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Current Opinion in
Structural Biology

Insights into the mechanism of prion propagation

Sarah Perrett[1] and Gary W Jones[2]

Proteins with prion properties have been identified in both mammals and fungi. The tractability of yeast as a genetic model has contributed significantly to our understanding of prion formation and propagation. A number of molecular chaperones have been found to modulate the ability of yeast prion proteins to propagate. The results of recent genetic and *in vitro* studies have shed light on the mechanism of prion propagation, the physical and structural basis of different prion strains and the species barrier, as well as the function and mechanism of the chaperones that interact with the prion proteins. Whether aspects of the mechanisms of formation, maintenance and clearance of prions are conserved between fungi and mammals remains to be seen.

Addresses
[1] National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, China
[2] Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland

Corresponding author: Perrett, Sarah (sarah.perrett@iname.com) and Jones, Gary W (Gary.Jones@nuim.ie)

Current Opinion in Structural Biology 2008, **18**:52–59

This review comes from a themed issue on
Folding and binding
Edited by Laura Itzhaki and Peter Wolynes

0959-440X/$ – see front matter
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DOI 10.1016/j.sbi.2007.12.005

Introduction

A number of human diseases have been attributed to protein misfolding or aggregation [1]. Misfolding can result in loss of normal protein function and so give rise to disease. Alternatively, as suggested in the case of amyloid diseases, the formation of stable, ordered aggregates (or 'amyloid') can have a toxic effect on cells. A complex network of quality control mechanisms exists within the cell to aid protein folding and assembly and to ameliorate the consequences of misfolded or damaged proteins [2]. Of particular importance is a class of proteins known as molecular chaperones that play a crucial role in ensuring correct protein folding and preventing accumulation of aggregated proteins within the cell [3].

Recent reviews in this journal have focused on sequence determinants of amyloid formation [4], atomic models of amyloid fibril structure [5] and on the role of intermediates in amyloid assembly [6]. The purpose of this review is to highlight papers published during the past two years that contribute to our understanding of the mechanism and structural basis of prion formation, as well as the role of chaperones in the propagation process, particularly focusing on yeast prion models. We also discuss how research on fungal prions contributes to understanding of mammalian prion disease.

The prion hypothesis

Prions are an interesting class of amyloidogenic proteins. A prion is an infectious protein: the prion form has a different structural conformation than the normal protein and is able to propagate this conformational change among other molecules of the same protein [7,8]. Prions are associated with a class of neurological diseases called transmissible spongiform encephalopathies, which include scrapie in sheep, bovine spongiform encephalopathy (BSE, or 'Mad Cow Disease') and the human form Creutzfeldt–Jakob disease (CJD) [8,9]. The BSE epidemic in the UK, followed by appearance of a new human form of the disease (variant CJD), has resulted in intense research effort in the prion field. However, more than a decade later, treatment for prion disease remains a distant hope and a number of basic questions regarding the disease mechanism remain unanswered, including the molecular mechanism of prion propagation, the structure or identity and cellular action of the neurotoxic species and the normal cellular function of the mammalian prion protein, PrP [9–11].

Wickner proposed that the genetic behaviour of the *Saccharomyces cerevisiae* non-Mendelian elements [*URE3*] and [PSI^+] could be explained if they were prion forms of the proteins Ure2 and Sup35, respectively [12]. In the meantime, much evidence has accumulated to support this proposal and further potential fungal prions have been identified, including Rnq1/[RNQ^+/PIN^+] in yeast and [Het-s] in the filamentous fungus *Podospora anserina* [13–15]. The fact that prions exist in yeast provides an ideal environment for detailed genetic analysis of factors affecting prion maintenance and has also facilitated characterization of the biochemical and biophysical properties of prion proteins [16]. The relative simplicity and tractability of yeast and other fungal systems has aided progress in understanding the more curious aspects of the prion phenomenon. In particular, studies on fungal prions have contributed convincing proof of the protein-only hypothesis of prion infectivity for [Het-s], [PSI^+], [*URE3*] [15] and recently also for [PIN^+] [17], a feat that has been more difficult to achieve for mammalian prions [18]. Another area in which research on fungal prions has contributed

significantly to the field is in the understanding of the physical and structural basis for prion infectivity, the barrier to cross-species transmission and the existence of different prion strains [19–22,23••,24•,25•]; the strain phenomenon being a well characterized but nevertheless puzzling characteristic of mammalian prions [8,9] and possibly of amyloids in general [26–29].

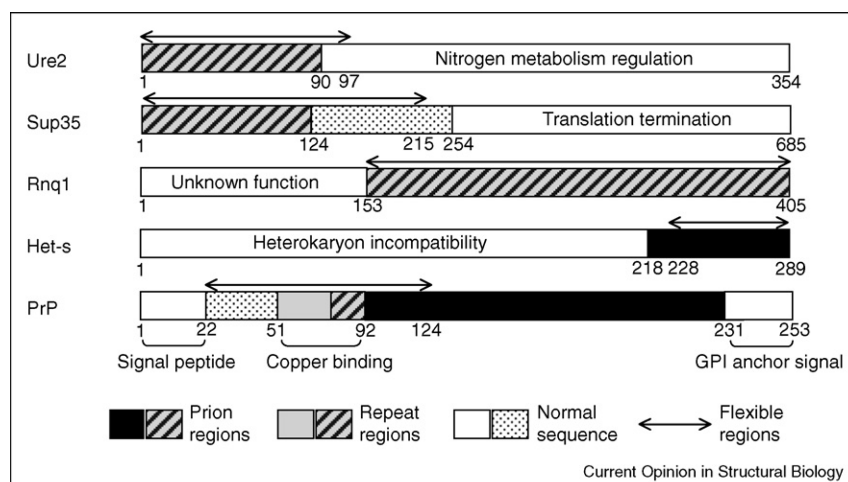
Structural aspects of prion proteins

The various prion proteins are related via the prion concept; however, comparison of the structural and functional properties of their native states shows limited similarity [16] (Figure 1). Ure2, Sup35 and Rnq1 each contain an Asn/Gln-rich region that conveys prion properties to the protein and hence is termed the prion domain. The amino-acid sequence of the Het-s protein lacks any obvious repetitive sequence region, although it is similarly divided into a globular functional region and a flexible prion domain. Nevertheless, Het-s prion domain fragments retain the ability to propagate as prions in yeast [30•]. The relationship between domain structure and function for the PrP protein is more complex. The PrP protein has a flexible N-terminal tail containing an octapeptide repeat region that may be involved in copper binding, but this flexible region only partially overlaps with the segment that is required for infectivity [9] (Figure 1). As further prion proteins are identified and characterized, it may become clear whether the presence of a flexible tail in the native state is a necessary feature in order to undergo a switch to a prion structure.

A great challenge in the amyloid field is to obtain detailed and reliable information about the structure of fibrillar forms, as these tend to defy conventional approaches to structure determination [31]. Recent X-ray diffraction studies on amyloid-like microcrystals formed from a short (7-residue) peptide of the Sup35 protein have provided an atomic resolution model of a possible building block for the structure of Sup35 fibrils (reviewed in reference [5]). Variations of this building block may form the basis of the structures of other amyloid protein fibrils, and this also provides a possible structural basis for prion strains [29]. However, this does not tell us whether the same structure exists in fibrils formed from the full-length protein. Recent approaches to this problem using the 253-residue NM fragment of Sup35 have included solid state NMR [32] and cysteine scanning [33]. A significant advance in structural detail was obtained in a recent NMR study of two strain variants of Sup35 NM fibrils, which suggests that the two strains share a common amyloid core involving the N-terminal Asn/Gln-rich region (residues 1–40), with strain-specific involvement of parts of the succeeding oligopeptide repeat region (residues 41–100) [24•]. This study, taken together with a recent genetic study [34], suggests that the existence of different [*PSI*+] strains has a structural basis, with different sequence regions providing core or variable structural elements of the fibril. Further, the relative ability of different prion strains to propagate may reflect the stability of that structure and its propensity to fragment into seeds [23••]. This in turn may provide an explanation for why relatively small variations in prion domain sequence can affect the ability of Ure2 homologues from closely related yeast species to stably propagate a prion state [35].

A further challenge in understanding the mechanism of prion propagation is mapping the pathway of structural changes involved in converting the native state into the aggregated prion form. The structure of the globular C-domain of Ure2 was solved some years ago, and its folding

Figure 1



Comparison of structural and functional properties of prion proteins. Domain structures of Ure2, Sup35, Rnq1, Het-s and PrP are shown. The functional regions are as indicated. Repetitive regions correspond to Asn/Gln-rich regions, except for PrP, in which residues 60–91 include four copies of an octapeptide repeat sequence PHGGGWGQ. Figure reproduced from reference [16] with permission from Elsevier.

mechanism has been studied in detail (reviewed in reference [16]). However, a clear picture of the role of folding intermediates in prion formation has yet to emerge. Use of solid state NMR [36], introduction of cysteine mutants [37] and H/D-exchange combined with mass spectrometry [38] have yielded some initial clues about the residues that may be involved in the Ure2 fibrillar structure, although at insufficient resolution to settle all current debates on this issue. Nevertheless, it seems clear that the C-domain of Ure2 maintains its native, enzymatically active fold within the fibrils [39], which also appears to be the case for Sup35 [40], indicating that prion fibrils may contain a mixture of the characteristics of amyloid and globular protein structures.

Role of chaperones in maintenance of prion propagation

Molecular chaperones play an important role in the cell, ensuring that proteins fold correctly to their native functional conformation [2,3]. Many chaperones are also classed as heat shock proteins (Hsps), as their expression is upregulated under conditions of cellular stress, when proteins are particularly likely to unfold and aggregate. Genetic studies have identified a number of chaperones that modulate the behaviour of yeast prions [41] (Table 1). Indeed, early evidence in support of the existence of prions in yeast [12] came from data showing that the protein disaggregase Hsp104 is required for efficient maintenance of [*PSI*$^+$] [42]. This strongly implied that efficient propagation of the [*PSI*$^+$] element was protein based.

Hsp104 is a protein disaggregase that provides a cellular defence against protein denaturation due to stress [43], but in addition it also plays an essential role in the formation and propagation of yeast prions [44]. Deletion or overexpression of Hsp104 can lead to prion curing (Table 1). A comprehensive mutagenesis study of Hsp104 has identified mutations located around the lateral channel of the Hsp104 hexamer as impairing prion propa-

Table 1

Summary of chaperone effects on prion propagation

Chaperone or co-chaperone	Cellular function	Effects on prion propagation when	
		Deleted	Overexpressed
Hsp104	Protein disaggregation and stress tolerance	Cures all known naturally occurring yeast prions	Efficiently cures [*PSI*$^+$] but not [*URE3*] or [*PIN*$^+$]/[*RNQ*$^+$]
Hsp70 (Ssa1-4)	Protein folding and stress tolerance. Bind to denatured proteins and prevent aggregation. Also involved in aspects of protein translocation and translation	Deletion of all 4 Ssa members is lethal, constitutes an essential gene family	Can cure some variants of [*PSI*$^+$] when co-chaperones co-expressed. Can counteract the [*PSI*$^+$] curing effect of Hsp104 overexpression. Ssa1 can cure [*URE3*] while Ssa2 cannot
Hsp70 (Ssb1/2)	Ribosome associated. Aid in folding of newly synthesized proteins	Ten-fold increase in spontaneous appearance of [*PSI*$^+$] in a [*PIN*$^+$] background	Can cure some weak variants of [*PSI*$^+$]
Hsp40 (Ydj1, Sis1, Apj1)	Deliver peptide substrates and stimulate ATPase activity of their relevant Hsp70 partner. Sis1 is involved in translation initiation	No effects of *YDJ1* or *APJ1* deletion. *SIS1* is essential	Ydj1 efficiently cures [*URE3*] but not [*PSI*$^+$]. All three can cure artificial [*PSI*$^+$] variants
Hsp110 (Sse1/2; related to Hsp70)	Nucleotide exchange factor for Hsp70 (Ssa and Ssb)	Reduced efficiency of propagation for some [*PSI*$^+$] variants. Cures [*URE3*]	Efficiently cures [*URE3*]
Fes1	Nucleotide exchange factor for Hsp70 (Ssa)	Reduced efficiency of propagation for some [*PSI*$^+$] variants. Cures [*URE3*]	No reported effects
Sti1	Aids in the Hsp70-Hsp90 protein folding cycle. Sti1 bridges Hsp70 to Hsp90 and regulates ATPase activity of both proteins	Reduced stability of [*URE3*]	Cures artificial [*PSI*$^+$] and weakens wild type variant

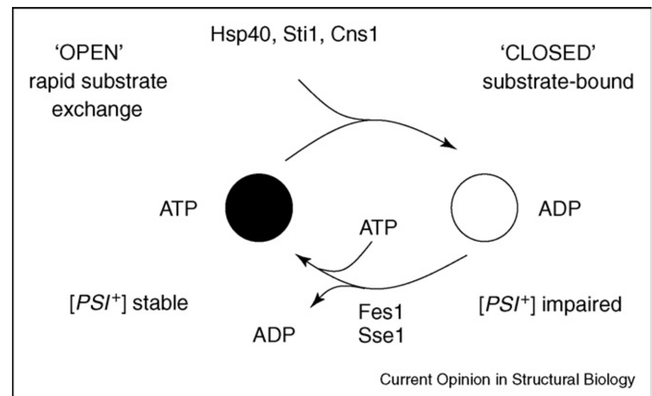
gation and also identified prion-specific effects for some Hsp104 mutants [45•,46•]. Further, a recent *in vitro* study has shed light upon the molecular mechanism by which Hsp104 binds to and processes different protein substrates [47•]. These studies demonstrate that Hsp104 not only processes heat-denatured and prion substrates differently, but also has an exquisite ability to distinguish between different prion substrates.

Hsp104 appears to play a dual role in both generation and propagation of yeast prions [44]. A recent *in vitro* study has shown that Hsp104 is required for the protein nucleation event that appears to be the precursor for prion formation [48••]. Furthermore, the action of Hsp104 in fragmenting amyloid fibrils produced by Sup35 ([*PSI*+]) and Ure2 ([*URE3*]) proteins showed that, in subsequent fibril-seeding assays, resulting products were non-functional for Sup35 propagation but very efficient at seeding Ure2. These *in vitro* data provide a possible explanation for the *in vivo* curing and non-curing abilities of Hsp104 overexpression for [*PSI*+] and [*URE3*], respectively [48••]. There remains some controversy, however, over the ability of Hsp104 to fragment Sup35 fibrils in the absence of other cofactors [48••,49,50•,51]. Apparently contradictory observations may reflect differences in reaction conditions as well as variations in the fibril or chaperone preparations used in different laboratories.

Studies focusing on the [*PSI*+] prion demonstrate that the role of Hsp104 in the appearance and propagation of prions *in vivo* reflects its inherent ability to recruit and remodel complexes that contain mature Sup35 protein [52••,53•]. Consistent with the role of Hsp104 as a protein disaggregase, it is the fracture and subsequent partitioning of prion fragments (propagons) to daughter cells that ensures prion propagation [53•]. Such an *in vivo* role for Hsp104 was indirectly questioned recently with the suggestion that cell growth may not be a requirement for [*PSI*+] curing by the prion-curing agent guanidine hydrochloride (GdnHCl) [54]. It is clear that the mechanism of curing by GdnHCl is through the inhibition of Hsp104 ATPase activity (reviewed in references [14,41,55]), and this seemed difficult to reconcile with the findings of Wu *et al.* [54]. However, in light of recent findings [56], it appears that the conclusions of Wu *et al.* [54] were based upon an artefact resulting from the alpha factor sensitivity of the strain used in their experiments so that growth-dependent curing occurred against a background of high levels of cell death. Hence, there seems to be an absolute growth requirement for GdnHCl-mediated curing of yeast prions.

In addition to the crucial role in propagation of yeast prions, Hsp104 from *Podopsora anserina* has also been shown to be required for the efficient propagation of the [Het-s] prion through the sexual cycle (which involves a single cell stage) but not the multi-cellular vegetative growth cycle [57].

Figure 2



Regulation of Hsp70 (Ssa1) reaction cycle by co-chaperones. Substrate binding is finely tuned by hydrolysis of ATP and nucleotide exchange. Stimulation of Hsp70 ATPase activity has been demonstrated for Ydj1, Sis1 (both of which are Hsp40s), Sti1 and Cns1. Genetic data suggest Cpr7 may also stimulate Hsp70 ATPase activity. Nucleotide exchange is facilitated by the action of Fes1 and Sse1/2. Figure adapted from references [41,60].

The role of Hsp104 in defence against cellular stress and protein denaturation is part of a multi-chaperone network that includes the action of Hsp70 and Hsp40 [43]. Recently, both *in vitro* and *in vivo* studies have demonstrated the importance and influence of the protein disaggregation suite of chaperones upon prion propagation [50•,58]. The development of an *in vitro* system to assess the fibril-forming ability of full-length Sup35 protein has allowed the analysis of the effects of chaperones on this process [50•]. While Hsp104 can stimulate fibril assembly in this assay, the Hsp40 family member Ydj1 has inhibitory effects, as is also seen for the effects of Ydj1 on Ure2 fibril assembly [59•]. The combination of the yeast Hsp70 protein Ssa1 with Ydj1 or another Hsp40 family member, Sis1, causes a severe inhibition of fibril assembly and can also inhibit the stimulatory effect of Hsp104 on fibril formation [50•]. The interplay between the chaperone disaggregation machinery is also seen *in vivo*, as mutants of Hsp104 can be isolated that can suppress the prion-impairment effects of an Hsp70 mutant [58].

Recent data further highlight the important role of Hsp70 and Hsp70 co-chaperones in modulation of yeast prion propagation. Fine-tuning of the Hsp70 ATPase cycle (Figure 2) appears to be a crucial factor in the efficient propagation of yeast prions [41,60]. Although clearly there is redundancy in the role of Hsp70 family members in aspects of prion propagation, there are also very distinct differences in how some highly homologous Hsp70 proteins act upon prion substrates [41,61]. Hsp70 co-chaperones consisting of Hsp40s, tetratricopeptide repeat (TPR) containing proteins and nucleotide exchange factors (NEFs) have all been shown to influence yeast prion propagation [41] (Table 1). The Hsp40 family members

Ydj1 and Sis1 have been implicated as playing a direct role in modulating propagation of [*URE3*] and [*RNQ*+], respectively [59•,62]. While the effects of Ydj1 and Sis1 on prion propagation are well established, the existence of 11 other J-domain containing proteins in the yeast cytosol raises the possibility that these proteins are also involved in aspects of prion propagation [63]. In addition, the newly identified Hsp70 NEF, Sse1 [64•,65•] can also have effects upon propagation of [*PSI*+] and [*URE3*] prions when overexpressed or deleted [60,66•] (Table 1).

One question to be addressed is whether Hsp70 co-chaperones affect prion propagation through an Hsp70-dependent or independent mechanism. The answer appears to be that both scenarios are possible. The *in vitro* ability of purified Ydj1 to inhibit Ure2 fibril formation suggests that the *in vivo* [*URE3*] curing ability of this co-chaperone is due to a direct role in preventing native Ure2 from being recruited into prion aggregates [59•]. By contrast, the *in vivo* curing by overexpression of the atypical Hsp70 family member *SSE1* appears to be absolutely dependent on its ability to interact and function as a NEF for Hsp70 (Ssa) proteins and thus suggests an indirect effect on prion propagation [66•].

Recent structural studies involving the Hsp70 chaperone family have produced detailed crystallographic data that will aid in future functional characterization of the role of Hsp70s and co-chaperones in prion propagation. Jiang *et al.* [67•] succeeded in producing the first detailed crystal structure for a Hsp70/Hsc70 chaperone that contains both the ATPase and peptide-binding domains. The identification of important residues at the interface between the ATPase and peptide-binding domains has allowed an understanding of the structural basis of inter-domain communication. These structural data have allowed interpretation of genetic Hsp70 mutant data and effects on prion propagation in terms of the importance of Hsp70 inter-domain communication and ATPase cycle regulation [60]. With the recent availability of a crystal structure for the Hsp70-related Sse1 [68•], the plethora of *SSE1* genetic data in relation to prion propagation, which will surely arise in coming years, can also undergo such scrutiny.

Relationship between mammalian and yeast prion propagation mechanisms

Research into mammalian prions on the one hand, and fungal prions on the other, has to a great extent progressed in parallel. Of course the mammalian and fungal prions are essentially unrelated in their native structure or function (Figure 1) and are linked only by their common ability to self-propagate a misfolded form of the protein. The yeast prions are not toxic to their host, although whether they are advantageous or deleterious to the fitness of the organism remains debatable [14,55]. However, the lack of apparent toxicity in yeast may reflect a delicate balancing of the toxicity threshold, modulated by sequence effects and the degree of interaction with other cellular cofactors [69–72]. Interestingly, exogenous Hsp104 expression (there is no direct mammalian homologue of Hsp104) has a protective effect against polyQ toxicity in mice [73]. Another interesting finding is that the Sup35 prion domain has a protective effect against polyQ toxicity in a drosophila model [74].

While the mechanism of neurotoxicity of mammalian PrP remains poorly understood, the picture that is emerging appears to rule out either simple gain of neurotoxic function (by the aggregated form of PrP, PrP^{Sc}) or simple loss of neuroprotective function (of the normal cellular form of PrP, PrP^{C}) [9,11]. Instead, it seems that PrP^{Sc} (or another species designated as PrP^{toxic}) recruits PrP^{C} into the toxicity mechanism—this has been referred to as a subversion-of-function mechanism [10]. The GPI anchor of PrP seems to play a crucial role in this subversion, as removal of the anchor allows accumulation of aggregated PrP, but without classic signs of prion-induced neurodegeneration [75]. This advance in understanding is crucial, as it explains why therapeutic targeting of PrP^{Sc} can at best only increase incubation times but cannot prevent neurodegeneration and further, it identifies prevention of recruitment of native PrP^{C} into the toxic form as a key target for therapeutic intervention [11].

Given the striking differences in the structure and physiology of mammalian and fungal prions, a hint that yeast and mammalian cells may act in similar ways to clear their prions is both unexpected and exciting. A number of tricyclic compounds identified as curing agents for yeast prions in a high-throughput screening assay were found to clear PrP^{Sc} in a mammalian cell model and conversely, compounds that had been identified in mammalian screens, were found to cure prions in yeast [76,77]. Unfortunately, drugs identified in cell-based assays have so far shown limited efficacy in animal models or treatment of end-stage human disease, perhaps as their target is inhibition of PrP^{Sc} accumulation, which in itself does not seem to lead to neurodegeneration [11]. However, this by no means negates the utility of yeast and other cell-based studies, as identification of the biological targets of such drugs may yet provide important information about the molecular mechanism of prion propagation and the cellular cofactors involved in this process [77,78]. Further, replacement of the Sup35 oligopeptide repeats with the oligopeptide repeats from PrP produces chimeric proteins that function as prions in yeast [79,80]. Such chimeric models may provide an important tool to identify and study factors that can modulate mammalian prion propagation. As understanding of prion behaviour in mammalian and fungal systems increases, no doubt further similarities and differences will come to light, which may in turn help us to understand the molecular basis of this intriguing phenomenon.

Conclusions

A number of structurally and functionally diverse proteins from fungi have been classed as prions because, like the mammalian prion protein PrP, they are able to undergo a heritable switch in conformation that can be propagated among other similar molecules. Research on these fungal proteins has made significant contributions to our understanding of the physical and structural basis of prion propagation, including perplexing characteristics of prions such as the phenomenon of strains and the related issue of the species barrier. The involvement of chaperones in the propagation of fungal prions is consistent with protein conformational change as the basis for the switch to a prion state. Further, study of chaperone–prion interactions has shed light on both the mechanisms of prion propagation and on the mechanisms by which chaperones recognise their substrates. Whether the mechanisms of formation, maintenance and clearance of prions are conserved between fungi and mammals remains to be seen. However, elucidation of the cellular action of anti-prion agents in both fungal and mammalian cells, together with the use of chimeras between well-studied fungal prion proteins and PrP, may provide important avenues towards curing prion disease.

Acknowledgements

Work in the Perrett laboratory is supported by grants from the National Natural Science Foundation of China (30470363, 30620130109, 30670428), the Chinese Ministry of Science and Technology (2006CB500703, 2006CB910903) and the Chinese Academy of Sciences (KSCX2-YW-R-119). Work in the Jones laboratory is supported by the Irish Health Research Board (RP/2004/227, RP/2006/111) and Science Foundation Ireland (RFP/2007/BICF493). Exchange between the laboratories has been supported by a grant from the China/Ireland Science and Technology Collaboration Research Fund administered by the Royal Irish Academy (CI-2004-01). We thank Mick Tuite, Ricardo Marchante, Tuomas Knowles and members of the Perrett and Jones laboratories for comments on the manuscript.

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