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14. ABSTRACT

We previously identified an example of structural inheritance whereby yeast mitochondria can exist in two alternative states thereby conferring two different sets of growth properties. In the [Leu+] state, rhozero yeast grow at rates indistinguishable from their mitochondrial DNA-containing rhoplus parent while [LeuP] rhozero cells exhibit slow growth and partial leucine auxotrophy. We have made progress in elucidating the difference between [Leu+] and [LeuP] strains and have also searched for additional examples of structural inheritance in yeast. Nfu1p is a mitochondrial protein that is involved in both Fe/S complex formation and mitochondrial chaperones, two functions that are consistent with their participation with [LeuP]. Relative to wild type strains, nfu1 strains show a substantially higher fraction of rhozero derivatives that are [LeuP]. These nfu1 [LeuP] rhozero strains also show a greater frequency of conversion to [Leu+]. New examples of structural inheritance were sought by examining the peroxisome and the vacuole, two potentially non-essential organelles. Using an unlinked non-complementation approach modified to uncover evidence of structural templating, we have been unable to find evidence to support the idea that either of these organelles provides an essential "seed" for the generation of additional copies of these structures. Our current efforts to find new examples of structural inheritance are leading us to examine other multi-subunit enzymes, such as the respiratory complexes, that might harbor structural information. Our studies of peroxisome biogenesis, while unfruitful as far as structural inheritance is concerned, have led to the surprising discovery that beta-oxidation is required to prevent the toxicity of fatty acids. This has led us to the hypothesis that a primary function of the yeast peroxisome, an organelle whose fundamental function has been elusive, is to coordinate membrane phospholipid composition through regulated degradation of fatty acids.

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Introduction

This proposal was funded to establish the molecular basis of what may prove to be a new type of structural inheritance and to identify additional examples of structural inheritance using yeast as a model system. By developing a better understanding of the molecular details of non-disease types of structural inheritance, aberrant cases, such as those caused by prions will be better understood. Two strains of genetically identical yeast differ phenotypically if a protein adopts the prion conformation in one and the non-prion conformation in the other. Thus, a structural difference, in this case due to the ability of certain proteins to adopt two folding states, one of which induces additional subunits of the protein to adopt that conformation, is manifest as a heritable difference. Having identified [*Leu*^{P}], a new example of structural inheritance in yeast, we now have made progress in understanding the factors that govern whether a cell adopts the [*Leu*^{P}] or [*Leu*$^+$] state. We summarize this progress in the body below.

As proposed, we have been attempting to identify additional examples of yeast structural inheritance, especially at the organellar level, that are perhaps more amenable to molecular dissection. This has led us to examine the vacuole and the peroxisome, two organelles that had been reported to be non-essential and therefore perhaps able to be analyzed using cytoduction, whereby non-nuclear yeast components can be transferred to a mating partner. The yeast vacuole, as summarized in the previous Annual Report, has not borne fruit in this regard; we have not found evidence for its structural inheritance and now believe vacuoles to be capable of templating themselves. Despite the large-scale screen for peroxisome-deficient yeast during the past year we have also been unable to show that this organelle exhibits structural inheritance. We summarize the peroxisome results below. A recent important study, in fact, concludes that peroxisomes are derived, not from pre-existing copies, but rather from the endoplasmic reticulum (Hoepfner, 2005).

We have been searching for and testing whether a variety of macromolecular complexes exhibit structural inheritance. For instance, as reported in the first update last year, it appears that pyruvate dehydrogenase is capable of self-assembly, *i.e.,* it does not exhibit structural inheritance. The body of this update summarizes our current thinking and strategy concerning this aspect of our work.

Despite the absence of structural inheritance in the peroxisome, our examination of this organelle has provided new insights into the function of this poorly understood organelle. As discussed in depth below, the fatty acid catabolism that occurs in the peroxisome appears to function, not primarily to provide energy from ingested fat, but rather to allow the ratio of saturated to unsaturated fatty acid in phospholipid to be adjusted upon changes in growth conditions. A case is made for the relevance of this modified research focus to the goals stated in our original proposal.

Body

We have separated this section of the annual report into three sections reflecting new results relating to the mitochondrial **[*Leu*^{P}]** phenotype (Aim 1 in the original proposal), experiments designed to identify new cases of structural inheritance (Aim 2 in the original proposal), and follow-up studies on peroxisomal function which have arisen out of Aim 2 of the original proposal.

Section 1: The [*Leu*^{P}] phenotype

When a ρ^+ (respiratory-competent) strain completely loses its mitochondrial DNA, two types of ρ° strains are obtained. One type of ρ°, termed [*Leu*$^+$], grows as well as does its ρ^+ parent while the other type of ρ°, named [*Leu*^{P}], grows two-fold more slowly on rich media and three-fold more slowly in the absence of leucine. The basis for our conclusion that the heritable difference between these two types of ρ° strains is due to a difference in mitochondrial structure is presented in our first paper on the subject (Lockshon 2002) and has been summarized in the 1st Annual Update.

The goal of determining the molecular basis of this heritable difference has led us to examine the effect of deletion of a variety of genes on the relative frequency of [*Leu*$^+$] *vs.* [*Leu*^{P}] appearance from a ρ^+ parent and on the dependency of the stability of the [*Leu*^{P}] state on these deletions. Since a genome-wide analysis of this property of ρ° derivatives did not seem feasible, we pursued a candidate gene approach.

Our attention turned to the mitochondrial chaperone proteins for two reasons. First, the chaperone Hsp104p exerts control over all known yeast prions. In our publication on [*Leu*^{P}], we attributed the failure of either deletion or overexpression of Hsp104p to influence [*Leu*^{P}] as evidence that [*Leu*^{P}] is a non-prion type of structural inheritance. This may be an over-simplification, however, since Hsp104p is a cytoplasmic protein. Its failure to influence [*Leu*^{P}] is possibly due to differential compartmentalization. A number of mitochondrial proteins, Jac1p, Hsp78p, Ssq1p and Nfu1p, have chaperone roles. In fact, Hsp78p is thought to function as the mitochondrial equivalent of Hsp104p. Second, a number of studies implicate mitochondrial chaperone activity in the synthesis and maturation of the covalent Fe/S complex (VOOS and ROTTGERS 2002). Interestingly, the reason that the mitochondrion is an essential organelle may be its unique ability to synthesize this cofactor (KISPAL *et al.* 2005). Fe/S is highly relevant to the leucine phenotype of [*Leu*^{P}] because the enzymatic activity of Leu1p, which catalyzes a step in leucine biosynthesis, has an absolute requirement for Fe/S. Indeed, deletion of Atm1p, which transports Fe/S out of the mitochondrion, causes complete leucine auxotrophy, presumably because Leu1p is cytosolic (KISPAL *et al.* 1999).

We are examining the influence of removal of Jac1p, Hsp78p, Ssq1p and Nfu1p proteins on the relative frequency with which [*Leu*$^+$] and [*Leu*^{P}] appear. Initial experiments employed the strains made by the deletion project. However, ρ° derivatives from this strain background (S288C) yielded, not [*Leu*$^+$] or [*Leu*^{P}] ρ° derivatives, but rather only slow-growing ρ° derivatives with little if any leucine growth

phenotype. We thus deleted *JAC1* and *NFU1* in the ρ^+ strain YDL121, which has a different genetic background (A364A) and was used in our original [Leu^P] study. Although *JAC1* is reported to be required for viability in other strain backgrounds, we were able to generate a *jac1Δ* strain in the A364A background. Whereas 9% of the spontaneous ρ° of the wild-type control strain were ρ^+ and 91% were [Leu^P], an *nfu1Δ* derivative of YDL121 yielded 45% [Leu^+] and 55% [Leu^P] ρ° progeny (**Figure 1**). In contrast, deletion of JAC1 did not alter the frequency of [Leu^P] progeny. This finding raises the possibility that the [Leu^P] phenotype might require proper Nfu1p function. Since *ssq1Δ* is synthetically lethal with *nfu1Δ,* perhaps *ssq1Δ* exerts a similar effect on [Leu^P] frequency. We are excited that this line of experiments will finally allow us to begin unraveling the molecular nature of the [Leu^P] phenotype.

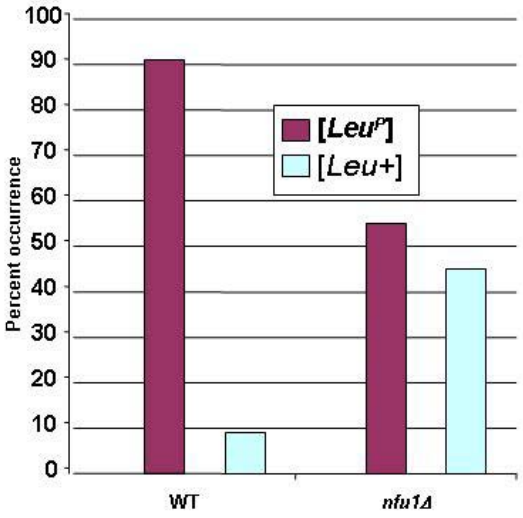

Figure 1. Deletion of NFU1 leads to enhanced frequency of [*Leu+*] generation. Strains were grown in media containing ethidium bromide to induce ρ° derivatives. These derivatives were then scored as [Leu^P] or [*Leu+*] by determining growth rate on media lacking leucine.

We have also initiated pilot studies to determine the feasibility of using unbiased screens to better understand the [Leu^P] phenotype. Initial pilot studies were required because this phenotype shows substantial strain specificity. We have examined the fate of ρ° derivatives of the strain BY4742. Our goal was to use BY4742 since this is the strain background in which the genome wide deletion set was constructed and would therefore be optimal for genetic screens. Unfortunately, ρ° derivatives in this background are uniformly slow growing on rich medium containing 2% glucose. Therefore, this strain is not optimal for screens. To perform a screen, we now feel that the best approach is to select for high copy suppressors of the [Leu^P] phenotype in the DL035 background. Overexpression of genes that result in rapid growth on 2% glucose and a [Leu^+] phenotype would be selected. This approach is complicated by the fact that this strain spontaneously becomes [Leu^+] at a significant frequency. Therefore finding suppressors may be complicated and we are currently considering the feasibility of this approach.

Section 2: The search for new cases of structural inheritance

Self-assembly asserts that all biological information is contained in the genome. While a number of exceptions have been described, prions are the first such exception understood in molecular detail. This proposal is based on the premise that there exist in yeast additional exceptions to self-assembly. We feel the genetic approach to discovering such exceptions, wherein such structures are modified or ablated *in vivo*, is the most promising. The challenge, then, is to identify ***non-lethal*** heritable changes in

structure. [Leu^P], a trait discovered inadvertently in the course of other studies, demonstrates that the yeast mitochondrion can exist in two alternative structural states.

We chose initially to examine two large and apparently non-essential structures in yeast: the vacuole and the peroxisome. As states in a previous annual report, we were able to rule out the vacuole as a site of structural inheritance. Here we describe studies of the peroxisome, which have led to a similar conclusion. Controversy has existed concerning the origin of the peroxisome and its inheritance since it was first discovered decades ago (PURDUE and LAZAROW 2001). As detailed in our First Annual Report, a variety of lines of evidence imply that a new peroxisome can only be made by using a pre-existing peroxisome as a template (HAZRA *et al.* 2002; SOUTH *et al.* 2001). Others have argued that the peroxisome's identity is defined by the localization of key peroxisomal-formation proteins in regions of ER which then bud off to create the nascent organelle. Based on the identity of the peroxisomal proteins of *S. cerevisiae*, β-oxidation (the catabolism of fatty acids to acetyl-CoA) is the only complete biochemical pathway that appears to be contained within this organelle. Since mutants lacking peroxisomes exist, the organelle is non-essential. It appeared therefore to be a good candidate for a self-templating cellular structure.

Intensive work in yeast over the past decade has identified dozens of proteins, the peroxins, which participate in the structure and function of the peroxisome (ECKERT and ERDMANN 2003). The phenotype used to identify *pex* genes in *S. cerevisiae* has been the inability of *pex* strains to grow on plates containing oleate (a fatty acid) as the sole carbon source. Pex3p and Pex19p in particular play a critical role in the organelle's structure: absence of either of them causes the peroxisome to disappear, yet complementation of either defect by re-introducing the gene causes the peroxisome to reappear. Reports of so-called protoperoxisomes (HAZRA *et al.* 2002) in these deletion strains allowed the notion of structural templating of the peroxisome to remain alive; the protoperoxisomes could be the "seed" on which newly formed peroxisomes were made.

If peroxisomes exhibit structural templating, we reasoned that there should exist (a) protein(s) in yeast that are responsible for the maintenance of even these residual protoperoxisomes. Deletions of a gene encoding such a protein should abolish peroxisomes even after the gene is re-introduced. Re-introducing peroxisomes *i.e.*, a template, by cytoduction after reintroducing the gene, however, should allow peroxisomes to be re-established. Based on the previous studies of *S. cerevisiae* peroxisomes, we reasoned that poor growth on oleate relative to glycerol (a non-fermentable carbon source which, like oleate, should not support the growth of respiratory-deficient mutants) would lack peroxisomes, the cellular location of the oleate-utilization (β-oxidation) machinery. Using robotic procedures, we identified ~150 of the ~4000 yeast deletion strains (each lacking a single non-essential gene) which grew poorly on plates containing oleate relative to growth on plates containing glycerol (**Table 1**). Most of the other *PEX* genes (including *PEX3* and *PEX19*) were identified in the screen, as were genes encoding proteins of unknown function and proteins involved in an impressive breadth of yeast physiology.

Table 1. Deletion strains identified in the oleate screen

ORF	GENE	PT	FUNCTIONAL CATEGORY	ADJ HIT
YJR105W	*ADO1*	++	adenosine kinase	
YNL197C	*WHI3*	+++	cell cycle	
YCR077C	*PAT1*	+++	cell wall	
YDR017C	*KCS1*	+++	cell wall	
YDR162C	*NBP2*	+++	cell wall	
YHL011C	*PRS3*	+++	cell wall	
YKL212W	*SAC1*	+++	cell wall	
YLR438W	*CAR2*	++	cell wall	
YMR238W	*DFG5*	+++	cell wall	
YOL081W	*IRA2*	+++	cell wall	
YOR360C	*PDE2*	+++	cell wall	
YNL215W	*IES2*	+++	chromatin remodeling	*PEX17*
YLR357W	*RSC2*	++	chromatin remodeling	
YOR038C	*HIR2*	++	chromatin remodeling	
YOL004W	*SIN3*	++	chromatin remodeling (HDAC)	
YOL012C	*HTZ1*	+++	chromatin remodeling (H2A var.)	
YDR334W	*SWR1*	+	chromatin remodeling (SWR-C)	
YLR085C	*ARP6*	+++	chromatin remodeling (SWR-C)	
YML041C	*VPS71*	+++	chromatin remodeling (SWR-C)	
YNL107W	*YAF9*	+++	chromatin remodeling (SWR-C)	
YEL033W		++	dubious ORF	*SPF1*
YGL152C		+++	dubious ORF	*PEX14*
YJL211C		+++	dubious ORF	*OPT1*
YLL020C		++	dubious ORF	*KNS1*
YNL120C		++	dubious ORF	*NCS1*
YOL050C		+++	dubious ORF	*GAL11*
YPR087W		++	dubious ORF	
YJL101C	*GSH1*	+++	glutathione synthesis	
YOR080W	*DIA2*	+	invasive growth	
YCR034W	*FEN1*	+++	lipid biosynthesis	
YKR067W	*GPT2*	+++	lipid biosynthesis	
YLR362W	*STE11*	++	mating	
YOR297C	*TIM18*	++	mitochondrial protein import	
YAL010C	*MDM10*	++	mitochondrial inheritance	*SPO7*
YLR368W	*MDM30*	+++	mitochondrial inheritance	
YOL076W	*MDM20*	+++	mitochondrial inheritance	
YMR060C	*SAM37*	+++	mitochondrial (Mdm10 cofactor)	
YPR131C	*NAT3*	++	mitochondrial (Mdm20 cofactor)	
YML120C	*NDI1*	+++	mitochondrial (oxidoreductase)	
YMR145C	*NDE1*	+++	mitochondrial NADH dehyd'ase	
YDL198C	*GGC1*	++	mitochondrial transport	
YPL060W	*LPE10*	+++	mitochondrial transport	
YPL270W	*MDL2*	++	mitochondrial transport	
YHR191C	*CTF8*	++	mitosis	
YOR073W	*SGO1*	+++	mitosis	
YJR074W	*MOG1*	+++	nuclear protein import	
YDR264C	*AKR1*	+++	palmitoyltransferase	*PEX10*
YAL051W	*OAF1*	+++	peroxisome	
YAL055W	*PEX22*	+++	peroxisome	
YDL065C	*PEX19*	+++	peroxisome	
YDR142C	*PEX7*	+++	peroxisome	
YDR244W	*PEX5*	+++	peroxisome	
YDR265W	*PEX10*	+++	peroxisome	*AKR1*
YDR329C	*PEX3*	+++	peroxisome	
YEL031W	*SPF1*	+++	peroxisome	*YEL033W*
YGL153W	*PEX14*	+++	peroxisome	*YGL152C*
YGL205W	*POX1*	+	peroxisome	
YGR077C	*PEX8*	+++	peroxisome	*PAC10*
YGR133W	*PEX4*	+++	peroxisome	*PHB1*
YIL160C	*POT1*	+++	peroxisome	
YIR004W	*DJP1*	+	peroxisome	
YKL197C	*PEX1*	+++	peroxisome	
YKR009C	*FOX2*	+	peroxisome	
YLR191W	*PEX13*	+++	peroxisome	
YMR026C	*PEX12*	+++	peroxisome	
YNL214W	*PEX17*	+++	peroxisome	*IES2*
YNL329C	*PEX6*	+++	peroxisome	
YOL044W	*PEX15*	+++	peroxisome	
YPL112C	*PEX25*	+	peroxisome	
YBR106W	*PHO88*	+++	phosphate transport	
YNL248C	*RPA49*	++	Pol I subunit	
YBL025W	*RRN10*	+++	Pol I transcription	
YGR104C	*SRB5*	++	Pol II mediator (head)	
YHR041C	*SRB2*	+++	Pol II mediator (head)	
YDL005C	*MED2*	+++	Pol II mediator (tail)	*PTC1*
YGL025C	*PGD1*	+++	Pol II mediator (tail)	
YOL051W	*GAL11*	+++	Pol II mediator (tail)	*YOL050C*
YBL058W	*SHP1*	+++	protein degradation	
YDL020C	*RPN4*	++	protein degradation	
YGR135W	*PRE9*	+++	protein degradation	
YHR111W	*UBA4*	+++	protein degradation	
YIL008W	*URM1*	+++	protein degradation	
YLR024C	*UBR2*	+++	protein degradation	
YNL119W	*NCS2*	++	protein degradation	*YNL120C*
YDL090C	*RAM1*	+++	protein farnesyltransferase	
YEL003W	*GIM4*	++	protein folding	
YGR078C	*PAC10*	++	protein folding	*PEX8*
YHR064C	*SSZ1*	+++	protein folding	
YOR265W	*RBL2*	++	protein folding	
YLL019C	*KNS1*	++	protein kinase	*YLL020C*
YNL298W	*CLA4*	+	protein kinase	
YDL006W	*PTC1*	+++	protein phosphatase	*MED2*
YER054C	*GIP2*	+++	protein phosphatase	
YBL027W	*RPL19B*	+++	ribosomal protein	
YBL072C	*RPS8A*	+++	ribosomal protein	
YDR025W	*RPS11A*	+++	ribosomal protein	
YDR418W	*RPL12B*	+++	ribosomal protein	
YHL033C	*RPL8A*	++	ribosomal protein	
YIL052C	*RPL34B*	+++	ribosomal protein	
YGR070W	*ROM1*	+	signal transduction	
YPL213W	*LEA1*	+++	splicing	
YJR104C	*SOD1*	++	superoxide dismutase	
YBR084W	*MIS1*	++	tetrahydrofolate metabolism	*SPT7*
YLR423C	*ATG17*	++	trafficking	
YPR032W	*SRO7*	++	trafficking	
YPR139C	*VPS66*	+++	trafficking	
YPR173C	*VPS4*	++	trafficking	
YIL128W	*MET18*	+++	transcription	
YDR216W	*ADR1*	++	transcription	
YMR179W	*SPT21*	++	transcription (histones)	
YBR081C	*SPT7*	++	transcription (SAGA)	*MIS1*
YJL140W	*RPB4*	+++	transcription (stress tolerance)	
YJL056C	*ZAP1*	+++	transcription (Zn and PI synth.)	
YHR206W	*SKN7*	+	transcription/oxidative stress	
YDL069C	*CBS1*	+++	translation	
YPR163C	*TIF3*	++	translation	
YJL212C	*OPT1*	+++	transporter	*YJL211C*
YAL009W	*SPO7*	+++	unclear	*MDM10*
YAR014C	*BUD14*	++	unclear	
YCR047C	*BUD23*	+++	unclear	
YCR063W	*BUD31*	+	unclear	
YFR035C		++	unclear	
YGL127C	*SOH1*	+++	unclear	
YGL173C	*KEM1*	+++	unclear	
YGR132C	*PHB1*	+++	unclear	*PEX4*
YHR100C		+++	unclear	
YJL184W	*GON7*	+++	unclear	
YJR118C	*ILM1*	+++	unclear	
YKR028W	*SAP190*	+++	unclear	
YLR021W		++	unclear	
YLR114C	*AVL9*	+	unclear	
YMR014W	*BUD22*	+	unclear	
YMR032W	*HOF1*	+	unclear	
YMR100W	*MUB1*	+++	unclear	
YNR018W		+	unclear	
YOL072W	*THP1*	+++	unclear	

A rating of the ratio of growth of on STY+glycerol relative to growth on STY+oleate ("PT") of strains deleted in the indicated ORFS: "+" (smallest oleate effect) to "+++" (largest). A protein's functional category was assigned based on the literature. "ADJ HIT" refers to the appearance in this table of a gene that is adjacent in chromosomal location to any other gene listed in the table. A line under the ORF indicates that the effect of its deletion on the localization of Pex11 was examined (see text).

We sought to identify the subset of hits in the oleate screen which, like *pex3Δ* and *pex19Δ* strains, lack a peroxisome. This was accomplished by crossing a GFP-fusion derivative of Pex11p into each of our positives and examining the fluorescence microscopically. Since Pex11p localized to peroxisomes, punctate fluorescence indicates maintenance of the organelle. Unfortunately, all the genes hit in the oleate screen, aside from *pex3Δ* and *pex19Δ* (which each showed diffuse fluorescence), showed punctate fluorescence.

Using this comprehensive approach, we have thus been unable to establish that the peroxisome, a seemingly good candidate, exhibits structural inheritance. Moreover, Hoepfner *et al.* (HOEPFNER *et al.* 2005) have recently provided convincing evidence using fluorescence microscopy that the peroxisome of *S. cerevisiae* is derived from the ER. We conclude, therefore, that the pursuit of structural inheritance in the peroxisome is no longer viable.

How, then, can a search for additional examples bear fruit? An approach that has long been of interest to us, but for which a general approach has thus far been elusive, is to examine yeast genes whose deletion causes a phenotype but which, when re-introduced into the deletion strain, are incapable of reversing this phenotype. We are examining one other poorly characterized but non-essential complex, the lipid particle. This structure is a site of sterol synthesis and fatty acid storage. Strains lacking four genes (*ARE1*, *ARE2*, *DGA1*, *LRO2*) fail to generate lipid particles. We are generating a strain lacking all four of these genes. We will then re-introduce the genes individually and collectively and determine whether lipid particles can be re-formed.

In addition, we are embarking on a literature-based approach to try and identify multi-protein complexes where structural inheritance might occur. We will identify multi-protein complexes (an extensive body of literature has come into being, both from standard and genome-wide protein purification studies) that exhibit easily screenable phenotypes when inhibited. We will then search the literature to determine whether genes encoding proteins in this complex have been cloned by complementation. For select complexes where complementation cloning is not reported, we will use the strains from the deletion set to verify this phenotype and use unlinked non-complementation to attempt to identify structural inheritance. We feel that with regard to new cases of structural templating, at a minimum, by the end of the granting period, we will have generated strong evidence against widespread structural templating. Alternatively, we will find complexes where templating exists and embark upon studies to gain a mechanistic understanding of this phenomenon.

Section 3: Peroxisome function and fatty acid toxicity

Our oleate screen and subsequent examination of the hits in greater detail is serving to shed much light on the function of the peroxisome. Briefly, we have established that for every deletion strain hit in

our oleate screen, poor growth on oleate is due, not to poor utilization of oleate, but to inhibition of growth by this unsaturated fatty acid. We hypothesize two general explanations for the inhibition of these deletion strains to be inhibited by oleate: One class of strains, such as the *pex* mutants, because they unable to catabolize oleate, allow toxic levels of unsaturated fatty acid to be incorporated into phospholipid. We hypothesize the other class of oleate-sensitive deletion strains to have defects in the sensing pathway that serves to adjust phospholipid fatty acid content. Mutants in this second class exhibit normal peroxisome induction and function in response to elevated oleate levels (**Figure 2**). Although the control of membrane fluidity is relatively well understood in a variety of bacteria, there are huge holes in our knowledge of such control in the eukaryotes. This work has direct bearing, not just on human heritable peroxisomal disorders, (FAUST *et al.* 2005) but on a host of human disorders which are related to membrane composition and fluidity (WANDERS and WATERHAM 2005). While the continuation of these studies in this alternative direction has no direct bearing on prion disease, they will certainly lead to a number of significant publications.

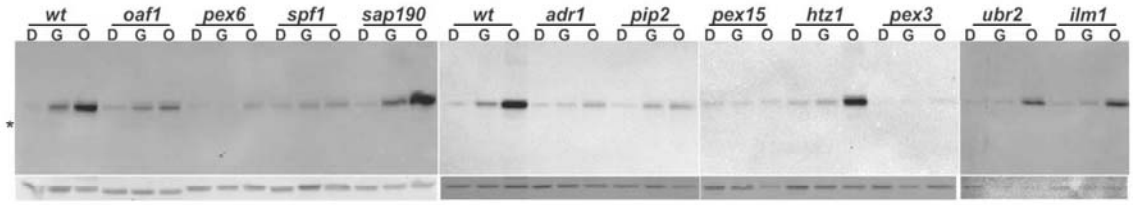

Figure 2. Induction of Pex11-GFP by glycerol and oleate in oleate-sensitive deletion strains. Protein extracts of strains containing Pex11-GFP and deleted in the indicated genes were made from portions of mid-log cultures after growth in STY+glucose (2%; **D**). The remaining cells were washed and transferred to STY+glycerol (3%). After 24 hr a portion of these cells were harvested (**G**). The remaining cells were then transferred to STY+oleate (0.1%) and harvested after 16 hr (**O**). Western blots were probed for GFP (upper panel) and subsequently probed for actin (lower panel). The asterisk shows the position of the actin band.

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Key Research Accomplishments

- Identification of a gene (*NFU1*) which modulates the rate of [*LeuP*] formation and inter-conversion to [*Leu+*]
- Concluded experiments ruling out structural inheritance as important for peroxisome biosynthesis and function
- Genome-wide screen for peroxisomal mutants and follow-up studies have uncovered links between β-oxidation, membrane lipid composition and mitochondrial function (Manuscript submitted).
- Conclusion of yeast mating studies leading to a submitted manuscript.
- Initiation of studies to examine a possible role for structural inheritance in the formation of multi-protein complexes in yeast.

Reportable Outcomes

Databases that will be made public in upcoming manuscripts or through lab website:

- Database of yeast gene deletions sensitive to oleic acid
- Database of yeast gene deletions that permit diploid mating

Manuscripts:

- Schmidlin, T., Kaeberlein, M., Lockshon, D., and B.K. Kennedy. Genome-wide screen identifies genes in yeast that are required to prevent mating in diploids. Manuscript submitted.
- Lockshon, D., Kerr, E.O., Surface, L., and B.K. Kennedy. Yeast lacking proper peroxisome function are sensitive to toxicity by the monounsaturated fatty acid oleate. Manuscript submitted.

Presentations:

• XXI International Conference on Yeast Genetics and Molecular Biology 2003, Gothenburg, Sweden, Dan Lockshon, Poster presentation.

A heritable structural alteration of the yeast mitochondrion.

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Prions have revived interest in hereditary change that is due to change in cellular structure. How pervasive is structural inheritance and what are its mechanisms? Described here is the initial characterization of [Leu P], a heritable structural change of the mitochondrion of *Saccharomyces cerevisiae* that often but not always accompanies the loss of all or part of the mitochondrial genome. Three phenotypes are reported in [Leu P] vs. [Leu+] strains: two-fold slower growth, three-fold slower growth in the absence of leucine, and a marked delocalization of nuclear-encoded protein destined for the mitochondrion. Introduction of mitochondria from a [Leu+] strain by cytoduction can convert a [Leu P] strain to [Leu+] and vice versa. Evidence against the Mendelian inheritance of the trait is presented. The incomplete dominance of [Leu P] and [Leu+], and the failure of *HSP104* deletion to have any effect suggest that the trait is not specified by a prion but instead represents a new class of heritable structural change.

• 2004 Yeast Genetics and Molecular Biology Meeting (July 27-31), Seattle, Dan Lockshon, Oral presentation and Poster presentation

Abstract #418A

Presentation: Poster
Topic: Epigenetic mechanisms

A genomic method for identification of structures in yeast which are incapable of self-assembly.

Daniel Lockshon, Brian Kennedy
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While prions have revived the concept that biological structures themselves are capable of 'encoding' biological information, additional phenomena show prions to be just one type of structural inheritance. We postulate the existence of non-essential structures in yeast which are incapable of self-assembly and which must therefore serve as templates for their own duplication. Our attempt to identify such structures centers around identification of pairs of non-essential genes whose deletion alleles fail to complement each other. Unlinked non-complementation of this type should occur between two genes whose products reside in a single non-essential structure and which must be expressed in order for the structure to be maintained. Our initial application of this strategy involves first, screening the set of yeast deletion strains for a phenotype that is already known to be the result of loss of a particular structure. Secondly, intercrossing of all such strains can then perhaps identify a doubly heterozygous deletion diploid which retains the phenotype. Candidates would then be subjected to a subsequent cytoduction step to attempt to rule out haploinsufficiency. The first structures (and phenotypes) we are examining using this protocol are the vacuole (strontium-sensitivity) and the peroxisome (inability to grow when the sole carbon source is a fatty acid) but this approach should also be applicable to any non-essential yeast structure whose loss results in a phenotype.

• XXII International Conference on Yeast Genetics and Molecular Biology August 2005, Bratislava, Slovakia, Dan Lockshon, Oral presentation and Poster presentation.

Integration of the peroxisome into the physiology of Saccharomyces cerevisiae.
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The identity and function of many components of the peroxisome of S. cerevisiae have been determined, yet we are just beginning to understand the integration of this organelle with the rest of yeast cell physiology. For example, how does exposure to fatty acids,

which are catabolized in the peroxisome, enhance peroxisomal (pex) protein levels? By screening the set of ~4800 yeast deletion strains for growth on oleate vs. glycerol as sole carbon sources, we identified ~140 genes, the deletion of which each cause impaired peroxisome function. In addition to most of the known pex genes, many proteins which reside in a variety of other cellular locales were identified. These hits suggest integration of peroxisome function with a wide variety of cellular processes. Data will be presented implicating PIP metabolism, fatty acid synthesis, Pol II function, chromatin structure and urmylation in peroxisome function. Surprisingly, many deletion strains identified in the screen grew more poorly on oleate plates than in the absence of any added carbon source. Experiments are in progress to test whether oleate growth inhibition of these strains is due to the oxidative stress response. Our genomic approach verifies that the genetic screens done previously have indeed identified most of the proteins that actually comprise the peroxisome. It is also permitting us to now appreciate the extent to which the function of this organelle depends on other aspects of the cellular machinery.

- **Seattle Area Yeast Meeting (2006) Dan Lockshon, Oral Presentation. Does the peroxisome participate in yeast membrane composition? (no abstract)**

Conclusions:

During the most recent period of support we have made progress in understand the molecular basis of [*Leu*^{P}] by determining that Nfu1p, a protein that participates in Fe/S formation in the mitochondrion, has a large effect on the frequency of [*Leu*^{P}] derivatives. In addition, our work has not provided any evidence that the peroxisome exhibits structural inheritance. Our work on the peroxisome has been fruitful in providing us with an opportunity to answer important questions concerning the network by which eukaryotes control membrane composition. One longstanding problem in biology is how the various membrane systems of a eukaryotic cell maintain their separate identities. This is in essence a problem of structural inheritance. A shift in our research agenda toward the study of membranes puts us in an excellent position to eventually address this problem. Lastly, we have continued our search for additional examples of yeast structural inheritance by examining the biogenesis of the lipid particle and by searching for genes not clonable by complementation and which therefore perhaps encode proteins that participate in structures which serve as templates for additional copies of themselves. It may be that the result of our studies is that structural inheritance is not a widespread phenomenon in yeast. Alternatively, we may yet identify examples that can become the subject of future mechanistic studies.

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