A

$10 \ \mu M \ Zn^{2+}$:	-	-	-	-	+	+	+	+
Phenanthroline (μM):	-	1	10	100	-	1	10	100
His-eIF4B ₂₈₀₋₅₂₇	-			-			-	
	1	2	3	4	5	6	7	8
GST-eIF4B ₃₂₀₋₅₂₇	-			-		-	-	-

В

10 µM Zn ²⁺ :	-	-	10	10	10	10	10	20	30	40
Phenanthroline (μ M):	-	90	-	10	30	60	90	90	90	90
His-eIF4B ₂₈₀₋₅₂₇	11	-	=	-	-	-	_	_	_	1
	1	2	3	4	5	6	7	8	9	10
GST-eIF4B ₃₂₀₋₅₂₇)		-	-	-	-	-	-	-	1

C



Supplementary Figure 1

Supplementary Figure Legend

Supplementary Figure 1.

Self-association of wheat eIF4B requires free zinc. In A and B, chelation of zinc by phenanthroline abolishes the zinc-mediated stimulation of eIF4B self-association but is reversed by excess zinc. GSTeIF4B₃₂₀₋₅₂₇ used in the pull-down assay is shown in the Coomassie-stained gel (bottom panel). Binding of His-eIF4B₂₈₀₋₅₂₇ to GST-eIF4B₃₂₀₋₅₂₇ in the presence of the indicated amount of zinc and/or phenanthroline was determined by Western analysis following binding of the latter to glutathione Sepharose resin (top panel). In B, the addition of excess zinc reverses the inhibition of eIF4B dimerization by phenanthroline. GST-eIF4B₃₂₀₋₅₂₇ used in the pull-down assay is shown in the Coomassie-stained gel (bottom panel). Binding of His-eIF4B₂₈₀₋₅₂₇ to GST-eIF4B₃₂₀₋₅₂₇ in the presence of the indicated amount of zinc and/or phenanthroline was determined by Western analysis following binding of the latter to glutathione Sepharose resin (top panel). In C, zinc reverses the inhibitory effect of phenanthroline on the zincmediated stimulation of eIF4B self-association. Zinc and the other indicated metal ions were added to binding reactions containing phenanthroline to measure their ability to reverse the inhibitory effect of phenanthroline effect on eIF4B self-association. Bound His-eIF4B₂₈₀₋₅₂₇ was resolved by SDS-PAGE and detected using Western analysis with anti-His antiserum (top panel). GST-eIF4B₃₂₀₋₅₂₇ used in the pulldown assay is shown in the Coomassie-stained gel (bottom panel).