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Short Communication

Expression of *DjXnp*, a novel member of the SNF2-like ATP-dependent chromatin remodelling genes, in intact and regenerating planarians

LEONARDO ROSSI[1], PAOLO DERI[2], ILARIA ANDREOLI[2], VITTORIO GREMIGNI[1], ALESSANDRA SALVETTI[1] and RENATA BATISTONI*[,2]

[1]*Dipartimento di Morfologia Umana e Biologia Applicata, Sezione di Biologia e Genetica and* [2]*Dipartimento di Fisiologia e Biochimica, Sezione di Biologia Cellulare e dello Sviluppo, Università di Pisa, Pisa, Italy*

ABSTRACT SWI/SNF-related complexes include proteins implicated in the regulation of gene expression by chromatin remodelling. We have identified in planarians, invertebrates well-known for their regenerative capability, the cDNA of a novel gene, *DjXnp*, which encodes a protein of 1,076 amino acids, containing seven helicase domains similar to those found in the SNF2-like family members. Sequence comparison reveals a significant degree of similarity of DjXNP with mammalian XNP/ATRX proteins. *In situ* hybridization experiments performed on intact and regenerating planarians demonstrated that *DjXnp* transcripts were distributed in mesenchymal cells and were especially abundant in nerve cells. During anterior regeneration, *DjXnp* was detected in the blastemal area where the nervous system is newly forming. This expression pattern reveals extensive similarities with that described for mammalian *XNP/ATRX*, suggesting that these genes may have a conserved function at the cellular level.

KEY WORDS: *planarians, regeneration, SWI/SNF, DNA helicase, XNP/ATRX*

The SNF2-like protein family includes DNA helicase/ATPases related to the SWI/SNF complexes involved in chromatin remodelling (Gorbalenya and Koonin, 1993; Eisen *et al.*, 1995). Within this family, XNP proteins are of special interest, since they may regulate the activity of specific genes in different chromosomal environments, as well as during neuronal development (Picketts *et al.*, 1996; Cardoso *et al.*, 1998). The human *XNP/ATRX* is expressed in the brain and other tissues. Mutations of this gene cause the ATR-X syndrome, an X-linked mental retardation disorder (Gibbons *et al.*, 1995). The murine homologue is involved in early stages of neuronal differentiation during embryogenesis (Gecz *et al.*, 1994; Stayton *et al.*, 1994). In addition to the helicase domains present in the C-terminal region, the mammalian XNP/ATRX proteins have an N-terminal region including three C2-C2 type zinc-finger motifs and a putative coiled coil domain (McDowell *et al.,* 1999; Cardoso *et al.*, 2000). This region, that corresponds to the exons 1-9 in the human gene, is absent in *Xnp-1*, a gene partially similar to *XNP/ATRX*, recently characterized in the nematode *C. elegans* (Villard *et al.*, 1999).

In a search for regulatory genes promoting differentiation of neoblasts - the totipotent stem cells of planarians (see Baguñà, 1998; Newmark and Sánchez-Alvarado, 2002) - by chromatin remodelling, we previously isolated in *Dugesia japonica* a cDNA fragment, *DjXnp*, showing similarity to human and mouse *XNP/ATRX* (Rossi *et al.*, 2001). The full-length sequence of *DjXnp* was established here by 5'/3' rapid amplification of cDNA ends (RACE). *DjXnp* is 3,734 bp long and has an open reading frame of 3,228 bp that encodes a protein of 1,076 amino acids, including seven putative helicase domains (Fig. 1A). The helicase domains of DjXNP share between 37% and 48% sequence identity with those found in known XNP-related proteins (Fig. 1B). A schematic alignment of these proteins is presented in Fig. 1C. Similarly to that found in *C. elegans* XNP-1 (Villard *et al.*, 1999) and *Drosophila* d-XNP, the whole DjXNP shows similarity only with the mammalian XNP/ATRX C-terminal region including the DNA helicase motifs (Appendix I). In these invertebrates, XNP proteins lack the N-terminal region,

Abbreviations used in this paper: ATRX, X-linked alpha-thalassemia with mental retardation; CNS, central nervous system; Dj, D. japonica; SWI/SNF, mating type switching/sucrose non-fermenting; XNP, X-linked nuclear protein.

***Address correspondence to:** Dr. Renata Batistoni. Dipartimento di Fisiologia e Biochimica, Sezione di Biologia Cellulare e dello Sviluppo, Universitá di Pisa, Via Carducci 13, 56010 Ghezzano (Pisa) Italy. Fax: +39-050-878-486. e-mail: batistoni@dfb.unipi.it

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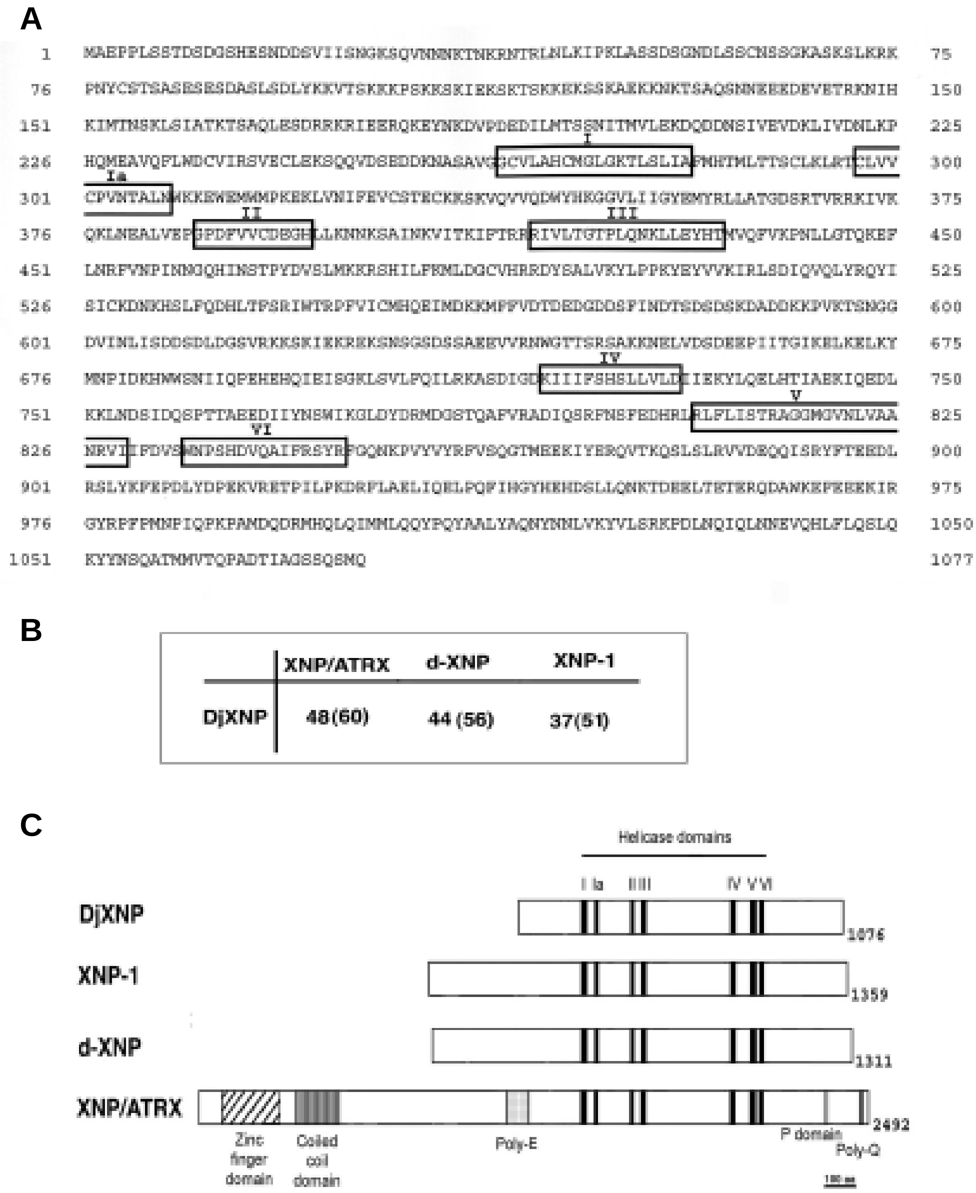


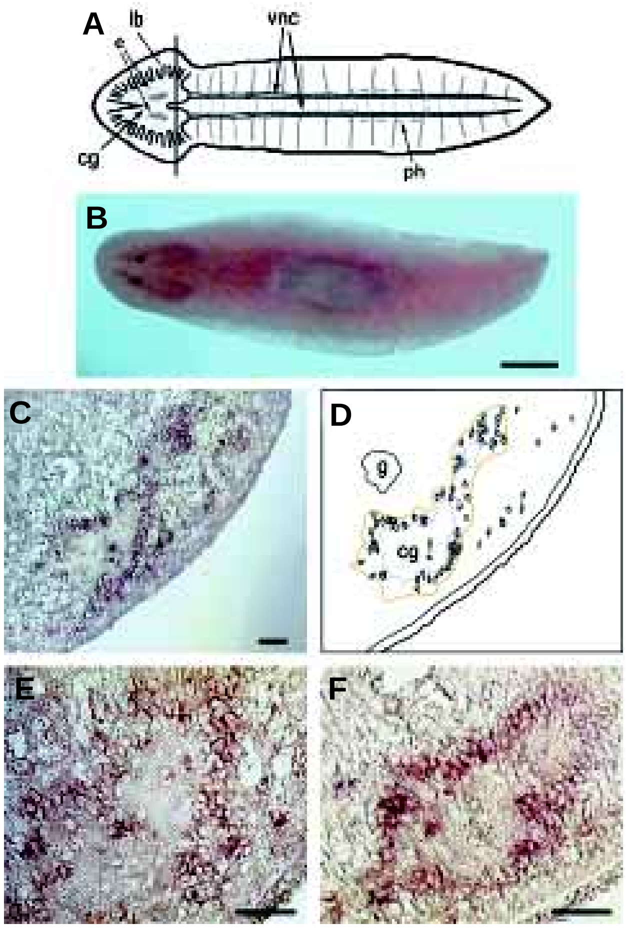
Fig. 1. Complete amino acid sequence of DjXNP, as deduced from nucleotide sequence analysis of *DjXnp* cDNA. *(Accession number AJ490823).* **(A)** *The DjXNP sequence is shown. The seven putative helicase domains I, Ia, II, III, IV, V, VI are boxed.* **(B)** *Pairwise comparisons of the DjXNP sequence region including the helicase domains with those of vertebrate and invertebrate XNP-related proteins:* C. elegans *XNP-1 (AF134186),* Drosophila *d-XNP (AF217802), human XNP/ATRX (U75653). The GAP program of the GCG software package was used. The degree of amino acid sequence identity and similarity (in brackets) is expressed as a percentage.* **(C)** *Schematic diagram of DjXNP compared with the XNP-related proteins indicated in B. Structural motifs are indicated. Numbers on the right refer to the amino acid residues of each protein.*

found in human and mouse XNP/ATRX. Although we cannot rule out a priori a phenomenon of convergent evolution, these findings suggest the possibility that the XNP-related genes derive from a common ancestral DNA helicase. The XNP/ATRX-specific N-terminal part might have in turn originated from one or more exon-shuffling events during mammalian or, possibly, vertebrate evolution (Villard *et al.*, 1997; Villard *et al.*, 1999).

The expression pattern of *DjXnp* was analyzed by *in situ* hybridization in intact and regenerating planarians. In addition to a diffuse transcription observed throughout the body mesenchyme, where differentiation of neoblasts ensures renewal of all cell types (see Baguñà, 1998; Newmark and Sánchez-Alvarado, 2002), *DjXnp* was highly expressed throughout the cephalic ganglia or 'brain'. This expression also extended to the proximal part of the brain lateral branches. Conversely, no preferential *Xnp* mRNA accumulation was observed in the two ventral nerve cords (VNC), which traverse the animal longitudinally (Fig. 2 A,B). In planarians the brain and VNC define morphologically the central nervous system (CNS), organized in distinct molecular domains (see Cebrià *et al.*, 2002 and references therein).

To investigate in detail the distribution of *DjXnp*-positive cells in the cephalic ganglia, we performed *in situ* hybridization directly on paraffin sections. *DjXnp* was expressed in a large number of nerve cells located along the periphery of the spongy regions of the cephalic ganglia (Fig. 2 C-E). The comparison with the expression pattern of the pan-neural marker synaptotagmin (Cebrià *et al.*, 2002) is shown in Fig. 2F.

During regeneration, the pattern of *DjXnp* expression in the amputated body fragments continued to be similar to that found in intact animals. *DjXnp* mRNA was never detected in the blastema of planarians regenerating a tail (Fig. 3 A-F). In planarians undergoing cephalic regeneration, *Xnp* expression was never observed in the blastema at 1 day after cutting (Fig. 3 G,L). In planarians regenerating a head, *DjXnp* mRNA accumulation was first seen in two bilaterally symmetrical blastemal regions at 3 days after cutting (Fig. 3 H,M). Later on, *DjXnp* appeared mainly localized in the area where the cephalic ganglia were forming (Fig. 3 I,N). The expression of *DjXnp* was also investigated by *in situ* hybridization on paraffin sections of planarians after 3 days of cephalic regeneration. Accumulation of *DjXnp* transcripts was detected in blastemal cells located close to the amputated nerve cords (Fig. 4 A,B). The analysis of synaptotagmin mRNA expression strongly supports the possibility that the *DjXnp*-positive cells represent blastema cells differentiating, or just differentiated, into brain nerve cells (Fig. 4C). On the whole, the



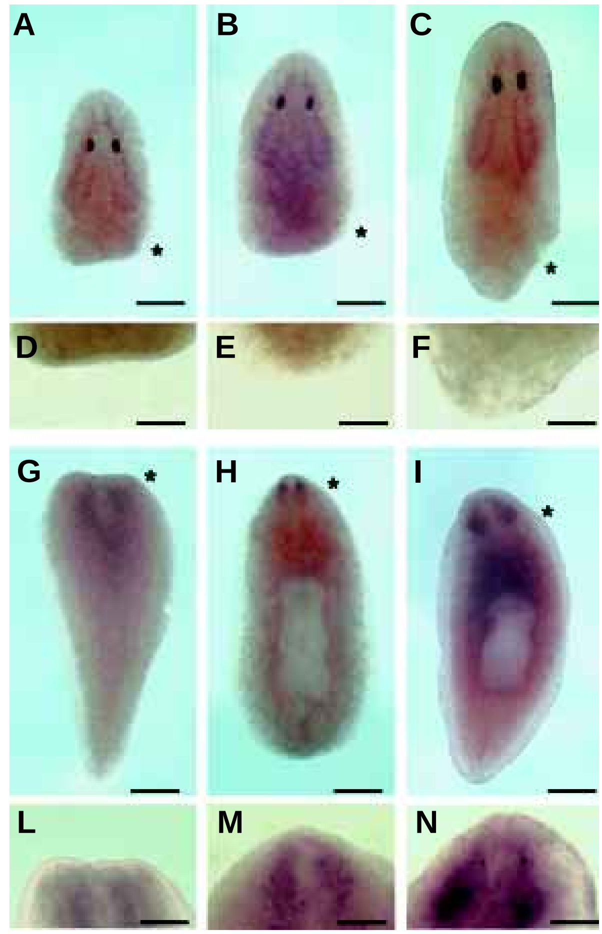


Fig. 2 (Left). *DjXnp* expression pattern in intact *D. japonica*, as detected by *in situ* hybridization. **(A)** *Diagram of the planarian body. The CNS is schematically represented, anterior is to the left. The plane of the sections depicted in C-F is indicated. cg, cephalic ganglia; e, eyes; lb, lateral branches; ph, pharynx; vnc, ventral nerve cords.* **(B)** *Ventral view of a planarian visualized by whole-mount* in situ *hybridization.* DjXnp *transcripts accumulated in the cephalic ganglia and in the proximal part of the lateral branches. (C-F)* In situ *hybridization on transverse sections.* **(C)** DjXnp*-expressing cells are distributed in the peripheral region of the cephalic ganglia.* **(D)** *Camera lucida drawing of the section depicted in (C), illustrating the various morphological structures. cg, cephalic ganglion; g, gut.* **(E)** *High magnification showing* DjXnp *mRNA accumulation in nerve cells.* **(F)** *Expression of the nerve cell marker* DjSyt *(Tazaki* et al.*, 1999; Cebrià* et al.*, 2002). Scale bars, 500 μm in B and 50 μm in C-F.*

Fig. 3 (Right). *DjXnp* expression pattern in regenerating *D. japonica*, as detected by whole mount *in situ* hybridization. *(A,D,G,L, 1 day post-amputation; B,E,H,M, 3 days post-amputation; C,F,I,N, 6 days post-amputation.* **(A,B,C)** *A planarian fragment regenerating a tail.* **(D,E,F)** *Magnified views of the regenerating areas indicated by an asterisk in A, B and C respectively.* **(G,H,I)** *A planarian fragment regenerating a head.* **(L,M,N)** *Magnified view of the regenerating areas indicated by an asterisk in G, H and I respectively. Scale bars, 500 μm in A-C and G-I, 200 μm in D-F and L-N.*

spatial and temporal expression patterns of *DjXnp* strongly suggest that this gene may be involved in differentiation and/or maintenance of nerve cells in planarians. Interestingly, *XNP/ATRX* is also associated to brain development in mammals (Gecz *et al.*, 1994; Stayton *et al.*, 1994). These data support the possibility that *Xnp*-related genes may share a similar role in the cells of distantly related organisms, and suggest that an ancient biochemical mechanism involving XNP-like proteins could have been conserved over the course of animal evolution.

Experimental Procedures

Planarians (Platyhelminthes, Tricladida) belonging to the asexual *Dugesia japonica* clonal strain GI (Orii *et al.*, 1993), were maintained and used as previously described (Salvetti *et al.*, 1998). The SMART RACE cDNA amplification kit (Clontech) was used to complete the *DjXnp* sequence. The 3' region (3' *DjXnp*) was isolated using the sequence-specific sense primer: 5'AATAAATTATTGGAATATCATACGATGG3'. The sequence-specific antisense primer 5'CCTTTTGTGTACCAAGAAG3' was used to amplify the 5' end (*5'DjXnp*). The PCR products were TA-cloned using pGEM-T easy vector (Promega). All clones were sequenced by automated fluorescent cycle sequencing (ABI). The DIG-RNA labeling kit (Roche) was used to produce *5'DjXnp* and synaptotagmin (*DjSyt*) digoxigenin (DIG)-labeled antisense and sense riboprobes. *In situ* hybridization procedures were perfomed as described by Agata *et al.* (1998) and Kobayashi *et al.* (1998).

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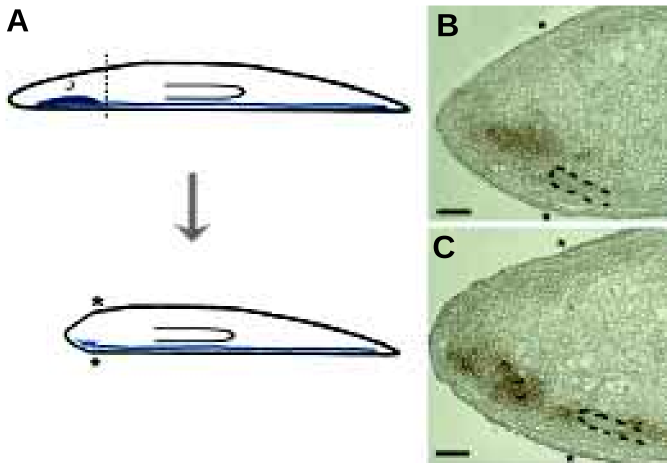

Fig. 4. Comparison between the expression patterns of *DjXnp* and *DjSyt* in *D. japonica* planarians regenerating a head, as detected by *in situ* hybridization on longitudinal sections, 3 days after cut. (A) *Schematic drawings of an intact planarian and a planarian regenerating a head. The CNS is schematically represented, anterior is to the left. The cutting site is indicated by a broken line. The blastema is delimited by two asterisks.* **(B)** DjXnp-*expressing cells accumulated in a blastema region close to an amputated nerve cord (outlined with a broken line).* **(C)** DjSyt *transcripts were observed in the blastema region where the cephalic ganglia are newly forming and in the cells of an old nerve cord (outlined with a broken line). Scale bars, 50 μm.*

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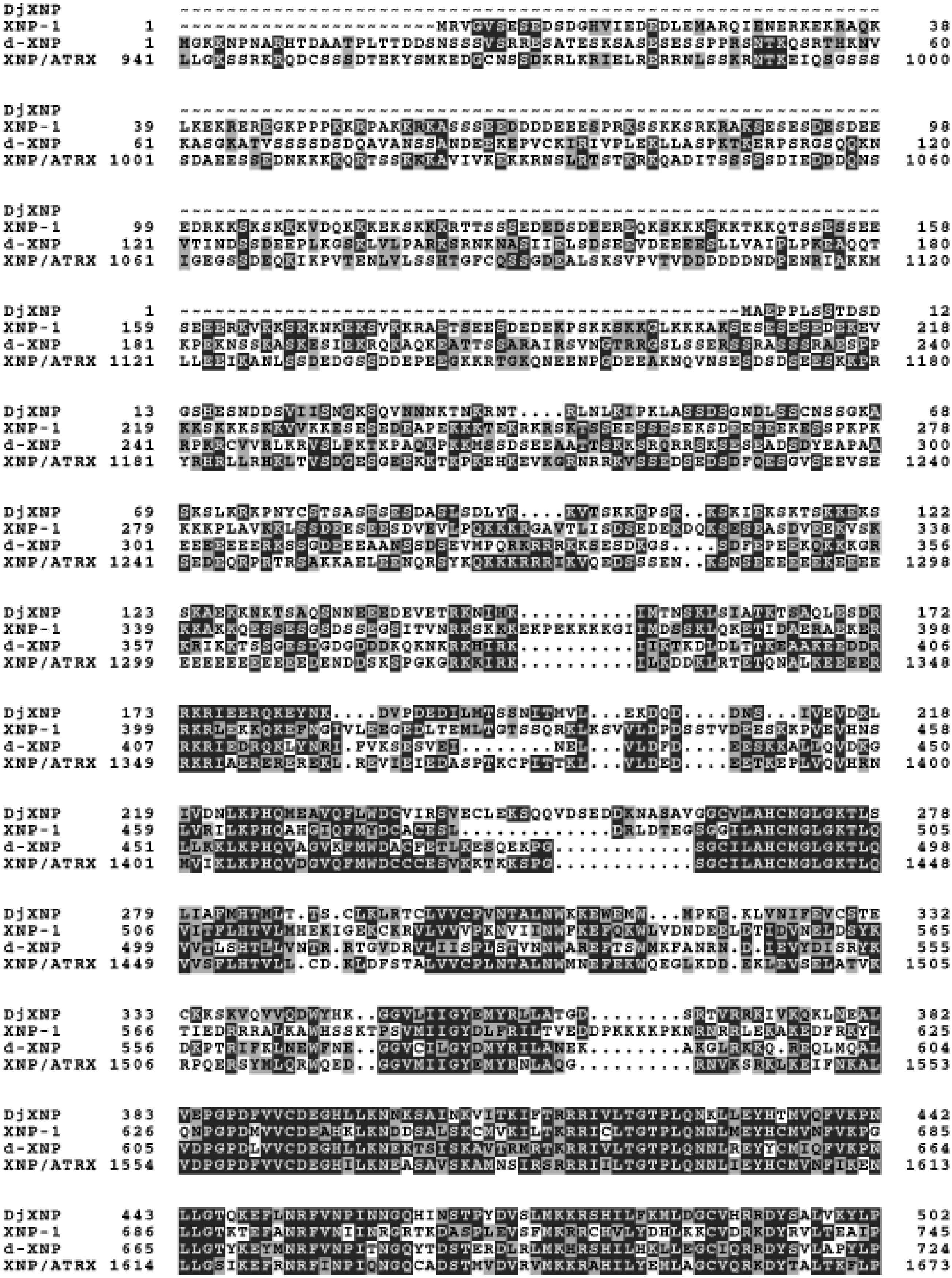
APPENDIX I (see opposite)

Comparison of the deduced amino acid sequence of DjXNP with those of other XNP proteins. Amino acid sequences were aligned using CLUSTAL W and visualised with BOXSHADE. The human XNP/ATRX sequence is shown from the amino acid position 941. Black backgrounds indicate identical residues and grey backgrounds indicate conserved residues.


DjXNP ~~~~~~
XNP-1 1 ~~~~~~~~~~~~~~~~~~~~~MRVGVSESEDSDGHVIEDEDLEMARQIENERKEKRAQK 38
d-XNP 1 MGKKNPNARHTDAATPLTTDDSNSSSVSRRESATESKSASESESSPPRSNTKQSRTHKNV 60
XNP/ATRX 941 LLGKSSRKRQDCSSSDTEKYSMKEDGCNSSDKRLKRIELRERRNLSSKRNTKEIQSGSSS 1000
DjXNP ~~~~~~
XNP-1 39 LKEKRERDGKPPPKKRPAKKRKASSSEEDDDDEEESPRKSSKKSRKRAKSESESDESDEE 98
d-XNP 61 KASGKATVSSSSDSDQAVANSSANDEEKEPVCKIRIVPLEKLLASPKTKERPSRGSQQKN 120
XNP/ATRX 1001 SDAEESSEDNKKKKQRTSSKKKAVIVKEKKRNSLRTSTKRKQADITSSSSSDIEDDDQNS 1060
DjXNP ~~~~~~
XNP-1 99 EDRKKSKSKKKVDQKKKEKSKKKRTTSSSEDEDSDEERSQKSKKKSKKTKKQTSSESSEE 158
d-XNP 121 VTINDSSDEEPLKGSKLVLPARKSRNKNASIIELSDSEEVDEEEESLLVAIPLPKEAQQT 180
XNP/ATRX 1061 IGEGSSDEQKIKPVTENLVLSSHTGFCQSSGDEALSKSVPVTVDDDDDDNDPENRIAKKM 1120
DjXNP 1 ---MAEPPLSSTDSD 12
XNP-1 159 SEEERKVKKSKKNKEKSVKKRAETSEESDEDEKPSKKSKKCLKKKAKSESESESEDEKEV 218
d-XNP 181 KPEKNSSKASKESIEKRQKAQKEATTSSARAIRSVNGTRRGSLSSERSSRASSSRAESPP 240
XNP/ATRX 1121 LLEEIKANLSSDEDGSSDDEPEEGKKRTGKQNEENPGDEEAKNQVNSESDSDSEESKKPR 1180
DjXNP 13 GSHESNDDSVIISNEKSQVNNNKTNKRNT....ELNLKIPKLASSDSGNDLSSCNSSGKA 68
XNP-1 219 KKSKKKSKKVVKKESESEDEAPEKKKTEKRKRSKKSSEESSSESEKSDEKEEEKESSPKPK 278
d-XNP 241 RPKKCVVRLKRVSLPKTKPAQKPKKMSSDSEEAAKTSKKSRQRRSKSESEADSDYEAPAA 300
XNP/ATRX 1181 YRHRLLRHKLTVSDGESGEEKKTKPKEHKEVKGRNRRKVSSEDSEDSDFQESGVSEEVSE 1240
DjXNP 69 SKSLKKKPNYCSTSASESESDASLSDLYK....KVTSKKKPSK..KSKIEKSKTSKKEKS 122
XNP-1 279 KKKPLAVKKLSSDEESEESDVEVLPQKKKRGAVTLISDSEDEKDQKSESEASDVEEKVSK 338
d-XNP 301 EEEEEEERKSSGDEEEAANSSDSEVMPQRKRRRKKSESDKGS....SDFEPEEKQKKKGR 356
XNP/ATRX 1241 SEDEQKPKTRSAKKAELEENQRSYKQKKKRRRIKVQEDSSSEN..KSNSEEEEEEKEEE 1298
DjXNP 123 SKAEKKNKTSAQSNNEEEDEVETRKNIHK..........IMTNSKLSIATKTSAQLESDR 172
XNP-1 339 KKAKKQESSESGSDSSEGSITVNRKSKKKEKPEKKKKGIIMDSSKLQKETIDAERAEKER 398
d-XNP 357 KRIKKTSSGESDGDGDDDKQKNKRKHIRK..........IIKTKDLDLTTKEAAKEEDDR 406
XNP/ATRX 1299 EEEEEEEEEEEDENDDSKSPGKGRKKIRK..........ILKDDKLRTETQNALKEEEER 1348
DjXNP 173 RKRIEERQKEYNK....DVPDEDILMTSSNITMVL...EKDQD....DNS...IVEVDKL 218
XNP-1 399 RKRLEKKQKEFNGIVLEEGEDLTEMLTGTSSQRKLKSVVLDPDSSTVDEESKKPVEVHNS 458
d-XNP 407 RKRIEDRQKLYNRI.PVKSESVEE........NEL...VLDFD....EESKKALLQVDKG 450
XNP/ATRX 1349 RKRIAEREREREKL.REVIEIEDASPTKCPITTKL...VLDED....EETKEPLVQVHRN 1400
DjXNP 219 IVDNLKPHQMEAVQFLWDCVIRSVECLEKSQQVDSEDDKNASAVGGCVLAHCMGLGKTLS 278
XNP-1 459 LVRILKPHQAHGIQFMYDCACESL.............DRLDTEGSGGILAHCMGLGKTLQ 505
d-XNP 451 LLKKLKPHQVAGVKFMWDACFETLKESQEKPG.............SGCILAHCMGLGKTLQ 498
XNP/ATRX 1401 MVIKLKPHQVDGVQFMWDCCCESVKKTKKSPG.............SGCILAHCMGLGKTLQ 1448
DjXNP 279 LIAFMHTMLT.TS.CLKLRTCLVVCPVNTALNWKKEWEMW...MPKE.KLVNIFEVCSTE 332
XNP-1 506 VITFLHTVLMHEKIGEKCKRVLVVVPKNVIINWFKEFQKWLVDNDEELDTIDVNELDSYK 565
d-XNP 499 VVTLSHTLLVNTR.RTGVDRVLIISPLSTVNNWAREFTSWMKFANRN.D.IEVYDISRYK 555
XNP/ATRX 1449 VVSFLHTVLL.CD.KLDFSTALVVCPLNTALNWMNEFEKWQEGLKDD.EKLEVSELATVK 1505
DjXNP 333 CKKSKVQVVQDWYHK..GGVLIIGYEMYRLLATGD.........SRTVRRKIVKQKLNEAL 382
XNP-1 566 TIEDRRRALKAWHSSKTPSVMIIGYDLFRILTVEDDPKKKKPKNRNRLEKAKEDFRKYL 625
d-XNP 556 DKPTRIFKLNEWFNE..GGVCILGYDMYRILANEK.........AKGLRKKQ.REQLMQAL 604
XNP/ATRX 1506 RPQERSYMLQRWQED..GGVMIIGYEMYRNLAQG..........RNVKSRKLKEIFNKAL 1553
DjXNP 383 VEPGPDFVVCDEGHLLKNNKSAINKVITKIFTRRRIVLTGTPLQNKLLEYHTMVQFVKPN 442
XNP-1 626 QNPGPDMVVCDEAHKLKNDDSALSKCMVKILTKRRICLTGTPLQNNLMEYHCMVNFVKPG 685
d-XNP 605 VDPGPDLVVCDEGHLLKNEKTSISKAVTRMRTKRRIVLTGTPLQNNLREYYCMIQFVKPN 664
XNP/ATRX 1554 VDPGPDFVVCDEGHILKNEASAVSKAMNSIRSRRRIILTGTPLQNNLIEYHCMVNFIKEN 1613
DjXNP 443 LLGTQKEFLNRFVNPINNGQHINSTPYDVSLMKKRSHILFKMLDGCVHRRDYSALVKYLP 502
XNP-1 686 LLGTKTEFANRFVNIINRGRTKDASPLEVSFMKRRCHVLYDHLKKCVDRKDYRVLTEAIP 745
d-XNP 665 LLGTYKEYMNRFVNPITNGQYTDSTERDLRLMKHRSHILHKLLEGCIQRRDYSVLAPYLP 724
XNP/ATRX 1614 LLGSIKEFRNRFINPIQNGQCADSTMVDVRVMKKRAHILYEMLAGCVQRKDYTALTKFLP 1673

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DjXNP      503  PKYEYVVKIRLSDIQVQLYRQYISICKDNKHS...........LFQDHLTFSRIWTRPFV   551
XNP-1      746  PKQEYVINVRQTERQCALYNAFLNDIVEDS........GLSKRLLPDYHMFSRIWTHPYQ   797
d-XNP      725  PKHEYVVYTTLSELQQKLYGYYM...TTHREQSGGDVVGKGARLFQDFQDLRRIWTHPMN   781
XNP/ATRX  1674  PKHEYVLAVRMTSIQCKLYQYYLDHLTGVGNNSEGGRGKAGAKLFQDFQMLSRIWTHPWC  1733

DjXNP      552  ICMHQEIMDKKMFLVDTDEDGDDSFINDTSDSDSKDADDKKPVKTSNGGDVINLISDDSD   611
XNP-1      798  LVLHEQRMERERVMREEAEEEAD.FIDDGDGSESESEGSFKSGSESDSGKSVVLSSDD..   854
d-XNP      782  LRVTSDNVIAKRLLSXDDSD.MEGFICDETDEDEAASNSSDSCETFKSDASMS......G   833
XNP/ATRX  1734  LQLDYISKENKGYF...DEDSMDEFIASDSDETS.................MSLSSDDYT  1773

DjXNP      612  LDGSVRKKSKIEKREKSNSGSDSSAE.EVVRNWGTTSRSAKKNELVDSDEEPIIT.GIKE   669
XNP-1      854  .EGSSKKKKNGNKPEIKKTAPQKKRKFLNSDDEDEEDGEDTAMAILQDGIRQSKRLAGEE   913
d-XNP      834  LAASSGKVKKRKTRNGNAGGGDSDSDLEMLGGLGGGSSVQK...................   874
XNP/ATRX  1773  ...KKKKKGKKGKKDSSSSGSGSDNDVEVIKVWNSRSRGGGEGNVDETGNNPSVSLKLEE  1830

DjXNP      670  LKELKYMKPID..KHWWS.NIIQPEHEHQIEISGKLSVLFQILRKASDIGDKIIIFSHSL   726
XNP-1      914  ADLRDTDTCPEYTGWFARLGLVKEEDRDDFALSNKLILLVEIIKKCEEIGDKLLVFSQSL   973
d-XNP      874  ...........DDPSEWWK.PFVEEREELNNVHHSPKLLILLRLLQQCEAIGDKLLVFSQSL   923
XNP/ATRX  1831  SKATSSSNPSSPAPDWYK.DFVTDADAEVLEHSGKMVLLFEILRMAEEIGDKVLVFSQSL  1889

DjXNP      727  LVLDIIEKYLQELHTIAEKIQEDLKKLNDSIDQSPTTAEEDIIYNSWIKGLDYDRMDGST   786
XNP-1      974  ESLTLIKRMLEYMAGTGQWFADGHEALNAEGEETW..........SWLEGEDYMTIDGSV  1023
d-XNP      924  QSLDVIEHFLSLVDSNTKNYE...............FEGDVGDFKGCWTSGKDYFRLDGSC   969
XNP/ATRX  1890  ISLDLIEDFLE..LASREKTEDKDKPLIYKGE............GKWLRNIDYYRLDGST  1935

DjXNP      787  QAFVRADIQSRFNSFEDHRLRLFLISTRAGGMGVNLVAANRVIIFDVSWNPSHDVQAIFR   846
XNP-1     1024  QSGKRDAVQTSFNDPLNLRARLMLISTRAGSLGTNMVAANRVIIFDACWNPSHDTQSLFR  1083
d-XNP      970  SVEQREAMCKQFNNITNLRARLFLISTRAGGLGINLVAANRVVIFDVSWNPSHDTQSIFR  1029
XNP/ATRX  1936  TAQSRKKWAEEFNDETNVRGRLFIISTKAGSLGINLVAANRVIIFDASWNPSYDIQSIFR  1995

DjXNP      847  SYRFGQNKPVYVYRFVSQGTMEEKIYERQVTKQSLSLRVVDEQQISRYFTEEDLRSLYKF   906
XNP-1     1084  VYRFGQTKPVYIYRFIAQGTMEERIYKRQVTKESTSMRVVDEAQIQRHYLGNDLTELYQF  1143
d-XNP     1030  VYRFGQIKPCYIYRLIAMGTMEQKVYERQVAKQATAKRVIDEQQISRHYNQTDLMELYSY  1089
XNP/ATRX  1996  VYRFGQTKPVYVYRFLAQGTMEDKIYDRQVTKQSLSFRVVDQQQVERHFTMNELTELYTF  2055

DjXNP      907  EPDLYD.....PEKVRETPILPKDRFLAELIQELPQFIHGYHEHDSLLQNKTDEELTETE   961
XNP-1     1144  TPSTFDPDVEISCAP......PKDRLLADVIHKNQHAVVDYIEHDTLFANVEDEKLTEQE  1197
d-XNP     1090  E.........LKPSTEREMPILPKDRLFAEILTEHEKLIFKYHEHDSLLEQEEHENLTEEE  1141
XNP/ATRX  2056  EPDLLEDPNSEKKKKRDTPMLPKDTILAELLQIHKEHIVGYHEHDSLLDHKEEEELTEEE  2115

DjXNP      962  RQDAWKEFEEEEKIRG.YRPFPMNPIQPKPAMDQDRMHQLQIMMLQQYPQYAALYAQNYNN  1020
XNP-1     1198  MKDAWTDYEKDKSGMPVRAQYAAPPMPGFPNGHIVGQNVQALLQNRMNQGIRVDQMQHDI  1257
d-XNP     1142  RKSAWAEYEAEK....TRTVQASQYMSYDRNAFGNQVMGQFGNASGSVTSNKIFGFRSDI  1197
XNP/ATRX  2116  RKAAWAEYEAEKKGL.TMRFNIPTGTNLPPVSFNSQTPYIPFNL...GALSAMSNQQLED  2171

DjXNP     1021  LVKYV.LSRKPDLNQIQLNNEVQHLFLQSLQKYYKSQATMMVTQPADTIAGSSQSMQ*--  1076
XNP-1     1258  LFKELQKMRIKDAGTAVKIVLLRNLLEQILPYIPDEKRGGMSEFNTHFIRLVHETDRKME  1317
d-XNP     1198  LLQLL.NMKISKDHQELNQNQVIQLVPTYLQQLYNEMKNGDPTMYKELLNLHSNIVHPSG  1256
XNP/ATRX  2172  LINQ...GREKVVEATNSVTAVRIQPLEDIISAVWKEKMNLSEAQVQALALSRQASQELD  2228

DjXNP           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
XNP-1     1318  TPADLERKSLESFKTVIKMVKMIPTCREPLARMTRDYPYLFA*~~~~~~~~~~~~~~~~~  1359
d-XNP     1257  HYMNPLLYANQNPNAAGYNQGTGGVPPMAGGSVAHGPPAAPAPGFEPDKVYEID*-----  1310
XNP/ATRX  2229  VKRREAIYNDVLTKQQMLISCVQRILMNRRLQQQYNQQQQQMTYQQATLGHLMMPKPPN  2288

DjXNP           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
XNP-1           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
d-XNP           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
XNP/ATRX  2289  LIMNPSNYQQIDMRGMYQPVAGGMQPPPLQRAPPPMRSKNPGPSQGKSM   2337
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