1 DEK influences the trade-off between growth and

2 arrest via H2A.Z-nucleosomes in Arabidopsis

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Abstract

The decision of whether to grow and proliferate or to restrict growth and develop resilience to stress is a key biological trade-off. In plants, constitutive growth results in increased sensitivity to environmental stress[1,2]. The underlying mechanisms controlling this decision are however not well understood. We used temperature as a cue to discover regulators of this process in plants, as it both enhances growth and development rates within a specific range and is also a stress at extremes. We found that the conserved chromatin-associated protein DEK plays a central role in balancing the response between growth and arrest in Arabidopsis, and it does this via H2A.Z-nucleosomes. DEK target genes show two distinct categories of chromatin architecture based on the distribution of H2A.Z in +1 nucleosome and gene body, and these predict induction or repression by DEK. We show that these chromatin signatures of DEK target genes are conserved in human cells, suggesting that DEK may act through an evolutionarily conserved mechanism to control the balance between growth and arrest in plants and animals.

Main

Plants are exposed to daily fluctuations in ambient temperature, which influence growth, development and fitness[3]. Being sessile organisms, plants frequently need to opt between growth and stress resilience, making them good systems to analyse how these trade-offs are made.

Recently, it has become clear that the chromatin landscape—i.e. the positioning of the nucleosome containing histone variants such as H2A.Z and H3.3 across the gene body—is correlated to the responsiveness of a gene to environmental variation[3–7]. For instance, hypersensitive environmental genes are enriched in H2A.Z-nucleosomes in their gene bodies, suggesting its involvement in the regulation of environmental response[4,7]. Warm temperature results in H2A.Z-nucleosome eviction and large-scale transcriptional activation in plants[8,9]. Furthermore, H2A.Z is removed from +1 nucleosomes of temperature-induced genes upon temperature increase allowing activation of expression by transcription factors[9]. The molecular mechanism underlying this response likely involves a re-organisation of the chromatin landscape by chromatin remodelling enzymes. In this paper, we demonstrate that DEK-DOMAIN CONTAINING PROTEIN3 (DEK3), the most abundant member of a family of four DEK-domain containing chromatin re-modellers in Arabidopsis[10], is a link between the chromatin landscape and environmental responsiveness.

The mammalian orthologue DEK is an oncoprotein involved in the development of cancer, inflammation and stem cell biology[11,12]. Because of its key role in cancer, DEK has been intensely studied in animals, and it has been described to have roles as an H3.3 chaperone, co-transcriptional regulator and in splicing[13–16]. Despite these studies, the genome-wide targets directly regulated by DEK are not known[17], and it is also unclear whether DEK serves as an activator or inhibitor of transcription. In Drosophila and humans DEK can serve as a H3.3 chaperone, and it remodels nucleosomes into more transcriptionally active chromatin through its histone chaperone activity[15]. By contrast, in mammalian cell lines DEK acts as a positive regulator of

77 heterochromatin formation, maintaining the balance between heterochromatin

78 and euchromatin *in vivo*[14]. DEK therefore appears to act as either an activator

79 or a repressor depending on the context.

80 In Arabidopsis, DEK was identified as a component of the nucleolus by mass

81 spectrometry[16]. DEK3 can change the topology of protein free DNA *in vitro*

82 and cause transcriptional repression of specific loci by increasing nucleosome

83 density[10]. Correct *DEK3* expression is critical for the degree of response of

84 the plants to some stress conditions, including high salinity and heat shock.

85 Plants with constitutively elevated levels of DEK3 are more sensitive to high

86 salinity, whereas plants deficient in *DEK3* are more salt tolerant[10].

87

88 To further understand the global effects of DEK3 on gene expression in

89 plants, we used chromatin immunoprecipitation DNA-sequencing (ChIP-seq)

90 and RNA-seq to investigate how DEK3 occupancy influences the

91 responsiveness of gene expression to temperature. We show that DEK3

92 affects temperature-dependent biological trade-offs in Arabidopsis, through

93 the up-regulation of stress response genes and by the suppression of thermo-

94 responsive induction of growth and development genes. Furthermore,

95 inherent chromatin landscape features are sufficient to predict whether a gene

96 will be up- or down-regulated by overexpression of DEK3. Additionally, we

97 show for the first time that DEK3 genetically and physically interacts with

98 H2A.Z and modifies H2A.Z-nucleosome distribution within the gene bodies of

99 DEK3 target genes. We suggest a model whereby feedback between the

100 chromatin landscape and chromatin re-modellers can affect trade-off between

101 growth and arrest, and therefore influence developmental plasticity.

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103

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Results

H2A.Z and DEK interact

Since increasing temperature does not cause H2A.Z-nucleosome loss *in vitro*[9], we sought to determine if other factors may contribute to this process in plants. To find H2A.Z interacting proteins, we performed H2A.Z affinity purification (from *HTA11::HTA11-FLAG* expressing lines) coupled with mass spectrometry. This approach identified three homologous DEK-domain containing chromatin re-modellers and 6 previously reported H2A.Z interactors[18] among 263 proteins found in complex with H2A.Z *in vivo* (Supp. Fig. 1a, Supp. Table.1). Two of them, DEK3 and DEK2, have been found among 7 proteins enriched significantly in samples collected at 27°C compared to 17°C (Fig. 1a, Supp. Table.1) suggesting a possible role in temperature pathway regulated through H2A.Z nucleosomes. There are four DEK-domain containing proteins in *Arabidopsis thaliana*, among them, DEK3 shows a strong and abundant expression in all plant organs[10]. Interestingly, DEK3 shows one of the highest fold change when comparing the binding affinities to H2A.Z complexes between 27°C and 17°C (Fig. 1a, Supp. Table 1). We confirmed the interaction of DEK3 with H2A.Z by co-immunoprecipitating H2A.Z- containing nucleosomes and H2A.Z protein (purified from plants), with purified DEK3 (Fig. 1b). This validation set up mimics *in vitro* binding experiments while preserving post-translational modifications of H2A.Z and DEK3. We find that H2A.Z purified from histone extract coimmunoprecipitated with DEK3 in the comparable levels to H2A.Z nucleosomes obtained from nuclear extract, despite reduced levels of other histones as determined by H3 levels (Fig. 1b). This suggests that other protein factors might not be necessary for the interaction between these two proteins.

Previously, immunopurification of DEK3 has identified its interaction with histones H3 and H4, but not with H2A and H2B[10]. The use of an antibody directed against all H2A variants in this previous study made the detection of H2A.Z very difficult as H2A.Z levels are only approximately 10 % of those of H2A[19].

138 **Perturbing *DEK* expression levels affects growth and stress response**

139 Temperature has a strong effect on plant growth and development. Growth in

140 high ambient temperatures (below the threshold of inducing widespread heat

141 stress), results in faster growth and accelerated development

142 (thermomorphogenesis), illustrated by increased hypocotyl length and early

143 flowering[20]. In contrast, heat stress induces metabolic imbalance,

144 accumulation of toxic by-products and, adversely influencing reproductive

145 growth and yield quality[21]. Plant resilience to stress conditions such as high

146 salinity and heat shock requires the correct level of DEK3[10]. Additionally, a

147 *DEK3* null allele (*dek3-2*) shows an exaggerated response to warmer (non-

148 stressful) temperature, while overexpression of *DEK3* reduces the thermal

149 responsiveness of seedlings, both as measured by elongation of hypocotyl

150 and flowering time (Fig. 1c-d, Supp. Fig. 1b-c). The altered responsiveness of

151 *DEK3* mis-expressing plants to temperature changes appears to be a general

152 property, since *DEK3* overexpressors are also unable to acclimate to cold

153 temperature and show increased sensitivity to cold stress, applied with and

154 without acclimation (Fig. 1e). *dek3-2* plants are less sensitive to freezing

155 without acclimation (Fig. 1e, middle panel), suggesting a key role of *DEK3* in

156 cold resistance pathways.

157 We observe similarly altered hypocotyl growth in *dek2* and *dek4* mutants,

158 suggesting a general role for this family of genes in controlling response to

159 temperature (Supp. Fig. 1d).

160

161 To further understand why plants with abnormal DEK3 protein levels exhibit

162 altered temperature responses, we investigated gene expression in *dek3-2*

163 and *35S::DEK3* plants at 17 ºC and 27 ºC. Since the ambient temperature

164 transcriptome is dynamic[22], we sampled the transcriptome over the 24 h day-

165 night cycle (Supp. Fig. 1e-g). Consistent with plant phenotypes, the largest

166 perturbation in gene expression occurs in *35S::DEK3* at 27 ºC (Supp. Fig. 1e).

167 The overexpression of *DEK3* caused ~4,860 genes to be miss-expressed in

168 at least one time-point compared to WT plants when grown at warm

169 temperature (Supp. table 2).

170   We used Principal Component Analysis (PCA) to further understand

171   differences in gene expression. Principle Components (PC) 1 and 2 together

172   could explain 54% of the gene expression variance and primarily separate the

173   samples based on time of day into day and night time samples (Supp. Fig. 1f).

174   Among the night samples, the expression of differentially expressed genes in

175   *35S::DEK3* plants at 27 ºC resembles the 17 ºC transcriptomes of all

176   genotypes rather than that of warm temperature transcriptomes (Supp. Fig.

177   1f). Indeed, this is consistent with the phenotype of *35S::DEK3* plants, which

178   at 27 ºC show perturbed hypocotyl elongation and flowering similar to the

179   plants grown at 17 ºC (Fig. 1c-d, Supp. Fig. 1b-c).

180   Hierarchical clustering of differentially expressed genes reveals three major

181   categories: the first group includes warm temperature induced genes in Col-0,

182   whose expression was induced during the day (first group: clusters 2 and 3)

183   or during the night (Second group: clusters 6 and 7), and genes whose

184   expression was specifically induced only by *DEK3* overexpression at 27 ºC

185   (third group: clusters 4 and 5) (Supp. Fig. 1g).

186   The first group is highly enriched for different GO terms related to metabolism,

187   translation and ribosome biogenesis, RNA methylation, nucleosome assembly

188   and histone modifications (Supp. Table 2); the second group of genes is

189   highly enriched for GO terms connected to shoot and meristem development,

190   regulation of growth, metabolism, transcription (RNA elongation and gene

191   silencing) and photosynthesis (Supp. Table 2). This cluster contains genes

192   encoding heat shock proteins such as *HSP70*, as well as genes related to

193   hypocotyl growth, like *LHY*. Genes in this group are more expressed in *dek3-2*

194   toward the end of the night; Genes in the third group respond only in

195   *35S::DEK3*, and are specifically enriched in GO terms related to response to

196   biotic stimuli and immune response (Supp. Fig. 1g, Supp. Table 2).

197

198   **DEK3 direct targets can be activated or repressed by elevated *DEK3***

199   **expression**

200   Since the effects of DEK3 on transcription may be indirect, we performed

201   CHIP-seq of *DEK3-CFP* expressed under its native promoter in *dek3-2*

202   mutant plants grown at 17 ºC and 27 ºC at the end of the night and day.

203   These time points are the most distinct according to a PCA analysis (Supp.

204   Fig. 1f), allowing us to capture the maximal diversity in DEK3 behaviour.

205   We observed DEK3 binding primarily in active chromatin states (Supp. Fig.

206   2a, the chromatin states 1, 3 and 7) characterized by open chromatin and

207   highly correlated with mRNA-encoding genes[23].

208   Since DEK3 primarily binds to DNA in shallow, broad peaks (not sharp peaks

209   like a transcription factor), a peak calling approach was not appropriate for

210   analyzing DEK3 binding profiles. Instead, we identified targeted genes by

211   clustering the DEK3 profiles over the gene bodies. DEK3 can be detected in

212   the majority of gene bodies with defined boundaries at the beginning and the

213   end of the genes (Fig. 2a, Supp. Fig. 2b). For many genes, there is an

214   enrichment for DEK3 in the 3' end of the gene (Fig. 2a, clusters 5 - 7), which

215   is consistent with its role as an H3.3 chaperone in *Drosophila* and

216   humans[14,15]. Since overexpression of DEK3 protein caused such a dramatic

217   effect on plants physiology and transcriptome at warm temperatures (Fig. 1c-

218   e, Supp. Fig. 1b-c, 1e-g), we checked its chromatin binding profiles and

219   compared them to the profiles of the native DEK3 (Supp. Fig. 2b). Since

220   native and overexpressed DEK3 bind widely throughout the genome (Fig. 2a,

221   Supp. Fig. 2b) it is not possible to make a quantitative comparison in their

222   binding to chromatin, however, it is clear that they have different binding

223   patterns. Overexpression of DEK3 results in extension of binding beyond the

224   ends of the gene body (Supp. Fig. 2b) which might contribute to the altered

225   gene expression seen in *35S::DEK3* (Fig. 1b-d, Supp. Fig.1b-c). We do not

226   observe a significant difference in the binding of DEK3 to chromatin that can

227   be associated with temperature or the time of day (Fig. 2a, Supp. Fig. 2b),

228   suggesting that other factors may be involved in temperature dependent gene

229   expression pattern of DEK3 targets during the day. Hence, we used all four

230   DEK3 ChIP-seq datasets from *DEK3::DEK3-CFP dek3-2* plants as biological

231   replicates for the following analysis in order to determine DEK3 direct targets

232   controlled by temperature.

233

234   We identified 2079 genes as potential direct DEK3 targets (Fig. 2b), as

235   defined by having a high DEK3 occupancy in both temperatures (clusters 3-5

236   in Fig. 2a) and being differentially expressed at least at one time point at 27

237   ºC when *DEK3* is perturbed (Supp. Table 2).

238

239   Since we observe the highest influence of DEK3 levels on gene expression

240   based on PCA and hierarchical cluster analysis at ZT0 (Supp. Fig. 1f-g), we

241   further analysed this timepoint. More than half of the potential direct DEK3

242   targets (1048 out of the 2079 genes) were differentially expressed at this time

243   point (Supp. Table 3). To analyse the mechanism of DEK3 transcription

244   regulation at this time point, we focused on these 1048 genes for further

245   analysis.

246

247   These DEK3 targets can be separated into three groups with distinct

248   transcriptional profiles (Fig. 2c, Supp. Table 3): Genes in Cluster 1 show

249   greatly enhanced expression at 27 ºC in *35S::DEK3* compared to Col-0 and

250   *dek3-2* (Fig. 2c). Since these genes are already expressed at low levels in

251   Col-0, it would be challenging to detect a significant reduction in expression in

252   *dek3-2* compared to WT plants (Fig. 2c).

253   This group is dominated by seven members of the AP2-type transcription

254   factors, mainly of the *ETHYLENE RESPONSE FACTOR (ERF)* class whose

255   expression is induced by DEK3 overexpression in combination with

256   temperature (Fig. 2d). Overall, the genes in this cluster are highly enriched for

257   stress genes (Supp. Fig. 2c). The miss-expression of the stress transcriptome

258   likely contributes to the observed enhanced sensitivity of *35S::DEK3* to

259   freezing (Fig. 1e).

260   Cluster 2 contains genes induced during the night at 27 ºC in Col-0. These

261   genes are up-regulated in *dek3-2* earlier in the night compared to Col-0, while

262   not induced at all when *DEK3* is overexpressed. For example, the

263   temperature responsive auxin biosynthesis gene *YUCCA8* that is necessary

264   for hypocotyl elongation at 27 ºC is directly repressed by DEK3. Prominent

265   transcription factors in cluster 2 include the AUXIN RESPONSE FACTORS

266   (ARF) 1 and 19, and the GATA transcription factors 2, 5 and 9. Many genes

267   also implicated in auxin signalling, for example *NAKED PINS IN YUCCA*

268   *MUTANT (NPY) 1, 3* and *5* are also repressed by DEK3. At the same time

269   these genes are induced even more during the night in *dek3-2* plants in

270 temperature dependent manner (Fig. 2d). Overall there is strong enrichment

271 for the GO-terms associated with growth, development and auxin (Supp. Fig.

272 2c), indicating that influence on growth caused by *DEK3* perturbations at 27

273 ºC is direct. The final cluster is heterogeneous, but it is the only cluster that

274 contains genes that decrease their expression levels at elevated

275 temperatures.

276

277 *DEK3* affects growth and survival in salinity stress[10], heat stress[10], elevated

278 ambient temperature and cold stress (Fig. 1c-e, Supp. Fig. 1b-c), indicating

279 that DEK3 controls genes playing a role in promoting stress responses and

280 inhibiting growth in a broad range of conditions. Consistent with this, using

281 publicly available transcriptomic data from the AtGenExpress consortium, we

282 found that the Cluster 1 genes were up-regulated and the Cluster 2 were

283 down-regulated in a wide range of abiotic stress conditions including UV-B,

284 salt, osmotic, cold, wounding, heat, drought, genotoxic and oxidative

285 stresses[24] (Supp. Fig. 4a).

286

287 **The activation or repression of DEK3 targets is related to gene body**

288 **H2A.Z**

289 *DEK3* mediates the correct expression of a set of genes that are widely

290 responsive to abiotic stresses, while simultaneously repressing the induction

291 of genes promoting growth (Fig. 2c, Supp. Fig. 2c). This raises the question of

292 how a single factor can both activate and repress gene expression in a locus

293 specific manner. This behaviour is similar to that reported in mammals, where

294 DEK behaves as repressor in some cases and as an activator in others[17,25].

295 Since chromatin state is correlated with the degree of gene

296 responsiveness[4,6,7], we investigated whether the binding pattern of H2A.Z,

297 H3.3 and DNA accessibility were predictive of whether a direct DEK3 target

298 would be activated ("stress" genes) or repressed ("growth" genes). To do this,

299 we built a conditional decision tree model that would take the distribution of

300 DEK3, H2A.Z and H3.3 (ChIP-seq) and the DNA accessibility (MNase-seq) in

301 WT as an input, and trained a model to predict whether a DEK3 bound gene

302 would be up- or down- regulated in the *35S::DEK3* plants at 27 ºC at the end

303   of the night (Fig. 3a, Supp. Fig. 3a-d), where the biggest difference in

304   expression could be observed (Supp. Fig. 1f).

305   Strikingly, the most important feature that distinguished between repressed

306   genes (referred to as the "growth" genes) and induced genes (referred to as

307   the "stress" genes) was the gene-body distribution of H2A.Z ($p<0.001$) with

308   most DEK3 up-regulated "stress" genes having H2A.Z in the gene body,

309   whereas the repressed "growth" genes did not have H2A.Z in the gene body.

310   These "growth" genes predominantly have +1 H2A.Z nucleosomes (Fig. 3a),

311   some "stress" genes were found to have both gene body H2A.Z and strong +1

312   H2A.Z. H3.3 and chromatin occupancy (via MNase-seq) also contribute to

313   differences in "growth", and "stress" genes. Specifically, genes with lower

314   H2A.Z levels, but open promoter regions are more likely to be "stress" genes

315   (Fig. 3a, node 2 and genes with lower levels of H3.3 are more likely to be

316   "growth" genes (Fig. 3a, node 6).

317

318   While the level of DEK3 binding does not determine whether a gene will have

319   H2A.Z in the gene body (Fig. 3a-b, Supp. Fig. 3e), perturbing *DEK3*

320   expression consistently changes the distribution of H2A.Z incorporation

321   around the +1 nucleosome and gene body (Fig. 3c-d, Supp. Fig. 3f). In *dek3-*

322   *2*, there is a larger ratio of gene body H2A.Z in "growth" genes compared to

323   WT plants (Fig. 3c-d (lower panel), Supp. Fig. 3f (lower panel)), which is

324   consistent with these genes being more easily induced in *dek3-2* compared to

325   Col-0 at 27 ºC (Supp. Fig. 4b (upper panel)). This might explain the

326   phenotypic differences between these plants (Fig. 1c-d, Supp. Fig. 1b-c).

327   Conversely, there is lower enrichment for H2A.Z in both the +1 position and

328   gene bodies of "stress" related genes in plants over-expressing *DEK3*

329   compared to WT background (Fig. 3c-d (upper panel), Supp. Fig. 3f (upper

330   panel)).

331   It has been shown that transcription of genes with H2A.Z in gene bodies is

332   more easily induced by environmental cues and stress[4,7]—these are the

333   genes that are induced by overexpression of *DEK3* and temperature (Fig. 3a,

334   node 1, Supp. Fig. 4b (lower panel)). In contrast, genes that have only +1

335   H2A.Z nucleosomes and H3.3 around TTS, are expected not to be easily

336   induced by environment but correlated with growth[4]. These genes are

337   suppressed by overexpression of DEK3 and induced in *dek3* in a temperature

338   specific way manner compared to Col-0 (Supp. Fig. 4b (upper panel)).

339

340   These observations are consistent with the interaction of DEK3 with H2A.Z

341   (Fig.1a-b, Supp. Fig. 1a), and with decreased expression of the "growth"

342   genes and increased expression of the "stress" related genes in the absence

343   of H2A.Z (Fig. 4d-e) or when its incorporation into chromatin is reduced (Fig.

344   4f-g). Given that H2A.Z is known to accumulate in the gene body of

345   environmentally responsive genes (such as in drought stress responsive

346   genes)[4,7,6], a relationship between DEK3 and H2A.Z may suggest a

347   mechanism by which DEK3 perturbations could affect H2A.Z distribution in +1

348   nucleosomes and gene bodies, and in this way control inducibility of gene

349   expression by environment. This pattern doesn't hold among strongly DEK3-

350   bound genes in other nodes (Fig. 3c, Supp. Fig. 3f), suggesting that the

351   chromatin landscape is likely to be changing as a result of the DEK3-H2A.Z

352   interaction, rather than as a consequence of changes in transcription.

353

354   ***DEK3* and *H2A.Z* interact genetically**

355   Our results suggest that DEK3 plays a role in driving H2A.Z-nucleosome

356   removal from the gene body of stress responsive genes. Supporting this

357   model (Supp. Fig. 4i), when H2A.Z levels or its deposition is perturbed in

358   mutants lacking H2A.Z or *ACTIN RELATED PROTEIN6 (ARP6)*, the

359   transcriptome shows similar mis-expression as in *35S::DEK3* (Fig. 4d-e), but

360   opposite to that of *dek3-2* (Fig. 4f-g). Furthermore, the *arp6-1* mutation is able

361   to partially suppress the effect of *dek3-2* on the transcriptome (Supp. Fig. 4g).

362   Since ARP6 might be present in several protein complexes including SWR1

363   and may have other roles[26,27], we also investigated the effect of removing

364   H2A.Z in plants on the *DEK3* dependent transcriptome. In the triple mutant for

365   *hta8,9,11*, we observe a consistent pattern over the time course, with up-

366   regulation of *35S::DEK3* activated genes and down-regulation of *35S::DEK3*

367   suppressed genes (Fig. 4c-e), similarly to what is observed in *arp6-1* (Fig. 4f-

368   g).

369

370 In general, "growth" related genes have less H2A.Z signal, and this is most

371 likely to be found near the TSS (Fig. 3a). These temperature dependent

372 genes are further induced by warm ambient temperature in *dek3-2* possibly by

373 increasing the levels of their H2A.Z gene body binding (Fig. 4d, Supp. Fig. 4b

374 (upper panel)). Reducing H2A.Z-nucleosome occupancy in the *h2a.z* and

375 *arp6-1* backgrounds slightly inhibits the temperature induction of "growth"

376 genes (Fig. 4d, 4f). However, when the levels of both proteins, H2A.Z and

377 DEK3, on chromatin are reduced in the *dek3-2 arp6-1* double mutant, the

378 temperature dependent activation of these genes is been impaired (Supp. Fig.

379 4g (upper panel)). In contrast, the "stress" related genes are enriched in

380 H2A.Z in their gene bodies (Fig. 3a) and up-regulated in the *35S::DEK3* plants

381 grown at 27°C, (Fig. 4e, Supp. Fig. 4b (lower panel)). These genes are also

382 up-regulated in *h2a.z* and *arp6-1* mutants in a temperature-dependent

383 manner (Fig. 4e, 4g), indicating that H2A.Z-nucleosome occupancy may

384 regulate their transcription. This increase is abrogated in *dek3-2 arp6-1*

385 plants, demonstrating the antagonistic interaction between DEK3 and ARP6

386 (Supp. Fig. 4g (lower panel)).

387

388 In light of the physical interaction between DEK3 and H2A.Z (Fig. 1a-b, Sup.

389 Fig. 1a) and in line with the transcriptional patterns of *dek3-2 arp6-1* double

390 mutants and *arp6-1* (Supp. Fig. 4g), we sought to determine if they also

391 interact genetically. The partial rescue of the *arp6-1* phenotype by the *dek3-2*

392 mutation at both an elevated temperature and cold stress (Fig. 4a, Supp. Fig.

393 4c-f) indicates that *ARP6* and *DEK3* might have opposite functions within the

394 same pathway. Over-expression of *DEK3* in *arp6-1* led to temperature-

395 dependent lethality (Supp. Fig. 4h), suggesting that normal levels of *DEK3*

396 expression are necessary for plants to survive with reduced H2A.Z-

397 nucleosomes when exposed to warm or cold temperature.

398

399 **H2A.Z deposition patterns in human DEK targets**

400 DEK proteins are conserved through evolution found in almost all higher

401 eukaryotes[29,30], and show similarity in domain structure between plants and

402 animals[12]. Three of the four plant DEK proteins, DEK2, DEK3 and DEK4,

403 have been found in complex with H2A.Z by mass spec analysis (Fig. 1a,

404 Supp. Fig. 1a, Supp. Table 1) and showed similar growth phenotypes in

405 response to warm temperature (Fig. 1d, Sup. Fig. 1d), suggesting functional

406 redundancy between them. We therefore asked if our finding that the pattern

407 of H2A.Z-nucleosome occupancy on the gene body may be predictive of how

408 a gene is regulated by DEK3 in Arabidopsis also applies in humans.

409 Previously, human DEK (hDEK) has been shown to both activate and repress

410 expression of the genes involved in cancer and hematopoiesis[30–38]

411 (summarised in Supp. Fig. 4j). We observe that hDEK up-regulated genes

412 possess mainly gene body H2A.Z, while hDEK down-regulated genes have

413 more +1 nucleosome H2A.Z binding (Supp. Fig. 4j, 4k). This suggests that the

414 interaction between DEK and H2A.Z may be functionally conserved, and that

415 the role of DEK in controlling the balance between growth and stress

416 resilience is conserved between plants and vertebrates. DEK in vertebrates

417 plays key roles in carcinogenesis[7,9,10,13,25,31,36–38,39], haematopoiesis[25,30,33] and

418 inflammation[11,46], processes involving a fine-control of proliferation and stress

419 resilience. It will be interesting to determine if these functions of DEK are also

420 mediated via H2A.Z-nucleosomes.

421

Discussion

423 We find that altered levels of DEK3, an Arabidopsis ortholog of the onco-

424 protein DEK, perturbs developmental programming in *Arabidopsis thaliana*

425 grown in warm temperature by influencing expression of environmental

426 (stress related) and growth related (developmental) genes (Sup. Fig. 2c, Sup.

427 Fig. 4b). The effect of DEK3 on the transcriptome and development appears

428 to be mediated at least in part by changes in the relative distribution of H2A.Z-

429 nucleosomes on the gene bodies of target genes (Fig. 3c-d, Sup. Fig. 3f).

430 These results may explain the dual effect of DEK3 on the transcription of its

431 target genes, shedding light on the longstanding question regarding the

432 influence of DEK on gene expression[12,17,25]. We also provide evidence for

433 physical and genetic interaction between DEK3 and H2A.Z in Arabidopsis

434 (Fig. 1a-b, Sup. Fig. 1a, Sup. Fig. 4c-g). Since DEK proteins are found in most

435 multicellular eukaryotes, as is the H2A variant H2A.Z, these findings may be

436   of broad relevance, and contribute towards understanding of hDEK as a

437   therapeutic target.

438

439   The chromatin landscape regulates accessibility of DNA to the transcriptional

440   machinery and transcription factors, controlling the transcriptome and how it

441   responds to a changing environment[47]. Incorporation of the histone variants,

442   H2A.Z and H3.3, into chromatin is frequently associated with responses to

443   environmental perturbations[4,7,8]. While it was reported previously that DEK is

444   a H3.3 chaperone and controls H3.3 deposition into chromatin[14], the effect of

445   altered levels of DEK3 on more than half of the warm temperature

446   transcriptome can be explained by initial pattern of H2A.Z distribution (Fig. 3a,

447   Sup. Fig. 3f). Previous work suggests that DEK may interact with H3.3[14] —

448   which is not inconsistent with this study as it is possible for a protein to have

449   multiple binding partners. It will be interesting to see if DEK3 is able to bind

450   H2A.Z and H3.3 simultaneously.

451

452   Our results suggest that DEK3 influences the chromatin landscape by

453   modulating the distribution of H2A.Z at specific genes thus controlling their

454   responsiveness to environmental signals (Fig. 3c-d, Sup. Fig. 3f). Our data

455   confirm and extend previous reports suggesting that the initial pattern of

456   H2A.Z on gene bodies is important for the proper induction of transcription in

457   response to environmental stress[4,7]. We show that the levels of H2A.Z on

458   gene bodies is altered by DEK3, influencing gene expression responsiveness

459   in response to environmental signals (Fig. 3). *DEK3* overexpression for

460   example depletes H2A.Z-nucleosome occupancy, leading to enhanced

461   transcriptional responses to higher temperature (Fig. 3c-d, Supp. Fig. 3f,

462   Supp. Fig. 4b).

463

464   DEK3 may influence H2A.Z distribution through its role in H3.3 incorporation

465   into chromatin. In animal systems, lack of DEK causes enhanced H3.3

466   incorporation into the chromatin by HIRA and DAXX/ATRX chaperones[14].

467   H3.3 incorporation into chromatin might in turn lead to elevated DNA

468   methylation and subsequent prevention of H2A.Z incorporation into gene

469   bodies[6]. This model is not consistent with our finding that increased levels of

470   H2A.Z occur in the gene bodies of *dek3* plants, and conversely,

471   overexpressing *DEK3* results in reduced gene body H2A.Z, at least in the

472   subset of the DEK3 target genes (Fig. 3a, 3c-d, Sup. Fig. 3f). These

473   observations suggest another mechanism of regulation. Interestingly, H3.3

474   distribution was able to explain the effect of *DEK3* expression on the warm

475   temperature transcriptome for only a subset of the genes (Fig. 3a, node 6),

476   while the H2A.Z pattern alone predicts the behaviour of more than half of the

477   DEK3 target genes (Fig. 3a). This effect could partially be explained by

478   possible physical interaction between DEK3 and H2A.Z (Fig. 1a-b). Even

479   though we could not exclude the possibility of indirect physical interaction

480   between these two proteins, our genetic studies confirm the biological

481   relevance of this interaction (Sup. Fig. 4c-f, Sup. Fig. 4h). Many cancers are

482   associated with impaired DEK levels[12,13,40,41,43,45,46,48–50,14,25,32–35,38,39], and it

483   will be interesting to determine if these show a similar relationship for

484   transcriptional response and the distribution of hDEK and H2A.Z-

485   nucleosomes.

486

487   Our work suggests a model for growth promoting and growth inhibiting

488   responses to environmental changes (Sup. Fig. 4i), where genes with high

489   levels of gene body H2A.Z are responsive to environmental induction, and this

490   sensitivity is enhanced by DEK which can facilitate a decrease in H2A.Z

491   occupancy and higher gene expression. Genes with low gene body H2A.Z

492   occupancy are more likely to be resistant to activation by environmental

493   signals in presence of normal DEK3 levels. For plants this is important for

494   example in tuning the response of the transcriptome to ambient temperature.

495   For plants it is of particular importance to keep a balance between

496   developmental and metabolic transcriptomes under different conditions.

497   Enhanced growth as a result of elevated temperature may cause a reduction

498   in size of adult plant and seed yield[51]. Additionally, plants need to attenuate

499   their development rate in order to provide appropriate response to biotic

500   stress to obtain optimised fitness[2]. However, it may also play a role in other

501   contexts, for example in the case of inflammation and cancer where changes

502   in the relative levels of nutrients and oxygen occur frequently, and cells must

503   respond appropriately.

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511 **Reference:**

512   1.   Todesco, M. *et al.* Natural allelic variation underlying a major fitness

513   trade-off in Arabidopsis thaliana. *Nature* **465**, 632–636 (2010).

514   2.   Huot, B., Yao, J., Montgomery, B. L. & He, S. Y. Growth-defense

515   tradeoffs in plants: A balancing act to optimize fitness. *Mol. Plant* **7**,

516   1267–1287 (2014).

517   3.   Zahraeifard, S. *et al.* Rice H2A.Z negatively regulates genes responsive

518   to nutrient starvation but promotes expression of key housekeeping

519   genes. *J. Exp. Bot.* **69**, 4907–4919 (2018).

520   4.   Coleman-Derr, D. & Zilberman, D. Deposition of histone variant H2A.Z

521   within gene bodies regulates responsive genes. *PLoS Genet.* **8**,

522   e1002988 (2012).

523   5.   Wollmann, H. *et al.* Dynamic deposition of histone variant H3.3

524   accompanies developmental remodeling of the Arabidopsis

525   transcriptome. *PLoS Genet.* **8**, e1002658 (2012).

526   6.   Wollmann, H. *et al.* The histone H3 variant H3.3 regulates gene body

527   DNA methylation in Arabidopsis thaliana. *Genome Biol.* **18**, 94 (2017).

528   7.   Sura, W. *et al.* Dual role of the histone variant H2A.Z in transcriptional

529   regulation of stress-response genes. *Plant Cell* tpc.00573.2016 (2017).

530   doi:10.1105/tpc.16.00573

531   8.   Kumar, S. V. & Wigge, P. a. H2A.Z-containing nucleosomes mediate

532   the thermosensory response in Arabidopsis. *Cell* **140**, 136–47 (2010).

533   9.   Cortijo, S. *et al.* Transcriptional regulation of the ambient temperature

534   response by H2A.Z-nucleosomes and HSF1 transcription factors in

535   Arabidopsis. *Mol. Plant* **10**, 1258–1273 (2017).

536 10.   Waidmann, S., Kusenda, B., Mayerhofer, J., Mechtler, K. & Jonak, C. A

537   DEK Domain-Containing Protein Modulates Chromatin Structure and

538   Function in Arabidopsis. *Plant Cell* **26**, 4328–4344 (2014).

539 11.   Pease, N. A., Wise-Draper, T. & Privette Vinnedge, L. Dissecting the

540   Potential Interplay of DEK Functions in Inflammation and Cancer. *J.*

541   *Oncol.* **2015**, (2015).

542 12.   Broxmeyer, H. E. *et al.* Concise Review: Role of DEK in

543   Stem/Progenitor Cell Biology. *Stem Cells* **31**, 1447–1453 (2013).

544   13.   Kappes, F. *et al.* The DEK oncoprotein is a Su(var) that is essential to
545   heterochromatin integrity. *Genes Dev.* **25**, 673–8 (2011).

546   14.   Ivanauskiene, K. *et al.* The PML-associated protein DEK regulates the
547   balance of H3.3 loading on chromatin and is important for telomere
548   integrity. *Genome Res.* **24**, 1584–1594 (2014).

549   15.   Sawatsubashi, S. *et al.* A histone chaperone, DEK, transcriptionally
550   coactivates a nuclear receptor. *Genes Dev.* **24**, 159–70 (2010).

551   16.   Sattler, M. & Valca, J. Recognition by DEK. **676**, 1961–1965 (2006).

552   17.   Sandén, C. *et al.* The DEK oncoprotein binds to highly and ubiquitously
553   expressed genes with a dual role in their transcriptional regulation. *Mol.*
554   *Cancer* **13**, 215 (2014).

555   18.   March-Díaz, R. & Reyes, J. C. The beauty of being a variant: H2A.Z
556   and the SWR1 complex in plants. *Mol. Plant* **2**, 565–77 (2009).

557   19.   Santisteban, M. S., Hang, M. & Smith, M. M. Histone Variant H2A.Z and
558   RNA Polymerase II Transcription Elongation. *Mol. Cell. Biol.* **31**, 1848–
559   1860 (2011).

560   20.   McClung, C. R., Lou, P., Hermand, V. & Kim, J. A. The Importance of
561   Ambient Temperature to Growth and the Induction of Flowering. *Front.*
562   *Plant Sci.* **7**, 1–7 (2016).

563   21.   Bita, C. E. & Gerats, T. Plant tolerance to high temperature in a
564   changing environment: scientific fundamentals and production of heat
565   stress-tolerant crops. *Front. Plant Sci.* **4**, 1–18 (2013).

566   22.   Jung, J.-H. *et al.* Phytochromes function as thermosensors in
567   *Arabidopsis*. *Science (80-.).* **354**, 886–889 (2016).

568   23.   Sequeira-Mendes, J. *et al.* The Functional Topography of the
569   Arabidopsis Genome Is Organized in a Reduced Number of Linear
570   Motifs of Chromatin States. *Plant Cell* **26**, 2351–2366 (2014).

571   24.   Kilian, J. *et al.* The AtGenExpress global stress expression data set:
572   Protocols, evaluation and model data analysis of UV-B light, drought
573   and cold stress responses. *Plant J.* **50**, 347–363 (2007).

574   25.   Capitano, Maegan L.; Broxmeyer, H. E. A role for intracellular and
575   extracellular DEK in regulating hematopoiesis. *Curr. Opin. Hematol.* **24**,
576   300–306 (2017).

577   26.   Yoshida, T. *et al.* Actin-related protein Arp6 influences H2A.Z-

578   dependent and -independent gene expression and links ribosomal

579   protein genes to nuclear pores. *PLoS Genet.* **6**, 10–17 (2010).

580 27. Berriri, S., Gangappa, S. N. & Kumar, S. V. SWR1 Chromatin-

581   Remodeling Complex Subunits and H2A.Z Have Non-overlapping

582   Functions in Immunity and Gene Regulation in Arabidopsis. *Mol. Plant*

583   **9**, 1051–1065 (2016).

584 28. Privette Vinnedge, L. M., Kappes, F., Nassar, N. & Wells, S. I. Stacking

585   the DEK: From chromatin topology to cancer stem cells. *Cell Cycle* **12**,

586   51–66 (2013).

587 29. Waldmann, T., Scholten, I., Kappes, F., Hu, H. G. & Knippers, R. The

588   DEK protein-an abundant and ubiquitous constituent of mammalian

589   chromatin. *Gene* **343**, 1–9 (2004).

590 30. Koleva, R. I. *et al.* HEMATOPOIESIS AND STEM CELLS C / EBP   and

591   DEK coordinately regulate myeloid differentiation. **119**, 1–3 (2012).

592 31. Sammons, M. *et al.* Negative regulation of the RelA/p65 transactivation

593   function by the product of the DEK proto-oncogene. *J. Biol. Chem.* **281**,

594   26802–26812 (2006).

595 32. Kim, K.-B. *et al.* Negative regulation of peroxiredoxin 6 (Prdx 6)

596   transcription by nuclear oncoprotein DEK during leukemia cell

597   differentiation. *Animal Cells Syst. (Seoul).* **18**, 318–323 (2014).

598 33. Hu, H. G., Illges, H., Gruss, C. & Knippers, R. Distribution of the

599   chromatin protein DEK distinguishes active and inactive CD21/CR2

600   gene in pre- and mature B lymphocytes. *Int. Immunol.* **17**, 789–796

601   (2005).

602 34. Zhang, Y. *et al.* The DEK oncogene activates VEGF expression and

603   promotes tumor angiogenesis and growth in HIF-1 a -dependent and -

604   independent manners. **7**, (2016).

605 35. Campillos, M., García, M. A., Valdivieso, F. & Vázquez, J.

606   Transcriptional activation by AP-2α is modulated by the oncogene DEK.

607   *Nucleic Acids Res.* **31**, 1571–1575 (2003).

608 36. Karam, M. *et al.* Chromatin Redistribution of the DEK Oncoprotein

609   Represses hTERT Transcription in Leukemias. *Neoplasia* **16**, 21-W14

610   (2014).

611   37.   Hu, H. gang, Scholten, I., Gruss, C. & Knippers, R. The distribution of
612   the DEK protein in mammalian chromatin. *Biochem. Biophys. Res.*
613   *Commun.* **358**, 1008–1014 (2007).
614   38.   Lohmann, F. *et al.* The DEK Oncoprotein Is a Critical Component of the
615   EKLF / KLF1 Enhancer in Erythroid Cells. **35**, 3726–3738 (2015).
616   39.   Riveiro-Falkenbach, E. & Soengas, M. S. Control of tumorigenesis and
617   chemoresistance by the DEK oncogene. *Clin. Cancer Res.* **16**, 2932–
618   2938 (2010).
619   40.   Smith, E. A. *et al.* DEK associates with tumor stage and outcome in
620   HPV16 positive oropharyngeal squamous cell carcinoma. **8**, 23414–
621   23426 (2017).
622   41.   Shibata, T. *et al.* DEK oncoprotein regulates transcriptional modifiers
623   and sustains tumor initiation activity in high-grade neuroendocrine
624   carcinoma of the lung. *Oncogene* **29**, 4671–4681 (2010).
625   42.   Khodadoust, M. S. *et al.* Melanoma proliferation and chemoresistance
626   controlled by the DEK oncogene. *Cancer Res.* **69**, 6405–6413 (2009).
627   43.   Lin, J. S. F. B. Y. Y. Y. Z. A. J. J. L. Z. DEK protein overexpression
628   predicts poor prognosis in pancreatic ductal adenocarcinoma. *Oncol.*
629   *Rep.* **37**, 857–864 (2016).
630   44.   Yu, L. *et al.* Critical role of DEK and its regulation in tumorigenesis and
631   metastasis of hepatocellular carcinoma. *Oncotarget* **7**, (2016).
632   45.   Privette Vinnedge, L. M. *et al.* The DEK oncogene promotes cellular
633   proliferation through paracrine Wnt signaling in Ron receptor-positive
634   breast cancers. *Oncogene* **34**, 2325–2336 (2015).
635   46.   Mor-Vaknin, N. *et al.* DEK-targeting DNA aptamers as therapeutics for
636   inflammatory arthritis. *Nat. Commun.* **8**, 14252 (2017).
637   47.   Asensi-Fabado, M. A., Amtmann, A. & Perrella, G. Plant responses to
638   abiotic stress: The chromatin context of transcriptional regulation.
639   *Biochim. Biophys. Acta - Gene Regul. Mech.* **1860**, 106–122 (2017).
640   48.   Kavanaugh, G. M. *et al.* The human DEK oncogene regulates DNA
641   damage response signaling and repair. *Nucleic Acids Res.* **39**, 7465–
642   7476 (2011).
643   49.   Deutzmann, A. *et al.* The human oncoprotein and chromatin
644   architectural factor DEK counteracts DNA replication stress. *Oncogene*

645   **34**, 4270–4277 (2015).

646   50.   Privette Vinnedge, L. M. *et al.* The human DEK oncogene stimulates β-

647   catenin signaling, invasion and mammosphere formation in breast

648   cancer. *Oncogene* **30**, 2741–52 (2011).

649   51.   Fischer, K., Zeilstra, I., Hetz, S. K. & Fiedler, K. Physiological costs of

650   growing fast: Does accelerated growth reduce pay-off in adult fitness?

651   *Evol. Ecol.* **18**, 343–353 (2005).

652

653

654

Fig. 1.

Chromatin protein DEK3 interacts with H2A.Z and plays a role in balancing the temperature response between growth and arrest in Arabidopsis

(a) Volcano plot showing the distribution of proteins identified by Mass Spec in complex with H2A.Z according to *p*-value and fold change binding. Horizontal line indicates significance level at *p*-value ≤ 0.05, and vertical lines shows fold change of 2.

(b) Validation of H2A.Z and DEK3 binding. Nuclear extracts from *35S::DEK3-CFP* expressing lines or from Col-0 were subjected to IP with anti-GFP antibodies, and mixed with H2A.Z-Flag purified from nuclear or histone plant extract. The western blot shows the presence of H2A.Z-Flag, the *35S::DEK3-CFP* and H3 in the immune complexes and in input lysates. The amount of protein in the input represents 1% of the amount of proteins used for IP.

(c) Boxplots summarising days to bolting of Col-0, *dek3-2*, *35S::DEK3_1* and *35S::DEK3_2* (independent over-expression lines). The plants have been grown at 17°C, 22°C and 27°C. *35S::DEK3_1* and *35S::DEK3_2* plants haven't bolt within 100 days when grown at 27°C. The following numbers of plants have been used (17/22/27°C): Col-0 9/18/23; *dek3-2* 12/12/24; *35S::DEK3_*1 18/12/24; *35S::DEK3_*2 18/12/24. Box and whisker plots show median, inter-quartile ranges and 95% confidence intervals (t-test p-values are summarized in Supp. Table 5).

(d) Boxplots summarising hypocotyl length (mm) of Col-0, *dek3-2*, *35S::DEK3_1* and *35S::DEK3_2* (independent over-expression lines). The seedlings have been grown for 7 days under short day photoperiod at 17°C, 22°C and 27°C. The following numbers of seedlings have been used (17/22/27°C): Col-0 55/54/46; *dek3-2* 55/57/58; *35S::DEK3_*1 16/18/16; *35S::DEK3_*2 13/7/17. Box and whisker plots show median, inter-quartile ranges and 95% confidence intervals (t-test statistic p-values are summarized in Supp. Table 5).

 (e) Summary of the cold sensitivity assays. Col-0, *dek3-2*, *35S::DEK3_1* and *35S::DEK3_2* (independent over-expression lines) seedlings have been grown for 21 days in SD at 22°C and either subjected to -6°C for 24hr, or subjected to -12°C for 4 days after 24 h acclimation at 4°C. The plants have been allowed to grow for a further 14 days under recovery conditions, to distinguish between plants that do and do not survive the treatment. All the pictures have been done at the same time and survival rates have been calculated. Percentages indicate survival rate. The survival rate of all control plants has been 100%. The following numbers of plants used in the assay: non-acclimated (survived/total) Col-0 19/27; *dek3-2* 23/27; *35S::DEK3_*1 16/27; *35S::DEK3_*2 14/27; acclimated (survived/total) Col-0 20/27; *dek3-2* 19/27; *35S::DEK3_*1 6/27; *35S::DEK3_*2 7/27.

Figure 1

**(a)**

(b)

(c)

(d)

(e)
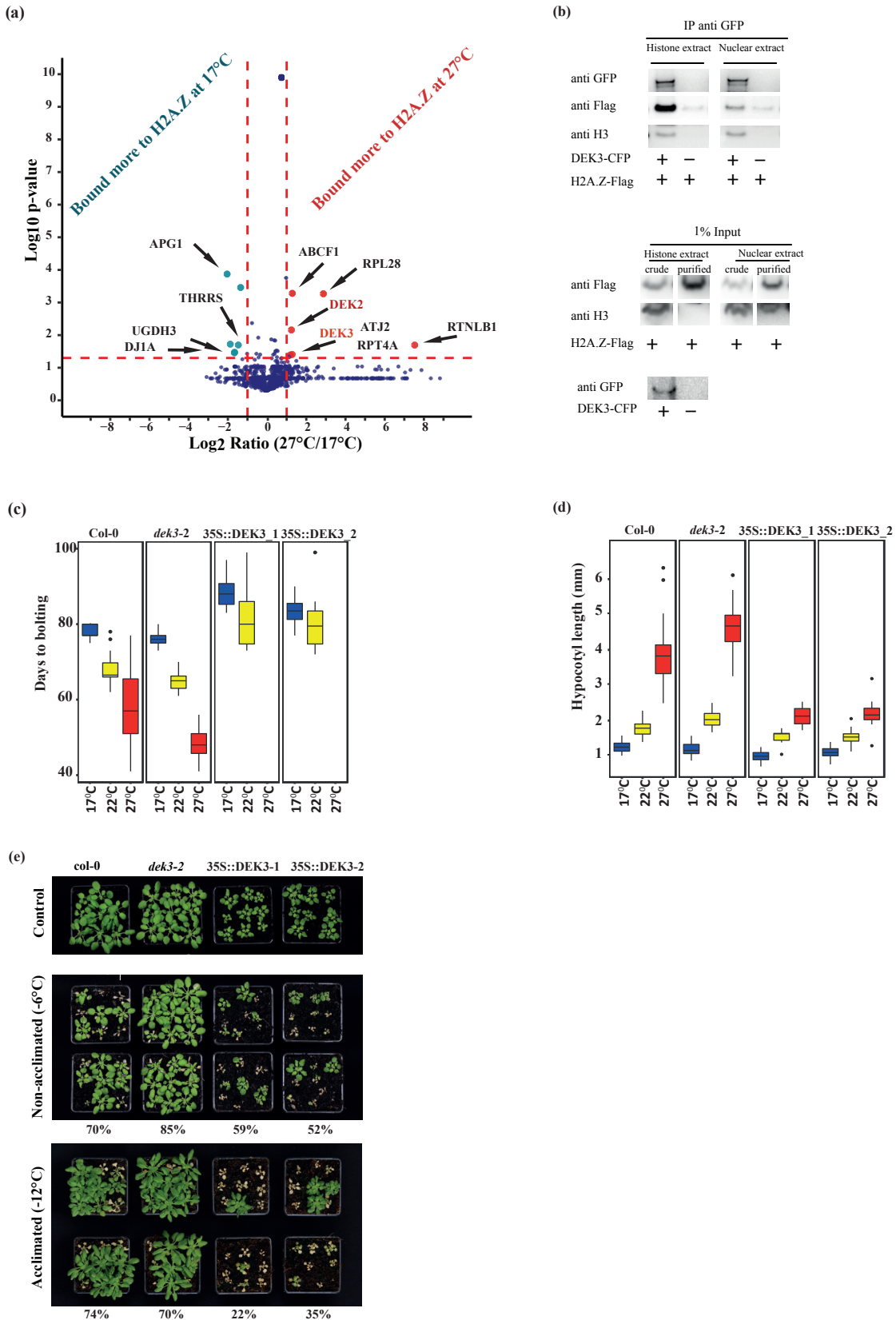
Fig.2.

DEK3 directly regulates the expression of "Growth" and "Stress" genes.

(a) ChIP-seq binding profiles of DEK3-CFP in seedlings grown at 17°C and 27°C and collected at the end of the night. The DEK3 binding patterns across all gene bodies (including 200 bp upstream of the transcription start site (TSS) and 200 bp downstream of the transcription termination site (TES) have been clustered. A large proportion of genes have an enrichment of DEK3 over their gene body, with clusters 3-5 showing the highest enrichment. The label colour bar on the right shows normalised read count, with the highest level in blue and lower in red.

(b) The overlap between the genes mis-expressed by perturbation in DEK3 levels (4,859 genes) and those that are bound directly by DEK3 (12,639 genes, cluster 3-5, from (a)). The overlap highlights DEK3's direct targets mis-regulated in warm temperature when DEK3 is overexpressed (2,079 genes).

(c) Transcriptional patterns of DEK3 direct targets at the end of the night (1,048 genes) which were hierarchically clustered into 3 groups based on their z-scores calculated from transcript per million (TPM) values in all time points. Up-regulated genes are in red and down-regulated genes are in blue. The sidebar to the left of the heatmap indicates the 3 clusters of differentially expressed genes. Black bars on the top indicate night, white bars day.

(d) Expression of direct DEK3 targets belong to AP2 and auxin related families as summarised in Supp. Table 2, in Col-0, *dek3-2, 35S::DEK3-CFP* plants grown at 17°C and 27°C under short photoperiod and collected at 8 different time points during 24 hr time course.
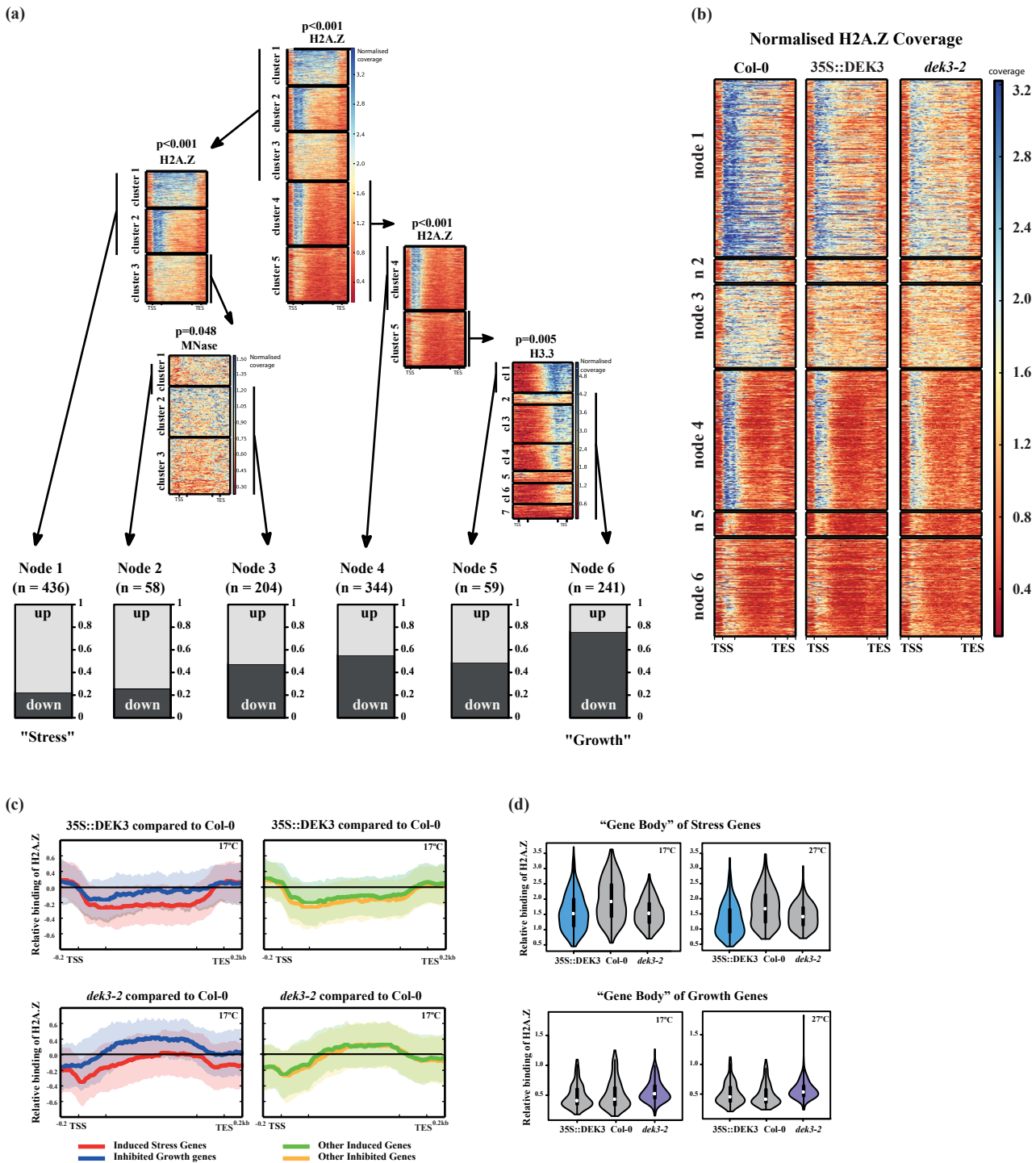
Figure 2

(a) DEK3::DEK3/End of Night

(b)

(c) Day Night

(d) AP2 Related Genes / Auxin Related Genes

Genotype
col
dek3-2
35S::DEK3
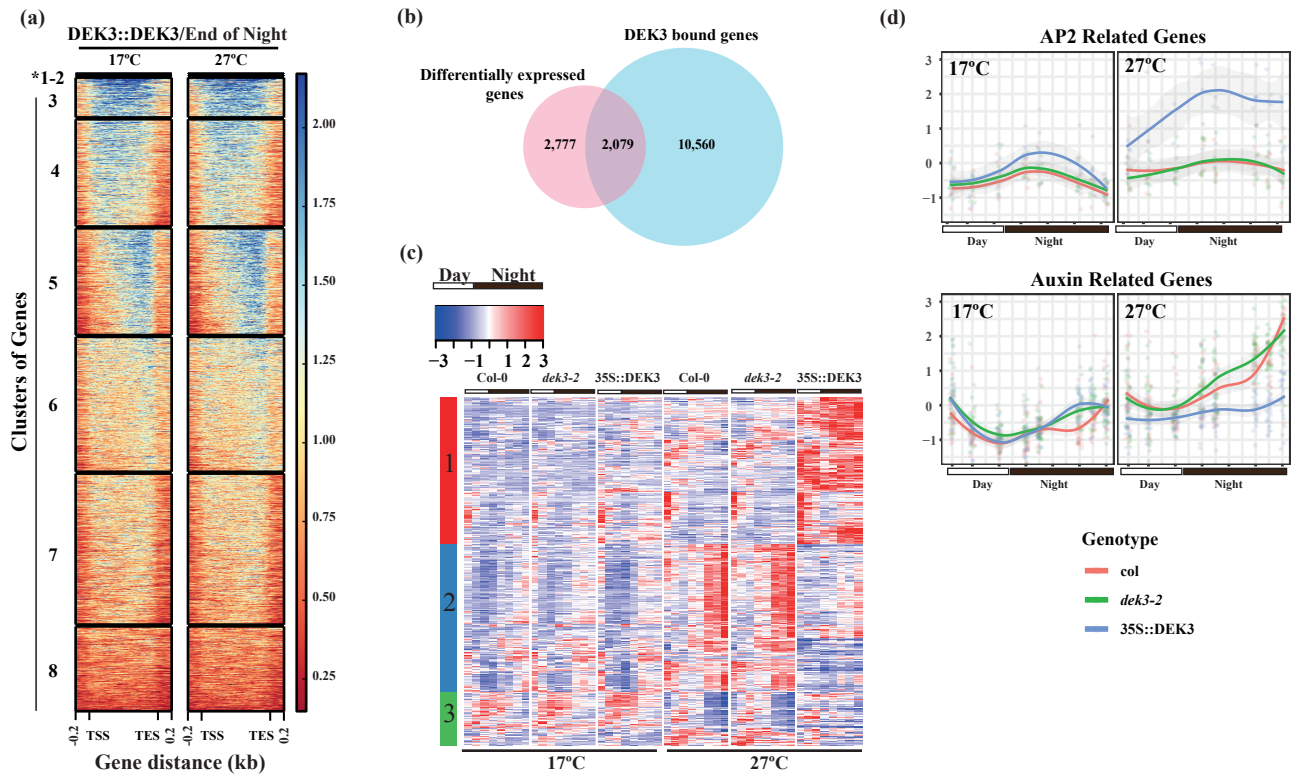
Fig. 3.

DEK3 affects H2A.Z distribution on target gene bodies

(a) The summary of decision tree predicting whether a DEK3 target gene is up- or down-regulated by overexpression of DEK3 in plants grown at 17°C. Each row in each of the clusters represents a gene, and each column represents a position along the gene body. At each branching point (node), the genes are split into two groups based on their ChIP-seq or MNase-seq profile. (b) The example ChIP-seq binding profiles of H2A.Z-Flag in Col-0, *dek3-2* and *35S::DEK3* plants grown at 27°C and collected at the end of the night. The binding profiles are ordered by the nodes identified by the decision tree. (c) Profiles of H2A.Z-Flag ChIP-seq coverage in *35S::DEK3* and *dek3-2* relative to Col-0 plants, depicted over the gene body, 200bp upstream (TSS) and downstream (TES). Plants were grown at 17°C and collected at the end of the night.

(d) The violin plot summarizing the distribution of natively expressed H2A.Z-Flag on the gene bodies of the "stress" and "growth" genes in Col-0, *dek3-2* and *35S::DEK3* plants grown at 17°C and 27°C (Collected at the end of the night).

Figure 3

(a)

p<0.001 H2A.Z

p<0.001 H2A.Z

p=0.048 MNase

p<0.001 H2A.Z

p=0.005 H3.3

Node 1 (n = 436)

Node 2 (n = 58)

Node 3 (n = 204)

Node 4 (n = 344)

Node 5 (n = 59)

Node 6 (n = 241)

"Stress"

"Growth"

(b) Normalised H2A.Z Coverage

Col-0   35S::DEK3   dek3-2

node 1
n 2
node 3
node 4
n 5
node 6

TSS   TES   TSS   TES   TSS   TES

(c)

35S::DEK3 compared to Col-0   35S::DEK3 compared to Col-0

dek3-2 compared to Col-0   dek3-2 compared to Col-0

Induced Stress Genes   Other Induced Genes
Inhibited Growth genes   Other Inhibited Genes

(d)

"Gene Body" of Stress Genes

"Gene Body" of Growth Genes

Fig.4.

DEK3 controls the expression of "growth" and "stress" genes antagonistically to H2A.Z

(a) Col-0, *dek3-2*, *arp6-1* and *dek3-2 arp6-1* seedlings were grown for 21 days in SD at 22°C and subjected to -12°C for 4 days after acclimation for 24 hr at 4°C. Percentages indicate survival rate. All control plants have survived. The numbers of plants used: acclimated (survived/total) Col-0 20/27; *dek3-2* 19/27; *arp6-1* 4/28; *dek3-2 arp6-1* 10/27.

(b) Venn diagram of overlap between the genes mis-expressed by perturbation in DEK3 levels (4,859 genes) and genes mis-expressed in *h2a.z* plants (3,609). The overlap highlights (1,079 genes) what are mis-regulated in both genotypes in warm temperature. (hypergeometric test p-value of the overlaps < 2.63e-65 for both groups).

(c) Expression profiles of DEK3 target genes in *35S::DEK3* and *h2a.z* genetic backgrounds were hierarchically clustered into two groups, based on the log2 ratio compared to the values of Col-0 in transcript per million (TPM). Plants were grown at 27°C and collected at 8 time points over 24 h. Up-regulated genes are shown in red and down-regulated genes are shown in blue. The sidebar on the left of the heatmap indicates the major clusters. Black bars on the top indicate night, white bars day.

(d) (e) Expression of "Growth"(d) and "Stress"(e) genes in *35S::DEK3* and *h2a.z* genetic backgrounds were hierarchically clustered, based on the log2 ratio compared to the values of Col-0 in transcript per million (TPM). Plants has been grown at 27°C and collected at 8 time points during 24 h. Up-regulated genes are shown in red and down-regulated genes are shown in blue. The sidebar on the left of the heatmap indicates major clusters. Black bars on the top indicate night, white bars day.

(f) (g) Expression, based on the log2 ratio compared to the values of Col-0 in TPM, of "Growth" (f) and "Stress" (g) genes in *dek3-2* and *arp6-1* genetic backgrounds were hierarchically clustered as in (f) and (g) respectively. Plants were grown at 17°C and 27°C, and collected at the end of the day (white bar) or at the end of the night (black bar). Up-regulated genes are shown in red and down-regulated genes are shown in blue. The sidebar on the left of the heatmap indicates the major clusters.

Figure 4

(a)

Col-0 *dek3-2* *arp6-1* *dek3-2 arp6-1*

Control

Acclimated (-12°C)

74% 70% 14% 37%

(b)

35S::DEK3 *h2a.z*

3778 1079 2530

(c)

-2 0 2

Day Night Day Night

log2(35S::DEK3/Col-0) log2(*h2a.z*/Col-0)

(d)

-2 0 2

"Growth Genes"

Day Night Day Night

log2(35S::DEK3/Col-0) log2(*h2a.z*/Col-0)

(e)

-2 0 2

"Stress Genes"

Day Night Day Night

log2(35S::DEK3/Col-0) log2(*h2a.z*/Col-0)

(f)

-2 0 2

"Growth Genes"

17°C 27°C 17°C 27°C

log2(*dek3-2*/Col-0) log2(*arp6-1*/Col-0)

(g)

-2 0 2

"Stress Genes"

17°C 27°C 17°C 27°C

log2(*dek3-2*/Col-0) log2(*arp6-1*/Col-0)