A fungal tolerance trait and selective inhibitors proffer HMG-CoA reductase as a herbicide mode-of-action

Joel Haywood 1,2, Karen J. Breese 2, Jingjing Zhang 2, Mark T. Waters 2, Charles S. Bond 2, Keith A. Stubbs 2 & Joshua S. Mylne 1,2

Decades of intense herbicide use has led to resistance in weeds. Without innovative weed management practices and new herbicidal modes of action, the unabated rise of herbicide resistance will undoubtedly place further stress upon food security. HMGR (3-hydroxy-3-methylglutaryl-coenzyme A reductase) is the rate limiting enzyme of the eukaryotic mevalonate pathway successfully targeted by statins to treat hypercholesterolemia in humans. As HMGR inhibitors have been shown to be herbicidal, HMGR could represent a mode of action target for the development of herbicides. Here, we present the crystal structure of a HMGR from Arabidopsis thaliana (AtHMG1) which exhibits a wider active site than previously determined structures from different species. This plant conserved feature enables the rational design of specific HMGR inhibitors and we develop a tolerance trait through sequence analysis of fungal gene clusters. These results suggest HMGR to be a viable herbicide target modifiable to provide a tolerance trait.

As herbicide resistance continues to rise, the efficacy of herbicides has diminished such that new modes of action are desperately needed. Only one new herbicide mode of action has been brought to market in almost 40 years. Weeds are yet to evolve significant resistance to cliomazone and bixlozone, two herbicides that disrupt isoprenoid biosynthesis by targeting the enzyme 1-deoxy-D-xylulose-5-phosphate synthase. Found in all kingdoms of life, isoprenoid biosynthesis is crucial for the synthesis of lipids, hormones, vitamins and defence compounds. The biosynthetic route differs between kingdoms; most animals, fungi, protists and archaea use a mevalonate (MVA) pathway, whereas most Gram-negative bacteria use a methylerythritol phosphate (MEP) pathway. Through a shared evolutionary history with cyanobacteria, plants use both pathways compartmentalised to the cytosol (MVA) or plastids (MEP). None of the known modes of action for any of the commercial herbicides affect the MVA pathway. An important enzyme in the MVA pathway is HMGR, which is a highly regulated, the rate-limiting enzyme of the MVA pathway and is the target of the group of hypercholesterolaemia therapeutics known as statins. Two classes (I and II) of HMGR have been defined based on the differences between the catalytic core domain structure, the presence of an N-terminal membrane domain of between two (plants) and eight (human) membrane-spanning helices in the majority of class I enzymes, and the varied NAD(P)H cofactor preference. HMGR regulation appears to be conserved between humans and plants with the N-terminus regulated by ubiquitination whereas catalytic core activity is regulated by phosphorylation. Many of the regulatory proteins differ, however, and this is further complicated by plants having multiple copies or isoforms plus a wide variety of external signals modifying expression, such as light and herbivory.

The first potent statin inhibitor of HMGR discovered was mevatatin, isolated from Penicillium citrinum in 1976. Lovastatin, isolated from Aspergillus terreus in 1978, became the first commercial statin in 1987. Second-generation statins have been semi-synthetic or synthetic products, but all statins competitively inhibit HMGR via a HMG-like moiety and a variable hydrophobic group that together give

Received: 30 May 2022
Accepted: 7 September 2022
Published online: 22 September 2022
Check for updates

1Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, Bentley, Perth, WA 6102, Australia. 2School of Molecular Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, Perth, WA 6009, Australia. Fax: +61 08 9266 9964; e-mail: joel.haywood@curtin.edu.au; josh.mylne@curtin.edu.au
affinities to HMGR that are 10,000-fold higher than HMG-CoA\(^{30}\). Lovastatin and mevastatin, as well as the semi-synthetic pravastatin and synthetic atorvastatin are all known to be herbicidal\(^{31-33}\). HMGR might have been overlooked as a herbicide target due to potential off-target risks arising from its conservation in humans and the antimicrobial activity of statins\(^{34}\), but recently developed selective insecticides against HMGR illustrate the potential to develop HMGR herbicides\(^{35}\).

Here, we solve crystal structures for a plant HMGR in apo form and complexed with a statin. These structures reveal a wider active site conserved in plants compared to other organisms. By rational design we develop statin derivatives with over 20-fold specificity for the plant over the human enzyme and, which importantly, retain herbicidal activity. By comparing the ATGMR structure to fungal HMGR genes in biosynthetic clusters for natural statins, we demonstrate a single amino acid change confers statin tolerance in vitro and in planta. Together these findings suggest HMGR is a viable target for herbicide development.

**Results**

**Statins range in herbicidal activity**

Previous studies have shown several statins to exhibit herbicidal activity against several plant species including *Lemma gibba*, *Raphanus sativus*, *Scoparia dulcis* and *A. thaliana*\(^{36-38}\). However, there is a lack of comparative data regarding the herbicidal efficacy of statins especially for second-generation, synthetic statins. To assess herbicidal activity, we treated a model dicot and a monocot (*A. thaliana* and *Eragrostis tef*, respectively) with a dose range of eight commercially available statins on soil, pre- and post-emergence (Fig. 1). All statins were more herbicidal against the dicot and in general were more effective post-emergence. In line with their physicochemical properties more closely matching those of post-emergence herbicides (Supplementary Fig. 1a). The synthetic statin rosuvastatin was the most herbicidal statin being lethal to *A. thaliana* at ~15 \(\mu M\) without formulation beyond including a wetting agent. Given that under the same conditions formulated glyphosate (Roundup\(^{\circ}\)) is lethal at ~3.5 \(\mu M\) (Supplementary Fig. 1b, c), we surmised that HMGR could represent a potential herbicide target.

**Crystal structure of ATGMR reveals scope for species selective compounds**

*A. thaliana* has two HMGR genes with different expression patterns, but ATGMR (At1g76490) is the most highly expressed\(^{39}\). The N-terminal transmembrane domains of HMGR are highly divergent between species and absent from class II HMGRs (Supplementary Fig. 2). By contrast, the conserved extracellular domain of ATGMR shares ~54% sequence identity with HsHMGR and strictly conserved catalytic residues (Supplementary Fig. 2). To develop plant-specific statins and mitigate off-target effects, we solved the crystal structure of the core domain of apo ATGMR and in complex with pitavastatin to resolutions of 1.9 and 2.1 \(\AA\), respectively, in space group 4, 2 2. Attempts were made to crystallise type I statins as described previously\(^{40}\), however, electron density for these ligands was ambiguous. The structure of the apo ATGMR displayed a single monomer in the asymmetric unit which through crystallographic symmetry forms a homotetrameric assembly (Fig. 2a), consisting of two canonical class I homodimeric HMGR folds, with high structural similarity to HsHMGR (PDB 1HWK, r.m.s.d. 1.1 \(\AA\) over 371 Ca atoms, Fig. 2b).

Closer inspection of the statin-binding pocket revealed two substitutions in ATGMR with respect to HsHMGR located at the hydrophobic CoA binding region of the active site pocket\(^{41}\), specifically, Ile\(^{657}/\)Leu\(^{657}\) and Ile\(^{661}/\)Val\(^{661}\) in ATGMR/HsHMGR respectively (Fig. 2c, d). Furthermore, a plant conserved Val to Pro (Pro\(^{236}/\)Val\(^{530}\) *A. thaliana*/human) substitution at the start of the L1-strand is the likely cause of conformational flexibility and lack of electron density in the Nα4-Lβ1 loop adjacent to the active site-delineating Lβ2-Lα1 loop (Fig. 2e, Supplementary Fig. 3a, b and Supplementary Table 1). This flexibility results in the loss of a type II β-turn found within the HsHMGR Lβ2-Lα1 loop that is stabilised by hydrogen bonding between a conserved Glu (Glu\(^{354}/\)Glu\(^{358}\) ATGMR/HsHMGR) and a Cys backbone amine (Cys\(^{367}/\)Cys\(^{367}\) ATGMR/HsHMGR) (Fig. 2f). This altered conformation of the Lβ2-Lα1 loop is not seen in any of the previous class I and II HMGR crystal structures\(^{41-45}\) and allows alternative conformations of the Cys\(^{367}\) residue (Fig. 2e, g, h). Importantly, the arrangement of the ATGMR Lβ2-Lα1 loop results in Glu\(^{354}\) being unable to form a hydrogen bond with the OS-hydroxyl group of the HMG moiety of statins or the equivalent thioester oxygen of HMG-CoA, as it is shifted 2.5 \(\AA\) away, creating a wider pocket (Fig. 2g). In this orientation it is more likely that Lys\(^{397}\) acts as a proton donor in the catalytic reduction of HMG-CoA to mevalonate as is suggested to occur in bacteria\(^{50}\) and with molecular dynamics and quantum mechanics/molecular mechanics simulations with HsHMGR\(^{51}\). Together, these differences increase the solvent-accessible area of the statin pocket from ~314 \(Å^3\) in HsHMGR to ~357 \(Å^3\) in ATGMR\(^{11}\).

The complex of ATGMR with pitavastatin (Fig. 3a, b) revealed a binding mode highly similar to fluvastatin in HsHMGR\(^{14}\) (Fig. 3c). Conserved polar interactions occur with the residues local to the cis
loop Arg296, Ser390, Asp396, Lys397, Lys398, Asn461 (HsHMGCR Arg590, Ser684, Asp690, Lys691, Lys692, Asn755) and a salt-bridge between the terminal carboxylate of the HMG moiety with Lys441 (HsHMGCR Lys735) (Fig. 3a).

The fluorophenyl group of pitavastatin maintains conserved stacking interactions with Arg296 (HsHMGCR Arg590) and hydrophobic interactions between the quinoline and cyclopropyl moiety with residues Leu268, Ile389, Leu558, Asp561 (HsHMGCR Leu562, Val683, Leu683, Asp686). This complex structure however also revealed two notable differences between the binding mode of class II statins in AtHMG1 and HsHMGCR; (i) loss of hydrogen bonding to the O5-hydroxyl group of the HMG moiety of statins from Glu265 (HsHMGCR Glu559), despite a slight shift of Glu265 towards the bound inhibitor (Fig. 3a and Supplementary Fig. 3c).
showing >20-fold higher specificity from human to plant when compared to atorvastatin, over HsHMGCR (IC50 890 nM ±143 nM) in vitro (Fig.4c, d). These AtHMG1 (HsHMGCR Ser565 and Ser661).

 Hydrogen bond donor (Fig.2e, g). Thus A. thaliana complementary Method 1) and assessed for herbicidal activity on soil with AtHMG1 in vitro by a activity (Fig.4). Compounds whereas side chains longer than the isopropyl group had reduced activity (Fig.4a, b). Dose-response curves confirmed compound 7 had switched preference from human to plant when compared to atorvastatin, showing >20-fold higher specificity for AtHMG1 (IC50 32 nM ±12 nM) over HsHMGCR (IC50 890 nM ±143 nM) in vitro (Fig. 4c, d). These molecules provide a framework for the future development of plant-specific HMGR inhibitors that might exhibit stronger herbicidal activity.

Exploiting biosynthetic gene clusters to engineer statin tolerance

The most commercially successful herbicides are often paired with a tolerance trait in crops. Statins produced from fungal biosynthetic gene clusters usually contain a copy of HMGR that imparts self-resistance, so we sought to determine the structural basis for this resistance. Sequence alignment of a HMGR gene (lurA) from the A. terreus genome revealed several mutations in the cluster-associated copy that were not present in the housekeeping copy (Supplementary Fig. 4). The corresponding residues of the AtHMGI crystal structure revealed a Leu (Leu296) to Thr mutation, whose equivalent was conserved in all A. terreus genomes in the NCBI database. The Leu to Thr mutation would likely disrupt the hydrophobic pocket essential for accommodating the decalin ring of natural statins (Fig. 5a), and so was incorporated into recombinant AtHMGI. The AtHMGI-L558T mutant was resistant to a range of statins (Fig. 5b) with >20-fold resistance to rosuvastatin in vitro (WT IC50 53 nM ±20 nM, L558T IC50 >1000 nM) (Fig. 5c). Without inhibitors, AtHMGI-L558T had reduced catalytic activity (WT Kap 69 µM ±19 µM and kcat 10.7 ± 1.0 s⁻¹, L558T Kap 24 µM ±16 µM and kcat 2.4 ± 0.3 s⁻¹) (Supplementary Fig. 5), but remained within the range of previously published rates for other class I and II HMGR enzymes.

To validate the potential of the L558T mutation for providing a plant tolerance trait, we overexpressed full-length AtHMGI (35 S::AtHMGI) and its equivalent with the L558T mutation (35 S::AtHMGI-L558T) in A. thaliana, using a cauliflower mosaic virus (CaMV) 35 S promoter. It has previously been shown that overexpressing AtHMGI in A. thaliana can give rise to a 40-fold rise in mRNA levels and a modest rise in resistance to lovastatin compared to non-transformed WT controls. Here we found with data collated from 19 independent T2 35 S::AtHMGI lines and 14 independent 35 S::AtHMGI-L558T T2 lines that both constructs conferred similar resistance to the selectable marker hygromycin (Fig. 6a, d). However, the 35 S::AtHMGI-L558T lines were over sixfold more resistant to rosuvastatin (ICso 300 µM vs ±18 µM) than 35 S::AtHMGI lines (ICso 46 µM ±5 µM) and more than 100-fold more resistant than non-transformed WT (ICso 3 µM vs ±1 µM) (Fig. 6b, d). Furthermore, analysis of the effects of rosuvastatin revealed 35 S::AtHMGI-L558T lines were up to 16-fold less sensitive to treatment than 35 S::AtHMGI lines (Fig. 6c). These results illustrate the potential for HMGR to have a tolerance trait and further validates the in vitro results (Fig. 5b, c).
Discussion

The relentless rise in herbicide-resistant weeds already poses a significant threat to global food security and as such, new herbicides with new modes of action are desperately needed. Moreover, as consumer attitudes shift, natural product 'bioherbicides' will rise in their appeal and currently in the USA enjoy an accelerated regulatory journey.

Herein, we have validated HMGR as a potential herbicide target. Using the HMGR crystal structure from a model plant we have demonstrated that, despite its overall sequence and structure conservation with HsHMGCR, differences in the architecture (especially the active site) can be exploited to develop plant-specific synthetic HMGR inhibitors. The progress herein provides a basis for the discovery of natural product statins that might be suitable bioherbicides.

The differences in the architecture of AtHMG1 that allowed for species selectivity largely arise from an unusual orientation of the Lβ2-Lα1 loop that is likely the result of increased flexibility in the neighbouring Nα4-Lβ1 loop. The atypical orientation of the Lβ2-Lα1 loop in AtHMG1 disrupts the hydrogen bonding network formed between the catalytic residues Glu265/Lys397/Asn461/Asp473 (HsHMGCR Glu559/Lys691/Asn755/Asp767), thereby retaining only those hydrogen bonds that stabilise the catalytic Lys via the adjacent Asn and Asp residues. The conserved location of the catalytic Lys between AtHMG1 and other class I and II HMGRs strongly suggests this residue is responsible for polarising the carbonyl oxygen of HMG-CoA substrate and mevaldehyde intermediate, and for performing the final protonation step. Glu265 is not in a favourable position to hydrogen bond to either the carbonyl oxygen nor the mevaldehyde oxygen of HMG-CoA. As a result, the Lβ2-Lα1 loop in AtHMG1 cannot form hydrogen bonds that stabilise the catalytic Lys via the Glu265 or Asn461, and for this reason, Glu265 is not a catalytic residue.

Fig. 4 | Modifying the isopropyl group of atorvastatin affects species selectivity. a Herbicidal activity of atorvastatin and its analogues (1–9) against A. thaliana with pre- and post-emergence treatments. The isopropyl moiety of atorvastatin is boxed in red. Modifications to the isopropyl region are shown. b Compounds 4 and 7 were selective in vitro for AtHMG1 (green bar) over HsHMGCR (grey bar) at 500 nM. n = 3 independent reactions with the mean ± s.d. Significance from two-tailed paired t test, values: compound 4 t = 5.536 df = 2 P = 0.03 (*) 95% CI [-60.92 to -7.635], compound 7 t = 16.93 df = 2 P = 0.003 (**) 95% CI [-34.37 to -20.44]. c In vitro inhibition of AtHMG1 (green plot) and HsHMGCR (grey plot) by 7 illustrating >20-fold selectivity for AtHMG1. n = 3 independent reactions with the mean ± s.d. except for a single point (AtHMG1 333 µM atorvastatin n = 2). Source data are provided as a Source Data file.

Article

https://doi.org/10.1038/s41467-022-33185-0

Nature Communications | (2022) 13:5563
HsHMGCR in silico simulations (HsHMGCR Glu559 and Asp767) might be hydrogen bond to Asp473 intermediate. Further molecular dynamics studies with AtHMG1, its expected to hydrogen bond and stabilise the mevaldyl-CoA scaffold. Modelling of atorvastatin along with compounds 60, 61, previous modelling studies have suggested the active site of HsHMGCR revealed a single dominant high-affinity binding mode with a large drop in affinity to the next most favourable binding mode. Atorvastatin exhibited the highest affinity followed by compound 7 and 4 (Supplementary Fig. 6) consistent with in vitro results (Fig. 4). Modelling with AtHMG1 revealed more varied poses of the analogues, with similar affinities between the most favourable and the next most favourable binding mode. These binding modes are possibly facilitated by a wider active site and flexibility in the L7 loop region (Supplementary Fig. 6) consistent with in vitro inhibition of WT (black plot) and L558T AtHMG1 (grey plot) by rosvastatin revealed the L558T mutation conferred >20-fold resistance, n = 3 independent reactions with the mean ± s.d. Source data are provided as a Source Data file.

Fig. 5 | A mutation found in a statin biosynthetic cluster confers statin resistance in vitro. a The hydrophobic pocket in AtHMG1 delineated by labelled residues (blue sticks) with pitavastatin (magenta sticks) bound, illustrating L558T mutation (grey bar) in comparison to wild-type (black bar) retained activity in vitro in the presence of statins: rosuvastatin (Ro.), pravastatin (Pr.), simvastatin (Si.), mevastatin (Me.), lovastatin (Lo.), fluvastatin (Fl.), atorvastatin (At.) and pitavastatin (Pi.), at 500 nM. n = 3 independent reactions with the mean ± s.d. **P < 0.001 ****P < 0.0001. b AtHMG1 with the L558T mutation (grey bar) in comparison to wild-type (black bar) retained activity in vitro in the presence of statins: rosuvastatin (Ro.), pravastatin (Pr.), simvastatin (Si.), mevastatin (Me.), lovastatin (Lo.), fluvastatin (Fl.), atorvastatin (At.) and pitavastatin (Pi.), at 500 nM. n = 3 independent reactions with the mean ± s.d. c In vitro inhibition of WT (black plot) and L558T AtHMG1 (grey plot) by rosvastatin revealed the L558T mutation conferred >20-fold resistance, n = 3 independent reactions with the mean ± s.d. Source data are provided as a Source Data file.

Fig. 6 | The L558T mutation gives resistance to rosvastatin in planta. Resistance to hygromycin (a) and rosvastatin (b, c) in 19 transgenic lines of 3S::AtHMG1 (blue) versus 3S::AtHMG1-L558T (red) compared to wild type (WT, black). Green pixels quantified and plotted as a percentage of no-inhibitor control. a, Both transgenic lines exhibited similar resistance to the hygromycin selectable marker, whereas WT was sensitive, mean ± 95% CI. b, 3S::AtHMG1-L558T transgenic lines were sixfold more resistant to rosvastatin than 3S::AtHMG1, mean ± 95% CI, but s.d. for WT. c, Susceptibility of transgens to rosuvastatin illustrated 3S::AtHMG1-L558T (red 80 µM n = 42) was up to 16-fold less susceptible to rosvastatin inhibition than 3S::AtHMG1 (blue 5 µM n = 54, 10 µM n = 54, 80 µM n = 57) when compared to untreated WT (black n = 12). Significance from one-way ANOVA with Dunnett’s multiple comparison correction against a common control performed, bars represent mean ± 95% CI. Values: 0 µM vs 80 µM 3S::AtHMG1-L558T q = 1.566 df = 214 P = 0.24 (ns) 95% CI [−27.00 to −5.299], 0 µM vs 80 µM 3S::AtHMG1 q = 2.395 df = 214 P = 0.04 (*) 95% CI [−31.92 to −0.4293], 0 µM vs 10 µM 3S::AtHMG1 q = 3.564 df = 214 P = 0.0013 (***) 95% CI [−39.82 to −8.3241], 0 µM vs 80 µM 3S::AtHMG1 q = 7.829 df = 214 P = <0.0001 (****) 95% CI [−68.29 to −36.95]. d, Representative image of resistance to hygromycin and rosvastatin from a single line of 3S::AtHMG1 and 3S::AtHMG1-L558T versus WT. Source data are provided as a Source Data file.

substrate thioester oxygen or the adjacent Asp773, which based on HsHMGCR in silico simulations (HsHMGCR Glu356 and Asp354) might be expected to hydrogen bond and stabilise the mevaldyl-CoA intermediate. Further molecular dynamics studies with AtHMG1, its substrate and cofactors might determine if the role of Glu356 is to hydrogen bond to Asp773, or to directly protonate the substrate as flucastatin (Supplementary Fig. 6) consistent with in vitro results (Fig. 4). Modelling with AtHMG1 revealed more varied poses of the analogues, with similar affinities between the most favourable binding modes. These binding modes are possibly facilitated by a wider active site and flexibility in the L7 loop region (Supplementary Fig. 3c) and might account for the difficulty we had in obtaining co-crystal structures for AtHMG1. The lower affinity for AtHMG1 than HsHMGCR for compounds 4 and 7 suggests further molecular dynamics simulations and crystallographic studies may be
necessary to help reveal the molecular basis of in vitro specificity (Fig. 4). Notably, selectivity over HsHMGCR was also obtained by targeting the same Lβ2-Lo1 loop region in *Manduca sexta* using gem-difluoromethylated HMGR inhibitors. Similar derivatives may also prove to be selective for plant HMGRs. Overall, the developed compounds provide a framework for further structure-based rational herbicide design targeting the Lβ2-Lo1 loop region of AtHMG1, which could be validated for selectivity in mammalian in vivo studies. Greater species selectivity might be obtainable by targeting the N-terminal domain of HMGR, which is highly divergent between humans and plants and is absent from class II HMGRs. A recent crystal structure of the regulatory elements that interact with the N-terminal domain in HsHMGCR and studies of compounds that increase HMGR degradation suggest that this could be an alternative mechanism to lower cholesterol levels. Future studies of the regulatory elements interacting with plant HMGR N-terminal domain and complexed crystal structures might in the same way also provide an avenue to develop more species-specific inhibitors of HMGR. The regulatory elements that control plant lipid metabolism might also provide new herbicial targets, just as the proprotein convertase subtilisin/kexin type 9 and angiopoietin-like 3 are providing new avenues for the treatment of hypercholesterolaemia.

By analysing the AtHMG1 crystal structure and sequences in fungal biosynthetic gene clusters, we identified a mutation conferring statin resistance without adversely affecting catalytic activity. Overexpressing this mutant protein in *A. thaliana* demonstrated its potential as a tolerance trait, but further investigations are needed. These could include (i) the efficacy of this protein mutant in different species; (ii) optimisation of expression and regulation, by modifying the N-terminal domain; (iii) its effects on sterol levels and seed set; and (iv) determining what HMGR inhibitor residues remain in the treated crop or soil. Future studies might also focus on other residues that potentially impart resistance, such as the end region of the SPF strand (residues 387–390), that show conservation in putative resistance genes from *Penicillium citrinum* and *Xylaria grammica* and could affect binding of the butyryl group of natural statins. We envisage that the development and discovery of new, natural product herbicides might also benefit from a similar approach to engineering resistance alleles from biosynthetic gene clusters containing compounds or targets of interest.

**Methods**

**Herbical activity assay**

Approximately 30 seeds of *A. thaliana* (accession Col-0) or *E. tef* were sown in 63 × 63 × 59 mm pots of Irish peat (Bord na Móna Horticulture Ltd, Newbridge, Ireland). Seeds were incubated in the dark for 3 days at 4 °C to synchronise germination. A single pre-emergence treatment (day 0) was performed when these seeds were transferred to a growth room at 22 °C with a 16:8 h light:dark photoperiod and 60% relative humidity. Two post-emergence treatments were performed following emergence of the seedlings (day 1) at days 4 and 7. Plants were watered daily and photographed on day 16. Treatments were conducted with *E. tef* (WT), and normalised response (L558T) non-linear regression model was determined by spectrophotometry. Data were normalised to a negative control to provide percentage inhibition.

**HMG-CoA reductase expression and purification**

An *E. coli* codon-optimised DNA sequence encoding the conserved extracellular region of AtHMG1 (Uniprot P14959, At5g6490, residues 121–392) was cloned into pQE30 (Qiagen) following an N-terminal His6-tag and tobacco etch virus cleavage site. The protein was expressed in the T7 SHuffle Express strain of *E. coli* (New England Biolabs) transformed with pREP4 (Qiagen) with the proteins expressed and purified as previously described. Briefly, cultures were grown in lysogenic broth containing 100 µg/mL ampicillin and 35 µg/mL kanamycin at 30 °C to an OD600 of 0.8–1.0. Cells were cooled to 16 °C before the expression was induced with 0.1 mM isopropyl β-D-thiogalactopyranoside. Following overnight culture, cells were harvested by centrifugation and lysed by ultrasonication in 100 mM HEPES (pH 7.5), 150 mM sodium chloride, 5 mM dithiothreitol, 0.1% Triton X-100. Lysed cells were then centrifuged (15,000×g) and the supernatant was incubated in 30 mL batches with Ni-NTA resin over-night at 4 °C. The resin was then washed with 50 mL of 100 mM HEPES (pH 7.5), 150 mM sodium chloride, 5 mM dithiothreitol followed by 50 mL of 100 mM HEPES (pH 7.5), 150 mM sodium chloride, 5 mM dithiothreitol, 20 mM imidazole. The protein was then eluted with 50 mL of 100 mM HEPES (pH 7.5), 150 mM sodium chloride, 5 mM dithiothreitol, 300 mM imidazole. Eluted protein was concentrated with a 30 kDa centrifugal filter unit (Millipore) and purified by size-exclusion chromatography (HiLoad 16/600 Superdex 200) in 100 mM HEPES (pH 7.5), 150 mM sodium chloride, 5 mM dithiothreitol. The protein was assessed for purity by SDS-PAGE, and protein concentration was determined by spectrophotometry.

**In vitro HMGR assay**

AtHMG1 described above and human HMGR (HsHMGCR, Uniprot P04035, residues 441–888, cloned as above) were purified as above, and activity was determined by spectrophotometric measurement of the decrease in absorbance at 340 nm that occurs with NADPH oxidation in the presence of substrate HMG-CoA (Sigma-Aldrich). Reactions were performed with an assay buffer consisting of 150 mM sodium chloride, 5 mM dithiothreitol, 50 mM HEPES pH 7.4 and 2% DMSO. For kinetics determinations, a final concentration of 150 nM enzyme was incubated at 37 °C in 300 µM NADPH and different concentrations of HMG-CoA. Non-linear regression analysis was performed with GraphPad Prism 9 by plotting the initial reaction rates, ict, interpolated from a standard curve against the substrate concentration. The Michaelis–Menten constant, Km, was determined by fitting the data with a Michaelis–Menten equation and values for Kcat were calculated by dividing Vmax by the molar enzyme concentration. To calculate relative specific activity, 500 nM of test compounds were pre-incubated at 37 °C with enzyme and 300 µM NADPH for 15 min before adding HMG-CoA to 200 µM. Resultant values were background subtracted and normalised to the average of the no-inhibitor control. For IC50 determinations, the same protocol was used, but varying inhibitor concentrations. Rosuvastatin data were plotted with a four-parameter (WT), and normalised response (LSSST) non-linear regression model and atorvastatin and analogues were plotted with a normalised response with variable slope non-linear regression model.

**Crystallisation and data collection**

The C-terminal core residues of AtHMG1 (residues 121–576) were cloned and purified as above. The core domain was concentrated to 10–15 mg/mL and used immediately for crystallisation. Crystal screening was performed with 96-well Intelli-Plates (Hampton Research) with 80 µL of reservoir solution using the sitting-drop vapour diffusion method at 16 °C. Crystals were obtained with a

---

https://doi.org/10.1038/s41467-022-33185-0
Crystallography data collection and refinement statistics

| Data collection | apo HMG1 | HMG1-pitavastatin |
|-----------------|----------|-------------------|
| **Space group** | 1 4 2 2  | 1 4 2 2           |
| **Unit cell dimensions** |          |                   |
| a, b, c (Å)     | 85.58, 85.58, 266.65 | 85.55, 85.55, 265.15 |
| α, β, γ (°)     | 90.00, 90.00, 90.00 | 90.00, 90.00, 90.00 |
| **Wavelength**  | 0.9537 | 0.9537            |
| **Resolution (Å)** | 1.7 | 2.1              |
| Rmerge (%)      | 11 (434) | 25 (267)         |
| Completeness (%)| 100 (99.9) | 60.9 (11.6)     |
| Redundancy (%)  | 13.3 (11.6) | 91.6 (60.7)     |
| CC1/2           | 1.00 (0.549) | 0.996 (0.341)  |

Reinforcement

| Resolution (Å) | 45.30-1.90 | 45.07-2.13 |
|----------------|------------|------------|
| No. of reflections | 39555 | 17133 |
| Rwork/Rfree | 20.7/24.3 | 22.0/26.1 |
| No. of atoms | 5398 | 5045 |
| Protein | 5307 | 4971 |
| Water | 91 | 19 |
| Ligand | 64 | 9 |
| Wilson B (Å²) | 51.0 | 12.8 |
| Average refined B-factor (Å²) | 51.0 | 9.4 |
| Protein only (Å²) | 51.0 | 9.4 |
| Water (Å²) | 53.5 | 12.8 |
| Ligand (Å²) | 45.0 | 9.4 |
| Bond lengths (Å) | 0.01 | 0.01 |
| Bond angles (°) | 1.36 | 1.55 |

Ramachandran analysis

| Favoured (%) | 97 | 94 |
| Allowed (%) | 3 | 6 |
| Outliers (%) | 0 | 0 |
| PDB accession | 7ULI | 8ECG |

*Numbers in parentheses refer to the highest resolution bin.

In planta statin resistance assay

DNA encoding the full-length AtHMG1 protein (UniProt P14891, residues 1–592) and the corresponding L5SST mutant were cloned into a derivative of the pMDC43 binary vector55 to yield 35S::AtHMG1 and 35S::AtHMG1-L5SST transgenes, respectively. These constructs were then introduced into Agrobacterium tumefaciens strain LBA4404 and separately used to transform A. thaliana by the floral dip method56,57. Seeds (T0) of transformed plants were collected and surface sterilised using 600 μL 70% ethanol, 750 μL 100% ethanol and soaked in 800 μL 50% bleach for 8 min, before washing with 500 μL sterile water and resuspension with 0.1% agar. Selection was performed on 30 μg/mL hygromycin growth medium (1% agar, 1% glucose, 0.45% Murashige & Skoog salts with vitamins, 0.3% 2-(morpholino)-ethanesulfonic acid (MES) (v/v), pH 5.7) in a growth room at 22 °C with 16:8 h light:dark photoperiod and 60% relative humidity. Surviving plants were transferred to 63 × 63 × 59 mm pots of Irish peat and grown to maturity in the same growth conditions. Seeds from plants with an adequate seed yield were then sterilised and selected again as described above with 30 μg/mL hygromycin growth medium. Seeds (T2) from 22 lines of 35S::AtHMG1 and 15 lines of 35S::AtHMG1-L5SST plants that exhibited approximately 3:1 segregation ratio of hygromycin resistant:sensitive were then sown (~15 seeds/well, n = 3 replicates), along with wild-type (WT) A. thaliana, on sterile 96-well microplates with 0.25 mL/well growth medium containing a low dose range serial dilution of 8–2000 μg/mL hygromycin and of 0.16–40 μM rosuvastatin (final concentration 2% DMSO) with respective media only controls, and then again on a second higher dose range of 195–50,000 μg/mL hygromycin and of 20–5120 μM rosuvastatin (final concentration 2% DMSO). Plates were sealed with porous tape and grown for a minimum of 10 days with the growth conditions described above. Plates were then imaged, and growth quantified using ImageJ as described above. Total green pixels were normalised against negative controls for the respective lines (2% DMSO and water) to provide percentage inhibition. Three of 22 lines of 35S::AtHMG1 and 1 of 15 lines of 35S::AtHMG1-L5SST plants were excluded from further analysis based on poor growth of the negative control or for displaying low hygromycin resistance. One of 19 35S::AtHMG1 lines and 2 of the 14 35S::AtHMG1-L5SST lines had data only for the higher dose range of hygromycin and rosuvastatin. For IC50 determinations, all data were respectively combined from 19 lines of 35S::AtHMG1, 14 lines of 35S::AtHMG1-L5SST and WT A. thaliana. Growth inhibition at varying concentrations of hygromycin and rosuvastatin were plotted with a four-parameter non-linear regression model using GraphPad Prism 9.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The refined structural protein models of apo HMG1 and HMG1-pitavastatin are available at PDB under accession codes 7ULI and 8ECG, respectively. Source data are provided with this paper.
References

1. Heap, I. The international survey of herbicide resistant weeds. Accessed 1 May 2022. https://www.weedscience.org (2022).
2. Shino, M., Hamada, T., Shigematsu, Y., Hirase, K. & Banba, S. Action mechanism of bleaching herbicide cyclopyrimate, a novel homogentisate solanesyltransferase inhibitor. J. Pestic. Sci. 43, 233–239 (2019).
3. Ferhatoglu, Y. & Barrett, M. Studies of clomazone mode of action. Pestic. Biochem. Physiol. 85, 7–14 (2006).
4. Lange, B. M., Rujan, T., Martin, W. & Croteau, R. Isoprenoid biosynthesis: the evolution of two ancient and distinct pathways across genomes. Proc. Natl Acad. Sci. USA 97, 13172–13177 (2000).
5. Summons, R. E., Jahnke, L. L., Hope, J. M. & Logan, G. A. 2-Methylbicyclics as biomarkers for cyanobacterial oxygenic photosynthesis. Nature 400, 554–557 (1999).
6. Brocks, J. J., Logan, G. A., Buick, R. & Summers, R. E. Archean molecular fossils and the early rise of eukaryotes. Science 285, 1033–1036 (1999).
7. Vranová, E., Coman, D. & Gruissem, W. Network analysis of the MVA and MEP pathways for isoprenoid synthesis. Annu. Rev. Plant Biol. 64, 665–700 (2013).
8. Rodríguez-Concepción, M. et al. Distinct light-mediated pathways regulate the biosynthesis and exchange of isoprenoid precursors during Arabidopsis seedling development. Plant Cell 16, 144–156 (2004).
9. Hemmerlin, A. et al. Cross-talk between the cytosolic mevalonate and the plastidial methylerthritol phosphate pathways in tobacco bright yellow-2 cells. J. Biol. Chem. 278, 26666–26676 (2003).
10. Lauter, O. et al. Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in Arabidopsis thaliana. Proc. Natl Acad. Sci. USA 100, 6866–6871 (2003).
11. Nagata, N., Suzuki, M., Yoshida, S. & Muraoka, T. Mevalonic acid partially restores chloroplast and etioplast development in Arabidopsis lacking the non-mevalonate pathway. Planta 216, 345–350 (2002).
12. Hoshino, Y. & Gaucher, E. A. On the origin of isoprenoid biosynthesis. Mol. Biol. Evolution 35, 2185–2197 (2018).
13. Zeng, L. & Dehesh, K. The eukaryotic MEP-pathway genes are evolutionarily conserved and originated from Chlamydia and cyanobacteria. BMC Genomics 22, 1–12 (2021).
14. Lichtenthaler, H. K., Schwender, J., Disch, A. & Rohmer, M. Biosynthesis of isoprenoids in higher plant chloroplasts proceeds via a mevalonate-independent pathway. FEBS Lett. 400, 271–274 (1997).
15. Goldstein, J. L. & Brown, M. S. Regulation of the mevalonate pathway. Nature 343, 425–430 (1990).
16. Jiang, S.-Y. et al. Discovery of a potent HMG-CoA reductase degrader that eliminates statin-induced reductase accumulation and lowers cholesterol. Nat. Commun. 9, 1–13 (2018).
17. Burg, J. S. & Espenshade, P. J. Regulation of HMG-CoA reductase in mammals and yeast. Prog. Lipid Res. 50, 403–410 (2011).
18. Yan, R. et al. A structure of human Scap bound to Insig-2 suggests how their interaction is regulated by sterols. Science 371, eabb2224 (2021).
19. Seydel, P. & Dörnburg, H. Establishment of in vitro plants, cell and tissue cultures from Oldenlandia affinis for the production of cyclic peptides. Plant Cell, Tissue Organ Cult. 85, 247–255 (2006).
20. Friesen, J. A. & Rodwell, V. W. The 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductases. Genome Biol. 5, 248 (2004).
21. Grundy, S. M. HMG-CoA reductase inhibitors for treatment of hypercholesterolemia. N. Engl. J. Med. 319, 24–33 (1988).
22. Bochar, D. A., Staffaflcher, C. V. & Rodwell, V. W. Sequence comparisons reveal two classes of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Mol. Genet. Metab. 66, 122–127 (1999).
23. Lawrence, C. M., Rodwell, V. W. & Staffaflcher, C. V. Crystal structure of Pseudomonas mevalonii HMG-CoA reductase at 3.0 angstrom resolution. Science 268, 1758–1762 (1995).
24. Istvan, E. S. & Deisenhofer, J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science 292, 1160–1164 (2001).
25. Learned, R. M. & Fink, G. R. 3-Hydroxy-3-methylglutaryl coenzyme A reductase from Arabidopsis thaliana is structurally distinct from the yeast and animal enzymes. Proc. Natl Acad. Sci. USA 86, 2779–2783 (1989).
26. Ragwan, E. R., Arai, E. & Kung, Y. New crystallographic snapshots of large domain movements in bacterial 3-hydroxy-3-methylglutaryl coenzyme A reductase. Biochemistry 47, 5715–5725 (2018).
27. Rodríguez-Concepción, M. & Boronat, A. Breaking new ground in the regulation of the early steps of plant isoprenoid biosynthesis. Curr. Opin. Plant Biol. 25, 17–22 (2015).
28. Endo, A., Kuroda, M. & Tsujita, Y. ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterogenesis produced by Penicillium citrinum. J. Antibiotics 29, 1346–1348 (1976).
29. Tobert, J. A. Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. Nat. Rev. Drug Discov. 2, 517–526 (2003).
30. Tabernero, L., Rodwell, V. W. & Staffaflcher, C. V. Crystal structure of a statin bound to a class I 3-hydroxy-3-methylglutaryl-CoA reductase. J. Biol. Chem. 278, 19833–19839 (2003).
31. Bach, T. J. & Lichtenthaler, H. K. Inhibition by mevinolin of plant growth, sterol formation and pigment accumulation. Physiologia Plant. 59, 50–60 (1983).
32. Kasahara, H. et al. Contribution of the mevalonate and meheterythritol phosphate pathways to the biosynthesis of gibberellins in arabidopsis. J. Biol. Chem. 275, 45188–45194 (2002).
33. Nkembo, M. K., Lee, J.-B., Nakagiri, T. & Hayashi, T. Involvement of 2-C-methyl-D-erythritol-4-phosphate pathway in biosynthesis of aphidicolin-like tetra cyclic diterpene of Scoparia dulcis. Chem. Pharm. Bull. 54, 758–760 (2006).
34. Jerwood, S. & Cohen, J. Unexpected antimicrobial effect of statins. J. Antimicrobial Chemother. 61, 362–364 (2008).
35. Zhang, Y. Y., Li, Y. M., Yin, Y., Chen, S. S. & Kai, Z. P. Discovery and quantitative structure-activity relationship study of lepidopteran HMG-CoA reductase inhibitors as selective insecticides. Pest Manag. Sci. 73, 1944–1952 (2017).
36. Brain, R. A. et al. Herbicidal effects of statin pharmaceuticals in Lemna gibba. Environ. Sci. Technol. 40, 5116–5123 (2006).
37. Shimada, T. L. et al. HIGH STEROL ESTER 1 is a key factor in plant sterol homeostasis. Nat. Plants 5, 1154–1166 (2019).
38. Enjuto, M. et al. Arabidopsis thaliana contains two differentially expressed 3-hydroxy-3-methylglutaryl-CoA reductase genes, which encode microsomal forms of the enzyme. Proc. Natl Acad. Sci. USA 91, 927–931 (1994).
39. Li, W. et al. Species-specific expansion and molecular evolution of the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) gene family in plants. PLOS ONE 9, e94172 (2014).
40. Vogeli, B., Shima, S., Erb, T. J. & Wagner, T. Crystal structure of archael HMG-CoA reductase: insights into structural changes of the C-terminal helix of the class-I enzyme. FEBS Lett. 593, 543–553 (2019).
41. Peacock, R. B. et al. Structural and functional characterization of dynamic oligomerization in Burkholderia cenocepacia HMG-CoA reductase. Biochemistry 58, 3960–3970 (2019).
42. Miller, B. R. & Kung, Y. Structural features and domain movements controlling substrate binding and cofactor specificity in class II HMG-CoA reductase. Biochemistry 57, 654–662 (2018).
43. Steussy, C. N. et al. A novel role for coenzyme A during hydride transfer in 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Biochemistry 52, 5195–5205 (2013).
Sarver, R. W. et al. Thermodynamic and structure guided design of statin based inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Medicinal Chem. 51, 3804–3813 (2008).

Park, W. K. et al. Hepatoselectivity of statins: design and synthesis of 4-sulfamoyl pyroles as HMG-CoA reductase inhibitors. Bioorg. Medicinal Chem. Lett. 18, 1151–1156 (2008).

Pfefferkorn, J. A. et al. Substituted pyroles as hepatoselective HMG-CoA reductase inhibitors: discovery of (3R,5R)-7-(2-(4-fluorophenyl)-4-isopropyl-5-(4-methylbenzylcarbamoyl)-2H-pyrazol-3-yl)-3,5-dihydroxyheptanoic acid (PF-3052334) as a candidate for the treatment of hypercholesterolemia. J. Medicinal Chem. 51, 31–45 (2008).

Pfefferkorn, J. A. et al. Design and synthesis of novel, conformationally restricted HMG-CoA reductase inhibitors. Bioorg. Medicinal Chem. Lett. 17, 4531–4537 (2007).

Pfefferkorn, J. A. et al. Design and synthesis of hepatoselective, pyrrole-based HMG-CoA reductase inhibitors. Bioorg. Medicinal Chem. Lett. 17, 4538–4544 (2007).

Istvan, E. S., Palntikar, M., Buchanan, S. K. & Deisenhofer, J. Crystal structure of the catalytic portion of human HMG-CoA reductase: insights into regulation of activity and catalysis. EMBO J. 19, 8135–8140 (2000).

Tabenero, L., Bochar, D. A., Rodwell, V. W. & Stauffacher, C. V. Substrate-induced closure of the flap domain in the ternary complex structures provides insights into the mechanism of catalysis by 3-hydroxy-3-methylglutaryl-CoA reductase. Proc. Natl Acad. Sci. USA 96, 7167–7171 (1999).

Oliveira, E. F., Cerequeira, N. M., Ramos, M. J. & Fernandes, P. A. QM/MM study of the mechanism of reduction of 3-hydroxy-3-methylglutaryl coenzyme A catalyzed by human HMG-CoA reductase. Catal. Sci. Technol. 6, 7172–7185 (2016).

Tian, W., Chen, C., Lei, X., Zhao, J. & Liang, J. CASTp 3.0: computed atlas of surface topography of proteins. Nucleic Acids Res. 46, W363–W367 (2018).

Roth, B. D. et al. Inhibitors of cholesterol biosynthesis. 1. trans-6-(2-51. Oliveira, E. F., Cerequeira, N. M., Ramos, M. J. & Fernandes, P. A. QM/MM study of the mechanism of reduction of 3-hydroxy-3-methylglutaryl coenzyme A catalyzed by human HMG-CoA reductase. Catal. Sci. Technol. 6, 7172–7185 (2016).

Tian, W., Chen, C., Lei, X., Zhao, J. & Liang, J. CASTp 3.0: computed atlas of surface topography of proteins. Nucleic Acids Res. 46, W363–W367 (2018).

Roth, B. D. et al. Inhibitors of cholesterol biosynthesis. 1. trans-6-(2-pyrrol-1-ylthio)-4-hydroxypropan-2-ones, a novel series of HMG-CoA reductase inhibitors. 1. Effects of structural modifications at the 2-and 5-positions of the pyrrole nucleus. J. Medicinal Chem. 33, 21–31 (1990).

Kennedy, J. J. et al. Modulation of polyketide synthase activity by accessory proteins during lovastatin biosynthesis. Science 284, 1368–1372 (1999).

Hutchinson, C. R. et al. Aspects of the biosynthesis of non-aromatic fungal polyketides by iterative polyketide synthases. Antonie Van Leeuwenhoek 78, 287–295 (2000).

Martin, J.-F., Garcia-Estrada, C. & Zeilinger, S. Biosynthesis and Molecular Genetics of Fungal Secondary Metabolites (Springer, 2014).

Theivagt, A. E., Amanti, E. N., Beresford, N. J., Tabenero, L. & Friese, J. A. Characterization of an HMG-CoA reductase from Listeria monocytogenes that exhibits dual coenzyme specificity. Biochemistry 45, 14397–14406 (2006).

Re, E. B., Jones, D. & Learned, R. M. Co-expression of native and introduced genes reveals cryptic regulation of HMG CoA reductase expression in Arabidopsis. Plant J. 7, 771–784 (1995).

Marrone, P. G. Pesticidal natural products—status and future potential. Pest Manag. Sci. 75, 2325–2340 (2019).

Haines, B. E., Wiest, O. & Stauffacher, C. V. The increasingly complex mechanism of HMG-CoA reductase. Acc. Chem. Res. 46, 2416–2426 (2013).

Haines, B. E., Steussy, C. N., Stauffacher, C. V. & Wiest, O. Molecular modeling of the reaction pathway and hydride transfer reactions of HMG-CoA reductase. Biochemistry 51, 7983–7995 (2012).

Abifadel, M. et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat. Genet. 34, 154–156 (2003).

Gautret, D. et al. ANGPTL3 inhibition in homozygous familial hypercholesterolemia. N. Engl. J. Med. 377, 296–297 (2017).

Hey, S, J. et al. Enhanced seed phytoester accumulation through expression of a modified HMG-CoA reductase. Plant Biotechnol. J. 4, 219–229 (2006).

Yan, Y. et al. Resistance-gene-directed discovery of a natural-product herbicide with a new mode of action. Nature 559, 415–418 (2018).

Xie, L. et al. Harzanic acid from Trichoderma afroharzianum is a natural product inhibitor of acetohydroxyacid synthase. J. Am. Chem. Soc. 143, 9575–9584 (2021).

Corral, M. G., Leroux, J., Stubbs, K. A. & Mylne, J. S. Herbicidal properties of antimalarial drugs. Sci. Rep. 7, 1–9 (2017).

Haywood, J. et al. Structural basis of ribosomal peptide macrocyclization in plants. elife 7, e32955 (2018).

Aragao, D. et al. MX2: a high-flux undulator microfocus beamline serving both the chemical and macromolecular crystallography communities at the Australian Synchrotron. J. Synchrotron Radiat. 25, 885–891 (2018).

Kabsch, W. XD. Acta Crystallogr. Sect. D. Biol. Crystallogr. 66, 125–132 (2010).

Winn, M. D. et al. Overview of the CCP4 suite and current developments. Acta Crystallogr. Sect. D. Biol. Crystallogr. 67, 235–242 (2011).

Mc Coy, A. J. et al. Phaser crystallographic software. J. Appl. Cry stallogr. 40, 658–674 (2007).

Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. Acta Crystallogr. Sect. D. Biol. Crystallogr. 66, 486–501 (2010).

Chen, V. B. et al. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr. Sect. D. Biol. Crystallogr. 66, 12–21 (2010).

Curtis, M. D. & Grossniklaus, U. A gateway cloning vector set for high-throughput functional analysis of genes in planta. Plant Physiol. 133, 462–469 (2003).

Clough, S. J. & Bent, A. F. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 16, 736–743 (1998).

Bechtold, N., Ellis, J. & Pelletier, G. In planta Agrobacterium-mediated gene transfer by infiltration of adult Arabidopsis thaliana plants. Comptes rendus de l’Academie des. Sci. Serie III, Sci. de la vie 316, 1194–1199 (1993).

Acknowledgements

The authors thank Grishma Vadlamani and Yit-Heng Chooi for their helpful comments. This research was undertaken in part using the MX2 beamline at the Australian Synchrotron, part of The Australian Nuclear Science and Technology Organisation, and made use of the Australian Cancer Research Foundation detector. J.H. was supported by an Australian Research Council Discovery Early Career Researcher Award (DE180101445) and funded in part by Ngenx Plants. This work was supported by an Australian Research Council Discovery Project DP190101048 to J.S.M., K.A.S. and J.H. and an ARC Linkage Infrastructure Equipment and Facilities Grant (LE190100123) to K.A.S.

Author contributions

J.H. and J.S.M. designed and coordinated the research. J.H., J.Z. and K.J.B. performed plant assays. K.J.B. and K.A.S. designed and synthesized atorvastatin analogues. M.T.W. made binary constructs used by J.H. and J.S.M. for plant transgenesis. M.T.W. made binary constructs used by J.H. and J.S.M. for plant transgenesis. J.H. analysed transgenic lines, made recombinant proteins, performed assays, and acquired crystals. J.H. and C.S.B. solved crystal structures. J.H. and J.S.M. wrote the manuscript with input from all authors.
