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Structure of complete Pol II-DSIF-PAF-SPT6 transcription complex reveals RTF1 allosteric activation

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Supplementary Material

Supplementary Note 1 | Cryo-EM data collection and processing and Model building

Supplementary Table 1 | Complete EC* BS3 crosslinking statistics and measured distances.

- A.** Summary of intra and inter complex crosslinks in complete EC*.
- B.** Crosslinks mapped to complete EC* and their distances as determined in Xlink analyzer⁷⁸.

Supplementary Table 3 | Modelling of PAF and RTF1 subunits and the SPT5 CTR.

Description of how regions of PAF and RTF1 were modelled in the complete EC*.

Supplementary Table 4 | RTF1 sequence alignment.

RTF1 sequences from the indicated organisms were aligned in MAFFT⁷⁶ and visualized in Jalview⁷⁷. Secondary structure elements observed in human or *S. cerevisiae* RTF1 are demarcated above the sequence alignment or as predicted by Psipred⁸⁰. Residues colored by percentage identity. Darker shades of blue indicate higher conservation.

Supplementary Note 1 | Cryo-EM data collection and processing and Model building

Cryo-EM data collection and processing

Three independent cryo-EM datasets were collected and merged. Cryo-EM data was collected on a FEI Titan Krios II transmission electron microscope operated at 300 keV. A K2 summit direct detector (Gatan) with a GIF quantum energy filter (Gatan) was operated with a slit width of 20 eV. Automated data acquisition was performed with FEI EPU software at a nominal magnification of 130,000x, corresponding to a pixel size of 1.049 Å/pixel. Image stacks of 40 frames were collected over 10 s in counting mode. The dose rate was 3.4-4.7 e⁻ per Å² per s for a total dose of 34-47 e⁻/Å². A total of 13,679 image stacks were collected (Dataset 1: 5557, Dataset 2: 5135, Dataset 3: 2987).

Frames were stacked, processed for motion correction, and CTF corrected with Warp¹. Particles were picked using a BoxNet2 algorithm as implemented in Warp. Particles from the three data sets were extracted with a box size of 360² pixels and merged, yielding a total of 611,983 particles. The combined particles were subjected to 3D-refinement in RELION 3.0² using a map of EC*³ that was low pass filtered to 30 Å resulting in a reconstruction with a resolution of 3.37 Å. IWS1, an SPT6 associating factor that is required for transcription through chromatin, was included during complex formation, however, we did not observe density for it in our maps. IWS1 does not have an effect on Pol II elongation activity in our RNA extension assays (data not shown). Successive rounds of 2D classification without image alignment were performed to remove non-aligning particles and contamination. The resulting 446,195 particles were CTF refined as implemented in RELION 3.0² fitting per particle defocus and astigmatism. CTF refined particles were subjected to global 3D refinement resulting in a reconstruction at 3.1 Å using the gold standard FSC 0.143 criterion (Map 1)⁴.

To improve densities for specific domains, we performed 3D classification without image alignment with applied soft masks around the region of interest. Masks were generated in Chimera⁵ and RELION 3.0² around the RTF1 Plus3 and helices that associate with the Pol II surface (Maps 2 and 3), CTR9 N-terminus (Map 6), CTR9 C-terminus (Maps 4 and 5), SPT6 core (Maps 9 and 10), and PAF1-LEO1 dimer (Maps 7 and 8). Our classification approach showed that CTR9, SPT6, and the PAF1-LEO1 dimer adopt at least two different conformations on the Pol II surface. The CTR9 and SPT6 conformations are similarly occupied and our model can be flexibly fit into either set of maps. Only one of the PAF1-LEO1 dimer conformations (Map 7) allows for unambiguous modelling of the dimerization domain. The other conformation appears to more heavily clash with the positioning of TFIIIF, however, its

lower resolution makes docking of the crystal structure unreliable. A composite map was generated for model refinement in Phenix⁶. Maps 1, 2, 3, 4, 6, 7, and 10 were combined using the Combine Focused Maps tool as implemented in Phenix⁶.

Model building

The structure of the complete EC* was determined by first placing the structure of EC* into Map A in Chimera (PDB ID: 6GMH)³ and locally adjusting it in Coot⁷. Clear additional density for RTF1 was observed adjacent to Pol II subunit RPB12. The crystal structure of the RTF1 Plus3 bound to a phosphorylated SPT5 CTR peptide was placed into the corresponding density in Map 2⁸ (PDB ID 4L1U) and flexibly fit with NAMDinator⁹. Four additional helices that anchor RTF1 to Pol II were modelled *de novo* using secondary sequence predictions from PSIPRED¹⁰ and a 2-residue extension to the previously determined RTF1 Plus3 structure. The register of the helices was confirmed by densities of bulky side chains of residues such as arginine and tyrosine. The SPT5 CTR is highly repetitive and a portion of the second and third repeat were used in the previously determined crystal structure⁸. Distances were better satisfied in our structure when we used the first and second repeat. Thus, the side chains of the SPT5 CTR were mutated to reflect the sequence of the first and second CTR.

To model CTR9, CDC73, and the N-terminal region of PAF1, we used a recent crystal structure of the *M. thermophila* complex (PDB ID 6AFO)¹¹ and threaded the human sequences through the *M. thermophila* structure in Phyre2¹² (CTR9: *M. thermophila* 30-964 corresponds to human 1-801; CDC73 *M. thermophila* 164-205 corresponds to human 217-256; PAF1 *M. thermophila* 13-119 corresponds to human 25-114). We then placed the threaded crystal structure into a locally refined map of particles selected for density corresponding to the N-terminus of CTR9 (Map 6). The crystal structure was then divided into three pieces. The first piece included CTR9 residues 446-801, CDC73 and PAF1 residues 25-49. This piece was merged with the trestle helix (CTR9 residues 807-892) and WDR61 from EC* (PDB 6GMH)³. The second piece included CTR9 residues 337-445 and PAF1 50-61. The final piece included CTR9 residues 2-371 and PAF1 residues 52-113. Each piece was separately refined and flexibly fitted using NAMDinator⁹ with standard settings (resolution cutoffs of 5 Å for the first piece, 8 Å for the second piece, and 10 Å for the final piece). The pieces were connected in Coot and a final round of NAMDinator⁹ was used to flexibly fit the ensemble into the density with a resolution setting of 8 Å followed by two rounds of Phenix real space refinement¹³.

Our cryo-EM densities show clear density for the triple barrel dimerization domain of the PAF1-LEO1. We were previously³ not able to fit a crystal structure of the domain into our densities (PDB ID: 4M6T)¹⁴. We thus carefully examined the crystal structure (PDB ID: 4M6T) and compared it to the crystal structure of human TFIIIF triple barrel domain (1F3U)¹⁵. The LEO1 portion of the PAF1-LEO1 crystal structure aligned well with one of the TFIIIF barrels. We used this alignment to trim away parts of the structure that followed the path of the triple barrel, resulting in a construct corresponding to residues 370-461 of LEO1 and residues 170-208 of PAF1. To model the remaining regions of LEO1 and PAF1, we compared the predicted secondary structure of PAF1 and LEO1 to the triple barrel structure of TFIIIF. We took the corresponding structured regions of TFIIIF and threaded the sequence of PAF1 or LEO1 through these sequences in Phyre2¹². Residues 462-497 of LEO1 correspond to residues 86-119 of RAP30. Residues 217-335 of PAF1 correspond to RAP74 residues 25-149. The threaded regions were aligned to the TFIIIF crystal structure in Pymol¹⁶, fitted to the appropriate density using the fitting function in Chimera⁵, renumbered in Coot⁷, and flexibly fitted into the appropriate density in a locally refined version of Map 7. Because we are not confident of side chain placements for the threaded model, the residues in this region are marked as unknown. Cross-linking data confirm the placement of the dimerization model. The N-terminus of PAF1 exits from the CTR9 solenoid and passes over the Pol II surface to the dimerization domain (residues 120-142). This region of PAF1 was modelled using crosslinking data and density for bulky side chains in Map 3. Additional modelling information for PAF, RTF1, and the SPT5 CTR can be found in Table 4.

The final model was refined against a composite map generated with Phenix using Maps 1, 2, 5, 7, 8, and 10. Five rounds of phenix.real space refine were performed with geometry minimization, adp, local grid search refinement, and secondary structure restraints were applied for nucleic acids and protein¹³. Local resolution maps were generated using the built-in local resolution tool in RELION 3.0. Figures were prepared with Pymol (version 2.3.2), Chimera (version 1.13.1)⁵, and ChimeraX (version 0.91)¹⁷.

Supplementary Note References

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Supplementary Table 1: I Complete EC* BS3 crosslinking statistics and measured distances.

A

Complete EC* components	Total number of unique crosslinks	Number unique crosslinks mappable to EC*+RTF1 structure
Total crosslinks	549	212
<i>Inter-complex crosslinks</i>		
Total	211	55
Pol II-Pol II	7	7
Pol II-DSIF	12	8
Pol II-SPT6	2	2
Pol II-PAF	18	3
DSIF-SPT6	9	3
PAF-SPT6	3	1
DSIF-DSIF	5	2
PAF-PAF	111	23
PAF-DSIF	2	0
Pol II-RTF1	17	4
DSIF-RTF1	2	0
PAF-RTF1	23	2
SPT6-RTF1	0	0
<i>Intra-protein crosslinks</i>		
Total	338	157
Pol II	52	46
DSIF	22	10
SPT6	45	27
PAF	180	53
RTF1	39	21

B

Protein1	Protein2	AbsPos1	AbsPos2	Distance (Å)
Rpb11	Rpb11	26	62	18.2701177
CTR9	CTR9	884	873	15.8310185
CTR9	CTR9	393	357	12.7578154
Rpb1	Rpb1	643	778	15.8741372
CTR9	CTR9	770	833	16.5989864
SUPT6H	SUPT6H	844	838	11.7155653
Rpb1	Rpb1	697	751	14.9866868
CTR9	CTR9	393	369	15.6775724
LEO1	LEO1	377	488	13.9384334
SUPT6H	SUPT6H	571	538	12.7927821
PAF1	PAF1	106	133	36.9260448
SUPT5H	SUPT5H	213	231	11.4994867
CDC73	CTR9	247	637	11.7826059
CTR9	CTR9	366	393	14.6879644

SUPT6H	SUPT6H	1229	1274	12.2702171
Rpb1	Rpb1	778	758	11.1438899
RTF1	Rpb3	504	155	20.2341138
CTR9	WDR61	798	140	20.8304766
RTF1	RTF1	380	486	16.865309
Rpb1	Rpb1	203	213	9.81079823
RTF1	RTF1	413	504	15.2439092
WDR61	WDR61	100	139	11.5615403
Rpb1	Rpb1	643	976	18.429499
WDR61	WDR61	29	97	17.3806937
Rpb2	Rpb2	264	327	10.4917664
Rpb1	Rpb1	778	751	12.5133107
PAF1	PAF1	126	133	12.1374015
Rpb1	Rpb1	697	758	11.7801122
SUPT6H	SUPT6H	1430	1454	18.2145837
RTF1	RTF1	413	555	37.9708956
Rpb2	Rpb2	821	815	18.3770435
SUPT5H	SUPT5H	306	276	9.55506039
Rpb1	Rpb1	285	331	15.6655533
CTR9	CTR9	844	833	16.9779451
Rpb1	Rpb1	778	767	8.27848307
SUPT5H	SUPT6H	344	1005	23.304636
Rpb2	Rpb2	413	458	17.2588393
SUPT6H	SUPT6H	1512	1430	14.5588075
CTR9	CTR9	773	833	10.4564323
SUPT5H	Rpb2	236	327	18.4509122
WDR61	WDR61	129	140	11.8117634
Rpb1	Rpb1	796	1135	19.9510937
CTR9	CTR9	695	729	11.4213953
CTR9	CTR9	868	873	8.54917125
Rpb2	Rpb2	327	337	11.2785632
Rpb1	Rpb1	910	976	11.6075266
Rpb2	Rpb2	427	421	10.4063816
CTR9	PAF1	444	62	20.0545422
SUPT6H	SUPT6H	626	622	7.70116108
SUPT5H	SUPT5H	597	627	14.1867435
Rpb2	Rpb2	134	427	13.3762301
Rpb1	Rpb1	910	918	13.7626781
CTR9	CTR9	777	770	15.458826
SUPT6H	SUPT6H	1452	1449	5.70046542

SUPT6H	SUPT6H	402	738	18.1947132
SUPT6H	SUPT6H	951	738	9.74593187
SUPT6H	SUPT6H	1243	1251	12.4442672
CTR9	CTR9	518	471	15.4846328
RTF1	RTF1	480	368	11.1956999
Rpb1	Rpb1	707	751	12.6311862
CTR9	CTR9	844	855	17.1138175
CTR9	CTR9	884	868	23.7933827
WDR61	CTR9	97	777	21.0289563
SUPT6H	SUPT6H	777	844	14.3226501
SUPT5H	Rpb2	439	1143	20.6347336
RTF1	RTF1	413	497	18.7352163
SUPT6H	SUPT6H	1512	1454	19.2114
Rpb4	Rpb4	137	90	17.118111
CTR9	CTR9	863	873	15.355936
RTF1	RTF1	413	368	21.0477934
RTF1	RTF1	413	518	27.8779423
Rpb1	Rpb1	910	919	12.5569339
LEO1	Rpb2	488	630	26.2414346
SUPT6H	SUPT6H	727	738	16.2980807
Rpb1	Rpb1	127	134	11.4131363
CTR9	CTR9	555	586	17.6551826
Rpb2	Rpb2	264	458	24.6715799
LEO1	PAF1	488	133	47.7876637
WDR61	WDR61	29	48	12.698096
CTR9	CDC73	645	247	10.546639
CDC73	Rpb8	247	146	18.9692735
Rpb1	Rpb3	697	199	17.1430967
RTF1	RTF1	454	397	16.1223204
CTR9	CTR9	282	252	15.4397472
CDC73	CTR9	257	637	12.7429389
CTR9	CTR9	187	213	13.9315388
Rpb3	Rpb3	225	2	17.084157
RTF1	RTF1	486	368	16.6115098
CTR9	CTR9	868	863	8.69519827
Rpb1	Rpb1	796	1133	26.2707536
CTR9	CTR9	660	637	16.5114528
SUPT5H	SUPT4H	274	116	13.9925943
Rpb2	Rpb2	192	815	18.8014873
CTR9	CTR9	216	187	12.0641331

SUPT6H	SUPT6H	1243	1269	12.7431808
Rpb1	Rpb1	32	226	18.4422321
Rpb2	Rpb2	340	332	12.6371317
Rpb11	Rpb11	37	70	5.4955805
RTF1	RTF1	531	518	9.2359934
Rpb7	Rpb7	123	153	34.7514714
RTF1	RTF1	518	368	36.1608577
Rpb3	Rpb11	225	47	15.9933716
RTF1	RTF1	413	424	8.26913605
CTR9	CTR9	403	187	45.7514495
SUPT4H	SUPT4H	109	102	11.0598696
CTR9	CTR9	863	855	12.4900681
Rpb3	RTF1	152	555	9.33903352
SUPT6H	SUPT6H	743	351	21.6503689
Rpb2	SUPT5H	264	236	28.7702123
SUPT5H	SUPT5H	597	578	17.8021609
Rpb2	PAF1	1010	126	13.8002299
SUPT6H	SUPT6H	1100	1135	22.2746827
Rpb1	Rpb1	1135	1125	11.1474495
Rpb1	Rpb1	1133	1125	11.9535016
CTR9	CTR9	784	770	15.6199666
Rpb2	Rpb2	210	494	17.9222026
Rpb6	SUPT6H	101	1327	42.7502083
Rpb1	Rpb1	1155	1219	15.3708025
PAF1	CTR9	106	216	8.08509511
Rpb2	Rpb2	942	934	6.83482304
Rpb2	Rpb2	264	457	27.4670717
CTR9	PAF1	246	68	6.57160992
Rpb1	Rpb1	619	1155	49.7560521
SUPT6H	SUPT6H	646	615	9.01789055
PAF1	CTR9	62	283	13.1880228
SUPT6H	SUPT6H	844	843	3.7983736
RTF1	RTF1	555	504	26.7173949
Rpb1	Rpb1	151	125	15.1210029
SUPT5H	SUPT6H	306	1005	42.0281042
RTF1	RTF1	499	555	33.116774
LEO1	PAF1	377	133	41.3958205
SUPT6H	Rpb6	1341	101	29.9738198
SUPT6H	SUPT6H	1454	1449	9.62992394
CTR9	PAF1	252	68	15.4210536

PAF1	CTR9	71	252	17.1252089
CTR9	CTR9	695	398	51.7317046
SUPT5H	Rpb1	439	32	21.295703
CTR9	CTR9	868	858	15.2798523
WDR61	WDR61	28	48	13.5374778
SUPT6H	SUPT5H	1229	274	94.7344045
RTF1	Rpb2	486	820	21.462501
Rpb11	Rpb11	62	23	17.0377161
Rpb1	Rpb2	1125	494	14.9694193
Rpb3	Rpb8	199	20	16.8477226
SUPT5H	SUPT5H	601	627	14.4685867
CTR9	CTR9	849	855	9.91867295
SUPT6H	SUPT6H	1005	898	18.494001
Rpb1	Rpb1	910	619	24.7952855
CDC73	CTR9	243	398	21.4612055
SUPT5H	Rpb11	213	26	121.776718
CTR9	CTR9	555	729	52.1491925
SUPT6H	SUPT6H	643	615	4.55319086
RTF1	RTF1	555	424	41.1924984
RTF1	RTF1	499	424	12.7187123
CTR9	PAF1	282	68	13.5274289
Rpb2	Rpb2	409	458	15.996939
PAF1	CTR9	68	283	15.3539993
LEO1	LEO1	488	514	63.0118094
SUPT5H	Rpb1	395	105	59.5513033
Rpb11	SUPT5H	62	274	111.319659
CTR9	CTR9	472	729	54.7780593
Rpb11	Rpb11	37	23	21.1147562
Rpb2	Rpb2	332	337	8.81617253
SUPT6H	SUPT6H	452	727	24.4580164
SUPT6H	SUPT6H	738	748	15.2648927
CTR9	PAF1	366	133	60.5313513
SUPT4H	SUPT4H	116	102	26.4194123
SUPT6H	WDR61	897	100	172.211489
WDR61	CTR9	254	742	17.0401322
Rpb1	Rpb1	125	132	10.6149207
RTF1	RTF1	497	424	15.3628103
LEO1	PAF1	461	196	18.7927394
Rpb4	Rpb7	94	155	27.0366276
CTR9	PAF1	713	34	20.7913357

LEO1	Rpb2	488	340	60.538748
CTR9	CTR9	849	833	24.8665136
PAF1	PAF1	71	106	24.6067471
RTF1	RTF1	413	512	29.2584873
LEO1	LEO1	488	517	67.4746692
CTR9	CTR9	872	855	25.8014951
SUPT6H	SUPT6H	1341	1327	25.1565166
CTR9	CTR9	660	587	27.6913987
CTR9	CTR9	713	742	11.5916551
Rpb2	Rpb2	436	421	14.2160376
RTF1	RTF1	555	513	25.1867331
CTR9	CDC73	660	257	11.110593
CTR9	WDR61	578	149	18.7442244
RTF1	LEO1	518	517	27.5102837
Rpb2	RTF1	151	518	23.5051885
Rpb5	Rpb1	41	1350	17.7068874
SUPT6H	SUPT6H	626	623	5.61212313
SUPT5H	SUPT5H	185	578	62.9741184
CTR9	CTR9	357	770	87.2037232
PAF1	PAF1	133	196	24.3226795
RTF1	RTF1	555	497	36.1249831
CTR9	CTR9	858	872	21.1219991
SUPT5H	SUPT5H	395	579	47.0743089
CTR9	CTR9	593	187	81.8687519
PAF1	PAF1	106	126	29.9129505
SUPT6H	SUPT6H	777	1135	16.5774192
CTR9	CTR9	544	521	18.382612
CTR9	CTR9	482	518	19.0723308
PAF1	CTR9	106	213	12.1862751
LEO1	RTF1	488	533	47.6947806
Rpb1	SUPT5H	445	627	28.4358126
RTF1	RTF1	504	518	14.9826527
SUPT6H	SUPT6H	419	646	44.0158656
CTR9	CTR9	586	872	117.355101
CTR9	CTR9	472	626	56.7268029
SUPT5H	SUPT5H	512	344	37.9343052
CTR9	CTR9	80	872	174.800028
SUPT5H	SUPT4H	268	116	5.42300701

Supplementary Table 3 | Modelling of PAF and RTF1 subunits and the SPT5 CTR.

Subunit/ Chain ID	Domain/ region	Residue range	Initial model	PDB template for initial model/ Chain	Modelling algorithm	Changes to initial model	Modelling confidence	Maps used	Map resolution for region (Å)
RTF1/R	N-terminus		Not modelled						
RTF1/R	Plus3	349-483	Crystal structure	4L1U/C		NAMDinator, manual correction, phenix. real_space _ refine	Atomic model	Maps 2, 3	3.5-7
RTF1/R	Pol II binding	484-600	<i>Ab initio</i>			manual correction, phenix. real_space _ refine	Atomic model 484- 504; 557- 590 Backbone trace 505- 556; 591- 600	Maps 2, 3	~4-6
CTR9/Q	TPRs	3-750	Homology	6AF0/A	Phyre2	NAMDinator, manual correction, phenix. real_space _ refine	Atomic model	Maps 4, 5, 6	~3.5-12
CTR9/Q	Vertex	751-806	Homology	6AF0/A	Phyre2	NAMDinator, manual correction, phenix. real_space _ refine	Atomic model	Maps 4, 5	~3.5-8
CTR9/Q	Trestle	807-892	Cryo-EM structure	6GMH/Q		manual correction, phenix. real_space _ refine	Atomic model	Maps 4, 5	~3.5-8
PAF1/V	N-terminus	1-24	Not modelled						
PAF1/V	CTR9 interacting region	25-113	Homology	6AF0/P	Phyre2	NAMDinator, manual correction, phenix. real_space _ refine	Atomic model	Map 7	~3.5-10
PAF1/V	Linker	120-142	<i>Ab initio</i>			manual correction, phenix. real_space _ refine	Atomic model	Map 2, 3	~3.5

PAF1/V	Triple barrel	170-208	Crystal structure	4M6T/A		NAMDinator, manual correction, phenix. real_space_refine	Atomic model	Map 7	~4-10
PAF1/V	Triple barrel	217-335	Homology	1F3U/B	Phyre2	NAMDinator, manual correction, phenix. real_space_refine	Backbone trace with unknown register	Map 7	~4-10
PAF1/V	C-terminus	336-531	Not modelled						
LEO1/U	N-terminus	1-369	Not modelled						
LEO1/U	Triple barrel	370-461	Crystal structure	4M6T/A		NAMDinator, manual correction, phenix. real_space_refine	Atomic model	Map 7	~4-10
LEO1/U	Triple barrel	462-497	Homology	1F3U/A	Phyre2	NAMDinator, manual correction, phenix. real_space_refine	Backbone trace with unknown register	Map 7	~4-10
LEO1/U	DNA loop/C-terminal extension	505-525	<i>Ab initio</i>			manual correction, phenix. real_space_refine	Backbone trace with unknown register	Map 7	~4-10
LEO1/U	C-terminus	526-666	Not modelled						
CDC73/X	N-terminal bundle and connector	1-111 and 112-217	Not modelled						
CDC73/X	Anchor helix	218-260	Homology	6AF0/C	Phyre2	NAMDinator, manual correction, phenix. real_space_refine	Atomic model	Maps 4, 5	~4-8
CDC73/X	Linker and Ras-like domain	261-226 and 337-531	Not modelled						
SPT5/Z	CTR linker	755-767	<i>Ab initio</i>			manual correction, phenix. real_space_refine	Backbone trace	Maps 2, 3	~5

SPT5/Z	CTR	780-790 (mutate d to 771- 781)	Crystal structure	4L1U/H		manual correction, phenix. real_space _refine	Atomic model	Map 2	~5
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Supplementary Table 4 | RTF1 sequence alignment.

Chd1 interacting region

sp O92541 RTF1_HUMAN 1-710	1MRGRLCVGRAAA-----AAAVAVPLAGQEGSPGGRRGSRGTTM--VKKRGRRVVDSDT--EDSGSDENIDQELLSLAKRKSSDSEEEKPPVSOPAAAS93	sp O92541 RTF1_HUMAN 1-710	94DSE--TSDSDDEWTFGSNKNKKKGKARK--IEKKGTMKKQANKTASGSSGSDKDS--AESAPEEGEVSDSD-----SNS162
sp A2AQ19 RTF1_MOUSE 1-715	1MRGRLCVGRAAAVAAVAAVAVPLAGQEGSGGVRGSRGTTM--VKKRGRRVVDSDT--EDSGSDENIDQELLSLAKRKSSDSEEEKPPVSOPAAAS98	sp A2AQ19 RTF1_MOUSE 1-715	99DSE--TSDSDDEWTFGSNKNKKKGKTRK--VEKKGAMKKQANKAAAGSSGSDSDS--AESAPEEGEVSDSE-----SS167
sp G1GX24 GLKX24_DANRE 1-701	1X-----WRSAALLFK-----HTHRGARLTMTVMVKRGRVVVDSDS--EDSGSDENIDQELLSLAKRKSSDSEEEKPPVSOPAAAS7	sp G1GX24 GLKX24_DANRE 1-701	76DSE--TSDSDDEWTVGGTKTKKKGPKSKGPKENNTVKRKGKASGSSGSDSDS--GESSAPEGEVSDSD-----SNS145
sp O9W261 RTF1_DROME 1-775	1M-----GKRTQSLIDNSSDSDSESETNLESDMSLAKRK-----GKRTQSLIDNSSDSDSESETNLESDMSLAKRK-----KPTAAKS45	sp O9W261 RTF1_DROME 1-775	46SSR--SDSDSDWA-----NKGAPSS--KKRKQPKPSRDSSSESNWDDSDDERARQSPAQTOQEHKPPEQASQAPQLSEQEEGEVSDSDSDSKSNS138
sp G5EB70 RTF1_CAEL 1-613	1M-----SS--ESASDEETKRRAPTS--DSDSDSADGAPKPGKPLST39	sp G5EB70 RTF1_CAEL 1-613	40DSS--ASDSQAE-----KPOAKPAKKTLTTRKRRATG--KPOAKPAKKTLTTRKRRATG--KPOAKPAKKTLTTRKRRATG--70
sp O9C950 MP5_ARATH 1-643	1M-----GQIENLLLEAGRTNSAGRSRHPPSPSRREGS33	sp O9C950 MP5_ARATH 1-643	34YSDGSSDSRDSDDEDRGYASRKPSGSOVPLKKRLEADERDRAAR--VLEGYGQGP-----SDR89
sp P53064 RTF1_YEAS7 1-558	1M-----SDIDEDLLLAGADESEEDQVLTTSAKRAK33	sp P53064 RTF1_YEAS7 1-558	34NND-----QSLSKKRRIEVS--VDDDEEDD-----YNP61
HMD			
sp O92541 RTF1_HUMAN 1-710	163SSSSD--SDSSDEEFDHGGYGEDLMGDEEDRARLEOMTEKEREQELFNRIEKREVLKRRFEIKKLIKLTAKKKEK-----KKKQEEQEKK250	sp O92541 RTF1_HUMAN 1-710	251KLTQIQ-----ESQVT--S-----HNKRRSKRDEK-----LDKKSQAMEELKAERE--290
sp A2AQ19 RTF1_MOUSE 1-715	168SSSSD--SDSSDEEFDHGGYGEDLMGDEEDRARLEOMTEKEREQELFNRIEKREVLKRRFEIKKLIKLTAKKKEK-----KKKQEEQEKK250	sp A2AQ19 RTF1_MOUSE 1-715	256KLTQIQ-----ESQVT--S-----HNKRRSKRDEK-----LDKKSQAMEELKAERE--295
sp G1GX24 GLKX24_DANRE 1-701	146SSSSD--SDSSDEEFDHGGYGEDLMGDEEDRARLEOMTEKEREQELFNRIEKREVLKRRFEIKKLIKLTAKKKEK-----KKKQEEQEKK233	sp G1GX24 GLKX24_DANRE 1-701	234KQSOVP-----DTQVMS--D-----HNKERRIKRDEK-----LDKKSQAMEELKAERE--274
sp O9W261 RTF1_DROME 1-775	139SSSGSDSSSSSDSEFDGGFDGLMGDEEDRRRLNGLSSEKRETEIYKRIEOREIMRTWEIERKLIKLRAGEKNOESKNKGERRAKKKEKREKKAKA240	sp O9W261 RTF1_DROME 1-775	241READAPLPTQASTSTLLDVEPKPSNEVRSASPLSTPALNRDAASTSAAVASIMPDDAASAGVSDYFHKERSKERKNVEANKTDOKRSNAMAALLKAKREG342
sp G5EB70 RTF1_CAEL 1-613	71-----SSD-----DQVDDDLFARKEDKARWKLTLEKEQELIERMARENAIAREELIAQOLAKKAKKSSEK-----GVKTEKRRKMSGG147	sp G5EB70 RTF1_CAEL 1-613	148SDAGSPK-----RKASDS-----DSEWDA--FHRPSDIRK-----HKEKNMADALKNRK--193
sp O9C950 MP5_ARATH 1-643	90EGDSSE-----ESDFGDLKYNEDRQKLAGMTFQEMILSERADKK-----GDMFTKELRSKRSE-----KTPVSKKETOTPLPAS163	sp O9C950 MP5_ARATH 1-643	164RGVRSAS-----R--SADRA-----G-----AKDDMLNELRAKRM--191
sp P53064 RTF1_YEAS7 1-558	62YSGNA--DYGSELEEEANPFLPGKYKBESDRCHLESPEMERETLTFERSQIMQKYQ-----ERKIFRARGDMK-----QQQRAKNDSDS143	sp P53064 RTF1_YEAS7 1-558	144RKTRAST-----R--SHAT-----G-----D1KASKLSQIRKORA--174
Plus3			
sp O92541 RTF1_HUMAN 1-710	291--KRKNRTALLAKKQ-----PLKTSEVYSDDEEEEE-----EDDKSSSEKSDRSRSTSSDDEEEKEEIPPKSQPVSLPPEELNRVRLSRH367	sp O92541 RTF1_HUMAN 1-710	367KLERWCHMPFEAKTIVTGCVRIGIGN--HNSKPYRVAEL--TGVVETAKVYQLGGRITNGQLRIENDQRV--FRLEFVSNQEFFTESEFMKWEAMFSAG463
sp A2AQ19 RTF1_MOUSE 1-715	296--KRKNRTALLAKKQ-----PLKTSEVYSDDEEEEE-----EDDKSSSEKSDRSRSTSSDDEEEKEEIPPKSQPVSLPPEELNRVRLSRH372	sp A2AQ19 RTF1_MOUSE 1-715	373KLERWCHMPFEAKTIVTGCVRIGIGN--HNSKPYRVAEL--TGVVETAKVYQLGGRITNGQLRIENDQRV--FRLEFVSNQEFFTESEFMKWEAMFSAG463
sp G1GX24 GLKX24_DANRE 1-701	275--KRKNRTALLAKKQ-----PLKTSEVYSDDEEEEE-----EDDKSSSVKSDRSRSTSSDDEEEKEEIPPKSQPVSLPPEELNRVRLSRH353	sp G1GX24 GLKX24_DANRE 1-701	353KLERWCHMPFEAKTIVTGCVRIGIGN--SNSKPYRVAEL--IDVETAKIYQLGSTRNGQLRIENDQTV--FRLEFVSNQEFFTESEFMKWEAMMIAG449
sp O9W261 RTF1_DROME 1-775	343KAKREEEERKMAEKDRDDKKEELDSVCSCKSAV--KLKASEIYSDSGSSDW--DEEPEPAGKRSRNSKASSESEDEEKARQPVHITTRDNLKRLSRY442	sp O9W261 RTF1_DROME 1-775	442KMERFVNLIFJESTVLCNCFVRSIGN--NGQKPYRVAEL--GVGVEIGKISYSGTTRNGRLKHKHQERV--FRLEFVSNQEFFTESEFMKWEAMMIAG449
sp G5EB70 RTF1_CAEL 1-613	194--EIEKNA--KNE-----ALSTDAVFGANSSS--SSQSDSRSSSSESPSERSEKDIYKKD--VDGLSELRRARLSRH266	sp G5EB70 RTF1_CAEL 1-613	266KLSMIHAPFFDSTVGCVRILGQGM--SGSGPYRIRWKI--GVGVEEKVYIELEGKTKTKILKCMQSGSERP--FRMQEVSNADEFQIIEFDWLLACKR--H362
sp O9C950 MP5_ARATH 1-643	192--KQDDPA--LRKLRSACKSGSGSRDFSSTRKPKLASNLSS--SSQSDSRSSQSDDESGNGMLSDDDRSDVP--TFEDVKEVTIRRS275	sp O9C950 MP5_ARATH 1-643	275KLERWCHMPFEAKTIVTGCVRIGIGN--HNSKPYRVAEL--TGVVETAKVYQLGGRITNGQLRIENDQRV--FRLEFVSNQEFFTESEFMKWEAMFSAG463
sp P53064 RTF1_YEAS7 1-558	175--RKNRHYSDNEDEDEEYR-----EEDKKGDEGSEYGDGEYNPFRDRDITDKREEVEEAEDEQREP-----EISDFNKLIRIGRS252	sp P53064 RTF1_YEAS7 1-558	252KLERWCHMPFEAKTIVTGCVRIGIGN--HNSKPYRVAEL--TGVVETAKVYQLGGRITNGQLRIENDQRV--FRLEFVSNQEFFTESEFMKWEAMFSAG463
Linker			
sp O92541 RTF1_HUMAN 1-710	368KLERWCHMPFEAKTIVTGCVRIGIGN--HNSKPYRVAEL--TGVVETAKVYQLGGRITNGQLRIENDQRV--FRLEFVSNQEFFTESEFMKWEAMFSAG463	sp O92541 RTF1_HUMAN 1-710	464MQLPTLDEINKKELSIKEALNYKENDQDIEEIVKEKERK--APPNYAMKKTQLLKKAMADELQDDKAKQIQDQLNELERAEALDRQTKNLSAISYI563
sp A2AQ19 RTF1_MOUSE 1-715	373KLERWCHMPFEAKTIVTGCVRIGIGN--HNSKPYRVAEL--TGVVETAKVYQLGGRITNGQLRIENDQRV--FRLEFVSNQEFFTESEFMKWEAMFSAG463	sp A2AQ19 RTF1_MOUSE 1-715	469MQLPTLDEINKKELSIKEALNYKENDQDIEEIVKEKERK--APPNYAMKKTQLLKKAMADELQDDKAKQIQDQLNELERAEALDRQTKNLSAISYI568
sp G1GX24 GLKX24_DANRE 1-701	364KLERWCHMPFEAKTIVTGCVRIGIGN--SNSKPYRVAEL--IDVETAKIYQLGSTRNGQLRIENDQTV--FRLEFVSNQEFFTESEFMKWEAMMIAG449	sp G1GX24 GLKX24_DANRE 1-701	450MQLPTLDEINKKEOSIKEAVNYKENDQIEDIVKEKERK--APPNYAMKKTQLLKKAMADESGDQKAKTLQDQLNELERAEALDRQTKNLSAISYI549
sp O9W261 RTF1_DROME 1-775	443KMERFVNLIFJESTVLCNCFVRSIGN--NGQKPYRVAEL--GVGVEIGKISYSGTTRNGRLKHKHQERV--FRLEFVSNQEFFTESEFMKWEAMMIAG449	sp O9W261 RTF1_DROME 1-775	539VQMPTIDLIAIKQNDIKKALNYEKDEDVDKIEEKNERN--RPTNYAMKKTCLMKERDAMLRGYDIAQDLGQQLDELINRASELDRKSHSTLNLISYI638
sp G5EB70 RTF1_CAEL 1-613	267KLSMIHAPFFDSTVGCVRILGQGM--SGSGPYRIRWKI--GVGVEEKVYIELEGKTKTKILKCMQSGSERP--FRMQEVSNADEFQIIEFDWLLACKR--H362	sp G5EB70 RTF1_CAEL 1-613	363GNLPTVIDMDKKQDIEKAIINHKYSDKEVDLMIKEKSKYTQ--VPRNFAMTKANWSKQELAQQRGDIRAEQIQTKIDELIRQADELEKRSKISAIJAFI462
sp O9C950 MP5_ARATH 1-643	276KLERWCHMPFEAKTIVTGCVRIGIGN--HNSKPYRVAEL--TGVVETAKVYQLGGRITNGQLRIENDQRV--FRLEFVSNQEFFTESEFMKWEAMFSAG463	sp O9C950 MP5_ARATH 1-643	376GRMPTQDISEKKEAIQRTNSFVSAETVKOMLEKKSASV--RPMNYAEKDRLRKELIAQSKNDEAGVERIKSKIQOLD--ASRNKGVYDKKALKLAEM473
sp P53064 RTF1_YEAS7 1-558	253FVAKFCFYHGFEDAMKGYGRVNVGTDKRTGKTSYRMVRI--ERVLF--LQKPNMKGKFTNQYFGVTKDKRKV--FQMYNF--SDGLLEAFEDVEYQVRLALDNSQ349	sp P53064 RTF1_YEAS7 1-558	350MIKPSLHLSLNKTKFEMVDEVNTPLTKTTEVHRHMRKGLSGTNAVLEKTVREKLOYAKETNNEKDIKAYSADLRNF--KRMVYVEKHHENDQDKKL451
Pincer 1			
sp O92541 RTF1_HUMAN 1-710	464MQLPTLDEINKKELSIKEALNYKENDQDIEEIVKEKERK--APPNYAMKKTQLLKKAMADELQDDKAKQIQDQLNELERAEALDRQTKNLSAISYI563	sp O92541 RTF1_HUMAN 1-710	564NQ---RNRWNIVE--SEKALVAESHNMKNQ--MDPFTTRQCKPTIVSNSRDP-----AVQAAI--LAQLNAKYGSV-----LPDAPKEMSKGQKDKDLNSK649
sp A2AQ19 RTF1_MOUSE 1-715	469MQLPTLDEINKKELSIKEALNYKENDQDIEEIVKEKERK--APPNYAMKKTQLLKKAMADELQDDKAKQIQDQLNELERAEALDRQTKNLSAISYI568	sp A2AQ19 RTF1_MOUSE 1-715	569NQ---RNRWNIVE--SEKALVAESHNMKNQ--MDPFTTRQCKPTIVSNSRDP-----AVQAAI--LAQLNAKYGSV-----LPDAPKEMSKGQKDKDLNSK654
sp G1GX24 GLKX24_DANRE 1-701	450MQLPTLDEINKKEOSIKEAVNYKENDQIEDIVKEKERK--APPNYAMKKTQLLKKAMADESGDQKAKTLQDQLNELERAEALDRQTKNLSAISYI549	sp G1GX24 GLKX24_DANRE 1-701	654NQ---RNRWNIVE--SEKALVAESHNMKNQ--MDPFTTRQCKPTIVSNSRDP-----SVHAAI--LAHNOYKSGSGAGQDN--SQQANKOLGQASQKDKD--TK640
sp O9W261 RTF1_DROME 1-775	539VQMPTIDLIAIKQNDIKKALNYEKDEDVDKIEEKNERN--RPTNYAMKKTCLMKERDAMLRGYDIAQDLGQQLDELINRASELDRKSHSTLNLISYI638	sp O9W261 RTF1_DROME 1-775	639ND---RNRKNVED--AEKALILEARANKGLKI--DDPFTTRITQPRMVGFKAKK--DEDDMOLAPL-----PPPGKGRPNEAGTSSASVR717
sp G5EB70 RTF1_CAEL 1-613	443KMERFVNLIFJESTVLCNCFVRSIGN--NGQKPYRVAEL--GVGVEIGKISYSGTTRNGRLKHKHQERV--FRLEFVSNQEFFTESEFMKWEAMMIAG449	sp G5EB70 RTF1_CAEL 1-613	463NH---RNRSKI--KDOVLSGOLKIEENSQ--DDPFTTRKGGVRMVSQSKSR-----LDGTLSA--SSSTTNLSDGG-----KDKSSSLAKPTQPPPS540
sp O9C950 MP5_ARATH 1-643	276KLERWCHMPFEAKTIVTGCVRIGIGN--HNSKPYRVAEL--TGVVETAKVYQLGGRITNGQLRIENDQRV--FRLEFVSNQEFFTESEFMKWEAMFSAG463	sp O9C950 MP5_ARATH 1-643	474NK---KNRAEFKNA--SEVKSITASLKAGEAG--YDPFSRWRTSSNYNGKNKGKDGNEEA--AAVAVETNGADAGAV--EATEAAL--EAAA--EAGKLID--TR567
sp P53064 RTF1_YEAS7 1-558	253FVAKFCFYHGFEDAMKGYGRVNVGTDKRTGKTSYRMVRI--ERVLF--LQKPNMKGKFTNQYFGVTKDKRKV--FQMYNF--SDGLLEAFEDVEYQVRLALDNSQ349	sp P53064 RTF1_YEAS7 1-558	452GELTSKRNKLNMNSIRNAEHVKKEDSNFNDSK--SDPFS--LTKT--KVYYQIEQK-----EENAKAKEIAQ-----EKLQEDKDAKDK57
Pincer 2			
sp O92541 RTF1_HUMAN 1-710	464MQLPTLDEINKKELSIKEALNYKENDQDIEEIVKEKERK--APPNYAMKKTQLLKKAMADELQDDKAKQIQDQLNELERAEALDRQTKNLSAISYI563	sp O92541 RTF1_HUMAN 1-710	710NQ---RNRWNIVE--SEKALVAESHNMKNQ--MDPFTTRQCKPTIVSNSRDP-----AVQAAI--LAQLNAKYGSV-----LPDAPKEMSKGQKDKDLNSK649
sp A2AQ19 RTF1_MOUSE 1-715	469MQLPTLDEINKKELSIKEALNYKENDQDIEEIVKEKERK--APPNYAMKKTQLLKKAMADELQDDKAKQIQDQLNELERAEALDRQTKNLSAISYI568	sp A2AQ19 RTF1_MOUSE 1-715	715NQ---RNRWNIVE--SEKALVAESHNMKNQ--MDPFTTRQCKPTIVSNSRDP-----AVQAAI--LAQLNAKYGSV-----LPDAPKEMSKGQKDKDLNSK654
sp G1GX24 GLKX24_DANRE 1-701	450MQLPTLDEINKKEOSIKEAVNYKENDQIEDIVKEKERK--APPNYAMKKTQLLKKAMADESGDQKAKTLQDQLNELERAEALDRQTKNLSAISYI549	sp G1GX24 GLKX24_DANRE 1-701	701NQ---RNRWNIVE--SEKALVAESHNMKNQ--MDPFTTRQCKPTIVSNSRDP-----SVHAAI--LAHNOYKSGSGAGQDN--SQQANKOLGQASQKDKD--TK640
sp O9W261 RTF1_DROME 1-775	539VQMPTIDLIAIKQNDIKKALNYEKDEDVDKIEEKNERN--RPTNYAMKKTCLMKERDAMLRGYDIAQDLGQQLDELINRASELDRKSHSTLNLISYI638	sp O9W261 RTF1_DROME 1-775	701PTSDSKYSLYS--LHDFDIDLPLVPNAEA--KSLVSSSNA--LPVKDGAPRR--SINLEDYKKRRG-----LI715
sp G5EB70 RTF1_CAEL 1-613	443KMERFVNLIFJESTVLCNCFVRSIGN--NGQKPYRVAEL--GVGVEIGKISYSGTTRNGRLKHKHQERV--FRLEFVSNQEFFTESEFMKWEAMMIAG449	sp G5EB70 RTF1_CAEL 1-613	717NQ---RNRKNVED--AEKALILEARANKGLKI--DDPFTTRITQPRMVGFKAKK--DEDDMOLAPL-----PPPGKGRPNEAGTSSASVR717
sp O9C950 MP5_ARATH 1-643	276KLERWCHMPFEAKTIVTGCVRIGIGN--HNSKPYRVAEL--TGVVETAKVYQLGGRITNGQLRIENDQRV--FRLEFVSNQEFFTESEFMKWEAMFSAG463	sp O9C950 MP5_ARATH 1-643	643NQ---RNRSKI--KDOVLSGOLKIEENSQ--DDPFTTRKGGVRMVSQSKSR-----LDGTLSA--SSSTTNLSDGG-----KDKSSSLAKPTQPPPS540
sp P53064 RTF1_YEAS7 1-558	253FVAKFCFYHGFEDAMKGYGRVNVGTDKRTGKTSYRMVRI--ERVLF--LQKPNMKGKFTNQYFGVTKDKRKV--FQMYNF--SDGLLEAFEDVEYQVRLALDNSQ349	sp P53064 RTF1_YEAS7 1-558	558APICOGAEHNQ-----LHDFELSLD--SLALQYGGPQGVQKAFMARKQLTEATVGCVRVAENDGKR--HGLT--TVSDYKRRRG-----L715
LATCH			
sp O92541 RTF1_HUMAN 1-710	650SASDLSSEDLFK-----VHDFQVKIDIQVPSSES-----KALAITSKAPPAKDGAPRR--SINLEDYKKRRG-----LI710	sp O92541 RTF1_HUMAN 1-710	710NQ---RNRWNIVE--SEKALVAESHNMKNQ--MDPFTTRQCKPTIVSNSRDP-----AVQAAI--LAQLNAKYGSV-----LPDAPKEMSKGQKDKDLNSK649
sp A2AQ19 RTF1_MOUSE 1-715	655SASDLSSEDLFK-----VHDFQVKIDIQVPSSES-----KALAITSKAPPAKDGAPRR--SINLEDYKKRRG-----LI715	sp A2AQ19 RTF1_MOUSE 1-715	715NQ---RNRWNIVE--SEKALVAESHNMKNQ--MDPFTTRQCKPTIVSNSRDP-----AVQAAI--LAQLNAKYGSV-----LPDAPKEMSKGQKDKDLNSK654
sp G1GX24 GLKX24_DANRE 1-701	641PTSDLSSEDLFK-----LHDFQVKIDIQVPSSES-----KALAITSKAPPAKDGAPRR--SINLEDYKKRRG-----LI710	sp G1GX24 GLKX24_DANRE 1-701	701NQ---RNRWNIVE--SEKALVAESHNMKNQ--MDPFTTRQCKPTIVSNSRDP-----SVHAAI--LAHNOYKSGSGAGQDN--SQQANKOLGQASQKDKD--TK640
sp O9W261 RTF1_DROME 1-775	718TDSKDYSLYS--LHDFDIDLPLVPNAEA--KSLVSSSNA--LPVKDGAPRR--SINLEDYKKRRG-----LI715	sp O9W261 RTF1_DROME 1-775	715NQ---RNRKNVED--AEKALILEARANKGLKI--DDPFTTRITQPRMVGFKAKK--DEDDMOLAPL-----PPPGKGRPNEAGTSSASVR717
sp G5EB70 RTF1_CAEL 1-613	541TOIKKKTDISS-----LHDFELSLD--SLALQYGGPQGVQKAFMARKQLTEATVGCVRVAENDGKR--HGLT--TVSDYKRRRG-----L715	sp G5EB70 RTF1_CAEL 1-613	463NH---RNRSKI--KDOVLSGOLKIEENSQ--DDPFTTRKGGVRMVSQSKSR-----LDGTLSA--SSSTTNLSDGG-----KDKSSSLAKPTQPPPS540
sp O9C950 MP5_ARATH 1-643	568APIGOGAEHNQ-----LHDFELSLD--SLALQYGGPQGVQKAFMARKQLTEATVGCVRVAENDGKR--HGLT--TVSDYKRRRG-----L715	sp O9C950 MP5_ARATH 1-643	474NK---KNRAEFKNA--SEVKSITASLKAGEAG--YDPFSRWRTSSNYNGKNKGKDGNEEA--AAVAVETNGADAGAV--EATEAAL--EAAA--EAGKLID--TR567
sp P53064 RTF1_YEAS7 1-558	558APICOGAEHNQ-----LHDFELSLD--SLALQYGGPQGVQKAFMARKQLTEATVGCVRVAENDGKR--HGLT--TVSDYKRRRG-----L715	sp P53064 RTF1_YEAS7 1-558	452GELTSKRNKLNMNSIRNAEHVKKEDSNFNDSK--SDPFS--LTKT--KVYYQIEQK-----EENAKAKEIAQ-----EKLQEDKDAKDK57