# **Supplementary Material**

SF1 and SF2 helicases: family matters

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**Current Opinion in Structural Biology, 2010** 

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Alignment of the helicase core of SF1 and SF2 proteins. SF1 and SF2 proteins from *H. sapiens* (Black), *S. cerevisiae* (violet), *E. coli* (green), and representative members of Flavi-Poty- and Pox-viruses (red) were identified and retrieved from Uniprot and NCBI databases. The helicase core sequences were aligned using ClustalW [1] and Seaview [2] alignment and visualization programs. The resulting alignments were further refined by hand using structural information. For clarity, large insertions are removed from the alignment. These sections are marked by cyan highlights of the number of deleted residues. Proteins are listed in alphabetical order by organism. Helicase motif designations are indicated at the top of the alignment. Characteristic residues in each motif are highlighted in yellow.

The numbering of the motifs is based largely on the original designation by Gorbalenya and Koonin [3]. Exceptions are as follows: motifs V, Va, and Vb were designated as a single motif V, and motif IIIa, present in SF1 proteins, but absent in most SF2 proteins. Motif IIIa in the SF1 was historically termed motif IV. However, structural comparisons of SF2 and SF1 proteins revealed that the position of motif IV in SF1 differed from the position of motif IV in SF2 proteins [4]. The SF1 motif corresponding to motif IV in SF2 was then termed motif IVa. For consistency, we chose here to assign the SF2 designation to both groups, i.e., the SF1 motif IVa is labeled here motif IV as well.

Several motifs that are primarily involved in nucleic acid binding show little sequence conservation (e.g. motifs Ib, Vb). In protein families with little structural information, appropriate designation of these motifs was ambigous. For this reason, no or only minimal highlighting of these motifs is shown. Similarly, motif Q is difficult to assign with limited structural information, and the corresponding sequence assignment should be viewed with care.

We only included certain viral SF2 proteins, even though many viruses encode SF1 and SF2 helicases [5,6]. However, sequence and structural information for most of these proteins is very limited. In contrast, the flaviviral NS3 proteins and the poxviral NPH-II helicase from vaccinia virus are among the biochemically and structurally best characterized SF2 helicases. In addition, flaviviridae NS3 and poxiviridae NPH-II, together with the CI helicases from potyviridae, bear interesting resemblance in sequence to the DEAH/RHA family. We therefore included in the alignment two viral proteins from each of these virus types (poxviruses, potyviruses, and three

flavivirus families). Comparing these well characterized proteins to other SF2 helicases may provide valuable insight into the function of other SF2 proteins.

Note: Because of the large size of the alignment layout, it is best viewed and printed at 400 % of its original size.

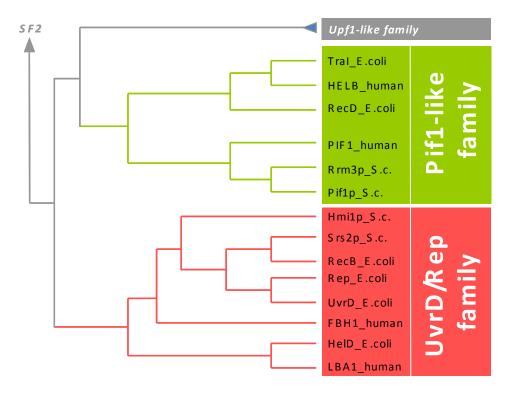
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mily	Rad3/Xpd		Ski2-like							DEAH/RHA								RIG-I-like			RecQ-like				
cerevisiae	Chl1p	Rad3p	Ski2p	Mtr4p	Hfm1p		Slh1p	Brr2p		Prp22p	Prp16p	Prp43	Dhr2p	Dhr1p	YL419p		Prp2p		Mph1p		Sqs1p	Hrq1p			
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cerevisiae																									

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Supplementary table TS1. List of eukaryotic proteins used in the SF12 alignment. Proteins from S. cerevisee and H. sepiens are listed by family, with homologous/orthologous proteins shown in the same column in each subtable. SUV3 homologs from both organisms could not be assigned to a family.

SF	1			2										
Family	UvrD/Rep	Pif1-like	Upf1-like	Swi/Snf	Rad3/Xpd	Ski2-like	DEAH/RHA	RIG-I-like	RecQ-like	DEAD-box	RecG-like			
E. Coli	RecB	Tral	Z1202	RapA	DinG	Q6XGE6	HrpA		RecG	DbpA	RecG			
	Rep	RecD	UL1		YoaA		HrpB		PriA	RhIB	PriA			
	UvrD								MFD	Dead	MFD			
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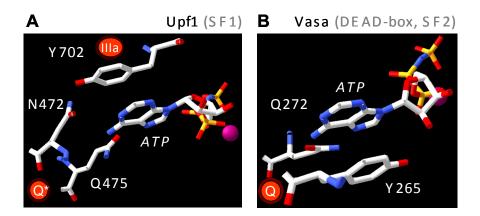
Supplementary table TS2. List of SP12 proteins from Ecoli used in the alignment. The highly similar type III restrictions enzymes BPP1 and BP15I could not be assigned to any family.



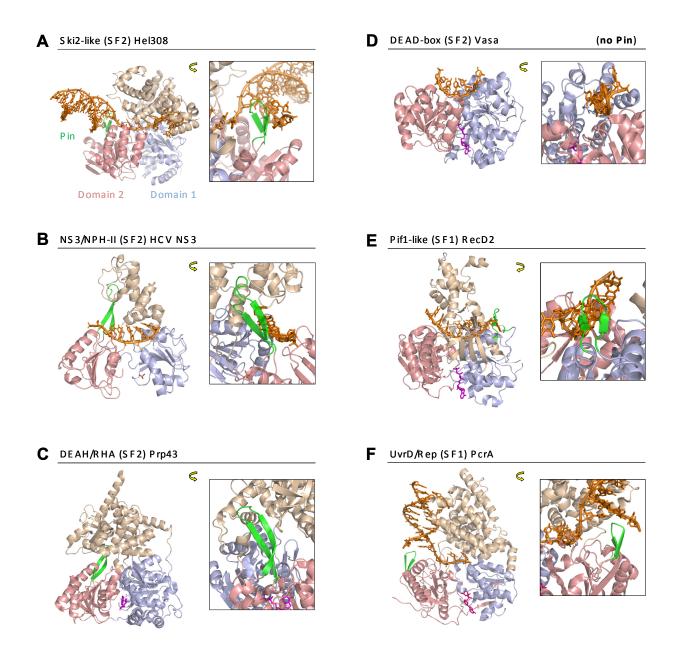
Cladogram for UvrD/Rep and Pif1-like families. The cladogram shows an example of the phylogenetic analyses for two SF1 protein families, based on the sequence alignment of the helicase core (Suppl. Fig.S1). The figure shows the result of an analysis using distance- and character-based program options (BioNJ, protpars) of Seaview4 [7,8]. Consistent grouping of proteins into family clusters, such as those seen here, were obtained with various phylogenetic methods. However, topologies of phylogenetic trees varied on occasion, dependent on the algorithm used. This variation is presumably due to the large number of unavoidable sequence gaps in the alignment (Suppl. Fig.S1). In several instances, branching was not supported by high bootstrap values. Consequently, the evolutionary relationship between individual family members and the phylogenetic relationship between the families (Fig.1) should thus be viewed with care. Nonetheless, the cladograms revealed several phylogenetic relations that are in excellent with structural data (Figs.3,4), for example the close relation of the NS3/NPH-II group to the DEAH/RHA proteins (Figs.3,4).

SF	F	Q	I	la	Ib	Ic	II	III	IIIa	IV	IVa	V	Va	Vb	VI
1	UPF1		<b>GPPGTGKT</b>	APSN AV	₽ <mark>₽</mark> ₽	E SA	DEARQ		<u>QY</u> Pw	UIPY Q		Y_ <del></del> ŢV <u>P</u>	<b>ECCEE</b>	<u>L</u> Ŀ <b>S</b> ≘VR	VALTRAK
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2	DEAD	PIPU		PIRELA	<u>GG</u>	<b>aTPGR</b> L		<b>ESAT</b>		LV YELK	HG COLOR	<u>√</u> § DvV	<u>arg-D</u> ,	NINYD	RIGRIGRA
	DEAH/ RHA			<b>QPRRYAA</b>	<u>GY</u> <sub>2</sub>	<b>™</b> T <b>Q</b> G <sub>W</sub> L	DE&HER	<b>MSAT</b> E		Ly LIG <sub>®</sub>	VLPLYS LP PRYFE	VYSTNIA	EISTI	¥VVD&G	QR <sub>@</sub> GRAGR <sub>W</sub>
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Characteristic sequence motifs for the individual SF1 and SF2 protein families. Sequence conservation on the family/group level of the characteristic helicase motifs is shown as sequence logo [9]. The height of the amino acid symbols reflects the sequence conservation and the color their chemical properties: polar - green, basic - blue, acidic - red, and hydrophobic -black. Sequence positions that are only conserved within a given family/group are not displayed.



The putative adenine stacking platform in motif IIIa of SF1 proteins. Sidechains of conserved residues that provide specificity for the adenosine base are shown from similar angles in structures of the SF1 helicase UPF1 [10] (A) and SF2 DEAD-box protein *Vasa* [11] (B). Atoms are colored as follows: C – white, O – red and N – blue. Residues of the Q-motif that contact the adenosine in a specific manner are positioned similarly in both structures (Q475, N472 in UPF1; Q272 in Vasa). A conserved aromatic residue stacking on the adenine base is located in motifs IIIa in UPF1, but in the Q-motif in Vasa (Y702 in UPF1, Y265 in Vasa). Notably, this residue approaches the adenine base from opposite direction in SF1 (top) and SF2 (bottom). In addition, motif IIIa contains a highly conserved arginine.



Characteristic "pins" in structures of SF1 and SF2 helicases. Representative structures of proteins from different SF2 and SF1 families, containing "pin" domains, a prominent  $\beta$ -hairpin. The helicase domain 1A is blue, 2A pink, accessory/ inserted domains are light-beige. The "pin" is green in all structures. ATP and nucleic acid, are magenta or orange, respectively. In the left panels, all structures are oriented as shown in **Fig.4**. The right panels show the structures rotated by roughly 90° (indicated by a yellow arrow), zoomed into the region containing the "pin". Panel

**D** shows the DEAD-box protein Vasa as example for a family lacking a "pin". The following structures are shown: (**A**) Hel308 (*Archaeoglobus fulgidus*) [12], (**B**) HCV NS3 (*Hepatitis C virus*) [13], (**C**) Prp43p (*S. cerevisiae*) [14], (**D**) Vasa (*D. melanogaster*) [15], (**E**) RecD2 (*D. radiodurans*) [15], (**F**) PcrA (*B. stearothermophilus*) [16]. We note that Rad3/XPD proteins, instead of a β-hairpin, have a 2-helix insertion in a similar location between motifs Va and VI [17]. It is unclear whether this motif serves a function similar to the "pin".

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