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A Genome-wide Survey and Systematic RNAi-based Characterization of Helicase-like Genes in *Caenorhabditis elegans*

Toshihiko Eki[1,2,*], Takeshi Ishihara[3,†], Isao Katsura[3], and Fumio Hanaoka[2,4,5]

Division of Life Science and Biotechnology, Department of Ecological Engineering, Toyohashi University of Technology, Toyohashi, Aichi 441-8580, Japan[1]; Cellular Physiology Laboratory, RIKEN, Wako, Saitama 351-0198, Japan[2]; Structural Biology Center, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan[3]; Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka 565-0871, Japan[4] and SORST, Japan Science and Technology Agency, Suita, Osaka 565-0871, Japan[5]

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Abstract

Helicase-like proteins play a crucial role in nucleic acid- and chromatin-mediated reactions. In this study, we identified 134 helicase-like proteins in the nematode *Caenorhabditis elegans* and classified the proteins into 10 known subfamilies and a group of orphan genes on the basis of sequence similarity. We characterized loss-of-function phenotypes in RNA interference (RNAi)-treated animals for helicase family members, using the RNAi feeding method, and found several previously unreported phenotypes. Fifty-one (39.5%) of 129 genes tested showed development- or growth-defect phenotypes, and many of these genes were putative nematode homologs of essential genes in a unicellular eukaryote, budding yeast, suggesting conservation of these essential proteins in both species. Comparative analyses between these species identified evolutionarily diverged nematode proteins as well as conserved family members. Chromosome mapping of the nematode genes revealed 10 pairs of putative duplicated genes and clusters of *C. elegans*-specific SNF2-like genes and *Helitrons*. Analyses of transcriptional profile data revealed a predominantly oogenesis- and germline-enriched expression of many helicase-like genes. Finally, we identified the *D2005.5*(*drh-3*) gene in an RNAi-based screen for genes involved in resistance to X-ray irradiation. Analysis of DRH-3 will clarify the potentially novel mechanism by which it protects against X-ray-induced damage in *C. elegans*.

Key words: *C. elegans*; comparative genomics; *drh-3*; helicase family; RNAi-based screen

1. Introduction

The helicase superfamily is made up of three functional classes, DNA helicases, RNA helicases, and chromatin remodeling ATPases, and the members of this family play a crucial role in various nucleic acid- and chromatin-mediated cellular reactions such as DNA replication, repair and recombination, pre-mRNA splicing, ribosome biogenesis, RNA interference, and chromatin remodeling.[1–5] Since these reactions are essential for maintenance, expression, and regulation of genetic information in the chromosome, dysfunctions of helicase genes may lead to genetic diseases, including cancers. Indeed, genetic mutations of the human RecQ-like BLM and the WRN DNA helicase result in the early development of various cancers and premature aging, respectively.[6] Most helicases share conserved amino acid sequence motifs, and these genes are classified into five families (SF1–SF5) on the basis of the occurrence and characteristics of conserved motifs.[7] Because of the biological importance of the helicase family, we conducted a comprehensive analysis of the functions of helicase family members in two model organisms: *Saccharomyces cerevisiae* and *Caenorhabditis*

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† Current address: Molecular Genetics Laboratory, Department of Biology, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka, Fukuoka 812-8581, Japan.

* To whom correspondence should be addressed. Tel. +81 532-44-6907. Fax. +81 532-44-6929. E-mail: eki@eco.tut.ac.jp

elegans. Previously, we examined loss-of-function phenotypes of yeast novel helicase-related genes, using gene knockout strains and characterized gene expression profiles by northern blotting.[8] In our previous study, we identified 21 uncharacterized genes including five essential genes *YDL031W*[*DBP10*], *YDL084W*[*SUB2*], *YKL078W*[*DHR2*], *YLR276C*[*DBP9*], and *YMR128W* [*ECM16*], and *YDL070W*[*BDF2*] and *YGL150C*[*INO80*] were later shown to be non-essential. Some of these novel genes were subsequently characterized to clarify their molecular functions, for example, *SUB2* in pre-mRNA splicing,[9] *DHR2*, *ECM16* and several DEAD-box genes such as *DBP9* in ribosome biogenesis,[10,11] *YOL095C*(*HMI1*) in the maintenance of mitochondrial DNA,[12] and *INO80* and *YDR334W*(*SWR1*) in chromatin remodeling and transcription.[13,14] Thus, comprehensive analyses focusing on the helicase superfamily can lead to the discovery of novel genes required for basic cellular reactions involved in cell proliferation, development, and aging.

In the current study, we have focused on helicase family members in a multicellular organism, the nematode *C. elegans*, using an RNA interference (RNAi) technique. Indeed, many novel multicellular specific proteins from other gene families have been discovered by RNAi-mediated comprehensive studies in *C. elegans*, including SR-related proteins,[15,16] proteins for the ubiquitylation system,[17] the forkhead proteins,[18] and G protein-coupled receptors.[19] Comparative analysis of helicase-like proteins in yeast and *C. elegans* will allow us to identify nematode-specific proteins that likely play an important role in multicellular organism-specific functions such as morphogenesis. Identification and characterization of these higher eukaryote-specific helicases will be useful in understanding the molecular mechanisms of genetic diseases caused by mutations of human helicase-like genes.

Here, we found 134 genes encoding putative helicase-like proteins in *C. elegans* and systematically prepared RNAi-treated animals for each of these genes to characterize their loss-of-function phenotypes. Fifty-one of 129 genes tested caused embryonic lethality or growth defects by RNAi, and these genes contained many putative homologs of yeast essential helicase-like genes. We identified two divergent gene clusters and 10 pairs of putative gene duplications on chromosomes and found germline- and oogenesis-enriched expression of many helicase-like genes. In addition, an RNAi-based screen was performed to identify genes required for resistance to X-ray irradiation, resulting in successful identification of the novel *D2005.5*(*drh-3*) gene.

2. Materials and methods

2.1. Sequence analyses

Identification of helicase-like proteins in *C. elegans* was performed as described in the legend of Table 1. Helicases were classified according to the yeast helicase-like protein subfamilies by Linder (http://www.medecine.unige.ch/~linder/helicases_list.html) with modifications (i.e. addition of the new subfamilies MPH1, PIF1, RAD3, and RECQ). Most orthologous proteins were identified from the InParanoid database[20] as described in the legend of Table 2. Homologous members in gene pairs and clusters were identified by homology search in *C. elegans* nucleotide sequence databases as described in the legend of Supplementary Table S4.

2.2. C. elegans strains and culture procedures

C. elegans wild-type strain Bristol N2 and the RNAi-hypersensitive *rrf-3* mutant strain NL2099 (*rrf-3*(*pk1426*) II)[21] (obtained from the *Caenorhabditis* Genetics Center) were used in this study. Animals were maintained at 20°C on nematode growth medium (NGM) agar plates seeded with the *Escherichia coli* OP50 strain, as described previously.[22]

2.3. Construction of recombinant DNA

Genomic DNA or cDNA fragments corresponding to helicase-like genes were cloned into the blunted *Eco*RI site of the double-stranded RNA (dsRNA) expression vector pPD129.36 (a kind gift of Dr A. Fire, Stanford University School of Medicine, USA). Insert DNA was directly amplified by PCR from a *C. elegans* embryo cDNA library (No. 937007, Stratagene, La Jolla, CA, USA) or genomic DNA (N2), using a gene-specific primer set for blunt-end cloning. PCR primers were purchased from Sawady (Tokyo) and Proligo LLC (Boulder, USA), and nucleotide sequences of the primers will be provided upon request. The nucleotide sequences of the resultant recombinant clones were determined by dye-terminator cycle sequencing.

2.4. Feeding RNAi

RNAi by feeding bacteria was performed using the N2 and the *rrf-3* strains, as described previously,[23] with the following modifications. In brief, the HT115(DE3) *E. coli* strain containing the pDP129.36 with a target gene-specific insert was grown overnight in 2× YT medium containing 100 μg/mL ampicillin (or 50 μg/mL carbenicillin and 12.5 μg/mL tetracycline) with stirring at 37°C. Aliquots of the culture (30 μL) were spread onto NGM agar in a Petri dish (Ø 6 cm) containing 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) and the indicated antibiotics and incubated at 37°C for 18 h for RNAi (RNAi plates). The next day, P0 animals at the fourth larval (L4) to young adult stages were placed onto the RNAi plates and fed the recombinant *E. coli* strain expressing dsRNA for over 18 h to avoid F1 progeny with leaky phenotypes. Subsequently, P0 animals were transferred onto new RNAi plates with bacteria-expressing dsRNA for 12 h to lay eggs (F1) and

Table 1. Summary of RNAi analyses of *C. elegans* helicase-like genes[a]

Subfamily	Gene	Transcript[b]	Protein ID	Insert DNA[c]	Phenotype of RNAi-treated nematode[d]	Phenotype code[e]	Growth retardation index[g]	X-ray sensitivity[h]	RNAi phenotype data (WormBase WS171)[i]	Highest homology matches (BLASTP analysis)[j]						
										S. cerevisiae	*E*-value	*H. sapiens*	*E*-value	*D. melanogaster*	*E*-value	*C. briggsae*	*E*-value
DEAD-box	*T07D4.4*	T07D4.4a	CE18219	C	WT (Clr Him)		0.90		WT	Dbp5p	3.0e-70	ENSP00000306117(DDX19A)	3.3e-115	CG7483-PA(eIF4AIII)	1.1e-64	CBP00777	5.7e-299
DEAD-box	*ZK686.2*	ZK686.2	CE34464	C	Gro		0.31		Gro Let Lva WT	Dbp6p	1.7e-37	ENSP00000333746(DDX51)	1.0e-48	CG9680-PA(Dbp73D)	7.2e-27	CBP00001	2.9e-247
DEAD-box	*H20J04.4*	H20J04.4b	CE39381	G	Gro Stp [Pvl]		0.48		Gro Rup Red_brood WT	Dbp8p	2.2e-90	ENSP00000247003(DDX49)	4.4e-118	CG9253-PA	4.6e-89	CBP21316	9.0e-252
DEAD-box	*C24H12.4*	C24H12.4a	CE27728	C	Lva		0.19		WT	Dbp9p	9.9e-96	ENSP00000258772(DDX56)	1.6e-105	CG1666-PA(Hlc)	2.4e-93	CBP01777	3.9e-307
DEAD-box	*Y94H6A.5*	Y94H6A.5b	CE37303	G	Gro		0.26		Lva WT	Dbp10p	3.8e-123	ENSP00000304072(DDX54)	8.8e-169	CG32344-PA	8.8e-150	CBP03399	0
DEAD-box	*cgh-1*	C07H6.5	CE00839	C	Adl Emb Pvl Rup Stp				Adl Emb Lva Pvl Rup Ste Stp WT	Dhh1p	6.0e-152	ENSP00000264018(DDX6)	2.6e-161	CG4916-PA(me31B)	1.6e-172	CBP02241	8.5e-226
DEAD-box	*Y71G12B.8*	Y71G12B.8	CE27030	G	Gro [Lva]		0.54	(-)	WT	Drs1p	5.3e-66	ENSP00000339633(DDX27)	5.1e-153	CG2173-PA(Rs1)	1.1e-146	CBP05301	0
DEAD-box	*Y55F3BR.1*	Y55F3BR.1	CE29884	G	Weak Gro [Gro Stp] (Emb)		0.83[0.80]	(-)	Gro Stp Loc_ab WT	Fal1p	5.6e-31	ENSP00000233084(DDX1)	1.8e-180	CG9054-PA(Ddx1)	2.3e-175	CBP03389	0
DEAD-box	*B0511.6*	B0511.6	CE26853	C	Lva		0.09		Let Lva WT	Has1p	3.5e-140	ENSP00000263239(DDX18)	2.0e-180	CG6375-PB(pit)	1.6e-178	CBP03010	3.0e-271
DEAD-box	*Y23H5B.6*	Y23H5B.6	CE25231	G	Gro		0.49		Emb Lva Stp Red_brood WT	Hca4p	1.3e-114	ENSP00000314348(DDX10)	5.7e-135	CG5800-PA	3.1e-118	CBP06620	1.5e-295
DEAD-box	*F55F8.2*	F55F8.2a	CE11190	C	Gro Pvl Sck Stp [Lva]		0.29		Bli Gro Lva Stp Red_brood WT	Prp28p	3.9e-40	ENSP00000328690(DDX24)	1.9e-115	CG9143-PA	1.9e-108	CBP18131	7.9e-203
DEAD-box	*F53H1.1*	F53H1.1	CE27427	G	Gro Sck		0.65	(+?)	Emb Gro Rup Pvl Ste Stp WT	Prp5p	5.6e-108	ENSP00000346236(DDX46)	2.1e-235	CG6227-PA	7.1e-232	CBP03983	0
DEAD-box	*R05D11.4*	R05D11.4	CE06239	G	WT		0.91		WT	Rok1p	4.9e-70	ENSP00000268854(DDX52)	9.1e-97	CG5589-PA	2.8e-88	CBP17618	7.4e-248
DEAD-box	*T26G10.1*	T26G10.1	CE00337	C	Lva Sck		0.04		Dpy Emb Gro Let Lva Pvl Ste Stp WT	Rrp3p	4.9e-102	ENSP00000350698(DDX47)	1.0e-154	CG9253-PA	2.8e-160	CBP02482	1.1e-240
DEAD-box	*ZK512.2*	ZK512.2	CE03821	G	Gro Lva		0.23		Gro Ste Dev_delay	Sph4p	2.3e-65	ENSP00000238146(DDX55)	2.2e-93	CG9630-PA	2.8e-84	CBP01738	4.6e-278
DEAD-box	*hel-1*	C26D10.2a	CE03025	C	Adl Emb Gro Let Lva Pvl Sck Ste Stp [Ste]				Adl Bmd Clr Emb Let Sck Ste Stp WT	Suh2p	1.3e-138	ENSP00000242776(DDX39)	1.6e-176	CG7269-PC(Hel25E)	1.1e-175	CBP05782	5.6e-222
DEAD-box	*F58G11.2*	F58G11.2	CE11402	G	WT		1.06		WT	Ded1p	8.7e-50	ENSP00000310870(DDX3X)	3.2e-53	CG9748-PA(bel)	1.8e-78	CBP17194	4.1e-181
DEAD-box	*C46F11.4*	C46F11.4	CE17559	C	WT		0.91		Ste WT	Dbp2p	2.6e-87	ENSP00000352308(DDX42)	3.3e-179	CG6418-PB	2.1e-184	CBP02456	0
DEAD-box	*F01F1.7*	F01F1.7	CE26887	C	Emb [Ste]				Emb Sck	Dbp2p	4.0e-82	ENSP00000310723(DDX23)	7.3e-226	CG10333-PA	2.9e-229	CBP08175	0
DEAD-box	*F58E10.3*	F58E10.3a	CE18785	C	Gro Lva Sck		0.19		Emb	Dbp2p	4.4e-124	ENSP00000216019(DDX17)	1.6e-159	CG10077-PA	2.2e-161	CBP22536	9.2e-282
DEAD-box	*H27M09.1*	H27M09.1	CE23832	C	weak Gro [Adl Gro Let Pvl Rup] (Clr Him)		0.78		Emb Lva WT	Dbp2p	1.1e-74	ENSP00000330349(DDX41)	8.3e-172	CG14637-PA(abs)	1.6e-168	CBP03123	0
DEAD-box	*Y54G11A.3*	Y54G11A.3	CE22474	C	WT		0.95		WT	Dbp2p	2.4e-84	ENSP00000238919(DDX43)	4.7e-103	CG10077-PA	4e-83	CBP04867	2.0e-230
DEAD-box	*F33D11.10*	F33D11.10	CE09901	C	Emb [Gro Sck]				Emb Mul Ste WT	Tif1p/Tif2p	5.7e-124	ENSP00000269349(DDX48)	3.7e-188	CG7483-PA(eIF4AIII)	3.4e-187	CBP21556	7.6e-206
DEAD-box	*F57B9.3*	F57B9.3	CE01338	G	Gro Lva Sck		0.17		Emb Lva Ste Loc_ab WT	Tif1p/Tif2p	2.8e-90	ENSP00000326381(EIF4A2)	1.8e-94	CG9075-PA(eIF4a)	5.9e-96	CBP03954	8.4e-120
DEAD-box	*inf-1*	F57B9.6a	CE01341	C	Emb				Emb Lva Loc_ab	Tif1p/Tif2p	4.7e-136	ENSP00000326381(EIF4A2)	4.6e-151	CG9075-PA(eIF4a)	1.3e-148	CBP03954	3.7e-206
DEAD-box	*mel-46*	T06A10.1	CE28485	G	not tested		0.96		Gro WT	Tif1p/Tif2p	8.5e-42	ENSP00000181534(DDX20)	2.1e-54	CG6539-PA(Dhh1)	8.1e-49	CBP03338	6.6e-208
DEAD-box	*Y65B4A.6*	Y65B4A.6	CE34419	C	Emb Lva Sck		0.10		Egl Lva WT	Tif1p/Tif2p	7.2e-124	ENSP00000269349(DDX48)	2.3e-188	CG7483-PA(eIF4AIII)	3.9e-186	CBP21556	3.6e-208
DEAD-box	*mut-14*	C14C11.6	CE06825	G	WT			(-)	WT	Dbp1p	6.3e-27	ENSP00000334167(DDX4)	1.7e-24	CG9748-PA(bel)	5.6e-23	CBP16557	4.3e-162
DEAD-box	*Y38A10A.6*	Y38A10A.6	CE28975	C	WT			(-)	WT	Dbp1p	3.5e-18	ENSP00000277804(DDX50)	2.8e-17	CG9748-PA(bel)	2.9e-17	CBP16557	7.9e-154
DEAD-box	*vbh-1*	Y54E10A.9c	CE37437	G	Gro Sck [Let, Pvl Dev_ab] (Him)		0.73		Emb WT	Dbp1p	7.5e-122	ENSP00000336725(DDX3Y)	2.7e-146	CG9748-PA(bel)	2.1e-143	CBP01103	2.0e-285
DEAD-box	*Y71H2AM.19*	Y71H2AM.19	CE38657	G	Lva		0.06	(+?)	Emb	Dbp1p	4.4e-134	ENSP00000310870(DDX3X)	1.2e-166	CG9748-PA(bel)	7.1e-166	CBP08424	1.7e-257
DEAD-box	*ZC317.1*	ZC317.1	CE15167	G	WT		0.87	(-)	WT	Dbp1p	1.3e-22	ENSP00000362687(DDX50)	1.5e-19	CG9748-PA(bel)	1.8e-18	CBP16557	1.2e-109
DEAD-box (glh)	*glh-3*	B0414.6	CE07736	C	WT				WT	Ded1p	4.2e-82	ENSP00000347087(DDX4)	3.4e-94	CG9748-PA(bel)	2.8e-86	CBP22748	2.2e-200
DEAD-box (glh)	*glh-2*	C55B7.1	CE09012	C	WT		0.86		WT	Dbp1p	6.7e-82	ENSP00000347087(DDX4)	8.5e-85	CG3506-PA(vas)	1.3e-96	CBP22748	2.6e-264
DEAD-box (glh)	*glh-4*	T12E5.3	CE29052	G	WT				WT	Dbp1p	8.3e-64	ENSP00000347087(DDX4)	1.7e-76	CG9748-PA(bel)	7.9e-73	CBP09018	2.0e-189
DEAD-box (glh)	*glh-1*	T21G5.3	CE25121	C	WT				WT	Dbp1p	6.4e-84	ENSP00000334167(DDX4)	6.1e-104	CG9748-PA(bel)	4.9e-95	CBP22748	3.2e-289
DEAD-box (DDX1-like)	*F20A1.9*	F20A1.9	CE35281	G	WT			(-)	Emb WT	ND		ENSP00000233084(DDX1)	4.3e-40	CG9054-PA(Ddx1)	5.4e-35	CBP23850	5.3e-97
DEAH-box	*rha-2*	C06E1.10	CE29563	C	Gro Lva		0.22	(-)	Age Emb Gro Let Lva Stp Red_brood WT	Ecm16p	7.4e-137	ENSP00000311135(DHX37)	1.0e-213	CG3228-PA(kz)	1.8e-204	CBP03724	0
DEAH-box	*Y37E11AM.1*	Y37E11AM.1	CE33853	G	WT			(-)	Dpy Emb Let Lva Loc_ab WT	Prp2p	7.1e-78	ENSP00000331907(DHX34)	5.3e-185	CG32533-PA	2.1e-174	CBP02030	0
DEAH-box	*rha-1*	T07D4.3	CE39177	C	WT			(-)	WT	Ylr419wp	5.5e-75	ENSP00000310700(DHX9)	1.6e-272	CG11680-PA(mle)	7.3e-259	CBP00773	0
DEAH-box	*mog-1*	K03H1.2	CE01027	C	Emb				Bmd Dpy Emb Let	Prp16p	3.6e-188	ENSP00000268482(DHX38)	0	CG32604-PA(l(1)G0007)	0	CBP16676	0
DEAH-box	*Y108F1.5*	Y108F1.5	CE31829	G	WT			(-)	Red_fat WT	Prp16p	4.4e-30	ENSP00000362416(DHX35)	1.3e-41	CG3225-PA	2.4e-27	CBP03695	5.6e-69
DEAH-box	*F52B5.3*	F52B5.3	CE05718	G	WT (Him)		0.90	(-)	Emb WT	Prp43p	4.5e-68	ENSP00000161863(YTHDC2)	1.3e-116	CG8915-PA	2.0e-96	CBP21397	0
DEAH-box	*F56D2.6*	F56D2.6a	CE01334	C	Gro Lva Sck Ste Stp		0.24	(-)	Emb Gro Lva Ste Dev_delay Loc_ab WT	Prp43p	1.7e-227	ENSP00000336741(DHX15)	9.0e-290	CG11107-PA	7.2e-282	CBP16424	0
DEAH-box	*mog-4*	C04H5.6	CE15592	C	Emb				Emb Let Lva Pvl Sck Ste Stp	Prp22p	1.2e-172	ENSP00000365613(DHX16)	8.5e-287	CG10689-PA	0	CBP04901	0
DEAH-box	*mog-5*	EEED8.5	CE01889	C	Emb				Emb Ste Loc_ab	Prp22p	7.5e-261	ENSP00000262415(DHX8)	0	CG8241-PA	0	CBP00651	0
DEAH-box	*T05E8.3*	T05E8.3	CE37043	C	Gro Stp [Pvl Sck]		0.61	(-)	Gro Pch Pvl Sck Stp WT	Prp22p	8.6e-99	ENSP00000225296(DHX33)	1.9e-124	CG4901-PA	7.9e-117	CBP17528	0
DEAH-box	*Y67D2.6*	Y67D2.6	CE27311	G	WT			(-)	WT	Prp22p	1.6e-135	ENSP00000252011(DDX35)	6.1e-200	CG3225-PA	1.7e-151	CBP03695	0
SKI2	*Y55B1AL.3*	Y55B1AL.3a	CE27019	G	WT			(-)	WT	Hfm1p	8.2e-32	ENSP00000295488	1.2e-141	CG7972-PA(mus301)	1.5e-125	CBP03650	0
SKI2	*W08D2.7*	W08D2.7	CE06562	C	Lva Sck		0.18		Emb Gro Let Lva Sck Ste Stp Loc_ab WT	Mtr4p	1.9e-162	ENSP00000230640(SKIV2L2)	7.7e-270	CG4152-PA(l(2)35Df)	1.3e-258	CBP14749	0
SKI2	*C08F8.2*	C08F8.2a	CE19689	C	Weak Gro Stp [Gro] (Emb)		0.86[0.68]	(+?)	Emb Gro Lva Stp Dev_delay Red_brood Transposon_ab WT	Suv3p	2.4e-70	ENSP00000343179(SUPV3L1)	4.1e-163	CG9791-PA	5.0e-160	CBP01637	0
SKI2	*Y46G5A.6*	Y46G5A.6	CE24303	C	WT		0.93	(-)	Emb Lva Ste Loc_ab Thin WT	Brr2p	9.7e-54	ENSP00000317123(ASCC3L1)	1.6e-114	CG5931-PA	7.8e-109	CBP22000	1.6e-152
SKI2	*C28H8.3*	C28H8.3	CE29195	C	WT (Dev_ab)			(+?)	WT	Ski2p	3.8e-28	ENSP00000260184	1.5e-119	CG10210-PA(tst)	1.2e-28	CBP02209	0
SKI2	*F01G4.3*	F01G4.3	CE40452	C	WT			(-)	WT	Ski2p	4.4e-168	ENSP00000364543	1.3e-213	CG10210-PA(tst)	7.2e-194	CBP15510	0
SKI2	*Y46G5A.4*	Y46G5A.4	CE21971	G	Emb [Lva]				Emb Lva Ste Loc_ab Thin WT	Slh1p	6.4e-308	ENSP00000317123(ASCC3L1)	0	CG5931-PA	0	CBP22000	0
SKI2	*Y54E2A.6*	Y54E2A.6	CE20305	G	WT			(-)	WT	Slh1p	1.8e-206	ENSP00000262902(ASCC3)	0	CG5205-PA	0	CBP01139	0
UPF1	*dna-2*	F43G6.1a	CE02219	G	WT			(-)	Emb WT	Dna2p	5.7e-84	ENSP00000351185(DNA2L)	1.3e-125	CG2990-PB	9.1e-101	CBP14508	0
UPF1	*C41D11.7*	C41D11.7	CE36108	C	WT			(-)	Ste WT	Hcs1p	6.1e-25	ENSP00000255078(IGHMBP2)	1.8e-33	CG1559-PA(Upf1)	8.5e-32	CBP21383	1.3e-129
UPF1	*emb-4*	Y80D3A.2	CE40670	G	Gro		0.75	(-)	Bmd Emb Loc_ab WT	Hcs1p	4.4e-21	ENSP00000156471(AQR)	0	CG31368-PA	0	CBP04765	1.9e-106
UPF1	*C05C10.2*	C05C10.2b	CE27795	C	WT			(-)	WT	Nam7p	5.7e-24	ENSP00000262803(UPF1)	3.2e-22	CG6967-PB	2.2e-20	CBP00296	0
UPF1	*smg-2*	Y48G8AL.6	CE28367	G	WT			(-)	WT	Nam7p	5.1e-210	ENSP00000262803(UPF1)	3.5e-271	CG1559-PA(Upf1)	1.9e-256	CBP02073	0
UPF1	*ZK1067.2*	ZK1067.2	CE06677	G	WT			(-)	WT	Nam7p	3.9e-24	ENSP00000244030(ZNFX1)	1.6e-114	CG6204-PA	2.8e-55	CBP00224	0
UPF1	*C44H9.4*	C44H9.4	CE35434	G	WT			(-)	WT	Sen1p	4.3e-15	ENSP00000317088	5.3e-20	CG1559-PA(Upf1)	8.3e-16	CBP13569	8.2e-266
UPF1	*K08D10.5*	K08D10.5	CE07357	C	WT			(+?)	WT	Sen1p	5.9e-11	ENSP00000244030(ZNFX1)	8.4e-10	CG7504-PA	4.3e-08	CBP24705	2.7e-62
UPF1	*R03D7.2*	R03D7.2	CE01610	G	WT			(-)	WT	Sen1p	1.0e-09	ENSP00000317088	3.2e-12	CG7504-PA	6.7e-10	CBP24705	7.4e-38
UPF1 (far related)	*C44H9.2*	C44H9.2	CE05419	G	WT			(-)	WT	ND		ND		ND		CBP13569	3.7e-199
SWI2/SNF2	*M03C11.8*	M03C11.8	CE34059	C	WT (Dev_ab)			(-)	Emb WT	Fun30p	2.8e-108	ENSP00000346217(SMARCAD1)	7.1e-143	CG5899-PA	3.0e-140	CBP19194	0
SWI2/SNF2	*T05A12.4*	T05A12.4a	CE37666	G	WT			(-)	Inc_mutation WT	Rad5p	9.2e-28	ENSP00000356473(SHPRH)	1.3e-57	CG7376-PA	1.6e-49	CBP07159	0
SWI2/SNF2	*C16A3.1*	C16A3.1c	CE30606	C	WT (Dev_ab)			(-)	WT	Isw1p	4.8e-29	ENSP00000349823(SMARCAL1)	8.1e-103	CG3753-PA(Marcal1)	2.7e-97	CBP16449	2.7e-193
SWI2/SNF2	*isw-1*	F37A4.8	CE29792	C	Weak Gro Stp [Gro Pvl]		0.87[0.70]	(-)	Emb Gro Pvl Rup Stp WT	Isw2p	1.0e-236	ENSP00000218157(SMARCA1)	0	CG8025-PC(Iswi)	0	CBP10391	0
SWI2/SNF2	*brf-1*	F15D4.1	CE15856	C	WT		0.93	(-)	WT	Mot1p	3.8e-120	ENSP00000265990(BTAF1)	7.9e-131	CG4261-PA(Hel89B)	2.6e-99	CBP00621	0
SWI2/SNF2	*C52B9.8*	C52B9.8	CE27114	C	WT			(-)	WT	Snf2p	4.0e-202	ENSP00000349788(SMARCA2)	2.2e-252	CG5942-PB(brm)	5.1e-253	CBP23032	0
SWI2/SNF2	*psa-4*	F01G4.1	CE05553	C	Emb Gro Sck Stp [Pvl Ste]				Emb WT	Sth1p	6.3e-220	ENSP00000265773(SMARCA2)	1.2e-283	CG5942-PB(brm)	0	CBP15438	0
SWI2/SNF2	*ssl-1*	Y111B2A.22	CE40241	G	Gro Stp [Lva Pvl Sck]		0.75	(+?)	Emb Stp Unclassified WT	Swr1p	7.9e-172	ENSP00000343042	6.3e-251	CG9696-PD(dom)	3.4e-244	CBP00109	0
SWI2/SNF2	*F59A7.8*	F59A7.8	CE11450	G	WT			(-)	Lva WT	Rad16p	7.5e-31	ENSP00000251165(TTF2)	4.3e-73	CG2684-PA(Ids)	1.1e-59	CBP09312	1.7e-150
SWI2/SNF2	*Y113G7B.14*	Y113G7B.14	CE23294	G	WT			(-)	WT	Rad16p	4.0e-26	ENSP00000251165(TTF2)	3.8e-27	CG2684-PA(Ids)	5.9e-27	CBP09312	1.3e-36
SWI2/SNF2	*F54E12.2*	F54E12.2	CE11083	C	WT			(-)	Emb WT	Ris1p	7.5e-59	ENSP00000251165(TTF2)	1.8e-116	CG10445-PA	2.6e-69	CBP14951	0
SWI2/SNF2	*T23H2.3*	T23H2.3	CE30397	G	WT			(-)	WT	Ris1p	8.1e-52	ENSP00000251165(TTF2)	1.8e-88	CG2684-PA(Ids)	1.3e-96	CBP09312	0
SWI2/SNF2	*rad-26*	C27B7.4	CE17477	C	WT			(-)	WT	Rad26p	1.1e-54	ENSP00000296477(RAD54L2)	2.3e-173	CG4049-PA	8.5e-161	CBP11938	0
SWI2/SNF2	*csb-1*	F53H4.1	CE33793	G	WT (Him)			(+?)	UV-induced Emb and apoptosis increased WT	Rad26p	1.5e-92	ENSP00000265899(ERCC6)	7.2e-99	CG9696-PE(dom)	2.4e-58	CBP10262	6.7e-257
SWI2/SNF2	*F53H4.6*	F53H4.6	CE33794	G	WT			(-)	WT	Rad26p	5.6e-42	ENSP00000265899(ERCC6)	1.7e-41	CG5942-PC(brm)	3.7e-16	CBP10262	1.5e-100
SWI2/SNF2	*snp-1*	B0041.7	CE17314	C	WT			(-)	Evl Ste Gonad development abnormal WT	Rad54p	1.0e-51	ENSP00000342402(ATRX)	7.5e-188	CG4548-PA(XNP)	2.0e-173	CBP05228	0
SWI2/SNF2	*rad-54*	W06D4.6	CE25143	C	WT		0.93	(+?)	Stress_ab WT	Rad54p	1.4e-159	ENSP00000262745(RAD54L)	8.4e-215	CG3736-PA(okr)	1.9e-204	CBP02997	0
SWI2/SNF2	*Y116A8C.13*	Y116A8C.13	CE35647	G	WT			(-)	Emb Stress_ab WT	Rad54p	8.4e-78	ENSP00000336606(RAD54B)	1.1e-100	CG3736-PA(okr)	1.0e-87	CBP00082	1.5e-275
SWI2/SNF2	*let-418*	F26F12.7	CE17716	C	Lva Pvl Sck Stp		0.21	(-)	Emb Lva Ste Pharyngeal_ab Unclassified WT	Chd1p	1.2e-130	ENSP00000369716(CHD3)	0	CG8103-PA(Mi-2)	0	CBP16569	0

Continued

Table 1. Continued

Subfamily	Gene	Transcript[b]	Protein ID	Insert DNA[c]	Phenotype of RNAi-treated nematode[d]	Phenotype code[e]	Growth retardation index[g]	X-ray sensitivity[h]	RNAi phenotype data (WormBase WS171)[i]	Highest homology matches (BLASTP analysis)[j]							
										S. cerevisiae	*E*-value	*H. sapiens*	*E*-value	*D. melanogaster*	*E*-value	*C. briggsae*	*E*-value
SWI2/SNF2	*H06O01.2*	H06O01.2	CE32454	G	WT			(+?)	WT	Chd1p	1.8e-187	ENSP00000284049(CHD1)	0	CG3733-PA(Chd1)	0	CBP08671	0
SWI2/SNF2	*tag-192*	T04D1.4	CE18196	C	WT			(-)	WT	Chd1p	7.4e-129	ENSP00000307304(CHD7)	0	CG3696-PA(kis)	0	CBP05229	0
SWI2/SNF2	*chd-3*	T14G8.1	CE03657	C	WT (Him)			(+?)	WT	Chd1p	6.0e-130	ENSP00000369716(CHD3)	0	CG8103-PA(Mi-2)	0	CBP16569	0
SWI2/SNF2 (far related)	*F19B2.5*	F19B2.5	CE20697	G	WT			(-)	WT	ND		ENSP00000308944(SMARCA3)	2.0e-08	CG3696-PA(kis)	2.6e-06	CBP14951	1.6e-06
SWI2/SNF2 (far related)	*C25F9.5*	C25F9.5	CE17469	G	WT			(-)	WT	ND		ENSP00000308944(SMARCA3)	9.2e-06	ND		ND	
SWI2/SNF2 (far related)	*M04C3.1*	M04C3.1a	CE18078	G	WT			(-)	WT	ND		ENSP00000308944(SMARCA3)	4.3e-06	CG2684-PA(Ids)	4.2e-07	ND	
SWI2/SNF2 (far related)	*Y43F8B.14*	Y43F8B.14	CE21898	G	WT			(-)	WT	ND		ND		CG2684-PA(Ids)	1.3e-07	ND	
SWI2/SNF2 (far related)	*C25F9.4*	C25F9.4	CE33975		not tested				WT	ND		ND		CG2684-PA(Ids-PA)	6.1e-07	ND	
SWI2/SNF2 (far related)	*M04C3.2*	M04C3.2	CE31973		not tested				WT	ND		ND		CG2684-PA(lds)	1.2e-06	ND	
MCM	*mcm-2*	Y17G7B.5a	CE19038	C	Emb Gro Lva Pvl Sck Stp [Ste]				Emb Pvl Ste	Mcm2p	6.6e-176	ENSP00000265056(MCM2)	8.7e-263	CG7538-PA(Mcm2)	1.5e-253	CBP04361	0
MCM	*mcm-3*	C25D7.6	CE08392	G	Emb				Emb Mul Pvl Ste	Mcm3p	2.1e-138	ENSP00000229854(MCM3)	5.1e-202	CG4206-PA(Mcm3)	5.0e-195	CBP08883	0
MCM	*mcm-4*	Y39G10AR.14	CE21767	G	Emb				Emb Nmo Lva	Cdc54p	4.3e-157	ENSP00000262105(MCM4)	2.2e-222	CG1616-PA(dpa)	4.2e-219	CBP01027	0
MCM	*mcm-5*	R10E4.4	CE03558	C	Emb				Emb Mul WT	Cdc46p	6.7e-135	ENSP00000216122(MCM5)	9.6e-241	CG4082-PA(Mcm5)	3.7e-230	CBP02973	0
MCM	*mcm-6*	ZK632.1a	CE00415	C	Emb [Pvl Ste]				Cyk Emb Mul Ste WT	Mcm6p	1.6e-164	ENSP00000264156(MCM6)	2.5e-232	CG4039-PA(Mcm6)	1.3e-217	CBP02437	0
MCM	*mcm-7*	F32D1.10	CE09874	C	Emb				Emb Mul Pvl Ste	Cdc47p	1.2e-137	ENSP00000307288(MCM7)	8.2e-187	CG4978-PA(Mcm7)	2.4e-187	CBP05176	0
PIF1	*C11G6.2*	C11G6.2	CE05256	G	WT			(-)	WT	Rrm3p	1.9e-16	ENSP00000268043(PIF1)	3.6e-09	CG3238-PA	7.9e-13	CBP06954	3.0e-10
PIF1	*F11C3.1*	F11C3.1	CE09347	G	WT			(-)	WT	Rrm3p	4.1e-06	ND		ND		ND	
PIF1	*Y116F11A.1*	Y116F11A.1	CE24155	G	WT			(-)	WT	Rrm3p	8.4e-16	ENSP00000268043(PIF1)	2.2e-09	CG3238-PA	8.0e-10	CBP06954	2.2e-08
PIF1	*pif-1*	Y18H1A.6	CE30311	G	WT			(-)	WT	Pif1p	2.3e-79	ENSP00000268043(PIF1)	5.9e-104	CG3238-PA	1.2e-97	CBP06954	2.3e-280
PIF1(*Helitron*)	*F33H12.6*	F33H12.6	CE17043	G	WT			(+?)	WT	Pif1p	2.0e-13	ENSP00000268043(PIF1)	2.0e-16	CG3238-PA	1.0e-17	CBP13991	3.5e-67
PIF1(*Helitron*)	*F59H6.5*	F59H6.5	CE20911	G	WT			(-)	WT	Pif1p	5.1e-13	ENSP00000268043(PIF1)	1.1e-15	CG3238-PA	1.4e-16	CBP13991	1.7e-70
PIF1(*Helitron*)	*Y16E11A.2*	Y16E11A.2	CE15283	G	WT			(-)	WT	Pif1p	1.5e-12	ENSP00000268043(PIF1)	5.3e-15	CG3238-PA	1.7e-16	CBP13991	4.3e-67
PIF1(*Helitron*)	*Y27F2A.5*	Y27F2A.5	CE26057	C	WT			(+?)	WT	Pif1p	1.1e-09	ENSP00000268043(PIF1)	1.2e-11	CG3238-PA	2.2e-10	CBP15482	2.8e-32
PIF1(*Helitron*)	*Y46B2A.2*	Y46B2A.2	CE21950	G	WT			(-)	WT	Pif1p	2.5e-13	ENSP00000268043(PIF1)	6.4e-16	CG3238-PA	8.5e-18	CBP13991	1.8e-68
PIF1(*Helitron*)	*ZK250.9*	ZK250.9	CE15283	G	WT			(-)	WT	Pif1p	1.5e-12	ENSP00000268043(PIF1)	5.3e-15	CG3238-PA	1.7e-16	CBP13991	4.3e-67
MPH1	*drh-2*	C01B10.1	CE33024	G	WT			(-)	Rde WT	Mph1p	2.5e-08	ENSP00000369197(DDX58)	2.1e-52	CG6493-PA(Dcr-2)	4.4e-09	CBP01380	2.2e-198
MPH1	*drh-3*	D2005.5	CE36120	C	Emb			(+)	Emb WT	Mph1p	2.7e-13	ENSP00000263642(IFIH1)	6.9e-47	CG6493-PA(Dcr-2)	1.3e-13	CBP16208	1.2e-267
MPH1	*drh-1*	F15B10.2	CE16988	C	WT			(-)	Rde WT	Mph1p	5.1e-13	ENSP00000263642(IFIH1)	1.8e-60	CG7922-PA	2.5e-16	CBP01380	0
MPH1	*dcr-1*	K12H4.8	CE25057	C	Weak Gro [Let Pvl Rup Deb_ab]		0.89[0.76]	(-)	Egl Esp Gro Let Pvl Rde Rup Loc_ab Unclassified WT	Mph1p	1.2e-10	ENSP00000343745(DICER1)	3.2e-257	CG4792-PA(Dcr-1)	3.6e-223	CBP05452	0
RAD3	*Y50D7A.2*	Y50D7A.2[*10]	CE33947	C	Emb Gro Let Lva Sck Stp		0.34	(-)	Emb WT	Rad3p	2.5e-161	ENSP00000221481(ERCC2)	4.5e-198	CG9433-PA(Xpd)	1.5e-197	CBP03819	1.2e-291
RAD3	*Y50D7A.11*	Y50D7A.11[*10]	CE35098	G	Gro Pvl Stp (Adl Emb Rup Sck)		0.62	(+?)	Rup Pvl Red_brood	Rad3p	5.0e-38	ENSP00000221481(ERCC2)	4.4e-51	CG9433-PA(Xpd)	7.2e-44	CBP03819	1.3e-83
RAD3	*bch-1*	F25H2.13	CE15894	G	WT			(-)	WT	Chl1p	3.3e-44	ENSP00000322287(RTEL1)	1.2e-117	CG4078-PA	2.1e-107	CBP14383	0
RAD3	*dog-1*	F33H2.1	CE17764	C	WT			(-)	Inc_mutation WT	Chl1p	5.8e-38	ENSP00000259008(BRIP1)	4.3e-102	CG4078-PA	1.2e-79	CBP11281	0
RAD3	*M03C11.2*	M03C11.2	CE03493	C	WT			(+?)	Emb WT	Chl1p	5.0e-77	ENSP00000309965(DDX11)	2.3e-118	CG11403-PA	4.7e-117	CBP19197	0
RECQ	*recq-5*	E03A3.2	CE00941	C	WT (Dev_ab)			(-)	Inc_mutation WT	Sgs1p	2.9e-61	ENSP00000317636(RECQL5)	2.9e-103	CG4879-PB(RecQ5)	1.6e-100	CBP04734	0
RECQ	*wrn-1*	F18C5.2	CE31791	C	WT			(-)	Age Clr Dpy Egl Lva Pvl Rup Small Unclassified WT	Sgs1p	1.5e-78	ENSP00000298139(WRN)	2.4e-114	CG6920-PA(mus309)	2.2e-69	CBP00715	0
RECQ	*K02F3.12*	K02F3.12a	CE33668	G	WT			(-)	WT	Sgs1p	2.4e-90	ENSP00000318727(RECQL)	3.0e-140	CG6920-PA(mus309)	1.3e-95	CBG13372	3.1e-261
RECQ	*him-6*	T04A11.6	CE31724	C	WT (Him)			(-)	WT	Sgs1p	3.0e-97	ENSP00000349859(BLM)	4.7e-128	CG6920-PA(mus309)	2.9e-132	CBP05142	1.3e-189
									Other helicase-like proteins								
RVB	*ruvb-1*	C27H6.2	CE08426	G	Emb Lva Sck [Let Pvl Ste]				Bmd Emb Gro Lva Pvl Stp WT	Rvb1p	3.3e-133	ENSP00000318297(RUVBL1)	6.5e-137	CG4003-PA(pont)	4.3e-140	CBP11169	2.5e-216
RVB	*ruvb-2*	T22D1.10	CE17254	G	Emb Lva Sck [Pvl Ste]				Cyk Emb Gro Mul Lva Pvl Sck Ste Stp	Rvb2p	6.5e-139	ENSP00000221413(RUVBL2)	4.9e-146	CG9750-PA(rept)	1.1e-144	CBP01546	3e-219
SSL2	*Y66D12A.15*	Y66D12A.15	CE28998	G	Emb			(+?)	Emb WT	Ssl2p	2.1e-205	ENSP00000285398(ERCC3)	1.1e-263	CG8019-PA(hay)	8.9e-262	CBP03250	0
KU70	*cku-70*	Y47D3A.4	CE28984		not tested				Age Unclassified WT	ND		ENSP00000353192(XRCC6)	3.4e-21	CG5247-PA(Irbp)	6.3e-08	CBP17736	1.7e-284
KU80	*cku-80*	R07E5.8	CE00660	G	WT			(-)	Growth_ab Reproduct_ab Unclassified WT	ND		ENSP00000329528(XRCC5)	9.1e-15	ND		CBP19221	2.8e-280
Twinkle-like	*F46G11.1*	F46G11.1	CE37646	G	WT			(-)	WT	ND		ENSP00000309595(PEO1)	1.9e-56	CG5924-PA	2.3e-41	CBP03429	1.6e-281
DNA pol theta-like	*polq-1*	W03A3.2	CE38982	G	WT			(+?)	WT	Brr2p	5.9e-20	ENSP00000264233(POLQ)	2.8e-110	CG6019-PA(mus308)	2.3e-128	CBP22167	8.6e-156
MOP-3-like	*nsh-1*	F20H11.2	CE20701	G	Gro Let Pvl Rup Sck Stp		0.49	(-)	Emb Gro Pvl Ste Stp Red_fat Thin WT	ND		ENSP00000267176(SBNO1)	0	CG3491-PA	6.8e-257	CBP03692	0
INTS6-like	*dic-1*	F08B4.1b	CE37220		not tested				Emb Lva Ste Loc_ab WT	ND		ENSP00000310260(INTS6)	2.3e-60	CG3125-PA(l(1)G0060)	1.3e-36	CBP19675	1.1e-151
Plant helicase-like	*F52G3.3*	F52G3.3	CE10858	G	WT			(-)	WT	ND		ND		ND		ND	
Plant helicase-like	*F52G3.4*	F52G3.4	CE10860	G	WT			(-)	WT	ND		ND		ND		CBP13991	3.1e-07

The genes encoding helicase-like proteins in *C. elegans* are summarized. For the classification of subfamilies, please refer to the classification of yeast helicase-like proteins (Table 2). The table here shows the gene names by subfamily, corresponding transcript, protein ID in WormBase (WS171), DNA source for the feeding RNAi construct, phenotypes of RNAi-treated nematodes and phenotype codes, sensitivity to X-ray irradiation in RNAi-treated worms, RNAi phenotype data as reported in WormBase (WS171), proteins with highest homology from *S. cerevisiae, H. sapiens, D. melanogaster*, and *C. briggsae* identified by BLASTP analyses, and corresponding *E*-values.

[a]Identification of helicase-like proteins in *C. elegans* was performed as described in what follows. In brief, the *C. elegans* protein data in WormBase[25] (release WS162) (http://www.wormbase.org/) were extensively searched with keywords such as 'helicase', and a sequence homology-based search was performed using the BLAST program, as described previously.[8] Amino acid sequences of 83 unique yeast helicase-like proteins (Table 2) and several known helicase-like proteins from mammals and plants [e.g. PEO1 (accession number NP_068602), MOP-3 (NP_060653), INTS6 (NP_036273) [also DDX26B [NP_872346]), and DNA polymerase theta (POLQ) (NP_955452) in humans] were used as queries for the BLAST search. Proteins with low similarities to a particular query sequence (*E*-value >1.0e-5) were checked for similarities to other helicase-like proteins by BLAST analysis. Additionally, amino acid sequences of candidate proteins were examined for helicase-related motifs (DEXDc [cd00046], HELICc [cd00079], etc.). Proteins without any helicase-related motifs were omitted from further analysis, with the exception of DIC-1, F20A1.9, and F52G3.3/F52G3.4, which are homologous to INTS6/DDX26B, DDX1, and Arabidopsis helicases, respectively.

RFC-like proteins and AAA+ proteins were omitted, with the exception of six subunits of the replicative MCM helicase and two RUVB-like proteins that are essential components of a chromatin remodeling complex. Questionable putative proteins with small sizes (less than 200 amino acid residues) were removed if they did not contain any helicase-related motifs, and six reported helicase-like pseudogenes were also omitted (Supplementary Fig. S1). Searches for helicase motifs in amino acid sequences and BLAST search for proteins and DNA were carried out using WormBase and NCBI databases.

[b]Transcripts with suffixes 'a', 'b', or 'c' encode the longest frame and were used for sequence analysis in this study.

[c]Source of insert DNA for feeding RNAi constructs: G, genomic DNA; C, cDNA.

[d]Adl, adult early lethal; Age, life span abnormal; Bli, blistered; Bmd, organism morphology abnormal; Clr, clear; Cyk, cytokinesis fails early embryo; Dpy, dumpy; Dev_ab, development abnormal; Dev_delay, developmental delay; Egl, egg laying abnormal; Emb, embryonic lethal; Esp, pathogen susceptibility increased; Evl; everted vulva; Gro, slow growth; Growth_ab, growth abnormal; Him, high incidence of male progeny; Inc_mutation, spontaneous mutation rate increased; Let, larval lethal; Loc_ab, locomotion abnormal; Lva, larval arrest; Mul, multiple nuclei early embryo; Nmo, nuclear morphology alteration early embryo; Pch, patchy coloration; Pvl, protruding vulva; Pharyngeal_ab, pharyngeal morphology abnormal; Rde, RNAi response abnormal; Rup, exploded through vulva; Red_brood, reduced brood size; Red_fat, fat content reduced; Reproduct_ab, reproductive system physiology abnormal; Sck, sick; Ste, maternal sterile; Stp, sterile progeny; Stress_ab, cell stress response abnormal; Transposon_ab, transposon silencing abnormal; WT, wild-type morphology. Parenthetic abbreviations indicate phenotypes in which RNAi-treated worms were observed on at least one occasion. Phenotypes observed in RNAi-treated rrf-3 mutant are shown in brackets.

[e]Phenotype code: The phenotypes of RNAi-treated nematodes are indicated by gray-scale coding: Emb in black, Lva and Gro in dark gray, and WT (no phenotype) in light gray. Empty code: no data (not tested). A phenotype code for the most intense phenotype is indicated.

[f]The growth retardation index is defined as the ratio of growth rate (μm/h) of progeny between RNAi-treated animals and control animals. Growth-defect phenotypes were tentatively classified as Lva with scores of less than 0.20, Gro with scores between 0.20 and 0.80, and WT with scores of more than 0.80. The index scores in brackets were derived from the experiments using the *rrf-3* mutant.

[g]Sensitivity to X-ray irradiation was assessed by visual inspection of treated worms and classified into the following groups: (+), sensitive; (+?), quasi-sensitive due to weak phenotypes of embryonic lethality, slow growth, or sickness, which was observed on at least one occasion in the experiment; (-), no sensitivity.

[h]RNAi phenotype data were derived from the currently reported data in WormBase (WS171)

[i]Information on homologous proteins of *H. sapiens, D. melanogaster*, and *C. briggsae* was obtained from WormBase (WS162) and BLASTP searches at NCBI. Queries of a number of *C. elegans* proteins were performed against human or *Drosophila* proteins to complete the table. The most similar budding yeast proteins were identified by a BLAST2P search in the *Saccharomyces* Genome Database. The proteins with *E*-values greater than 1e-5 were omitted and denoted as ND (not detected). Some human and fly proteins are indicated with corresponding gene names in parentheses. Human protein sequences were derived using Ensembl Translation ID (release 40). Human gene names with square brackets are not approved by the HUGO gene nomenclature committee.

[j]These genes may be a split single gene encoding the *C. elegans* ortholog ERCC2, since *Y50D7A.11* encodes a peptide (197 amino acid residues) that is similar to the N-terminal region of human ERCC2 (760 amino acid residues), and *Y50D7A.2* encodes an N-terminal-truncated ERCC2-like peptide with 606 amino acids (data not shown).

Table 2. Comparison of loss-of-function phenotypes of helicase-like genes in *S. cerevisiae* and *C. elegans*

Subfamily	*C. elegans* protein[a]	*E*-value of BLASTP analysis in WormBase (WS159)[a]	Phenotype code of RNAi-treated nematode[b]	*S. cerevisiae* ORF[c]	*S. cerevisiae* protein	Phenotype code of knockout strain[d]	Function of yeast protein[c]
DEAD-box subfamily							
DEAD-box	T07D4.4a	1.8e-70		YOR046C	Dbp5p		Nucleo-cytoplasmic RNA transport
DEAD-box	ZK686.2	5.9e-37		YNR038W[e]	Dbp6p		Ribosome biogenesis (60S)
DEAD-box	H20J04.4b	7.7e-90		YHR169W	Dbp8p		Ribosome biogenesis
DEAD-box	C24H12.4a	5.2e-75		YLR276C	Dbp9p		Ribosome biogenesis
DEAD-box	Y94H6A.5a	5.2e-130		YDL031W	Dbp10p		Ribosome biogenesis
DEAD-box	C07H6.5 (CGH-1)	2.1e-151		YDL160C	Dhh1p		Decapping and mRNA turnover
DEAD-box	Y71G12B.8	9.2e-112		YLL008W	Drs1p		Ribosome biogenesis
DEAD-box	B0511.6	3.1e-157		YMR290C	Has1p		Ribosome biogenesis
DEAD-box	Y23H5B.6	2.2e-115		YJL033W	Hca4p		Ribosome biogenesis, pre-rRNA maturation (40S)
DEAD-box	F53H1.1	6.5e-111		YBR237W	Prp5p		Pre-mRNA splicing
DEAD-box	F55F8.2a	6.4e-44		YBR142W	Mak5p		Ribosome biogenesis (60S)
DEAD-box	R05D11.4	3.8e-72		YGL171W	Rok1p		Ribosome biogenesis, pre-rRNA maturation (40S)
DEAD-box	T26G10.1	4.4e-114		YHR065C	Rrp3p		rRNA maturation (40S)
DEAD-box	ZK512.2	2.5e-68		YFL002C	Spb4p		Ribosome biogenesis, pre-rRNA maturation (60S)
DEAD-box	C26D10.2a (HEL-1)	1.9e-141		YDL084W	Sub2p		Pre-mRNA splicing, mRNA export
DEAD-box	F33D11.10	4.1e-130		YDR021W	Fal1p		Ribosome biogenesis, pre-rRNA maturation (40S)
DEAD-box	Y65B4A.6	8.6e-130					
DEAD-box	F57B9.6a (INF-1)	1.6e-135		YKR059W	Tif1p		Translation initiation
		1.6e-135		YJL138C	Tif2p		Translation initiation
DEAD-box	F01F1.7	1.2e-68		YDR243C	Prp28p		Pre-mRNA splicing
DEAD-box	F58E10.3a	1.1e-136		YNL112W	Dbp2p		RNA stability, ribosome biogenesis
DEAD-box	Y71H2AM.19	2.5e-139(Dbp1p)/2.0e-137(Ded1p)		YOR204W[f]	Ded1p		Translation initiation
DEAD-box	Y54E10A.9a (VBH-1)	9.9e-129(Dbp1p)/2.1e-126(Ded1p)		YPL119C	Dbp1p		Translation initiation
DEAD-box	F01F1.7/F53H1.1	2.6e-19/4.9e-19		YGL064C[g]	Mrh4p		Maintenance of mitochondrial DNA
DEAD-box	F58E10.3a	2.3e-88		YGL078C	Dbp3p		Ribosome biogenesis, pre-rRNA maturation (60S)
DEAD-box	B0511.6	2.0e-43		YDR194C	Mss116p		Mitochondrial gene expression
DEAD-box	B0511.6	2.3e-38		YKR024C	Dbp7p		Ribosome biogenesis (60S)
DEAD-box	C14C11.6 (MUT-14)[h]						
DEAD-box	C46F11.4						
DEAD-box	F57B9.3						
DEAD-box	F58G11.2						
DEAD-box	H27M09.1						
DEAD-box	T06A10.1 (MEL-46)						
DEAD-box	Y38A10A.6						
DEAD-box	Y54G11A.3						
DEAD-box	Y55F3BR.1						
DEAD-box	ZC317.1						
DEAD-box (glh)	B0414.6 (GLH-3)						
DEAD-box (glh)	C55B7.1 (GLH-2)						
DEAD-box (glh)	T12F5.3 (GLH-4)						
DEAD-box (glh)	T21G5.3 (GLH-1)						
DDX1-like	F20A1.9						
DEAH-box subfamily							
DEAH-box	C06E1.10 (RHA-2)	2.7e-146		YMR128W	Ecm16p		Ribosome biogenesis (40S)
DEAH-box	K03H1.2 (MOG-1)	5.5e-207		YKR086W	Prp16p		Pre-mRNA splicing
DEAH-box	F56D2.6a	1.3e-227		YGL120C	Prp43p		Pre-mRNA splicing
DEAH-box	C04H5.6 (MOG-4)	1.6e-176		YNR011C	Prp2p		Pre-mRNA splicing

Continued

Table 2. Continued

Subfamily	*C. elegans* protein[a]	*E*-value of BLASTP analysis in WormBase (WS159)[a]	Phenotype code of RNAi-treated nematode[b]	*S. cerevisiae* ORF[c]	*S. cerevisiae* protein	Phenotype code of knockout strain[d]	Function of yeast protein[c]
DEAH-box	EEED8.5 (MOG-5)	3.0e-252		YER013W	Prp22p		Pre-mRNA splicing
DEAH-box	T07D4.3 (RHA-1)	7.3e-82		YLR419W			Unknown
DEAH-box	EEED8.5 (MOG-5)	7.6e-121		YKL078W	Dhr2p		Ribosome biogenesis (40S)
DEAH-box	F52B5.3						
DEAH-box	T05E8.3						
DEAH-box	Y108F1.5						
DEAH-box	Y37E11AM.1						
DEAH-box	Y67D2.6						
SKI2 subfamily							
SKI2	W08D2.7	1.1e-173		YJL050W	Mtr4p		Ribosome biogenesis, pre-rRNA processing (60S), nuclear RNA degradation (?), mRNA transport (?)
SKI2	C08F8.2a	8.2e-70		YPL029W	Suv3p		Mitochondrial RNA degradation
SKI2	F01G4.3	3.2e-181		YLR398C	Ski2p		dsRNA killer propagation, cytoplasmic 3'-5' RNA degradation
SKI2	Y46G5A.4	0		YER172C	Brr2p		Pre-mRNA splicing
SKI2	Y54E2A.6	3.2e-210/3.4e-316(Y46G5A.4)		YGR271W	Slh1p		Regulation of translation?
SKI2	Y54E2A.6	2.5e-58		YGL251C	Hfm1p		Crossover control in meiosis
SKI2	C28H8.3						
SKI2	Y46G5A.6						
SKI2	Y55B1AL.3						
UPF1 subfamily							
UPF1	F43G6.1b (DNA-2)	4.7e-86		YHR164C	Dna2p		DNA replication, Okazaki fragment maturation
UPF1	Y48G8AL.6 (SMG-2)	5.1e-212		YMR080C	Nam7p		RNA stability, nonsense-mediated RNA decay
UPF1	Y48G8AL.6	2.0e-40		YKL017C	Hcs1p		DNA replication?
UPF1	Y48G8AL.6	5.4e-44		YLR430W	Sen1p		tRNA-, snRNA-, snoRNA-maturation
UPF1	Y48G8AL.6	8.2e-56		YER176W	Ecm32p		Translation termination
UPF1	C05C10.2						
UPF1	C41D11.7						
UPF1	C44H9.4						
UPF1	K08D10.5						
UPF1	R03D7.2						
UPF1	Y80D3A.2 (EMB-4)						
UPF1	ZK1067.2						
UPF1 (far related)	C44H9.2						
SWI2/SNF2 subfamily							
SWI2/SNF2	M03C11.8	2.2e-114		YAL019W	Fun30p		DNA repair?
SWI2/SNF2	F37A4.8 (ISW-1)	3.0e-207		YBR245C	Isw1p		Chromatin remodeling, transcription
		4.4e-249		YOR304W	Isw2p		Chromatin remodeling, transcription
SWI2/SNF2	F15D4.1 (BTF-1)	3.6e-122		YPL082C	Mot1p		Transcription
SWI2/SNF2	Y111B2A.22 (SSL-1)	2.0e-181		YDR334W	Swr1p		Chromatin remodeling, DNA repair
SWI2/SNF2	H06O01.2	4.0e-180		YER164W	Chd1p		Chromatin remodeling, transcription
SWI2/SNF2	W06D4.6 (RAD-54)	6.9e-160		YGL163C	Rad54p		DNA repair, DNA recombination
SWI2/SNF2	F53H4.1 (CSB-1)	9.8e-91		YJR035W	Rad26p		Transcription coupled repair
SWI2/SNF2	F01G4.1 (PSA-4)	6.9e-220(Snf2p)/1.4e-212(Sth1p)		YOR290C	Snf2p		Chromatin remodeling, transcription
SWI2/SNF2	C52B9.8	1.0e-205(Snf2p)/1.3e-190(Sth1p)		YIL126W	Sth1p		G2 control, chromatin remodeling, transcription
SWI2/SNF2	W06D4.6 (RAD-54)	3.6e-106		YBR073W	Rdh54p		DNA repair, DNA recombination

SWI2/SNF2	F01G4.1/C52B9.8	2.2e-105/9.8e-102	YFR038W	Irc5p		Unknown
SWI2/SNF2	Y111B2A.22 (SSL-1)	3.7e-113	YGL150C	Ino80p		Chromatin remodeling, transcription, DNA repair
SWI2/SNF2	F54E12.2	9.5e-46	YBR114W	Rad16p		DNA repair
SWI2/SNF2	F54E12.2	2.3e-46	YLR032W	Rad5p		Post-replication repair
SWI2/SNF2	F54E12.2	5.3e-65	YOR191W	Ris1p		Chromatin structure, gene silencing
SWI2/SNF2	B0041.7 (XNP-1)					
SWI2/SNF2	C16A3.1					
SWI2/SNF2	C27B7.4 (RAD-26)					
SWI2/SNF2	F26F12.7 (LET-418)					
SWI2/SNF2	F53H4.6					
SWI2/SNF2	F54E12.2					
SWI2/SNF2	F59A7.8					
SWI2/SNF2	T04D1.4 (TAG-192)					
SWI2/SNF2	T14G8.1 (CHD-3)					
SWI2/SNF2	T23H2.3					
SWI2/SNF2	Y113G7B.14					
SWI2/SNF2	Y116A8C.13					
SWI2/SNF2 (far related)	C25F9.5					
SWI2/SNF2 (far related)	F19B2.5					
SWI2/SNF2 (far related)	M04C3.1					
SWI2/SNF2 (far related)	Y43F8B.14					
SWI2/SNF2 (far related)	C25F9.4					
SWI2/SNF2 (far related)	M04C3.2					
MCM subfamily						
MCM	Y17G7B.5a (MCM-2)	1.2e-180	YBL023C	Mcm2p		DNA replication
MCM	F32D1.10 (MCM-7)	3.1e-143	YBR202W	Cdc47p		DNA replication
MCM	C25D7.6 (MCM-3)	8.3e-141	YEL032W	Mcm3p		DNA replication
MCM	ZK632.1a (MCM-6)	7.5e-171	YGL201C	Mcm6p		DNA replication
MCM	R10E4.4 (MCM-5)	1.2e-137	YLR274W	Cdc46p		DNA replication
MCM	Y39G10AR.14 (MCM-4)	1.8e-160	YPR019W	Cdc54p		DNA replication
PIF1 subfamily						
PIF1	Y18H1A.6 (PIF-1)	9.8e-83	YML061C	Pif1p		Maintenance of mitochondrial DNA and telomeres
		2.0e-80	YHR031C	Rrm3p		rDNA replication, Ty1 transposition
PIF1	C11G6.2					
PIF1	F11C3.1					
PIF1	Y116F11A.1					
PIF1 (*Helitron*)	F33H12.6					
PIF1 (*Helitron*)	F59H6.5					
PIF1 (*Helitron*)	Y16E11A.2					
PIF1 (*Helitron*)	Y27F2A.5					
PIF1 (*Helitron*)	Y46B2A.2					
PIF1 (*Helitron*)	ZK250.9					
MPH1 subfamily						
MPH1	D2005.5 (DRH-3)	1.0e-12	YIR002C	Mph1p		DNA repair
MPH1	C01B10.1 (DRH-2)					
MPH1	D2005.5 (DRH-3)					
MPH1	F15B10.2 (DRH-1)					
MPH1	K12H4.8 (DCR-1)					
RAD3 subfamily						
RAD3	Y50D7A.2	8.8e-161	YER171W	Rad3p		DNA repair, transcription
RAD3	M03C11.2	9.1e-64	YPL008W	Chl1p		Chromosome segregation

Continued

Table 2. Continued

Subfamily	*C. elegans* protein[a]	*E*-value of BLASTP analysis in WormBase (WS159)[a]	Phenotype code of RNAi-treated nematode[b]	*S. cerevisiae* ORF[c]	*S. cerevisiae* protein	Phenotype code of knockout strain[d]	Function of yeast protein[c]
RAD3	Y50D7A.11						
RAD3	F25H2.13 (BCH-1)						
RAD3	F33H2.1 (DOG-1)						
RECQ subfamily							
RECQ	T04A11.6 (HIM-6)	2.3e-99		YMR190C	Sgs1p		DNA repair, DNA recombination
RECQ	E03A3.2 (RCQ-5)	1.6e-63					
RECQ	F18C5.2 (WRN-1)	1.8e-78					
RECQ	K02F3.12	1.3e-89					
Other helicase-related proteins							
RVB	C27H6.2 (RUVB-1)	1.1e-132		YDR190C	Rvb1p		Chromatin remodeling, transcription
RVB	T22D1.10 (RUVB-2)	2.2e-138		YPL235W	Rvb2p		Chromatin remodeling, transcription
SSL2	Y66D12A.15	1.2e-206		YIL143C	Ssl2p		DNA repair, transcription
KU70	Y47D3A.4 (CKU-70)	>0.01		YMR284W	Yku70p		DNA repair, telomere maintenance
KU80	R07E5.8 (CKU-80)	0.0023		YMR106C	Yku80p		DNA repair, telomere maintenance
YLR247C	T05A12.4a	5.1e-32		YLR247C			Unknown
YDR291W[j]	F18C5.2	1.4e-07		YDR291W	Hrq1		Unknown
YDR332W	R05D11.4	9.1e-08		YDR332W	Irc3p		Unknown
HPR5	Y55B1BR.3	1.3e-06		YJL092W	Hpr5p		DNA repair
HMI1	NDi			YOL095C	Hmi1p		Maintenance of mitochondrial DNA
Y'-Hel1	ND			YBL111C			Unknown
Y'-Hel1	ND			YBL113C			Unknown
Y'-Hel1	ND			YDR545W	Yrf1-1p		Unknown
Y'-Hel1	ND			YEL077C			Unknown
Y'-Hel1	ND			YER190W	Yrf1-2p		Unknown
Y'-Hel1	ND			YFL066C			Unknown
Y'-Hel1	ND			YGR296W	Yrf1-3p		Unknown
Y'-Hel1	ND			YHL050C			Unknown
Y'-Hel1	ND			YHR218W			Unknown
Y'-Hel1	ND			YHR219W			Unknown
Y'-Hel1	ND			YIL177C			Unknown
Y'-Hel1	ND			YJL225C			Unknown
Y'-Hel1	ND			YLL066C			Unknown
Y'-Hel1	ND			YLL067C			Unknown
Y'-Hel1	ND			YLR466W	Yrf1-4p		Unknown
Y'-Hel1	ND			YLR467W	Yrf1-5p		Unknown
Y'-Hel1	ND			YML133C			Unknown
Y'-Hel1	ND			YNL339C	Yrf1-6p		Unknown
Y'-Hel1	ND			YOR396W			Unknown
Y'-Hel1	ND			YPL283C	Yrf1-7p		Unknown
Y'-Hel1	ND			YPR204W			Unknown
Twinkle-like	F46G11.1						
DNA pol theta-like	W03A3.2 (POLQ-1)						
MOP-3-like	F20H11.2 (NSH-1)						
INTS6-like	F08B4.1b (DIC-1)						
Plant helicase-like	F52G3.3						
Plant helicase-like	F52G3.4						

Loss-of-function phenotypes of helicase-like proteins in *S. cerevisiae* and *C. elegans* are summarized according to subfamily for comparison between species.[a]*C. elegans* proteins putatively orthologous to the yeast helicase-like proteins and *E*-values of the BLAST analyses are shown. *C. elegans* proteins were identified by BLASTP analysis and database

search against the InParanoid database (version 4.0 updated April 2005, http://inparanoid.cgb.ki.se/). Several putative orthologs were identified as reciprocal best BLAST hits with an *E*-value <1.0e-30 between *S. cerevisiae* and *C. elegans*. Suffixes 'a' and 'b' indicate variants with the highest homology to the yeast protein.[b]Phenotype code (*C. elegans*): The phenotypes of RNAi-treated nematodes are indicated by gray-scale coding: Emb in black, Lva and Gro in dark gray, and WT (no phenotype) in light gray. Empty code: no data (not tested). A phenotype code for the most intense phenotype is indicated.[c]Classification and functions of yeast helicase-like proteins are according to the yeast RNA helicase database by Linder and colleagues.[d]Phenotypes of the corresponding knockout strains were mainly obtained from the *Saccharomyces* Genome Database and our previous report[8] and shown by phenotype codes: lethal in black, slow growth in dark gray, and viable in light gray, no data in white.[e]The proteins surrounded with bold lines are a putative orthologous pair based on BLASTP scores, but were not in the InParanoid database. The Ku70 and Ku80 homologs in yeast and nematodes are described in the *Saccharomyces* Genome Database and WormBase.[f]Two pairs of yeast proteins (Snf2p and Sth1p, Ded1p and Dbp1p) with two *C. elegans* orthologs are surrounded by dashed lines.[g]The yeast proteins with BLAST scores lower than that of the putative homologs or without any sequence homologies to *C. elegans* proteins are indicated in separated box for each subfamily.[h]*C. elegans* proteins without significant similarities to yeast helicase-like proteins are also indicated separately.[i]ND, not detected. Several *C. elegans* proteins with *E*-values greater than 1e-10 when compared with the Y'-Hel1 proteins were omitted because the similarities were to low-complexity regions in the amino acid sequences.[j]Twenty-five budding yeast-specific proteins including subtelomere-specific helicase-like proteins and four yeast proteins (Hrq1p, Hpr5p, Hmi1p, and Irc3p) and six *C. elegans* (higher eukaryote)-specific proteins (DIC-1, NSH-1, POLQ-1, F46G11.1, F52G3.3, and F52G3.4) were detected.

then removed. The eggs on this second RNAi plate were used for phenotypic analyses of F1 progeny. HT115(DE3) with vector alone was used as control bacteria for mock RNAi treatments. For double-RNAi treatment, 50 μL of culture suspension equally mixed with growing bacteria for each target gene was seeded on RNAi plates for dsRNA expression.

2.5. Phenotypic analyses of RNAi-treated animals

The hatching rate of the eggs was determined as described previously.[24] RNAi-treated animals for 12 h were transferred onto a new RNAi plate to lay eggs for 12 h. F1 eggs laid on the RNAi plate were cultured for 24 h. Subsequently, the numbers of hatched larvae and dead eggs were scored to determine the hatching rate. The experiments were repeated at least twice. Growth of hatched F1 progeny was monitored by body length measurements as described previously.[22] The growth-defect phenotypes were tentatively classified as larval arrest (Lva), slow growth (Gro), and normal growth (WT), using the growth retardation index, as described in the legend of Table 1. Brood size of RNAi-treated animals was examined in two ways as described in the legend of Supplementary Table S2. X-ray sensitivity assay of RNAi-treated animals was performed as described in the legend of Tables 3 and 4.

3. Results

3.1. Identification of helicase-like genes in C. elegans

In this study, we have expanded our analyses of helicase family members from unicellular eukaryote *S. cerevisiae* to a multicellular animal, the nematode *C. elegans*. A sequence homology search, with known helicase-like proteins as the queries, identified 134 gene products in the recent *C. elegans* protein data in the public nematode database WormBase[25] (release WS162). These proteins were classified into 10 subfamilies (DEAD-box, DEAH-box, SKI2, UPF1, SWI2/SNF2, MCM, PIF1, MPH1, RAD3, and RECQ) on the basis of a modified classification of yeast helicase-like proteins and one group of 'other helicase-like proteins' containing 11 orphan proteins (Table 1). Three subfamilies (DEAD-box, DEAH-box, and SKI2) contain many proteins involved in aspects of RNA metabolism, including ribosome biogenesis, pre-mRNA splicing, RNA degradation, and translation.[2,3] A number of the proteins in the MCM,[26] PIF1,[27] RAD3, and RECQ[6] subfamilies play roles in DNA-mediated reactions, and the SWI2/SNF2 members act primarily in chromatin remodeling and/or DNA metabolism.[4,5] The total number of helicase-like proteins in *C. elegans* (134 proteins) was greater than the number of yeast helicase-like proteins (103 proteins including 21 subtelomeric helicase-like proteins).[28] Among the genes identified were six nematode homologs of mammal- and

Table 3. Influence of X-ray irradiation on the viability of F1 progeny from RNAi-treated animals

X-ray dose (Gy)	Hatching rate (%)			
	Control	*D2005.5* (RNAi)	*rad-51* (RNAi)	*Y66D12A.15* (RNAi)
0	91.8 ($n = 622$)	32.5 ($n = 382$)	62.4 ($n = 86$)	1.1 ($n = 451$)
40	62.8 ($n = 756$)	0.6 ($n = 313$)	3.2 ($n = 313$)	0.0 ($n = 574$)

The cDNA fragments corresponding to *D2005.5* and *rad-51*(*Y43C5A.6*) were amplified from phage cDNA clones yk331a2 and yk401c3 (a kind gift of Dr Y. Kohara, National Institute of Genetics, Japan), respectively, by PCR with the primer set yk5′-F (5′-TGGCGGCCGCTCTAGAACTAGTGGATC-3′) and yk3′-SmaR (5′-TTCCCGGGTGAATTGTAATACGACTCACTATAG GGCG-3′). These cDNAs were used for X-ray-induced embryonic lethality assay. The genomic DNA fragment (∼2.3 kb) corresponding to *Y66D12A.15* was amplified from *C. elegans* genomic DNA (N2 strain) by PCR using the primer set Y66Dex1-3F (5′-AAGCTTGAAAAACCCAGAAAAATGGCA-3′) and Y66Dex1-3R (5′-TTCCACTCCAACCTTGGTCGCATCGGC-3′). These fragments were cloned into the dsRNA expression vector, and the nucleotide sequences were confirmed by sequencing. Four young adult worms were fed bacteria-expressing dsRNA to the target gene on an RNAi plate for 18 h and were subsequently X-ray-irradiated (Radioflex 320CG, RIGAKU, Tokyo) at a rate of 2 Gy/min. Irradiated animals were transferred onto a fresh RNAi plate, cultured for 2 days to lay eggs and then removed. After 24 h, the hatching rate of eggs laid on the plate was determined. The total numbers of eggs counted are indicated in parentheses.

Table 4. Influence of X-ray irradiation on the growth of F1 progeny from RNAi-treated animals

	Body length (mm)		
	Control	*D2005.5* (RNAi)	*gei-17* (RNAi)
Mock irradiated	0.932 ± 0.062 ($n = 40$)	0.923 ± 0.076 ($n = 32$)	0.919 ± 0.116 ($n = 43$)
Significance relative to control (*P*-value)		0.521	0.560
X-ray irradiated (40 Gy)	0.992 ± 0.076 ($n = 56$)	0.726 ± 0.208 ($n = 18$)	0.826 ± 0.211 ($n = 31$)
Significance relative to control (*P*-value)		<0.0001	<0.0001

The genomic DNA fragment corresponding to *gei-17*(*W10D5.3*) was amplified using the primer set W10D5.3-F (5′-CGCTTCCACTTCCATTCTACGATG-3′) and W10D5.3-R (5′-GGCCATTCCAGATGGAGATGAGCC-3′). The *D2005.5* cDNA fragment (∼1.5 kb) was amplified from a *C. elegans* embryo cDNA library using the primers D1-BF (5′-CCGGGATCCA TCGTTGATCTGATGCCTGCGATGG-3′) and ZAP-R (5′-GAATTGTAATACGACTCACTATAGGGC-3′). The *D2005.5* cDNA and *gei-17* genomic DNA fragment were used for an X-ray-induced growth retardation assay. The growth of larvae from RNAi-treated animals was monitored by determining the mean body length of the animals. The mean ± standard deviation values of body length of animals at 3 days after X-ray or mock irradiation were determined and are indicated . Numbers of animals measured are in parentheses. Statistical significance of the differences in mean body length between control and RNAi-treated animals in each group was analysed by Student's *t*-test (significance at $P < 0.05$) using the software package JMP IN5.1.2J (SAS Institute, Cary, USA).

plant-specific helicase-like genes (*polq-1, nsh-1, dic-1, F46G11.1, F52G3.3*, and *F52G3.4*) and five *C. elegans*-specific SNF2-like genes (*C25F9.4, C25F9.5, M04C3.1, M04C3.2*, and *Y43F8B.14*). Six *Helitrons*, a novel class of mobile genetic elements encoding a 'rolling circle' replication protein and a helicase[29,30] (*F33H12.6, F59H6.5, Y16E11A.2, Y27F2A.5, Y46B2A.2*, and *ZK250.9*), were also identified.

Drosophila melanogaster, *Homo sapiens*, and *Caenorhabditis briggsae* proteins homologous to each *C. elegans* protein are presented in Table 1. Most helicase-like genes were well conserved between *C. elegans* and *C. briggsae*, with the exception of the *C. elegans*-specific SNF2-like genes, several PIF1-like genes including the *Helitrons*, and plant helicase-like gene homologs (*F52G3.3* and *F52G3.4*). Although homologs of the *Helitrons*, *F52G3.3*, and *F52G3.4* were detected in plants (data not shown), these three gene groups were not conserved in humans or flies. We detected putative human and fly homologs of many genes from the known subfamilies, as well as the orphan genes, but we were unable to find putative counterparts of several DEAD-box genes including four *glh* genes, some of the UPF1- and SWI2/SNF2-like genes, and most of the PIF1 members, in addition to the *C. elegans*-specific SNF2-like genes and two plant helicase-like genes (Table 1).

3.2. Phenotypic analyses of C. elegans RNAi-treated for helicase-like genes

Of the 134 genes identified in this study, 49 corresponded to genes of known function; however, the functions of the remaining genes are unknown. Therefore, we used the feeding RNAi method to identify loss-of-function phenotypes of uncharacterized helicase-like genes to aid in ascertaining the function of the gene products. We

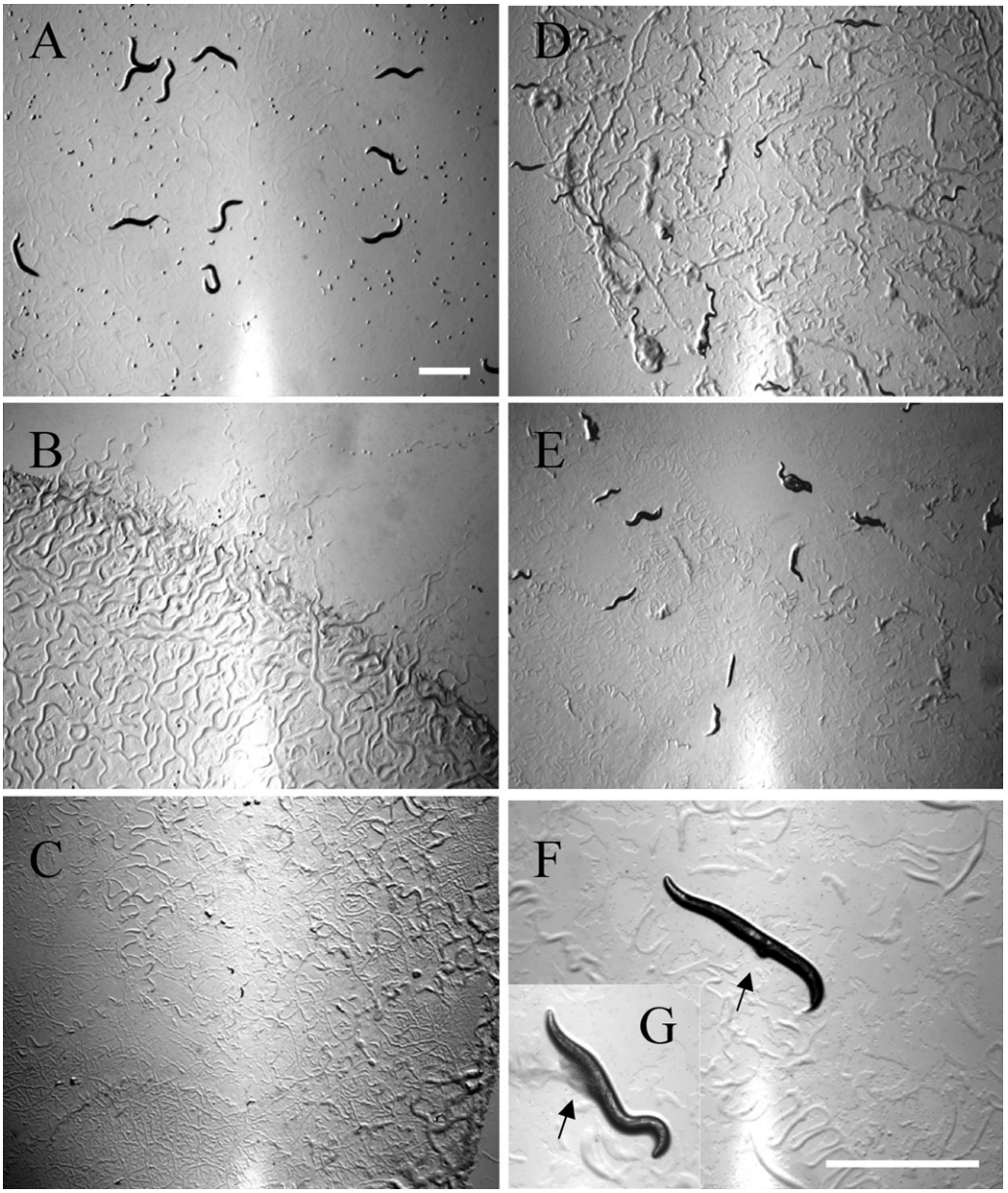

Figure 1. Typical phenotypes of F1 progeny from nematodes RNAi-treated for helicase-like genes. Typical images of the F1 progeny from eggs laid by RNAi-treated P0 animals on RNAi plates for control [vector alone (**A**)], *mcm-6*(*ZK632.1*) RNAi (**B**), *W08D2.7* RNAi (**C**), *ZK686.2* RNAi (**D**), *Y50D7A.11* RNAi (**E**), and *cgh-1* (*C07H6.5*) RNAi [(**F**) and (**G**) in a threefold enlarged image] are shown. The progeny were cultured on RNAi plates supplemented with dsRNA-expressing bacteria for 3 days after laying, and images were then captured. The RNAi phenotypes shown are embryonic lethal (Emb in Table 1) (B), larval arrest (Lva) (C), slow growth (Gro) (D), slow growth and sterile progeny (Gro Stp) (E), and protruding vulva (Pvl) (F and G). Arrows indicate protruded vulva (F) and resultant abdominal burst (G). Bar: 1 mm.

prepared 129 dsRNA expression constructs with cDNA or genomic DNA fragments of the target genes, and *E. coli* transformants expressing dsRNA were fed to P0 animals to examine the RNAi-induced phenotypes of the resultant F1 progeny. Typical culture images of the F1 progeny 3 days after hatching are shown in Fig. 1. The control progeny from mock-treated P0 animals grew to adults and laid F2 eggs (Fig. 1A). In contrast, eggs laid by RNAi-treated animals for *mcm-6*, encoding a subunit of the replicative MCM helicase,[26] exhibited an embryonic lethal phenotype (Fig. 1B). RNAi for the uncharacterized genes *W08D2.7* and *ZK686.2* encoding a yeast Ski2p-like protein and a DEAD-box protein caused larval growth arrest (Fig. 1C) and growth retardation (Fig. 1D), respectively, suggesting that both gene products are essential for development and/or larval growth. RNAi for *Y50D7A.11* which encodes an ERCC2-like protein caused a progeny sterile phenotype (no F2 eggs in the culture) with growth retardation (Fig. 1E). In addition to embryonic lethality and sterility,[31] we observed an

RNAi-induced developmental abnormality (a protruding vulva phenotype in Fig. 1F) and increased mortality (Fig. 1G) among F1 survivors of *cgh-1*(*RNAi*) animals.

In this study, we examined primarily embryonic lethality and growth-defect phenotypes. Fig. 2 shows growth curves of F1 larvae from animals treated with for 22 helicase members. Growth of progeny from *T26G10.1*(RNAi) and *B0511.6*(RNAi) was almost completely arrested (Fig. 2); however, RNAi-induced growth retardation was variable in progeny among the targeted genes. For example, growth rates of progeny from *F58E10.3*(*RNAi*), *Y23H5B.6*(*RNAi*), and mock-treated animals were calculated to be 3.4, 8.7, and 17.6 μm/h, respectively (Fig. 2). The level of growth defects in the progeny is represented as the growth retardation index in Table 1.

We compared our results with RNAi phenotype data in the public WormBase (WS171) and most phenotypes were in agreement (Table 1). Furthermore, we successfully obtained several new phenotypes; for instance, RNAi for two DEAD-box subfamily members, *C24H12.4* and *Y71G12B.8*, resulted in larval arrest and slow growth, respectively. RNAi for *Y66D12A.15*, which encodes an ortholog of the human ERCC3-like protein, and *psa-4*, which is required for embryonic development,[32] resulted in embryonic lethality in the current study. [These phenotypes in *Y66D12A.15*(*RNAi*) and *psa-4*(*RNAi*) were not previously present in the database (WS162), but have been recently confirmed in the updated version (WS171) during revision of the manuscript.] On the other hand, it was reported that RNAi for *dna-2, F20A1.9, F52B5.3, F59A7.8, M03C11.2, M03C11.8, Y116A8C.13*, and *Y37E11AM.1* caused an embryonic lethal phenotype, but no growth defect and/or visible abnormalities were observed in our experiments even using the RNAi-hypersensitive *rrf-3* mutant[33,34] as a host (data not shown). The discrepancies of RNAi experiments are summarized in Supplementary Table S1.

In this study, we examined effects of suppression of 39 germline- or oocyte-expressed helicase-like genes on brood size by feeding RNAi from L1 stage or from L4 stage (Supplementary Table S2). Reduction in brood size was observed in *F55F8.2*(*RNAi*) and *T05E8.3*(*RNAi*) animals in the L4 RNAi experiments. In the L1 RNAi experiments, suppression of *F56D2.6* and *C08F8.2* caused significant reductions of brood size; however, reduction was due to sterility in P0 animals by *F56D2.6* RNAi and to embryonic lethality by *C08F8.2* RNAi (data not shown). Reduced brood size in *C08F8.2*(*RNAi*) and *F55F8.2*(*RNAi*) animals has been observed previously by others.[35]

3.3. *An RNAi-mediated screen for helicase-like genes involved in resistance to X-ray irradiation*

We tried to identify genes that play important roles in specific conditions—in this case, X-ray irradiation—and applied the feeding RNAi technique to screen for genes involved in protection against X-ray-induced DNA damage. Assuming that dysfunctions in candidate genes would cause hypersensitivity to X-rays, animals RNAi-treated for 87 helicase-like genes that were dispensable for embryonic survival were tested for their X-ray sensitivity. RNAi-treated P0 animals were irradiated with X-rays (40 Gy), and the hatching rate of the resultant F1 progeny was examined. Several candidate genes were detected, but only *D2005.5*(*drh-3*) RNAi reproducibly enhanced the sensitivity to X-rays (Table 1). The hatching rate of the F1 progeny from *drh-3*(*RNAi*) animals without X-ray irradiation was 32.5% due to embryonic lethality induced by RNAi alone; however, the viability of F1 progeny from irradiated *drh-3*(*RNAi*) animals markedly decreased to 0.6% (Table 3). A similar X-ray hypersensitivity was observed in *rad-51*(*RNAi*) animals, in which DNA double-strand break repair and meiotic homologous recombination were suppressed.[36,37] X-ray-induced growth retardation was also observed in the F1 progeny from *drh-3*(*RNAi*) animals. The F1 progeny from *drh-3*(*RNAi*) and *gei-17*(*RNAi*) animals without irradiation developed normally. However, F1 larvae from irradiated animals exhibited a slow-growth phenotype (Table 4). The *gei-17* gene encodes a putative E3 SUMO ligase that participates in embryonic DNA damage responses in *C. elegans*, and the *gei-17*(*RNAi*) embryo is sensitive to other DNA-damaging agents.[38,39]

4. Discussion

4.1. *Identification of helicase family members and RNAi-based phenotypic analyses in C. elegans*

This is the first survey of members of helicase-like genes in *C. elegans*. In this study, several novel members of helicase family were identified by a systematic BLAST-based homology search, including two plant helicase-like genes of *F52G3.3* and *F52G3.4* and five *C. elegans*-specific SNF2-like genes. It should be noted that the current total number of helicase-like genes (134 genes) is tentative. For example, both *Y50D7A.2* and a neighboring gene, *Y50D7A.11*, may be a split single gene encoding the *C. elegans* ortholog ERCC2 (see the legend of Table 1).

In this study, we identified 51 helicase-like genes that are required for viability and/or developmental growth of *C. elegans*. This percentage (39.5% of 129 genes tested) was significantly higher than the number of phenotype-positive genes from several genome-wide RNAi analyses (∼10%[35,40] to 27%[41]), suggesting the biological importance of helicase-like genes in cellular function. The number of genes required for embryonic development and/or larval growth was variable among the subfamilies. For example, many members of the DEAD-box (63.9% of the members), DEAH-box (54.5%), MCM (100%), and MPH1 (50%) subfamilies exhibited development- and

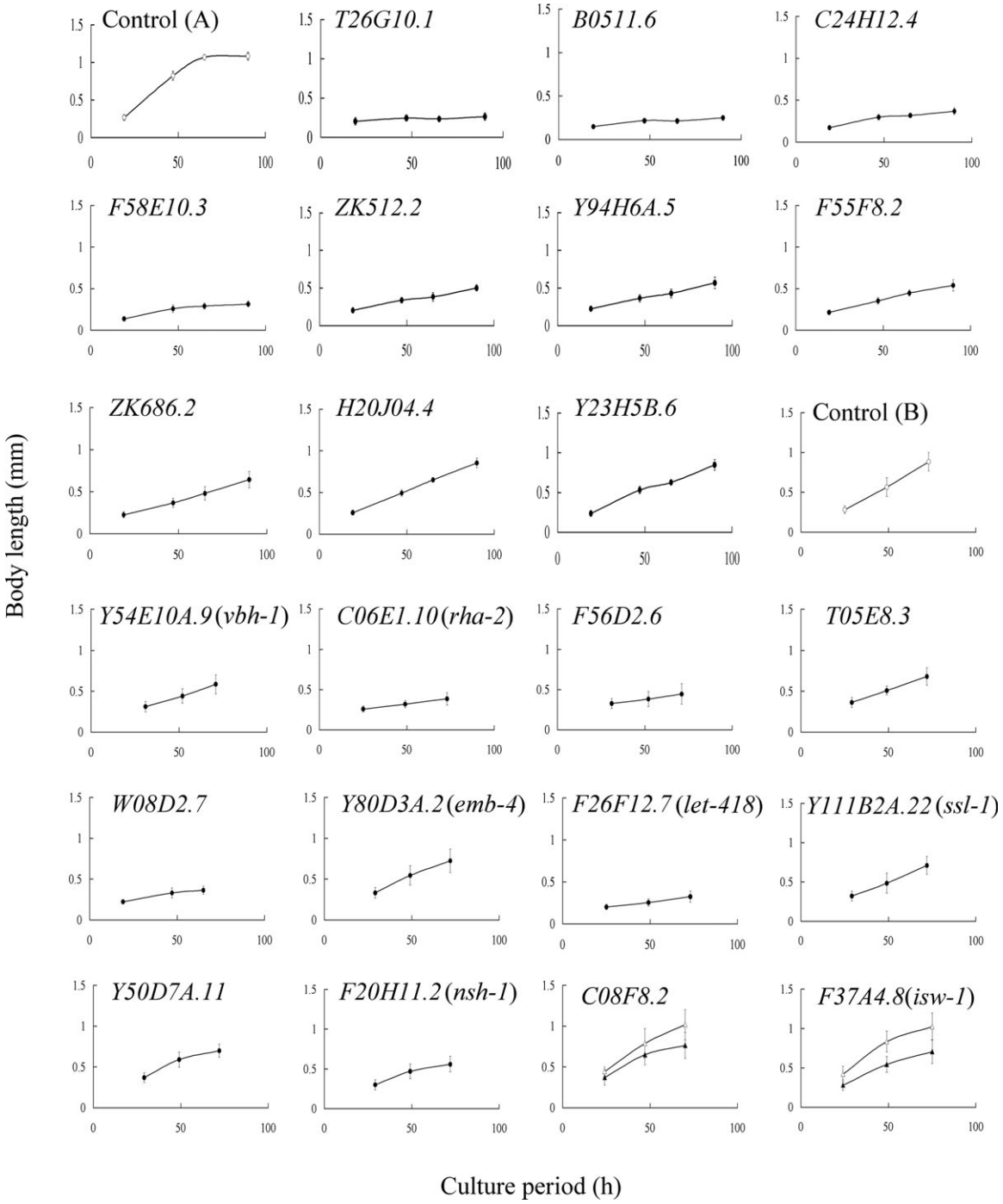

Figure 2. Influence of RNAi treatment of helicase family genes on larval growth. The growth of F1 larvae from eggs laid by RNAi-treated P0 animals was monitored by measuring the body length of progeny. The resultant growth curves of progeny of animals (N2 strain) that were RNAi-treated for the indicated 10 genes (*T26G10.1* to *Y23H5B.6*) in the DEAD-box subfamily are shown together with the growth curve of progeny without RNAi-treatment [control (A)]. The growth curves obtained from RNAi experiments for the genes in other subfamilies are shown with their control growth curve [control (B)] as follows: *Y54E10A.9*(*vbh-1*) from the DEAD-box subfamily; *C06E1.10*(*rha-2*), *F56D2.6*, and *T05E8.3* from the DEAH-box subfamily; *W08D2.7* from the SKI2 subfamily; *Y80D3A.2*(*emb-4*) in the UPF1 subfamily; *F26F12.7*(*let-418*) and *Y111B2A.22*(*ssl-1*) from the SWI2/SNF2 subfamily; *Y50D7A.11* from the RAD3 subfamily; and *F20H11.2*(*nsh-1*) as an orphan member, respectively. Experiments for *C08F8.2* (SKI2 subfamily) and *F37A4.8*(*isw-1*) (SWI2/SNF2 subfamily) indicated in bold letters were carried out using the *rrf-3* mutant as a host because of weak slow-growth phenotypes of the RNAi-treated N2 animals, and the resultant growth curves of progeny of control (open triangle) and RNAi-treated (closed triangle) animals are shown. The calculated growth rate for each population was 17.5 μm/h [control (A)], 0.7 (*T26G10.1*), 1.5 (*Y71H2AM.19*), 3.3 (*B0511.6*), 3.4 (*C24H12.4*), 4.0 (*ZK512.2*), 4.5 (*Y94H6A.5*), 5.1 (*F55F8.2*), 5.5 (*ZK686.2*), 8.4 (*H20J04.4*), 8.7 (*Y23H5B.6*), 12.5 [control (B)], 6.8 (*Y54E10A.9*(*vbh-1*)), 2.7 (*C06E1.10*(*rha-2*)), 3.0 (*F56D2.6*), 7.4 (*T05E8.3*), 3.2 (*W08D2.7*), 9.1 (*Y80D3A.2*(*emb-4*)), 2.6 (*F26F12.7*(*let-418*)), 9.1 (*Y111B2A.22*(*ssl-1*)), 7.6 (Y50D7A.11), 6.0 (*F20H11.2*(*nsh-1*)), 8.5 (*C08F8.2*) and 12.5 (*rrf-3* control), and 8.3 (*F37A4.8*(*isw-1*)) and 11.8 (*rrf-3* control).

growth-defects by RNAi. In contrast, relatively few of the PIF1 (0%), RECQ (0%), UPF1 (10%), or SWI2/SNF2 (15.4%) subfamily members showed such defects (Supplementary Table S3). These results are consistent with our previous phenotypic analysis using knockout strains of yeast helicase-like genes.[8] The difference in the incidence of these phenotypes among the subfamilies could be accounted by the biological roles in the members in each subfamily. It is interesting that the suppressions of half of the genes (*dcr-1* and *drh-3*) in the MPH1 subfamily cause growth-defects or embryonic lethality. This suggests biological importance of RNAi in viability and larval growth in *C. elegans* because both gene products act in RNAi.[42,43] We found some discrepancies in RNAi-induced phenotypes between our experiments and the studies reported in WormBase (Supplementary Table S1). We newly found larval arrest and slow-growth phenotypes caused by *C24H12.4* RNAi and *Y71G12B.8* RNAi, respectively. *C24H12.4* and *Y71G12B.8* encode putative homologs of the yeast DEAD-box proteins Dbp9p and Drs1p, respectively. Since both yeast proteins are required for ribosomal RNA biogenesis[11,44] and essential for viability in yeast,[3,8] our observations on both genes are probably significant (see the legend of Supplementary Table S1 for other discrepancies).

4.2. Loss-of-function phenotypes of helicase family members diverged in C. elegans

The putative *C. elegans* orthologs of yeast helicase-like proteins were identified and are shown in Table 2 with their loss-of-function phenotypes. The MCM subfamily members[26] and two RUVB-like proteins[45] were completely conserved and required for viability in both species. The DEAD-box, DEAH-box, SKI2, UPF1, and SWI2/SNF2 subfamilies contained two classes of proteins, those that were conserved in both species and those that were species-specific. For example, 21 putative orthologous pairs of the DEAD-box members were well conserved. In contrast, we could not detect any putative nematode homologs corresponding to the four yeast proteins Mrh4p, Dbp3p, Dbp7p, and Mss116p, or any yeast homologs of 15 nematode proteins. Budding yeast contain one or two members of the PIF1, MPH1, RAD3, and RECQ subfamilies; however, the number of members in each of these subfamilies had increased in *C. elegans*, and many of these divergent proteins are conserved in humans (Table 1). Twenty-five budding yeast-specific proteins and six higher eukaryote-specific proteins were also detected (Table 2). We found a high degree of conservation of loss-of-function phenotypes for homologs in both organisms. The majority (20 of 22 proteins) of putative *C. elegans* orthologs of yeast essential DEAD-box members caused embryonic lethality or growth-defect phenotypes by their depletion (Table 2). Similar phenotypic conservation of essential homologs in both species was also found in members of the DEAH-box, SKI2, MCM, and RAD3 subfamilies, as well as in the RUVB-like and SSL2-like proteins, suggesting that these putative conserved orthologs play similar essential cellular roles in *C. elegans* as in their yeast counterparts (Table 2). Interestingly, depletions of the subfamily members which have diverged in *C. elegans* were rarely able to induce growth-defect phenotypes (i.e. only nine of 67 genes tested across the subfamilies).

Because of the detection of diverged members in *C. elegans*, we assigned all helicase-like genes and pseudogenes to the six *C. elegans* chromosomes to examine the distribution of the extranumerary genes in the genome. The BLAST-based sequence homology searches identified 10 candidate gene pairs, three highly related gene pairs, and two gene clusters in the helicase-like genes (Supplementary Table S4) and mapped to chromosomes (Supplementary Fig. S1). Six putative gene pairs (*glh-1* and *glh-2*, *glh-4* and *T08D2.3*, *F33D11.10* and *Y65B4A.6*, *F57B9.3* and *inf-1*, *F53H1.1* and *Y73B3B.5*, and *mut-14* and *ZC317.1*) belonged to the DEAD-box subfamily (Supplementary Fig. S1A). At least two partners of the paired genes were pseudogenes (*T08D2.3* and *Y73B3B.5*). Four pairs of putative duplicated genes were identified among the SWI2/SNF2 (*let-418* and *chd-3*), SKI2 (*Y46G5A.4* and *Y46G5A.6*), PIF1 (*C11G6.2* and *Y116F11A.1*), and MPH1 (*drh-1* and *drh-2*) subfamilies (Supplementary Figs S1C, D, G and H). In addition, two clusters of *C. elegans*-specific SNF2-like genes and *Helitrons* in the PIF1 subfamily were found on the terminal regions of chromosomes V and II, respectively, suggesting that these genes might have been generated by a few rounds of gene duplications (Supplementary Figs S1C and G; see the figure legend).

RNAi for the subfamily members diverged in *C. elegans* poorly induced growth-defect phenotypes (Table 2). This phenomenon may indicate a functional redundancy with paralogous proteins or diverged members in *C. elegans*. In fact, two diverged DEAD-box members, GLH-1 and GLH-4, are known to play redundant functions in germline development. Kuznicki *et al.*[46] showed that double RNAi for *glh-1/4* was required for significant sterile phenotype. Since some of paired proteins (e.g. LET-418 and CHD-3) have redundant functions,[47] we assumed that the products of duplicated genes with unknown function (i.e. Y67D2.6 and Y108F1.5, or C11G6.2 and Y116F11A.1) may be the case. However, none of the detectable phenotypes in animals treated with double RNAi for these paired genes were observed (data not shown).

4.3. Expression profiles of helicase-like genes in C. elegans and influence of RNAi for germline-enriched genes

We examined the expression profiles of the helicase-like genes, using four published genome-wide expression

studies of *C. elegans* genes[48–51] (Supplementary Table S2). Reinke *et al.*[51] identified germline-enriched and sex-regulated genes and classified the genes into several expression categories. Assignment of helicase-like genes in each subfamily to each expression category revealed that many helicase-like genes (58 of 134 genes) were categorized as 'intrinsic' and 'oogenesis-enriched' genes (Supplementary Table S5A). The fraction of helicase-like genes (21.6%) in the oogenesis-enriched category is significantly higher than that of *C. elegans* genes in general (5.7% of the total genes), suggesting an expression bias for helicase family members in oogenesis in hermaphrodites. Dominant expression of helicase-like genes in the embryonic stages was also detected in another study[50] (Supplementary Table S5B). The data reported by Jiang *et al.*[48] show sex-biased expression of the helicase-like genes (12.7% of helicase-like genes versus 27.6% of total *C. elegans* genes in male; 32.1 versus 24.7% in hermaphrodites in Supplementary Table S5C). This is also consistent with the high proportion of helicase-like genes in the oogenesis-enriched genes. Assignment of the helicase-like genes to the *C. elegans* gene expression map[49] also indicates that helicase-like genes were relatively concentrated (1.9- to 4.2-fold) in six mountains (2, 5, 7, 11, 20, and 25) out of 46 mountains, and mountains 2, 7, and 11 contain predominantly oocyte- and germline-enriched genes[49] (Supplementary Table S6). In addition, the dominant expression of PIF1 or SKI2 members in males is interesting, because three of three and three of six genes in the PIF1 and SKI2 subfamilies, respectively, appeared in the 'male dominant groups' (Supplementary Table S5C), and this may implicate these genes in male-specific functions such as spermatogenesis. Most *Helitrons* of the PIF1 members were poorly expressed in the aforementioned studies.

Since oogenesis-enriched expression of many helicase-like genes suggests potential roles of their gene products in the development and proliferation of germ cells, we examined the effect of suppression of 39 germline-enriched genes on brood size by RNAi, and reduced reproductive capacity in *F55F8.2*(*RNAi*) and *T05E8.3*(*RNAi*) animals was detected (Supplementary Table S2). *F55F8.2* encodes a homolog of yeast-splicing factor Prp28p, and the *T05E8.3* gene product is similar to yeast Prp22p and a putative homolog of human DHX33 (Table 1). This indicates that both gene products play important roles in the reproduction in *C. elegans*. In the L1 RNAi analysis, suppression of *F56D2.6* and *C08F8.2* caused a significant reduction in brood size because of sterility in P0 animals and of embryonic lethality, respectively, suggesting that *F56D2.6*, encoding a putative homolog of yeast-splicing factor Prp43p, is required for reproduction, and as a putative homolog of yeast mitochondrial RNA helicase Suv3p, C08F8.2 plays an essential role in embryonic viability. In the previous study by Colaiacovo *et al.*,[52] four helicase-like genes have been identified in an RNAi-based screen for genes involved in chromosome morphogenesis and nuclear organization in *C. elegans* germline. We also found several detectable phenotypes in *dcr-1*(*RNAi*) and *ruvb-2*(*RNAi*) animals, but did not find weak phenotypes reported in *rha-1*(*RNAi*) and *C27B7.4*(*RNAi*) animals (Table 1).

4.4. Identification of drh-3 gene involved in resistance to X-ray-induced DNA damage

In this study, we succeeded in identifying *D2005.5*(*drh-3*) as a gene for protection against X-ray irradiation. Four Dicer-like proteins in the MPH1 subfamily, including DRH-3, have recently been shown to play an important role in RNAi.[43] It remains to be resolved why depletion of the RNAi factor DRH-3 causes X-ray hypersensitivity in *C. elegans*. Previous RNAi-based studies have shown that some helicase family members are implicated in resistance to X-ray-induced DNA damage. Boulton *et al.*[53] showed that RNAi of *rad-54* and *Y116A8C.13* caused DNA repair defect phenotypes. Recently, van Haaften *et al.*[54] performed a genome-wide screen for *C. elegans* genes that protect cells against ionizing radiation to identify three helicase-like genes *D2005.5*, *Y80D3A.2*, and *isw-1*. Although *D2005.5* was commonly detected in the screens by us and van Haaften *et al.*, we failed to find marked X-ray-dependent phenotypic defects in the progeny of *Y80D3A.2*(*RNAi*) or *isw-1*(*RNAi*) animals (Table 1). Several genes including *rad-54* or RecQ-like helicase genes are thought to be involved in DNA repair of X-ray-induced DNA damage; however, these genes were not always detected in previous RNAi-based screens. In order to isolate more candidate genes, several technical improvements in the screens may be required, including more sensitive assay systems (e.g. use of reporter animals[55]), RNAi-based screens using *rrf-3* mutants,[34] or soaking RNAi-mediated screens.[41]

In conclusion, we have identified helicase-like genes in *C. elegans* and characterized loss-of-function phenotypes of these genes. The results obtained from phenotypic analyses, comparative analyses, chromosome mapping, and a study of the expression patterns of helicase family members will be useful for studying helicase-mediated molecular reactions governing dynamic regulation of DNA, RNA, and chromatin. Furthermore, characterization of DRH-3 will elucidate functional interactions between the resistance to X-ray irradiation and RNAi.

Supplementary material

Supplementary Data: Supplementary data are available online at dnaresearch.oxfordjournals.org.

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