LETTER

Proto-genes and *de novo* gene birth

Anne-Ruxandra Carvunis[1,2,3], Thomas Rolland[1,2], Ilan Wapinski[4], Michael A. Calderwood[1,2], Muhammed A. Yildirim[5], Nicolas Simonis[1,2]†, Benoit Charloteaux[1,2,6], César A. Hidalgo[7], Justin Barbette[1,2], Balaji Santhanam[1,2], Gloria A. Brar[8], Jonathan S. Weissman[8], Aviv Regev[9,10], Nicolas Thierry-Mieg[3], Michael E. Cusick[1,2] & Marc Vidal[1,2]

Novel protein-coding genes can arise either through re-organization of pre-existing genes or *de novo*[1,2]. Processes involving re-organization of pre-existing genes, notably after gene duplication, have been extensively described[1,2]. In contrast, *de novo* gene birth remains poorly understood, mainly because translation of sequences devoid of genes, or 'non-genic' sequences, is expected to produce insignificant polypeptides rather than proteins with specific biological functions[1,3–6]. Here we formalize an evolutionary model according to which functional genes evolve *de novo* through transitory proto-genes[4] generated by widespread translational activity in non-genic sequences. Testing this model at the genome scale in *Saccharomyces cerevisiae*, we detect translation of hundreds of short species-specific open reading frames (ORFs) located in non-genic sequences. These translation events seem to provide adaptive potential[7], as suggested by their differential regulation upon stress and by signatures of retention by natural selection. In line with our model, we establish that *S. cerevisiae* ORFs can be placed within an evolutionary continuum ranging from non-genic sequences to genes. We identify ~1,900 candidate proto-genes among *S. cerevisiae* ORFs and find that *de novo* gene birth from such a reservoir may be more prevalent than sporadic gene duplication. Our work illustrates that evolution exploits seemingly dispensable sequences to generate adaptive functional innovation.

Both genome-wide surveys and analyses of individual cases have shown that *de novo* gene birth has occurred throughout the evolution of many lineages, potentially affecting species-specific adaptations and evolutionary radiations[1,2,5,6,8,9]. Genes are thought to emerge *de novo* when non-genic sequences become transcribed, acquire ORFs and the corresponding non-genic transcripts access the translation machinery[1,2,4,5,8]. However, it is hard to reconcile this proposed mechanism with expectations that non-genic sequences should lack translational activity and, even if translated, should encode insignificant polypeptides[1,3,4,6]. Evidence of associations between non-genic transcripts and ribosomes has suggested that non-genic sequences may occasionally be translated, which could provide raw material for natural selection[6]. It has also been speculated that genes that originate *de novo* could initially be simple and gradually become more complex over evolutionary time[4]. These ideas are consistent with reports showing that genes that emerged recently are shorter, less expressed and more rapidly diverging than other genes[1,10–13]. We developed an integrative evolutionary model whereby *de novo* gene birth proceeds through intermediate and reversible proto-gene stages, mirroring the well-described pseudo-gene stages of gene death (Fig. 1a)[14].

We investigated this model at genome scale in the context of *de novo* gene birth in *S. cerevisiae*[8,10]. In *S. cerevisiae*, a minimal length threshold of 300 nucleotides was originally used to delineate ORFs likely to be

genes from non-genic ORFs occurring by chance in non-genic sequences[15]. The resulting gene catalogue has undergone numerous adjustments[16], with currently ~6,000 ORFs annotated as genes and ~261,000 unannotated ORFs containing at least three codons considered to be non-genic ORFs (Supplementary Fig. 1). Non-genic sequences are broadly transcribed in *S. cerevisiae*[17], their overexpression is mostly non-toxic[18], and the corresponding transcripts can associate with ribosomes, often at AUG start codons[6,19]. We reasoned that translation of non-genic ORFs could be more common than expected. Such translation events would not systematically lead to *de novo* gene birth, as the corresponding polypeptides would not necessarily have specific biological functions. Instead, upon translation, non-genic ORFs would become proto-genes (Fig. 1b). Proto-genes would provide adaptive potential[6] by exposing genetic variations that are usually hidden in non-genic sequences. A subset of proto-genes could occasionally be retained over evolutionary time, for instance if providing an advantage to the organism under specific environmental conditions. Retained proto-genes could gradually evolve the characteristics of genes, whereas other proto-genes might lose the ability to be translated. Such a reservoir of proto-genes would allow evolutionary innovations to be attempted without affecting existing genes.

This evolutionary model leads to the following predictions: (1) the structural and functional characteristics of *S. cerevisiae* ORFs (for example, length, expression level or sequence composition) should reflect an evolutionary continuum ranging from non-genic ORFs to genes; (2) many non-genic ORFs should be translated; and (3) ORFs that emerged recently should occasionally have adaptive functions retained by natural selection.

To examine these predictions, we estimated the order of emergence of *S. cerevisiae* ORFs (Fig. 1c). Annotated ORFs were classified into ten groups based on their conservation throughout the Ascomycota phylogeny (Supplementary Fig. 2). Of ~6,000 annotated ORFs, ~2% are found only in *S. cerevisiae* (ORFs[1]) (Supplementary Fig. 2)[10] and ~12% are found only in the four closely related *Saccharomyces sensu stricto* species (ORFs[1–4]). The ~88% of annotated ORFs found outside of this group (ORFs[5–10]) are well characterized and can confidently be considered genes. ORFs[1–4] are poorly characterized and their annotation as genes is debatable (Supplementary Fig. 2)[16,20]. The weak conservation of ORFs[1–4] suggests that they emerged recently, which we corroborated using gene duplication events to control for relative time of emergence (Supplementary Fig. 3). We estimate that over 97% of ORFs[1–4] originated *de novo* rather than by cross-species transfer, which could also explain their weak conservation (Supplementary Information). ORFs[1–4] often partially overlap ORFs[5–10], which seems incompatible with cross-species transfer, or preferentially lie within

[1]Center for Cancer Systems Biology (CCSB) and Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, Massachusetts 02215, USA. [2]Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA. [3]UJF-Grenoble 1/CNRS/TIMC-IMAG UMR 5525, Computational and Mathematical Biology Group, Grenoble F-38041, France. [4]Department of Systems Biology, Harvard Medical School, Boston, Massachusetts 02115, USA. [5]Center for International Development and Harvard University, Cambridge, Massachusetts 02138, USA. [6]Unit of Animal Genomics, GIGA-R and Faculty of Veterinary Medicine, University of Liege, 4000 Liege, Wallonia-Brussels Federation, Belgium. [7]The MIT Media Lab, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142, USA. [8]Howard Hughes Medical Institute, Department of Cellular and Molecular Pharmacology, University of California, San Francisco, and California Institute for Quantitative Biosciences, San Francisco, California 94158, USA. [9]Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, USA. [10]Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA. †Present address: Laboratoire de Bioinformatique des Génomes et des Réseaux (BiGRe), Campus Plaine, Free University of Brussels, 1050 Brussels, Wallonia-Brussels Federation, Belgium.
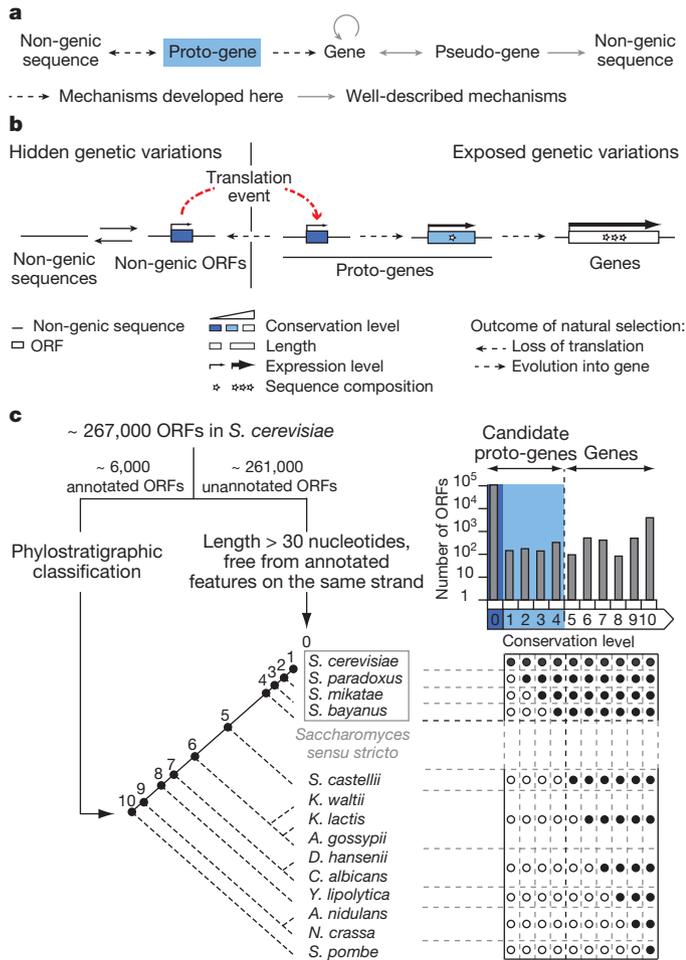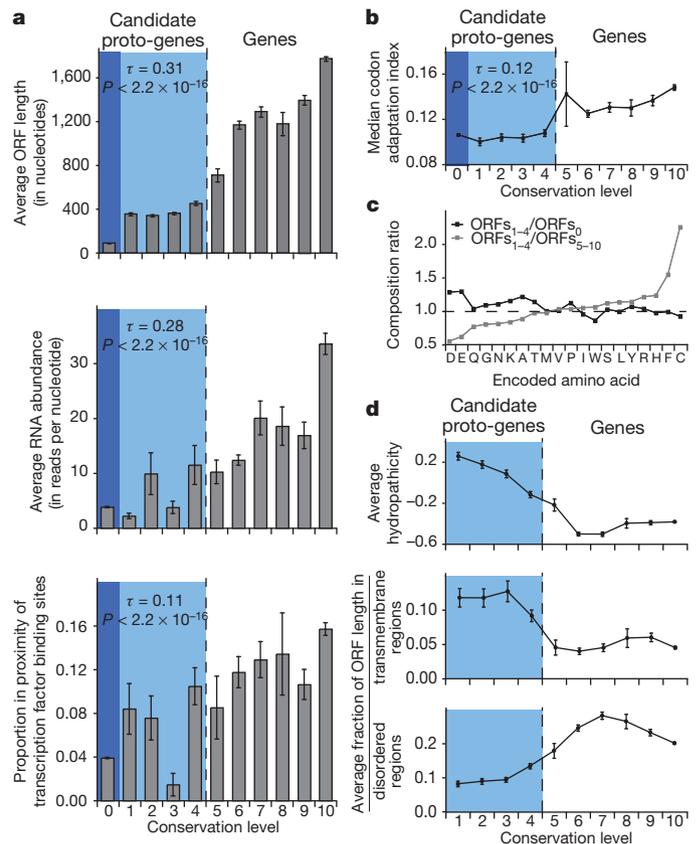
Figure 1 | From non-genic sequences to genes through proto-genes.
a, Proto-genes mirror for gene birth the well-described pseudo-genes for gene death. Circular arrow indicates gene origination from pre-existing genes, such as through gene duplication. Pseudo-genes are highly related to existing genes but have accumulated disabling mutations and translation of functional proteins is no longer possible[14]. The premise that pseudo-gene formation represents irreversible gene death has been challenged by reports of pseudo-gene resurrection[14] (bidirectional arrow). After enough evolutionary time pseudo-gene decay renders them indistinguishable from non-genic sequences (unidirectional arrow). Whereas pseudo-genes resemble known genes, proto-genes resemble no known genes. Proto-genes arise in non-genic sequences and either revert to non-genic sequences or evolve into genes (bidirectional arrow). There can be no reversion of genes to proto-genes (unidirectional arrow) as gene decay engenders pseudo-genes. **b**, Details of the proposed model for the gradual emergence of protein-coding genes in non-genic sequences via proto-genes. Solid arrows indicate the reversible emergence of ORFs in non-genic transcripts, or of transcripts containing non-genic ORFs. Examples where transcript appearance precedes ORF appearance have been described[1,2,8], but the reverse order of events cannot be ruled out. Arrows representing expression level symbolize transcription (hidden genetic variations) or transcription and translation (exposed genetic variation). The variations in width of these arrows reflect changes in expression level resulting, at least in part, from changes in regulatory sequences. Sequence composition refers to codon usage, amino acid abundances and structural features. **c**, Assigning conservation levels to *S. cerevisiae* ORFs. Conservation levels of annotated ORFs were assigned according to comparisons along the reconstructed phylogenetic tree, by inferring their presence (filled circles) or absence (open circles) in the different species according to the phylostratigraphy principle (Supplementary Information)[1]. Top right, number of ORFs assigned to each conservation level (logarithmic scale). *A. gossypii, Ashbya (Eremothecium) gossypii; A. nidulans, Aspergillus nidulans; C. albicans, Candida albicans; D. hansenii, Debaryomyces hansenii; K. lactis, Kluyveromyces lactis; K. waltii, Kluyveromyces (Lachancea) waltii; N. crassa, Neurospora crassa; S. pombe, Schizosaccharomyces pombe.*

subtelomeric regions whose instability may facilitate *de novo* emergence (Supplementary Fig. 4). In addition to classifying ORFs$_{1-10}$, we assigned a conservation level of 0 to ~108,000 unannotated ORFs longer than 30 nucleotides and free from overlap with annotated features on the same strand (ORFs$_0$) (Supplementary Information). ORFs$_0$ and ORFs$_{1-4}$ constituted our initial list of candidate proto-genes.

To test the evolutionary continuum prediction, we first verified that ORF conservation level correlates positively with length and expression level (Fig. 2a and Supplementary Fig. 5)[1,10–12]. These correlations suggest that genes evolve from non-genic ORFs that lengthen and increase in expression level over evolutionary time. A negative correlation between ORF length and expression level[21] was observed among

Figure 2 | Existence of an evolutionary continuum ranging from non-genic ORFs to genes through proto-genes. a, Length (top; error bars represent s.e.m.), RNA expression level (middle; error bars represent s.e.m.), and proximity to transcription factor binding sites (bottom; error bars represent standard error of the proportion) of ORFs correlate with conservation level (Supplementary Table 4). P and τ, Kendall's correlation statistics. Estimation of RNA abundance from RNA-Seq[25] in rich conditions. The positive correlation between proximity to transcription factor binding sites and conservation level is shown for a window of 200 nucleotides and holds when considering windows of 300, 400 and 500 nucleotides (Kendall's $\tau = 0.14$, 0.16, 0.17, respectively; $P < 2.2 \times 10^{-16}$ in each case). **b**, Codon bias increases with conservation level (Supplementary Table 4). Codon bias estimated using the codon adaptation index (Supplementary Information). P and τ, Kendall's correlation statistics. Error bars represent s.e.m. The large s.e.m. observed for ORFs$_5$ may be related to the whole genome duplication event (Supplementary Fig. 3). **c**, Relative amino acid abundances shift with increasing conservation level. For each encoded amino acid, the ratio between its frequency in ORFs$_{1-4}$ and its frequency in ORFs$_{5-10}$ (grey), or the ratio between its frequency in ORFs$_{1-4}$ and its frequency in ORFs$_0$ (black), is plotted. Enrichment of cysteine in proteins encoded by ORFs$_{1-4}$ relative to those encoded by ORFs$_{5-10}$ ($P < 1.8 \times 10^{-150}$, hypergeometric test) corresponds to 3.6 ± 0.1 residues (mean, s.e.m.) per translation product. **d**, Predicted structural features of ORF translation products correlate with conservation level. ORFs$_0$ were not included in these analyses as their short length hinders the reliability of structural predictions. Error bars represent s.e.m.

ORFs$_{5-10}$, but not among ORFs$_{1-4}$ (Supplementary Fig. 5). Thus, some ORFs may increase in expression level at different rates than they increase in length over evolutionary time. Lengthening of ORFs could occur by loss of stop codons, possibly following translational read-through, by shift of start codons or by duplication followed by fusion with other ORFs[10,22]. Increase in ORF expression level could be mediated by recruitment of existing regulatory elements[1]. The proportion of ORFs located in the vicinity of transcription factor binding sites increases with conservation level, suggesting that novel regulatory elements could also emerge (Fig. 2a)[1].

In line with a study of codon evolution in metazoans[23], we observed a positive correlation between codon usage bias and conservation level (Fig. 2b). Relative abundances of amino acids in proteins encoded by ORFs$_{1-4}$ show levels intermediate between those in proteins encoded by ORFs$_{5-10}$ and in hypothetical translation products of ORFs$_0$ (Fig. 2c), similar to observations in bacteria[24]. Probably owing to this biased sequence composition, ORFs$_{1-4}$ exhibit a higher hydropathicity, a higher tendency to form transmembrane regions and a lower propensity for intrinsic structural disorder[10] than ORFs$_{5-10}$ (Fig. 2d). Taken together, our observations support the existence of an evolutionary continuum ranging from non-genic ORFs to genes.

To assess the extent of non-genic translation, we searched for signatures of translation of ORFs$_0$ at genome scale in a ribosome footprinting data set generated in both rich and starvation conditions[25]. In this data set, ~1% of sequencing reads could not be mapped to ORFs$_{1-10}$. We developed a stringent pipeline to detect unequivocal translation signatures for ORFs$_0$ located on transcripts associated with ribosomes (Fig. 3a and Supplementary Fig. 6). We found that 1,139 of ~108,000 ORFs$_0$ show such evidence of translation (ORFs$_0^+$). This number is significantly higher than expected if the ribosome footprinting assay was non-specific, or if the presence of ribosomes on non-genic transcripts was unrelated to the presence of ORFs$_0$ (Fig. 3b). These ORFs$_0^+$ are enriched in adenine at position -3 from the start codon, which probably favours translation initiation (Fig. 3c and Supplementary Information). We verified that ORFs$_0^+$ did not originate from gene duplication or cross-species transfer and are not genes that have failed to be annotated due to their short length (Supplementary Information). The 1,139 ORFs$_0^+$ therefore appear to be translated non-genic ORFs.

We detected strong differential translation of ORFs$_0^+$ and ORFs$_{1-4}$ in starvation or rich conditions, whereas most ORFs$_{5-10}$ are translated in both conditions (Fig. 3d and Supplementary Fig. 6). We found that the binding sites of four transcription factors involved in mating and stress response are preferentially located close to ORFs$_0^+$ and ORFs$_{1-4}$ (Supplementary Table 1) and that ORFs$_{1-4}$ are enriched in the Gene Ontology term "response to stress" (Supplementary Table 2). Recently emerged ORFs may provide adaptive functions in response to environmental stress.
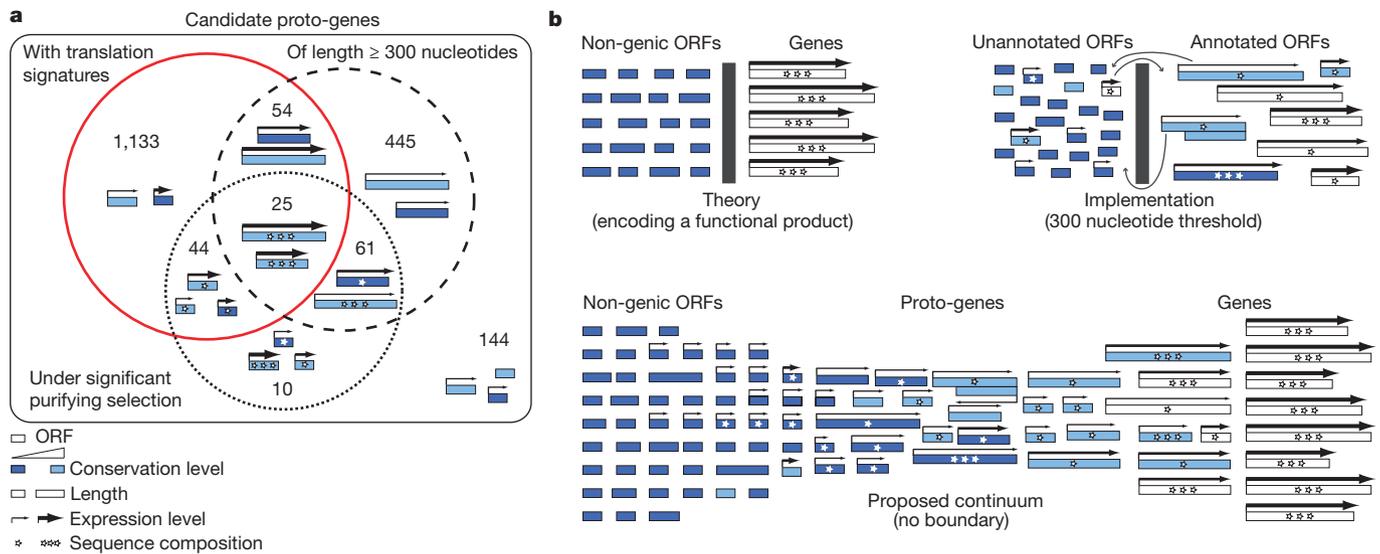
Retention by natural selection was measured by comparing the genome sequences of eight *S. cerevisiae* strains to evaluate the tendency of ORF sequences to be purged of non-synonymous mutations (purifying selection) relative to expectations under neutral evolution. Most ORFs$_0^+$ and ORFs$_{1-4}$ do not exhibit a significant deviation from neutral evolution, yet ~3% of ORFs$_0^+$ and 9–25% of ORFs$_{1-4}$ appear under purifying selection (Fig. 3e). This fraction increases with conservation level, in line with the proposed evolutionary continuum (Supplementary Fig. 7 and Supplementary Information). Our observations suggest that recently emerged ORFs occasionally acquire adaptive functions that are retained by natural selection, in agreement with findings in primates and with evolutionary models derived from inter-species comparisons[12,13,26].

Overall, our results show that *de novo* gene birth could proceed through proto-genes. From the initial comprehensive set of candidate proto-genes (all ORFs$_0$ and ORFs$_{1-4}$), we excluded ORFs$_0$ that seem to lack translation signatures according to our stringent pipeline (Supplementary Fig. 6). The 25 ORFs$_4$ that are longer than 300
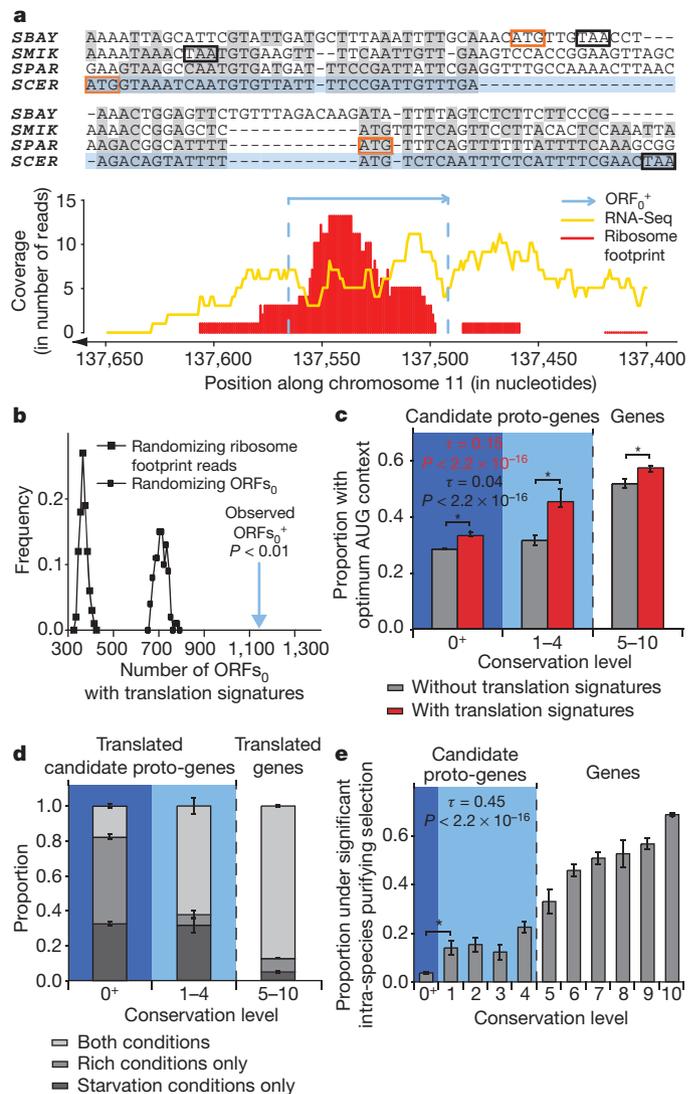
Figure 3 | Translation and adaptive potential of recently emerged ORFs. a, Example of an ORF$_0^+$ showing signatures of translation in starvation conditions. Syntenic regions in *Saccharomyces sensu stricto* species are aligned. Orange and black boxes indicate in-frame start and stop sites, respectively. SCER, *S. cerevisiae*; SPAR, *S. paradoxus*; SMIK, *S. mikatae*; SBAY, *S. bayanus*. **b**, Significance of the observed number of ORFs$_0^+$. Distribution of the number of ORFs$_0$ expected to show signatures of translation if the ribosome footprinting assay were non-specific (as modelled by randomizing footprint reads positions 100 times; squares), or if the presence of ribosomes on non-genic transcripts were not related to the presence of ORFs$_0$ (as modelled by randomizing ORFs$_0$ positions 100 times; circles). *P*, empirical *P* value. **c**, AUG context of ORFs with and without translation signatures. The presence of an adenine at position -3 from the start codon indicates optimum AUG context (Supplementary Information). *P* and τ, Kendall's correlation statistics. Asterisks mark significant differences between ORFs with and without translation signatures (*P* < 0.05, Fisher's exact test). **d**, Candidate proto-genes tend to undergo condition-specific translation. **e**, Signatures of intra-species purifying selection. The positive correlation (Supplementary Table 4) holds when only considering ORFs that are free from overlap with ORFs$_{1-10}$ (Supplementary Fig. 7), and is not entirely driven by the interdependence between strength of purifying selection and expression level (Supplementary Information)[29,30]. Asterisk marks a significant difference in proportion of ORFs under significant intra-species purifying selection between ORFs$_0^+$ and ORFs$_1$ (*P* = 0.0001, hypergeometric test). *P* and τ, Kendall's correlation statistics. Error bars represent standard error of the proportion in all panels.

nucleotides, show signatures of translation and are under purifying selection, can confidently be considered genes despite being weakly conserved (Fig. 4a and Supplementary Fig. 8). The remaining 1,891

a Candidate proto-genes

With translation signatures

Of length ≥ 300 nucleotides

1,133   54   445

25

44   61

144

Under significant purifying selection

10

☐ ORF

◼— Conservation level

☐ Length

↦ ► Expression level

✦ ••• Sequence composition

b Non-genic ORFs   Genes

Theory (encoding a functional product)

Unannotated ORFs   Annotated ORFs

Implementation (300 nucleotide threshold)

Non-genic ORFs   Proto-genes   Genes

Proposed continuum (no boundary)

Figure 4 | Identification of proto-genes in a continuum ranging from non-genic ORFs to genes. a, Characterization of candidate proto-genes (ORFs$_0^+$ and ORFs$_{1-4}$). Venn diagram not drawn to scale. **b**, The binary model of annotation (top) and the proposed continuum (bottom).

ORFs (1,139 ORFs$_0^+$ and 752 ORFs$_{1-4}$) present characteristics intermediate between non-genic ORFs and genes, meeting our proto-gene designation (Supplementary Table 3). We propose to place these ORFs in a continuum where strict annotation boundaries no longer have to be set (Fig. 4b).

Gene birth mechanisms involving re-organization of pre-existing genes, notably following gene duplication, have long been regarded as the predominant source of evolutionary innovation[1,2]. Since the split between *S. cerevisiae* and *S. paradoxus*, sporadic gene duplications have generated between one and five novel genes[27]. In contrast, 19 of the 143 ORFs$_1$ that arose *de novo* during the same evolutionary period were found under purifying selection. Therefore, *de novo* gene birth seems to be more prevalent than previously supposed[3,10,12], in agreement with recent estimations in humans and other primates[1,9]. The involvement of proto-genes in *de novo* emergence of protein-coding genes in *S. cerevisiae* probably holds for other species and may extend to RNA genes and regulatory elements. Examination of translation program remodelling upon stress, in light of our evolutionary model, may further understanding of phenotypic diversity and plasticity of cellular systems[7,28].

METHODS SUMMARY

Detection of translation signatures. The mapping of ribosome footprint reads to ORFs does not necessarily indicate full-length, ORF-specific translation events[6,25]. To model the number of ORFs$_0^+$ expected if the detected presence of ribosomes on non-genic sequences was not related to the presence of ORFs$_0$, we randomized the positions of ORFs$_0$ while maintaining their length distribution and the observed positions of RNA-Seq and footprint reads. To model the number of ORFs$_0^+$ expected if footprint reads observed outside of annotated ORFs were non-specific, we randomized the positions of footprint reads throughout non-genic sequences while maintaining the length distribution of footprint reads, the positions of RNA-Seq reads and the positions of ORFs$_0$. We optimized three parameters with regard to these two null models: (1) the proportion of ORF length covered in RNA-Seq and footprint reads was fixed at 50% minimum; (2) the factor by which the number of footprint reads per nucleotide in the ORF should be higher than the number of footprint reads per nucleotide in surrounding up- and down-stream windows was fixed at a minimum of 5; and (3) the size of these windows was fixed at 300 nucleotides. Any two ORFs$_0$ that partially overlap on the same strand and show translation signatures in the same experimental conditions were both eliminated from the set of ORFs$_0$ considered to show translation signatures.

Significant purifying selection signatures. We estimated the number of synonymous mutations per synonymous site (dS) and the number of non-synonymous mutations per non-synonymous site (dN) for each ORF present without disruptive mutations in eight *S. cerevisiae* strains. The likelihood of the dN/dS ratio was determined under two distinct null models: assuming neutral evolution (the rates of synonymous and non-synonymous substitutions are equal) and not assuming neutral evolution. All ORFs with dN/dS < 1 and $P < 0.05$ (chi-squared distribution of likelihoods with one degree of freedom) were considered to be subject to significant purifying selection.

1. Tautz, D. & Domazet-Loso, T. The evolutionary origin of orphan genes. *Nature Rev. Genet.* **12**, 692–702 (2011).
2. Kaessmann, H. Origins, evolution, and phenotypic impact of new genes. *Genome Res.* **20**, 1313–1326 (2010).
3. Jacob, F. Evolution and tinkering. *Science* **196**, 1161–1166 (1977).
4. Siepel, A. Darwinian alchemy: human genes from noncoding DNA. *Genome Res.* **19**, 1693–1695 (2009).
5. Khalturin, K., Hemmrich, G., Fraune, S., Augustin, R. & Bosch, T. C. More than just orphans: are taxonomically-restricted genes important in evolution? *Trends Genet.* **25**, 404–413 (2009).
6. Wilson, B. A. & Masel, J. Putatively noncoding transcripts show extensive association with ribosomes. *Genome Biol. Evol.* **3**, 1245–1252 (2011).
7. Jarosz, D. F., Taipale, M. & Lindquist, S. Protein homeostasis and the phenotypic manifestation of genetic diversity: principles and mechanisms. *Annu. Rev. Genet.* **44**, 189–216 (2010).
8. Cai, J., Zhao, R., Jiang, H. & Wang, W. De novo origination of a new protein-coding gene in *Saccharomyces cerevisiae*. *Genetics* **179**, 487–496 (2008).
9. Wu, D. D., Irwin, D. M. & Zhang, Y. P. De novo origin of human protein-coding genes. *PLoS Genet.* **7**, e1002379 (2011).
10. Ekman, D. & Elofsson, A. Identifying and quantifying orphan protein sequences in fungi. *J. Mol. Biol.* **396**, 396–405 (2010).
11. Lipman, D. J., Souvorov, A., Koonin, E. V., Panchenko, A. R. & Tatusova, T. A. The relationship of protein conservation and sequence length. *BMC Evol. Biol.* **2**, 20 (2002).
12. Wolf, Y. I., Novichkov, P. S., Karev, G. P., Koonin, E. V. & Lipman, D. J. The universal distribution of evolutionary rates of genes and distinct characteristics of eukaryotic genes of different apparent ages. *Proc. Natl Acad. Sci. USA* **106**, 7273–7280 (2009).
13. Cai, J. J. & Petrov, D. A. Relaxed purifying selection and possibly high rate of adaptation in primate lineage-specific genes. *Genome Biol. Evol.* **2**, 393–409 (2010).
14. Zheng, D. & Gerstein, M. B. The ambiguous boundary between genes and pseudogenes: the dead rise up, or do they? *Trends Genet.* **23**, 219–224 (2007).
15. Oliver, S. G. *et al.* The complete DNA sequence of yeast chromosome III. *Nature* **357**, 38–46 (1992).
16. Fisk, D. G. *et al. Saccharomyces cerevisiae* S288C genome annotation: a working hypothesis. *Yeast* **23**, 857–865 (2006).
17. Nagalakshmi, U. *et al.* The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science* **320**, 1344–1349 (2008).
18. Boyer, J. *et al.* Large-scale exploration of growth inhibition caused by overexpression of genomic fragments in *Saccharomyces cerevisiae*. *Genome Biol.* **5**, R72 (2004).
19. Brar, G. A. *et al.* High-resolution view of the yeast meiotic program revealed by ribosome profiling. *Science* **335**, 552–557 (2012).
20. Li, Q. R. *et al.* Revisiting the *Saccharomyces cerevisiae* predicted ORFeome. *Genome Res.* **18**, 1294–1303 (2008).
21. Jansen, R. & Gerstein, M. Analysis of the yeast transcriptome with structural and functional categories: characterizing highly expressed proteins. *Nucleic Acids Res.* **28**, 1481–1488 (2000).

22. Giacomelli, M. G., Hancock, A. S. & Masel, J. The conversion of 3′ UTRs into coding regions. *Mol. Biol. Evol.* **24,** 457–464 (2007).
23. Prat, Y., Fromer, M., Linial, N. & Linial, M. Codon usage is associated with the evolutionary age of genes in metazoan genomes. *BMC Evol. Biol.* **9,** 285 (2009).
24. Yomtovian, I., Teerakulkittipong, N., Lee, B., Moult, J. & Unger, R. Composition bias and the origin of ORFan genes. *Bioinformatics* **26,** 996–999 (2010).
25. Ingolia, N. T., Ghaemmaghami, S., Newman, J. R. & Weissman, J. S. Genome-wide analysis *in vivo* of translation with nucleotide resolution using ribosome profiling. *Science* **324,** 218–223 (2009).
26. Vishnoi, A., Kryazhimskiy, S., Bazykin, G. A., Hannenhalli, S. & Plotkin, J. B. Young proteins experience more variable selection pressures than old proteins. *Genome Res.* **20,** 1574–1581 (2010).
27. Gao, L. Z. & Innan, H. Very low gene duplication rate in the yeast genome. *Science* **306,** 1367–1370 (2004).
28. Hayden, E. J., Ferrada, E. & Wagner, A. Cryptic genetic variation promotes rapid evolutionary adaptation in an RNA enzyme. *Nature* **474,** 92–95 (2011).
29. Pal, C., Papp, B. & Hurst, L. D. Highly expressed genes in yeast evolve slowly. *Genetics* **158,** 927–931 (2001).
30. Drummond, D. A., Raval, A. & Wilke, C. O. A single determinant dominates the rate of yeast protein evolution. *Mol. Biol. Evol.* **23,** 327–337 (2006).

Author Contributions A.-R.C., I.W., M.E.C. and M.V. conceived the project. A.-R.C. led the project and performed most of the analyses. T.R. evaluated cross-species transfer events, optimized the ribosome footprint analysis pipeline and assisted in other analyses. I.W. designed the conservation level tool and calculated most of the purifying selection statistics. M.A.C., C.A.H., A.R. and N.T.-M. advised on the research. M.A.Y. aligned the sequencing reads. B.S. predicted disordered and transmembrane regions and assisted in the cross-species transfer analyses. N.S. and B.C. assisted in analyses. G.A.B. and J.S.W. shared their expertise in ribosome footprinting data analysis and provided the meiosis ribosome footprinting raw and processed data. A.-R.C., T.R., M.E.C. and M.V. designed the figures. All authors contributed to writing the manuscript.

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