MINIREVIEW

Yeast as a Model System for Mammalian Seven-Transmembrane Segment Receptors (43720)

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Abstract. **Investigators have used the budding yeast *Saccharomyces cerevisiae* as a model system in which to study the β-adrenergic receptor, the T-cell receptor pathway, initiation of mammalian DNA replication, initiation of mammalian transcription, secretion, the CDC2 kinase system, cell cycle control, and aging, as well as the function of oncogenes. This list continues to grow with the discovery of an immunoglobulin heavy-chain binding homologue in yeast, an Rb binding protein homologue, and a possible yeast arrestin. Yeast is relatively easy to maintain, to grow, and to genetically manipulate. A single gene can be overexpressed, selectively mutated or deleted from its chromosomal location. In this way, the *in vivo* function of a gene can be studied. It has become reasonable to consider yeast as a model system for studying the seven transmembrane segments (7-TMS) receptor family. Currently, subtypes of the β-adrenergic receptor are being studied in yeast. The receptor and its G_{α}-G-protein, trigger the mating pheromone receptor pathway. This provides a powerful assay for determining receptor function. Studies expressing the muscarinic cholinergic receptor in yeast are underway. The yeast pheromone receptor belongs to this receptor family, sharing sequence and secondary structure homology. An effective strategy has been to identify a yeast pathway or process which is homologous to a mammalian system. The pathway is delineated in yeast, identifying other genetic components. Then yeast genes are used to screen for human homologues of these components. The putative human homologues are then expressed in yeast and in mammalian cells to determine function. When this type of "mixing and matching" works, yeast genetics can be a powerful tool.** [P.S.E.B.M. 1994, Vol 206]

One of the basic themes shared by living organisms is their ability to respond to change in their external environment. Cells have answered this need by the plasma membrane receptor. Cells monitor the external conditions and translate environmental changes into physiological change. Theoretically, the first primitive plasma membrane receptors have been changed, remodeled, and adapted for specific functions, throughout evolution. Upon first examination these receptor systems appear quite diverse in the ligands they bind and in the type of signal they send into the cell. However, we are finding that some of these receptor systems are functionally conserved from humans to yeast. In fact, some of the components of these receptor systems can be "mixed and matched" to function in mammalian cells and in the budding yeast *Saccharomyces cerevisiae*.

The plasma membrane receptors satisfies the need for the transmission of information from the environment through the chemical and physical barrier of the lipid bipolar layer to effect changes deep within the cell. Several families of membrane receptors have

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evolved. The purpose of this review is not to conduct an exhaustive discussion of all membrane receptors families, but to focus on one such family, the G-protein-linked receptors. This family of receptors has seven helical transmembrane segments (7-TMS receptors). These receptors show homologous transmembrane domains, but show very little homology in the connecting loop domains, except between closely related subtypes. Several reviews of sequence and secondary structure homology between these receptor systems can be found (1–8). An extensive study of sequence homology of these receptors has been completed (9). These results suggest that, based on sequence homology and on secondary structure, the rhodopsin, α_1, α_2, β_1 and β_2 adrenergic, the M_1 and M_2 muscarinic cholinergic receptors, as well as the substance K receptor are closer evolutionarily than other receptors such as the yeast α-factor receptor, and the nicotinic acetyl choline receptors. Except in cases where receptor molecules have apparently been derived from a common ancestor, such as the subtypes of the adrenergic receptor, it is not clear which G-protein-linked 7-TMS receptors share a "recent" common origin. This reviewer believes that it is conceivable that these receptors may have evolutionarily converged to this common functional theme. This is not hard to imagine, when one considers there is considerable "cross-talk" between receptors and G-proteins. There appear to be several G-proteins which can be activated by a single type of receptor. There is also cross-talk between the elements which desensitize receptors. Many of these receptors are acted on by homologous as well as heterologous systems which dampen their signal. It is conceivable that a receptor could be recruited, serving some new function, during evolutionary time. This attention to the evolution of receptor is central to the development of the yeast α-factor receptor system as a model for mammalian systems. Based on sequence homology of the yeast receptor and its G-protein, one would not expect other receptors and other G-proteins to work in the yeast signal pathway, but they do. This is why this concept of cross-talk is vital to our understanding the yeast model.

Another common characteristic of these receptor systems is that they are associated with a G-protein which is composed of three subunits: α, β, and γ. These G-protein subunits show sequence homology when α subunits are compared to α subunits, β with β, and γ with γ, from one receptor system to another. For instance, the vertebrate family of 7-TMS receptor G_α subunits are about 45% identical (10, 11), with the GTP binding site highly conserved (12). The yeast β and γ subunits are about 30%–40% identical to the rhodopsin β and γ subunits (13). G-proteins have common features. The receptors transduce a signal by causing an exchange of GTP for GDP on the larger α component of a G-protein and by the dissociation of the α component from the βγ component. At this point the signal is amplified with dozens of G-proteins being activated by the same occupied receptor. In most cases, some component of the G-protein is isoprenylated and presumably associates with the cytoplasmic side of the plasma membrane. In yeast, the γ subunit is farnesylated, and this is necessary, presumably, for anchoring the β/γ complex to the plasma membrane (14). It is widely believed that both the α and the βγ subunits have effectors. Some of these effector targets are not known. In the case of the rhodopsin receptor, the β/γ subunit is an inhibitor of the G_α subunit (15). The target of the α unit is a cGMP phosphodiesterase and the $G_{\beta\gamma}$ target appears to be phospholipase A_2 (16). The target for the β_2-adrenergic receptor's G_α component is adenylate cyclase while the $G_{\beta\gamma}$ component is inhibitory and its target may be the inositol phosphate pathway. The specific effector molecule acted on by the $G_{\beta\gamma}$ subunit in the yeast α-factor pathway is unknown, but the pathway is triggered by the $G_{\beta\gamma}$ component (17) and the G_α protein is inhibitory. In some instances the G_α component can inhibit adenylate cyclase, as is the case with some muscarinic and α-adrenergic receptors. Different subtypes of receptors can be inhibitory or stimulatory to adenylate cyclase as seen in the A_1 and A_2 adenosine receptors of the brain, respectively (18, 19). In addition, the same G-protein component can be inhibitory under one set of conditions and stimulatory under another, as is the case for the muscarinic receptor (20). The complexity of G-proteins and their function may prove to be enormous. In addition to cross-talk between G-proteins, their receptors and the components of different pathways. It has been postulated that different G_α and $G_{\beta\gamma}$ components may form different combinations of G-proteins. The A_1 adenosine receptor has an α1 and an α2, Gα subunit which can both combine with the same β/γ subunit (21), although the functional significance of this is not well understood. Dissecting the web of G-protein interactions with receptors and effectors may prove to be a formidable task. This is why the yeast G-protein-linked receptor system may be helpful as a "testing ground" in the study of mammalian receptor systems. Extensive screening was unable to identify other receptor linked G-proteins in yeast, other than these associated with the α-factor receptor. A very effective strategy for determining genetics of different cellular processes in mammalian cells has been to identify homologous systems in yeast, then return to yeast to test those genes for functional homology.

Yeast as a Model System

S. cerevisiae has proven to be an ideal organism for genetic analysis because of the relative ease with

which a single gene can be manipulated. More importantly, most of the basic processes and pathways involved in growth and metabolism in mammalian cells are conserved from yeast to humans. The list of functionally interchangeable systems between yeasts and humans is growing. These include human c-RAS and yeast c-RAS (22), mammalian GAP (G-protein GTP'ase activating protein) gene which can replace *IRA*1 in yeast to regulate c-RAS (23), and a possible FOS/JUN transcriptional regulation system in yeast. Yeast has a c-*JUN* homologue, *GCN*4, also a transcriptional regulator (24, 25). A fusion protein, with the *GCN*4 DNA binding region replaced by the c-*JUN* binding region, functions to replace the wild type *GCN*4 in yeast (26). Further, the mating-specific pheromone/receptor transcriptional factor (PTRF), now known to be a complex which includes proteins encoded by *MCM*1, STE12 and *MAT*1α gene, binds to an upstream activator element in yeast. This upstream region, the pheromone response element (PRE), contains the serum response element (SRE), associated with the mammalian c-FOS. When the SRE is inserted upstream to yeast genes, it places them under the mating response control of yeast PTRF (27, 28). Work is in progress using yeast as a model system for studying the effects of different agonists on subtypes of the β-adrenergic receptor (29, 30). These studies use cell cycle arrest or α-factor-like arrest to assay the effects of a specific drug on a particular receptor expressed in yeast. G_α subunits from various mammalian receptor systems have been expressed in yeast. They are able to inhibit the βγ subunits of the receptor pathway from triggering cell cycle arrest. However, once these G_α subunits are, presumably, coupled with the yeast β/γ complex they are unable to interact with the α-factor receptor and respond to α-factor stimulation (31). There, the G_αs are similar enough to form aggregation with yeast β/γ, but not similar enough to interact "correctly" with the α-factor receptor. Just recently, the human gene involved in cystic fibrosis (CRF) was fused to the yeast gene STE6. STE6 is a membrane channel protein necessary for the export of a-factor. The CRF gene is a membrane chloride channel and when fused to STE6 it functions allowing a-factor to be exported as measured by mating efficiency (32). When a mutated CRF is fused to STE6, the same mutation which caused cystic fibrosis, the channel no longer functions and a-factor can not leave the cell. A yeast system, apparently homologous to the T-cell receptor activation pathway in humans has been identified (33, 34). Currently, yeast is being used as a model system for studying immunosuppressive drugs, like cyclosporin A, FK506, and rapamycin. Yeast has become a model for initiation of replication in mammalian cells. The 2 μm yeast plasmid is autonomously replicated in mammalian cells, if some SV40 genes are expressed, such as T antigen (35). This initiation of replication has been mapped to the yeast origin ARS1. This demonstrates that human replication initiation factors are functionally conserved. A yeast protein SPL1 protein has domains which share sequence homology with mammalian c-myc (36). A yeast heat shock protein belonging to the HSP70 family is homologous to the immunoglobulin heavy chain binding protein (37). A yeast protein which is functionally similar to the immunoglobulin heavy-chain enhancer-binding protein NF-μE3, has been identified (38). There appears to be a type of ^{53}P cell cycle control system in yeast. The human ^{53}P protein combines with human CDC2 to inhibit proliferation in yeast (39). Apparently, yeast has an Rb-type system. The yeast protein *MSI*1 is related to a retinoblastoma-binding protein (48kd) (40). This yeast protein is a negative regulator of RAS and if human Rb and the 48kd protein are expressed in yeast they suppress the heat-shock sensitivity of the yeast *ira*1 mutant and *RAS2*^{val19} strains. These are just some examples of how yeast are being used as models for studying human genes and their functions.

The Rhodopsin Receptor System

The rhodopsin receptor is a 7-TMS receptor which is located in the outer segments of photoreceptor cells, rod cells. The receptor protein tranverses the plasma membrane and has a pocket which holds the chromophore 11-cis-retinol. Absorption of light causes 11-cis-retinol to isomerize to all trans-retinal. This in turn causes a conformational change in the apoprotein rhodopsin (opsin). This change activates the G-protein, transducin. The G-protein's G_α component exchanges GTP for GDP and dissociates from the $G_{\beta\gamma}$ subunits. It then activates cGMP phosphodiesterase, lowering the levels of cGMP (41). Ultimately, this results in opening of calcium membrane channels and depolarization of the photoreceptor cell. The all trans-retinal leaves the rhodopsin apoprotein and is recycled (42).

The signal can be turned off in the absence of light stimulation by the reconstitution of 11-cis-retinol/rhodopsin, hence the reassociation of the G-protein and the hydrolysis of GTP to GDP on the G_α subunit. Under conditions of continuous stimulation, the signal is attenuated by a system which involves the phosphorylation of the C-terminal tail of activated all trans-retinal rhodopsin, or meta II rhodopsin. This type of homologous desensitization of the rhodopsin receptor is carried out by a specific rhodopsin kinase (RK) (43, 44). Rhodopsin kinase adds up to seven phosphate groups onto the tail of rhodopsin (45). Heterologous desensitization also occurs by phosphorylation of the activated receptor by protein kinase A (PKA) and by protein kinase C (PKC) (46). Phosphorylation is insufficient to stop the signal and a phosphoprotein, arrestin, must bind the phosphorylated receptor. It is also

believed that this binding of arrestin protects meta II from being hyperphosphorylated which would prevent its removal and recycling (47, 48). Arrestin must dissociate before all trans-retinal can leave to be recycled. There is some evidence that arrestin may also interact with the effector protein of transducin, the cGMP phosphodiesterase to inhibit its function (49). This has not yet been demonstrated to produce a significant dampening of the rhodopsin receptor pathway. In Bovine retina, two distinct arrestins, which associate with rhodopsin, have been identified (50).

Visual arrestin has been studied extensively. Several functional domains have been identified with regard to binding to activated rhodopsin. There is a phosphorylation recognition domain which binds specifically to a phosphorylated rhodopsin C-terminal, an activation regulation domain which interacts with those regions of the rhodopsin molecule which change conformation upon light activation, a hydrophobic interacting domain. Arrestin has a regulatory domain in the C-terminus which allows the hydrophobic domain to move and bind to the activated rhodopsin molecule, and the N-terminus of arrestin interacts specifically with the phosphorylated C-terminus of rhodopsin to effect a conformational change in arrestin upon binding (51). The function of arrestin is perhaps more complicated than just the desensitization of receptors. Visual arrestin in rod cells has been shown to activate phospholipase C (52). Visual arrestin moves from the inner segments of the rod cells to the outer segments in response to light (53). In fact, it has been demonstrated that arrestin enters the nucleus of rod cells in a light-dependent manner (54). Our work has shown that visual arrestin may also be involved in DNA replication (55).

Visual arrestin has also been studied for its role as a target for the autoimmune disorder known as Uveitis. Specific and distinct regions of arrestin are involved in T-cell recognition, response of uveitogenic proliferation and adaptive transfer response (55, 58), although acting as an antigen in an autoimmune disease is not thought to be the normal function of arrestin.

The Adrenergic Receptor Family

Other members of 7-TMS receptor family are the adrenergic receptors. These receptors include the α_1 and α_2 subtypes as well as the β_1 and β_2 adrenergic receptors. Most of these receptors are ubiquitous throughout mammalian tissues. The most extensively studied of these receptors is the β_2 adrenergic receptor. These receptors are activated by ligand binding. Activation of the receptor protein causes the dissociation of a G-protein and the exchange of GTP for GDP on the G_α subunit. The dissociated G_α subunit acts on adenylate cyclase. Adenylate cyclase activation increases cellular levels of cAMP, which is one of the second messengers used in signal transduction pathways. The signal can be stopped if the receptor is no longer occupied by agonist. The GTP is hydrolyzed by the G_α component and the unoccupied receptor allows for the aggregation of the trimer G-protein.

Once stimulated the cell has several ways to dampen the signal. One is to internalize the receptor protein. Another, more immediate mechanism, is heterologous and homologous desensitization. Heterologous desensitization involves phosphorylation of the receptor by PKA and PKC, as seen in the desensitization of the rhodopsin receptor. Homologous desensitization is achieved by phosphorylation of the C-terminal of the receptor by a specific β-adrenergic receptor kinase, βARK1 and βARK2 (59, 60). This kinase is specific for the occupied receptor which has lost the G_α component but is still coupled to its $G_{\beta\gamma}$ subunit (61) and is activated, in part, by autophosphorylation. This autophosphorylation is occupied receptor-dependent. Phosphorylation of the receptor is not sufficient for the attenuation of the signal, a β-arrestin is required, β-ARR (62). Again, as in the desensitization of the rhodopsin receptor by arrestin, the β-ARR binds the phosphorylated receptor to prevent its interaction with its G-protein. Arrestin will not bind to a receptor which has been phosphorylated by PKA or PKC (63). *In vitro,* βARK phosphorylates light-bleached meta II rhodopsin and RK phosphorylates the occupied β_2 adrenergic receptor (64, 65). This phosphorylation, of different receptors was several fold less efficient than the phosphorylation of the correct receptors, as expected. It was also discovered that the two arrestins, β-ARR and visual arrestin could also work in the rhodopsin and β_2-adrenergic systems, respectively. Hence, we have a functional interchangeability. The β1, 2, and 3 arrestins are around 70%–80% identical to each other and are about 50% identical to visual arrestin (50).

The Muscarinic Cholinergic Receptor Family

This family of receptors trigger a signal pathway by interaction with a G-protein. Subtypes of these receptors include the M_1 and M_2 groups of receptors. Cholinergic and adrenergic receptors all display neurotransmission abilities. Some of these G-proteins are considered stimulatory G_s and some inhibitory G_i. Some of these G-proteins can act as stimulators and inhibitors of adenylate cyclase under different conditions (21). These receptors are also desensitized by phosphorylation of the receptor protein. When visual arrestin and β-arrestin are added to these receptor systems they desensitize the occupied phosphorylated receptor (66). Recently, the M5 muscarinic receptor protein was expressed in yeast. It is synthesized and inserted into the membrane, however, the initial results

indicate that it does not function through the α-factor receptor pathway (67). It may be necessary to coexpress its G-protein, as seen for the adrenergic receptor in yeast. There are limitations to these kinds of mixing and matching experiments.

The Yeast Mating Pheromone Receptor

Since most investigators are not as familiar with the yeast receptor system as with the rhodopsin or adrenergic receptor systems, a more in-depth review is in order. The mating pheromone α-factor receptor system in particular has been extensively studied in *S. cerevisiae*. Yeasts have a haploid and a diploid life cycle. The haploid cell can mate (fuse), with another haploid cell to produce a diploid. Mating occurs between cells of opposite mating types, a type and α type. It is an elaborate and specific process which involves the production of mating pheromones and the recognition of the opposite mating type pheromones, via a-factor and α-factor plasma membrane receptors (68, 69). The fusion of opposite mating types is also dictated by cell-type specific cell surface agglutinins (70). In addition, cells must be at the same point in the cell cycle for a successful diploid to be formed.

Most yeast in the wild are homothallic. That is coming from a single clone. The a- and α-type cells are basically the same cells but are different allele at the MAT locus. Each cell can switch mating types with every other cell cycle in the haploid life cycle. An a cell becomes an α cell and vice versa. This mating type switch is controlled by a double stranded DNA endonuclease known as HO (71). This cleaves the a or α allele out the MAT locus. A copy of the opposite mating type gene is then used to replace it. The copies of the two mating type genes which are to the left and right of the MAT locus near the end of Chromosome 3 are silent. Their expression is inhibited by several factors including the switch (SWI) genes. SWI4 and SWI6 are of particular importance to this discussion because they are believed to be involved in initiation of DNA replication (72). It has been shown that the transcription factor SWI6 moves into the nucleus to directly or indirectly initiate replication. This is one example of a component of the α-factor receptor pathway participating in replication.

The cell type is determined by the a-genes expressed only in a cells and α set expressed only in α cells. The expression of these genes is controlled by the allele at the MAT locus. Examples of these mating type specific genes are pheromone receptors *STE*2, the α-factor receptor expressed in the a cell (73, 74) and *STE*3 the gene coding for the a-factor receptor in α cells (75, 76). Genes expressed only in a cells include a-factor (MFa1) (77). *BAR*1, a gene which codes for a secreted protease which digests extracellular mating pheromone, is expressed only in a cells (78). Genes expressed only in α type haploids are *STE*3, the a-factor receptor, and MFα1, the α-factor coding gene. The cell surface agglutinins are also type specific. The a-specific genes are expressed constitutively. Although produced, the a-1 message is not needed. The MAT α allele codes for α-1 and α-2. The α-specific genes are activated by α-1, while the a-specific genes are inhibited, in part, by α-2 (77).

There is a set of genes which are expressed in both a and α haploid cells. These haploid specific genes are also under the control of the products of the MAT locus. These genes are repressed in part by the a-1/α-2 complex in the diploid. All of the a-specific, α-specific, and diploid-specific genes depend on a transcription activator *MCM*1. This protein binds a-specific, α-specific, and diploid-specific promoters and may interact with either α-1 or α-2, and a-1 or a-1/α2 (77, 79). *MCM*1 is another known component of the α-factor receptor pathway which is involved in the initiation of DNA replication. *MCM*1 and other similar proteins *MCM*2 and 3 are involved in DNA replication (80). Haploid specific genes include genes coding for the receptor's G-protein which associates with the pheromone preceptor, G_α GAP (GPA1/SCG1), G_β *STE*4 and G_γ *STE*18 (81). It has been experimentally determined through the use of mutants for each of the G-protein subunits and the pheromone receptor, that binding of ligand results in the dissociation of this G-protein complex from the occupied receptor. This in turn causes the exchange of GTP for GDP by the G_α subunit. This results in the dissociation of the α from the β/γ components. Although it is presumed that the α subunit has some function, it is the β/γ component which activates the α-factor receptor system pathway. This leads to cell cycle arrest in late G1 at a point named START along with a host of physical changes as well as the expression of a battery of genes necessary for mating.

There are several ways that a signal from an occupied yeast receptor is dampened. One way is the internalization of the receptor itself which is dependent on the presence of a C-terminal domain (82, 83). The C-terminus is hyperphosphorylated in a fashion similar to the phosphorylation of the rhodopsin and adrenergic receptors (84, 85). Another factor which is required for the effective desensitization of this response includes the protein product of the *SST*2 gene (86, 87). The mechanism by which these factors dampen the signal is not yet understood, however, it does not act at the receptor/G-protein level. The mechanism which relies on the phosphorylation of the C-terminal domain of the receptor would require some receptor specific kinase. A specific α-factor receptor kinase has not yet been isolated. Additionally, researchers have not been able to clone and sequence the yeast arrestin. Based on the work in our labora-

tory, one would expect that a yeast arrestin would share a high degree of sequence homology with visual arrestin, since several monoclonal antibodies directed against S antigen cross-reacted with a putative 48 kd protein (55). One possible explanation for ours and other laboratories inability to identify a homologue by hybridization screening might be that, other than a few small regions, the yeast arrestin may share little or no sequence homology with the visual or β-arrestins.

Yeast Arrestin-like Protein

A yeast arrestin-like protein was initially identified as part of a complex implicated in DNA replication. This replicative complex is a large multienzyme complex having a molecular weight of about two million (88). The yeast arrestin is phosphorylated as part of the replicative complex in a manner dependent on the cell division cycle gene (CDC7) (55). CDC7 kinase is necessary for cells to traverse the G_1/S boundary of the cell cycle and initiate replication (89, 90). Arrestin may serve as the "trigger" for initiation of replication. In addition, authentic bovine retinal arrestin stimulates DNA polymerase I activity, the primary enzyme involved in DNA synthesis during replication in yeast (55). These findings tie the yeast arrestin in with cell cycle control and replication.

We have proposed a model of how arrestin might function. Our model begins with yeast arrestin binding to a stimulated α-factor receptor and being sequestered to the plasma membrane. This would make arrestin unavailable in the nucleus. Release from α-factor arrest allows arrestin to enter the nucleus. Once in the nucleus it becomes part of the large replicative complex. We propose that this presence or absence of arrestin may provide another level of cell cycle control. Once it becomes part of the replicative complex, it is phosphorylated, either directly by the CDC7 kinase component or by some kinase downstream. In short, arrestin would be the trigger for cells to enter S-phase and initiate replication. We expressed bovine visual arrestin from an inducible promoter in yeast. Our preliminary results also show that bovine arrestin acts specifically to prevent α-factor arrest, making cells ~100-fold more resistant to α-factor. Precedence exists for movement of a growth-regulating protein from the cytoplasm to the nucleus (91), and it has been suggested that arrestin translocates into the nucleus of photoreceptor cells in response to light (50). Remember, *MCM*1 and SWI6 are both components of the α-factor receptor pathway and necessary for initiation of replication. SWI6 moves from the cytoplasm into the nucleus for the beginning of S-phase, just as we propose yeast arrestin might.

The α-Factor Pathway

A discussion of the α-factor receptor pathway is germane to this review since the putative yeast arrestin may be involved in cell cycle arrest and in initiation of replication. Other genes in the mating pathway which are expressed in both mating cell types include *STE*20, *STE*5, which form a protein kinase cascade with *STE*11 and *STE*7 (92–95), and with *KSS*1 and *FUS*3. These genes continue this kinase cascade, with one result being the activation of *STE*12, the transcription factor (96). The mating pathway apparently branches at some point above *STE*12, which is a transcriptional regulator (97, 98). One pathway controls pheromone specific cell cycle arrest, and includes *FAR*1. *KSS*1 and *FUS*3 are redundant genes which share sequence homology with the mammalian mitogen-activated protein (MAP) kinase (99, 100). These genes appear to have some overlapping functions; however *FUS*3 is needed to cause a pheromone type arrest (101). *FUS*3 may function in cell cycle arrest after α-factor stimulation, through the G_1 cyclins. The G_1 cyclins are involved in forming a protein kinase complex with CDC28. They are believed to be in direct physical contact with CDC28 and thereby activate it, allowing cells to pass through START. The cell cycle arrest part of the α-factor response is in part the result of the inhibition of G_1 cyclins by *FUS*3 (102, 103). Directly or indirectly *FUS*3 inhibits the G_1 cyclins. Initially *FUS*3 was thought to inhibit the activity of CLN3 by some posttranscriptional mechanism and inhibit transcription of CLN1 and 2, but now it is believed that *FUS*3 has a more general inhibitory role, in addition to its direct role in α-factor arrest. CLN1, 2, and 3, have a large degree of functional overlap and only one is needed for cells to divide.

It has been determined that *FUS*3 acts in the α-factor pathway by phosphorylating *FAR*1 (104). Once phosphorylated, *FAR*1 can associate with CDC28-CLN2 kinase. Phosphorylation of *FAR*1 is also CDC28-CLN2 dependent. This represents a type of positive feedback control of *FAR*1 and cell cycle arrest. Elimination of *FAR*1 results in the inability of cells to arrest at START in response to α-factor, but those cells still show induction of α-factor pathway specific genes. The α-factor response can be dissected into two branching pathways, the cell cycle arrest pathway and the induction of genes necessary for successful mating. *FAR*1 is a link between the α-factor pathway and cell cycle arrest at START.

Apparently, this model is simplistic. There are other genes associated with START which are part of the α-factor pathway. Some CDC37 alleles which are temperature sensitive cause the cells to arrest at START and shmoo. Shmooing is a change in the shape of the cell prior to mating. This arrest is very similar to CDC28 arrest. This shmoo arrest, in the absence of α-factor, is observed with a temperature shift in both haploid types and in the diploid. The haploid cells can still mate after CDC28 or CDC37 arrest (105). There

are CDC28 mutant alleles which allow the cell to enter S-phase, initiate replication but arrest as a "dumbbell phenotype" at non-permissive temperature. Other mutant alleles are defected in both initiation of replication and nuclear division.

Another set of CDC/START mutants are CDC36 and CDC39. Although these genes seem to regulate passage through START on the bases of nutritional requirements and function in the heat shock response pathway, they cause a shmooing arrest at START in the haploid cell and not in the diploid (106). What is very interesting about these temperature sensitive mutant alleles is that they can also cause cell cycle arrest at random points throughout the cell cycle in the STE mutants. This suggests that mutations in either of these two genes not only cause metabolic defects and that an intact α-factor receptor system is necessary to arrest cells at START. CDC36 and CDC39 do not show the karyogamy defect as do CDC28 and CDC37. The final complication to this story is that CDC36 and CDC39 are needed for desensitization of the α-factor response (107). The products of these genes apparently does not work by attenuating receptor G-protein interaction, as postulated for yeast arrestin. In the desensitization of the α-factor pathway, CDC36 and CDC39 operate at START in the cell cycle.

The Family of Arrestins

It is now evident that visual arrestins and adrenergic arrestins are part of a much larger family of functionally similar proteins which uncouple G-proteins from their receptors. Two visual arrestins have been cloned from *Drosophila* which are 42% identical to human arrestin (108). Apparently, bovine retinal rod cells have 4 different arrestins, not one (109). Arrestin and arrestin-like molecules have been identified in the pineal gland of humans (110). The presence of arrestin in nonvisual tissues appears to be the norm and not the exception. An arrestin in nucleated red blood cells has been identified and it binds mammalian rhodopsin to attenuate the stimulated receptor (111). Arrestin-like molecules have been found in the choroid plexis of the brain of quails (112), in the mammalian lung (113), in tobacco plant cells (114), and in yeast (55).

Summary

The possible value of yeast as an organism for studying mammalian 7-TMS receptor systems has been recognized (115). "Mixing and matching" of receptors and receptor components in yeast has its limitations, but when it works it provides a beautiful system for studying receptor function. In addition, the yeast mating pheromone's kinase cascade, its resultant transcriptional control of batteries of genes, and ultimately the receptor system's participation in initiation of DNA replication may be conserved and may have a counterpart in mammalian growth control receptors. The study of this entire receptor system and the identification of homologous systems in mammalian cells may provide a major short cut to understanding growth and cell cycle control.

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