confirmed that at least 8 more supernova remnants emit the OH maser, among 66 observed (8).

References

1. H. Weaver *et al.*, *Nature* **208**, 29 (1965); E. Gunderman, thesis, Harvard University, Cambridge, MA (1965).
2. J. M. Moran *et al.*, *Astrophys. J. Lett.* **152**, L97 (1968).
3. F. Yusef-Zadeh, K. I. Uchida, D. Roberts, *Science* **270**, 1801 (1995).
4. D. A. Frail, W. M. Goss, V. I. Slysh, *Astrophys. J.* **424**, L111 (1994).
5. For example, see K. Tatematsu *et al.*, *ibid.* **351**, 157 (1990).
6. Y.-L. Huang, R. L. Dickman, R. L. Snell, *ibid.* **302**, L63 (1986).
7. M. Elitzur, *ibid.* **203**, 124 (1976).
8. D. A. Frail, private communication.

The Molecular Biology of Rice

Ko Shimamoto

Rice provides the main source of food for 50% of the world's population and so may be the most important plant on Earth. It is also becoming an increasingly attractive plant for study by biologists. Rice has 24 chromosomes, and its genome is 4.3 megabases, one-tenth the size of the human genome and only three times that of *Arabidopsis thaliana*, a favorite plant of molecular biologists. A monocotyledonous plant, rice (*Oryza sativa* L.) belongs to the grass family (Poacea), which includes maize, sorghum, wheat, barley, and oats. A better understanding of the genetics and the biology of rice can therefore also help to improve these other food crops. New developments in two areas of rice molecular biology—genome analysis and transformation—will greatly influence future improvement of this important crop.

Researchers at the Rice Genome Research Program in Tsukuba, Japan, have recently constructed a genetic map of rice that contains 1383 markers, of which 883 are derived from expressed genes (*1*). The average interval between markers is 300 kb. Approximately 20,000 complementary DNAs (cDNAs) derived from a variety of tissues and cells cultured under different conditions were partially sequenced. This is the largest body of information on expressed plant cDNAs currently available and so will facilitate gene identification in other plants. One important finding to emerge from the analysis of cereal genomes is that, for much of the genome, the order of genes is well conserved among major cereals (*2*). This synteny extends to even the nucleotide level of the chromosome (*3*), suggesting that once genes with products of interest to agriculture are isolated from rice, counterparts in wheat, maize, and other cereals can be easily identified. Because the genome size of wheat is 34 times larger, and that of maize is 6 times larger, than the genome size of rice, information from rice may be crucial in finding genes in other cereals. A recent study in *Science* indicates that this synteny extends to quantitative trait loci that determine variation in complex phenotypes such as seed size, seed dispersal, and flowering time in the cereal genomes (*4*). These findings reinforce the notion that grasses can be considered a single genetic system (*5*).

Rice: A valuable model system. [Photograph by Ronald R. Johnson/The Image Bank]

The first successful positional cloning of cereal genes is reported by Song *et al.* in this issue of *Science* (*6*). The gene cloned is *Xa21*, which confers resistance to leaf blight, one of the major bacterial diseases of rice. The gene has novel features compared with other recently identified disease resistance genes in plants: *Xa21* has leucine-rich repeats, which are important for protein-protein interaction, and a serine-threonine kinase domain, which transmits a signal to quickly activate the plant's defense mechanisms against pathogen attack. The authors of this report are also isolating genes that determine resistance to rice blast, a fungal disease, and flowering time.

An actively transcribed retrotransposon has recently been described in rice. Tos17 is a member of the rice retrotransposon family and is actively transcribed in tissue culture (*7*). Many copies of this gene are inserted in numerous different sites in the chromosomes during tissue culture. Therefore, if a large number of plants are generated from cultured rice cells, some plants may have Tos17 in some of their genes. Indeed, insertion of this element in three different genes has recently been demonstrated (*7*). This finding implies that once a large pool of plants derived from cultured cells are prepared, mutants can be identified that have insertions in genes whose sequences are already known. This reverse genetic approach should become extremely powerful in defining the functions of genes with sequence information already in the database.

Functional analysis of isolated genes and crop improvement by molecular biological approaches require a simple and reliable method to transform plant cells. Rice is the first cereal in which fertile transgenic plants were obtained and is by far the easiest cereal to transform at present (*8*). Two methods are used for rice transformation: protoplast (cells without a cell wall) transformation, either by electroporation or polyethylene glycol (*9*), and particle bombardment of embryos (*10*). In both, transformed cells must be selected and plants regenerated from them. With these methods, rice with improved resistance to viral and fungal pathogens and insect pests has been generated (*11*). Transgenic rice has also provided information on promoter elements of monocot genes that confer tissue-specific and inducible expression (*12*).

Although these two methods are routinely used in laboratories worldwide, they are still laborious and time consuming. A simpler method is desirable. Monocots, including rice, are thought to be a poor host for *Agrobacterium* infection. Thus, *Agrobacterium* transformation, which is the most widely used method for plant transformation, has not been effectively used for rice and other monocots. But callus tissues from the scutellum of the mature embryo are highly competent for *Agrobacterium* infection in the presence of acetosyringone, an inducer of gene transfer from the bacterium to plant cells. By this means transgenic plants can be produced with high frequencies (*13*). This finding is important in three ways. First, cell culture and selection steps are much simpler than the two other methods. Second, the same transformation vector system can now be used for dicots such as *Arabidopsis* and tobacco and for rice, a monocot. Lastly, this method does not require any special equipment. This is particularly important for the progress of rice biotechnology in many rice-growing countries. This transformation method will likely open up a new era for the genetic engineering of rice.

Development of a saturated molecular map

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and a simple method to transform rice will undoubtedly speed up identification of the genes that determine important agricultural traits of rice—flower and leaf development, photoperiod sensitivity of flowering, and resistance to various pests and pathogens. For instance, we know little about flower development of rice at present. If the determinants of the number of flowers were known, we could increase the number of seeds. Similarly, if we understood how the flower is induced by a light signal, we may be able to generate rice with much shorter growth periods. There will likely be much excitement about the molecular biology of rice in the near future.

References

1. N. Kurata *et al.*, *Nature Genet.* **8**, 365 (1994).
2. S. H. Hubert *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4251 (1990); S. Ahn and S. D. Tanksley, *ibid.* **90**, 7980 (1993); R. Shields, *Nature* **365**, 297 (1993); N. Kurata *et al.*, *Bio/Technology* **12**, 276 (1994).
3. R. P. Dunford *et al.*, *Nucleic Acids Res.* **23**, 2724 (1995); A. Kilian *et al.*, *ibid.*, p. 2729.
4. A. H. Paterson *et al.*, *Science* **270**, 1714 (1995).
5. J. L. Bennetzen and M. Freeling, *Trends Genet.* **9**, 259 (1993).
6. W.-Y. Song *et al.*, *Science* **270**, 1804 (1995).
7. H. Hirochika, unpublished results.
8. K. Shimamoto, *Curr. Opin. Biotechnol.* **5**, 158 (1994).
9. K. Shimamoto *et al.*, *Nature* **338**, 274 (1989); S. K. Datta *et al.*, *Bio/Technology* **8**, 736 (1990).
10. P. Christou *et al.*, *Bio/Technology* **9**, 957 (1991); L. Li *et al.*, *Plant Cell Rep.* **12**, 250 (1993).
11. T. Hayakawa *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 9865 (1992); H. Fujimoto *et al.*, *Bio/Technology* **11**, 1151 (1993); W. Lin *et al.*, *ibid.* **13**, 686 (1995).
12. J. Kyozuka *et al.*, *Plant Cell* **6**, 799 (1994); Y. Yin and R. N. Beachy, *Plant J.* **7**, 969 (1995).
13. Y. Hiei *et al.*, *Plant J.* **6**, 271 (1994).

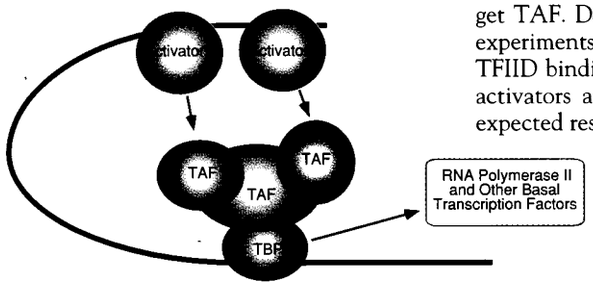
Mechanisms of Gene Activation

Stephen Buratowski

The main question facing the eukaryotic gene expression field is the same today as it has been for many years. How do regulatory transcription factors (which include the products of many proto-oncogenes, genes essential for proper differentiation and development, and key components of signal transduction pathways) increase the amount of messenger RNA produced by their target gene promoters? Clear answers to this question have been waiting for a better understanding of the basal transcription machinery—RNA polymerase II and the accessory factors that position polymerase at the transcription initiation site. A great deal of recent evidence points to the basal transcription factor complex TFIID as a key component in the response to transcription regulators. Some of the most persuasive experiments yet that show TFIID is a target of activators are presented in two papers by Sauer *et al.* in this issue (*1*).

Transcription regulatory proteins typically contain two functional domains: a DNA binding domain that recognizes specific sequences within its target promoters and an activation domain that is required for transcription stimulation. On the basis of the prokaryotic transcription paradigm, activation domains of regulatory proteins are predicted to make direct protein-protein contacts with one or more components of the basal transcription machinery. This contact could stabilize binding of the basal machinery to the promoter or increase the rate of a kinetically slow step (as some prokaryotic activators stabilize association of RNA polymerase with the promoter DNA or increase the rate of DNA strand separation within the transcription complex). A great deal of effort has gone into identifying the targets of eukaryotic activation domains, and certainly no shortage of candidates exists. In vitro protein interaction studies have detected activator interactions with nearly every component of the basal transcription complex. However, very few of these contacts have been shown to occur in the context of the transcription complex or are even correlated with transcription stimulation.

The case for activator-TFIID interactions is much stronger. TFIID consists of the TATA binding protein (TBP) and approximately 10 other TBP-associated factors (TAFs). Whereas TBP is sufficient for promoter binding and basal transcription, the presence of the TAFs confers the ability to respond to activators. Thus, TAFs have been postulated to be coactivators in transcription. In vitro protein interaction assays show that different activators can contact specific TAFs. The caveat of these results is that the interactions are seen between two proteins removed from the context of the transcription complex. In the biochemical tour-de-force leading up to the two papers in this issue (both from the same set of authors), Sauer *et al.* (*1*) have expressed each of the individual TAFs and reconstituted the TFIID complex from recombinant subunits. More importantly, they have assembled subcomplexes of TFIID to test whether the absence of a particular TAF affects activation. As predicted, in vitro transcription stimulation by an activator absolutely requires the presence of the contacted TAF subunit. This correlation strongly supports the hypothesis that the TFIID subunits are targets for transcription activation domains.



One mechanism of synergistic transcription activation. Two simultaneous contacts by activators can cause synergistic activation of transcription, leading to stabilized binding of TFIID to the promoter and, possibly, conformation changes in the complex. As a result of this interaction, association of the remaining basal transcription factors is enhanced.

The experiments of Sauer *et al.* (*1*) extend earlier results by reproducing a key feature of eukaryotic transcription: synergistic activation of transcription by multiple activator proteins. Multiple activators at a promoter in vivo or in crude in vitro systems lead to a greater than additive increase in the amount of transcription. Sauer *et al.* show that the ability of two activators to work synergistically correlates with simultaneous contacts between each activator and its target TAF. Deoxyribonuclease I footprinting experiments show a dramatic increase of TFIID binding to the promoter when both activators are present. This is exactly the expected result if the two activators are stabilizing the same complex. The additive free energy of the individual protein contacts leads to an exponential increase in the equilibrium binding constant ($\Delta G = -RT\ln K$, where ΔG is the change in the Gibbs free energy, R is the gas constant, T is the absolute temperature in degrees Kelvin, and K is the equilibrium binding constant). Therefore, at least some transcription activation appears to be simply the result of cooperative binding and recruitment of TFIID. This idea is supported by in vivo experiments in which a protein fusion between a regulatory factor DNA binding domain and TBP bypasses the need for an activation domain (*2*).

So, are the TAFs merely passive targets

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