Secondary Metabolism: The Acetate Pathway to Fatty Acids, Macrolides and Polyketides

Lecture 7
Biofuels and Bioproducts

Bronx Community College - 2017
Chemistry and BioEnergy Technology for Sustainability NSF ATE 1601636

Outline

- Fatty Acid Synthesis
  - Overview
  - Review of Claisen Condensation and β oxidation
  - FAS Mechanism (@ Molecular and Macromolecular Levels)
  - Fatty Acids, Triglycerides and Biodiesel fuels
  - Prostaglandins and examples of Fatty Acids in Medicine

- Macrolide antibiotics (example = Erythromycin)
- Polyketides, Aromatic and Linear

- Photos from Bronx Biodiesel Lab

Slide Images modified from: Paul Dewick *Medicinal Natural Products: A Biosynthetic Approach* (3rd Ed. Wiley, 2009)

Relationship between Fatty Acids, Macrolides and Polyketides

CLAISEN REACTION → "Poly-β-keto-ester"

LIPIDS

- ***Complete reduction*** = *Saturated* FAs
- ***Partial reduction*** = *Unsaturated* FAs (Z-cis)
- Triglycerides (Fats vs. Oils)
- Phospholipids
- Oleic -> linoleic -> α-linoleic -> γ-linoleic
- Omega 3 FAs
- aracadonic acid, leukotrienes, prostaglandins
- acetylenic FAs, branched FAs
- Biodiesel...

MACROLIDES

Varying amounts of reduction/dehydration

Examples = Erythromycin (azithromycin)

"Statins", "Avermectins", polyene antifungals, immunosuppressants

POLYKETIDES

No reduction of keto-groups

Sets chain length for chemical elaboration

Can be aromatized (e.g. tetracyclines)

Fatty Acid Synthase (FAS) Enzymes

- ***Animals*** = (Type I) Multifunctional enzyme with seven discrete, functional *domains* providing all catalytic activity. All domains are on a single polypeptide, encoded by a single gene. The enzyme is a homo-dimer and both units are required for activity.

- ***Fungi*** = (Type I) Multifunctional enzyme, seven domains are distributed over two non-identical polypeptides (α and β). Enzyme is a dodecamer ($\alpha_6\beta_6$).

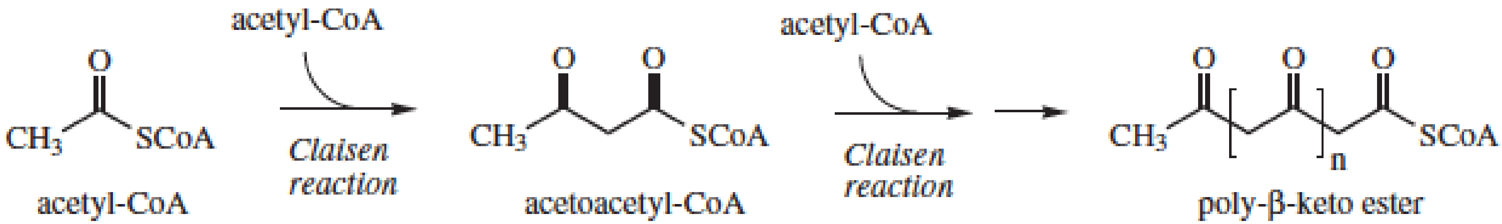
FAS Enzymes Contd.

- ***Bacteria and Plants***
  - Type II FAS Enzymes
  - An assembly of *separable* enzymes
  - Encoded by seven *different* genes

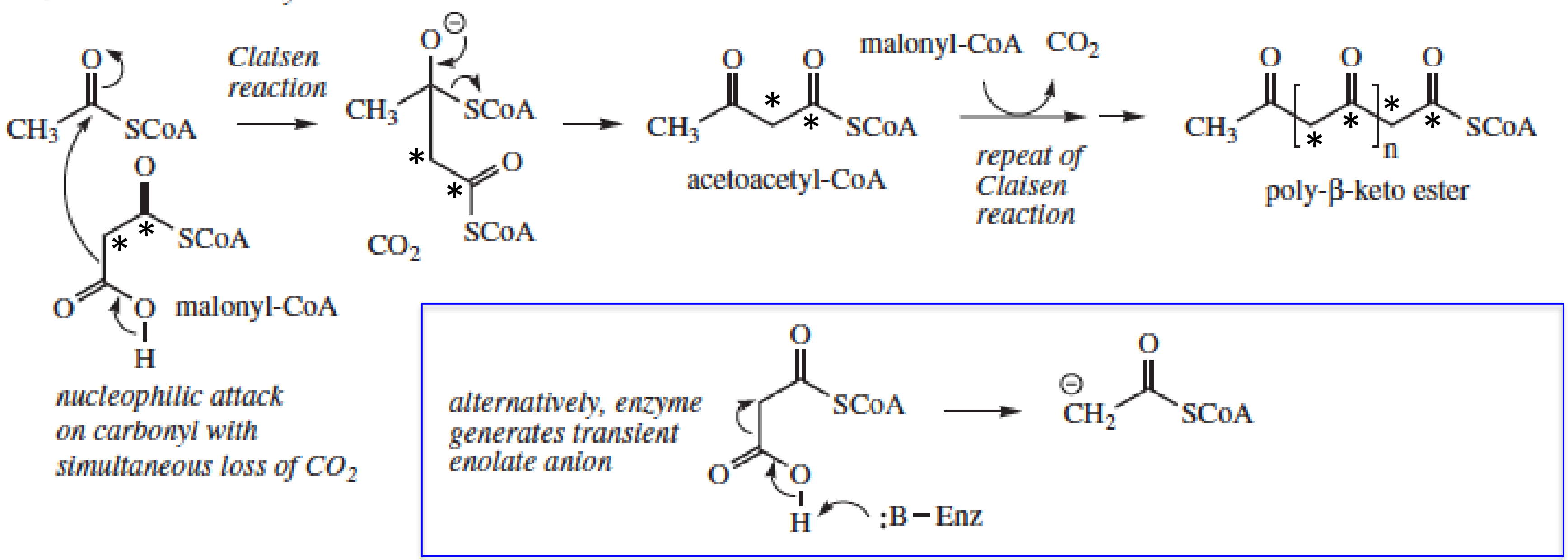
Both Type I and II FAS enzymes employ same mechanism to grow the FA chain

The Claisen Condensation

Claisen reaction: acetyl-CoA



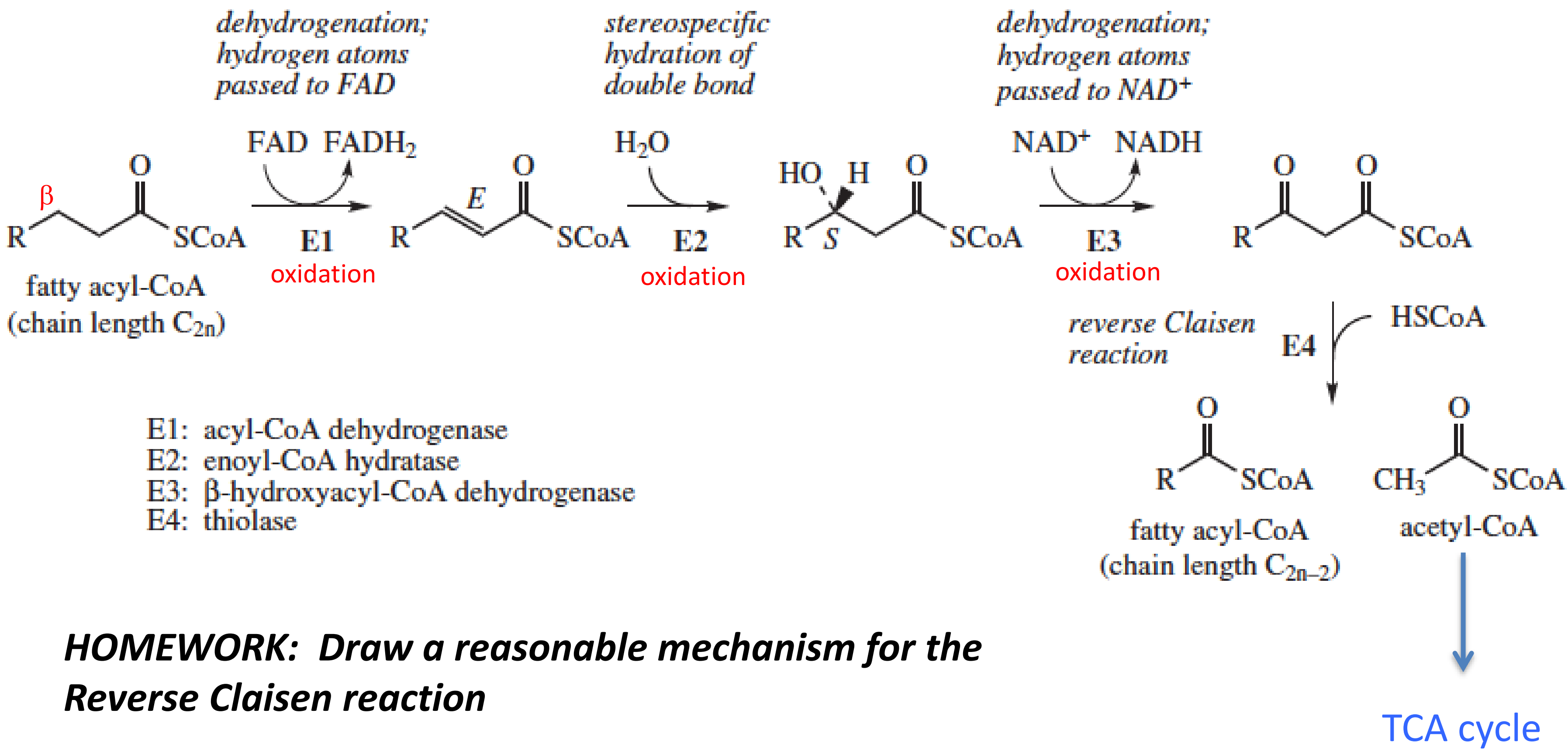
Claisen reaction: malonyl-CoA



Mechanistic Evidence:

- It is known that the condensation occurs between malonyl co-A and acetyl co-A
- $^{13/14}C$ evidence shows that no CO_2 from malonyl co-A goes to β-keto product

β Oxidation: a Reverse Claisen reaction

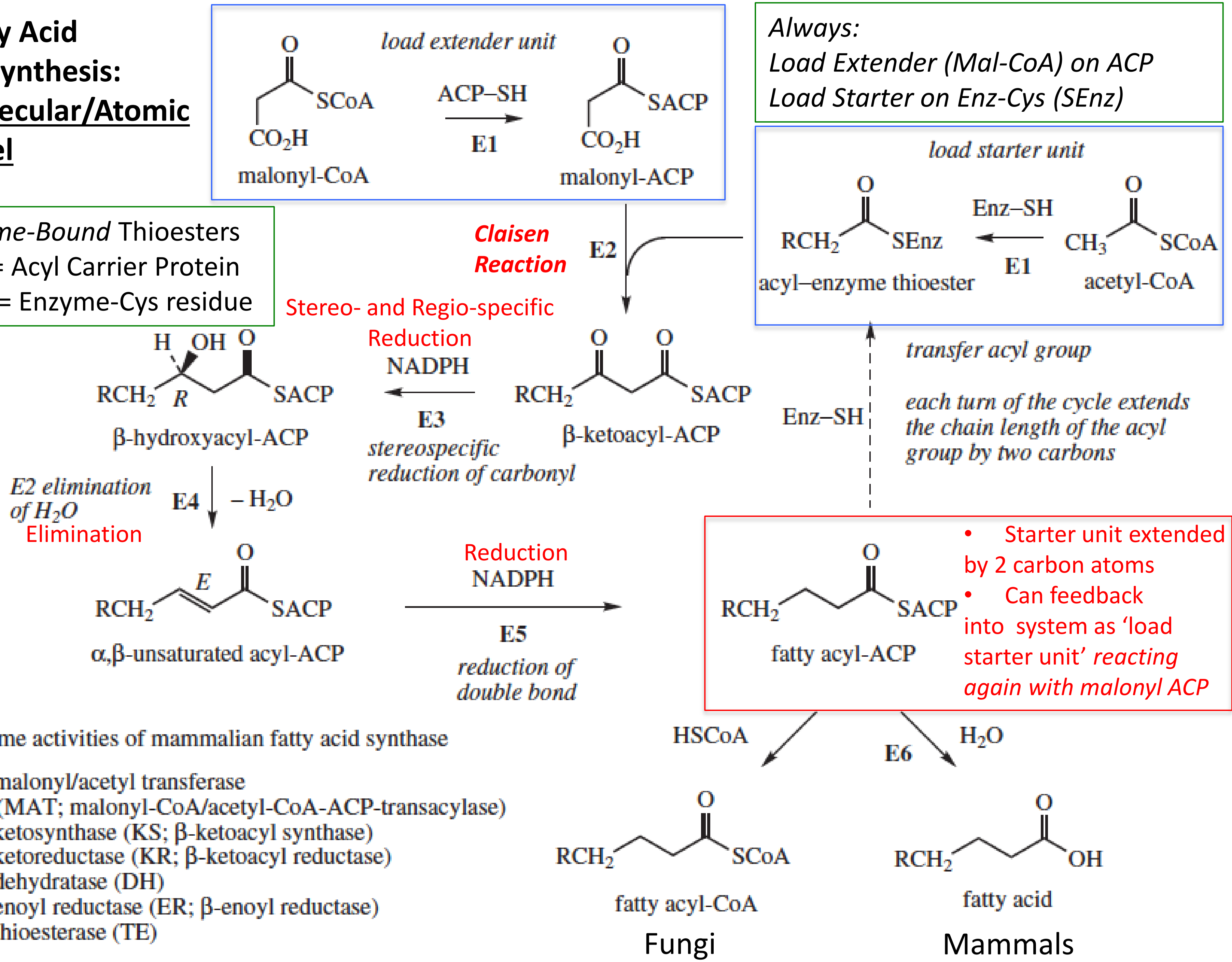


HOMEWORK: Draw a reasonable mechanism for the Reverse Claisen reaction

Fatty Acid Biosynthesis: Molecular/Atomic Level

Always:
Load Extender (Mal-CoA) on ACP
Load Starter on Enz-Cys (SEnz)

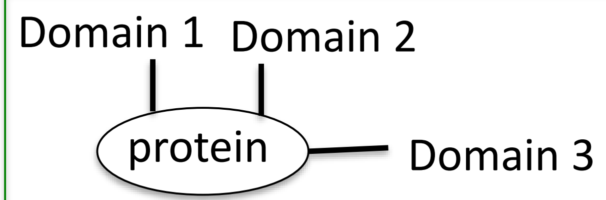
Enzyme-Bound Thioesters
ACP = Acyl Carrier Protein
SEnz = Enzyme-Cys residue



Enzyme activities of mammalian fatty acid synthase

E1: malonyl/acetyl transferase (MAT; malonyl-CoA/acetyl-CoA-ACP-transacylase)
E2: ketosynthase (KS; β-ketoacyl synthase)
E3: ketoreductase (KR; β-ketoacyl reductase)
E4: dehydratase (DH)
E5: enoyl reductase (ER; β-enoyl reductase)
E6: thioesterase (TE)

Fatty Acid Biosynthesis: Protein/Domain Level



Example Domains:

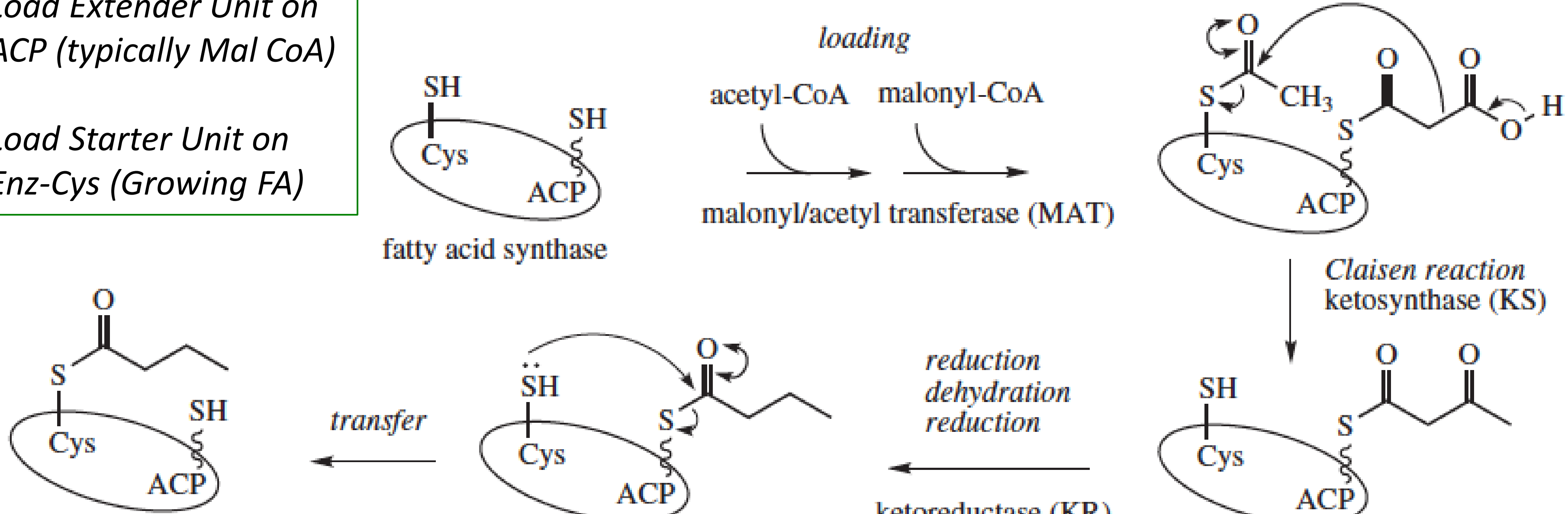
Domain 1 = Ketosynthase (KS) Domain

Domain 2 = ACP Binding Site

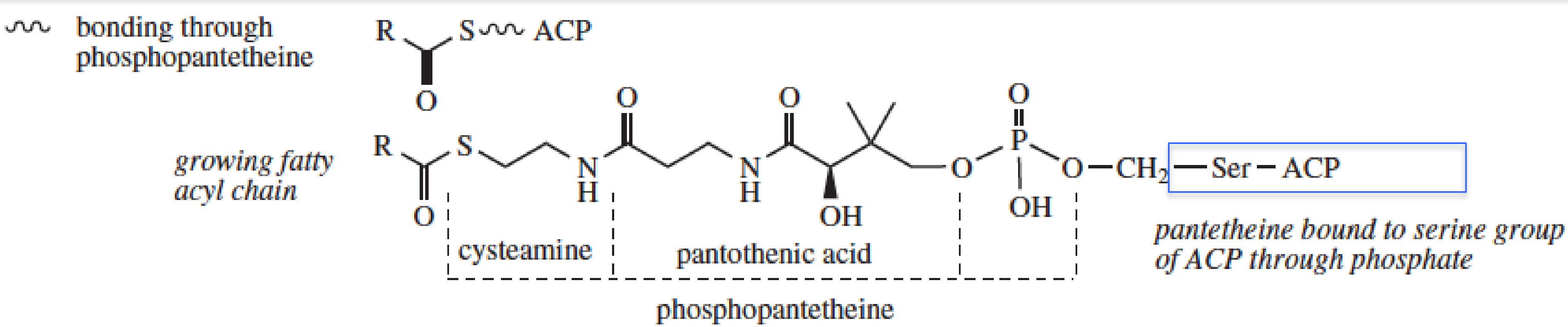
Domain 2 = Ketoreductase (KR) Domain

Load Extender Unit on ACP (typically Mal CoA)

Load Starter Unit on Enz-Cys (Growing FA)

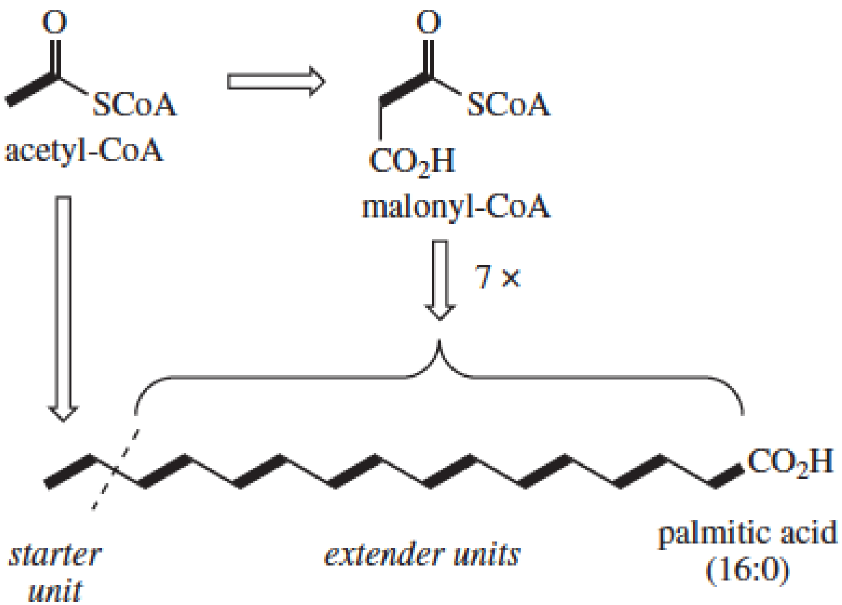


Form malonyl ACP - and continue extension by 2C unit…

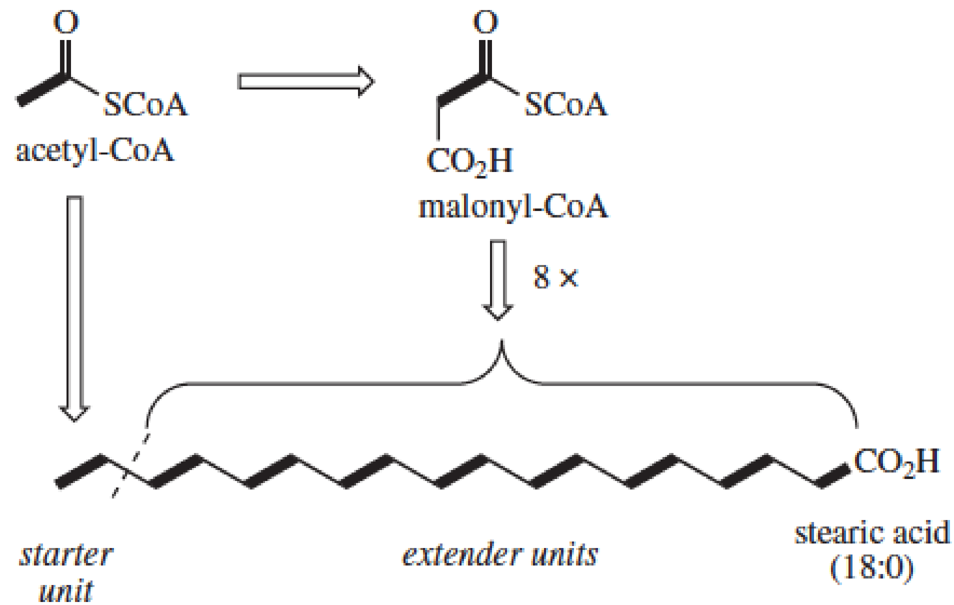


Some Common Fatty Acids

Palmitic Acid:
Principal component of Palm Oil

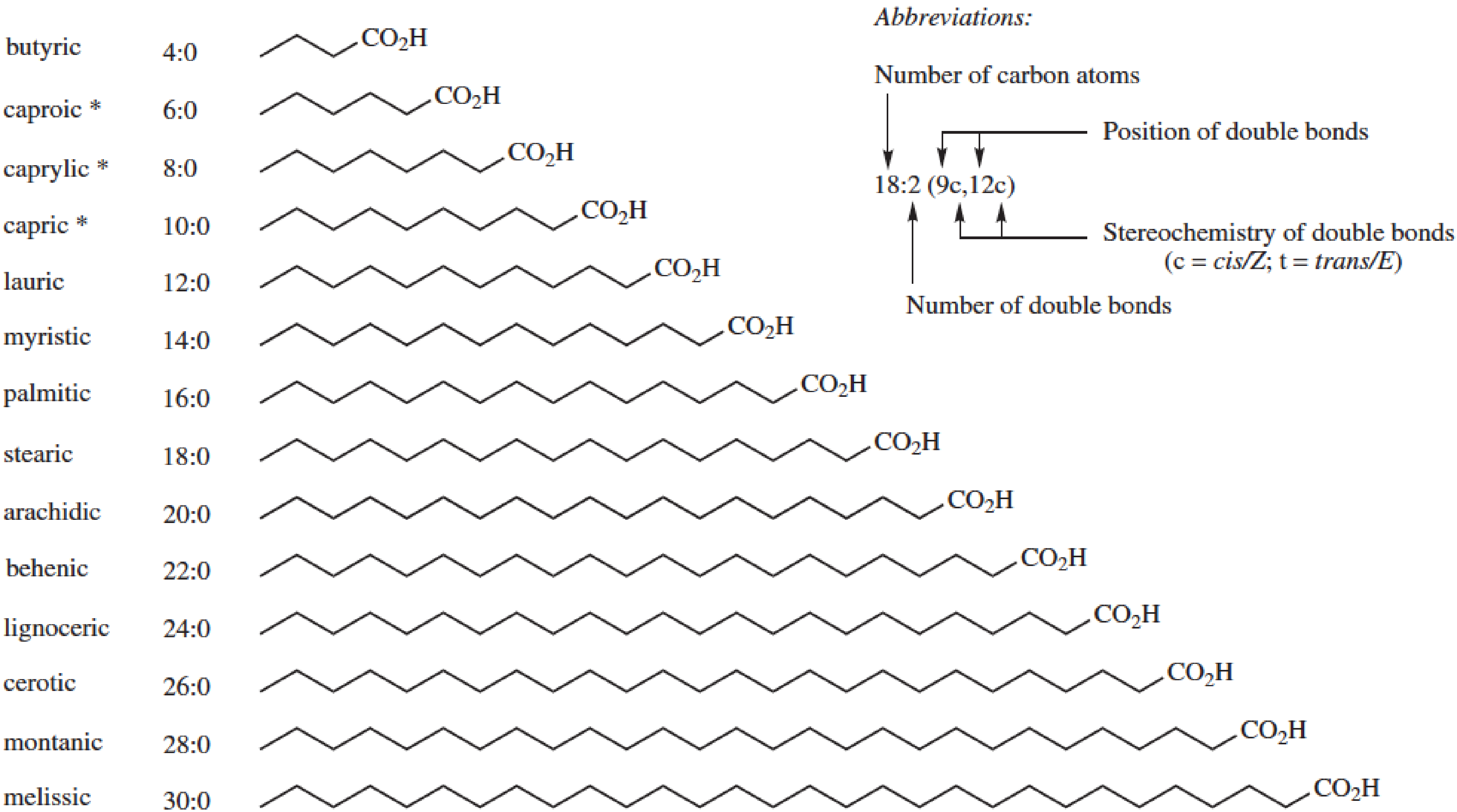


Stearic Acid:
Principal component of Tallow



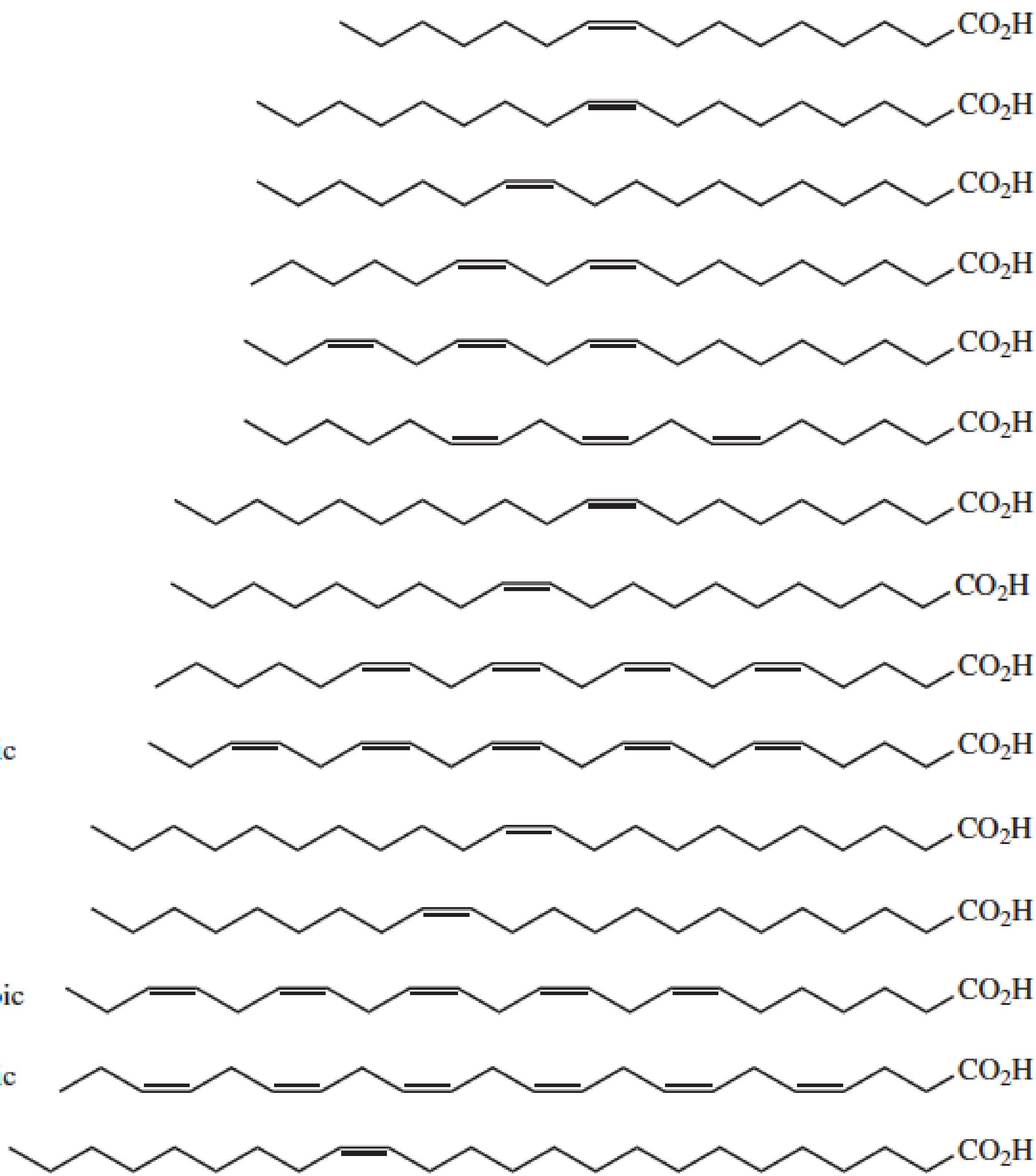
- 2C Acetate units derived from acetyl coA (starter unit) and malonyl CoA - CO_2 (extender units)
- ***Most natural FAs are C_{16}-C_{18}*** but can be C_4-C_{30}
- Rare examples of odd-numbered FAs (e.g. propionic acid C_3 starter unit)

Saturated Fatty Acids (*aka fats*) and FA Nomenclature



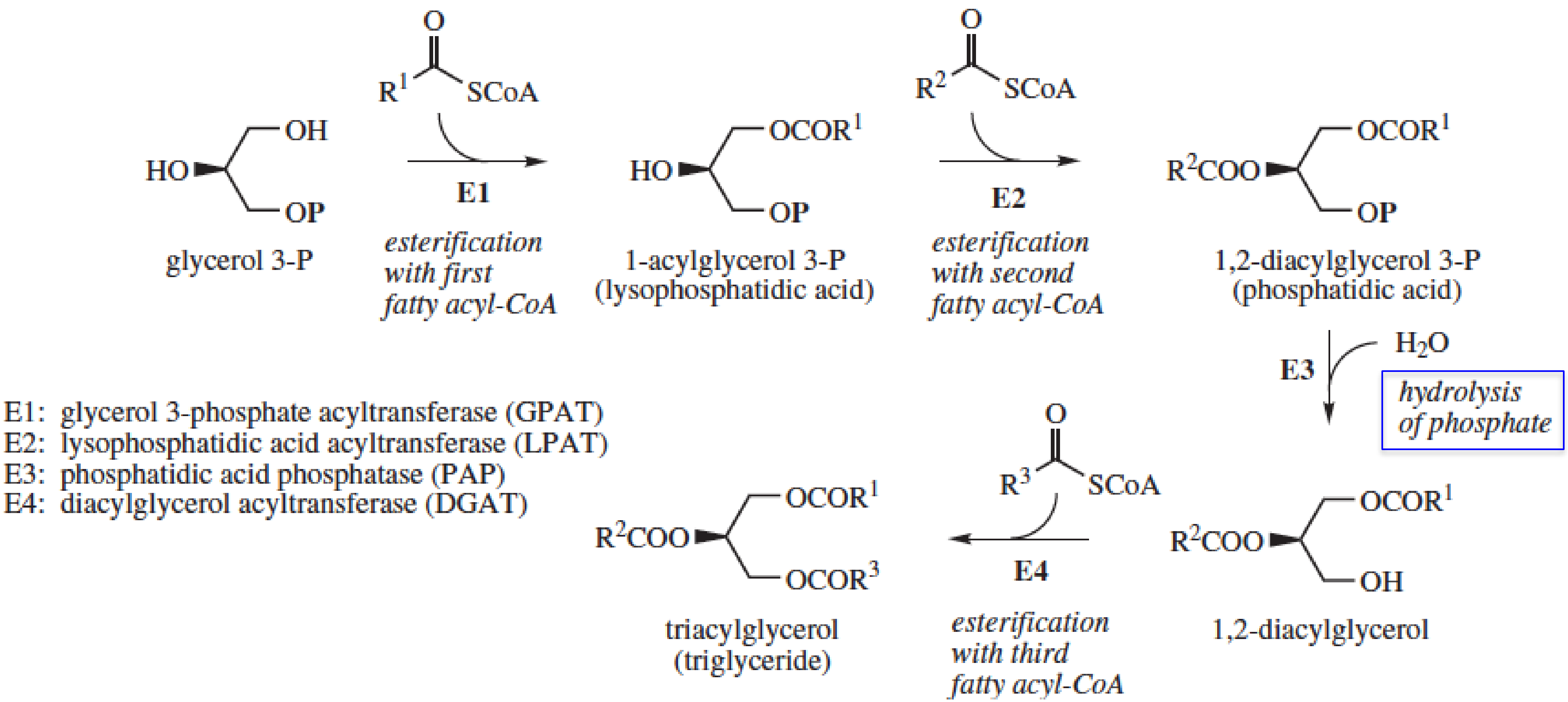
* To avoid confusion, systematic nomenclature (hexanoic, octanoic, decanoic) is recommended

Unsaturated and Polyunsaturated Fatty Acids (*aka oils*) – can you spot the ω-3s?



palmitoleic	16:1 (9c)
oleic	18:1 (9c)
cis-vaccenic	18:1 (11c)
linoleic	18:2 (9c,12c)
α-linolenic	18:3 (9c,12c,15c)
γ-linolenic	18:3 (6c,9c,12c)
gadoleic	20:1 (9c)
gondoic	20:1 (11c)
arachidonic	20:4 (5c,8c,11c,14c)
eicosapentaenoic (EPA)	20:5 (5c,8c,11c,14c,17c)
cetoleic	22:1 (11c)
erucic	22:1 (13c)
docosapentaenoic (DPA)	22:5 (7c,10c,13c,16c,19c)
docosahexaenoic (DHA)	22:6 (4c,7c,10c,13c,16c,19c)
nervonic	24:1 (15c)

Triglyceride Biosynthesis



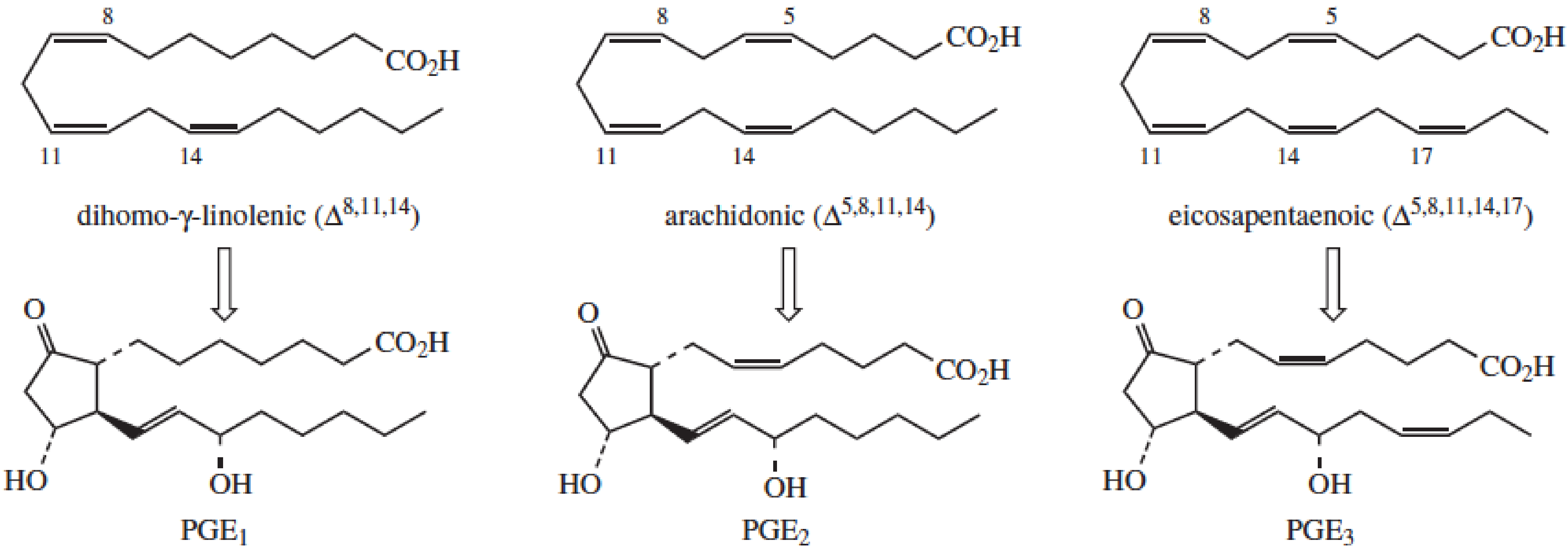
- Most FAs are bound as glycerol triesters (triglycerides) and can be common or mixed FAs
- In nature most triglycerides are mixed and therefore isomers can form (central atom is chiral)
- If the phosphate remains intact, phospholipids results
- Phospholipids are important components of cell membranes
- e.g. Platelet activating factor (in mammals) nM potency for e.g. blood clotting (Ginkgolides = selective antagonist)

Fuel Properties of Fats/Oils: Think Biodiesel!

	FAME	Melting point ˚C	Boiling point ˚C	Kinematic Viscosity (mm^2/s)	Cetane number	Oil stability Index (h)	Density g/cm^3
FFA							
	C8:0	16.7	239.7				0.910
	C10:0	31.6	269.0				0.893
	C12:0	43.2	298.9	2.43	67	>40	0.880
	C14:0	54.4	250.5	3.30	66	>40	0.862
	C15:0	51:53	257.0				0.842
	C16:0	62.9	351.0	4.38	86	>40	0.853
	C16:1	-0.1		3.67	51	2.1	0.894
steric	C18:0	69.6	383.0	5.85	101	>40	0.847
oleic	C18:1	13:14	360.0	4.51	59	2.5	0.895
linoleic	C18:2	−5:−12	230.0	3.65	38	1.0	0.900
α-linoleic	C18:3			3.14	23	0.2	
	C20:0	75.5	328.0				0.824
	C22:1	33.8	381.5		74		0.860
	C22:6	−44.0	446.7				0.943

Fakhry, et al. *J. Water Resource Protection* 5, 9, 2013

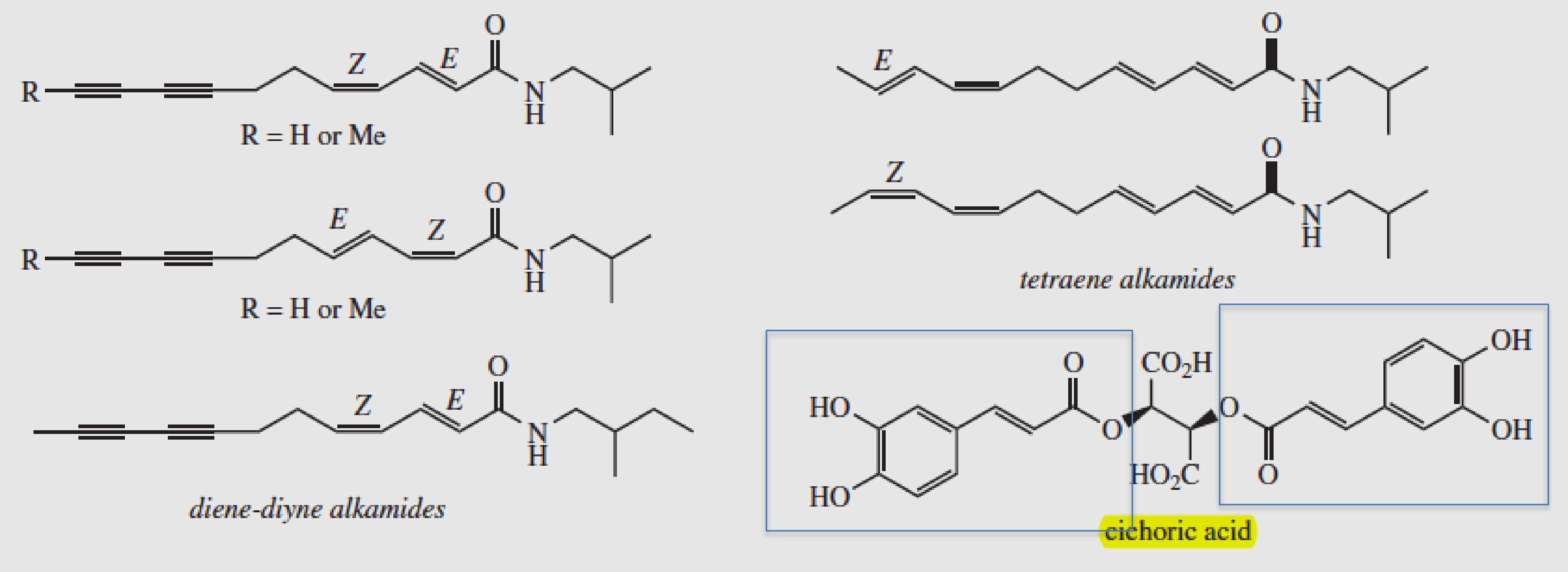
Prostaglandins



- Prostaglandins responsible for diverse physiological functions including smooth muscles contraction/relaxation, (uterus, cardiovascular, intestinal, bronchial), inhibit gastric acid secretion, control blood pressure, suppress platelet aggregation, mediators of inflammatory response,
  fever and allergy.
- γ-linolenic acid, aracadonic acid and omega 3 FAs are precursor to prostaglandin E biosynthesis
- Secondary messenger compounds -> modulating transmission of hormone stimulation and metabolic response.
- Have been "holy grail" of drug targets for years, but chances of unwanted side effects are very high since they have so many interrelated functions.
- NSAIDS inhibit early steps in prostaglandin biosynthesis -> transformation of unsaturated FAs into cyclic peroxidases (free radical mechanism involving COX enzymes)

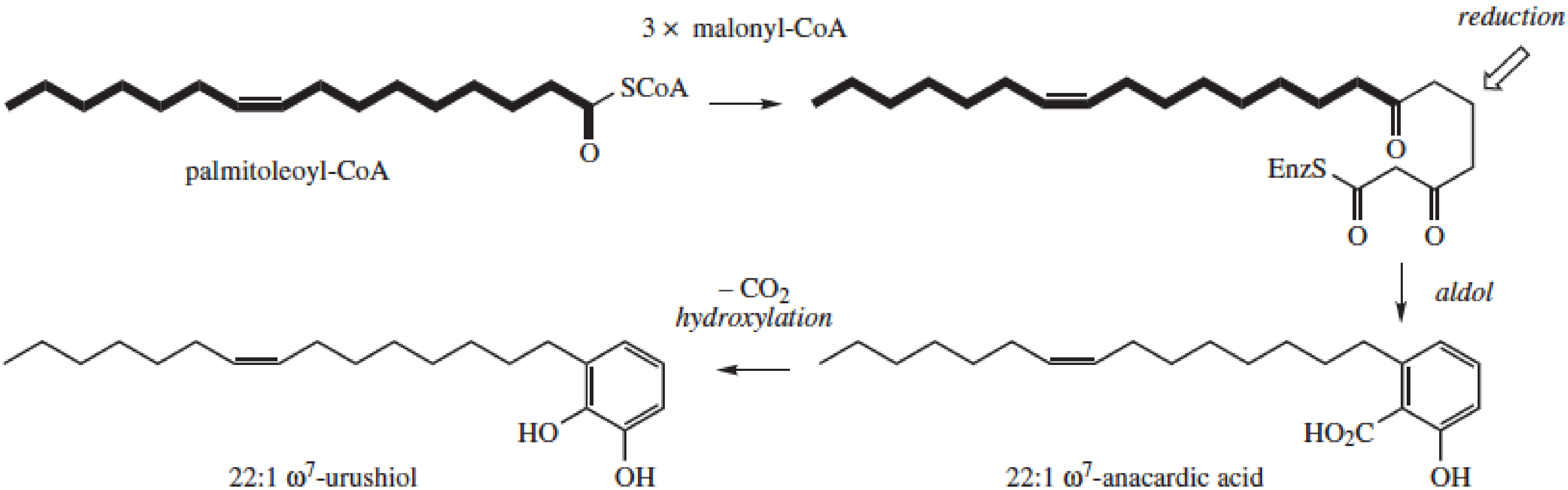
Entire biosynthetic mechanism -> page 60

Interesting Fatty Acids from *Echinacea*



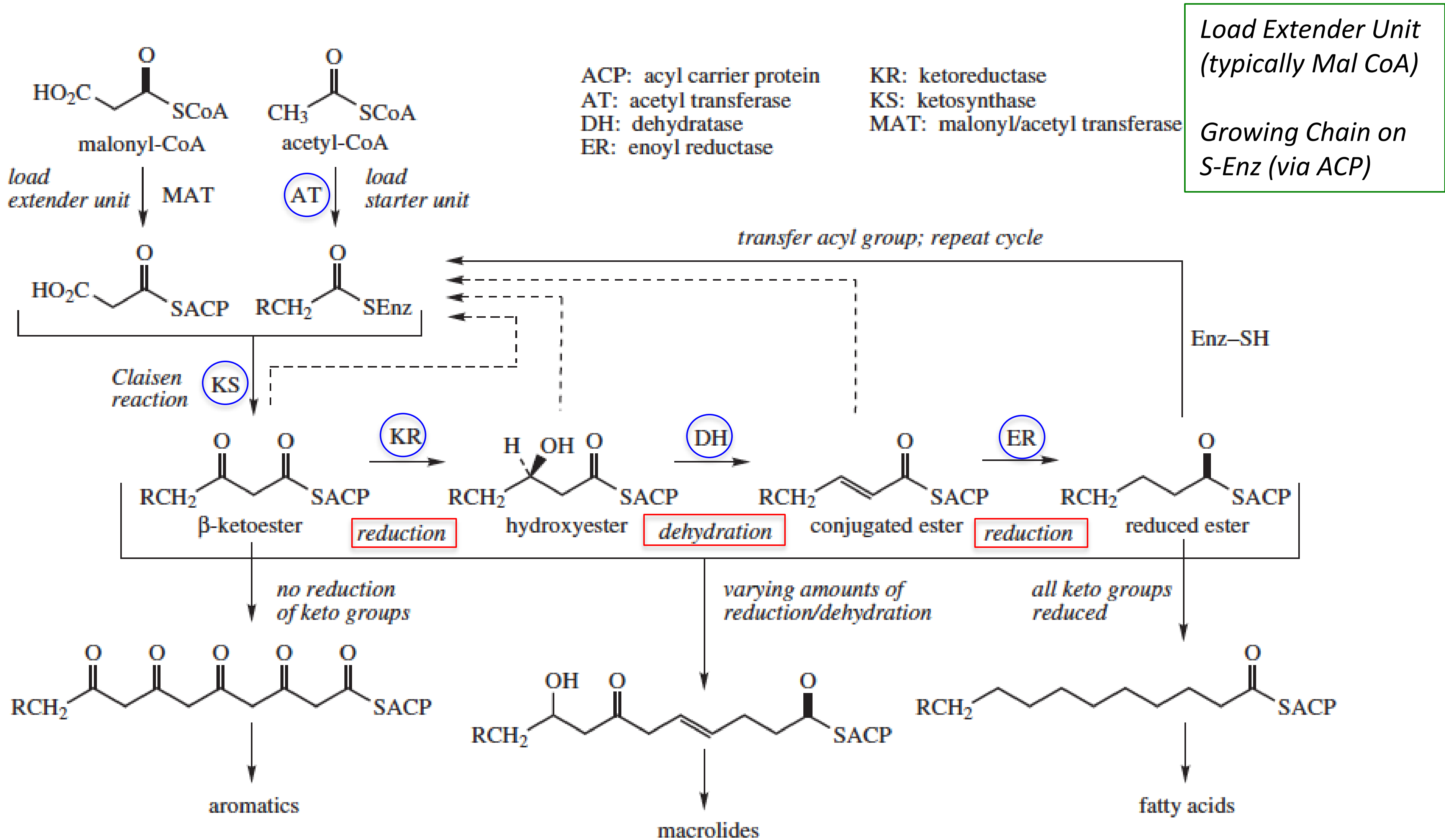
- Commonly used for immunostimulatory effects (vs. the common cold)
- The bio-activity has been linked to combinations of the above FAs (synergism)
- Immunostimulatory, anti-inflammatory, anti-bacterial, anti-viral
- Alkylamides derived from valine and isoleucine (comprise ~ 0.6% w/w of plant roots)
- Diene-diynes degrade significantly during drying and prolonged storage
- Caffeic acid is from shikimic acid pathway (mixed biosynthesis) and a lignin precursor

Poison Ivy/Oak



Mechanism of action: Oxidized to quinone and attacked by nucleophilic groups of protein

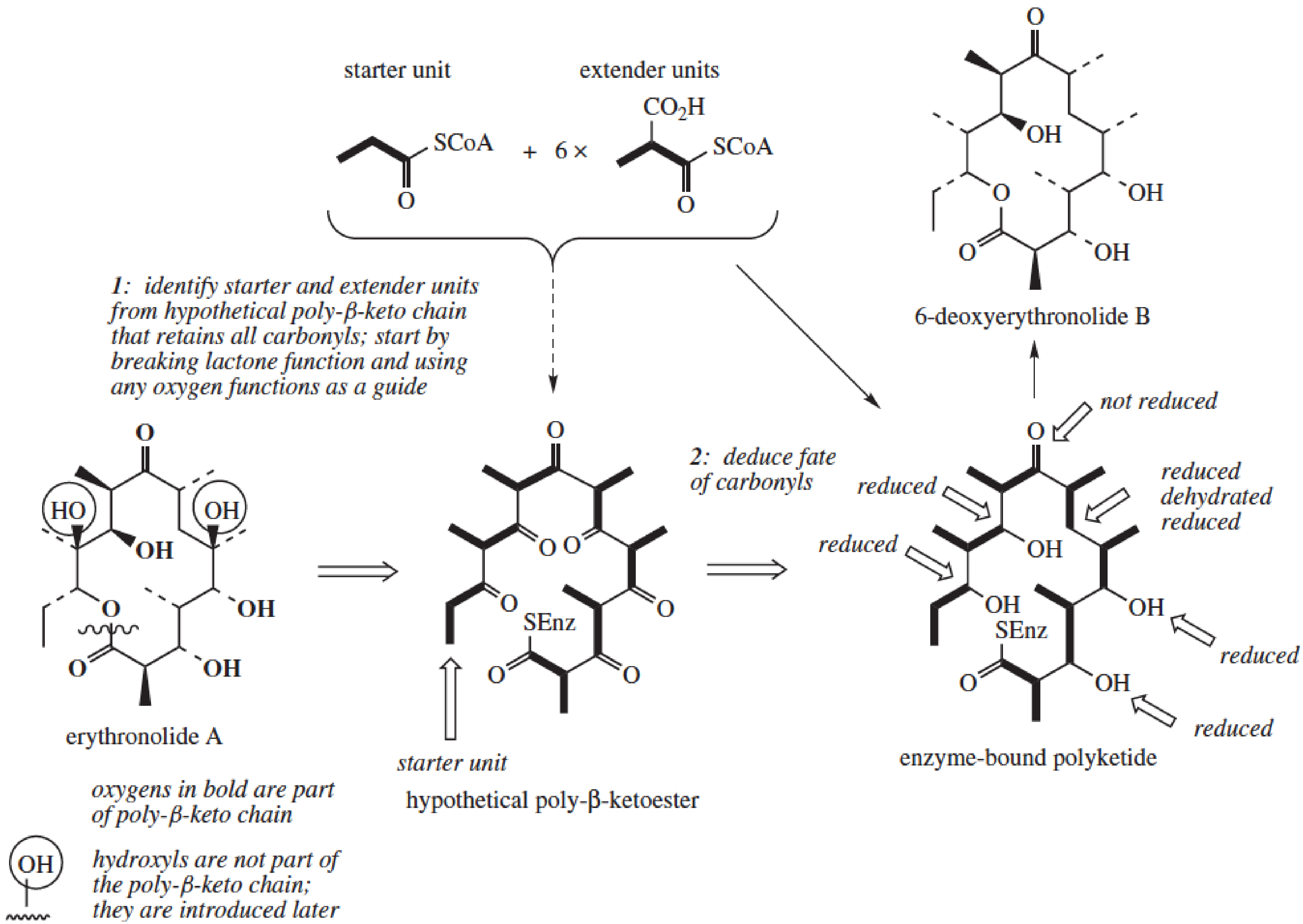
Polyketide Synthases (PKS) allow for Differentiation between Aromatics, Macrolides and FAs



Type II PKS (bacteria only)

Type I PKS (bacteria, fungi)

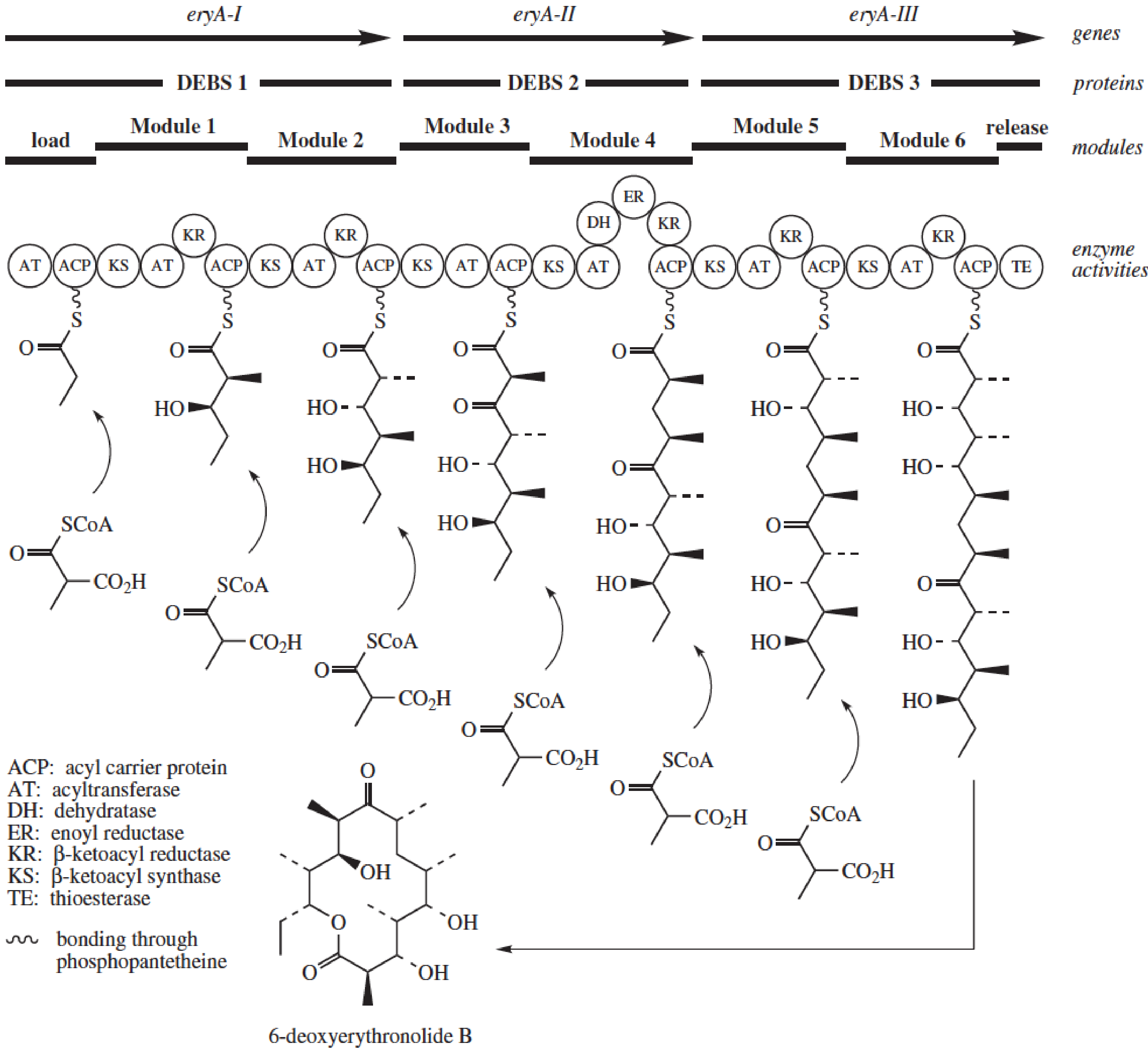
Erythromycin Retro-biosynthesis

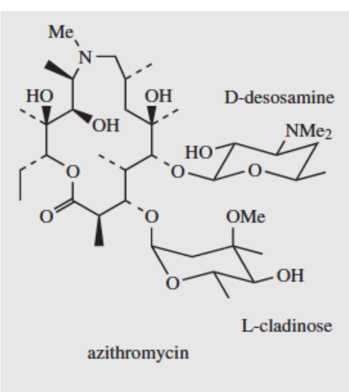


PKS Enzymes

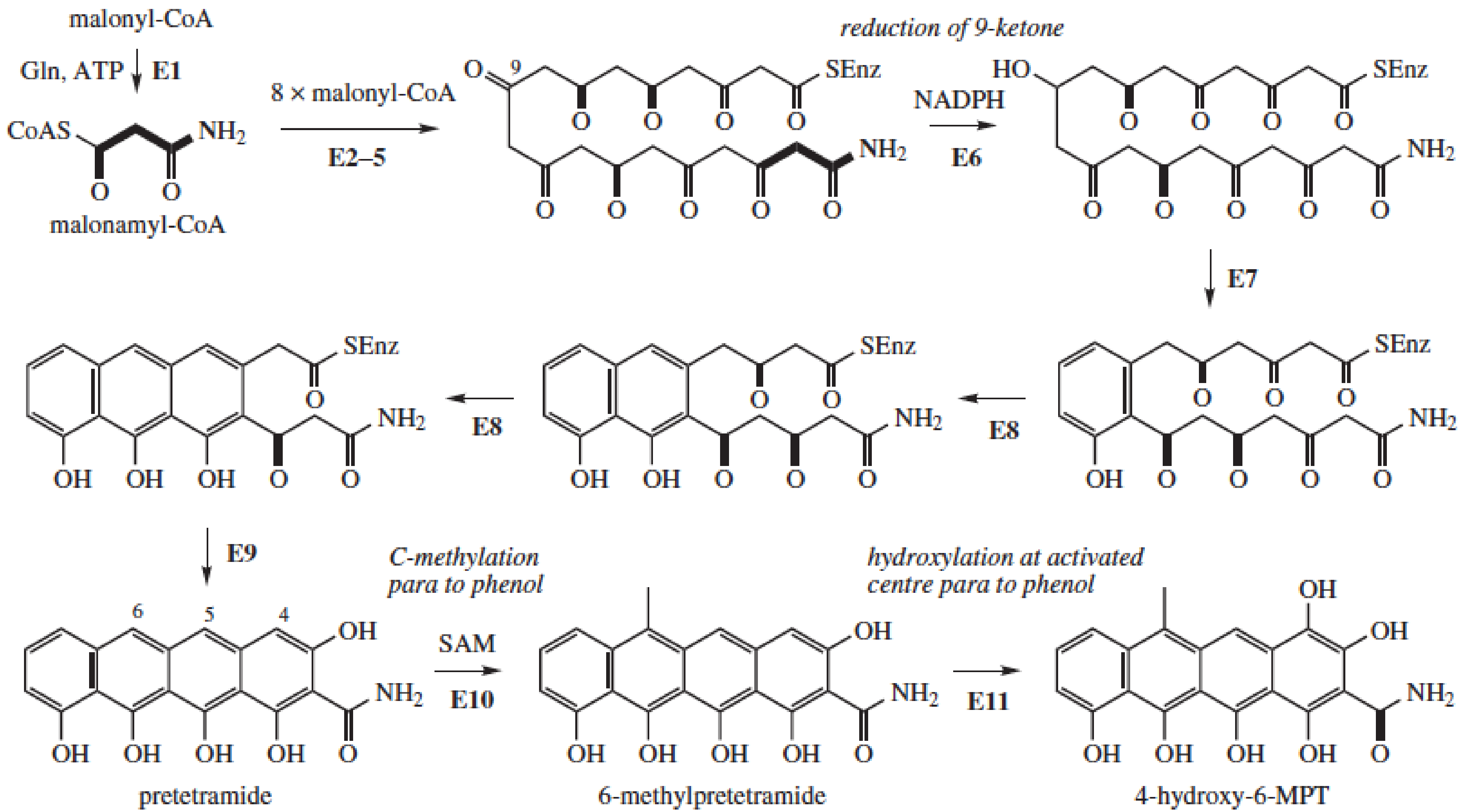
- Modular
- Genes encode proteins, encode modules
- ACP long swinging arm
- Stereospecific
- Engineer-able!

e.g. Z-Pack (azithromycin)





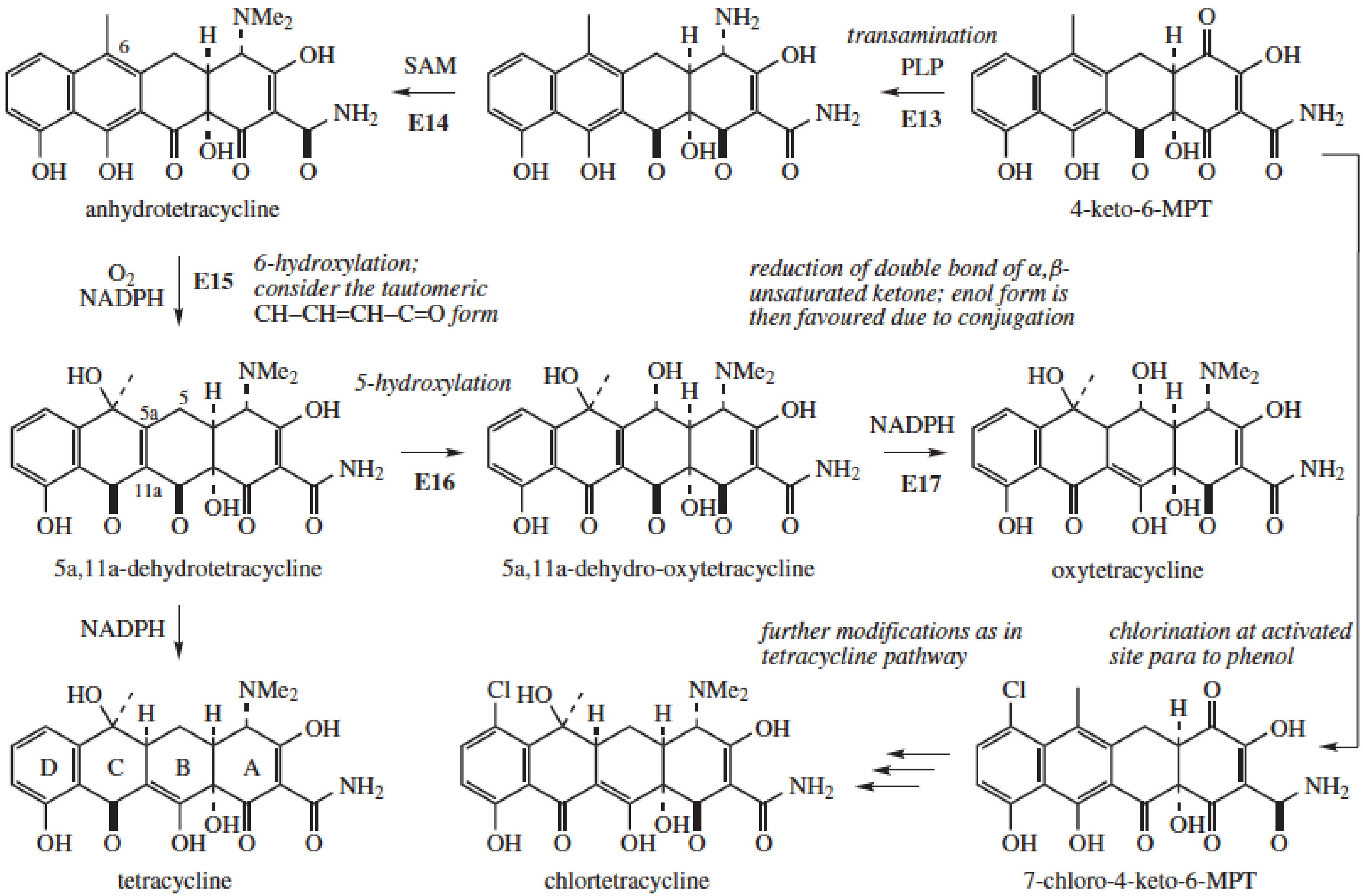
Tetracycline Part 1



E1: OxyD (amidotransferase)
E2: OxyA (ketosynthase)
E3: OxyB (chain length factor)
E4: OxyC (ACP)
E5: OxyP (acyltransferase)
E6: OxyJ (9-ketoreductase)

E7: OxyK (aromatase)
E8: OxyN (cyclase)
E9: OxyI (cyclase)
E10: OxyF (methyltransferase)
E11: OxyL (oxygenase)
E12: OxyG (oxygenase)

Tetracycline Part 2



E13: OxyQ, OxyR (aminotransferase)
E14: OxyT (*N*-methyltransferase)
E15: OxyS (oxygenase)
E16: OxyE (oxygenase)
E17: TchA (reductase)

Linear Polyketides

- Formed by “cascade” reactions

- e.g. cyclic polyether toxins formed from long chain polyepoxides

- Dinoflagellate “red tides”