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Transcription activator like effector nucleases (TALENs): A new, important, and versatile gene editing technique with a growing literature.

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ABSTRACT

Transcription activator like effector nucleases (TALENs) is a new and powerful technique in genetic engineering that can delete deleterious genes or add beneficial genes to organisms. It is being widely studied to improve crops and livestock, and is also being investigated clinically. Comparing the details of how both TALENs and its competitor, CRISPR-Cas9, function, reveals the potential advantages of TALENs. The growing literature, besides covering the scientific and technical aspects of TALENs, also includes pertinent information on regulatory aspects and the public's perception and acceptance of TALENs.

For several decades researchers have been benefiting mankind with biotechnology and genetic engineering. These techniques include editing genes to eliminate undesired traits, and editing or adding genes to incorporate desired traits in organisms. (Engelhaupt 2012). When a new development or technique in vital fields of research like these become important enough to generate its own substantial literature, librarians and information specialists need to become aware of this field and its literature so they can adequately serve their students, scientists, researchers, and patrons who may need information about this field.

An innovation in gene editing is the versatile technique, transcription activator like effector nucleases, commonly known by its abbreviation TALENs. This still developing technique is showing its potential by being applied to develop improved livestock, and crops, such as soybeans with no trans fats (Pennisi 2016a), and has also already been applied clinically saving the lives of two infants with leukemia (Cross 2017).

Another gene editing technique and the main competitor to TALENs is CRISPR-Cas9. CRISPR which stands for clustered regularly interspaced palindromic repeats, and Cas9 which stands for CRISPR associated protein 9, has generated thousands of publications, and has been described in the library literature (Butler 2016). The number 9 is simply the designation for one member, the most useful one, of this family of proteins. To fully understand the applications, and potential advantages of TALENs compared to the more well-known CRISPR-Cas9, we need to understand the details of each of these techniques to see their strengths and weaknesses.

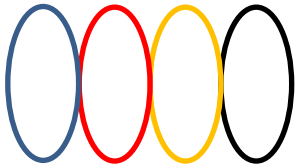
First, we need to start with a brief primer on genes and the genetic code, hopefully just a review for many readers. We have known for decades that DNA, the genetic material, the stuff of genes, is a long chain where each link contains one of 4 different building blocks. These building blocks, usually referred to as bases, are commonly abbreviated as A, C, G, and T. The complete DNA of an organism is known as its genome. The exact order of these bases and the direction in which they are read determines what each gene will produce. The main product of genes are proteins which are the visible stuff of organisms, and also enzymes, the proteins that are the amazingly efficient biological catalysts that build and maintain the structures of organisms, and are responsible for the chemical reactions that maintain life. Proteins consist of chains of amino acids, and each amino acid is coded by a specific three letter sequence (codon) of DNA bases.

TALENs are based on a discovery made when Xanthomonas, a well-known bacterium, pathogenic to many plant species, was being investigated (Bonas et al 1989). This bacterium causes a plant's cells to grow abnormally large. In the early 2000's researchers determined how Xanthomonas causes this damage (Penissi 2012). This bacterium produces proteins called transcription activator like effectors (TALEs) that bind to specific DNA sequences in the affected plant, and then adversely affect expression of the plant's gene.

Each TALE consists of a string of nearly identical small protein units, each unit containing 33 to 35 amino acids (Chandrasegaran & Carroll 2016). The only difference in the sequence of

amino acids within these units is at positions 12 and 13 which is variable. Each of these variable units recognizes and binds to one of the four bases in DNA. Most TALEs contain between 13 and 28 of these small protein units. Figure 1 schematically shows a portion of a TALE binding to a portion of a DNA sequence. The TALE protein units that bind to each of the four DNA bases are shown in different colors. The DNA bases are indicated by their letters.

Figure 1 A representative portion of a TALE bound to its corresponding DNA



The breakthrough in the practical application of TALEs to genetic manipulation occurred when two discoveries were made. The first is that these protein units can easily and inexpensively be synthesized (Pannisi 2012) then joined together so that synthetic TALEs can be created to recognize and target any desired sequence of DNA. The second discovery is that an enzyme that cuts DNA, known as a nuclease, can be attached to the TALE (Pannisi 2012). This combination of a synthetic TALE with its attached nuclease is a transcription activator like effector nuclease (TALEN). This targeting can be done in living cells, cultured cells, stem cells, or fertilized eggs (Chandrasegaran & Carroll 2016).

After the nuclease cuts DNA, the cell has naturally occurring repair mechanisms which are enzymes that rejoin the broken strands of DNA. This repair mechanism is error prone, and sometimes the repair is done incorrectly and a mutation, which is a change in the DNA, results. The most common types of mutations are the insertion of a base, deletion of a base, or substituting one base for another. Here is a simple example of an insertion mutation and its effect on a gene.

Figure 2a shows the first five amino acids (UniProtKB) and their corresponding codons (Nelson & Cox 2008) in one of the subunits of the protein, human hemoglobin. To make it easier to visualize the relationships between the codons and their corresponding amino acids, each of these codons are a different color.

Figure 2a Human Hemoglobin Sequence

DNA: ATGGTACTCAGTCCAGCG
Amino Acid: M·····V·····L·····S·····P·····A

Now suppose that this DNA is cut by a nuclease at the second C, and when this break is repaired a mutation occurs, an extra base, T, is inserted after the second C. Figure 2b shows this new mutated sequence. The newly introduced codon TAG does not code for any amino acid. Instead it is a signal to stop the growth of this chain of amino acids, which means that no protein is produced. Deletion and substitution mutations can produce the same effect, inactivating the expression of a gene. While introducing this particular mutation in humans would be fatal, there are situations where inactivating a gene is advantageous. One such application where a mutant is deliberately selected after a TALEN is applied, is inactivating a gene in wheat that makes this plant susceptible to a mildew infection (Pennisi 2016a). Another application of a TALEN is turning off two genes in soybeans to allow the beans to produce healthier cooking oil (Chang 2017, Pennisi 2016a).

Figure 2b Mutated Human Hemoglobin Sequence

DNA: ATGGTACTCTAGTCCAGCG
Amino Acid: M·····V·····L

When a TALEN cuts DNA, instead of simply allowing the organism's repair mechanism to rejoin the broken strands of DNA, an additional DNA sequence corresponding to a desired gene can be added and then be incorporated into the organism's genome. A real-life application being investigated using this technique is correcting the genetic defect in sickle cell anemia (Tasan et al. 2016). In this disease a mutation of a single base changes a codon for the normal amino acid glutamic acid to the codon for another amino acid, valine. This change adversely affects the function of hemoglobin and the health of people stricken with this disease (Nelson & Cox 2008). The deleterious gene can be eliminated and a corrected gene added in its place.

Similar to the discovery of TALEs in the late 1980's, in the 1990's researchers observed something strange and unexpected in the DNA of bacteria. On each side of some genes they found short sequences of DNA that did not code for any amino acids. These sequences read the same in either direction, that is, they were palindromes. These unexpected sequences were named clustered regularly interspaced palindromic repeats (CRISPR). CRISPRs aroused interest because if they were not coding for proteins, what could be their role? A closer examination of the genes between the CRISPRs showed they were DNA sequences from viruses. During a previous viral infection, the bacteria had incorporated some of the viral DNA. Yes, bacteria, not just animals, can be infected with viruses.

The CRISPR sequences that surrounded the viral DNA formed an immune system for the bacteria, so the next time this virus infected the bacteria, the bacteria would be able to defend against it. CRISPRs frame the viral DNA sequences and are a recognition site to signal Cas9 to perform its role, which as we will see below is to cut DNA.

Here is where a second class of molecules comes into play, RNAs. RNAs are a family of substances chemically related to DNA, and are part of the cellular machinery that converts the genetic code from DNA to biosynthesize proteins. A key property of both DNA and RNA is that bases on one chain pair with bases on another chain. Specifically, A is attracted to and pairs with T, and C is attracted to and pairs with G. These attractions can be between two chains of DNA, two chains of RNA, or two chains, one consisting of DNA and another of RNA. The only difference in this pairing is a slight one. In RNA the chemically similar base known as U substitutes for T.

When a virus that had previously infected the bacteria invades again, the bacteria's immune system produces two RNAs. One RNA containing a sequence based on the CRISPR sequence fits into Cas9. The second RNA which also fits into Cas9 matches the viral DNA that was stored between two CRISPR sequences. Matching means that the RNA sequence consists of bases, where each base pairs with the corresponding base on DNA, as shown in Figure 3 which is a partial sequence of a DNA RNA chain.

Figure 3 A Viral DNA RNA Pairing

Viral DNA ·····A·····C·····G·····T·····G·····C·····
RNA ·····U·····G·····C·····A·····C·····G·····

Since this second RNA matches the DNA sequence of the invading virus, in addition to fitting into Cas9, it also binds to the DNA of the invading virus. The combination of Cas9 with its two RNAs bound to the invading viral DNA activates Cas9 enabling it to perform its role, which is to cut the DNA of the invading virus, inactivating it. Cas9 is a nuclease.

The breakthrough in CRISPR-Cas9 was the discovery that this system can be engineered to make it independent of having to first recognize either CRISPR or viral DNA sequences. Researchers were able to synthesize guide RNAs. These RNAs while still being able to fit into Cas9 could also contain any additional desired RNA, specifically sequences that match the DNA sequences of genes that they wanted to target and modify. Like TALENs, this targeting can be done in living cells, cultured cells, stem cells, or fertilized eggs (Chandrasegaran & Carroll 2016).

Similar to when the TALEN nuclease cuts DNA, when Cas9 cuts DNA, the cell's naturally occurring repair mechanisms rejoin the broken strands of DNA. Just like in TALENs, this repair mechanism is error prone, and sometimes the repair is done incorrectly and a mutation results. Also, as with TALENs, an additional DNA sequence corresponding to a desired gene can be added and then be incorporated into the organism's genome.

Another gene editing technique, an older one known as zinc fingers, is used less often because it is difficult to design. It is also more expensive because a single company controls almost all of the intellectual property rights (Pennisi 2012).

A major challenge in applying TALENs is that while TALENs are designed to target a specific and desired DNA sequence, other places on the genome may have a similar DNA sequence, causing the TALEN to also bind at this additional location. This off-target binding may produce undesired and unpredictable consequences when the TALEN's nuclease cuts DNA at this unintended location (Chandrasegaran & Carroll 2016). This imprecision may have evolved because of the ongoing evolutionary battle between the invading Xanthomonas, where its TALE attaches to the plants genome causing disease, and the plants' response to this invasion. A plant may respond to this invasion by evolving, changing its DNA sequence at the site targeted by the TALE. A Xanthomonas containing a TALE with the ability to bind to this new and non-exact sequence will have an evolutionary advantage (Chandrasegaran & Carroll 2016).

A similar phenomenon also takes place in CRISPR-Cas9 where the synthesized guide RNA may bind to an unintended sequence allowing Cas9 to cut the genome at this off-target location. This imprecision evolved to give a protective advantage to the host bacteria. Viruses evolve rapidly and the ability of the bacteria's CRISPR-Cas9 to recognize a new and slightly different viral sequence will enable the bacteria to survive the viral attack.

So, the issue facing researchers is which technique, TALENs or CRISPR-Cas9, can better limit this off-targeting. The technical challenges in controlling TALENs or CRISPR-Cas9 are different. TALENs are a protein DNA recognition system, while CRISPR-Cas9 is an RNA DNA recognition system. The chemistries of each are different. Also different are the nucleases in each system, each with its own properties and specificities. At this time as pointed out by Pennisi (2016a) and by Chandrasegaran & Carroll (2016) in their exhaustive, scholarly review of the literature, TALENs are inherently more target specific.

The method with the most precision and least doubt about deleterious off-target effects is more likely to be accepted by researchers, clinicians, governments, and the public. Regardless of any potential benefits of genetic modifications of foods, regulators and the public must accept these benefits, otherwise there may be little market for these enhanced products. Many people are squeamish about any kind of genetic manipulation of their foods as shown by many European countries that have banned cultivation of genetically modified crops (Chang 2017). Related to this issue is how governments choose to regulate TALEN modified crops and inform the public when they are consuming such products. The United States Department of Agriculture currently distinguishes between plants that have had genes added to them, and plants where genes have been simply removed or turned off. The latter are exempted from the regulations that apply to genetically modified organisms (Hall 2016; Pennisi 2017b). Consumers now face the prospect of eating foods with deleted genes, without their knowing this. On the other hand other countries such as Canada have stricter regulations. Canada regulates any crop that has been genetically modified.

Yet another issue is the patenting and licensing of this technology, and the disputes that may arise. While so far there have been no hassles in TALENs patents (Hall 2016; Pennisi 2016a), patent and licensing clashes regarding CRISPR-Cas9 may take years to resolve (Contreas & Sherkow 2017; Cohen 2017)

Considering all the scientific, technical, agricultural, regulatory, medical, business, commercial, socioeconomic, public perception and acceptance aspects of TALENs, many different types of information resources need to be made available to patrons, depending on their specific needs. We mediators need to be aware of and prepared to search multiple and diverse sources of information.

Methods and results

The appropriate database search strategy for TALENs is:
"transcription activator like effector nuclease*" OR talen OR talens

The literature sometimes describes TALENs as "transcription activator-like effector nuclease*". But searching this phrase should not be necessary because hyphens are read as spaces in databases. This phrase is truncated to include the singular and plural using the wild card symbol appropriate for the database or database family being searched. The abbreviated terms talen and talens are included because even a quick perusal of the literature shows that in the database records for many articles, only the abbreviation is present. Note that talen must not be truncated. Doing so will retrieve many articles containing the word talent. Searching the terms talen and talens will be precise only in databases that search titles, abstracts, and indexing terms in their default fields. If the default search field also includes authors, many false drops will be recovered because Talen and Talens are author surnames. These author name false drops can be eliminated by adding:
NOT (talen[author] OR talens[author])
to your search. But this strategy is not advisable because it will exclude relevant articles on TALENs where by coincidence an author has the name Talen or Talens.

The size and rapid growth of the TALENs literature is illustrated in Table 1. This data does not include all the publications on TALENs, only those from journals that meet the strict criteria for inclusion in this database (Testa 2016). Nonetheless, this figure clearly indicates the trend of how this field is progressing. Even though TALENs were first reported in the literature in 2010 (Christian et al. 2010), a few articles mentioning TALENs are retrieved in earlier years, because

some authors in totally unrelated fields used TALEN as an abbreviation for a chemical substance with a long name.

Table 1.TALENs articles in Science Citation Index Expanded (September 2017)

Year	Number of Articles
2007	3
2008	1
2009	3
2010	3
2011	16
2012	53
2013	196
2014	333
2015	294
2016	308
2017	159

To show the diversity of databases containing references on TALENs and the references unique to each database, a multi-file search was performed in selected databases in the STN family of databases from 2010 to the present, September 2017. More databases than the ones searched here are likely to contain references on TALENs. This search just emphasizes the many databases that contain articles on TALENs, and how you can use this STN tool. The databases included here are: BIOSIS Previews, CABA, Chemical Abstracts Plus, Chemical Business NewsBase, Chemical Engineering And Biotechnology Abstracts – Verfahrenstechnische Berichte, Chemical Industry Notes, Ei Compendex Dissertation Abstracts, Embase, Elsevier BIOBASE, Food Science and Technology Abstracts, MEDLINE, Science Citation Index.

In these databases, the default search fields do not include authors. At this time, only MEDLINE and Chemical Abstracts have controlled terms for TALENs. MEDLINE has the newly introduced MeSH term "Transcription Activator-Like Effector Nucleases" with over 80 articles indexed with this term. Chemical Abstracts has a Chemical Abstracts Registry Number

(Weisgerber 1997) for TALENs (1400268-71-4), and this number is included in the search because not only are there over 1400 articles in Chemical Abstracts indexed with this number, but also because Chemical Abstracts, Registry Numbers can also be searched in these 7 databases; BIOSIS Previews, CABA, Chemical Business NewsBase, Chemical Engineering And Biotechnology Abstracts – Verfahrenstechnische Berichte, Chemical Industry Notes, Embase, and MEDLINE. Controlled terms may be added to other database as TALENs become more widely studied.

=> file medline cin biosis dissabs fsta caba caplus compendex esbiobase ceaba-vtb embase cbnb scisearch
=> search (transcription activator like effector nuclease? OR talen OR talens OR 1400268-71-4)
=> search L1 and py=>2010
=> set duporder file
=> duplicate remove L2

This series of command activates the duplicate removal feature in STN. This feature shows the number of unique citations in each of the databases searched. This process first compares all of the citations retrieved from these databases and eliminates all duplicates. The system then collects and makes available for display all of the unique records that are present in the first database on the list. Then the system looks into the second database on the list and notes which records are not present in the first database. Then the third database is compared to the two above it for unique records. The successive databases in the list are analyzed in the same manner. This procedure displays the maximum number of unique records in the first database on the list, and the least number of records from the last database.

Keeping in mind that in STN multi-file searches you can list the databases in any order, it makes economic sense to print or display as many citations as possible from the database that has the least expensive cost. The first database searched here is MEDLINE which costs only 41 cents to display each citation with its abstract. This display cost rises in each succeeding database to $19.45 for a single record with its abstract in Science Citation Index.

In this search the databases were searched from the least expensive to the most expensive based on their per record display cost. But when choosing the database order, the searcher needs

to consider other costs such as connect hour costs, and search term costs and hourly cost options in Chemical Abstracts Plus and Chemical Industry Notes. The search and display costs for any STN database can readily and inexpensively be obtained with these commands when logged on to STN:

=> file stnguide

=> s databasename/dbn;d cost

When searching any subject we need to see if the database contains a controlled term for that subject. The database's thesaurus can be searched for controlled terms. Another way to find controlled terms is to perform a search that focuses on TALENs by limiting the above search to article titles, and then examining the index terms to see if a relevant controlled term has been applied.

This multi-file search in STN (Table 2) shows that even databases where references on TALENs would not be expected, like the engineering database EI Compendex, have unique references. So searchers need to consider all databases available to them especially from vendors that have multi-file search capabilities. Such searching can also be done in databases from the large and diverse number of databases provided by EBSCO Information Services (2017). While duplicates are automatically removed when searching, the number of references unique to each database is not tabulated.

Table 2 TALENs coverage in selected STN databases

Database	Number of unique citations
MEDLINE	1252
Chemical Industry Notes	3
BIOSIS Previews	841
Dissertation Abstracts	57
Food Science and Technology Abstracts	5
CABA	55
Chemical Abstracts Plus	1058
EI Compendex	10
Elsevier BIOBASE	35
Chemical Engineering And Biotechnology Abstracts	2

Embase	804
Chemical Business NewsBase	34
Science Citation Index	145

Since TALENs are being applied to crops, one would expect the United States Department of Agriculture's free database, AGRICOLA (https://agricola.nal.usda.gov/) to contain pertinent articles. This database currently contains more than 100 articles on TALENs.

Since genetic engineering generates regulatory issues, we can expect that TALENs may also generate such issues. Good places to check how the U.S. government is regulating or planning to regulate TALENs are the Code of Federal Regulations (McKinney 2006) and its daily update the Federal Register (Relyea 2011). The documents from these publications along with documents and records from many other federal agencies can readily be searched in the Advanced Search Mode at the Search Government Publications link at FDsys – Federal Digital System (https://www.gpo.gov/fdsys/search/home.action). As of September 2017 the Code of Federal Regulations currently has no entries on TALENS, while the Federal Register has more than 80 entries. However many of these are false drops where talen is the name of a company. This database gives you the option of selecting a range of dates (TALENs were first reported in 2010) and the ability to select entries from the relevant government agencies; the Food and Drug Administration and the Department of Health and Human Services. Furthermore, the gateway to U.S. government's science information, https://www.science.gov/, has hundreds of references on TALENs.

Newspapers are also an abundant source of articles on TALENs. Searching the full name for TALENs yields a manageable number of newspaper reference in the Lexis-Nexis database. But the search terms talen or talens yields hundreds with many false drops. Adding a subject specific search term will eliminate nearly all of the false drops.

Regarding the important area of patents, nearly all patent searches need to be a combination of text terms along with appropriate classification codes (Simmons & Kaback 1996). To show the challenge of finding correct classification codes for TALENs, a search performed in September 2017 in PATENTSCOPE (World Intellectual Property Organization 2017) searching just the full name for TALENs and limiting the search to titles yielded 19 entries. But the classification codes for these patents that clearly focus on TALENs showed no clear pattern and

contained no terms that suggest TALENs. Furthermore, a search in the Cooperative Patent Classification (Espacenet 2017), the current official classification system in the world's major countries, for both the abbreviated and full names for TALENs retrieved several codes, but none specifically mention TALENs. Apparently TALENs is cross referenced to these codes. Consequently it is difficult for non-experts to know which of these terms need to be included in a search. Furthermore, several different and intricate patent databases must be searched to insure that all patents in any area are retrieved (Simmons & Kaback 1996). So, while any experienced searcher can readily retrieve nearly all relevant articles in bibliographic databases, given the challenge of finding the correct classification terms and the intricacies of the patent databases, we must heed the still pertinent caveat of Nancy Lambert, among many others, who advise that in many situations it is best to turn to patent searching experts (Shrode 1997).

Regarding books, Books In Print (http://www.booksinprint.com/) searching from the advent of TALENs in 2010 to the present lists just these three books on TALENs.

Kuhn R, Wurst W, Wefers B, editors. 2016. TALENs: Methods and Protocols. New York: Humana Press.

Yamamoto T, editor. 2015. Targeted Genome Editing Using Site-Specific Nucleases ZFNs, TALENs, and the CRISPR/Cas9 System. New York: Springer.

Doudna JA, Sontheimer EJ, editors. 2014. The Use of CRISPR/cas9, ZFNs, TALENs in Generating Site Specific Genome Alterations. Amsterdam: Elsevier.

This small number of books is not a disadvantage to researchers in this field. Rather it confirms that most researchers in both established and developing fields in the biological and chemical sciences rely on scientific journals as their primary source of knowledge. In journal articles published in both the established field of structural biology and in the then developing field of combinatorial chemistry fewer than 6% of the cited references were to books (Lascar & Mendelsohn 2001; Barnett 2002).

Conclusion

Because of ongoing uncertainties about public and regulatory acceptance of TALENs, researchers need to be aware of all issues related to genetic engineering. In addition their literature needs will go well beyond just the scientific and technical sources, and should also include vital and relevant information about other genetic engineering aspects of TALENs. Librarians and information professionals need to take advantage of these many varied and diverse resources on TALENs to adequately, efficiently, and best serve their patrons.

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