- **retroplasmids**, which are linear or circular plasmids that encode a reverse transcriptase. DNA and RNA polymerases, or reverse transcriptase, encoded by plasmid DNA sequences are used to maintain and propagate the plasmid.

True plasmids are mostly **cryptic** (that is, neutral) passengers in nature, but some linear plasmids (notably of *Podospora anserina*) insert into mitochondrial DNA and cause **mycelial senescence**. Most linear plasmids exhibit typical virus characteristics as far as structure, replication and function are concerned even to the extent that plasmid-free strains may contain plasmid remnants integrated into their mitochondrial DNA. These different groups of true plasmids probably arose independently of one another and may have a different evolutionary origin from that of the mitochondrial host-genome. They were either vertically transmitted from the original endosymbiont that gave rise to the mitochondrion or invaded the mitochondrion at various times during fungal evolution (Hausner, 2003).

Although most ***mitochondrial*** plasmids are cryptic and symptomless, cytoplasmic plasmid DNA is responsible for the **killer phenomenon** in the yeast *Kluyveromyces lactis* by coding for a killer toxin, which kills cells lacking the plasmid (cells hosting the killer plasmid are immune to the toxin). These plasmids reside in the cytoplasm and have an expression system independent of both nucleus and mitochondrion. Plasmids of *Kluyveromyces lactis* can be transferred to other yeasts (including *Saccharomyces cerevisiae*), conferring the killer/immunity phenotypes (Fichtner *et al*., 2003). This shows that the plasmids are autonomous replicons, which can be expressed in a wide range of host yeasts.

The *Kluyveromyces lactis* killer plasmid toxin is chemically and functionally different from killer toxins produced in *Saccharomyces cerevisiae* (especially wine yeasts), which are encoded by double-stranded RNA (dsRNA) virus. Different killer toxins, K1, K2, K28, and Klus, have been described; each being encoded by a 1.5- to 2.3-kb double-stranded M satellite RNA located in the cytoplasm. These M satellite dsRNAs require larger helper virus (generally called L-A virus) for maintenance; L-A belongs to the Totiviridae family, and its dsRNA genome of 4.6 kb codes for proteins that form the virions that encapsidate separately the L-A or M satellites (Rodríguez-Cousiño *et al*., 2011; Rodríguez-Cousiño & Esteban, 2017).

Both budding yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*) have been used for studies of plant, animal and human viruses. Many RNA viruses and some DNA viruses replicate in yeasts. As many of the fundamental eukaryotic cell functions are highly conserved from yeasts to higher eukaryotes, these easily-cultivated fungi offer many unique advantages in virus research over 'higher' eukaryotes and are particularly suited to study the impact of viral activities on cell function during virus-host interactions (Zhao, 2017). **Mitoviruses** are simple RNA **mycoviruses** that replicate in host mitochondria and are frequently found in fungi; genomics approaches are now being used to study them (Kotta-Loizou, 2019; Nibert *et al*., 2019).

Virus-like particles (**VLPs**) have been observed in electronmicrographs of many fungi. They are very similar in appearance to small spherical RNA viruses, but there is little evidence that these particles are effective in hypha-to-hypha infection. Many of the observed VLPs are presumably degenerate or defective viruses that can only be transmitted by hyphal fusions. No vectors are known for fungal viruses; transmission seems to depend on hyphal fusions. Unexpectedly, virus infections of fungi usually cause no recognisable phenotype. The exception is a mycovirus of *Agaricus bisporus*, which causes **La France disease** and ruins the crop. Diseased crops contain three virus particles and require up to 10 different RNA molecules to produce infective particles; as though some are defective viruses and others are helper viruses, or perhaps different viruses perform complementary, but essential, functions (Frost & Passmore, 1980). In a study of the infection on commercial mushroom farms in Poland, the virus particles were found in 120 of 200

samples tested; this level of La France disease could be a threat to the mushroom industry (Borodynko *et al*., 2010).

Saccharomyces cerevisiae also harbours retrovirus-like elements, as retrotransposons (now called **transposable elements** or **TEs**) able to integrate into the nuclear genome by targeting particular chromatin structures. The transposable elements of Saccharomyces cerevisiae consist of **LTR** (**L**ong **T**erminal **R**epeat) retrotransposons called **Ty elements** belonging to five families, Ty1 to Ty5. They take the form of either full-length coding sequences or non-coding solo-LTRs corresponding to remnants of former transposition events. The first cytoplasmic plasmid to be observed was the so-called **two-micron DNA** of *Saccharomyces* (Ty1). The name refers to the contour length of the circular DNA molecules in electronmicrographs; it has a base composition similar to nuclear DNA and quite different from mtDNA. There can be 50 to 100 two-micron DNA molecules per diploid cell, amounting to something like 3% of the nuclear DNA. The two-micron DNA molecules are transmitted to buds independently of both nuclei and mitochondria. The two-micron circular DNA carries inverted repeat sequences at either end of two different unique sequence segments; this structure implies that it inserts itself as a whole into the yeast chromosome (Bleykasten-Grosshans & Neuvéglise, 2011; Bleykasten-Grosshans *et al*., 2013; Stukenbrock & Croll, 2014).

So far, we have described nucleic acid molecules that encode features segregating in the cytoplasm. In the final decade of the twentieth century, however, great attention was given (and continues to be given) to a ***proteinaceous hereditary element***, called a **prion protein**. The attention devoted to prions derives from their ability to cause diseases in mammals: ***scrapie*** in sheep, bovine spongiform encephalopathy (***BSE***) in cattle; and in humans, ***kuru*** and new variant Creutzfeldt-Jakob disease (***nvCJD***). In these cases, the pathogenic agent is a variant of a normal membrane protein (the prion protein) that is encoded in the mammalian genome.

Prions are infectious proteins, which means that they are altered forms of a normal cellular protein that may have lost their normal function but have acquired the ability to modify the normal form of the protein into the same abnormal configuration as themselves. The variant prion protein folds abnormally and in addition causes normal prion proteins to fold abnormally so that the proteins aggregate in the central nervous system and cause the encephalopathy; the aggregated proteins are called **amyloids**.

Several prion-forming proteins have been identified in fungi, mostly in the yeast *Saccharomyces cerevisiae*. We have already referred briefly (see Section 7.5 and Table 3) to the infectious ***het-s*** (heterokaryon incompatibility) phenotype of *Podospora anserina* as a protein that adopts a prion-like form to function properly as part of the self/nonself recognition system that ensures that only related hyphae share resources. The prion form of het-s can convert the non-prion form of the protein in a compatible mate after hyphal anastomosis. However, when an incompatible mycelium mates with a prion-containing mycelium, the prion causes the incompatible hyphal compartments to die when a programmed cell death response is triggered by interaction between specific het alleles (Paoletti & Clavé, 2007; Bidard et al., 2013; Paoletti, 2016).

First among several prions identified in *Saccharomyces cerevisiae* is the PSI^+ form of the Sup35p protein. Sup35p is an essential yeast protein involved in the termination of translation. In the [PSI^+] state, Sup35p adopts the structural conformation that causes it to direct the refolding of native molecules into a form that can aggregate into filaments of discarded nonfunctional protein.

This depletes the cytoplasm of functional translation terminator and results in translation errors; which is the [PSI^+] phenotype that is inherited by daughter cells following budding, and is infectious following cell fusion, in which case it propagates by autocatalytic conversion of the normal form of the protein.

The prion phenotype is officially described as '***increased levels of nonsense suppression***' because the prion suppresses nonsense-mutations by allowing the mutant genes to produce functional proteins; that is, the translation error is to translate the mutant code as working protein. Another unusual trait identified in yeast in the 1990s was called [**URE3**], which results from the prion form of the normal cellular protein Ure2p, which is a nitrogen catabolite repressor. Note that the names for yeast prions are normally shown in square brackets to indicate that they segregate in a non-mendelian manner. Two more recent discoveries are [$MOT3^+$], the prion form of a nuclear transcription factor, which as a prion causes transcriptional derepression of anaerobic genes; and [GAR^+], the normal function of the proteins being as components of plasma membrane proton pumps but as a prion it causes resistance to glucose-associated repression. For a list of prions refer to the Wikipedia page ***Fungal prion*** [at https://en.wikipedia.org/wiki/Fungal_prion], which seems to be regularly updated.

The part of the Sup35p protein that makes it a prion (the prion determining domain) is a glutamine/asparagine-rich amino-terminal region that contains several oligopeptide repeats. Removal of these repeats eliminates the ability of Sup35p to propagate PSI^+ and expanding the repeat region increases the spontaneous occurrence of PSI^+. Although deleting the analogous repeats from BSE prion protein does not prevent prion propagation and transmission in experimental mice, expansion of the repeat region does increase the spontaneous appearance of spongiform encephalopathies by several orders of magnitude in humans. It is likely that the oligopeptide repeats give the prion protein the intrinsic tendency to acquire a conformation that enables the protein to refold and self-propagate its conformation, so effectively polymerising with sister molecules to form the fibrous proteinaceous deposits called amyloids. Database searches for regions with amino acid content comparable to the yeast prions has revealed numerous such domains in eukaryotes, but these are lacking from prokaryotes (Alberti *et al*., 2009; King *et al*., 2012).

Fungal prions are generally benign; indeed, some confer a potential advantage to the fungus. Although it has been claimed that [URE3] and [PSI^+] are 'diseases' of yeast caused by laboratory cultivation, some [PSI^+] cells actually fare better than their prion-free siblings when subjected to adverse conditions. When 700 wild strains of *Saccharomyces* were genetically screened for unknown prion elements, one-third of the strains were found to harbour them (Halfmann *et al*., 2012). These 'natural' prions created diverse and often beneficial phenotypes. Evidently, fungal prions govern heritable traits in nature, but in a manner that confers the capacity on the fungus to adapt quickly and reversibly to variable environments. It has been suggested that the amyloid-folding promoted by fungal prions is a widespread and evolutionarily conserved mode of ***signal transduction***, which is based on the transmission of an amyloid-fold from a STAND receptor protein to an effector protein (Daskalov *et al*., 2012). Fungal prions potentially provide a model for understanding disease-causing prions of humans and animals, particularly by identifying the sequence features and mechanisms that enable prion domains to switch between functional and amyloid-forming states [for information about disease-causing prions of humans and animals refer to website of the ***U.S. Department of Health & Human Services Centers for Disease Control and Prevention*** at https://www.cdc.gov/prions/index.html].

Chapter 7.10 References and further reading

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