Genetic and molecular approaches to synthesis and action of the yeast killer toxin

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Summary. The K1 killer toxin of *Saccharomyces cerevisiae* is a secreted, virally-coded protein lethal to sensitive yeasts. Killer yeasts are immune to the toxin they produce. This killer system has been extensively examined from genetic and molecular perspectives. Here we review the biology of killer yeasts, and examine the synthesis and action of the protein toxin and the immunity component. We summarise the structure of the toxin precursor gene and its protein products, outline the proteolytic processing of the toxin subunits from the precursor, and their passage through the yeast secretory pathway. We then discuss the mode of action of the toxin, its lectin-like interaction with a cell wall glucan, and its probable role in forming channels in the yeast plasma membrane. In addition we describe models of how a toxin precursor species functions as the immunity component, probably by interfering with channel formation. We conclude with a review of the functional domains of the toxin structural gene as determined by site-directed mutagenesis. This work has identified regions associated with glucan binding, toxin activity, and immunity.
Key words. Saccharomyces cerevisiae; protein toxin; yeast toxin precursor; protease processing; lectin; (1 → 6)-β-D-glucan; receptor; resistant mutants; spheroplasts; ion-permeable channels; site-directed mutagenesis; toxin functional domains.

Introduction

The *Saccharomyces cerevisiae* K1 killer system is one of many described among the yeasts (see review by Young[47]), although the structure and action of the protein toxins produced by most of these killers have not been examined in any detail. Because *Saccharomyces cerevisiae* is a convenient organism to use genetically, and because the killer phenotype is easy to detect, the system has been exploited to pursue the genetic basis of maintenance of the killer virus in host cells and the expression of its toxic gene product. Some comprehensive reviews cover these topics[8,41,44]. The problem of toxin action has been primarily studied by genetic approaches, and this biased approach could profit from more detailed biochemical work. One purpose of this review is to convey the message that genetic and mutational dissection of the toxin structural gene is now at a point where precise biochemical and biophysical work could proceed on the pore-forming mechanism of the protein.

Killer biology

The presence of killer strains of *Saccharomyces cerevisiae* was discovered by Makower and Bevan[29]. The K1 killer they found has been the archetype in subsequent work. Such killers contain a virus and secrete a virally-coded protein toxin that kills yeasts of the same species lacking the virus. Killer strains are not killed by the toxin they produce and are said to be immune. At least three different viruses can confer a killer phenotype in *Saccharomyces cerevisiae*. All produce different proteins, the so-called K1, K2 and KT28 toxins, and each immunity system is specific for only one toxin type. The presence of an antagonistic killer phenotype in natural yeast populations may confer a competitive advantage. Some support for this view comes from a survey of killers among yeasts colonizing cactus fruits, where the results suggest that the killer yeasts can exclude non-killers from this habitat[39]. Killer yeasts present a problem in the contamination of industrial fermentations[30] and have been shown in the laboratory to be efficient competitors with non-killers in some situations[48]. The killer system can be used as a dominant selectable marker for genetic transformation and plasmid maintenance and in the latter application serves as an anticontaminant trait for yeasts used in industrial fermentations[16,43].

Viral nature. The detailed virology of the killer system is beyond the scope of this article and will just be summarised here (see Wickner[44] for a review). The *Saccharomyces cerevisiae* K1 killer virus system is composed of two distinct double-stranded RNA species: L (large) and M (medium), that are separately packaged by a common major capsid protein into the virus-like particles of the mycovirus. In laboratory situations the virus is normally vertically transmitted by vegetative cell division or through sexual fusion where it behaves as a classic cytoplasmically transmitted genetic element[38]. Recently conditions have been developed where it is possible to transmit the virus in a quasi-infectious way to yeast cells using modified DNA transformation procedures[20].

The killer and immunity phenotypes conferred by the virus have allowed extensive genetic analysis of nuclear genes necessary for replication and maintenance of the virions and of others that determine virion copy number. Other studies have explored the molecular biology of replication of the dsRNA genome and its packaging into capsids. In many ways this system is a paradigm for the interaction of a stably maintained virus with a eukaryotic host cell.

Identification and structure of the K1 toxin gene

The cytoplasmic inheritance of the killer character [38] and correlation of the presence of the M1 double-stranded RNA molecule with the K1 killer phenotype [2, 42] provided the first evidence that the toxin genome was virally-based. Subsequent work showing that the in vitro translation product from denatured purified M1 dsRNA was immunoprecipitated by antiserum raised against the purified secreted toxin [5, 33], provided strong evidence that the toxin gene was encoded by this dsRNA molecule. Cloning the killer gene by making a cDNA copy of part of the M1 dsRNA, sequencing this DNA and comparing its encoded protein sequence with that of the purified toxin, showed that this was indeed the case [4, 37]. In further work the cDNA gene was used in expression studies in *Saccharomyces cerevisiae* [22, 28], where plasmids containing the gene with an appropriate promoter, conferred both the K1 killer and immunity phenotypes on sensitive host yeast strains.

The outline of the toxin precursor protein encoded by the gene is shown in figures 1 and 2. An amino-terminal signal peptide of some 26 amino acid residues precedes an 18 residue proregion. The α and β subunits of the secreted toxin follow in the precursor and are separated by an interstitial glycosylated region called the γ peptide [5, 27, 50]. Production of mature toxin from this precursor clearly requires extensive processing and this has been a major focus of effort (see section on biogenesis below). Examination of the toxin sequence shows that the α subunit, of 103 amino acid residues, with a pI of 5.35, has two hydrophobic domains capable of spanning a membrane and these are likely to be part of a channel-forming component (see mutations in the toxin gene section below). In contrast, the smaller β subunit, of 83 amino acid residues, has a pI of 3.94 and is hydrophilic with the COOH-terminal half being relatively acidic. The toxin has no known homology to any other protein including the K2 toxin (see below). The mature toxin has a calculated molecular weight of 20,658 and exists as a disulphide bridged $\alpha-\beta$ dimer of pI 4.34 which is reasonably soluble in aqueous buffers. Studies of the protein in non-denaturing systems indicate that the dimer is capable of forming aggregates [9], though whether these are necessary for channel formation is unknown. When the disulphide bonds are reduced, the α subunit is insoluble in aqueous

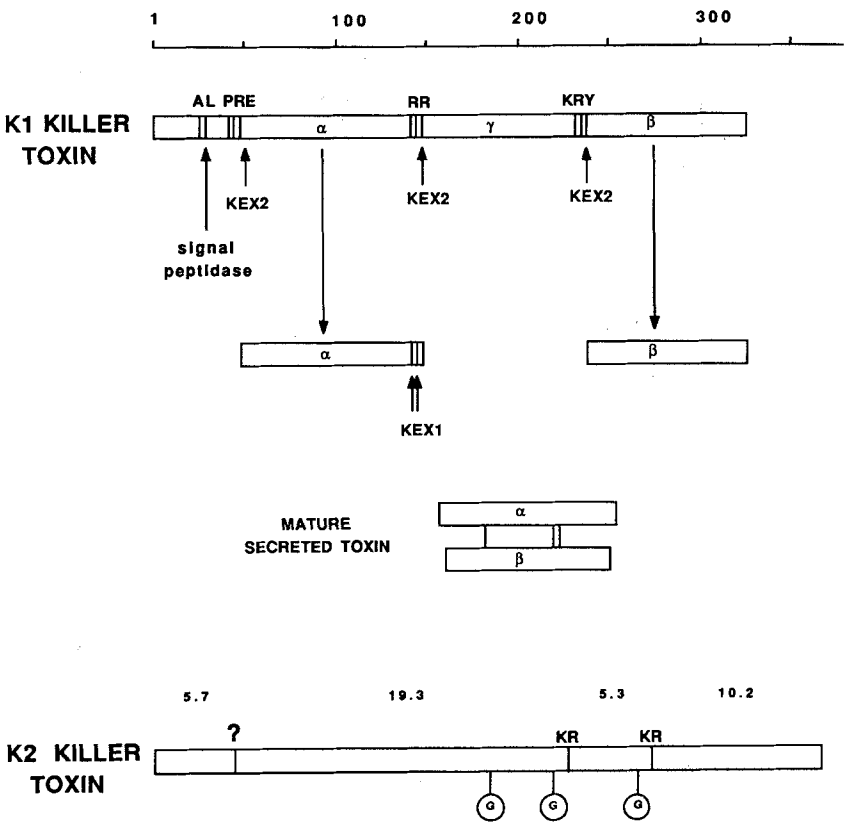

Figure 1. Outline of structure and processing of the K1 and K2 killer toxins. The upper panel represents the K1 toxin-immunity precursor with the amino acid residue number at the top. Processing sites in the precursor are indicated with the single letter amino acid code. The probable signal peptide site is indicated, as are the sites of cleavage by the KEX2 endoprotease following basic residues which generate the α and β toxin subunits. COOH-terminal removal of arginine residues from the α subunit by the KEX1 gene product completes the processing. The mature secreted toxin is shown below with an arbitrary assignment of disulphide bonds. At the bottom is an outline of the K2 toxin-immunity precursor structure. Possible processing sites for the KEX2 product following pairs of basic residues are indicated, as are sites for attachment of N-linked polysaccharides with circled G's. Note the similarity in overall structure with the K1 precursor. The molecular weights (in thousands) of the major components are shown. These probably correspond to the 21 k and 9 k secreted species that comprise the toxins. See text for further details.

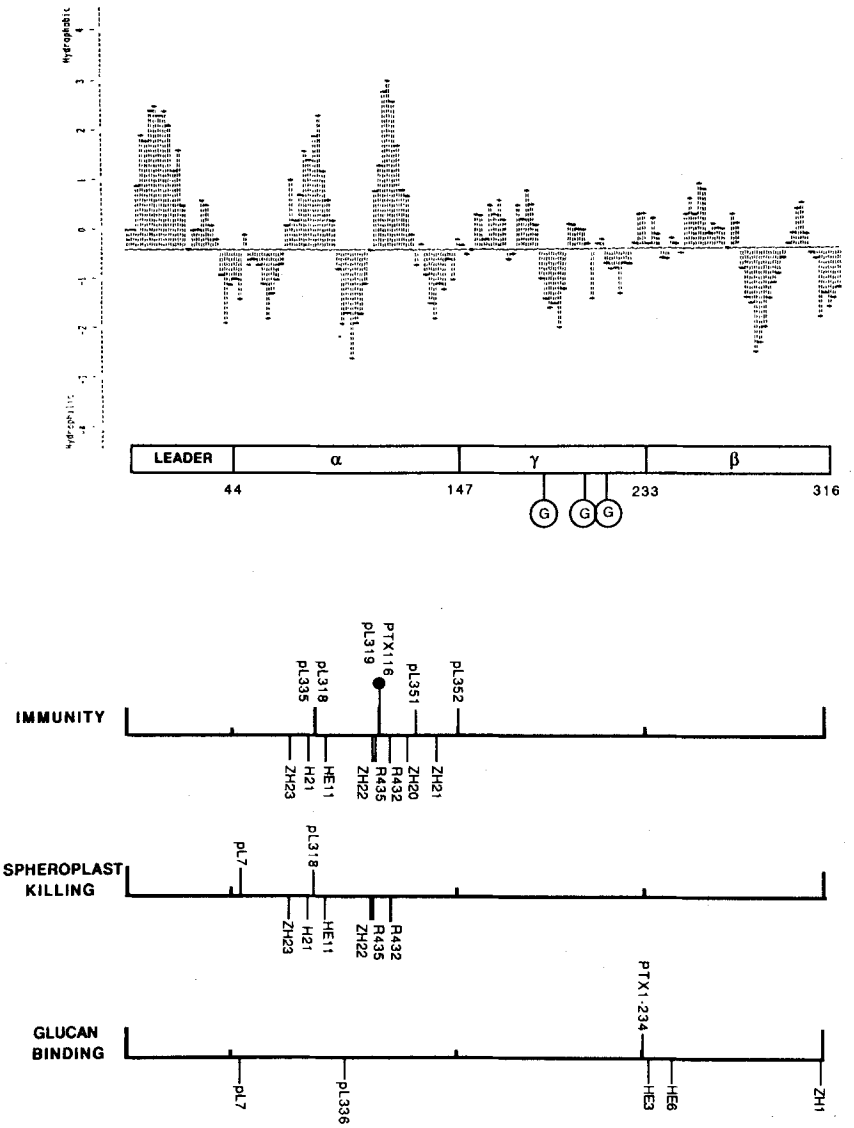

Figure 2. Functional domains of the K1 killer toxin. The structure of the precursor and its hydropathic profile are shown at the top. Note the two hydrophobic regions in α thought to be involved in channel formation. Below are grouped mutations which lead to defects in immunity, channel formation as measured by spheroplast killing, and the lectin-like glucan binding. Individual mutations are shown by number, | lines represent point mutations or insertions and ↑ indicates a nonsense mutation. Mutations shown above the line are published[3,40], those below the line are from the unpublished work of H. Zhu. See text for further details. The circled G's represent sites of attachment of N-linked glycosyl residues.

buffers, perhaps indicating a role for the β-subunit in maintaining the solubility of the toxin in aqueous media.

K2 killer toxin

The K2 toxin is a protein coded by a small viral dsRNA species termed M2. The portion of the M2 dsRNA which codes for the K2 toxin activity and immunity to K2 toxin has recently been cloned, sequenced and expressed (Thomas, Dignard and Whiteway, unpublished work). The primary nucleic acid sequence shows no identity with that coding for the sequence of the K1 toxin, and this non-identity extends to the protein level. This lack of identity was unexpected considering the apparent similarity of action of the toxins at the physiological (D. Rogers, Ph.D. thesis, University of London 1976), and wall receptor levels. The organization of the polypeptide encoded by the K2 toxin gene is shown in figure 1. This precursor appears to have a similar overall structure to that of the K1 toxin, with possible toxin subunits separated by pairs of basic residues. Using an antibody raised against a LacZ::K2 fusion protein, an immunoreactive intracellular species with an apparent molecular weight of 43,000 has been detected. This likely corresponds with a glycosylated intracellular precursor. The same antibody recognises a 21,000 extracellular species and reacts very weakly with a 9,000 species, which may be subunits derived from the precursor, as indicated in figure 1. Unlike the K1 toxin, these putative toxin subunits do not appear to be associated by disulphide bonds, as the separate subunits are detected on Western

blots of sodium dodecylsulphate polyacrylamide gels run in the absence of mercaptoethanol. The 21,000 molecular weight species appears to be glycosylated, as it is reduced in size by treatment with endoglycosidase H.

Biogenesis and secretion of toxins

The finding that the mature secreted K1 toxin was made as a larger precursor that was processed in the yeast secretory pathway allowed detailed analysis of this system which turns out to be remarkably similar to that seen in the maturation of many eukaryotic hormones and neuropeptides[8]. Identification of endoplasmic reticulum and later secretory pathway events has relied heavily on conditional lethal, temperature sensitive, yeast mutants which block procession of secretory and plasma membrane proteins along the secretory pathway at the restrictive temperature. Analysis of toxin precursors by immunoprecipitation with antitoxin antibody demonstrated that an amino terminal signal peptide is necessary for entry into the yeast endoplasmic reticulum[27]. The protoxin is glycosylated in the endoplasmic reticulum and the precursor is proteolytically processed to mature toxin late in the pathway, probably at a late Golgi step[6,10,27]. The system has allowed a definition of the components of the protease processing system, the first for any eukaryote. This work is summarised in figure 1 where the primary event is an endoproteolytic cleavage following pairs of basic residues in the precursor by the product of the yeast KEX2 gene, an endoprotease with homology with subtilisin[24,26,32,45]. A subsequent event involves a carboxypeptidase B-like activity which removes the COOH-terminal basic residues of the α chain by the product of the KEX1 gene, a serine carboxypeptidase with homology with the yeast vacuolar protease, carboxypeptidase Y[19]. Following such processing, the mature toxin is secreted via the constitutive secretory pathway to the growth medium.

Expression of the native M2dsRNA-coded K2 toxin also requires the presence of both the KEX2 and KEX1 genes, as does expression of a cDNA copy of the gene from the yeast alcohol dehydrogenase promoter (Whiteway, Dignard and Thomas, in preparation). It thus seems certain that the K2 precursor is processed at Lys-Arg sites, but whether the pattern is similar to K1 with KEX2 cleavage following the 221 and 268 pairs of residues, with release of an interstitial γ equivalent and KEX1-dependent removal of residues 221 and 220, remains to be determined.

Functional studies of toxin action

Early physiological and mutant studies[12,13] suggested at least two steps of toxin action: binding to the cell wall and action at the cell membrane. More recent work on mutants with alterations in the toxin structural gene and on nuclear gene mutations that cause defects in components necessary for toxin action extend this view.

Cell wall receptor. Extracellular proteins often recognize target cells by binding to specific receptor molecules at the cell surface. The K1 toxin behaves in this way and binds to a cell wall (1 → 6)-β-D-glucan, which is at least a component of this surface receptor and is necessary for toxin action. An important approach here was the isolation of killer toxin resistant mutants. These are obtained by a simple selection, a population of sensitive cells is treated with toxin and survivors screened for resistance. Early studies[1] isolated two killer resistant mutants called *kre*1 and *kre*2 that had a reduced level of toxin binding to cells. Binding of toxin to a trypsin-resistant, periodate-sensitive, cell wall component implicated a glucan as a receptor in sensitive cells. Further binding studies[11] identified an abundant cell wall receptor (1.1×10^7/cell) with an association constant of 2.9×10^{-6} M that was missing from *kre*1 mutant cells. Binding to this receptor was rapid and energy-independent[36]. This wall receptor could be solubilised with zymolyase (an endo-(1 → 3)-β-D-glucanase) and such solubilised extracts from sensitive cells competitively inhibited toxin action, whereas solubilised wall extracts from a *kre*1 resistant mutant did not. Evidence identifying (1 → 6)-β-D-glucan as the receptor came from several sources[23]. Fractionation and selective degradation of the cell wall receptor, measured by its ability to competitively inhibit toxin action, implicated this glucan. In addition, the (1 → 6)-β-D-glucan component of *kre*1 mutants was reduced to approximately half of that found in isogenic wild type cells. Use of purified polysaccharides from a range of organisms as competitive inhibitors of toxin action showed that only those containing (1 → 6)-β-D-glycan linkages were effective. In addition (1 → 6)-β-D-glucans (such as pustulan) coupled to a dextran matrix bound the toxin avidly, allowing an affinity purification. Such binding of K1 toxin to a pustulan-Sepharose column was pH-dependent with an optimum at pH 4.7. This binding step may be responsible for the known narrow pH range around pH 4.7 where the toxin is active on yeast cells.

While these results identify the (1 → 6)-β-D-glucan component as a wall receptor, the exact nature of the lectin-like interaction of the toxin with this glucan remains unclear. We have some preliminary information that the glucan binding domain of the toxin protein spans both toxin subunits (see section on mutagenesis of the toxin gene below). However, we do not know the precise structure of the (1 → 6)-β-D-glucan receptor, how it is assembled into the overall structure of the yeast cell wall, or how the receptor mediates toxin action – perhaps by facilitating passage of the toxin through the cell wall to the plasma membrane?

KRE genes. Identification of genes which lead to an altered glucan wall receptor should provide insight into the synthesis and assembly of the β-glucans themselves. These polymers which are widespread in nature have intrinsic interest and their synthesis remains poorly understood. We have recently begun a molecular analysis of

the KRE1 and KRE2 genes and of three other genes KRE4, 5 and 6 that lead to toxin resistance when mutated, and which appear to be involved in cell wall glucan synthesis. It is anticipated that such an analysis will provide molecular information on the components involved. The KRE1 gene has been cloned and sequenced (Boone and Bussey, Yeast *4* (1988) S434, and J. Cell Biol., in press). It codes for a 32,000 molecular weight serine, threonine-rich protein with an NH2-terminal signal peptide. The KRE1 gene product traverses the yeast secretory pathway and is found extracellularly as a heavily O-glycosylated species. The gene is not essential for vegetative growth of yeast. Null mutants grow somewhat more slowly, but cells have a normal size and shape. Such null mutants are toxin resistant, have a reduced level of (1 → 6)-β-D-glucan and oversecrete cell wall proteins into the growth medium[15] (and Boone and Bussey, J. Cell Biol., in press). At the ultrastructural level, electron micrographs of cross sections of the yeast cell wall show that an outer electron dense region is missing in the mutants. Analysis of the residual (1 → 6)-β-D-glucan in the *kre*1 mutants suggests that it is altered in structure from the wild type polymer. This would explain why despite the presence of (1 → 6)-β-D-glucan, an effective functional receptor is missing in the *kre*1 mutants. Linkage data, C^{13} NMR spectroscopy, and molecular sizing indicate that the mutant (1 → 6)-β-D-glucan is of lower molecular weight and has a higher proportion of (1 → 3)-β-D-glucose linked residues in both the backbone and branches than the wild type structure. The results of our work are consistent with the KRE1 gene product being responsible for extended synthesis of the glucan polymer to generate a structure that is a functional toxin receptor.

Action of other toxins at the cell wall level. Patterns of cross resistance of K1-resistant mutants to other killer toxins indicate that (1 → 6)-β-D-glucan is widely used as a component of a wall receptor for these proteins. Work of Al-Aidroos[1] and Rogers and Bevan[34] showed that the *kre*1-1 mutant allele was resistant to many yeast toxins. These included the K2 toxin of *Saccharomyces cerevisiae* and toxins from the genera *Candida, Torulopsis, Pichia, Kluyveromyces* and *Debaryomyces*. Examples so far found of toxins that can kill *kre*1 mutants are the KT28 toxin of *Saccharomyces cerevisiae* and the *K. lactis* killer toxin[21]. The KT28 toxin has been shown to bind to a cell wall mannan receptor on sensitive *Saccharomyces cerevisiae*[35]. Little is known about the receptor for the *K. lactis* toxin. In contrast, *kre*2 mutants appear to have a narrow spectrum of resistance, being found resistant only to K1 and *C. albicans* killers[1,34]. Unfortunately, the basis for this specific toxin binding defect in *kre*2 mutants remains unknown.

Sensitivity of spheroplasts. Spheroplasts of *Saccharomyces cerevisiae* were found to be sensitive to the K1 toxin. This sensitivity was shown to be independent of wall receptor binding by the demonstration that spheroplasts of receptor defective mutants were toxin-sensitive[1,13]. The action of the toxin on cells was seen as at least a two-stage process, with binding to wall receptors necessary in cells to allow the toxin access to the plasma membrane where the events found in spheroplasts would occur. Some recent work amplifies this view and suggests that the cell wall receptors can determine the specificity of toxin action on sensitive yeasts. On whole cells the K1 toxin has only a narrow host range, killing sensitive cells of *Saccharomyces cerevisiae* and *Torulopsis glabrata*[14,49]. However, examination of toxin action on spheroplasts shows that the K1 toxin is far less discriminating, and can kill yeast spheroplasts from the genera *Candida, Kluyveromyces* and *Schwanniomyces*, while cells of such yeasts are toxin insensitive (Zhu and Bussey, Appl. envir. Microbiol. *55* (1989) 2105–2107).

Physiology of toxin action at the plasma membrane

Initial studies on the physiology of intoxicated cells indicated that growing yeast cells were most susceptible[46] and that loss of ATP, potassium and cellular metabolites occurred following a lag after toxin addition[13,36], and yeast cells were seen to shrink in volume with such treatment[7]. Examination of the toxin-dependent loss of potassium showed that such effects were energy-dependent, suggesting an electro-chemical membrane potential was essential for toxin action[36]. Later work addressed more immediate effects which follow toxin addition. The toxin was shown to inhibit leucine transport and cotransport of protons[18]. In addition proton pumping to the medium from glucose fed cells was also inhibited. In a further study the same group[17] showed that toxin reduced the proton gradient across the membrane of yeast cells, with acidification of cell contents and potassium efflux. Based on passive proton permeability studies, it was argued that cell acidification was due to an increase in proton permeability in intoxicated cells. These experiments suggest that the toxin in some way perturbs an energized membrane state, but whether it acted to inhibit some component of the proton pump or more directly by forming a protein channel was unclear. Work on another killer toxin from *Pichia kluyverii* provides the only direct evidence for a killer toxin forming a protein channel in a lipid bilayer. Work of the group of Middelbeek[31] showed that this killer toxin (as yet uncharacterized) had physiological effects on *Saccharomyces cerevisiae* very similar to that of the K1 toxin described above. Using crude preparations of the *Pichia kluyverii* toxin, Kagan[25] showed that it could form ion permeable channels in planar phospholipid bilayers. These channels were relatively non-selective for common physiological cations and anions, were weakly voltage-dependent, and had a bimodal conductance distribution centered around 140 and 220 picoSiemens. Kagan suggested that these channels were sufficient to cause the in vivo effects of the *Pichia kluyverii* and *Saccharomyces cerevisiae* killer toxins. The toxin-induced channels would cause a 'leak pathway' for major ions such as K^+ and H^+ and dissipate the normal

ionic gradients including the proton gradient necessary for physiological transport across the plasma membrane. Kagan calculated that the toxin channels had approximately the right conductance to account for the K+ efflux data[31,36] and also for the time course of the ion exchanges seen. The killer toxin channels have a conductance two orders of magnitude greater than found with the bacterial colicins, but the yeast cell has 1,000 times the volume of the bacterial cell, and this would be consistent with the fact that the killer toxin takes about 10 times longer to dissipate the yeast cells membrane potential compared to colicin action on a bacterial cell.

While the in vitro pore work of Kagan provides good evidence that killer toxin from *Pichia kluyverii* can form pores, direct definitive evidence is still missing for the *Saccharomyces cerevisiae* K1 toxin, although some preliminary (and unpublished) results by Kagan did demonstrate in vitro channels for this toxin. The more recent structural and mutant work, discussed below, should provide an excellent opportunity to study the pore-forming nature of the K1 toxin.

Mutations in the toxin gene

The availability of the toxin precursor gene and an expression system in yeast have made directed mutational change and testing for altered function a relatively straightforward process. Preliminary work on defining functional domains of the killer toxin, through site-directed mutagenesis[3,9,40] has been published. These mutants and a more extensive series that have been analysed (Zhu and Bussey, unpublished work) are summarised in figure 2 with tentative domain assignments. One of the limitations of the approach has been that because of the complexity of biogenesis of the toxin not all mutants are processed and secreted. Another potential problem is lethality of some mutants through retention of functional toxin with a defective immunity component; use of an inducible promoter for expression of the precursor gene has been employed to avoid such a bias[40].

Defects in processing or secretion of toxin. A set of mutations in the signal region lead to reduced levels of toxin secretion[27] (and Lolle, unpublished results). Others, especially insertion mutants scattered throughout the precursor also have this phenotype[3]. The γ region is glycosylated (see fig. 2) and recently the role of the three asparagine-linked glycosylation sites have been examined (Vernet and Thomas, unpublished). Seven mutants were made, replacing the three Asn residues with the nonglycosylatable Gln residue in all combinations. These mutants were independently examined for phenotype following transformation to a sensitive yeast strain. Colonies harbouring the mutants were slightly smaller than those with the wild type toxin gene. The reduction in colony size directly correlated with the number of mutated glycosylation sites, with the triple mutant being the most affected. This triple mutant had a wild type level of the immunity phenotype, but the amount of toxin activity was reduced approximately 20% over the wild type. This reduced production could be accounted for by the slower growth rate of the cells. These data indicate that the non-glycosylated precursor is still functional, and that the glycosylation that normally occurs in the endoplasmic reticulum is not essential for folding, transfer through the secretory pathway or processing of the toxin.

Binding to the cell wall. The lectin-like domain of the toxin that binds to the (1 → 6)-β-D-glucan receptor can be mapped by screening mutants for defects in this function. Such mutants should fail to kill yeast cells but could retain the ability to kill spheroplasts. A series of mutations in the β subunit and two in α have lost the ability to bind to the wall, are inactive on whole cells, yet kill spheroplasts and define a glucan binding domain which appears to span both subunits. The simple expectation that β alone contains the lectin domain seems improbable from these results.

Channel formation. Regions necessary to form the putative membrane channel have been identified operationally by assaying mutant toxins for ability to kill yeast spheroplasts. It can be seen from figure 2 that all mutants defective in this function map to the α subunit. This seems a reasonable conclusion as a series of mutations spanning the β coding region retain the ability to kill spheroplasts. Many of these presumed channel-defective mutants are within the two prominent hydrophobic domains of the α protein, spanning residues 72–91 and 112–177, and strongly suggest that these regions are essential for the membrane action of the toxin. It remains uncertain whether both α and β subunits are involved in the channel forming process or whether the dimer dissociates and α alone are sufficient for channel formation.

Immunity domain. The finding that immunity was conferred by the toxin precursor gene[22,28] led to studies mapping immunity to the α subunit[3,40]. This work and some additional mutants (Zhu, unpublished) are shown in figure 2. These site-directed mutations altering immunity define a region of the α subunit that overlaps remarkably with the region that is thought to be involved in membrane channel formation. This seeming paradox of immunity and toxin action being conferred by similar or overlapping domains of the protein was resolved by several models[3]. The features of these models are that the toxin precursor or some other incompletely processed nontoxic α-containing product, competitively interfere with mature toxin action. This could be by occupying a necessary receptor on the plasma membrane or directly by interfering with channel formation. The precise protein product that confers immunity has not been determined, but some mutants (see fig. 2) which fail to allow precursor processing and toxin secretion retain immunity, suggesting that the precursor can function as an immunity component. In addition, strains defective in the processing proteases encoded by the KEX2 or KEX1

genes retain immunity. Possible alternative processing of the protoxin precursor to an immunity component has been suggested, based on the finding of toxin precursor fragments of intermediate size in cell extracts[40], but so far this notion lacks compelling experimental support.

Prospects for further work

The presence of a killer phenotype in the well characterized *Saccharomyces cerevisiae* system has served as a genetic window into the molecular and cellular biology of a range of cellular processes such as viral interactions with the host; protein secretion; and cell surface assembly. The cell biology of such processes is far from being fully understood, and further study of genes already found and of their interactions, and the use of the system to generate new genes promises a continued harvest. This emphasis on genetics has led to only limited work on the molecular basis of the killing reaction itself. The wide range of other protein toxins made by killer yeasts also remains unexplored and may repay study with information at the basic and applied levels. Finally, the use of killer systems built into strains for industrial fermentations such as wine and beer production should find continued use in reducing the risks of contamination by unwanted yeasts.

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Conclusion

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According to the endosymbiotic theory at least two organelles of present eukaryotes, namely mitochondria and chloroplasts, originate from the symbiosis of a protoeukaryote with gram-negative prokaryotes. This theory is supported by the presence of a double membrane, DNA, a protein synthesizing machinery and functionally related proteins in the corresponding membranes of mitochondria and chloroplasts as well as in gram-negative prokaryotes. However, since the early days of this symbiosis a lot of time has passed, and many changes must have occurred which confuse today's picture. For instance, many originally mitochondrial genes must have moved to the nucleus, since today more than 90% of all mitochondrial proteins are encoded by nuclear DNA. Similarly, proteins which may have a common origin, like the porins of the outer membrane, might have diverged according to the needs of their host organism. For most bacteria it must have been useful to have small, cation-selective pores with an exclusion limit of 600–800 D, whereas the pores of mitochondria and chloroplasts were selected to be larger (exclusion limit around 6000 D and 7000–13,000 D, respectively [1]) and anion selective. Both kinds of channels are voltage-gated (VDAC) and consist of multiple membrane-spanning β-sheets [2].

Features which clearly distinguish between the mitochondrial and bacterial porins that the present review focusses on are the absence of sequence homology and the differences in their tertiary structure (trimers for bacterial [10] and monomers or dimers for mitochondrial porins [6]). Why the different requirements? Bacterial porins control the passage of various ions and hydrophilic solutes up to the size of ATP. Their expression is dependent on the osmolarity of the surrounding media [1]. In contrast, mitochondria have pores with an exclusion limit ten times as large, and the necessity for this is not fully understood. The absence of porin from yeast mitochondria only causes transient respiratory problems in a few yeast strains, suggesting the presence of alternative pores [5]. Since the alternative pores found in porinless mutants have an exclusion limit of about 600 D, the most important mitochondrial substrates which cross the outer membrane must either be rather small or pass through specialized channels rather than unspecific pores. Since the respiratory problems of porinless yeast mutants seem to be caused by a reduction in the levels of mitochondrial cytochromes, an involvement of porin in the biogenesis of these proteins has to be considered. Thus the rate-limiting step could be the import into mitochondria of either the apocytochromes themselves, or of the precursors of heme which later associates with them inside the mitochondria. In fact, cytochrome *c* binds to mitochondrial porin in vitro [6]. Whether this interaction is also meaningful in vivo and has biological significance like the binding of hexokinase to porin [3] will become clear once the outer membrane receptor(s) for mitochondrial protein import has(have) been identified.

Other differences between bacterial and mitochondrial porins are due to the reverse orientation of the mitochondrial and the bacterial membranes: bacterial porins are