Defining Statement

Cellulases are enzymes (EC # 3.2.1.4) that catalyze the hydrolysis of the \(\beta-1, 4\) glucosyl linkages present in the insoluble linear glucose homopolymer, cellulose. Cellulases are important industrial enzymes and they play a major role in the global carbon cycle. They are produced by all plants, many fungi, some bacteria, and a few animals.

Introduction

Cellulases are enzymes that catalyze the hydrolysis of the \(\beta-1, 4\) linkages present in the linear glucose polymer, cellulose (EC # 3.2.1.4). They are members of a class of enzymes called glucoside hydrolases and these enzymes are cataloged into sequence-related families on the CAZy website: http://www.cazy.org/.

Plant Cell Wall Cellulose

The substrate for true cellulases in nature is the cellulose present in plant cell walls. Plant cellulose is very difficult to degrade, because it is insoluble and contains highly ordered crystalline regions in which the cellulose chains are closely packed and are inaccessible to enzymes. In the cell wall, cellulose is coated by other sugar polymers called collectively, hemicellulose, which is primarily a mixture of xyloglucan, xylan, and mannan, whereas in secondary cell walls lignin is also present. Cellulose is the most abundant natural polymer with about \(10^{12}\) tons produced by plants each year. Cellulose is synthesized by membrane-bound complexes of cellulose synthases as parallel bundles of about 30 cellulose chains and each chain can contain...
20,000 glucose residues. These bundles are called microfibrils and they interact to form fibrils, which interact to form wood fibers.

**Cellulose Turnover**

Some cellulose is recycled by fire or by photodegradation but cellulases play an important role in the carbon cycle by degrading cellulose in dead plant material. Aerobic microorganisms, both bacteria and particularly fungi, carry out much of the cellulose degradation occurring in soils. However, some anaerobic bacteria and a few anaerobic rumen fungi also degrade cellulose, either in the rumen compartment of ruminants such as cows, sheep, and deer, or in association with termites or in soil (Clostridium thermocellum, Clostridium cellulovorans, and Acetivibrio cellulosolvens). Almost all cellulytic microorganisms secrete their cellulases outside their cell wall, as bacteria and fungi are unable to transport insoluble materials, such as cellulose, inside the cell. The soluble sugars produced by the hydrolysis of cellulose are transported inside the cell and metabolized.

**Animal Cellulases**

It is now clear that some cellulytic insects produce their own cellulases and also harbor symbiotic cellulytic microorganisms. It is not exactly known how the cellulases produced by the different organisms present in insect guts function in cellulose degradation. A recent paper described sequencing the metagenome from the hindgut of a termite, which showed that many different plant cell wall-degrading enzymes from many different microorganisms were present. Some cellulytic termites (Macrotermitinae) utilize aerobic symbiotic cellulytic fungi (Termitomyces) to break down plant material in their nests and then eat the fungi and residual plant material. There also are a few other animals that produce cellulases, including several types of mollusks particularly snails, slugs, and shipworms. Cellulytic mollusks play an important role in plant cell wall degradation by grinding up the biomass material into small particles, thus making it much easier for the cell wall-degrading enzymes in their digestive system to access and hydrolyze the sugar polymers. In effect, the animals are pretreating the biomass they ingest, making it more accessible to their enzymes and to those of their symbiotic microorganisms.

**Plant Cellulases**

Plants produce cellulases, and different plant cellulases have quite different roles in plant physiology; some plant cellulases clearly function in degrading cellulose, such as those involved in fruit ripening or leaf abscission in the fall. Others are involved in growth and remodeling of plant cell walls and their exact role in these processes is not clear. Finally, there is a membrane-bound cellulase, which is required for cellulose biosynthesis. Its exact role in this process is not known, although it might release cellulose chains from cellulose synthetase. Most plant cellulases do not contain a carbohydrate-binding module (CBM); however some of those that clearly function in cellulose degradation do contain one or more CBMs.

**Cellulase Kinetics**

The specific activities of individual cellulases on crystalline cellulose are much lower than those of most enzymes, because of the recalcitrance of cellulose. However, in terms of catalytic enhancement, cellulases are very active enzymes, as the half-life of crystalline cellulose in water at neutral pH is estimated to be about 100 million years. It takes concentrated sulfuric acid at 125°C to hydrolyze native cellulose at a reasonable rate. When cellulases are assayed on low-molecular-weight soluble substrates, they show normal Michaelis–Menten kinetics and some of them have high specific activities, showing that they are basically similar to other enzymes. However, when cellulases are assayed on insoluble substrates they have very different properties, as the assays are usually nonlinear with time and with the amount of enzyme. Different studies have produced different explanations for this behavior. The nonlinearity of *Thermobifida fusca* Cel6A on amorphous cellulose was shown to be due to the heterogeneity of the substrate.

**Cellulase Assays**

An important step in studying any enzyme is the development of a specific assay for the enzyme. Most cellulase assays measure the production of reducing sugars from a high-molecular-weight form of cellulose, as every cleavage event produces a new reducing end and long polymers have few ends per mg. Endocellulases can also be assayed by measuring the decrease in the viscosity of carboxymethyl cellulose (CMC), which they cause by reducing the size of CMC due to internal cleavages. Another way to assay cellulases is to use a particle counter to measure the increase in the number of cellulose particles produced by a cellulase incubated with a uniform-sized cellulose preparation. This assay gives different kinetics of hydrolysis than measuring the increase in reducing ends, as it is linear with both time and enzyme. This assay requires particles that are larger than 100 microns in diameter, because smaller particles did not show an increase in particle number during hydrolysis.
even though there was an increase in reducing ends. It is not clear exactly how particles are fragmented but it may involve the cellulose pore structure.

**Cellulase Diversity**

There are two known cellulase mechanisms: hydrolysis with retention of the stereochromy of the anomeric hydroxyl group, and hydrolysis with inversion of the anomeric hydroxyl group. One important difference between these mechanisms is that most retaining enzymes can catalyze transglycosylation and hydrolysis, whereas no known inverting enzyme catalyzes transglycosylation. However, there is no obvious difference in the hydrolytic activities of cellulases that utilize the different mechanisms. Cellulases are named by the family number of their catalytic domain (CD), followed by a capital letter that is assigned based on the order in which family members were discovered in a given organism, with A being used for the first, and so on.

Although cellulose is a homopolymer of glucose, with only a single type of linkage (β-1, 4), cellulases are very diverse in their sequence structures and mechanisms. There are 14 cellulase families listed on the CAZy website and several of these families (10, 26, 51, 74) mainly contain other types of glycosyl hydrolases, with only a few members in each family having cellulase activity but, even if these families are excluded, there are still ten cellulase families. All members of a glycosyl hydrolase family have the same basic protein fold and utilize the same catalytic mechanism but their substrate specificities and mode of attack can be quite different. Because there are many amino acid sequences that can give the same protein fold, several families share the same fold, even though the sets of sequences in each family show little similarity between the families.

There are nearly twice as many cellulase families, compared to the next largest group of hydrolases, the seven families of xylanases. Furthermore, there are seven different protein folds among the known cellulase structures, and a structure has not been determined for any family. There are two possible reasons for this cellulase diversity. One reason is that the actual substrate of most cellulases is not pure cellulose but rather plant cell walls, which are extremely diverse and complex, containing many other components, some of which are bound to the cellulose fibrils. The other reason is that cellulose itself is quite complex with both crystalline and amorphous regions. Because cellulose is insoluble, cellulases have to bind to a cellulose particle, rather than to a single cellulose molecule and there are differences in the interactions between cellulose chains created by the detailed structures of cellulose particles. It appears that cellulases are under positive selection because when the DNA and protein sequences of two related cellulase genes were compared, there were nearly as many DNA changes that caused an amino acid change (nonsynonymous) as DNA changes that did not change the amino acid (synonymous).

**Functional Cellulase Classes**

There are three functionally different types of cellulases: endocellulases, also called endoglucanases, exocellulases, also called cellobiohydrolases (EC # 3.2.1.91), and processive endocellulases, which were discovered later. All endocellulase CDs whose structures have been determined have an open active site, as would be expected, because they are able to bind to the interior of long cellulose molecules. In contrast, all exocellulases have their active sites in a tunnel, consistent with their processive activity. In the case of glycosyl hydrolase family GH-48 enzymes, only part of the active site is in the tunnel, but these enzymes are just as processive as family GH-7 enzymes, in which the entire active site is in a tunnel.

Two classes of exocellulases exist: one class attacks the nonreducing end of a cellulose molecule, cleaving off cellobiose residues, and all known members of this class are in family GH-6. Members of the other class attack the reducing end of a cellulose chain, cleaving off cellobiose residues, and all aerobic fungal members of this class are in family GH-7, whereas the bacterial members are in family GH-48. The anaerobic fungal reducing end attacking exocellulases are also in family GH-48, rather than in family GH-7, which is surprising because family 7 exocellulases are more active than those in family GH-48. There are a few published reports claiming that enzymes from other families are exocellulases but these reports are not well documented and they are probably endocellulases. The most studied example is CBHA from *C. thermocellum*, which has a crystal structure, but unlike other exocellulases, its active site is not in a tunnel. A careful study clearly showed that CBHA is an endocellulase like most other family 9 cellulases. There are several assays, including determination of the percentage of insoluble reducing sugars that are produced, lack of reduction in the viscosity of CMC, and the determination of the products of cellohexose hydrolysis, that clearly distinguish between exocellulase and endocellulase activity. All of them should be used to give a clear result before an enzyme is called an exocellulase.

All well-documented processive endocellulases are in family GH-9, which is the largest cellulase family and includes most plant cellulases, animal cellulases, many bacterial cellulases, but very few fungal cellulases. Processive endocellulases have an open active site cleft, like all endocellulases, but in addition they contain a family 3c CBM, which is rigidly attached to the C-terminus of the CD. The two domains are oriented so that a cellulose chain can bind...
simultaneously to both domains. The family 3c CBMs, present in processive endocellulases, differ from family 3a and 3b CBMs, in that they lack the conserved aromatic residues, which cause high affinity for cellulose. Although 3c CBMs bind very weakly to cellulose, it has been shown that they are required for the processive activity of these enzymes, which sequentially cleaves off cellotetraose residues from the nonreducing end of the cellulose fragment produced in the original endocellulolytic cleavage.

A fourth enzyme, called cellobiase or β-glucosidase, is required to completely hydrolyze cellulose to glucose. β-Glucosidase hydrolyzes the soluble oligosaccharides, produced by cellulases, to glucose. Many aerobic fungi secrete a β-glucosidase as part of their crude cellulase, whereas most cellulolytic aerobic bacteria do not, and their β-glucosidases are usually cytoplasmic. Some organisms, mainly anaerobic bacteria, contain a cytoplasmic cellobiase phosphorylase, also called dextrin phosphorylase, which converts cellobiase and soluble dextrans to glucose and glucose-1-phosphate, conserving the energy in the cellobiase linkage.

**Synergism**

In both aerobic and anaerobic organisms, certain cellulases can act synergistically on crystalline cellulose with the specific activity of some mixtures being 10 times more than that of any single cellulase in the mixture. Synergism is usually seen only in the digestion of substrates that contain crystalline cellulose, probably because there are only a few regions in this substrate that are accessible to each cellulase, causing access to the substrate to limit the rate of hydrolysis. It seems likely that synergism occurs only when two cellulases attack different regions of the cellulose surface and each one creates new sites of attack for other enzymes in the mixture. There is no evidence that synergism requires interactions between the synergizing cellulases, as cellulases from unrelated organisms show similar synergism to those from the same organism. All good endocellulases appear to act synergistically with any exocellulase, but most endocellulases do not show synergism with each other. Exocellulases show synergism with other exocellulases, but only if they attack different ends of the cellulose chain. Processive endocellulases can show synergism with all other types of cellulase. Cellulose pretreated with an endocellulase is a better substrate for most exocellulases, than is untreated cellulose, but the reverse is not true. It has been shown in synergistic mixtures of an exocellulase and an endocellulase that endocellulase activity is increased as much in the mixture as is exocellulase activity. Therefore, it may be that when an exocellulase degrades a cellulose chain, it disrupts some regions in adjacent cellulose molecules, making them more available to an endocellulase. However, over time, the molecules in the disrupted regions may reform their interactions with adjacent chains, so that an endocellulase may only be able to attack the transiently disrupted chains when it is present along with the exocellulase.

**Mechanisms of Cellulose Degradation**

There appear to be at least three different ways by which cellulolytic microorganisms degrade cellulose. Most aerobic cellulolytic microorganisms use the free cellulase mechanism in which they secrete a set of individual cellulases (six to ten), each of which contains a CBM joined by a flexible linker peptide to the CD. Additional domains of unknown function are often present in cellulases. The CBM can be N-terminal or C-terminal to the CD and the location probably does not affect enzyme activity. Certain cellulases act synergistically to degrade crystalline cellulose so that the mixture can be 15 times more active than any single cellulase. In several aerobic microorganisms, the cellulase mixture contains one member of each class of exocellulase and multiple endocellulases, with the exocellulases making up more than 50% of the total cellulase protein. However, as more cellulolytic microorganisms are studied the variations in the cellulases in the mixtures have become larger.

Most anaerobic microorganisms use the cellulosomal mechanism producing large (>1 million MW) multienzyme complexes, called cellulosomes, which are usually bound to the outer surface of the microorganism. Only a few of the enzymes in cellulosomes contain a CBM, but the scaffoldin protein that contains multiple cohesin domains does contain a family 3 CBM, which binds the cellulosome to cellulose. Enzymes that are present in cellulosomes contain a small domain, called a dockerin domain, that binds them to the scaffoldin protein by binding very tightly to a cohesin domain. The dockerin domain is joined to the CD by a flexible linker peptide, usually at its C-terminus. Processive cellulosomes are major cellulosomal enzymes, although bacterial cellulosomes do not contain an exocellulase that attacks the nonreducing end of cellulose. However, they do contain an exocellulase that attacks the reducing end of the cellulose and several processive endocellulases, which attack the nonreducing end. Cellulosomal enzymes also act synergistically and cellulosomes contain a large number of different types of plant cell wall-degrading enzymes, with 72 different cellulosomal genes (dockerin containing) identified in *C. thermocellum*. An important unanswered question about cellulosomes is do the cellulosomal enzymes bind randomly to the cohesin domains of a given scaffoldin molecule or is there a mechanism that binds them in a defined way? It appears that in a given organism, all the different dockerins on the enzymes have the same affinity for all the different cohesin domains, so that if there is self-assembly, binding is likely to be random, creating a vast number of different types of cellulosomes. The cellulosomal mechanism is as effective in cellulose
degradation as the free enzyme mechanism, even though it would be expected that the large size of cellulosomes would make it difficult for them to access cellulose that is present in small pores, which contain a large amount of the total cellulose surface area in plant biomass. Cellulase in these pores can be accessed by free cellulases.

The third strategy appears to be used by at least two cellulytic bacteria: *Cytophaga hutchinsonii*, an aerobe and *Fibrobacter succinogenes*, an anaerobe. The DOE Joint Genome Institute has determined the DNA sequence of the *C. hutchinsonii* genome (http://genome.jgi-psf.org). *C. hutchinsonii* codes for a number of cellulase genes, most of which do not encode a CBM, none of which encodes a dockerin domain, and all of which appear to code for endoglucanases. Thus, this organism does not code for any processive cellulases, which are major components of the cellulases of most other cellulytic microorganisms. These results clearly distinguish *C. hutchinsonii* from most other well-studied aerobic and anaerobic cellulytic microorganisms. The genome sequence of the ruminal bacterium *F. succinogenes* also has been determined. This organism also does not code for any known processive cellulases and only one of its many endocellulases that have been cloned and sequenced binds to cellulose. *F. succinogenes* does not encode dockerin domains and a scaffoldin gene has not been identified. *F. succinogenes* grows very rapidly on cellulose, so that its cellulose-degrading mechanism is very efficient. One possible mechanism for cellulose degradation by these two organisms is the one proposed for starch degradation by *Bacteroides thetaiotaomicron*. In this mechanism, starch is bound to a complex present in the outer membrane and individual molecules are transported into the periplasmic space, where they are degraded by starch-degrading enzymes. This mechanism would not require processive cellulases, as individual cellulose molecules would be readily degraded by endoglucanases. If this is the process by which cellulose is degraded, it will be very interesting to determine the mechanism by which the outer membrane proteins are able to bind and transport individual cellulose molecules. It is possible that this information would allow the design of new cellulases or cellulose-modifying proteins, which would be able to increase the rate of cellulose degradation by free cellulases or cellulosomes.

**Carbohydrate-Binding Modules**

CBMs play an important role in the ability of cellulases to degrade crystalline cellulose. They have little or no effect on the activity of most cellulases on soluble cellulose derivatives such as CMC or oligosaccharides, or on insoluble amorphous cellulose, although the activity of processive endoglucanases on amorphous cellulose is reduced when the binding CBM is removed. A major role of the CBM is to anchor the cellulase to insoluble cellulose, so that the CD remains close to the substrate long enough to allow the CD to bind a segment of a cellulose molecule into its active site. The flexible linker that separates the CBM from the CD allows the CD to access regions of the cellulose, adjacent to the bound CBM. Some workers have proposed that a CBM can also disrupt the structure of cellulose, making cellulose chains more accessible to the CD but this is still controversial. This activity would be equivalent to the Cx activity proposed by Reese, in his early discussion of the nature of cellulases. CBMs have also been reported to target cellulases to specific regions of cellulose, presumably to regions where they will be most active. It has been shown that family 2 CBMs can diffuse on the surface of cellulose without dissociation, giving them the ability to readily access new regions of a cellulose particle after the CD has hydrolyzed the cellulose near the original site of binding. Other CBMs that bind to crystalline cellulose may also have this ability, because they appear to bind in a similar way.

There are many CBM families with 45 listed on the CAZy website (http://afmb.cnrs-mrs.fr/CAZY/fam/acc_CBM.html). Not all CBMs bind cellulose, because many families contain chitin-binding domains, xylan-binding domains, or mannan-binding domains. Some CBMs can bind to several polymers, whereas most are specific for only one. Labeled CBMs can be used to stain plant materials, and different members of a given family can give very different staining patterns, showing that there is even greater binding specificity than is seen with pure substrates. Almost all enzymes that have high activity on insoluble substrates contain a substrate-binding domain, in addition to a CD, so that the presence of such a domain is a general property of enzymes that degrade insoluble substrates.

All fungal cellulase CBMs are in family 1 and they are small, containing about 30 amino acids. Most aerobic bacterial cellulase CBMs are in family 2 and they are larger, containing about 120 amino acids. The CBMs on cellulosomal scaffoldins are in family 3. Most of the CBMs in these three families bind to crystalline cellulose and have a relatively flat binding surface that usually contains three aromatic residues spaced so they can bind to three adjacent glucose residues in a cellulose molecule. They also contain a number of residues, which can hydrogen bond to the cellulose chain. Site-directed mutagenesis has shown that the aromatic residues are essential for high-affinity binding whereas the other residues play a secondary role. The CBMs in families 4 and 6 bind to single cellulose molecules and their binding sites are in a groove and again aromatic residues are important for binding. A number of cellulases contain multiple CBMs and in some cases they are from the same family and in other cases they are from different families. It has been shown that the affinity of a protein containing two CBMs can be significantly higher than a protein with only one. An atomic force microscopy study of the binding of a family 1 CBM to bacterial cellulose found that the bound CBM was present in aggregates, and not as
Cellulase Substrate Preference

Several studies have analyzed the properties of the cellulase that remained after significant hydrolysis by a pure cellulase to identify the preferred sites of attack for that cellulase (amorphous or crystalline regions), as well as to determine how the enzyme has changed the average chain length of the cellulose. A study of *Trichoderma reesei* Cel7A, an exocellulase, and *T. reesei* Cel7B, an endocellulase, found as expected that Cel7A did not cause large changes in the cellulose chain length whereas Cel7B did. A study of four *Cellulomonas fimii* cellulases acting on Sigma cellulose found that the endocellulase Cel5A rapidly reduced the chain length of cellulose, whereas another endocellulase Cel6A had a lesser effect on chain length. Both enzymes increased the crystallinity of the residual cellulose, suggesting that they preferentially degrade amorphous regions in the cellulose. The two exocellulases tested, Cel6B and Cel48A, had no effect on chain length and Cel6B increased the crystallinity of the residual cellulose, whereas Cel48A decreased its crystallinity. Four synergistic mixtures were tested and none of them caused significant differences in crystallinity, showing that both crystalline and amorphous regions were attacked at about the same rate. Another study of comparable enzymes from *T. fusca* showed that both the endocellulase Cel5A and the exocellulase Cel6B primarily digested amorphous cellulose, whereas the processive endocellulase Cel9A digested both types of cellulose, although Cel9A also preferred amorphous regions.

Cellulase Regulation

Extensive studies on the regulation of cellulase synthesis in *T. reesei* have been conducted, and it appears that this regulation is very complex. Glucose strongly represses cellulase synthesis and the β-1,2 linked glucose disaccharide, sophorose, induces synthesis. A number of transcription factors have been identified in *T. reesei*, which can bind to cellulase promoters, and some of these are activators and some are repressors. The exact mechanisms that regulate cellulase synthesis are still not completely understood. Cellulase synthesis in *T. fusca* and many cellulolytic bacteria is regulated by at least two mechanisms: induction by cellulbiose or laminariobiose (β-1,3-glucose disaccharide), and repression by any good carbon source including cellulbiose. This is reasonable, as the extracellular enzymes secreted by *T. fusca* grown on cellulose make up about 50% of the total protein in the culture. If there is sufficient sugar for growth or no cellulose present, there is no reason to synthesize cellulases. The cellulbiose required for induction is produced from cellulose by the uninduced level of secreted cellulase, which is mainly Cel6A. CelR is a regulatory protein that is a member of the Lac I gene family, and it binds to a 14 base inverted repeat sequence: TGGGAGCGCTCCCA. This sequence is upstream of the start site of all six *T. fusca* cellulase genes coding for secreted cellulases, as well as the genes for a number of other secreted proteins induced by growth on cellulose as well as an operon that codes for a cytoplasmic β-glucosidase and a putative cellulbiose transport system. The CelR gene is just upstream of this operon. The binding of CelR to the regulatory sequence is inhibited by cellulbiose, as expected. Because laminariobiose also induces cellulase synthesis, it also probably binds to CelR but this has not been tested. At this point of time it is not known whether induction by laminariobiose is useful for *T. fusca*, or whether it is an accidental result of the low specificity of the CelR sugar-binding site. A puzzling finding is that the Cel6B and Cel48A genes, which each encode exocellulases, have a second CelR-binding site that is about 200 bases upstream of the start site of the gene and this site is not present in the other cellulase gene upstream sequences. These two cellulases are made in equal amounts and together they make up over 70% of the secreted cellulase protein. At this point of time there is no information about the mechanism by which good carbon sources inhibit cellulase synthesis in *T. fusca* but there are no upstream sequences common to all the cellulbiose inducible genes besides the CelR-binding site.

Cellulase Uses

Cellulases are currently the third largest industrial enzyme, in terms of commercial value, because of their use in cotton processing (stonewashing denim), paper recycling, as detergent enzymes, in juice extraction and as animal feed additives. However, cellulases will become the largest volume industrial enzyme, if ethanol, butanol, or some other fermentation product of sugars, produced from biomass, becomes a major transportation fuel, as
seems likely. Currently, all industrial cellulases are almost produced from aerobic cellulolytic fungi, such as Hypocrea jecorina (T. reesei) or Humicola insolens. This is due to the ability of these organisms to produce extremely large amounts of crude cellulase (as much as 130 g l\(^{-1}\)), the high specific activity of their crude cellulase on crystalline cellulose, relative to other crude cellulases, and the ability to genetically modify these strains, so as to tailor the set of enzymes they produce to give optimal activity for specific uses. Research to increase the activity of \(T. \text{reesei}\) cellulases in degrading pretreated biomass have identified a family 61 enzyme from another organism that increases activity several fold. This is a surprising result as the studied family 61 cellulases have low activity and \(T. \text{reesei}\) produces several family 61 enzymes.

Studies are underway in several laboratories to produce cellulases in plants. This could lower the cost of cellulase, if they can be expressed at a high level (greater than 10\% of total plant protein) without changing the overall yield of plant biomass. A major advantage of cellulase production in plants is that the amount of enzyme produced can be easily adjusted to demand, by varying the acreage of the crop. In contrast, building new fermenters is capital intensive and there is currently no other product that could utilize the amount of fermenter capacity that would be required for large-scale production of cellulases for cellulosic ethanol, if the process changed or the demand dropped.

See also: Enzymes, Industrial (overview); Ethanol; Forest Products: Biotechnology in Pulp and Paper Processing; Xylanases

**Further Reading**


**Relevant Websites**

http://www.cazy.org/ – CAZY, Carbohydrate-Active Enzymes
http://genome.jgi-psf.org – JGI, Eukaryotic Genomics