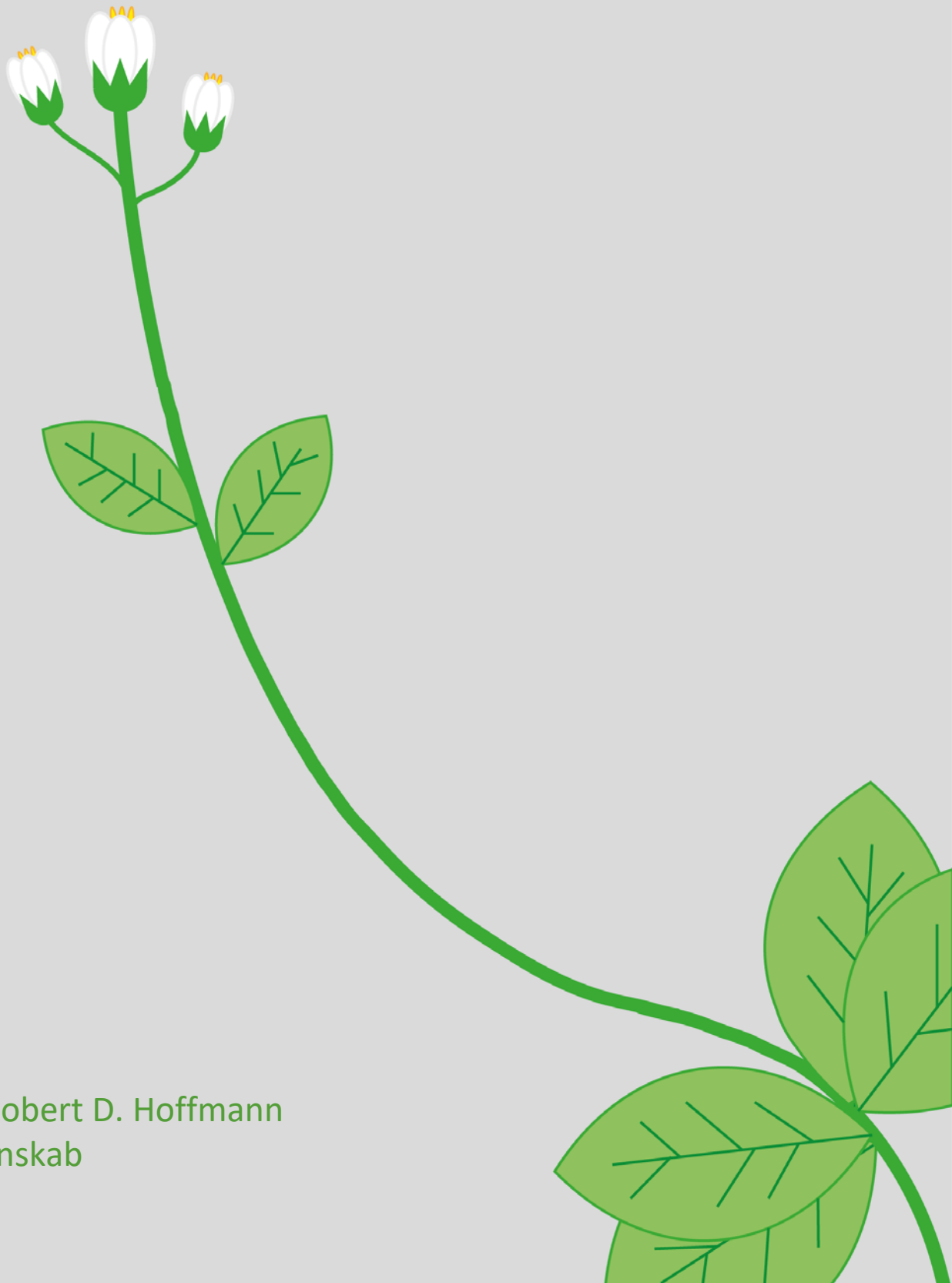
VITAMIN D3 IN ARABIDOPSIS THALIANA

By Claudia C. Lassen – Sukkertoppen Gymnasium

Projekt Forskerspirer 2017



Contact: Robert D. Hoffmann
Naturvidenskab

Claudia C. Lassen
Sukkertoppen Gymnasium

Contents

Claudia C. Lassen
Sukkertoppen Gymnasium

1 Introduction

NASA has plans for inhabiting Mars. With the prospect of NASA sending humans to Mars in the 2030's, this future moves closer and closer. An important part of this project is supplying the astronauts with a nutritious diet and this includes vitamins. NASA will supply their astronauts through dietary supplements, which require energy to produce compared with a product that could grow on Mars [5]. Therefore, a genetically modified plant that produced the sufficient amount of vitamin D_3 without using up scarce resources would be beneficial. The plant *Arabidopsis thaliana* is an excellent choice because of its well-known genome, its ability to self-pollinate, and its relatively short growth cycle at five to eight weeks [18].

When in space, astronauts will not get enough sunlight to produce the necessary amount of vitamin D_3. The major role of vitamin D_3 in humans is maintaining calcium homeostasis. It is therefore very important for the development of a healthy skeleton [12]. A lack of vitamin D has been shown to lead to increased risk of hypertension, autoimmune diseases, diabetes and cancer [9]. Dietary supplements are therefore needed to ensure a healthy skeleton.

Vitamin D_3 is synthesised in our skin from the precursor 7-dehydrocholesterol, also known as provitamin D_3. The photosynthesis of previtamin D_3 from provitamin D_3 takes place in the dermis with the use of solar ultraviolet light at wavelengths between 295-300 nm [11][16]. This undergoes thermal isomerization to become vitamin D_3 and is then - by a vitamin D_3 binding protein - transported to the circulatory system [10].

1.1 Aims

This project could eventually supply permanent inhabitants on Mars with an easy supply of vitamin D_3. My goal is to utilize plants' ability to build complex organic molecules from water and carbon dioxide, and to gain information on how this could be used in the space industry.

2 Background

2.1 Synthesis in humans to insert into *A. thaliana*

The precursor for most human sterols is lanosterol and the pathway from lanosterol to vitamin D_3 has seven steps and is done by nine different enzymes. The synthesis from lanosterol to

provitamin D_3 involves the removal of three methyl groups, the migration of a double bond and the reduction of two double bonds [20]. Afterwards, UVB light and a thermal isomerization changes it to vitamin D_3 [xyxz]. This pathway can be seen in A1. There can be variations in the pathway, as the reduction of carbon-24 can happen between any of the other steps [27]. Vitamin D_3 is biologically inactive and must undergo two hydroxylations before it becomes active. The first is in the liver, where it is hydroxylated into $25OHD_3$, which is the most common form found in the circulatory system in vertebrates. Then it is hydroxylated in the kidneys to $1\alpha,25(OH)_2D_3$ [12].

2.2 Synthesis of 7-dehydrocholesterol in *A. thaliana*

Higher plants do not synthesise 7-dehydrocholesterol. Sterol synthesis in higher plants start with cycloartenol while in animals and fungi, it starts with lanosterol [4]. To make use of the human pathway in *A. thaliana* and produce 7-dehydrocholesterol, we must first mutate it so it can produce lanosterol. A point mutation in the gene oxidosqualene-cycloartenol synthase (*CAS1*) on chromosome 2 changes the production from 100% cycloartenol to 24% lanosterol, 20% parkeol and 56% cycloartenol. The mutation is called I481V and will change the 481th amino acid from valine to isoleucine [28]. The sequence for valine is ATC and the most common for isoleucine in *A. thaliana* is GTT [19]. This is the mutation we will use.

3 Methods

3.1 PCR Mutagenesis to create lanosterol

First DNA must be extracted from the plant. To do this, the cell walls must be broken, then the cell membrane must be disrupted. Afterwards, the DNA must be separated from other tissue and proteins. The procedure from [22] will be followed for this. Then *CAS1* (Arabidopsis gene identifier AT2G07050) will be amplified using PCR. For this, we need nucleotides, DNA polymerase and primers. The primers used will be: 5'atgtggaaac tgaagatcgc'3 and 5'tcattctcct tgttgcaata ata'3. This is based on the gene sequence seen in A2 [25].

When the gene has been amplified it is ligated into the directional pENTR vector and in a second step transferred into the vector pMDC32 by LR clonase (Invitrogen). To introduce the mutation site-directed PCR mutagenesis will be used. The primers 5'ca cggttggccc gtttctgact gcac'3 and

5'gtgc agtcagaac gggccaaccg tg'3 will mutate valine to isoleucine [14]. The vector contains the strong constitutive promoter CaMV35S which works well in eudicots [6], the kanamycin resistance gene for selection in *E. coli* and *A. tumefaciens*, and the hygromycin resistance gene for selection in Arabidopsis.

The vector is then inserted into the *E. coli* TOP 10 strain by heat-shock transformation. If this method does not work as this vector is over 10Kb, the electropolation transformation method will be used [1]. Then transformed bacteria are selected on agar plates containing ampicillin as a selection marker. On the next day, 10 positive colonies will be re-streaked on fresh selective plates and grown over night. The plasmids are then extracted using a mini-prep kit. To control that the vectors are correct, they will be sent to sequencing.

To transform the plants, we will first insert the vectors by electropolation into *Agrobacterium tumefaciens* strain GV3101::pMP90 [13][7]. These are selected with the use of kanamycin. Flowering *A. thaliana* plants are dipped into a solution of *A. tumefaciens* according to the protocol described in [3]. This will make about 1% of the plants transformed [3]. These are selected with hygromycin and the F1 generation can be screened for being homozygous for the T-DNA insert.

This method has been tested before as cited earlier and should therefore work if executed correctly. This method has many steps, and if anything is done wrong at any point in the procedure it will take longer to complete, especially if something goes wrong in *A. thaliana*.

3.2 Pathway insertion

A. thaliana already possesses a C4-demethylation complex [17][21] and can therefore omit to insert the genes for the enzymes involved in this process. The genes will be inserted into a vector as in 3.1, only this time there will need to be inserted around 1200 nucleotides per enzyme. There are six enzymes in total, and these can be viewed in A4.

There are many factors where this project can run into problems. First, the genes might not be transcribed. This is avoided by using a strong constitutive promoter. Second, they might not get translated into a protein. Translation is the subject of much research, but is such a complex system that it is not entirely understood yet. It is thought that micro-RNA's play a big part in the role of translation because they break down a lot of mRNA [26]. Third, the enzymes' place in the cell is often given by the ER and this might lead to the enzymes getting either broken down or not being

in the same area. Lastly, the product vitamin D_3 might get broken down by the cell. This is however not a big problem as CAS1 resides in the vacuole where the point mutation will be [15]. As the vacuole is the storage area in the cell this might mean the cell will not pay attention to unneeded products.

The method described in 3.1 will be used to complete this part of the project as well.

3.3 Analytical methods

Vitamin D_3 is the first product to analyse. This will be done by a mix of high performance liquid chromatography (HPLC) at 265 nm which is one of the more used analytical methods [12][2], mass spectrophotometry (MS), and nuclear magnetic resonance (NMR). NMR lacks the sensitivity of MS and HPLC, but is a valuable tool for structure elucidation [12]. The procedures that will be followed are [24] for HPLC, and [8] for MS.

If vitamin D_3 cannot be found in larger quantities than normal [12] then this analysis will be done on the other products as well. If no products can be found the activity of the inserted genes will be examined with a northern blot made according to this protocol [23].

4 Plan

4.1 Stage one

Month	**Week**	**Day**	**Tasks**
December			Order the needed gene sequences, vectors, and organisms, and start growing a 1.000 A. thaliana.
January	1		Prepare E. coli.
	2	Mon	Extract and purify PCR product.
		Tue	Insert gene into vector, make point mutation and insert into E. coli.
		Wed	Extract vectors and send to sequencing.
		Fri	Insert vector into A. tumefaciens.
	3	Mon	Floral dip
	4		Select transformed A. thaliana (around 10) and pollinate.

February			Plant a 1.000 seeds
March			Grow A. thaliana
April			Select A. thaliana (if selected correctly before all of them)

4.2 Stage two

Month	**Week**	**Day**	**Tasks**
May			Order the needed gene sequences, vectors, and organisms, and start growing a 1.000 A. thaliana.
June	1		Prepare E. coli.
	2	Mon	Extract and purify PCR product.
		Tue	Insert gene into vector and insert into E. coli.
		Wed	Extract vectors and send to sequencing.
		Fri	Insert vector into A. tumefaciens.
	3	Mon	Floral dip
	4		Select transformed A. thaliana (around 10) and pollinate.
July			Plant a 1.000 seeds
August			Grow A. thaliana
September			Select A. thaliana (if selected correctly before all of them)

4.3 Stage three

As it cannot be predicted whether the occurrence of vitamin D_3 will be present, I cannot predict the timeframe for how long this stage will take. Therefore, I have chosen the pessimistic way of doing things and made this schedule presuming there was never any signs of products or expression.

Month	**Week**	**Day**	**Tasks**
September	1		Prepare samples for analysis.
	2		NMR for all products in succession
	3		HPLC for all products in succession

	4		MS for all products in succession
October	1		Northern Blotting to test for gene expression.

4.4 Budget

Materials	**Price**
Primers	200 kr.
Polymerase and dNTPs for PCR	1000 kr.
A. thaliana, E. coli and A. tumefaciens	Free
Growing plants in climate chamber	600 kr.
Sequencing	3500 kr.
Mini prep kit	1500 kr.
Gateway Cloning Kit	4000 kr.
Use of NMR, HPLC, and MS apparatus'	4000 kr.
General chemicals, media, and consumables	5000 kr.
Sum	19.800 kr.

Conclusion

This project's completion could be able to supply astronauts in space with a producing organism that does not require organic molecules to create them. We might be able to use this thought process to make plants synthesise expensive and time-consuming molecules without damaging their ability to grow. If this works it could make way for a new way to synthesise not only dietary supplements, but other molecules as well. Even a step on the way to the utilization of plants in this way would be a success. Maybe it could be synthesised so eating one *A. thaliana* plant would provide you with half your daily vitamin intake. This is of course still future speculation, but bacteria and fungi are being used very competently. I believe we are closer to using plants in this way as well.

Claudia C. Lassen
Sukkertoppen Gymnasium

Acknowledgements

Contact:

Robert D. Hoffmann

Post Doc, Section for Transport Biology, Department of Plant and Environmental Sciences, University of Copenhagen

Jacob Mejlsted

Bachelor Student, Biotechnology, Technical University of Denmark

Rasmus John Normand Frandsen

Associate Professor, Department of Biotechnology and Biomedicine, Technical University of Denmark

Mikako Sasa

Science Manager, Novozymes A/S

This project would not be where it is without help from these busy and generous people. I would like to give my gratitude to Robert D. Hoffmann for helping me direct my work even when my work process was inefficient at times. Second, Jacob Mejlsted helped get this project on its feet in its early days and without him this idea would never have formed. A huge thank you to Rasmus Frandsen who explained to me some procedures in biotechnology so I could work further with the project. Finally, my thoughts go out to Mikako Sasa. Her work and enthusiasm has been a tremendous motivation, and talking with her and her department was a great help.

Reference List

[1] Addgene. n.d. Bacterial Transformation [Internet]. Addgene the nonprofit plasmid repository; [cited 2017 Oct 29]. Available from: https://www.addgene.org/protocols/bacterial-transformation/

[2] Byrdwell WC. 2009. Comparison of Analysis of Vitamin D3 in Foods Using Ultraviolet and Mass Spectrometric Detection. J. Agric. Food Chem. 57, 2135-2146.

[3] Clough SJ, Bent AF. 1998. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. The Plant Journal, 16: 735–743. doi:10.1046/j.1365-313x.1998.00343.x

[4] Darnet S, Rahier A. 2004. Plant sterol biosynthesis: identification of two distinct families of sterol 4α-methyl oxidases. Institut de biologie moléculaire des plantes, Université de Strasbourg, France.

[5] Davis-Street J, Neasbitt L, Smith SM, Zwart SR. 2012. Space Nutrition. NASA.

[6] Fujii Y, Kodama Y. 2015. In planta comparative analysis of improved green fluorescent proteins with reference to fluorescence intensity and bimolecular fluorescence complementation ability. Plant Biotech. 32(1): 81-87. 10.5511/plantbiotechnology.15.0120a

[7] Griffiths AJF, Miller JH, Suzuki DT, et al. 2000. An Introduction to Genetic Analysis [Internet]. 7th edition. New York: W. H. Freeman. Recombinant DNA technology in eukaryotes; [cited 2017 Oct 29]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK22002/

[8] Gundry RL, White MY, Murray CI, et al. 2009. Preparation of Proteins and Peptides for Mass Spectrometry Analysis in a Bottom-Up Proteomics Workflow. Current protocols in molecular biology. doi:10.1002/0471142727.mb1025s88.

[9] Holick MF. 2004. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am. J. Clin. Nutr. 80, 1678S-1688S.

[10] Holick MF, Maclaughlin JA, Clark MB, Holick SA, Potts Jr JT, Anderson RR, Blank IH, Parrish JA, Elias P. 1980. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science Vol. 210, Issue 4466, pp. 203-205.

[11] Hume EM, Lucas NS, Smith HH. 1927. On the absorption of vitamin D from the skin. Department of Experimental Pathology, Lister Institute, London.

[12] Jäpelt RB. 2011. Vitamin D in plants – occurrence, analysis, and biosynthesis. PhD Thesis. National Food Institute, Technical University of Denmark.

[13] Koncz C, and Schell J. 1986. The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of Agrobacterium binary vector. Mol. Gen. Genet. 204:383- 396.

[14] Laursen K. 2016. Site Directed Mutagenesis by PCR [Internet]. Addgene, Cornell University; [cited 2017 Oct 29]. Available from: http://blog.addgene.org/site-directed-mutagenesis-by-pcr

[15] Lin X, Kaul S, Rounsley S, et al. 1999. Sequence and analysis of chromosome 2 of the plant Arabidopsis thaliana [Internet]. NCBI Genome Project; [cited 2017 Oct 29]. Available from: https://www.ncbi.nlm.nih.gov/nuccore/NC_003071.7?report=genbank&from=2924360&to=2930751

[16] MacLaughlin JA, Anderson RR, Holick MF. 1982. Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin. Science 216, 1001-1003.

[17] Mialoundama AS, Jadid N, Brunel J, et al. 2013. Arabidopsis ERG28 Tethers the Sterol C4-Demethylation Complex to Prevent Accumulation of a Biosynthetic Intermediate That Interferes with Polar Auxin Transport. The Plant Cell. 25(12):4879-4893. doi:10.1105/tpc.113.115576.

[18] NASC. n.d. Growing Arabidopsis [Internet]. The European Arabidopsis Stock Centre; [cited 2017 Oct 29]. Available from: http://arabidopsis.info/InfoPages?template=newgrow;web_section=arabidopsis

[19] Nakamura Y. 2007. Codon Usage Database; Arabidopsis thaliana [Internet]. Department of Plant Gene Research, Kazusa. NCBI; [cited 2017 Oct 29]. Available from: http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=3702

[20] Porter FD, Herman GE. 2011. Malformation syndromes caused by disorders of cholesterol synthesis. J. Lipid Res. 52, 6-34.

[21] Rahier A, Darnet S, Bouvier F, Camara B. 2012. The Sterol C4-Demethylation in Higher Plants. Institut de Biologie Moléculaire des Plantes, CNRS, UPR2357, Strasbourg, cedex, France

[22] Rogers SO, Bendich AJ. 1988. Extraction of DNA from plant tissues. Departments of Botany and Genetics, University of Washington, Seattle, WA 98195, USA.

[23] Ross A. n.d. Northern Blotting Protocol. Beggs' Lab.

[24] Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. 2011. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. African Journal of Traditional, Complementary, and Alternative Medicines. 8(1):1-10.

[25] Shinn P, Chen H, et al. 2002. Arabidopsis thaliana CAS1 mRNA [Internet]. [cited 2017 Oct 30] Available from:
https://www.ebi.ac.uk/Tools/dbfetch/emblfetch?style=html&id=BT001118&Submit=Go

[26] Vasudevan S, Tong Y, Steitz JA. 2007. Switching from Repression to Activation: MicroRNAs Can Up-Regulate Translation. Science Vol. 318, Issue 5858, pp. 1931-1934.

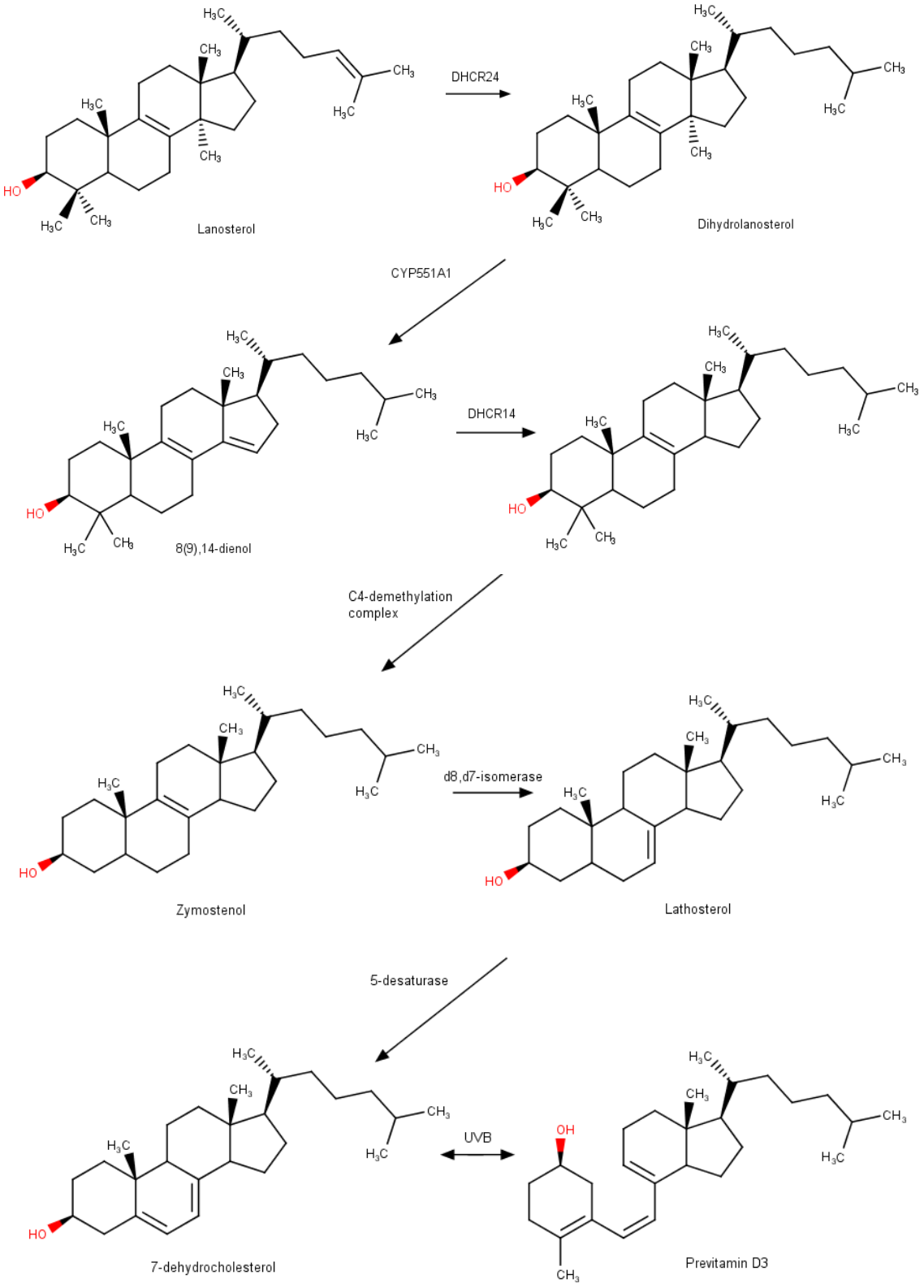
[27] Waterham HR, Koster J, Romeijn GJ, Hennekam R, Vreken P, Andersson HC, FitzPatrick DR, Kelley R. 2001. Mutations in the 3β-hydroxysterol Δ 24-reductase gene cause desmosterolosis, an autosomal recessive disorder of cholesterol biosynthesis. Am. J. Hum. Genet. 69, 685-694.

[28] Wu T, Griffin JH. 2002. Conversion of a Plant Oxidosqualene-Cycloartenol Synthase to an Oxidosqualene-Lanosterol Cyclase by Random Mutagenesis. Department of Biological Science and Technology, National Chiao Tung University, Taiwan, and Department of Chemistry, Stanford University, California.

Claudia C. Lassen
Sukkertoppen Gymnasium

A Attachments

A1 Vitamin D_3 pathway



A1 shows the Kandutsch-Russell pathway. [20] This is the pathway inserted into *A. thaliana* to convert lanosterol to provitamin D_3.

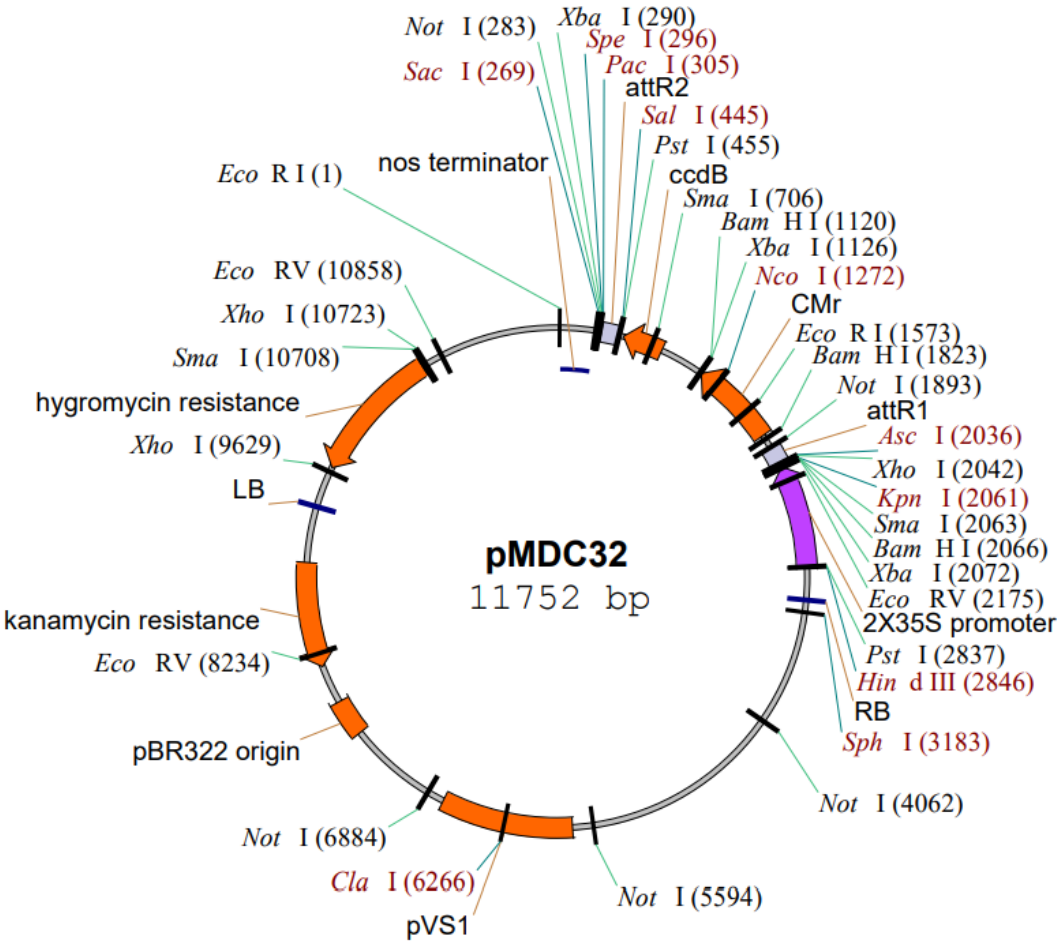
A2 CAS1 gene

```
atgtggaaac tgaagatcgc ggaaggaggt agtccatggc ttagaaccac caataatcac        60
gtcggaagac agttttggga gttcgatccg aatctcggta ctcctgagga tctcgccgcc       120
gtcgaagaag ctaggaagtc tttttcggat aatcgattcg tgcagaaaca tagcgccgat       180
ctgcttatgc gccttcagtt ttcaagagaa aatttgatta gcccagtttt acctcaagtc       240
aaaatcgaag acactgatga tgttacagag gagatggtgg aaaccacgtt aaagaggggt       300
ctagatttct attcaactat acaggcacac gacgggcact ggccaggtga ttatggtggt       360
cctatgtttc ttctcccagg actgataatt acactctcca taactggagc actgaataca       420
gtattgtcgg aacaacataa acaagaaatg cgccgttatc tctataatca ccagaatgag       480
gacggaggtt ggggtttaca tattgagggc cctagcacca tgtttgggtc tgtgttgaac       540
tatgttactc taaggttgct tggagaagga cctaacgatg gagatggaga tatggagaaa       600
ggacgagact ggatactaaa tcatggtggt gctaccaata ttacatcttg ggggaaaatg       660
tggctatcgg tacttggagc ttttgaatgg tccggaaata acccactgcc acctgagata       720
tggcttctcc catatttcct gccaatacat ccaggaagga tgtggtgcca ttgtcgaatg       780
gtgtacttgc cgatgtcgta tttgtatgga aaaaggtttg tgggtcccat aacgtccact       840
gttttatcac tgagaaagga gcttttcaca gtaccatatc atgaagtcaa ctggaatgaa       900
gcacgcaacc tttgcgcaaa ggaggattta tactacccac atccacttgt gcaagatatt       960
ctttgggcat cacttcataa gattgttgag cctgttctga tgcgatggcc tggtgcaaat      1020
ttgagagaaa aggctataag aaccgcaata gaacatattc attatgaaga tgagaatact      1080
aggtacatct gcataggtcc cgtgaacaag gtattaaata tgctttgctg ttgggtagaa      1140
gacccaaact cagaggcttt caagttgcac ctaccaagaa tccatgactt tctctggtta      1200
gctgaagatg gaatgaagat gcagggttat aacggaagcc agctatggga tacaggtttt      1260
gctattcaag cgattttggc aactaacctc gtcgaagaat atgggcccgt tttggaaaaa      1320
gcacattcat ttgtcaagaa ttcccaggtg ttagaagact gccctggaga tctgaattac      1380
tggtatcgcc acatttctaa aggggcttgg cctttctcaa ctgcagatca cggttggccc      1440
atctctgact gcaccgcaga aggactgaaa gctgctcttt tgctatccaa agttcccaag      1500
gcgattgttg gtgaaccaat agatgcaaaa cggttatatg aagctgttaa tgttatcatt      1560
tctttacaga atgcagatgg aggcctcgca acatatgagc tcaccaggtc ataccttgg       1620
ttagagctaa tcaacccagc agaaaccttt ggcgatattg ttattgatta tccttacgtg      1680
gaatgtacat cagctgctat ccaagctttg atatcatttc gaaagctgta tcctggtcat      1740
cgaaagaagg aagtagatga gtgcattgag aaggcggtta agttcattga atccattcaa      1800
gcagcagatg gctcatggta tggatcatgg gctgtttgct tcacgtatgg tacgtggttt      1860
ggagtgaaag ggctggtagc tgttggaaaa acattgaaaa actctccaca tgttgctaaa      1920
gcttgtgaat ttctattgtc gaaacaacaa ccttcgggcg gctggggaga aagctatctt      1980
tcatgtcaag acaaggtcta ttcaaacctt gatggcaaca gatctcacgt cgtgaataca      2040
gcatgggcta tgctcgcact cattggtgct gggcaagctg aggtagaccg gaaaccacta      2100
```

```
caccgggctg caagatactt gattaatgct caaatggaga atggtgattt tccacaacag      2160
gaaataatgg gagtcttcaa taggaactgc atgataacat atgccgcgta tcgaaacatt      2220
tttccgatat gggctttggg ggagtaccgt tgtcaggtat tattgcaaca aggagaatga      2280
```

A2 shows the mRNA for the *CAS1* gene. The location of the mutation I481V is highlighted with green.

A3 Vector pMDC32



A3 shows the vector mentioned in 3.1.

A4 Pathway enzyme structure

DHCR24:

LEFVLIHQRWVFVCLFLLPLSLIFDIYYYVRAWVVFKLSSAPRLHEQRVRDIQKQVREWKEQGSKTFMCTGRPGW
LTVSLRVGKYKKTHKNIMINLMDILEVDTKKQIVRVEPLVTMGQVTALLTSIGWTLPVLPELDDLTVGGLIMGTGI
ESSSHKYGLFQHICTAYELVLADGSFVRCTPSENSDLFYAVPWSCGTLGFLVAAEIRIIPAKKYVKLRFEPVRGLEAI
CAKFTHESQRQENHFVEGLLYSLDEAVIMTGVMTDEAEPSKLNSIGNYYKPWFFKHVENYLKTNREGLEYIPLRH
YYHRHTRSIFWELQDIIPFGNNPIFRYLFGWMVPPKISLLKLTQGETLRKLYEQHHVVQDMLVPMKCLQQALHT
FQNDIHVYPIWLCPFILPSQPGLVHPKGNEAELYIDIGAYGEPRVKHFEARSCMRQLEKFVRSVHGFQMLYADCY
MNREEFWEMFDGSLYHKLREKLGCQDAFPEVYDKICKAARH

CYP51A1:

MLLLGLLQAGGSVLGQAMEKVTGGNLLSMLLIACAFTLSLVYLIRLAAGHLVQLPAGVKSPPYIFSPIPFLGHAIAF
GKSPIEFLENAYEKYGPVFSFTMVGKTFTYLLGSDAAALLFNSKNEDLNAEDVYSRLTTPVFGKGVAYDVPNPVFL
EQKKMLKSGLNIAHFKQHVSIIEKETKEYFESWGESGEKNVFEALSELIILTASHCLHGKEIRSQLNEKVAQLYADL
DGGFSHAAWLLPGWLPLPSFRRRDRAHREIKDIFYKAIQKRRQSQEKIDDILQTLLDATYKDGRPLTDDEVAGML
IGLLLAGQHTSSTTSAWMGFFLARDKTLQKKCYLEQKTVCGENLPPLTYDQLKDLNLLDRCIKETLRLRPPIMIM
MRMARTPQTVAGYTIPPGHQVCVSPTVNQRLKDSWVERLDFNPDRYLQDNPASGEKFAYVPFGAGRHRCIGE
NFAYVQIKTIWSTMLRLYEFDLIDGYFPTVNYTTMIHTPENPVIRYKRRSK

DHCR14:

MAPTQGPRAPLEFGGPLGAAALLLLLPATMFHLLLAARSGPARLLGPPASLPGLEVLWSPRALLLWLAWLGLQA
ALYLLPARKVAEGQELKDKSRLRYPINGFQALVLTALLVGLGMSAGLPLGALPEMLLPLAFVATLTAFIFSLFLYMK
AQVAPVSALAPGGNSGNPIYDFFLGRELNPRICFFDFKYFCELRPGLIGWVLINLALLMKEAELRGSPSLAMWLV
NGFQLLYVGDALWHEEAVLTTMDITHDGFGFMLAFGDMAWVPFTYSLQAQFLLHHPQPLGLPMASVICLINA
TGYYIFRGANSQKNTFRKNPSDPRVAGLETISTATGRKLLVSGWWGMVRHPNYLGDLIMALAWSLPCGVSHLL
PYFYLLYFTALLVHREARDERQCLQKYGLAWQEYCRRVPYRIMPYIY

Δ7,Δ8-isomerase:

MTTNAGPLHPYWPQHLRLDNFVPNDRPTWHILAGLFSVTGVLVVTTWLLSGRAAVVPLGTWRRLSLCWFAVC
GFIHLVIEGWFVLYYEDLLGDQAFLSQLWKEYAKGDSRYILGDNFTVCMETITACLWGPLSLWVVIAFLRQHPLR

FILQLVVSVGQIYGDVLYFLTEHRDGFQHGELGHPLYFWFYFVFMNALWLVLPGVLVLDAVKHLTHAQSTLDAK
ATKAKSKKN

5-desaturase:

MDLVLRVADYYFFTPYVYPATWPEDDIFRQAISLLIVTNVGAYILYFFCATLSYYFVFDHALMKHPQFLKNQVRREI
KFTVQALPWISILTVALFLLEIRGYSKLHDDLGEFPYGLFELVVSIISFLFFTDMFIYWIHRGLHHRLVYKRLHKPHHI
WKIPTPFASHAFHPIDGFLQSLPYHIYPFIFPLHKVVYLSLYILVNIWTISIHDGDFRVPQILQPFINGSAHHTDHHM
FFDYNYGQYFTLWDRIGGSFKNPSSFEGKGPLSYVKEMTEGKRSSHSGNGCKNEKLFNGEFTKTE