

iBiology.org Teaching Tools

Chaitan Khosla's Lecture Part 1:

An Introduction to Polyketide Assembly Lines

Teaching Tools were prepared by Becky Anderson and Ben Barsi-Rhyne.

Contents

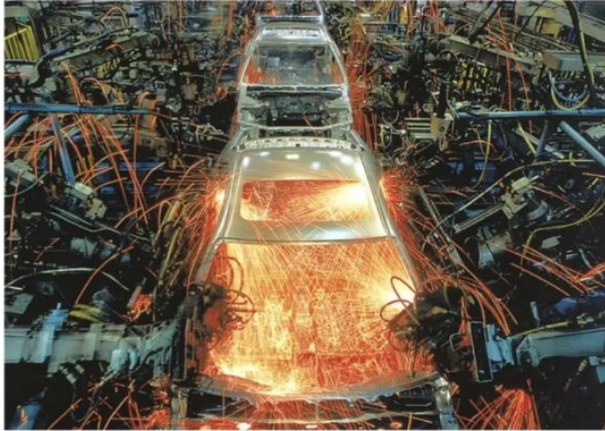
1. Keywords and Terms
 2. Lecture Notes
 3. Review Questions
 4. Answers to Review Questions
 5. Discussion Questions
 6. Answers to Discussion Questions
 7. Questions for Discussion Paper
 8. Answers to Questions for Discussion Paper
-

1. Keywords and Terms

Polyketide, DEBS, translocation, acyl transfer, SAXS

2. Lecture Notes

Assembly-Line Biosynthesis of Polyketide Antibiotics: Part 1



Chaitan Khosla
Depts of Chemistry and Chemical Engineering
Stanford University

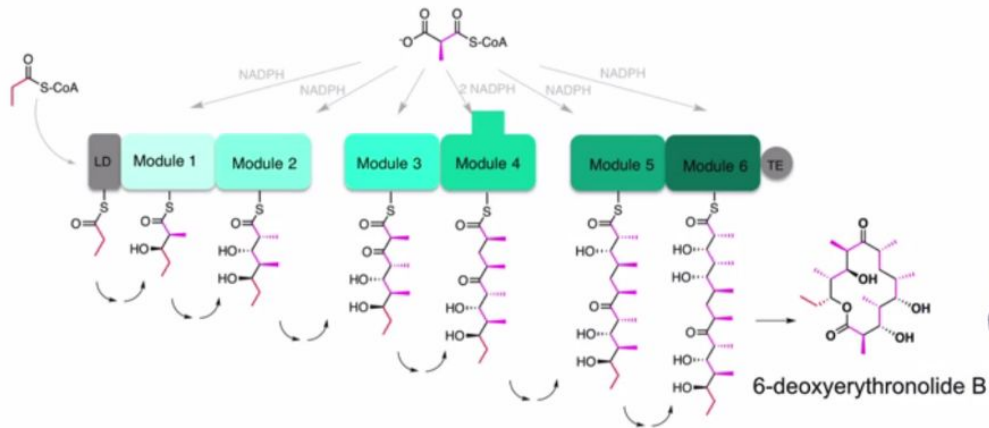


0:00-3:50 min

Automobile assembly lines build cars using a series of waystations. At each waystation, there are catalysts that perform specific tasks independently of upstream and downstream waystations. These assembly lines are modular, so by altering the catalysts or inputs at certain waystations the same assembly line can create different outputs (ie different cars).

This is analogous to the way in which a class of antibiotics, called polyketide antibiotics, are synthesized in nature.

Assembly Line Polyketide Antibiotic Biosynthesis: The 6-Deoxyerythronolide B Synthase (DEBS)



Cortes, et al. *Nature* (1990)
Donadio, et al. *Science* (1991)



iBiology.org

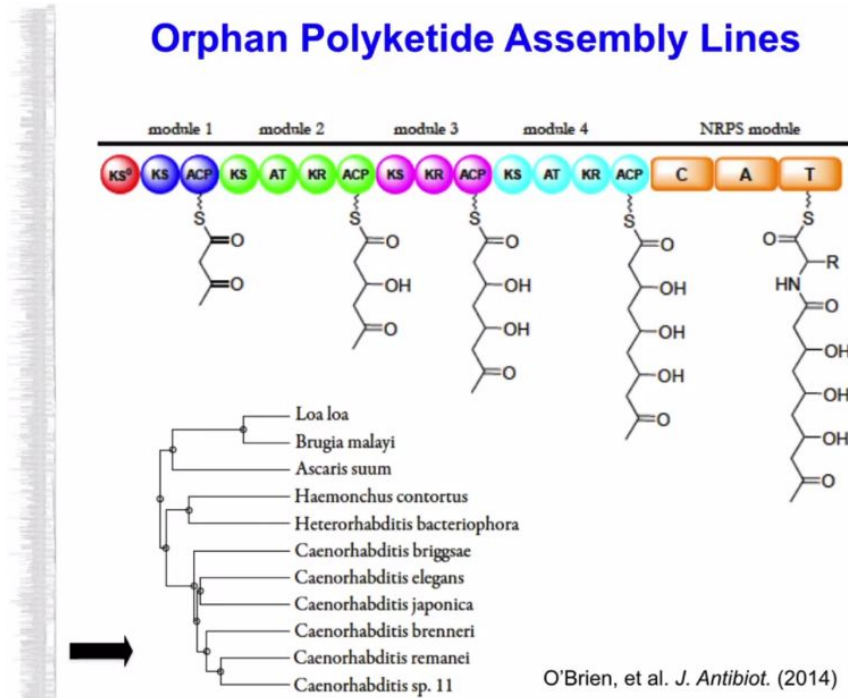
3:50-9:15 min

DEBS (6-Deoxyerythronolide B Synthase) is an assembly line consisting of multiple enzymes in 6 modules. These modules function together to make a key intermediate in the biosynthesis of the important antibiotic erythromycin.

DEBS uses precursors that are available from metabolic processes, and each module incrementally adds precursors to a growing polyketide chain to create the highly complex final product 6-deoxyerythronolide B.

Many other complex antibiotics are also made using a similar biosynthetic assembly line strategy.

Orphan Polyketide Assembly Lines

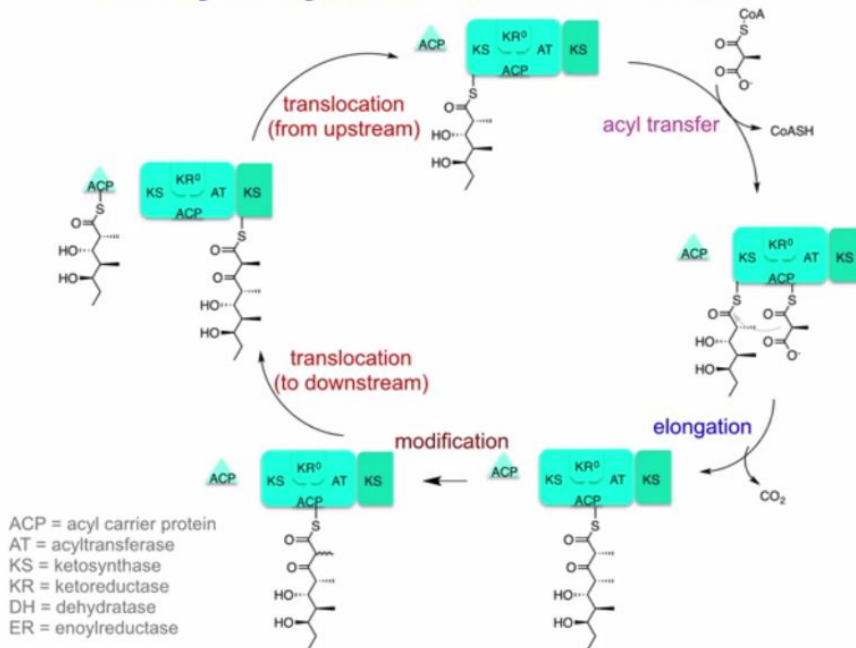


iBiology.org

10:25-17:25 min

DEBS was the first of these assembly lines to be discovered, and only a few others were cloned and sequenced during the first two decades of this field of study. However, around the mid-2000s it became much easier to sequence genomes, and this led to a huge expansion in the number of sequenced assembly lines. The vast majority of these are called “orphan” assembly lines because their function in nature is unknown. Given this abundant sequence information, the current challenges are to figure out what these assembly lines are doing and how to manipulate them to create specific molecules of interest.

Catalytic Cycle of Module 3 of DEBS



17:25 – 28:05 min

To address these challenges, it is necessary to understand how an assembly line like DEBS works. Each module undergoes a catalytic cycle that consists of the following events (using module 3 as an example):

Translocation: the ACP of the upstream module 2 transfers the polyketide chain to the KS of module 3.

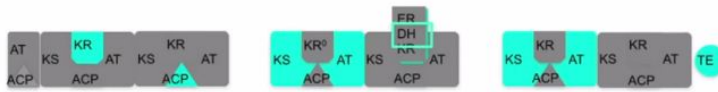
Acyl transfer: the AT of module 3 selects a precursor and transfers it onto the ACP.

Elongation: a carbon-carbon bond is formed between the precursor and the polyketide chain, which extends the chain. This is the key energy-giving step, as the release of CO₂ drives this catalytic cycle forward.

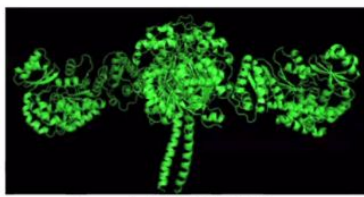
Modification: KR₀ catalyzes racemization.

Translocation: the polyketide chain is transferred to the downstream module 4.

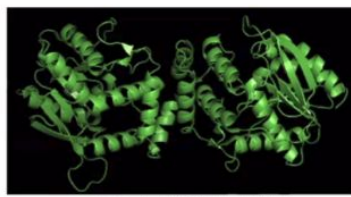
The Pieces



Keatinge-Clay, et al. (2006) *Structure*

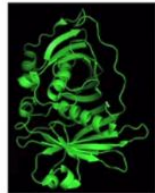


Tang, et al. (2006) *PNAS*



Tsai, et al. (2001) *PNAS*

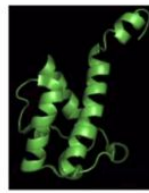
ACP = acyl carrier protein
 AT = acyltransferase
 KS = ketosynthase
 KR = ketoreductase
 DH = dehydratase
 ER = enoylreductase



Keatinge-Clay (2008) *J. Mol. Biol.*



Broadhurst, et al. (2003) *Chem. Biol.*



Alekseyev, et al. (2007) *Prot. Sci.*

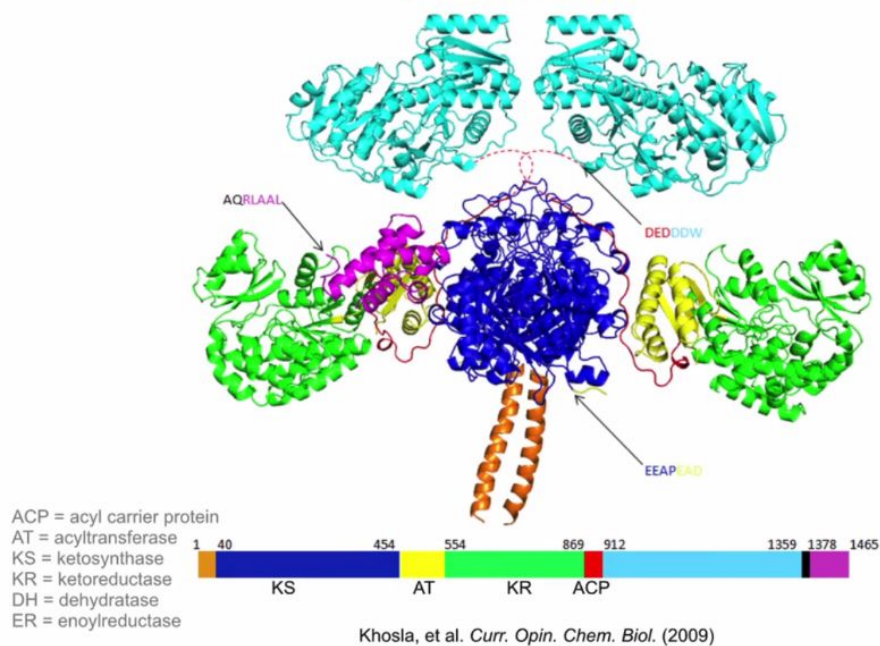


28:05 – 30:55 min

To further our knowledge of how polyketide assembly lines function, it is useful to gain an understanding of their 3-dimensional structures.

Using X-ray crystallography and NMR, the atomic structures of many portions of DEBS have been solved. This was done by extracting these portions out of the assembly line and analyzing them individually. DEBS is repetitive and uses the same active sites over and over throughout the assembly line, and the domains in the different modules are very homologous to one another. Therefore, solving the structure of a prototypical domain gives insight into the structure of that domain within all the different modules.

The Module



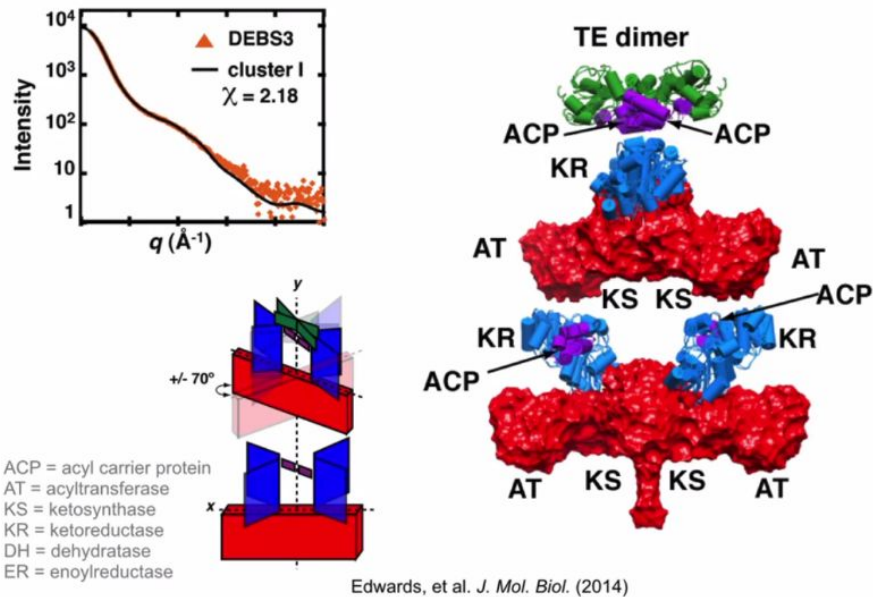
30:55 – 32:30 min

Starting from the atomic structures of the individual domains, it is possible to build models for how each domain might be arranged within a module.

The relative orientations of the domains with respect to one another in this model are somewhat speculative.

However, the atomic structure of a homolog of the polyketide synthase (the vertebrate fatty acid synthase) has been solved, and comparison with this structure provided the basis for this model.

SAXS Analysis of Two Successive Modules



32:30 – 34:20 min

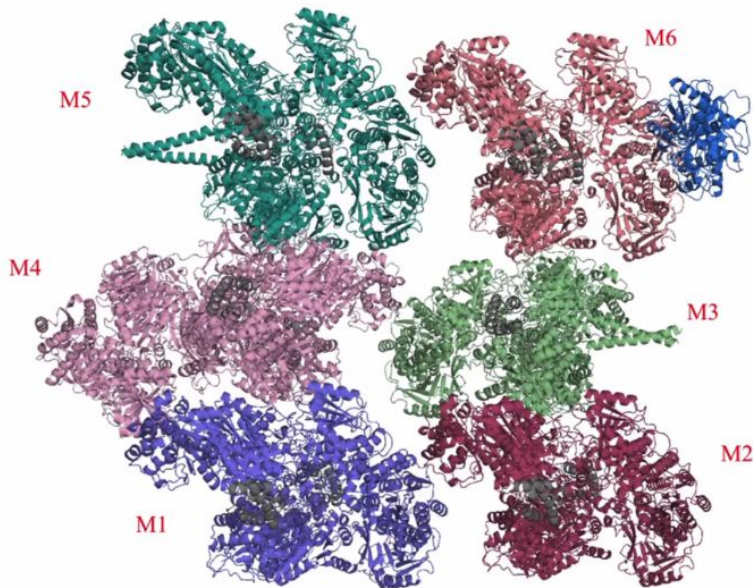
In order to gain additional data regarding the 3-dimensional structures of modules, low-resolution methods can be used.

One such method is small angle X-ray scattering (SAXS).

This technique can be used to analyze a protein's size and shape. Therefore, SAXS analysis can help to refine the models that were constructed based on homology with the fatty acid synthase.

An advantage of low-resolution methods is that they can be used to study larger portions of the assembly line, such as two adjacent interacting modules.

The DEBS Assembly Line



34:20 – end

By combining all of these structural models of individual domains and modules, it is possible to construct a model of how the entire DEBS assembly line might be arranged in 3-dimensional space.

The current model predicts that the growing polyketide chain would move through the assembly line in a serpentine pattern.

3. Review Questions

1. Polyketide biosynthesis is analogous to which of the following:
 - a. A game of chess
 - b. An automobile assembly line
 - c. Driving a car
 - d. None of the above
2. SAXS is a technique that can be used to study which of the following?
 - a. The growth rate of bacterial cells.
 - b. The atomic structure of small proteins.
 - c. The metabolic processes occurring within bacteria.

- d. The size and shape of larger proteins.
3. Why is DEBS important?
- a. It creates a key intermediate in the synthesis of the antibiotic erythromycin.
 - b. It is required for proper cellular metabolism, and without it bacteria cannot live.
 - c. It degrades the antibiotic ampicillin, allowing bacteria to survive ampicillin treatment.
 - d. It is an essential component of the bacterial cell wall.
4. Which of the following is true?
- a. The exact 3-dimensional structure of DEBS is known.
 - b. Only the structures of certain domains of DEBS are known.
 - c. The structure of DEBS is completely unknown.
5. Put these events in order (using the catalytic cycle of DEBS module 3 as an example):
- a. Acyl transfer (2)
 - b. Modification (4)
 - c. Translocation from the ACP of module 2 (1)
 - d. Translocation to the KS of module 4 (5)
 - e. Elongation (3)
6. True or False: DEBS has many repetitive elements, and therefore solving the structure of one domain can give insight into the structure of homologous domains.
- a. True
 - b. False

4. Answers to Review Questions

- 1. B
- 2. D
- 3. A
- 4. B
- 5. C, A, E, B, D

6. A

5. Discussion Questions

1. What is significant about the way polyketide antibiotics are synthesized? Why might such a system have evolved in nature?
2. Why would it be useful to understand the individual components of polyketide biosynthesis assembly lines?
3. What do all of the DEBS modules have in common? What implication does this have for the study of DEBS?
4. What techniques have been used to analyze the structures of polyketide assembly lines like DEBS? What advantages/disadvantages do these different techniques have? How could structural models be useful?
5. If you were to join the field of polyketide assembly lines, what would you want to study?

6. Answers to Discussion Questions

1. Polyketide antibiotics are synthesized using “assembly lines” consisting of multiple modules. Each module performs a specific task in a manner that is independent from what is happening upstream and downstream of that module. Therefore, the same assembly line can produce different outputs simply by changing a catalyst or input at a specific module. Such a system would allow for the rapid evolution of novel functions. For example, a mutation in one of the modules could result in the creation of a completely new product, which could be advantageous for the bacteria.
2. Each module carries out a unique part of the overall biosynthetic process. Understanding how each domain functions within a module in a well-known assembly line like DEBS may make it easier to predict what “orphan” assembly lines are doing. Furthermore, it may be possible to alter these domains or mix-and-match entire modules to promote the synthesis of a product of interest, or prevent the creation of an unwanted product.
3. All of the DEBS modules share the same types of catalytic domains, such as the ketosynthase and acyltransferase. Each of these domains is highly homologous to the same domains in the other modules. Therefore, solving the atomic structure of at least one prototypical domain is very informative and gives insight into the structure

of all of the homologous domains. This greatly facilitates the analysis of the structure of an extremely large assembly line like DEBS.

4. To analyze the atomic structure of catalytic domains, X-ray crystallography and NMR have been performed. These techniques can be used to study structure at the atomic level, however they can only be used on relatively small samples. Therefore, to use these techniques the domains were analyzed independently instead of in the context of their module. SAXS analysis is a lower-resolution method that gives information about a protein's general size and shape. While SAXS cannot be used to study proteins at the atomic level, the advantage of such lower-resolution techniques is that they can be used to study much larger samples, such as two adjacent modules. By combining these types of analyses (along with comparison to homologs), it is possible to make models of the structure of the entire DEBS assembly line. These models are useful because it is important to understand the physical interactions between the domains/modules in order to begin manipulating them to generate a product of interest.
5. Possible answers may include:
 - a. I would like to further study DEBS. Since so much is already understood about this assembly line, it would be the easiest to use this as a starting point to then manipulate to create new products.
 - b. I would like to study "orphan" assembly lines. Since it is unknown what these assembly lines do, studying these assembly lines could be very informative. I may discover that some of them are generating products that would be useful for medicine or other applications.

7. Questions for Discussion Paper

Walker, MC. et al. Expanding the fluorine chemistry of living systems using engineered polyketide synthase pathways.

Science. 2013 Sep 6; 341(6150): 1089–1094.

1. What was the overall goal of this research? Why would achieving this goal be useful?

2. How are the experiments shown in figures 2A and 2B similar? What is different about them?
3. Describe the experimental set-up depicted in Figure 3. What are the two main results from this experiment?
4. What is the purpose of making the different mutations shown in Figures 4C and D? How do these mutations affect the DEBS modules, and how is this useful?
5. What traits of *E. coli* are important for the *in vivo* chain extension reactions using fluoromalonyl-CoA?

8. Answers to Questions for Discussion Paper

1. The goal of this research was to develop a method of introducing fluorine into small molecules that could serve as building blocks for complex fluorinated products. Specifically, fluoromalonyl-CoA could act as an extender unit for polyketide biosynthesis, enabling the introduction of fluorine into structurally complex and biologically active compounds. This would be useful because many pharmaceutical compounds contain at least one fluorine atom. Organofluorines are also used in diverse fields including diagnostics and agriculture.
2. They are similar because they both result in the production of fluoromalonyl-CoA. However, they use different pathways to create this product. In 2A, a two-step reaction is used starting from fluoroacetate. This is important because fluoroacetate is produced naturally by certain bacteria. In 2B, CoA is directly ligated to fluoromalonate.
3. This experiment tests whether a fluorinated extender can be used in a chain elongation reaction. Fluoromalonyl-CoA was used as an extender to lengthen an acetyl-CoA starter. This reaction was catalyzed by a simple ketosynthase NphT7 and the ketoreductase PhaB. The two main results are that the ketosynthase could efficiently use fluoromalonyl-CoA as an extender to create acetofluoroacetyl-CoA, and that the ketoreductase was capable of reducing this fluorinated product. Since chain elongation and ketoreduction are key steps in the catalytic cycle of polyketide synthases, it was important to verify that both of these processes could occur with the fluorinated substrate.
4. These mutations were made in order to alter the specificity of the DEBS modules. Normally these modules have a high preference for methylmalonyl-CoA over

fluoromalonyl-CoA. However, the mutations shown in these figures confer a preference for fluoromalonyl-CoA. This is useful because by combining these mutated and normal DEBS modules, fluorine could be incorporated in a site-specific manner. Importantly, in 4D they find that a two-step chain elongation reaction can occur even when fluorine is incorporated in the first step, indicating that fluorinated intermediates can be tolerated in downstream reactions.

5. The DEBS modules have a preference for methylmalonyl-CoA over fluoromalonyl-CoA, however *E. coli* contain almost no methylmalonyl-CoA. While they do have a sizable pool of malonyl-CoA, this is much less efficiently incorporated by the DEBS modules than fluoromalonyl-CoA is. Therefore, fluoromalonyl-CoA would face little competition as an extender unit. Another useful aspect of performing *in vivo* chain extension reactions is that *E. coli* continually generate ATP through normal metabolic processes, whereas ATP needed to be added to all of the *in vitro* reactions.