UNIQUE RADIOPROTECTIVE DAMAGE SUPPRESSOR PROTEIN (DSUP): COMPARATIVE SEQUENCE ANALYSIS

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Tardigrades are one of the most stress-resistant complex organisms on the Earth, of particular interest is their high radioresistance. The protein Dsup (Damage suppressor) recently discovered in *R. varieornatus* is directly related to a decrease of the level of nuclear DNA damage by ionizing radiation; however, the mechanism of this process is not fully understood. To better understand the function and origin of the Dsup protein, a comparative analysis of the amino acid sequence of this protein with the amino acid sequences of proteins in open databases was carried out. We showed that other DNA-binding proteins of tardigrades have the highest similarity for the Dsup protein, including histone proteins and proteins involved in the response to stress, as well as the SRP40 yeast protein. Thus we hypothesize that Dsup could arise from some ancestral histone-like protein.

УНИКАЛЬНЫЙ РАДИОПРОТЕКТОРНЫЙ БЕЛОК DSUP (DAMAGE SUPPRESSOR): СРАВНИТЕЛЬНЫЙ АНАЛИЗ ПОСЛЕДОВАТЕЛЬНОСТИ

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Тихоходки являются одними из самых стрессоустойчивых сложных организмов на Земле, особенный интерес представляет их высокая радиорезистентность. Недавно открытый в *R. varieornatus* белок Dsup (Damage suppressor) напрямую связан со снижением уровня повреждений ядерной ДНК ионизирующим излучением, однако механизм этого процесса до конца неясен. Чтобы лучше понять функции и происхождение белка Dsup, был проведен сравнительный анализ аминокислотной последовательности этого белка с аминокислотными последовательностями белков в открытых базах данных. Мы показали, что наибольшее сродство с белком Dsup имели другие ДНК-связывающиеся белки тихоходок, в том числе белки гистонов и белки, вовлеченные в ответ на стресс, а также белок дрожжей SRP40. Таким образом можно предположить, что Dsup мог возникнуть в результате изменений в гистоноподобном белке предшественнике.

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Tardiagrades (water bears) are among most extremotolerant multicellular organisms, which are able to withstand various harsh environmental conditions and multiple stress factors. Thus study of mechanisms of tardigrade stress-tolerance is highly valuable for genetics, molecular biology, medicine, biotechnology and astrobiology. Several pathways lead to tardigrade stress-resistance including cryptobiosis and abundance of specific metabolites [1,2]. Recent comparative genomics studies revealed group of *Eutardigrada* unique proteins that reduce damage of various cellular components during stress [2,3]. Among them the most perspective protein is the nuclear localized protein Damage suppressor (Dsup) of *Ramazzottius varieornatus* that was discovered in *YOKOZUNA-1* strain cultivated from dry moss (Sapporo, Japan) [4]. This tardigrade could survive even in open space or after treatment with high doses of ionizing radiation (~5 kGy) [3,4]. Dsup protein directly contributes to radioresistance through unique and not fully explored mechanism, that makes essential the search for it's evolutionary origin and determination of homologous proteins [3,5]. This work aims to verify the group of most evolutionary close proteins to Dsup in genomes and proteomes of tardigrades and other organisms to predict functional features of this unique protein and mechanism of radioresistance.

Materials and Methods

In this work we performed Basic Local Alignment Search (BLAST) with BLAST NCBI, EMBL BLAST+ and PSI-BLAST to identify protein sequence matches and reveal proteins homologous to Dsup. BLAST searches were based on nucleotide (*RvY*_17224) and amino acid (P0DOW4) sequences. Search was performed against sequences of all proteins and genes in UniProtKB, Swiss-Prot, TrEMBL databases and separately for tardigrade and human taxonomic IDs. Conserved domains were additionally investigated by NCBI Conserved Domains against database of superset CDD v3.19 ($p\text{-val}<0.01$). Multiple Sequence Alignment (MSA) and phylogram were obtained with Clustal Omega.

Results and Discussion

The highest protein sequence identity matches to Dsup was identified for representatives of class *Eutardigrada R. varieornatus* (*R. v.*) and *H. dujardini* (*H. d.*). Among nine tardigrades proteins we determined mitochondria targeting peptide 3 (ATPM3) (Table 1) (required for extremotolerance) [6] and late embryogenesis abundant proteins (LEA2, LEA7) (also abundant in stress-tolerant organisms [2]). It is important that Dsup demonstrates high sequence similarity to tardigrades histone H1 proteins as HistoneH1_1 (*R. v.*), HistoneH1_2 (*R. v.*) and putative histone H1-delta A0A1W0WGV1 (*H. d.*), that may spread the light on the origin of this protein and peculiarities of DNA-binding activity. The highest levels of sequence similarity to Dsup were observed for uncharacterized DNA-binding proteins A0A1D1UTB4 (*R. v.*) with 30 %, A0A1W0X4B1 (*H. d.*) with 28 % and A0A1W0XB17 (*H. d.*) with 29% (Table 1). The last one was also described as Dsup-like and have the same radioprotective properties [3]. Search against non-tardigrades species demonstrated a small amount of DNA-binding proteins with high level of sequence similarity to Dsup. However, among them we determined *S. cerevisiae* suppressor protein SRP40 with 22% sequence similarity. Remarkably that SRP40 functions as chaperone of small nucleolar ribonucleoprotein particles and it is immunologically and structurally close to rat Nopp140 (evolutionarily conserved chaperone) [7].

Conclusion

Detailed search for sequences similar to Dsup protein allowed us to find possible links between this protein and some other proteins contributing to stress-resistance (*R. v.*). It should be noted that the most part of these proteins appear to be able to bind to DNA and, in some cases, can take part in the response to stress. The Dsup protein can nonspecifically bind to DNA and nucleosomes [8], and we hypothesize that Dsup could arise from some ancestral histone-like protein. To validate mechanism of radioresistance induced by Dsup and uncover practical potential of using of this protein further experiments with classical complex model organisms are required [9,10].

Table 1. Mining for most similar sequences to Damage suppressor protein

Protein	Protein Description	Organism	Length, am.	Score (Bits)	Identities, %
Dsup	Damage supressor protein Dsup, DNA-binding, related to extremotolerance	*R. varieornatus* (tardigrade)	445	-	-
ATPM3	Tardigrade-unique protein with predicted mitochondria targeting peptide 3, related to extremotolerance	*R. varieornatus* (tardigrade)	896	75	23
SRP40	Suppressor protein SRP40, DNA-binding	*S. cerevisiae* (yeast)	406	87	22
A0A1W0XB17	Uncharacterized protein (mentioned as Dsup-like)	*H. dujardini* (tardigrade)	328	85	29
HistoneH1_1	Histone H1_1 protein, DNA-binding	*R. varieornatus* (tardigrade)	400	98	27
HistoneH1_2	Histone H1_2 protein, DNA-binding	*R. varieornatus* (tardigrade)	474	82	23
A0A1W0WGV1	Histone H1-delta (Putative), DNA-binding	*H. dujardini* (tardigrade)	306	64	25
LEA2	Late Embryogenesis Abundant 2, related to extremotolerance	*R. varieornatus* (tardigrade)	517	64	25
LEA7	Late Embryogenesis Abundant 7, related to extremotolerance	*R. varieornatus* (tardigrade)	348	62	25
A0A1D1UTB4	Uncharacterized proteins, best match identity and length	*R. varieornatus* (tardigrade)	412	71	30
A0A1W0X4B1	Uncharacterized proteins, DNA-binding	*H. dujardini* (tardigrade)	306	69	28

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