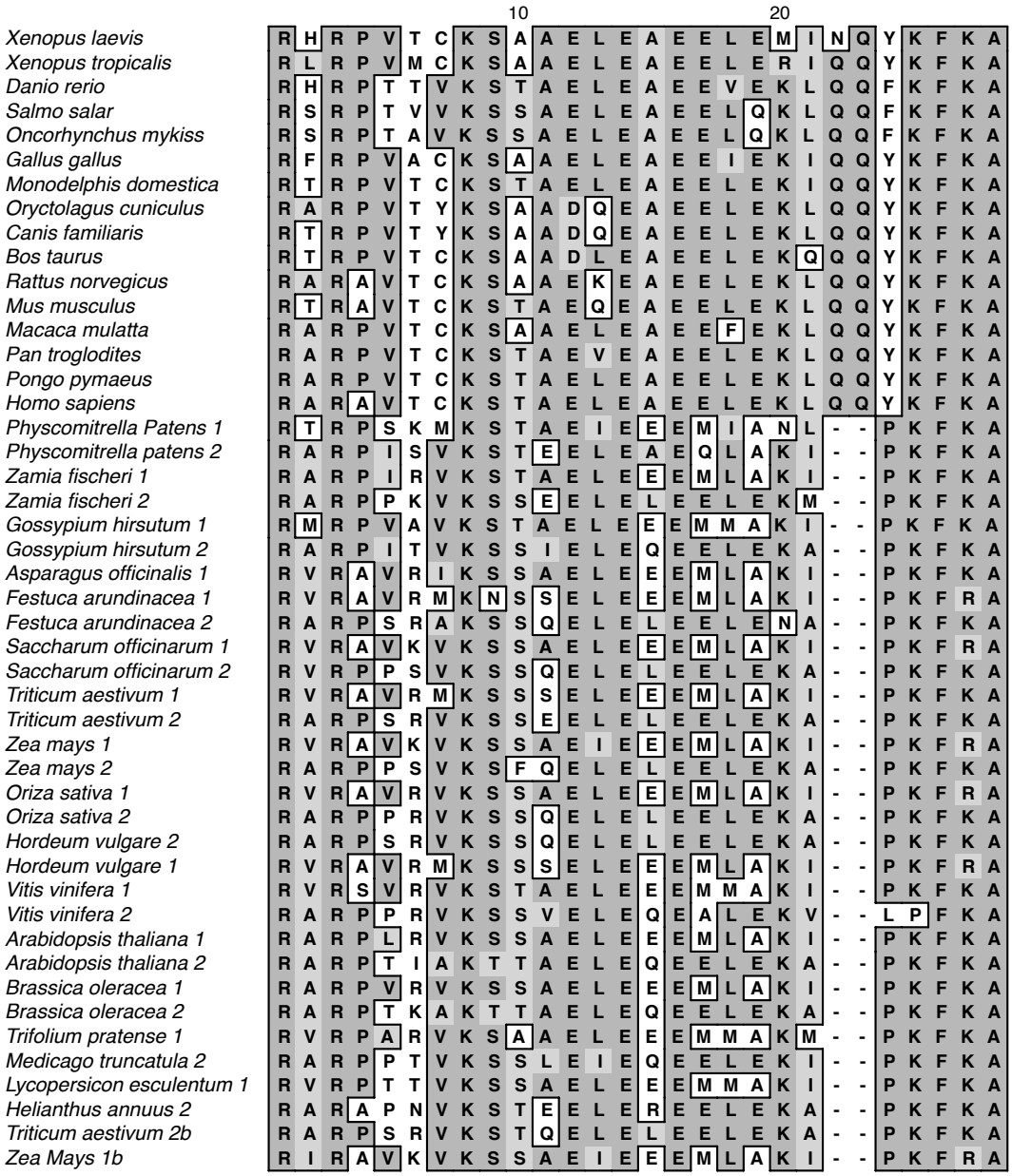
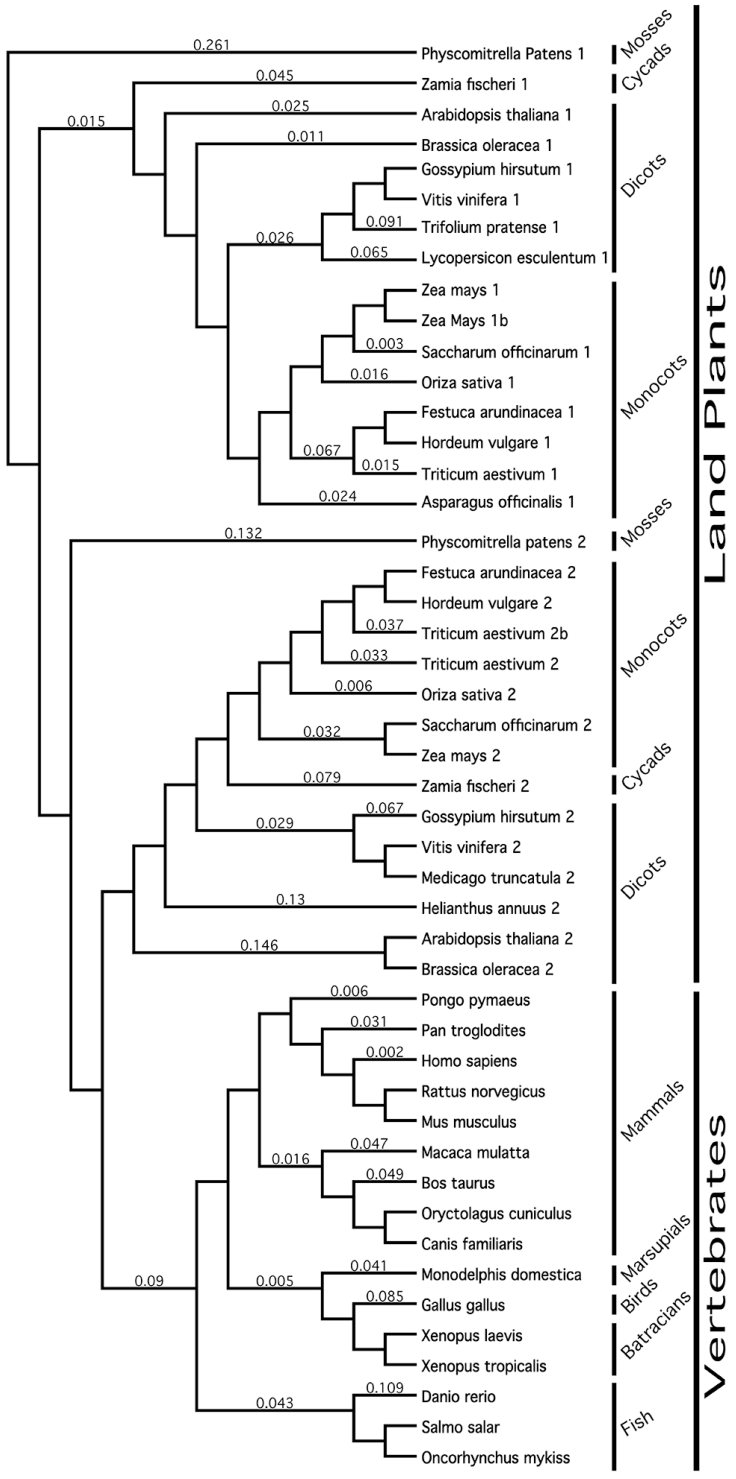
SUPPLEMENTAL FIGURE 1 ONLINE



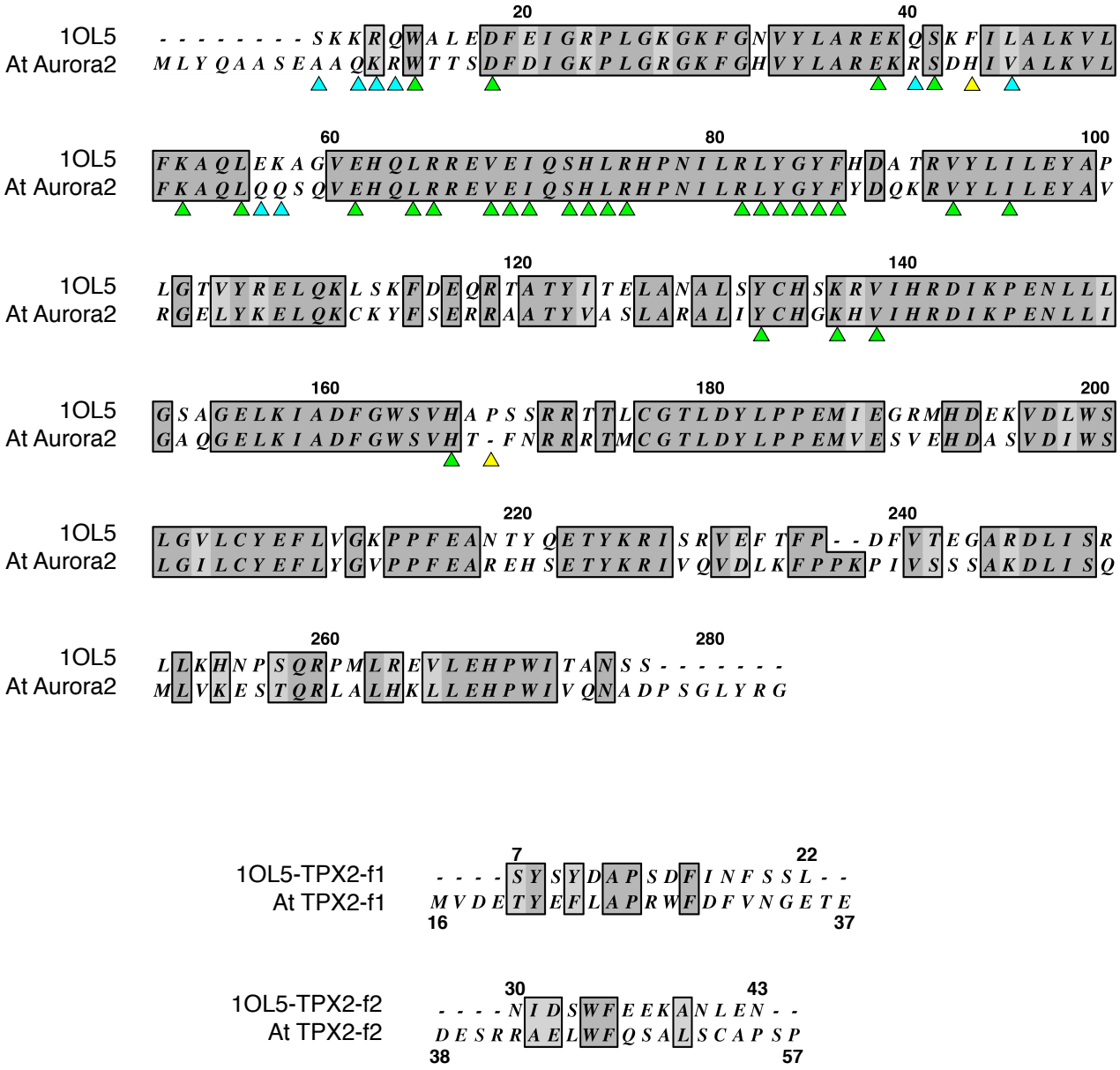
Alignment of the TPX2 Signature Motifs.

Blast searches identified TPX2 motifs in the protein of various animals and plants, but not in insects, worms or fungi. All researched species that were used to make a relationship tree in Supplemental Figure 2 are shown.



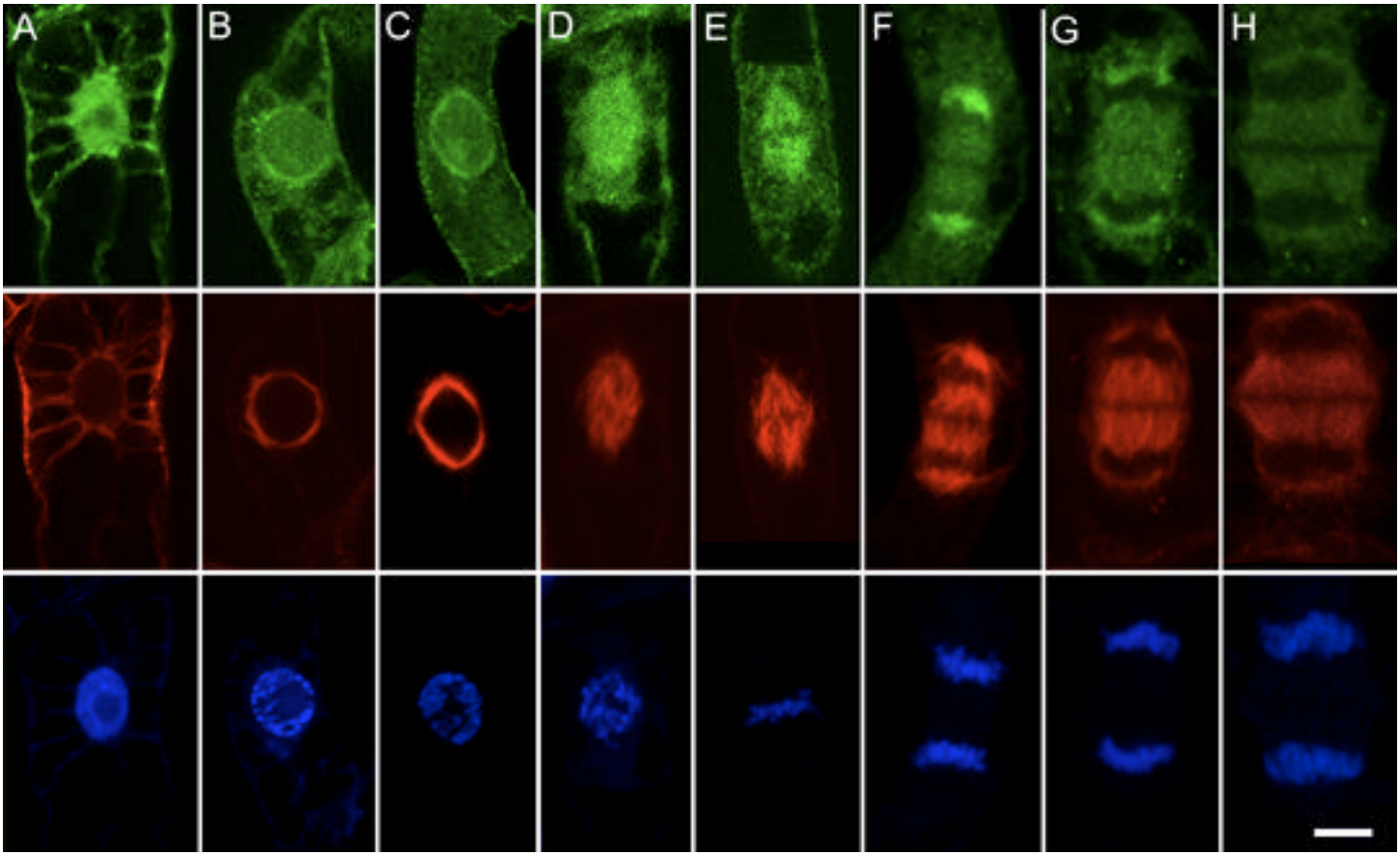
Phylogram of Eukaryote TPX2 Signatures.

The relationship tree was constructed on both At TPX2 signature sequences using the Neighbor Joining method and Poisson distance correction. All land plants, including the moss Physcomitrella, possess two copies of the TPX2 signature, suggesting that the duplication occurred before land plant speciation. The TPX2 signature found in vertebrates is more closely related to the second plant signature than to the first one. Interestingly, evolution is quite well respected despite the short length of the signature. Number 1 and 2 refer to the signature positions from N- to C-terminus of TPX2 found in plant species.



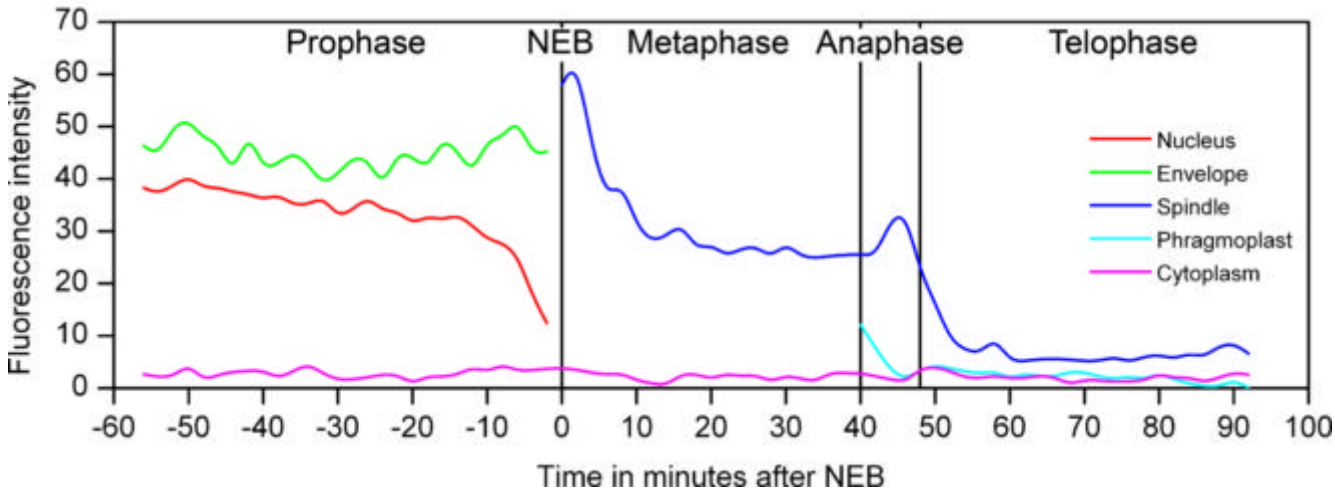
Sequence Alignment of Arabidopsis and Human Aurora and TPX2.

Shown are the amino acid sequence alignment of At Aurora2 with the crystallographic partial sequence of Hs Aurora A (1OL5; top) and the alignment of At TPX2 with the two crystallographic sequences of Hs TPX2 that interact with HsAurora A in the crystal (bottom). The 38 amino acids from human Aurora A indicated by triangles (▲, colored according to Fig. 2A) are involved in the interaction with Hs TPX2 and show a variation of their accessible surface when calculated with or without the presence of TPX2. The full length At Aurora2 sequence, which may have conserved this function, shows 74% identity and 95% similarity to the human Aurora A sequence.



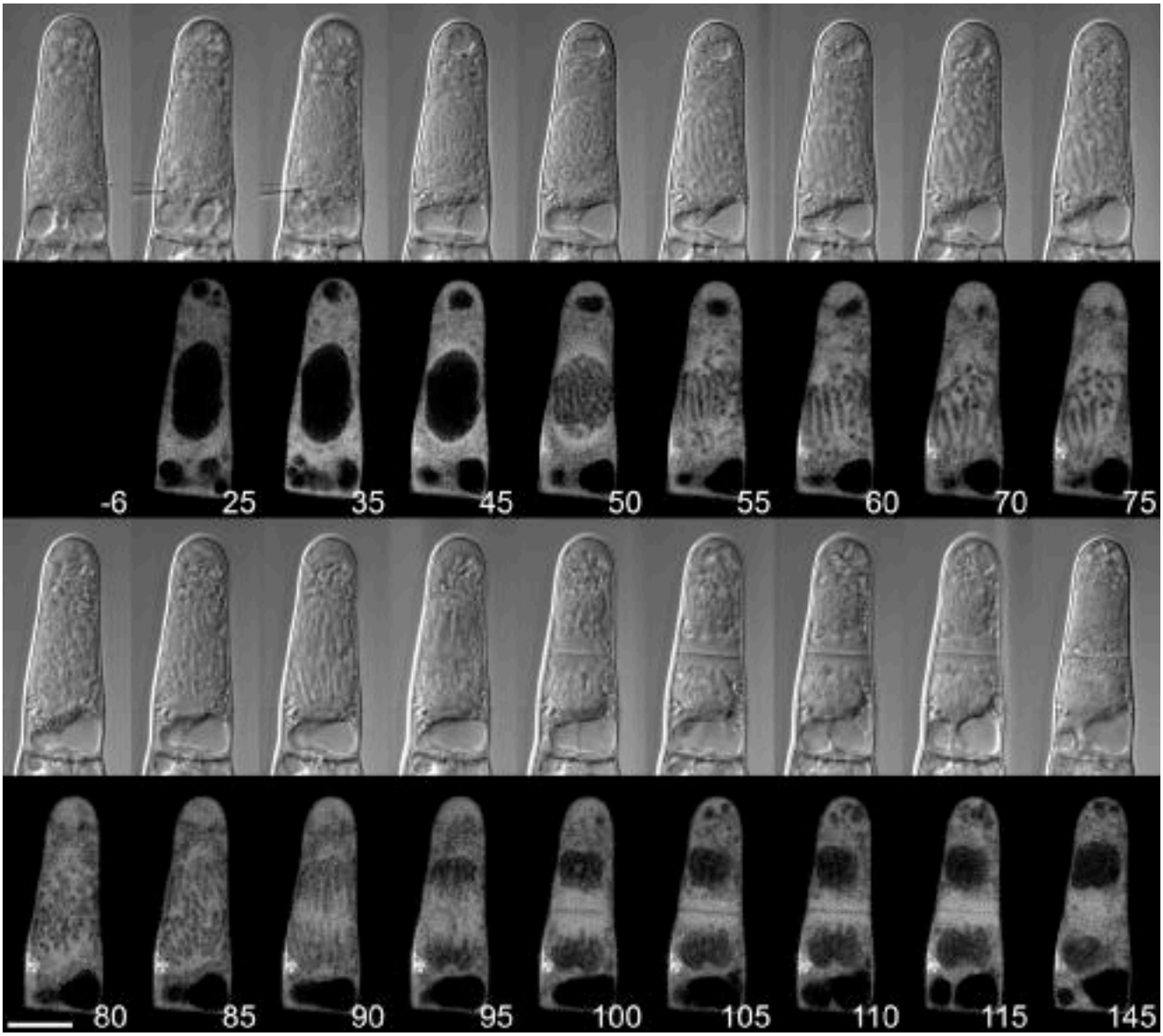
At TPX2 Localization during Cell Division in Immunolabeled BY-2 cells.

(A-H). Endogenous TPX2 (Top), microtubule (middle) and dapi (bottom) staining of cycling cells. TPX2 localization is intranuclear in late G2 (A) and perinuclear in early prophase (B). In late prophase (C) it forms two polar crescents outside of the nucleus. After NEB the spindle becomes labeled (D, E). The polar labeling progressively disappears during anaphase-telophase transition (F-H).



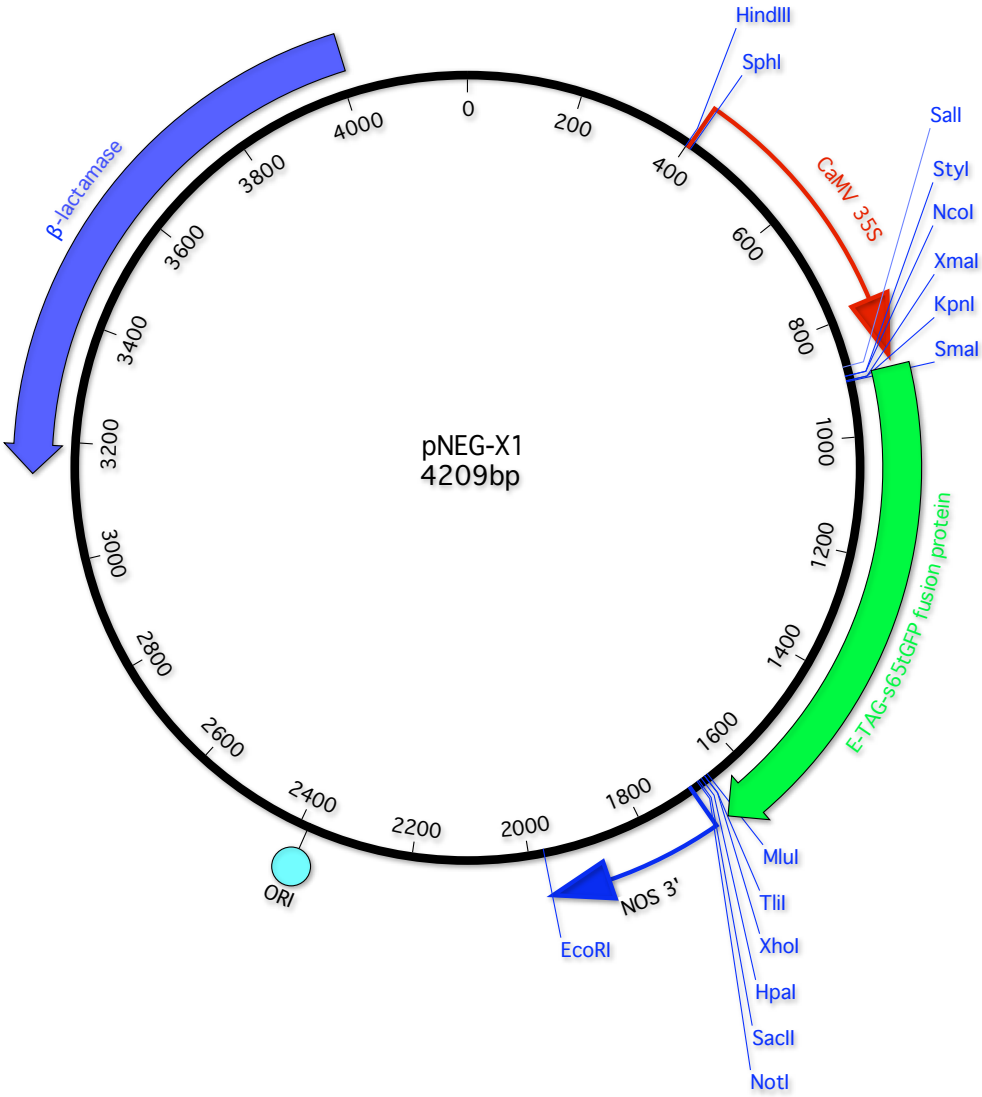
Dynamics of At TPX2 during Cell Division.

GFP-At TPX2 fluorescence intensity graph of various BY-2 cell parts during prophase, mitosis and cytokinesis. The nuclear fluorescence is quickly reduced before NEB. The peak at the onset of metaphase is due to the intense labeling of the first microtubule bundles in the plain of focus (see also Figure 3 K at T=3'). Thereafter, the protein becomes distributed over many newly polymerized microtubules. At the end of anaphase the protein is focused to the shortening kinetochore fibers and is then clearly degraded. All fluorescence intensities were measured as the mean pixel intensities in 50 x 50 pixel boxes of the cell in movie S3, except the nuclear envelope measurements, which were based on a 15 x 15 pixel box. All data were corrected to the background intensity.



Microinjection of TRITC-dextran.

As a control, TRITC conjugated dextran (160 kDa) was microinjected in a *Tradescantia virginiana* stamen hair cell during late prophase. The inert polysaccharide distributes throughout the cytoplasmic volume of the cell, but is excluded from the nucleus and the vacuole. Just before NEB (35 to 45 min after injection) a fluorescent halo becomes visible around the nucleus. This coincides with the (organelles excluding) pro-spindle that is formed at that time. The dextran is again excluded from the reforming nucleus during telophase due to the restricted accessible volume between the decondensing chromosomes. Needle concentration was 0.5 mg/ml, time in minutes after injection and the bar is 20 μm.



Multiple cloning site 5' end of eGFP

```
          SalI            NcoI KpnI  XmaI
ACAATTACAGTCGACTCTAGAGGATCC ATG GTA CCC GGG GCT CCA GTG CCT TAT CCA GAT CCA CTT GAA CCT CGC ATG GTG AGC
TGTTAATGTCAGCTGAGATCTCCTAGG TAC CAT GGG CCC CGA GGT CAC GGA ATA GGT CTA GGT GAA CTT GGA GCG TAC CAC TCG
                            M   V   P   G   A   P   V   P   Y   P   D   P   L   E   P   R   M   V   S
                                                    E-Tag                                   eGFP
```

Multiple cloning site 3' end of eGFP

```
                                                                MluI    XhoI   HpaI SacII NotI
GCC GCC GGG ATC ACT CAC GGC ATG GAC GAG CTG TAC AAA CGC GTC TCG AGT TAA CCGCGGCGGCCGCCCGGCTGCAGATC
CGG CGG CCC TAG TGA GTG CCG TAC CTG CTC GAC ATG TTT GCG CAG AGC TCA ATT GGCGCCGCCGGCGGGCCGACGTCTAG
 A   A   G   I   T   H   G   M   D   E   L   Y   K   R   V   S   S   *
                         eGFP
```

Map and multiple cloning sites of pNEG-X1.

Map of the pUC derivative pNEG-X1 showing the position of AmpR resistance gene, ORI, CaMV 35S promoter, E-tag-eGFP coding frame, NOS terminator as well as localization of important single cutter restriction enzymes. 5' and 3' MCS sequences are detailled below the map showing also the peptide sequence of the E-tag and translation frame.

Combined Results of Experiments Concerning GFP:At TPX2 Expression.

GFP-constructs	Protein size in kDa	At TPX2 expression / labeling				Organism	Number of cells	Illustrations
		Nuclear only	Nuclear + Cytoplasmic	Nuclear excluded	Microtubule (interphase and/or mitosis)			
full length 1-758	113	60%	5%	35%	yes	Arabidopsis root meristem	300	Fig 3J
1-758	113	-	4%	96%	yes	*N. plumb*a. protoplasts	25	-
1-758	113	-	100%	-	yes	*N.bentha*. leaves	300	-
1-758	113	80%	20%	-	yes	BY-2 cells	80	Fig 3K
1-758 + colchicin 10µM	113	98%	2%	-	no	BY-2 cells	50	-
1-758 + oryzalin 2µM	113	96%	4%	-	no	BY-2 cells	25	-
1-758 + latrunculin B 10µM	113	82%	18%	-	yes	BY-2 cells	50	-
1-579	92	-	100%	-	yes	BY-2 cells	72	Fig 4B
1-463	79	90%	10%	-	no	BY-2 cells	62	Fig 4A
1-303	61	80%	20%	-	no	BY-2 cells	64	Fig 4A
1-104	39	-	100%	-	no	BY-2 cells	30	Fig 4C
1-403 + 463-758	106	87%	13%	-	yes	BY-2 cells	46	Fig 4A
1-303 + 463-758	95	70%	30%	-	yes	BY-2 cells	34	Fig 4A
163-463	61	80%	20%	-	no	BY-2 cells	60	Fig 4A
220-463	55	100%	-	-	no	BY-2 cells	18	Fig 4A
220-463 NLS1 mut	55	-	100%	-	yes	BY-2 cells	22	Fig 4B
303-463	45	-	100%	-	no	BY-2 cells	50	Fig 4C
ChS/1-72	79	11%	34%	55%	no	BY-2 cells	18	Fig 4D
ChS/59-72	72	-	16%	84%	no	BY-2 cells	38	Fig 4D
ChS/220-303	80	100%	-	-	no	BY-2 cells	22	Fig 4A
463-758	62	100%	-	-	no	BY-2 cells	32	Fig 4A
220-303 + 463-758	72	100%	-	-	no	BY-2 cells	25	Fig 4A
610-758	44	100%	-	-	no	BY-2 cells	18	Fig 4A
610-758 NLS2 mut	44	-	-	100%	yes	BY-2 cells	30	Fig 4B
303-610	63	-	-	100%	yes	BY-2 cells	25	Fig 4D
303-610 + LMB	63	-	100%	-	no	BY-2 cells	25	-
463-610	44,5	-	-	100 %	yes	BY-2 cells	25	Fig 4D
463-610 NES mut	44,5	-	100%	-	no	BY-2 cells	21	Fig 4C
463-610 + LMB	44,5	-	100%	-	no	BY-2 cells	25	Fig 5B
550-630	37	-	100%	-	no	BY-2 cells	18	Fig 4C
684-758	21	-	-	-	yes	BY-2 cells	11	Fig 4B

Accession numbers

Animal organism	**Accession number**	**Plant organism**	**Accession number**
Bos Taurus	NP_001092368 (2)	*Arabidopsis thaliana*	AT1G03780 (1,2)
Canis familiaris	XP_850934 (2)	*Asparagus officinalis*	CV461179 (1)
Danio rerio	BX927210.9 (2)	*Brassica oleracea*	DY025756 (1,2)
Gallus gallus	NP_989768 (2)	*Festuca arundinacea*	DT685973 (1,2)
Homo sapiens	BAG50902 (2)	*Gossypium hirsutum*	ES817692 (1) DR462351 (2)
Macaca mulatta	XP_001109645 (2)	*Helianthus annuus*	BU028081 (2)
Monodelphis domestica	XP_001364540 (2)	*Hordeum vulgare*	CK123033 (1,2)
Mus musculus	EDL05995 (2)	*Medicago truncatula*	BQ147900 (2)
Oryctolagus cuniculus	ENSOCUP00000002784 (2)	*Lycopersicon esculentum*	BE462309 (1)
Oncorhynchus mykiss	BX085216.3 (2)	*Oryza sativa*	CK070246 (1,2)
Pan troglodites	XP_514566 (2)	*Physcomitrella Patens*	XP_001755041 (1,2)
Pongo pymaeus	NP_001125744 (2)	*Saccharum officinarum*	CA249551 (1) CA293532 (2)
Rattus norvegicus	NP_001101260 (2)	*Trifolium pratense*	BB912562 (1)
Salmo salar	DY736807.1 (2)	*Triticum aestivum*	CD924467 (2) BE637442 (2b)
Xenopus tropicalis	AAI35343 (2)	*Vitis vinifera*	CAO69775 (1,2)
Xenopus laevis	AAH68637 (2)	*Zamia fischeri*	DY030926 (1,2)
		Zea mays	BQ487079 (1) CF015305 (1b) CF036158 (2)
		Oryza sativa	CK070246 (1,2)
		Physcomitrella Patens	XP_001755041 (1,2)

Accession numbers corresponding to various organism sequences in which a TPX2 signature is present. (1) and (2) refers to the closest similarity to respectively the first and second signature motif found in Arabidopsis thaliana. (1,2) refers to sequences including both signatures.

List of primers used in generating various At TPX2 constructs

GFP-constructs	Oligos used for GFP fusions	
in pNEGX1	**forward**	**reverse**
1-758	GTCTCGAGTATGGAAGCAACGGCGGAG	GCGGCCGCTTATCTCATCTGACCAGC
463-758	GTCTCGAGTGAAAGTAAAGGAGAAATG	GCGGCCGCTTATCTCATCTGACCAGC
1-579	GTCTCGAGTATGGAAGCAACGGCGGAG	GCGGCCGCAGGTTCTGGTTTTGGTGG
1-463	GTCTCGAGTATGGAAGCAACGGCGGAG	GCGGCCGCAAATATCTTTTTGTTCAA
1-303	GTCTCGAGTATGGAAGCAACGGCGGAG	GCGGCCGCGCTTGTTTGGAACTTCTT
1-104	GTCTCGAGTATGGAAGCAACGGCGGAG	GCGGCCGCAGAAGGCTGCGATTGTAA
1-403	GTCTCGAGTATGGAAGCAACGGCGGAG	GCGGCCGCTGAAGCTATTGAAGATGT
163-463	GTCTCGAGTCCCAAACCACCAATGCAG	GCGGCCGCAAATATCTTTTTGTTCAA
220-463	GTCTCGAGTACTACCAATCTGATTCAA	GCGGCCGCAAATATCTTTTTGTTCAA
220-463 NLS1 mut	GTCTCGAGTACTACCAATCTGATTCAA	GCGGCCGCAAATATCTTTTTGTTCAA
303-463	GTCTCGAGTAGCACGCGAGACCTATTC	GCGGCCGCAAATATCTTTTTGTTCAA
463-758	GTCTCGAGTTTTGAAAGTAAAGGAGAA	GCGGCCGCTTATCTCATCTGACCAGC
220-303	GTCTCGAGTACTACCAATCTGATTCAA	GCGGCCGCGCTTGTTTGGAACTTCTT
610-758	GTCTCGAGTATGGAGACAGAAGAAGCC	GCGGCCGCTTATCTCATCTGACCAGC
610-758 NLS2 mut	GTCTCGAGTATGGAGACAGAAGAAGCC	GCGGCCGCTTATCTCATCTGACCAGC
303-610	GTCTCGAGTAGCACGCGAGACCTATTC	GCGGCCGCTCTCCTCCTCTCTTCCCT
463-610	CACGCGTGTTTGAAAGTAAAGGAGAA	GCGGCCGCTCTCCTCCTCTCTTCCCT
463-610 NES mut	CACGCGTGTTTGAAAGTAAAGGAGAA	GCGGCCGCTCTCCTCCTCTCTTCCCT
550-630	GTCTCGAGTAAATTAGGAGATGTAAAG	GCGGCCGCTGGGTCCTCTTTTATAAC
684-758	GTCTCGAGTATGGTGGAGGAAGAGAGA	GCGGCCGCTTATCTCATCTGACCAGC
in pKC-GFP-CHS		
ChS/1-72	CACGCGTGGGGGCGGAGGGGGATGGAAG--CAACGGCGGAG	TCTAGATTATTAGGCCTCTACCTTGAACGA
ChS/59-72	CACGCGTGGGGCGGAGGGGGCCTAGAAT--CAAAGCAAGG	TCTAGATTATTAGGCCTCTACCTTGAACGA
ChS/220-303	CACGCGTGGGGCGGAGGGGGTCAAGCCA--TCAAAAGGC	TCTAGATTATTACAGTGTCAGCTTAGGTCG
GFP-constructs	**Oligos used for Mutagenesis**	
in pNEGX1	**forward**	**reverse**
220-463 NLS1 mut	CAAGCCATCAAttGGCAAAAGCTAG	CTAGCTTTTGCCaaTTGATGGCTTG
610-758 NLS2 mut	GAACCAGTACAAtgGATACCGTGAAG	CTTCACGGTATCcaTTGTACTGGTTC
463-610 NES mut	CTGTCTTtGATATATTCGACAAGtTCTCACTG	CAGTGAGAaCTTGTCGAATATATCaAAGACAG

Restriction sites corresponding to XhoI (red), NotI (blue), MulI (pink) and XbaI (green) are shown upstream of the TPX2 coding sequence. Lower case letters correspond to mutagenized bases.