*, 10234-10244.

[113] van den Bremer, E.T.J.; Jiskoot, W.; James, R.; Moore, G.R.; Kleanthous, C.; Heck, A.J.R.; Maier, C.S. Probing metal ion binding and conformational properties of the colicin E9 endonuclease by electrospray ionization time-of-flight mass spectrometry. *Protein Sci.*, **2002**, *11*, 1738-1752.

[114] Hannan, J.P.; Whittaker, S.B.; Davy, S.L.; Kuhlmann, U.C.; Pommer, A.J.; Hemmings, A.M.; James, R.; Kleanthous, C.; Moore, G.R. NMR study of Ni2+ binding to the H-N-H endonuclease domain of colicin E9. *Protein Sci.*, **1999**, *8*, 1711-1713.

[115] van den Bremer, E.T.J.; Keeble, A.H.; Visser, A.J.W.G.; van Hoek, A.; Kleanthous, C.; Heck, A.J.R.; Jiskoot, W. Ligand-induced changes in the conformational dynamics of a bacterial cytotoxic endonuclease. *Biochemistry*, **2004**, *43*, 4347-4355.

[116] Czene, A.; Németh, E.; Zóka, I.G.; Jakab-Simon, N.I.; Körtvélyesi, T.; Nagata, K.; Christensen, H.E.M.; Gyurcsik, B. The role of the N-terminal loop in the function of the colicin E7 nuclease domain. *J. Biol. Inorg. Chem.*, **2013**, *18*, 309-321.

[117] Czene, A.; Tóth. E.; Németh, E.; Otten, H.; Poulsen, J.C.; Christensen, H.E.; Rulíšek, L.; Nagata, K.; Larsen, S.; Gyurcsik, B. A new insight into the zinc-dependent DNA-cleavage by the colicin E7 nuclease: a crystallographic and computational study. *Metallomics*, **2014**, *6*, 2090-2099.

[118] Cheng, Y-S.; Hsia, K-C.; Doudeva, L.G.; Chak, K-F.; Yuan, H.S. *J. Mol. Biol.*, **2002**, *324*, 227-236.

[119] Chak, K-F.; Safo, M.K.; Ku, W-Y.; Hsieh, S-Y.; Yuan, H.S. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 6437-6442.

[120] Hsieh, S-Y.; Ko, T-P.; Tseng, M-Y.; Ku, W-Y.; Chak, K-F.; Yuan, H.S. *EMBO J.*, **1997**, *16*, 1444-1454.

[121] Dennis, C.A.; Videler, H.; Paupit, R.A.; Wallis, R.; James, R.; Moore, G.R.; Kleanthous, C. *Biochem J.*, **1998**, *333*, 183-191.

[122] Ko, T-P.; Liao, C-C.; Ku, W-Y.; Chak, K-F.; Yuan, H.S. *Structure*, **1999**, *7*, 91-102.

[123] Kleanthous, C.; Walker, D. *Trends Biochem. Sci.*, **2001**, *26*, 624-631.

[124] Kolade, O.O.; Carr, S.B.; Kühlmann, U.C.; Pommer, A.; Kleanthous, C.; Bouchcinsky, C.A.; Hemmings, A.M. *Biochimie*, **2002**, *84*, 439-446.

[125] Németh, E.; Kožíšek, M.; Schilli, G.K.; Gyurcsik, B. Preorganization of the catalytic Zn2+-binding site in the HNH nuclease motif – a solution study. *J. Inorg. Biochem.*, **2015**, http://dx.doi.org/10.1016/j.jinorgbio.2015.03.017.

[126] Németh, E.; Körtvélyesi, T.; Kožíšek, M.; Thulstrup, P.W.; Christensen, H.E.M.; Asaka, M.N.; Nagata, K.; Gyurcsik B. Substrate binding activates the designed triple mutant of the colicin E7 metallonuclease. *J. Biol. Inorg. Chem.*, **2014**, *19*, 1295-1303.

[127] Shlyapnikov, S.V.; Lunin, V.V.; Perbandt, M.; Polyakov, K.M.; Lunin, V.Y.; Levdikov, V.M.; Betzel, C.; Mikhailov, A.M. Atomic structure of the Serratia marcescens endonuclease at 1.1 A resolution and the enzyme reaction mechanism. *Acta Crystallogr., Sect. D.*, **2000**, *56*, 567-572.

[128] Friedhoff, P.; Kolmes, B.; Gimadutdinow, O.; Wende, W.; Krause, K.L.; Pingoud, A. Analysis of the mechanism of the Serratia nuclease using site-directed mutagenesis. *Nucl. Acids Res.,* **1996**, *24*, 2632-2639.

[129] Shi, Z.; Chak, K-F.; Yuan, H.S. Identification of an essential cleavage site in ColE7 required for import and killing of cells. *J. Biol. Chem.*, **2005**, *280*, 24663-24668.]

[130] Németh, E.; Körtvélyesi, T.; Thulstrup, P.W.; Christensen, H.E.M.; Kožíšek, M.; Nagata, K.; Czene, A.; Gyurcsik, B. Fine tuning of the catalytic activity of colicin E7 nuclease domain by systematic N-terminal mutations. *Protein Sci.*, **2014**, *23*, 1113-1122.

[131] Hsia, K.C.; Li, C.L.; Yuan, H.S. Structural and functional insight into sugar-nonspecific nucleases in host defense. *Curr. Opin. Struct. Biol.*, **2005**, *15*, 126-134.

[132] Gyurcsik, B.; Czene, A.; Jankovics, H.; Jakab-Simon, N.I.; Ślaska-Kiss, K.; Kiss, A.; Kele, Z. Cloning, purification and metal binding of the HNH motif from colicin E7. *Protein Expr. Purif.*, **2013**, *89*, 210-218.

[133] Bueren-Calabuig, J.A.; Coderch, C.; Rico, E.; Jimenez-Ruiz, A.; Gago, F. Mechanistic insight into the catalytic activity of betabetaalpha-metallonucleases from computer simulations: Vibrio vulnificus periplasmic nuclease as a test case. *Chembiochem*, **2011**, *12*, 2615-2622.

[134] Keenholtz, R.A.; Mouw, K.W.; Boocock, M.R.; Li, N.S.; Piccirilli, J.A.; Rice, P.A. Arginine as a general acid catalyst in serine recombinase-mediated DNA cleavage. *J. Biol. Chem.*, **2013**, *288*, 29206-29214.

[135] Silva, P.J.; Schulz, C.; Jahn, D.; Jahn, M.; Ramos, M.J. A tale of two acids: when arginine is a more appropriate acid than H3O+. *J. Phys. Chem. B.*, **2010**, *114*, 8994-9001.

[136] Midon, M.; Gimadutdunow, O.; Meiss, G.; Friedhoff, P.; Pingoud, A. Chemical rescue of active site mutants of S. pneumoniae surface endonuclease EndA and other nucleases of the HNH family by imidazole. *ChemBioChem*, **2012**, *13*, 713-721.

[137] Németh, E.; Schilli, G.K.; Nagy, G.; Hasenhindl, C.; Gyurcsik, B.; Oostenbrink, C. Design of a colicin E7 based chimeric zinc-finger nuclease. *J. Comput. Aided Mol. Des.*, **2014**, *28*, 841-850.

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