Correction

Correction for “Demonstration of the importance for cellulose hydrolysis of CelS, the most abundant cellulosomal cellulase in Clostridium thermocellum,” by David B. Wilson, which appeared in issue 42, October 19, 2010, of Proc Natl Acad Sci USA (107:17855–17856; first published October 4, 2010; 10.1073/pnas.1012746107).

The author notes that the title of his manuscript appeared incorrectly. The title should appear as “Demonstration of the importance for cellulose hydrolysis of CelS, the most abundant cellulosomal cellulase in Clostridium thermocellum.” Additionally on page 17855, middle column, first paragraph, line four, Clostridium should instead read Clostridium. The online version has been corrected. This error does not affect the conclusions of the article.

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Demonstration of the importance for cellulose hydrolysis of CelS, the most abundant cellulosomal cellulase in Clostridium thermocellum

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Aerobic and anaerobic bacteria use different mechanisms to degrade cellulose, with many aerobic microorganisms secreting a synergistic set of individual enzymes that includes multiple cellulases, most of which contain a carbohydrate-binding module (CBM) in addition to their catalytic domain (free enzyme mechanism). Anaerobic microorganisms usually produce a similar set of enzymes, but they are present in large multienzyme complexes (>1 million molecular weight) called cellulosomes (cellulosomal mechanism) (1). Most of the cellulosomal enzymes do not contain a CBM, but the scaffoldin protein in the cellulosome does contain a CBM. At this time, there are still a number of unanswered questions about how cellulosomes are able to degrade cellulose so well, despite their large size, which should limit their access to the cellulose surface, much of which is present in narrow pores (2). One suggestion is that enzyme proximity on the cellulosomes increases the rate of cellulose degradation, but it would seem that enzyme proximity would be most effective if enzymes were incorporated in a specific order in cellulosomes. All of the different cohesin domains in the scaffoldin protein have equal affinity for all of the dockerin domains present on the cellulosomal enzymes so that it is thought that the enzymes are randomly incorporated into cellulosomes, but this has not been proven. Synergism between cellulases that have different sites of attack (i.e., endocellulases and exocellulases) is important for crystalline cellulose degradation by both mechanisms, because the specific activity of a synergistic mixture can be up to 15 times that of the most active individual cellulase (3).

Importance of Exocellulases for Crystalline Cellulose Hydrolysis

Exocellulases are known to play a major role in the hydrolysis of crystalline cellulose in both the free enzyme mechanism and the cellulosomal mechanism (1). There are two classes of exocellulases: one class, found in families GH-7 and GH-48, attacks the reducing ends of cellulose molecules, processively releasing cellobiose, whereas the other class, found in family GH-6, attacks the nonreducing ends in the same way (4). Exocellulases from the different classes show synergism on crystalline cellulose (4). Many aerobic microorganisms produce an exocellulase from each class. However, Clostridium thermocellum, an anaerobic, cellulolytic, thermophilic soil bacterium, which is the most studied cellulosomal microorganism (5), only produces a single cellulosomal exocellulase, originally called CelS. The lack of a nonreducing end attacking exocellulase may result from the fact that anaerobic microorganisms get much less ATP from a glucose molecule than aerobic organisms and therefore need to produce longer oligomers from cellulose hydrolysis than aerobic microorganisms to obtain enough energy to grow on cellulose (6). Longer oligomers produce more ATP, because the ATP-binding cassette transporter that takes the oligomers into the cell uses one ATP per oligomer, regardless of the length of the oligomer (6), and C. thermocellum contains a phospholipase that produces one ATP for each β 1–4 linkage in the oligomer (6). Replacing a nonreducing end attacking exocellulase with a processive endoglucanase that also attacks the nonreducing end of cellulose chains, but that produces cellobiose, significantly increases the average size of the oligomers produced from cellulose. C. thermocellum produces three such endocellulases, and because they have similar activities, it is not known why there are multiple enzymes with this activity. CelS is the most abundant cellulosomal enzyme in C. thermocellum cells grown on cellulose (7, 8), and CelS has been shown to be important for crystalline cellulose hydrolysis in reconstituted cellulosomes and designer cellulosomes (7, 9).

Daniel et al. develop a method for deleting genes in C. thermocellum and use it to knockout the CelS gene.

Lack of CelS Specifically Reduces the Growth Rate and Cell Yield of C. thermocellum on Cellulose

In PNAS, Daniel et al. (10) develop a method for deleting genes in C. thermocellum and use it to knockout the CelS gene (10). The knockout strain grows on cellulose at about the same rate and with the same cell yield as the wild-type (WT) strain. However, it grows on cellulose at about one half the rate of the WT strain, and cellulosomes from this strain degrade cellulose at about one half the rate of WT cellulosomes. This result is consistent with what was found with a related enzyme CelF from C. cellulolyticum, where RNAi was used to inhibit CelF synthesis (11). The final biomass yield of the mutant strain grown on cellulose is 60% of the WT strain, although both strains completely degraded the cellulose in the medium. C. thermocellum also produces a noncellulosomal GH-48 exocellulase, but it was shown that this enzyme is expressed at a low level by the knockout strain; additionally, a double knockout strain lacking both genes behaved the same as the single knockout strain. Proteomic studies showed that, in addition to eliminating CelS, the deletion strain had change in the amounts of four other cellulosomal proteins, increasing three and decreasing one. However, two of these proteins are noncatalytic, and the other two were present in such low amounts that these changes would not be expected to have much effect on cellulosomal activity.

Two possible explanations for the reduced biomass production from cellulose by the knockout strain are that a larger fraction of the energy derived from cellulose hydrolysis may be used to make cellulosomes to compensate for their lower specific activity or that the average chain length of the cellulose-oligomers produced by the cellulosomes...
may be lower for the mutant than for WT cellulosomes, which would reduce the amount of ATP available from the cellulose to the mutant cells. Because the primary product of CelS is cellobiose, one would expect that removing it would increase the average chain length of the oligomers produced by the knockout cellulosomes. However, there are strong synergistic interactions occurring between the different enzymes on the cellulosomes, and therefore, it is possible that the average length of the oligomers produced by the knockout cellulosomes was decreased because of a change in synergism. The knockout approach should help answer some of the questions that remain about the role of the many different proteins that are present on cellulosomes, especially those that currently have no known function.