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Ndt80 activates an isoform of *ORC1* and *SMA2* via a bi-directional MSE motif

**Ndt80 activates the meiotic *ORC1* transcript isoform and *SMA2* via a bi-directional middle sporulation element in *Saccharomyces cerevisiae***

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**Summary**

The origin of replication complex subunit *ORC1* is important for DNA replication. The gene is known to encode a meiotic transcript isoform (*mORC1*) with an extended 5'-untranslated region (5'-UTR), which was predicted to inhibit protein translation. However, the regulatory mechanism that controls the *mORC1* transcript isoform is unknown and no molecular biological evidence for a role of *mORC1* in negatively regulating Orc1 protein during gametogenesis is available. By interpreting RNA profiling data obtained with growing and sporulating diploid cells, mitotic haploid cells, and a starving diploid control strain, we determined that *mORC1* is a middle meiotic transcript isoform. Regulatory motif predictions and genetic experiments reveal

that the activator Ndt80 and its middle sporulation element (MSE) target motif are required for the full induction of *mORC1* and the divergently transcribed meiotic *SMA2* locus. Furthermore, we find that the MSE-binding negative regulator Sum1 represses both *mORC1* and *SMA2* during mitotic growth. Finally, we demonstrate that an MSE deletion strain, which cannot induce *mORC1*, contains abnormally high Orc1 levels during post-meiotic stages of gametogenesis. Our results reveal the regulatory mechanism that controls *mORC1*, highlighting a novel developmental stage-specific role for the MSE element in bi-directional *mORC1/SMA2* gene activation, and correlating *mORC1* induction with declining Orc1 protein levels. Because eukaryotic genes frequently encode multiple transcripts possessing 5'-UTRs of variable length, our results are likely relevant for gene expression during development and disease in higher eukaryotes.

Keywords

*NDT80*, *SUM1*, *ORC1*, *SMA2*, 5'-UTR, MSE, meiosis, sporulation, isoform, bi-directional promoter

## Introduction

DNA replication in budding yeast is a multi-step process initiated by the origin of replication binding complex (ORC), which includes six subunits.<sup>1</sup> *ORC1* encodes a conserved ATPase essential for the mitotic cell cycle.<sup>2,3</sup> While Orc1 functions during pre-meiotic DNA replication and protects repetitive ribosomal DNA (rDNA) sequences from becoming unstable during meiotic recombination, no role is known for the protein during middle and late stages of meiosis and spore formation.<sup>4</sup> The mitotic isoform of *ORC1* is divergently expressed with the long non-coding RNA *XUT1538*, which belongs to a class of regulatory lncRNAs that are targeted by the cytoplasmic 5'-3' exoribonuclease Xrn1.<sup>5</sup> Paradoxically, *ORC1* expression is strongly induced in diploid cells that enter meiotic M-phase,<sup>6,7</sup> and this induction pattern coincides with the transcriptional activation of divergently expressed meiosis-specific *SMA2*. This gene is important for the spore membrane pathway that ensures proper encapsulation of haploid nuclei into spores.<sup>8-10</sup> Bi-directional transcription patterns, which may involve pairs of mRNAs, long non-coding RNAs (lncRNAs) or a combination of both, have been described as an intrinsic property of yeast promoters, but the regulatory mechanisms underlying this phenomenon are often not understood.<sup>9, 11, 12</sup>

An earlier RNA- and ribosome profiling study of yeast sporulation reported that *ORC1* encodes a meiotic isoform with an extended 5'-untranslated region (UTR) that was predicted to inhibit Orc1 translation during post-meiotic stages of spore development *via* upstream open reading frames (uORFs).<sup>13</sup> However, Orc1 protein levels during meiosis and gametogenesis have not

been determined, and the transcription factors that control the expression of *mORC1* during growth and development are unknown.

Meiotic M-phase requires middle genes that are specific for the process and genes that function during mitosis and meiosis. The transcriptional activator Ndt80 induces both types of genes *via* direct interaction with MSEs,<sup>14</sup> while Sum1 represses meiosis-specific genes, including *NDT80*,<sup>15</sup> during vegetative growth either alone or by recruiting the histone deacetylase Hst1 to a sequence motif that overlaps certain MSEs.<sup>16,17</sup> *NDT80* is transcriptionally activated during meiotic prophase I in a two-step process, whereby the gene is first de-repressed prior to meiotic M-phase I, when Ume6 and Sum1 activities are progressively down-regulated, and then strongly induced *via* an auto-activating loop when cells trigger the meiotic divisions;<sup>18</sup> reviewed in.<sup>19</sup> Ndt80 target promoters were identified in a large-scale *in vivo* protein-DNA binding assay of samples from sporulating cells.<sup>20</sup> This experiment, together with position weight matrices (PWMs), which represent patterns such as transcription factor target motifs in DNA sequences, identified genes that are likely regulated by Ndt80.<sup>21,22</sup>

In this study we report that cells switch to a long *ORC1* transcript isoform containing an extended 5'-UTR (*mORC1*) prior to entry into meiotic M-phase, while starvation alone fails to induce this transcript. Importantly, we show that in meiosis Ndt80 directly activates *mORC1* together with the divergently expressed *SMA2* locus *via* its bi-directional MSE target motif, while Sum1 acts as a mitotic repressor for both transcripts. Finally, we demonstrate that Orc1 protein becomes undetectable when cells finish pre-meiotic DNA replication and start expressing *mORC1*, while Orc1 remains detectable in an MSE deletion mutant that fails to induce the long

isoform. These findings agree with large-scale ribosome profiling data.<sup>13</sup> Our data suggest a novel role for the Ndt80 activator in yeast meiosis, which is to down-regulate Orc1 protein via induction of an untranslatable transcript isoform. The results therefore highlight an interesting regulatory design that enables an activator to repress a target gene product during eukaryotic cell differentiation

## Results

### *Datasets and experimental rationale*

In earlier work, we used tiling arrays to determine the transcriptome of diploid budding yeast during fermentation, respiration and sporulation in comparison to vegetative growth of haploid cells.<sup>9,23,24</sup> Initially, we focussed on meiotic lncRNAs and later on developmentally regulated transcript isoforms with extended 5'-UTRs. Published tiling array data are available at the ReproGenomics Viewer (RGV, [rgv.genouest.org](http://rgv.genouest.org); Figure S1<sup>25</sup>) and the Saccharomyces Genomics Viewer (SGV, [sgv.genouest.org](http://sgv.genouest.org)<sup>26</sup>). Furthermore, we interpreted DNA strand-specific RNA-Sequencing data from mitotically growing haploid and diploid wild type versus *xrn1* mutant cells in S288C, W303 and SK1 strain backgrounds,<sup>5</sup> and our unpublished RNA-Sequencing data (not DNA strand-specific) from *MATa/α* and *MATα/α* cells cultured in YPD, YPA and SPII media (E. Becker, M. H. Guilleux, K. Waern, M. Snyder and M. Primig et al., in preparation).

The 5'-UTR expression analysis by Lardenois, Liu *et al.* included a non-exhaustive list of early, middle and late transcript isoforms, which lacked the meiotic isoform *mORC1* because the segmentation algorithm used to analyse tiling array data failed to detect it.<sup>9,23</sup> The *ORC1* locus is, however, an interesting case: its mRNA is cell cycle regulated in mitotically growing cells and

strongly induced during meiotic development, although the the protein it encodes is *a priori* dispensable after pre-meiotic DNA replication is finished.

*Diploid yeast cells express divergent ORC1/XUT1538 transcripts in mitosis and mORC1/SMA2 only in meiosis but not starvation*

Diploid cells growing asynchronously in the presence of glucose (YPD) or acetate (YPA) and synchronized haploid cells undergoing a full mitotic cell cycle express only the mitotic *ORC1* transcript isoform (to which we also refer as the short isoform; Figure 1A), while the 5'-extended *mORC1* isoform is undetectable. We also observed a faint signal corresponding to what appeared to be an lncRNA divergently expressed from the *ORC1* promoter. In fact, this RNA turned out to be the Xrn1-sensitive unstable transcript *XUT1538*.<sup>5</sup> We note that the activity of Xrn1 is strong in S288C and W303 but attenuated in SK1 (Figure 1B). This indicates that the *ORC1* promoter is bidirectional during vegetative growth.

*MATa/α* cells cultured in sporulation medium (SPII) induce *mORC1* when they exit pre-meiotic DNA replication and enter M-phase. This coincides with the transcriptional onset of divergently expressed *SMA2*, which overlaps the constitutively expressed antisense lncRNA *SUT292* (Figure 1C). A Northern blot by Brar et al. 2012 suggests that SK1 cells exclusively express the short *ORC1* transcript isoform during vegetative growth and pre-meiotic DNA replication when Orc1 is needed. Critically, at the onset of meiotic M-phase approximately six hours after transfer into sporulation medium, cells completely switch to expressing the long transcript isoform. Note that the meiotic isoform has the size predicted for a full-length *ORC1* transcript with a 5'-extended UTR (see Figure 5C in reference<sup>13</sup>). Genomics data thus reveal a complex regulatory pattern

involving five transcripts: haploid and diploid cells undergoing mitotic growth express *SUT292* and divergent *ORC1/XUT1538* transcripts downstream of it, while middle meiotic cells continue to express *SUT292* but co-induce divergent *mORC1/SMA2* transcripts *via* a developmentally regulated bi-directional promoter element (Figure 1D).

We have previously reported that early and middle meiotic isoforms are not induced by starvation alone, since they typically do not accumulate to normal (or even detectable) levels in sporulation-deficient *MAT $\alpha$ / $\alpha$*  control cells.<sup>23</sup> In the case of *ORC1*, tiling array data and RNA-Sequencing data from starving SK1 *MAT $\alpha$ / $\alpha$*  cells cultured in sporulation medium indicate that they do not induce the long isoform. We conclude that *mORC1*'s transcriptional activation or its stability (or both) depend on meiosis (Figure 2A, B).

*Divergent promoters driving isoforms pair them with ubiquitous transcripts or developmentally stage-specific mRNAs*

We find that the expression of *mORC1/SMA2* during gametogenesis is likely not an isolated case. Further examples include *ORC3/SPO75* (which overlaps antisense *MMMI*), *PEX32/POP7* (which overlaps antisense *CUT028*), *PCMI/SOM1* (for which *mSOM1* overlaps antisense *HHY1*) and *IWR1/YDL114W*; see [sgv.genouest.org](http://sgv.genouest.org), [rgv.genouest.org](http://rgv.genouest.org) and the Yeast Promoter Atlas at [ypa.csbb.ntu.edu.tw/](http://ypa.csbb.ntu.edu.tw/).<sup>27</sup> Meiotic *IWR1* (*mIWR1*) is not detectable by tiling arrays in haploid cycling cells (Figure S2A top and bottom panels). The tiling array data indicate the presence of an unknown weakly expressed SUT-type antisense transcript that overlaps *IWR1*; however, the function of this transcript, if it has any at all, is presently unclear. High-throughput data for *mIWR1*, *IWR1* and *YDL114W* obtained with SK1 are reproduced by RT-PCR assays using



samples from the distantly related strain JHY222 (which is derived from the standard background S288C<sup>9,28</sup>), indicating that the phenomenon is not strain-specific but generally occurs in budding yeast (Figure S2B). We note that *mIWR1* accumulates to lower levels than *YDL114W*, which may reflect distinct RNA synthesis rates or decay rates. These results concur with the finding that yeast promoters are intrinsically bi-directional.<sup>11,12</sup>

*mORC1/SMA2 repression in mitosis requires Sum1 while their full induction in meiosis depends on Ndt80*

A search for regulatory motifs in the *ORC1* promoter region identified an MSE immediately upstream of *mORC1* (Figure 3A, B). Given the base composition of *mORC1*'s MSE it is likely bound by the meiotic activator Ndt80 and the mitotic repressor Sum1, which is consistent with *ORC1* transcript isoform's middle meiosis-specific expression pattern (Figure 3C).<sup>17</sup>

We next sought to prove that the predicted promoter element is indeed biologically active. To this end, we first designed combinations of oligonucleotide primers for RT-PCR assays to validate tiling array data and RNA-Seq data in wild type cells, and to study the expression of mitotic and meiotic isoforms encoded by *ORC1* in the absence of the *Ndt80* activator and *Sum1* repressor. None of the gene deletions affected the mitotic isoform in JHY222 cells cultured in rich media (YPD, YPA) and sporulation medium (SPII) at bi-hourly time points (2h-10h) (Figure 4A). To the contrary, we found that *mORC1* was moderately de-repressed in *sum1* cells cultured in rich medium (YPD) and sporulation medium (SPII), while it was nearly undetectable in *ndt80* mutant cells cultured in rich media and sporulation medium under the conditions used (Figure 4A). We next assayed the divergently transcribed *SMA2* gene and found a broadly similar

induction pattern in JHY222 wild type cells as compared to tiling array data obtained in the SK1 background (Figure 4B). As expected, *SMA2* mRNA did not accumulate to normal meiotic levels in the absence of *NDT80* and was elevated in *sum1* mutant cells cultured in growth, pre-sporulation, and sporulation media (Figure 4B). These results are consistent with a role for Ndt80 and Sum1 in the regulation of *mORC1*. The observed lack of *mORC1* induction in *ndt80* mutant cells could, however, be an indirect effect because *ndt80* cells arrest during pachytene stage of meiotic prophase I, which might impair the transcription of the long *ORC1* isoform.<sup>14</sup>

#### *Ndt80 and Sum1 directly act on mORC1/SMA2 via an MSE element*

The results described above complement earlier work where we predicted an MSE in the intergenic region of *ORC1* and *SMA2*, which was reported to be bound by Ndt80 *in vivo*.<sup>9,20</sup> The combined results are consistent with -- but do not prove -- a direct role for Ndt80/MSE. To provide unambiguous evidence for a novel function of Ndt80 in activating a meiotic *ORC1* transcript isoform, we deleted the MSE (in a congenic strain background for technical reasons related to selectable marker genes; Figure 5A) and found that *mORC1* indeed failed to be induced in middle meiosis, while the mutation did not alter the mitotic isoform's expression level (Figure 5B). Consistently, *SMA2* mRNA also failed to be meiotically induced in the absence of a functional MSE in the gene's promoter region. (Figure 5C). We note that a low level of MSE-independent *SMA2* expression appear to be mediated by at least one other promoter element. This is, however, likely insufficient for Sma2 function since the *ORC1* MSE deletion strain displays a sporulation phenotype similar to the one previously reported for the *sma2* mutant: cells progress through the meiotic divisions but mostly fail to form asci because the nuclei are

not properly packaged (Figure 6A, B).<sup>8,10</sup> The simplest explanation is that Sum1 contributes to the repression of *mORC1* and *SMA2* during mitotic growth, while Ndt80 activates the transcripts from middle meiosis onwards by directly interacting with a bi-directional MSE present in the *ORC1* promoter.

#### *mORC1* expression and *Orc1* protein levels are negatively correlated

Our findings, together with the prediction by Brar et al., that the long isoform of *ORC1* may inhibit protein translation, raises the interesting possibility that Ndt80 represses *Orc1* after pre-meiotic DNA replication by activating a transcript isoform that sequesters ribosomes at its 5' end via uORFs; (reference<sup>13</sup>; Figure S3). The extended *ORC1* 5'-UTR contains two such uORFs encoding proteins of 113 and 64 amino acids, respectively, that are in frame with the main ORF (Figure 7A). We reasoned that the induction of *mORC1* should correlate with declining *Orc1* protein levels as cells enter meiotic M-phase and found this indeed to be the case. Importantly, we detected the *Orc1* protein during and after M-phase in the *ORC1*<sup>MSE $\square$</sup>  deletion strain that cannot induce *mORC1* (Figure 7B, C; Figure S4). These findings are consistent with the regulatory design proposed in Figure 8: *mORC1* and *SMA2* are repressed in mitosis by the Sum1 complex and activated in meiosis by Ndt80, which enables *Sma2* but not *Orc1* protein to accumulate when cells exit meiosis and enter gamete formation.

## **Discussion**

The yeast meiotic transcriptome comprises classical early, middle and late mRNAs, meiotic transcript isoforms that possess either 5'- or 3'-extended UTRs, and lncRNAs.<sup>6,7,9,23,29,30</sup> These findings raise the question if the transcriptional regulatory network, which controls

developmental stage-specific mRNAs, also contributes to the regulation of meiotically induced mRNA isoforms and lncRNAs. In this report, we begin to unravel the regulatory mechanism controlling the meiotic isoform of *ORC1*, which is co-induced with divergent *SMA2* when diploid cells undergo meiosis and gametogenesis. We also present evidence supporting the conceptually new model that the activator Ndt80 negatively regulates post-meiotic Orc1 protein levels by inducing the long isoform of *ORC1*, which inhibits translation via an extended 5'UTR.

*The ORC1 promoter drives divergent mRNA/lncRNA expression in mitotically growing cells*

It is unclear what role, if any, the divergent lncRNA in the *ORC1* locus might play during growth and development. It is perhaps noteworthy that the transcript, although annotated as *XUT1538*,<sup>5</sup> also shows features typical for two other types of lncRNAs, since it is detectable in wild type cells (SUTs<sup>12</sup>) and it accumulates in the absence of Rrp6 (CUTs<sup>31</sup>). Given the considerable overlap between these transcript classes, especially in the cases of SUTs and XUTs, more work is needed to understand the molecular mechanisms governing their variable synthesis and decay rates.

*Establishing developmental stage-specific middle meiotic isoform expression*

Contrary to early meiotic transcript isoforms present in mitotic *ume6* cells,<sup>23</sup> one would not expect middle meiotic transcript isoforms such as *mORC1* to strongly accumulate in a fermenting *sum1* mutant because their activator Ndt80 is undetectable in cells cultured in rich medium. Indeed, we find that *mORC1* is weakly de-repressed in fermenting JHY222 *sum1* cells and during incubation in sporulation medium. For *SMA2* the level of mitotic accumulation in *sum1* cells is elevated as compared to *mORC1*, which might be due to distinct RNA half-lives.

Taken together, our results are consistent with a role for Sum1 in repressing *mORC1* and *SMA2* during mitotic growth *via* the putative target sequence within the *ORC1*<sup>MSE</sup> (see Figure 3C and Figure 8). In addition, it is conceivable that *SMA2* expression is partially inhibited during mitosis by *SUT292* and *XUT1538* *via* well-established antisense- and promoter interference mechanisms<sup>32,33</sup>; for review see reference<sup>34</sup>.

*A new role for Ndt80 in the activation of a meiotic transcript isoform that inhibits translation*

One might expect *ORC1* to be transcriptionally repressed when cells exit pre-meiotic DNA replication, because there is no further need for assembling an origin recognition complex at autonomously replicating sequence (ARS) elements. Yet, earlier work with microarrays containing probes for the 3'-regions of ORFs shows that *ORC1* gene expression strongly increases as cells progress through meiotic development.<sup>6,7</sup> Recent studies using tiling arrays and RNA-Sequencing helped explain this puzzling fact: cells induce a long transcript isoform with an extended 5'-UTR proposed to inhibit Orc1 translation.<sup>13,29</sup> However, neither microarrays nor RNA-Seq experiments unambiguously show that the 5'-extended isoform is synthesized through to the same transcription termination site (TTS) as the short isoform. We propose that data in previously published work and this study are consistent with the notion that both isoforms use a common TTS as the model in Figure 8 implies.<sup>13,23</sup>

A key question that we sought to answer is which regulator activates *mORC1* and *SMA2* at the onset of meiotic M-phase. The presence of an MSE prompted us to assay *mORC1* induction in an *ndt80* mutant strain and we found that the long transcript isoform does not accumulate to normal levels in the absence of Ndt80. In spite of the predicted MSE's presence in the promoter, this

effect could still be indirect because *ndt80* mutant cells arrest at the pachytene checkpoint prior to entry into M-phase and therefore simply might be unable to induce *mORC1*. Two lines of evidence argue against this interpretation and in favour of our model (Figure 8). First, a high-throughput protein-DNA binding assay based on chromatin immunoprecipitation and microarrays (ChIP-Chip) showed that Ndt80 binds the *ORC1* upstream region *in vivo*.<sup>20</sup> Second, deleting the MSE in the *ORC1* promoter prevents normal induction of the meiotic *ORC1* isoform and strongly reduces *SMA2* expression during sporulation. We currently do not know why we detect low levels of *SMA2* in the MSE mutant strain. Another weak promoter element might mediate basal expression or the mRNA might be unusually stable in meiotic cells.

An intriguing aspect of the model in Figure 8 is that Ndt80 could potentially drive bi-directional transcription of mRNA/isoform pairs *via* MSEs that both have a biological function. Such a novel role for Ndt80 is consistent with earlier reports suggesting that yeast promoters typically mediate bi-directional transcription.<sup>11,12</sup> Our findings raise the possibility that promoters driving the expression of divergent transcript may have brought about an evolutionary advantage: cells need to induce *SMA2* given its important role in sporulation,<sup>8,10</sup> while *ORC1* is not involved in late meiotic processes. Therefore, the induction of an extended isoform, which inhibits Orc1 translation *via* uORFs, represents an elegant solution for down-regulating a protein without the need for repressing the promoter. In addition, we speculate that this mechanism, which keeps the *ORC1* promoter chromatin in an open configuration during the entire process of gametogenesis, may also allow for rapid induction of *ORC1* during spore germination and initiation of the first round of mitosis.

*ORC1* is a model locus suitable to study the regulation of 5'-extended developmental stage specific transcripts and their role in controlling protein levels when cells switch from growth to development. Our findings extend the known roles of Ndt80/Sum1 to the transcriptional control of middle meiotic transcript isoforms. Bearing in mind that the DNA binding fold of Ndt80 was suggested to be evolutionarily linked to the major tumour suppressor TP53,<sup>35</sup> our results are potentially relevant for transcriptional mechanisms implicated in development and disease in humans.

## **Experimental procedures**

### *Yeast strains*

The tiling array data were produced with wild type SK1 *MATa/α* and sporulation deficient *MATa/α* control strains. RT-PCR assays were done with samples from SK1 *MATa/α* and JHY222 *MATa/α* as published.<sup>23</sup> The expression of the long *ORC1* isoform was analysed in JHY222 *MATa/α ndt80* and *sum1* homozygous deletion strains and JHY338 *MATa/α ORC1*<sup>MSE</sup> (Table 1). Yeast strains were cultured at 30°C in standard rich medium with glucose (YPD) or acetate (YPA) and sporulation medium (SPII).

### *Yeast Sc<sub>1lg</sub> tiling array data and RNA-Sequencing data*

In this study, we employed unpublished non-DNA strand-specific RNA-Sequencing data that were produced using the Illumina GAII system. Duplicate samples from wild type SK1 *MATa/α* and meiosis-deficient *MATa/α* control cells were cultured in rich medium (YPD), pre-sporulation medium (YPA), and sporulation medium (SPII, 4h, 6h, 8h; Becker et al., in

preparation). Furthermore, we interpreted published Yeast Sc\_tlg GeneChip expression data from duplicate samples of asynchronously growing SK1 *MATa/α* cells cultured in rich medium (YPD) or pre-sporulation medium (YPA), and differentiating cells cultured in sporulation medium (SPH). In addition, dividing and starving meiosis-deficient *MATα/α* cells were used as a control.<sup>9</sup> SK1 is a strain background commonly employed in genetic and genomic analyses of meiosis because of its efficient sporulation properties.<sup>7</sup> Published Sc\_tlg GeneChip data from single samples of synchronized cells undergoing mitotic growth and division were obtained with the W101 *MATa* strain.<sup>24</sup> Mitotic gene expression is typically studied in haploid cells because of well-established cell synchronization protocols.<sup>36,37</sup> Graphical displays of tiling array data are available online at SGV (Saccharomyces Genome Viewer, [sgv.genouest.org](http://sgv.genouest.org)<sup>26</sup>) and RGV (ReproGenomics Viewer, [rgv.genouest.org](http://rgv.genouest.org)<sup>25</sup>). A DNA strand-specific RNA-Seq dataset was used to interpret the transcriptomes of asynchronously growing haploid wild type and *xrn1* temperature sensitive mutants in the S288C, W303, and SK1 backgrounds, and diploid SK1 wild type and *xrn1* mutant cells.<sup>5</sup>

#### *RT-PCR assays*

Total RNA was isolated using the hot phenol method as described.<sup>7</sup> Briefly, cell pellets were treated with hot phenol (65°C) and phenol/chloroform (1:1). Total RNA was precipitated overnight with two volumes of 100% ethanol and 0.1 volume of 3 M NaOAc (pH 5) at -80°C. The RNA was digested with 2 units of DNaseI for 30 min at 37°C, and then 2 μg of RNA was reverse transcribed into cDNA using reverse transcriptase and random primers supplied in the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). 1 μl of cDNA was



amplified for 28 cycles (denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min) using Taq DNA Polymerase (Qiagen). PCR products were run on a 2% agarose gel in 1×TAE buffer containing GelRed DNA dye (Biotium) and photographed using the Gel Doc XR+ imaging system (Bio-Rad).

#### *Prediction of MSEs*

We screened a 2 kb region upstream of the annotated *ORC1* locus (Chr13:14210-142210) using the *Match* tool of the TRANSFAC professional database.<sup>38</sup> We employed the MSE motif M01515 with cut-off scores minimizing false positives. The logo was produced with the R package *seqLogo*.<sup>39,40</sup> A single MSE motif was predicted with a core score of 1.00 and a matrix score of 0.949.

#### *MSE deletion*

The predicted Ndt80 target site MSE in the *ORC1* promoter region was deleted using the 50:50 genome editing method as recently described.<sup>41,42</sup> Genomic PCR was used to screen deletion strains for successful integration/excision events. For this study we analysed two independent isolates that were verified by DNA sequencing. We note that the construction of this strain required the *ura3Δ0* auxotrophic marker present only in the JHY338 background, which is derived from prototrophic JHY222. Oligonucleotide sequences are given in Table 3.

#### *Sporulation landmarks*

Diploid cells were cultured in growth medium, presporulation medium and sporulation medium, harvested, and fixed in ethanol as described.<sup>7</sup> The percentage of bi-, and tetranuclear cells and asci was determined using a standard manual cell counter.

#### *Light- and fluorescence microscopy*

Yeast cells were stained with DAPI (Interchim) at 5 µg/ml and inspected using a Zeiss AxioImager fluorescence microscope (Zeiss). Pictures were taken with an AxioCam camera using default settings of AxioVision software (Zeiss).

#### *Protein analysis*

Protein extracts were prepared and analysed by Western blotting as published.<sup>9</sup> Briefly, 35 µg of a total protein extract was loaded on a 4-20% SDS-PAGE gradient gel, and run first at 60V for 30 minutes and then at 120 V for one hour. Proteins were transferred onto a PVDF membrane (Millipore) at 60mA for 2.5 hours using a semi-dry electroblotter (Hoefer). The membrane was blocked in 5% milk (Regilait) for one hour at room temperature, and incubated over night at 4°C on a shaker with the primary polyclonal anti-Orc1 antibody (Santa Cruz) at a dilution of 1:200. A monoclonal antibody against Pgk1 (Invitrogen) was used at 1:15'000. Secondary anti-goat and anti-mouse antibodies (ThermoScientific) diluted at 1:30'000 or 1:5000, respectively, were incubated at room temperature for one hour, before the signal was revealed using an ECL kit (General Electric) and the ChemiDoc XRS imaging system (Bio-Rad). Band intensities were quantified using Quantity One 1-D analysis software (Bio-Rad).

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**Table 1. Yeast strains.**

<b>Strain ID</b>	<b>Background and genotype</b>	<b>Reference</b>
MPY1	JHY222 <i>MATa/MATα</i> <i>HAP1/HAP1</i> <i>MKT1(D30G)/MKT1(D30G)</i> <i>RME1(INS</i> <i>308A)/RME1(INS</i> <i>308A)</i> <i>TAO3(E1493Q)/TAO3(E1493Q)</i>	<sup>9</sup>
MPY392	JHY222 <i>MATa/MATα</i> <i>HAP1/HAP1</i> <i>MKT1(D30G)/MKT1(D30G)</i> <i>RME1(INS</i> <i>308A)/RME1(INS</i> <i>308A)</i> <i>TAO3(E1493Q)/TAO3(E1493Q)</i> <i>rrp6::kanMX4/rrp6::kanMX4</i>	
NKY155 1	SK1 <i>MATa/MATα</i> <i>ho::LYS2/ho::LYS2</i> <i>ura3/ura3</i> <i>lys2/lys2</i> <i>leu2::hisG/leu2::hisG</i> <i>arg4-Nsp/arg4-Bgl</i> <i>his4x::LEU2-URA3/his4B::LEU2</i>	<sup>7</sup>
NKY471	SK1 <i>MATα/MATα</i> <i>ho::LYS2/ho::LYS2</i> <i>ura3/ura3</i> <i>lys2/lys2</i>	
MPY454	W101 <i>MATa</i> <i>ho::lys5 gal2</i>	<sup>24</sup>
MPY631	JHY222 <i>MATa/MATα</i> <i>HAP1/HAP1</i>	This study

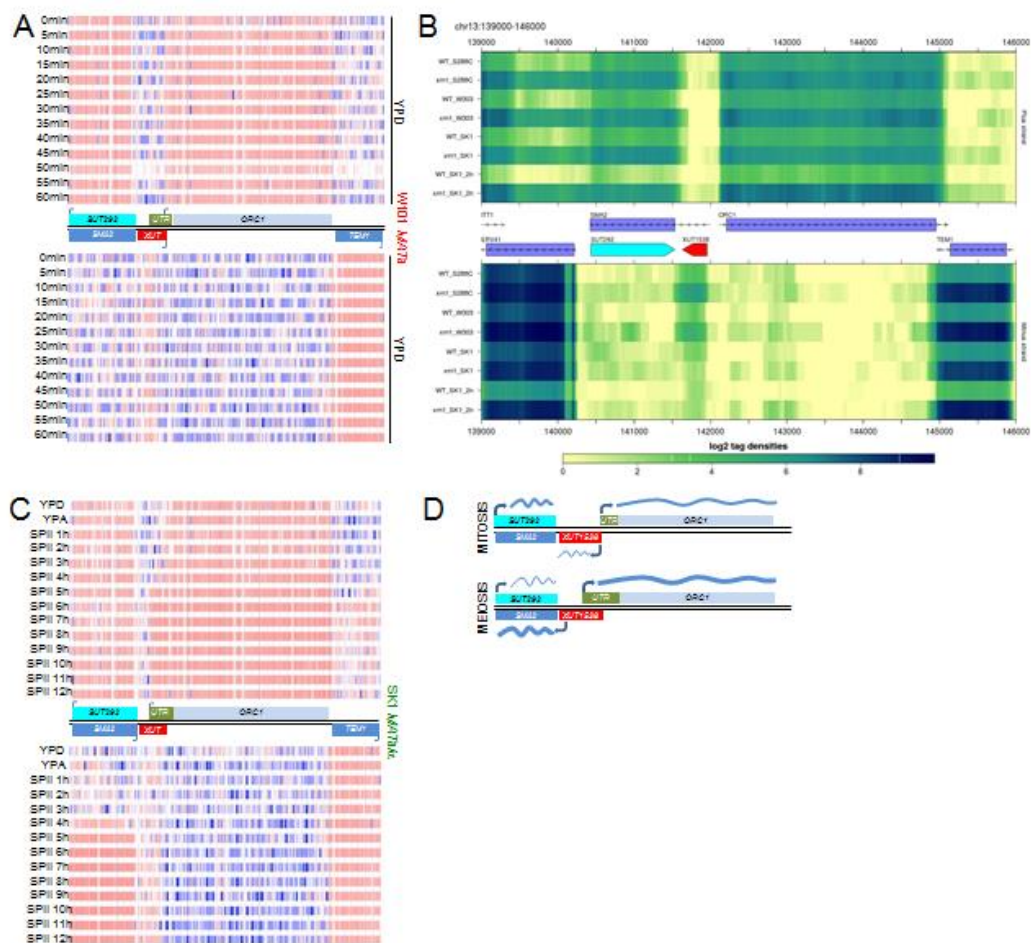
	<i>MKT1(D30G)/MKT1(D30G)</i>	<i>RME1(INS</i>	
	<i>308A)/RME1(INS</i>	<i>308A)</i>	
	<i>TAO3(E1493Q)/TAO3(E1493Q)</i>		
	<i>sum1::kanMX4/sum1::kanMX4</i>		
MPY553	JHY222 <i>MATa/MATα</i>	<i>HAP1/HAP1</i>	This study
	<i>MKT1(D30G)/MKT1(D30G)</i>	<i>RME1(INS</i>	
	<i>308A)/RME1(INS</i>	<i>308A)</i>	
	<i>TAO3(E1493Q)/TAO3(E1493Q)</i>		
	<i>ndt80::kanMX4/ndt80::kanMX4</i>		
MPY742	JHY338 <i>MATa/MATα</i> <i>ura3/ura3</i> <i>leu2/leu2</i> <i>lys2/+</i>		This study
	<i>his3/+</i>		
MPY794	JHY338 <i>MATa/MATα</i> <i>ura3/ura3</i> <i>leu2/leu2</i> <i>lys2/+</i>		This study
	<i>his3/+ mseΔ<sup>ORC1</sup>/mseΔ<sup>ORC1</sup></i>		

**Table 2. Oligonucleotides for RT-PCR assay.**

<b>Target genes</b>	<b>Forward primer</b>	<b>Reverse primer</b>	<b>Size (bp)</b>
<i>ORC1</i>	5'- TCGATGGAGGTCAGAAGAG A-3	5'-TTCGGCTAATTCTGCAGTGA- 3'	353
<i>mORC1</i>	5'- AGGACTGCTATGGGGCATT -3'	5'-TTCGGCTAATTCTGCAGTGA- 3'	789
<i>SMA2</i>	5'- CGTCTGATTGTGTGGGGTGT- 3'	5'-GGGCATTTCCTGTGTGCTTG- 3'	538

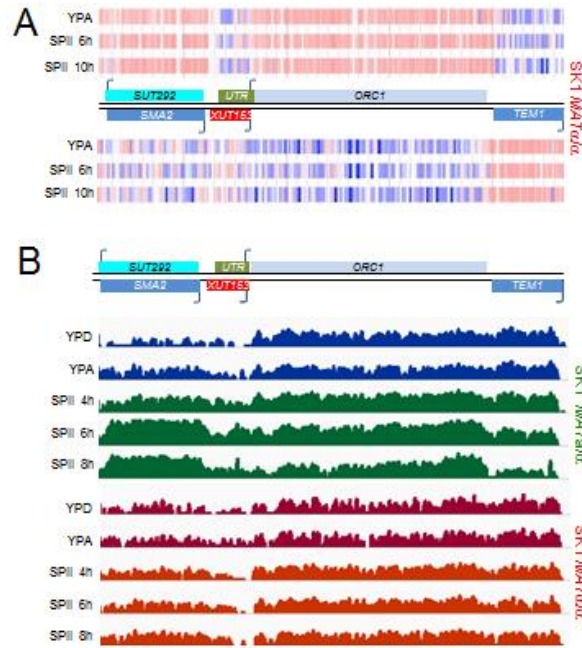
**Table 3. Oligonucleotides to construct and validate MSE deletion.**

<b>Purpose</b>	<b><i>ORC1</i> 50:50-U2</b>	<b><i>ORC1</i> D2-50</b>
MSE deletion	<p>5'-</p> <p>TAAGTGCATGGTATGGAGTG</p> <p>TATAATGGTTTATAATTTCC</p> <p>CCTAAGATAAATGTTCTCCC</p> <p>AAAAATTTACCAAGAAAAA</p> <p>AAATTAAGAATACTACACAC</p> <p>GTACGCTGCAGGTCGAC-3'</p>	<p>5'-</p> <p>TGTGTAGTATTCTTAATTTT</p> <p>TTTTCTTGGTAAATTTTGG</p> <p>GAGAACATTTATCGATGAA</p> <p>TTCGAGCTCG-3'</p>
	<b>Forward primer</b>	<b>Reverse primer</b>
Validation of MSEΔ <sup><i>ORC1</i></sup>	<p>5'-</p> <p>TGGTATGGAGTGTATAATGG</p> <p>-3'</p>	<p>5'-</p> <p>CCCAAGCATCAATTGTGTA</p> <p>G-3'</p>



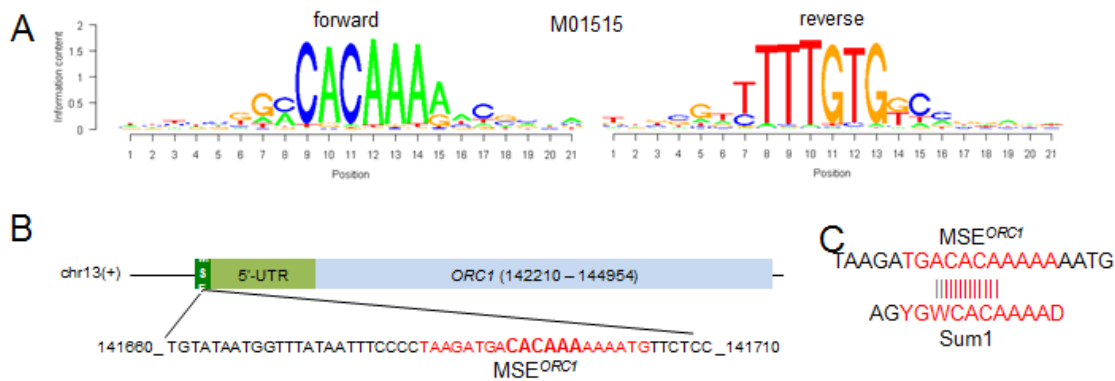
**Figure 1. *ORC1* isoform expression during growth and differentiation.** (A) Color-coded heatmaps generated with RGV version 1.0, show DNA strand-specific *Sc\_tlg* tiling array expression data ordered in rows for samples and columns for each oligonucleotide probe (blue is low, red is high; bicolor pivot 3.9 on the log scale). The strain background is shown to the right in red, time points are given in minutes to the left. A schematic represents the loci (shades of blue for ORFs and SUT, green for the UTR, and red for XUT) on both DNA strands (black lines). Arrows indicate transcription start sites. Note that the data shown, which cover one mitotic cycle, are part of a larger experiment reported in reference<sup>24</sup>. (B) A heatmap shows RNA-

Sequencing data for three wild type strains (WT S288C, W303 and SK1) and corresponding strains lacking Xrn1 activity (*xrn1*) given to the left as in panel B. All strains are haploid unless their DNA content is indicated (2n). Cells were cultured in YPD. The complete dataset was reported in reference<sup>5</sup>. (C) A heatmap like in panel A shows samples from diploid wild type cells cultured in rich media (YPD, YPA) and sporulation medium (SPII) taken at the time points indicated in hours (h). The strain is indicated to the right in green. Genome-wide data are from reference<sup>9</sup>. (D) A schematic summarizes the mitotic (top) and meiotic (bottom) expression profiles of *SMA2* and *ORC1* (dark and light blue rectangles, respectively) and the lncRNAs *SUT292* and *XUT1538* (blue and red rectangles). Transcripts are shown as wavy blue lines. Black lines represent the top and bottom DNA strands. Arrows indicate transcription start sites.

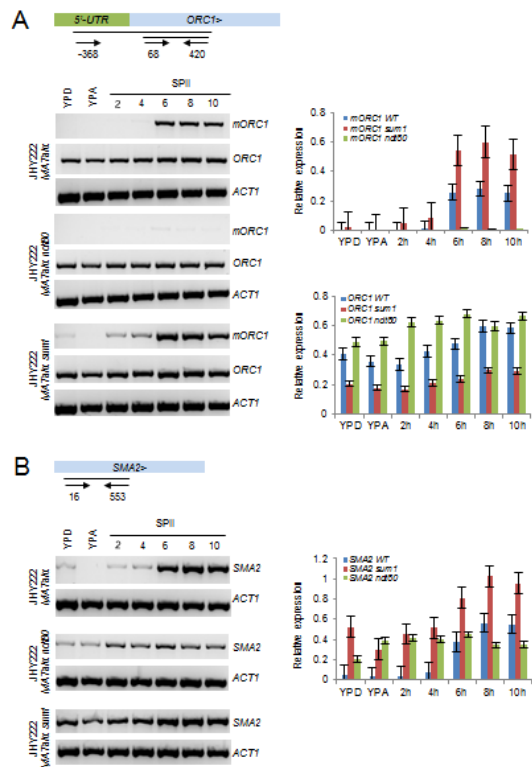


**Figure 2. *mORC1* induction requires sporulation.** (A) Tiling array data for *ORC1* are shown as in Figure 1 for sporulation deficient SK1 *MATα/α* cells cultured in pre-sporulation medium (YPA) and sporulation medium (SPII, 4, 6, 8h). (B) A schematic shows the region containing the *ORC1* locus as in Figure 1. RNA-Sequencing data (not DNA strand-specific) are given as a color-coded histogram (IGV version 2.3.40 set at log scale data range min 0 and max 800) for cells cultured rich media in blue (YPD, YPA) and for sporulation medium in green (SPII) as shown to the left. The wild type (SK1 *MATa/a* in green) and sporulation deficient control strains (SK1 *MATα/α* in red) are indicated to the right.





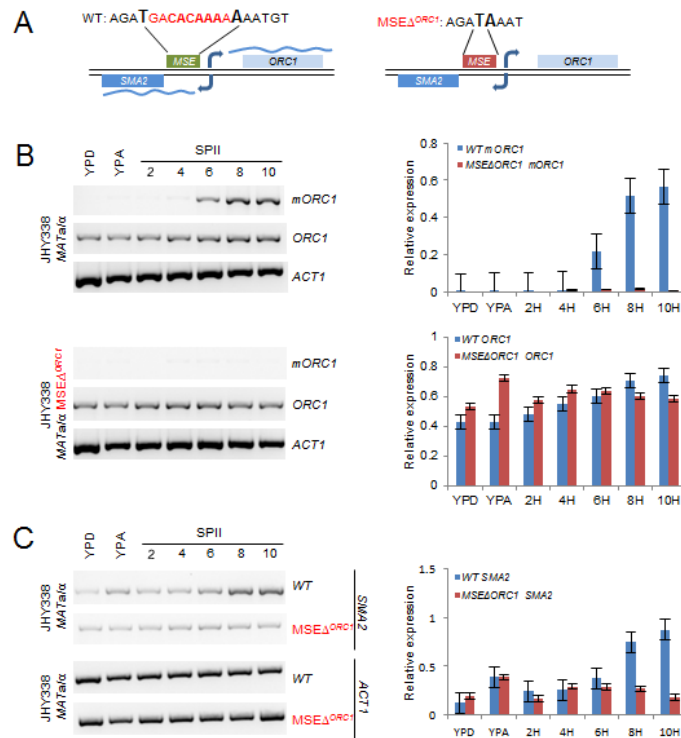
**Figure 3. MSE prediction.** (A) Logos of the predicted MSE (M01515) are shown as graphs plotting information content (y-axis) versus position for each base in the sequence for forward (left) and reverse (right) DNA strands. (B) A schematic represents the MSE in dark green, the 5'-UTR in light green and *ORC1* in light blue. A black line represents the top DNA strand (+). The chromosome number is indicated. The base coordinates and the base composition of the 5'-*mORC1* region, which contains a predicted MSE (bases are shown in red with the core bases enlarged and in bold), are shown at the bottom. (C) The predicted *ORC1* MSE is aligned with the Sum1 target motif; a vertical line indicates base matches and similarities. Bases in the core sequence are given in red.



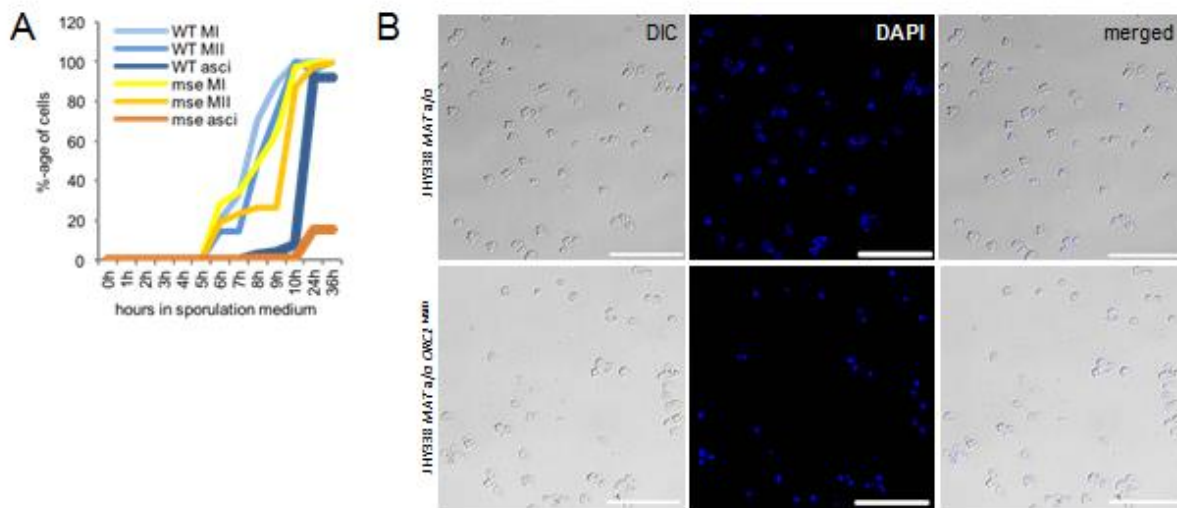
**Figure 4. Ndt80-dependent *mORC1/SMA2* expression.** (A) A schematic shows the *ORC1* 5'-UTR in green and the ORF in light blue; > indicates the transcriptional direction. Small arrows symbolize oligonucleotide primers and black lines represent PCR products. Their coordinates with respect to the first base in the ATG start codon are given. The output of RT-PCR assays is shown for *ORC1* isoforms (*mORC1*, *ORC1*) and *ACT1*. The wild type, *ndt80* and *sum1* strain backgrounds are shown to the left. Cells were harvested in rich media (YPD, YPA) and sporulation medium (SPII) at the bi-hourly time points indicated at the top. Two bar graphs show quantified signals from RT-PCR assays in panel A for *mORC1* (top) and *ORC1* (bottom) for the wild type (blue), *sum1* (red) and *ndt80* (green) strains given in the legends. Relative expression levels (y-axis) are plotted against samples (x-axis) as shown. Bars indicate the values obtained in duplicate experiments. (B) A schematic on top shows the *SMA2* locus and the position of

oligonucleotide primers (arrows) beneath a black line indicating the PCR fragment. The output of RT-PCR assays for *SMA2* in wild type, *ndt80* and *sum1* strains is shown and bar diagrams are given as in panel A.

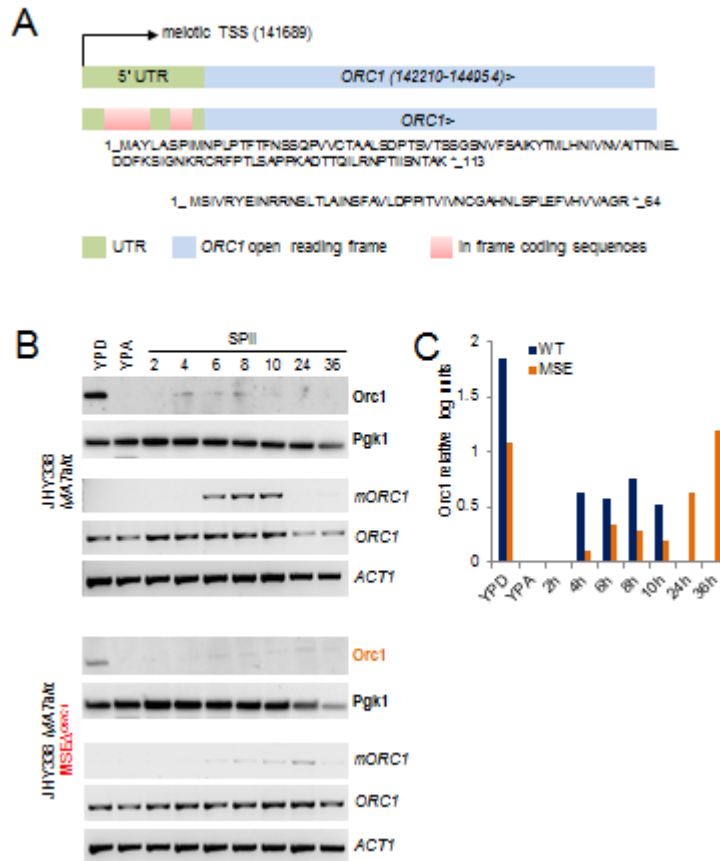
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**Figure 5. Bi-directional MSE-dependent *mORC1/SMA2* expression.** (A) A schematic shows the wild type and MSE mutant sequences upstream of *ORC1*. The deleted sequence is given in red, flanking bases are enlarged and given in bold. The locus is symbolised as in Figure 1C. (B) The output of RT-PCR assays with samples from wild type cells versus cells lacking the MSE upstream of *mORC1* ( $MSE\Delta^{ORC1}$ ) is shown for the *ORC1* isoforms and for *ACT1*. RT-PCR signals are given as bar diagrams as in Figure 5. (C) RT-PCR data are given for *SMA2* and *ACT1* in wild type (WT) and motif deletion strains ( $MSE\Delta^{ORC1}$ ) as in panel B.



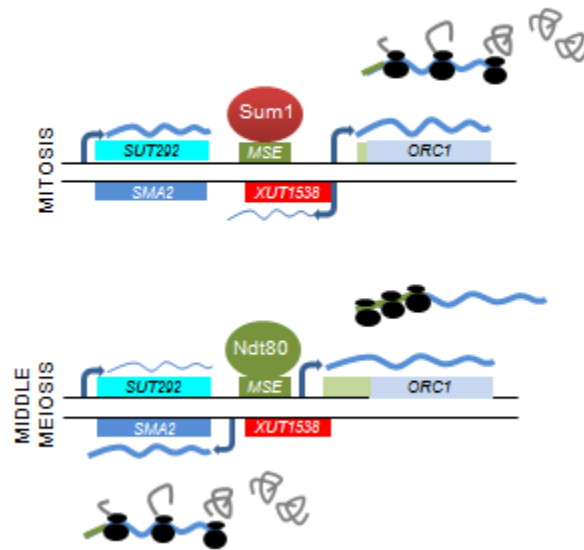
**Figure 6. Phenotypic analysis of the MSE deletion mutant.** (A) A graph shows the percentage of wild type and MSE mutant cells cells (y-axis) at the bi-nuclear (MI), tetra-nuclear (MII) and ascus stage over time in sporulation medium shown in hours (x-axis). (B) Representative images of wild type (top) and MSE deletion (bottom) strains are shown using differential interference contrast (DIC, left), fluorescent staining of DNA (DAPI, middle) or both (merged, right). The strains are given to the left. A bar indicates 50 $\mu$ m.



**Figure 7. *ORC1* RNA versus *Orc1* protein levels.** (A) A schematic shows the *ORC1* locus in blue and the extended 5'UTR in grey at the top. Genome coordinates for the *ORC1* ORF and the meiotic transcription start site (TSS) are given. Two in frame upstream ORFs located in the 5'-UTR are shown in red at the bottom. The amino acid sequences are indicated and an asterisk represents the stop codon. (B) Cells from wild type (JHY388 *MATa/α*) and MSE mutant (JHY338 *MATa/α* MSEΔ<sup>*ORC1*</sup>) strains were cultured in growth media (YPD, YPA) and sporulation medium (SPII) at the time points indicated in hours. As shown to the right, protein samples were analysed for *Orc1*, using *Pgk1* as a loading control. RNA samples were assayed for the long isoform (*mORC1*), and the short isoform (*ORC1*), using *ACT1* as a loading control. (C) A color-

coded graph shows quantified log-transformed units (y-axis) representing Orc1 protein levels in panel B for samples from growing and sporulating cells (x-axis). Samples from wild type (WT) cells are shown in black, those from mutant ( $MSE\Delta^{ORC1}$ ) cells are shown in orange.

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**Figure 8. A model for mORC1/SMA2 induction in meiosis.** A schematic depicts the mitotic (top) and meiotic (bottom) regulation of *ORC1* and *SMA2* shown as light and dark blue rectangles, respectively, by Sum1 (dark red) and Ndt80 (green). Mitotic and meiotic *ORC1* 5'-UTRs are shown in green. *SUT292* and *XUT1538* are given in blue and red, respectively. The MSE is given as a light green rectangle. Transcripts are shown as wavy lines for which the thickness represents the expression level. Black lines represent the top and bottom DNA strands.