During the 1950s and the 1960s, cases of non-mendelian cytoplasmically mediated inheritance were reported in many organisms. The detection of mutations located in the mitochondrial or chloroplastic genomes, as well as the presence of epimorphic nucleic acid has clarified the nature of many of the genetic determinants involved. However, some are still mysterious. In many cases, their highly infectious properties suggest that they are not of a truly genetic nature, but of an epigenetic nature. Recent findings showing that two such yeast elements, \([\text{PSI}]\) and \([\text{URE3}]\), bear close resemblance to mammalian prions has prompted a novel interest in these phenomena. In the ‘protein-only’ prion hypothesis, the infectious element is an abnormal conformational state of a cellular protein, which can trigger the conversion from the normal to the abnormal form. This has clearly been demonstrated for the \([\text{PSI}]\) element.

Because of their coenocytic structure (Box 1), filamentous fungi stand as very good models to detect and study such cytoplasmic and infectious particles (Fig. 1). Cellular continuity is a main characteristic of these organisms. Hyphal septae have pores and hyphae can be connected by anastomoses. Therefore, a single infectious element is able to contaminate a large area of mycelium, and so its effects are detectable without the need of selection. In this review, we focus on phenomena observed in filamentous fungi that do not seem to be connected with any kind of nucleic acid. Hence, senescence phenomena, in which mitochondrial dysfunction has been observed, will not be discussed, despite the fact that the real nature of the responsible determinants is still mysterious.

**Non-conventional infectious elements in filamentous fungi**

Old data (most often in French) described phenomena involving non-conventional infectious factors in filamentous fungi. Recently, it was shown that two yeast cytoplasmic determinants are similar to known mammalian prions, in that their different states are attributed to conformational changes of normal cellular proteins. In the light of this discovery, fungal elements are now being reconsidered. This review presents four elements that affect vegetative incompatibility, conidiogenesis, morphology and cell growth. Recently, one element has been shown to be a prion analogue. The status of the others is not clear. We consider the view that non-conventional inheritance might be initiated by the appearance, in the cytoplasm, of a metabolite or a macromolecule whose production involves a positive regulatory loop.

**Box 1. Glossary**

- **Anastomosis (pl. anastomoses)**: Process by which two hyphae fuse to create a network, resulting in the exchange of cytoplasmic constituents and in some cases of nuclei.
- **Ascospore**: A spore produced in an ascus and resulting from meiosis in Ascomycetes.
- **Coenocytic (or syncytial)**: Characteristics of organisms for which a cellular continuity exists. In filamentous fungi, continuity is ensured by perforated hyphal septae and anastomoses.
- **Conidium (pl. conidia)**: Specialized non-motile cell involved in asexual dispersion.
- **Conidiogenesis**: The process of conidium formation.
- **Hypha (pl. hyphae)**: One filament constituted of successive cells separated by septae.
- **Hyphal septum (pl. hyphal septa)**: Cell wall between two cells of a hypha. In most fungi, it possesses a special structure that ensures a cytoplasmic junction between two contiguous cells.
- **Mycelium (pl. mycelia)**: A mass of hyphae.
- **Propagule**: Any kind of cell that is involved in the dispersion of the fungus.
- **Thallus (pl. thalli)**: The vegetative body of a thallophyte. In filamentous fungi, it is equivalent to the mycelium.
The barrage phenomenon in Podospora anserina

Rizet described the first instance of fungal non-conventional inheritance when studying the "barrage" reaction at the contact area between the two-strains s and S (renamed [Het-s] and [Het-S], Ref. 9), of Podospora anserina. This "barrage" is the result of a vegetative incompatibility reaction promoting cell death. [Het-S] and [Het-s] strains differ by the nature of the polymorphic allele that is present at a locus, now called het-s. When [Het-S] or [Het-s] strains are self-crossed, the resulting progeny are uniformly resistant with [Het-s] or [Het-S], respectively. Upon crossing a [Het-S] strain with a [Het-s] strain, the progeny are composed of two [Het-S] strains and two unexpected [Het-s*] (formerly denoted s*) strains that are not reactive with either [Het-S] or [Het-s]. Interestingly, from these [Het-s*] strains, one can spontaneously obtain true [Het-s] strains at low frequency. Anatomoses between [Het-s*] and [Het-s] strains invariably promote the transformation of the [Het-s*] mycelium into a [Het-s] strain independent of nuclear clear transmission. Anatomoses are required to promote the transformation. Additionally, crosses between [Het-s] and [Het-s*] yield only [Het-s] strains when [Het-s] is the female partner, and mostly [Het-s*] strains when the female partner is [Het-s*] (Ref. 7). Involvement of mitochondria was excluded when the first mitochondrial mutant was obtained, because the transformation and the mitochondrial mutation did not propagate similarly in heterokaryons. To account for these properties, it was suggested that the protein encoded by het-s, which is able to induce and regulate its own synthesis, is present in strain [Het-s] and absent in strain [Het-s*] (Ref. 7).

Recent observations suggest that the transformation of [Het-s*] into [Het-s] is a prion phenomenon: (1) the het-s allele is necessary for the propagation of the transformation from [Het-s*] to [Het-s]. Indeed, a strain containing the null allele het-s (which therefore lacks the pHET-s polypeptide) is unable to transmit the [Het-s] state to a [Het-s*] culture; (2) overexpression of the pHET-s polypeptide increases the probability of the transition; (3) transformation occurs in the absence of translation; and (4) the pHET-s polypeptide is present in similar amounts and has similar electrophoretic mobility in [Het-s] and [Het-s*] strains. However, the polypeptide present in the [Het-s] strain is more resistant to protease K digestion. In both types of strain, the pHET-s polypeptide is present either as monomers or multimers (di-, tri- and tetramers). Interaction between the pHET-s monomers is confirmed in yeast with the two-hybrid system; in addition, interactions between the pHET-s monomers or multimers (di-, tri- and tetramers) are conserved in all these polypeptides and whether the same mechanism accounts for all the self-propagating conformational changes. Second, it suggests that the ability of protein to catalyse or "seed" their own conformational modification is a property of proteins more widespread than presently thought. Pre-existing "seeds" could be needed to perform proper folding, as observed for the self-folding of hp60 proteins and the conformational changes of the SUP35p in the [PSI] phenomenon or the PrP protein of mammals. In the case of Podospora anserina, crosses with the het-S allele result in absence of the "seed", thus revealing the phenomenon. However, if the protein is essential, like hp60, "seeds" are always present and continuously transform the newly synthesized proteins. The third reason is the particular properties of the pHET-s polypeptide, namely: (1) its ability to interact with pHET-s, and not pHET-s*,

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Non-conventional infectious elements

The same strain of N. haematococca displays two different morphological modifications caused by two specific cytoplasmic and infectious factors, the "Anneau" (A; left) and the "Secteur" (S; middle). A morphological modification very similar to the "Secteur" has been described in several fungal species, including C. pallescens (right).

In fungi, the cytoplasmic and infectious nature of determinants is defined by two characteristics: (a) their transmission into a recipient mycelium following anastomosis and (b) their non-mendelian segregation during meiosis. (a) To demonstrate transmission through anastomosis, one usually performs "barrage experiments" (as exemplified by the "Secteur" of N. haematococca). When a small inoculum of the donor mycelium (that contains the determinant) is put at the periphery of a growing mycelium A; no determinant Mycelium B; no determinant Cytoplasm without determinant Cytoplasm with determinant Männlinen (b) Non-mendelian segregation is observed following a cross between a mycelium that contains the determinant with one that does not. Usually, ascus analysis shows that the nuclear determinant contains the determinant with one that does not. During the contamination process, the donor nuclei do not invade the recipient mycelium and hence the determinant is usually presented the characteristic phenotype promoted by the determinant. During the contamination process, the donor nuclei do not invade the recipient mycelium and hence the determinant is usually presented the characteristic phenotype promoted by the determinant. The same strain of N. haematococca can display two different morphological modifications caused by two specific cytoplasmic and infectious factors, the "Anneau" (A; left) and the "Secteur" (S; middle). A morphological modification very similar to the "Secteur" has been described in several fungal species, including C. pallescens (right).

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Reviews

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to start the incompatibility reaction and (2) its effect during meiosis in converting [Het-s] to [Het-s*]. The pHET-S and pHET-αpHET-s proteins differ by 14 amino-acid substitutions. Only one of these is responsible for the incompatibility reaction. None has yet been analysed for the [Het-s] to [Het-s*] converting effect of the het-S allele. Analysis of these two properties via a combination of genetic and biochemical approaches should yield interesting data.

The closed system of cytoplasmic variation in Aspergillus glaucus

Subhak Sharpe reported the second instance of a cytoplasmic and infectious factor, in Aspergillus glaucus. It involved sectors that differ from the surrounding mycelium by several characteristics, particularly enhanced conidiogenesis. It was readily infectious through anastomoses, but it was not clear whether it could infect resting mycelium like the Podostroma transition from [Het-s+] to [Het-s-], or only the cells of the growing margin like the elements described in the next section. It was shown that a mosaic of cells that do or that do not contain the determinant formed the sectors. This could be transmitted via the conidia or the ascospores and curiously segregated in nearly a 1:1 manner in sexual crosses. In this system, it was not clear what prevented the factor from invading the totality of the mycelium. No further studies were carried out and we are left with conjectures about its nature. Interestingly, this phenomenon was not the sole report on the role of cytoplasm in conidiation of Aspergillus.

The morphological modifications of numerous species

The third example that we discuss is that of the mycelium morphological modification present in numerous ascomycetous and basidiomycetous species. When grown on appropriate media, their reproductive propagules (being either asexual or sexual spores) generate thalli with dense aerial hyphae. Often, at the periphery of the growing cultures, sectors with a modified morphology develop randomly (Fig. 2). These are characterized by a reduced amount (or absence) of aerial hyphae and an intense pigmentation that diffuses in the medium. Four fungal species have been carefully looked at, Carexularia pallescens (M.J. Vicariot-Hugonnet, 1965, PhD thesis, pp. 1–58, Université de Paris-Sud, Centre d’Orsay), Pestalozzia annulata, Hypomycetes ipomoeae, and, especially, Nectria harnatococca (M.J. Dubois-Bayezy, 1979, PhD thesis, pp. 1–163, Université de Paris-Sud, Centre d’Orsay).

In this latter species, two types of modifications can appear in the same culture, the ‘Secteur’ and the ‘Anneau’ (Fig. 2). The frequency of these modifications varies substantially with culture conditions, especially temperature. Once initiated, these modifications spread through anastomoses, but only in the cells of the growing edge. The modifications are characterized by a speed that dictates their form. The ‘Secteur’ determinant travels at twice the growth rate and generates a typical sector. The ‘Anneau’ determinant travels at 20 times (around 4 mm h−1) the rate of the growing hyphae and the resulting ‘Secteur’ is a ring that surrounds the culture. Contamination experiments have shown that each determinant is transmissible to heterogeneous cultures (Fig. 1) and is specific to one modification. Upon subculture with mass hyphal transfer, the modified morphology can be continually maintained. However, fragmentation of a modified culture allows the restoration of normal morphology in a varying proportion.

FIGURE 3. Alternate metabolite states as hereditary determinants

(a) The first description, but not proposition, that a positive regulatory loop can generate a hereditary determinant is that of Novick and Weiner. They showed that, when grown in a medium that contains a limiting, constant and specific concentration of a gratuitous inducer of the lactose operon, an E. coli population is composed of cells that are either in a non-induced state (top) or a fully induced state (bottom). It happens because entry of lactose in the cell is greatly facilitated by the periplasm encoded by the lacY gene. This generates a self-maintained regulatory circuit, by which induced cells stay induced (because of efficient entry of lactose) and non-induced cells remain so, until a stochastic activation of the lactose operon occurs. They also showed that a dynamic equilibrium is reached (b) despite the constant induction of cells. Indeed, non-induced cells divide faster than induced ones allowing for a constant replacement in the population of the cells that have made the transition towards the fully induced state. In this lactose operon system, a single positive loop is sufficient to generate the alternative states. A double reciprocal negative action might achieve the same generation of alternative states.

FIGURE 4. Growth modification

Some mutant strains of Pestalozzia annulata (here the AS6-5 strain) might spontaneously present sectors with an altered morphology, in which hyphal growth rates and morphology are modified (C) or normal (B). Unlike the ‘Secteurs’ depicted in Fig. 2 that develop in wild type, this ‘Crippled Growth’ is restricted to mutant strains that have elevated translation accuracy.
of subcultures. This suggests that the modified mycelia are mosaics of cells that may or may not contain the determi-
nant and might, therefore, be in a state of dynamic equi-
librium (Fig. 3). Asexual conidia can transmit both factors; ascospores never do so. From all the above data, it was
concluded that each modification is caused by a specific
cytoplasmic and infectious factor.

Both 'wild' and 'mutant' sectors are under the control of
nuclear genes\cite{25}. Two kinds of mutations have been
detected. Some do prevent expression of both modifi-
cations and these map to at least four loci. Expression of
both determinants seems thus to involve a common path-
way. The other mutations prevent specifically the for-
mation of either one of the modifications. They are
located at a unique locus for the 'Secteur' (the S locus) and
at another unlinked single locus for the 'Anneau' (the A
locus). Two kinds of mutant alleles were detected, those
that completely abolish the production of the determin-
ants (a or s alleles) and those that promote its constitu-
tive expression (a* or s*). Interestingly, in the latter cases,
the mutant mycelia present a red colour but are able to
produce aerial hyphae to the same extent as wild type. It is
supposed that these loci are directly involved in the gener-
aton of the determinants. Cloning of the A and S genes is
now under way (S. Graziati, P. Silard and M.J. Daboussi,
unpublished). It would not be surprising if a membrane
component is involved, in view of the amazing speed (an
order of magnitude higher than that of the barrage phe-
nomenon in P. anserina) at which the 'Anneau' factor travels through the anastomoses.

The similar phenomena observed in the other three
fungi have not been studied thoroughly, but it seems that
development of the sectors follows roughly the same rules
as above. However, optima of temperature for appearance
of the modification are variable and it was suggested that
two events are required to allow the formation of sectors in
P. anulata and C. pallidosa\cite{25} (M.J. Vicariot-Hugonnier,
1965, PhD thesis, pp. 1–58, Université de Paris-Sud, Centre
d’Orsay). Therefore, despite a similar morphology (Fig. 2),
these sectors might be generated by different mechanisms.

The Crippled Growth in Podospora anserina

The final case that we discuss of cytoplasmic and infectious
fungus is the recently discovered C determinant that
causes growth impairment (Crippled Growth as opposed
to Normal Growth, Fig. 4) in some mutant strains of
Podospora anserina\cite{30}. Unlike the above determinants,
its propagation through anastomoses is not very efficient
since only half the contamination experiments succeed in
the best cases. The responsible element is efficiently transmit-
ted through mitosis but not through meiosis. It is induced
in stationary phase and cured by various stresses. Whereas
the latter property is well established for the yeast element
[PSI] (Ref. 25), the former one is unique to the C element.

Presence of C can be detected during stationary phase
in all strains that have been tested, including the wild type,
but it propagates during the growth phase only in strains
that display increased translation accuracy (AS strains). Two
models can be proposed to account for the role of trans-
laction fidelity. First, translation accuracy might be directly
involved in the generation of the element. It could increase
during stationary phase accounting for induction of C dur-
ing this period, but during active growth, fidelity would be
too low, except in AS strains, and hence the element
might not always be due to a nucleic acid mutation. A second
hypothesis is that a translation or post-translational error might
control the production of a factor involved in the elimi-
nation of the determinant. In AS strains, this hypothetical
factor might be involved in a regulatory circuitry that
controls the propagation of the C determinant. It is clear that transition from
Normal Growth to Crippled Growth displays very differ-
ent properties from the barrage phenomenon and is thus
likely to be caused by a somewhat different mechanism.

Biological significance

It is worth remembering that stable alternative metabolic
states can be heritable in some cases (Fig. 3)\cite{30}. This was first
proved and discussed for the all or none \(\beta\)-galactosidase
induction observed at low inducer concentrations in E. coli\cite{27}. In
truth, a positive regulatory loop, at any level of a metabol-
olite or macromolecule production could be sufficient to
promote with some stability alternative states\cite{27}. In fact,
the conformational changes undergone by prion proteins is
formally identical to such processes because, in a sense, it can
be viewed as a loop at the level of the protein folding.

Are these phenomena mere exceptions or of general
occurrence in living cells? Two lines of reasoning suggest
that the latter proposition is probably correct. First, in
view of the complexity of the cell regulatory network, it is
likely that unwanted positive loops could arise during evo-
lution with high frequency along with mutations in the com-
ponents of the network. Second, in many of the organisms
that were genetically studied for cytoplasmic inheritance,
yeast, Aspergillus, Podospora or paramecia, several of
these phenomena have been described.

Once the loop has appeared, two possibilities follow.

The phenomenon might be positively selected by natural
selection. It would thus commit the cell to engage in a
primitive ‘differentiation’ process\cite{27}. Appearance of the
sectors in many fungal strains isolated from nature (J.
Chevaugeon, unpublished) strongly indicates that they are
positively selected and are thus akin to a true differentiation.
This proposal is confirmed by the fact that several differ-
entiation pathways, like sex determination in Drosophila
melanogaster\cite{29} or dauer formation in Caenorhabditis ele-
gans\cite{30}, do present a regulatory circuitry that can generate
alternative states. The process might be deleterious, at least
in some conditions. Control systems as the one postulated
for Crippled Growth might then be set up. If such systems
are not present, we have proposed that activation of the
loops could lead in part to ageing\cite{27}.

Genetics has so far been mostly concerned with the
inheritance of the individual constituent of the cell (e.g. ‘one
gene = one enzyme’ paradigm). The phenomena we have
discussed show that it is also important to study the inher-
ance of structure and of regulatory circuitry. Sudden and
stable phenotypic change, especially in a mitotic lineage,
might not always be due to a nucleic acid mutation.

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pathway might apply to other signaling pathways as well, and might help explain how multicellular organisms downstream signaling components. Mechanisms of specificity used by the RTK/RAS/MAP kinase signaling have been proposed to explain signaling specificity. Specificity can arise at the level of the receptor, through the pleiotropic nature of these pathways has prompted the question of how they can preserve the specificity of a signal biological decisions are mediated by this signaling pathway.

transcription factors by MAP kinase3. A wide variety of signaling cascade, culminating in the regulation of nuclear pathway: the receptor tyrosine kinase (RTK)/RAS/MAP kinase signaling cascade as a model to discuss various hypotheses that have been proposed to explain signaling specificity. Specificity can arise at the level of the receptor, through the modulation of signaling kinetics, through the interaction of different signaling pathways, and at the level of downstream signaling components. Mechanisms of specificity used by the RTK/RAS/MAP kinase signaling pathway may apply to other signaling pathways as well, and might help explain how multicellular organisms are able to generate tissues of diverse forms and functions from a small set of common signaling pathways.

The molecular basis by which commonly used signaling pathways are able to elicit tissue-specific responses in multicellular organisms is an important yet poorly understood problem. In this review, we use the receptor tyrosine kinase (RTK)/RAS/MAP kinase signaling cascade as a model to discuss various hypotheses that have been proposed to explain signaling specificity. Specificity can arise at the level of the receptor, through the modulation of signaling kinetics, through the interaction of different signaling pathways, and at the level of downstream signaling components. Mechanisms of specificity used by the RTK/RAS/MAP kinase signaling pathway may apply to other signaling pathways as well, and might help explain how multicellular organisms are able to generate tissues of diverse forms and functions from a small set of common signaling pathways.

A great mystery in biology is the question of how equi-potent cells subsequently acquire distinct tissue-specific properties. In recent years, it has become clear that cellular signaling pathways play an integral role in the process of tissue differentiation, and one striking finding is that a handful of conserved signaling pathways seem to be used repeatedly to specify a wide variety of tissues. However, the pleiotropic nature of these pathways has prompted the question of how they can preserve the specificity of a signal in their ability to elicit tissue-specific responses. This review focuses on one such pleiotropic signaling pathway: the receptor tyrosine kinase (RTK)/RAS/MAP kinase signaling cascade. Activation of this cascade is mediated by growth factor ligands (e.g. EGF, NGF), that bind to and activate specific RTKs (Refs 1, 2). Receptor activation results in the initiation of a RAS/MAP kinase signaling cascade, culminating in the regulation of nuclear transcription factors by MAP kinase3. A wide variety of biological decisions are mediated by this signaling pathway. For instance, RAS proteins are essential for embryonic development in vertebrates4, as well as neuronal and adipo-
cytogenesis in Podospora anserina. Genetics 151, 67–95


21. Beisson, J. (1977) Non-nucleic acid inheritance and biological decisions in biology is the question of how equi-potent cells subsequently acquire distinct tissue-specific properties. In recent years, it has become clear that cellular signaling pathways play an integral role in the process of tissue differentiation, and one striking finding is that a handful of conserved signaling pathways seem to be used repeatedly to specify a wide variety of tissues. However, the pleiotropic nature of these pathways has prompted the question of how they can preserve the specificity of a signal in their ability to elicit tissue-specific responses. This review focuses on one such pleiotropic signaling pathway: the receptor tyrosine kinase (RTK)/RAS/MAP kinase signaling cascade. Activation of this cascade is mediated by growth factor ligands (e.g. EGF, NGF), that bind to and activate specific RTKs (Refs 1, 2). Receptor activation results in the initiation of a RAS/MAP kinase signaling cascade, culminating in the regulation of nuclear transcription factors by MAP kinase3. A wide variety of biological decisions are mediated by this signaling pathway. For instance, RAS proteins are essential for embryonic development in vertebrates4, as well as neuronal and adipo-cytogenesis in cell culture assays5. Furthermore,