Characterization of targeted missense *Zea mays Histidine Kinase1* mutations in *Saccharomyces cerevisiae* reveals residues important for signaling activity

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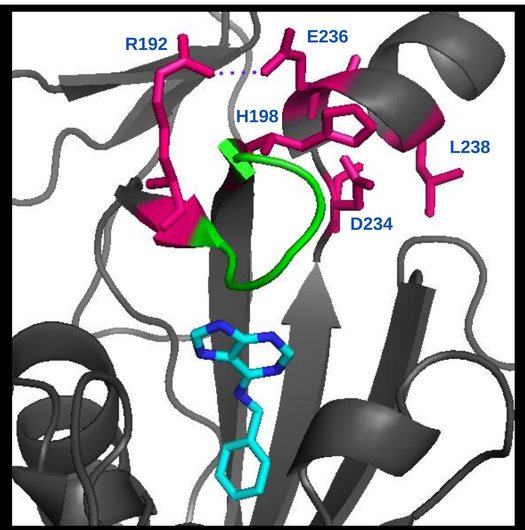
Abstract

Cytokinins (CKs) regulate a diverse assortment of processes in plants, including cellular division, vasculature differentiation, and meristem maintenance. CK perception and response is regulated through a two-component signal transduction system consisting of histidine-kinase receptors, histidine phosphotransferase proteins and response regulators. Two-component signaling systems are highly conserved in bacteria, fungi and plants and allow organisms to sense and respond to diverse stimuli. *Zea mays Histidine Kinase1 (ZmHK1)*, a CK receptor, was identified as the gene underlying the semi-dominant mutant *Hairy Sheath Frayed1 (Hsf1)*. *Hsf1* plants are marked by outgrowths of proximal leaf tissue (sheath, auricle, and ligule) in the distal leaf blade, reduced leaf size, and increased leaf pubescence. Three specific missense mutations in the CK binding domain of ZmHK1 define all the *Hsf1* alleles identified to date. Each mutation causes increased ligand binding affinity and CK hypersignaling, giving rise to the *Hsf1* phenotype. Using a *Saccharomyces cerevisiae* reporter strain where the endogenous his-kinase receptor gene has been functionally replaced by plasmid derived copies of the heterologous *ZmHK1* gene, we tested the ability of a series of 18 targeted missense ZmHK1 mutations generated specifically in the ligand binding domain to promote *Hsf1*-like CK hypersignaling. Yeast harboring these different mutant *ZmHK1* genes were grown with and without different CKs to quantitatively analyze their ability to bind and signal. Some mutations led to *Hsf1*-like hypersignaling, while others showed no significant changes in activity or completely disrupted signaling. These targeted amino acid changes are providing insight as to which residues are critical for ligand recognition, binding, and signaling. Our current characterization and analysis will be presented.

Structure model of the ZmHK1 CK receptor binding domain



Mutation	Location	Wild Type Amino Acid	Mutated Amino Acid
ZmHK1-AEWL	238	Leucine	Phenylalanine
ZmHK1-AEWL LW	238	Leucine	Tryptophan
ZmHK1-H198E	198	Histidine	Glutamic Acid
ZmHK1-1603	236	Glutamic Acid	Lysine
ZmHK1-1603 ER	236	Glutamic Acid	Arginine
ZmHK1-D234K	234	Aspartic Acid	Lysine

Figure 1. Model of the ZmHK1 receptor and CK ligand (6-benzlyaminopurine, 6-BAP) in blue/aqua in the binding pocket. Mutated residues are shown in magenta, with the loop forming one face of the binding pocket in green. Details of the different ZmHK1 mutations are listed in the table, with EMS-derived mutants highlighted in yellow and site-directed engineered mutants in white.

Rationale for engineered mutagenesis of specific residues

The residues selected for site-directed mutagenesis were based on predicted intramolecular interactions from the homology structure model of the ZmHK1 CHASE domain. The model predicts interaction of the amino acids mutated by EMS and other residues nearby with residues in the loop domain. These interactions are presumed to stabilize the position of the loop domain affecting the shape of the binding pocket. The EMS and engineered mutations are expected to disrupt the intramolecular interactions and destabilize the position of the loop, potentially altering ligand binding. For example, R192 is predicted to form a H-bond with E236, the residue mutated to K in ZmHK1-1603. Similar electric charge changes were made for E236 and for H198, which was suggested to interact with D234. Likewise, ZmHK1-AEWL is L238F change; thus, L238 was replaced by other hydrophobic ringed residues.

Using a yeast assay system to monitor ZmHK1 activity

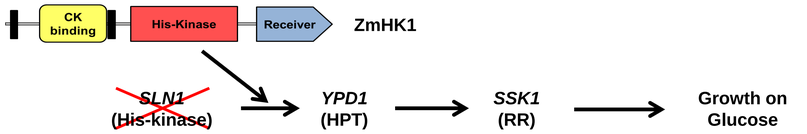


Figure 2. To assay his-kinase activity, the endogenous yeast HK (*SLN1*) is replaced by wild type or mutant versions of the maize ZmHK1 CK receptor. Signaling and activation of downstream components is dependent on CK binding. The ability to grow on glucose media is dependent on the presence of CK; in its absence there is no signaling and no growth, unless ZmHK1 is hypersignaling.

ZmHK1 activity is characterized by specific yeast growth patterns

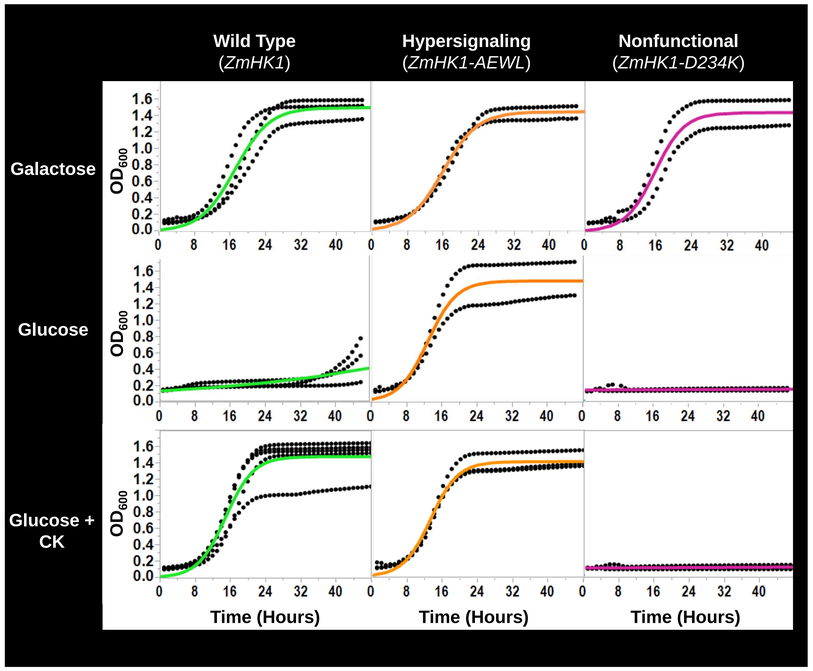


Figure 3. Different yeast growth patterns define changes in ZMHK1 activity. Growth was monitored hourly for 48 hours on galactose media (positive control, top), glucose media (negative control, middle), and glucose media supplemented with CK (1 µM 6-BAP, bottom). Mutants were classified into three categories based on their growth pattern. All yeast lines grow in galactose media. Wild type ZmHK1 lines (left) only grow on glucose supplemented with CK. Hypersignaling ZmHK1 lines (center) are able to grow in glucose media with or without CK. Nonfunctional ZmHK1 lines (right) do not grow in glucose media supplemented with CK. Using these criteria, 18 targeted ZmHK1 mutants were tested for their ability to grow in glucose with and without CK.

We tested 18 engineered ZmHK1 mutants in the yeast system for their ability to grow on glucose media with and without CK. Three mutants were identified as having *Hsf1*-like hypersignaling activity.

Hypothesis: Individual hypersignaling mutants display the same level of growth no matter the amount of CK supplied.

Mutant / Media	*ZmHK1-AEWL*	*ZmHK1-AEWL LW*	*ZmHK1-H198E*	*ZmHK1-1603*	*ZmHK1-1603 ER*	*WT ZmHK1*
1 µM 6-BAP	A	A	A	A	A	A
100 nM 6-BAP	A	A	A	A	B	B
Glucose	A	A	A	B	B	C

Table 3. Test of null hypothesis that amount of CK supplied has no effect on growth within individual mutants. Bonferroni corrections were made for multiple comparisons (critical p-value = .05/18 = 0.002777778, 5% overall error rate). Levels not connected by the same letter are significantly different. Three mutants - ZmHK1-AEWL, ZmHK1-AEWL LW, and ZmHK1-H198E - do **not** differ in growth in presence or absence of CK, indicating that signaling occurs independent of ligand. Two mutants - ZmHK1-1603 and ZmHK1-1603 ER - grow on glucose media without CK present, but have increased growth when supplied with even a small amount of CK. WT ZmHK1 has a steady increase in growth rate as CK concentration is increased in media.

Some hypersignaling mutants signal at constitutively high levels, while others show increased signaling when supplied with CK.

Hypothesis: When grown in the same media, hypersignaling mutants grow at the same rate as one another.

Mutant / Media	1 µM 6-BAP	100 nM 6-BAP	Glucose
ZmHK1-AEWL	A	A	A
ZmHK1-AEWL LW	A	A	A
ZmHK1-H198E	A	A B	B
ZmHK1-1603	A	B C	C
ZmHK1-1603 ER	A	C	C
WT ZmHK1	A	D	D

Table 2. Test of null hypothesis that all hypersignaling mutants show the same growth pattern when placed on the same media. Bonferroni corrections were made for multiple comparisons (critical value =.05/15 = .00333, 15% total error rate). Levels not connected by the same letter are significantly different in growth rate in their respective media. At a concentration of 1 µM 6-BAP, all ZmHK1 mutants and WT are saturated with CK and no difference in growth rate can be observed. As the concentration of CK decreases, differences in growth rate are observed.

Hypersignaling mutants differ in growth pattern when placed in the same media. Saturation occurs by a concentration of 1 µM 6-BAP.

Conclusions

- Use of the liquid culture assay improved our ability to identify subtle growth rate differences not detectable with the colony plate assay.
- Both EMS-derived and targeted engineered mutations in the ZmHK1 CHASE domain assayed in the yeast his-kinase system produced a variety of growth patterns suggesting distinct modifications to ZmHK1 activity.
- Many of the engineered mutations, that generated residue substitutions chemically similar to the EMS-derived mutations, reproduced a hypersignaling phenotype in yeast.
- Novel residues near the loop domain were identified that when mutated resulted in a hypersignaling phenotype in yeast.
- Hypersignaling occurs over a range of activities suggesting distinct changes to ligand binding affinities.
- Further characterization of wild type and mutant ZmHK1 with and without ligand are expected to reveal essential insights into how these structural changes modulate receptor function.

Acknowledgements

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