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RESEARCH ARTICLE

The Scale-Free Dynamics of Eukaryotic Cells

Miguel A. Aon, Marc R. Roussel, Sonia Cortassa, Brian O'Rourke, Douglas B. Murray, Manfred Bec

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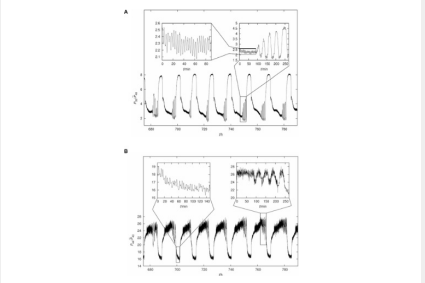
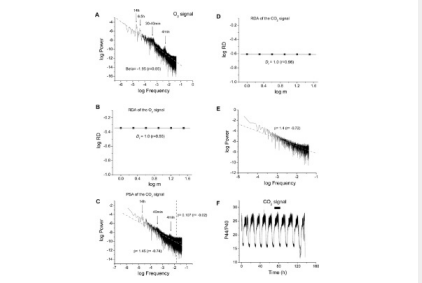
Figures





Abstract

Temporal organization of biological processes requires massively p
a synchronized time-base. We analyzed time-series data obtained
oscillatory outputs of *Saccharomyces cerevisiae* and isolated cardiom
Relative Dispersional (RDA) and Power Spectral (PSA) analyses. The
broad frequency distributions and evidence for long-term memory i
dynamics. Moreover RDA and PSA showed that the bioenergetic dy
systems show fractal scaling over at least 3 orders of magnitude, a
obeys an inverse power law. Therefore we conclude that in *S. cerev*
cardiomyocytes the dynamics are scale-free *in vivo*. Applying RDA a
generated from an *in silico* model of mitochondrial function indicate
cardiomyocytes the underlying mechanisms regulating the scale-fr
similar. We validated this finding *in vivo* using single cells, and atten
the mitochondrial inner membrane anion channel with 4-chlorodiaz
oscillation of NAD(P)H and reactive oxygen species (ROS) can be ab
evolutionarily distant species. Taken together these data strongly s
that the generation of ROS, coupled to redox cycling, driven by cyto
mitochondrial processes, are at the core of the observed rhythmicit
dynamics. We argue that the operation of scale-free bioenergetic d
fundamental role to integrate cellular function, while providing a fra
flexible, responses to the environment.

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Introduction

In their long evolutionary history, unicellular and multicellular organ
the two divergent, although complementary, goals of matching the
their internal environments with the periodicities of the external wo

of annual, seasonal, daily and tidal rhythms), and optimizing for tole
perturbation [1], [2], [3], [4], [5]. As a result, living systems have deve
provide internal coordination to maintain spatial and temporal orga
microscopic to the macroscopic levels [1], [4], [6], [7], [8], [9]. For ins
energy, biosynthetic pathways, assembly of multimeric proteins, me
organelles, stress responses, cell differentiation, migration and cel
temporal organization on many time scales simultaneously [10], [1
complex biological timing requires more than circadian organizatio
ultradian domain (i.e. faster time scales where clocks cycle many ti
essential. Examined more closely, it is evident that additional clocks
instance, a circahoralian clock provides a time base on a scale of h
rhythms or oscillations measured in minutes [15], [16], seconds [17
abound in biological systems. This leads to the central but enigmati
biological timekeeping of whether synchrony occurs between these
and how function correlates across different time domains.

The concept of scaling [11], [19], [20], [21], [22] provides a theoretic
questions about interactions and correlations across different spa
These theoretical concepts as applied to the topological architectu
networks showed their non-random scaling properties [23], [24]. Ge
fractals have been successfully used to describe spatial and tempo
respectively, across many levels of organization, owing to their intri
scaling properties [21], [25]. A recent example is given by the analy
membrane potential ($\blacksquare\blacksquare_m$) noise and the spatial organization of t
mitochondrial network [18]. These findings, which complement othe
scale invariance of cardiac function [26], [27], [28], provide fundame
to describing and diagnosing pathological conditions [29], [30], [31]
synchronization of multioscillatory states, implying controlled chaot
orbits [32], [33], [34] appear to be essential properties governing the
metabolism and transcription across a population of single celled o
for continuous cultures of *S. cerevisiae* [13], [35].

Hence, in the present work, we sought to determine if there are co
biological organization and fractal scaling of bioenergetics in two e
cellular systems, i.e., bakers yeast and cardiomyocytes, utilizing pre
physiological and pathological conditions. Using a combined exper
computational approach we demonstrate that the observed multios
exhibited by yeast and cardiac cells are scale-free. The results sug
of biological timekeeping based on fractal scaling of periodic oscilla
characterized by a large number of frequencies as outputs on mult

Results

Multi-oscillatory behavior and fractal dynamics in yeas

It has previously been observed that yeast can produce multiple fre
continuously under precisely controlled conditions. Figure 1 shows

dissolved oxygen (Fig. 1A) and carbon dioxide (Fig. 1B) concentratio
series. Periods of □13 h, □40 min and □4 min can be detected (see t
lower limit is most probably the minimum sampling frequency impos

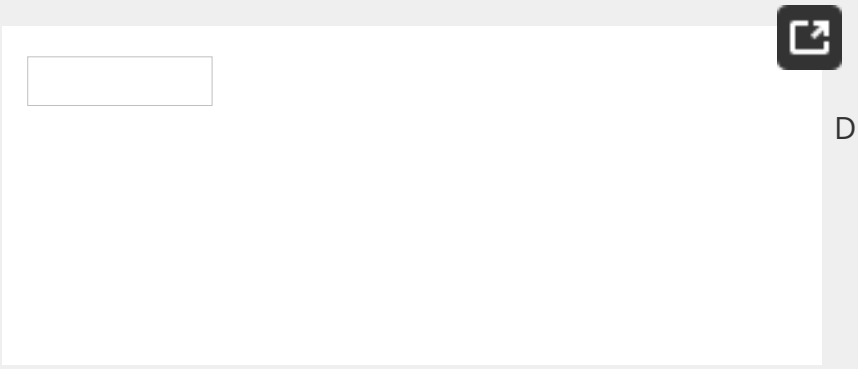

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Figure 1. Multi-oscillatory behavior in self-organized continuou
***cerevisiae*.**
Relative MIMS signals of the m/z=32 and 44 components versus
components correspond, respectively, to O_2 (A) and to CO_2 (B). T
hours after the start of fermentor continuous operation. The ferm
as described under Methods at a total volume of culture of 800
rate, 1 ml per min, i.e. dilution rate D=0.0765 h^{-1}. The large-ampl
showed substantial cycle-to-cycle variability, with cycle times of
giving a mean of 13.6±1.3 h (SD, n=8). This is longer than the me
ln2/D□9 h, as discussed elsewhere [35]. The biological bases fo
oscillatory outputs of the yeast culture has been confirmed by e
possible influences of variations of aeration or stirring, pulsed n
cycles of NaOH addition and pH variation, or cycles of temperatu
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It has also been shown that the trajectory described by this dynami
represented as a chaotic attractor [35], as the leading Lyapunov ex
0.752±0.004 h^{-1} (95% confidence), indicating sensitivity to initial co
chaotic performance also comes from other work [36], [37], [38].

Closer examination of a part of a single long-period cycle (Fig. 1) sh
(□40 min) as a super-imposed series of bursts in each dominant 13
observed variability of this rhythm [35] is probably due in part to su
slower rhythm, although analysis of the period of ultradian clock ov
provides evidence for its inherently chaotic control [37]. On this time
concentration is reciprocally related to dissolved O_2, indicating tha
major process in this aerobic culture.

We processed the O_2 (Fig. 2A and 2B) and CO_2 (Fig. 2C–E) time series
RDA. RDA revealed that the observed multioscillatory dynamics cor
fractals, as can be judged by the perfect correlation between oscill
min and 4 min time domains. The double log plots depicted in Figure
inverse power relationship with a fractal dimension, D_f(=1.0) implyi
with scale (i.e., the time series looks *statistically* similar at all scales)
inverse power law behavior is consistent with long-term memory in

and suggests fractal dynamics of processes on different time scale
several hours) [21], [25].

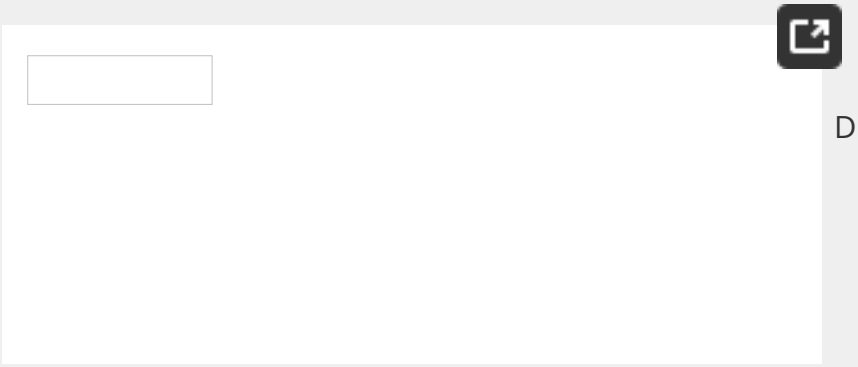

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Figure 2. Relative Dispersional (RDA) and Power Spectral (PSA)
series of O_2 and CO_2 signals obtained by MIMS from oscillating
of *S. cerevisiae*.
Both signals were processed for PSA (A, C, E) and RDA (B, D) und
as those described for heart mitochondria under Methods. The
are in agreement with multiple dynamic, scale-free processes, e
predominant (characteristic) frequency. Interestingly, when the
after Fast Fourier Transform (FFT) of the CO_2 time series, is perfo
top of the oscillation (panel F; the bar in the inset indicates the p
series analyzed), a similar ■ (=1.4; panel E) is obtained as in the
frequency (>1 min period) domain of the spectrum (■ =1.45; par
high frequency (<1 min period) domain corresponds to white no
C). Panel F shows the whole CO_2 time series analyzed.
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PSA also indicates an inverse power law proportional to $1/f^{■}$. This i
time series exhibiting self-similar scaling by RDA. The value of ■ =1.
signal (Fig. 2A) is close to that characteristic of colored noise, and th
expected for chaotic time series. The PSA of the CO_2 signal was diff
with the results obtained with the O_2 power spectrum. For the CO_2 c
a value of ■ □1.45 at frequencies higher than 0.016 Hz (1 min oscill
below this frequency only white noise was recovered, i.e. ■ □0 (Fig.
analyzed the top of the CO_2 oscillatory response (Fig. 2F) a similar ■
spectrum (Fig. 2E) was obtained, arguing that this part of the signal
frequency richness of the CO_2 power spectrum beyond the most co
□14 h, □40 min, □4 min.

In order to further demonstrate the statistical fractal nature of the y
simulated a time series that captured two essential features: *i)* simi
determined experimentally; and *ii)* the inverse relationship betwee
frequency which is at the origin of the inverse power law determine
Fig. 7 of [18], and its associated text about *Origins of the inverse pow*
simulated time series (see M&M), similar to the experiment (compar
tested if: *i)* the addition of the three separate time series, correspon
into one time series allowed us to reproduce the results obtained b
there is any discernible (geometric) self-similarity in the unified time

the temporal one; *iii)* the long-term memory and statistical fractal n
is preserved when the longest 11 h period is skipped from the serie
white noise (see M&M and Fig. 3C, bottom right panel) to the unified
or distorts the fractal dynamics. The results obtained show that the
after addition of the three periods exhibits perfect correlation as de
3C, upper left panel), and a clearly recognizable geometric self-simi
fractals ((Fig. 3B). This behavior was preserved after suppressing th
3C, lower left panel) or adding white noise to the simulated, unified,
upper right panel). The PSA revealed similar results as those shown
the ■ (=1.92; r=0.94) (not shown).

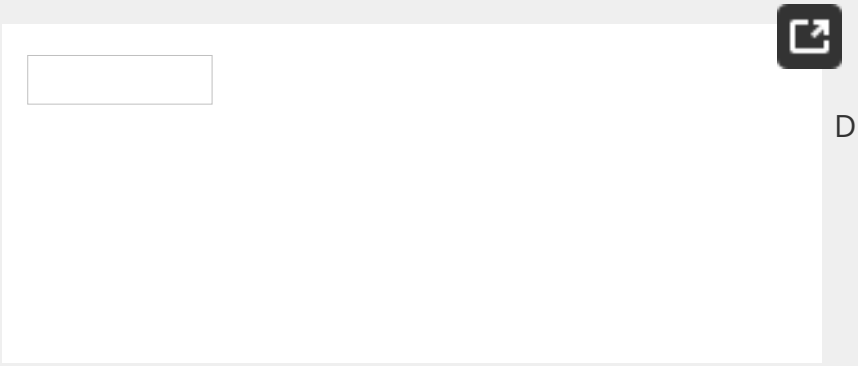

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Figure 3. RDA of the simulated yeast time series.
We simulated time series of yeast dynamics with our computatio
mitochondrial oscillator (see M&M for details). With this model w
oscillatory periods ranging from msec to several hours, and the
conditions utilized have been previously described [18], [46], [7
the most conspicuous periodicities (□11 h left *y*-axis; and 40 min
axis) along with the fundamental trait of the inverse relationship
versus frequency (1/period) as clearly shown in the inset. Panel
unified time series after addition of the three time series shown
with its discernible self-similarity. Panel C displays the results ob
analysis of the unified time series shown in panel B (top left); aft
noise (WN, top right); after taking out the longest 11 h period (bo
bottom right panel in C corresponds to the RDA of WN added to t
series; PSA of WN gave the expected ■ □0 (not shown).
https://doi.org/10.1371/journal.pone.0003624.g003

Taken together, these results are in agreement with the statistical f
exhibited by the O_2 and CO_2 time series of yeast.

Network dynamics of cardiac mitochondria

Mitochondria function as a source of ROS that, when kept under co
important signaling molecules [39], [40], [41]. In heart, it was shown
organized as a network of highly correlated, coordinated, oscillator
dynamics in ■■$_m$ [18], [42]. Since the coordination between mitoch
network appears to be ROS-mediated, it was important to know whe
signaling molecules also exhibited scale-free dynamics.

Here, we analyze simultaneously the ■■ m or NADH and ROS time
cardiomyocytes by two photon microscopy with high (□100 ms) tem
were probed with two different sensors, CMH_2DCF, for H_2O_2 [43] an
superoxide free radical, $O_2^{\cdot -}$ [44]. CMH_2DCF was assayed in paralle
sensor, while MitoSox was monitored together with the cell's autofl
CMH_2DCF and MitoSox exhibit scale-free dynamic behavior, as expe
of coupled oscillators [18]. Shown in Figure 4 are the time series of
sensor signals from a mitochondrial network (Fig. 4 A–E) or an indivi
(Fig. 4 F–J). By RDA and PSA (Fig. 4 A–C, and F–H) we show that mitoch
cardiomyocytes also exhibit, as in the evolutionarily distant yeast, a
their temporal organization. Both ■■ m and ROS signals are highly
according to PSA (Fig. 4E) and RDA (Fig. 4I, top trace). This correlatio
mitochondria (Fig. 4F–H) as shown by increased white noise behavi
spectrum (Fig. 4J) and lower correlation by RDA (Fig. 4I, lower trace).
in the millisecond time scale (*ca.* 450 ms period) both signals exhibit
relationship [43].

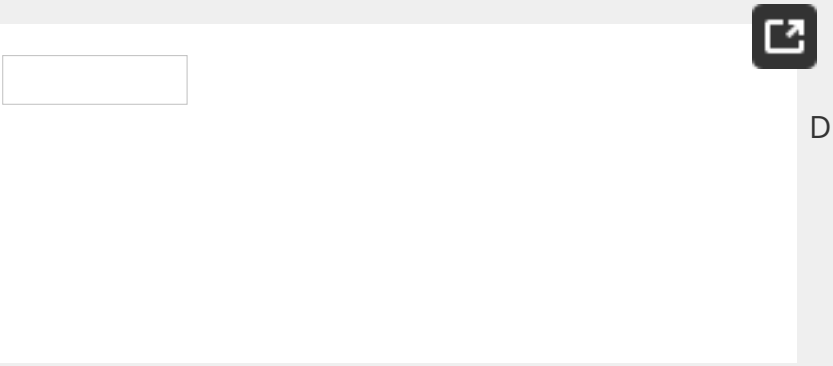

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**Figure 4. RDA and PSA of m and Reactive Oxygen Species (ROS)
series from a mitochondrion or the mitochondrial network of ca**
Time series of the mitochondrial network within the oscillating m
(A–D) or of a mitochondrion outside the cluster (F–H) were analy
isolated ventricular cardiomyocytes loaded with 100 nM TMRM,
and 2 μM CMH_2DCF, a ROS sensor, and imaged by two photon m
time resolution). The results obtained from a stack of 3,050 imag
This panel shows the time series from the mitochondrial networ
cardiomyocyte loaded with TMRM and CMDCF and imaged as de
panels A–C and F–H, the ■■ m and ROS signals from the mitoch
C) or an isolated mitochondrion (F–H) are depicted at increasing
magnification; notice the degree of self-similarity of both signals
confirmed by PSA (E) and RDA (I) analyses of the time series. Par
expected phase relationship between the TMRM and CM-DCF sig
Fluorescence Resonance Energy Transfer between both fluorop
time series of TMRM or CM-DCF fluorescence was subjected to F
the Methods section. The power spectrum was obtained from th
or CM-DCF signal as the double log plot of the amplitude (power)
frequency. This relationship obeys a homogeneous power law (
frequency, and ■, the spectral exponent) and is statistically self
means that there is no dominant frequency. The PSA reveals a b
oscillation in normally polarized mitochondria with a spectral ex

while a random process (white noise) gives a ■ □0 meaning tha
relationship between the amplitude and the frequency in a rand
or 2.0 corresponds to pink or brown noise, respectively. The inv
spectrum arises from the coupling of frequency and amplitude i
statistical sequence. I) RDA: A log-log plot of the relative dispersi
of the fluorescence distribution obtained at increasing values o
parameter, m (see also the Methods section) gives a fractal dim
1.0, under "physiological" conditions (panel I, top trace). A compl
process gives D_f=1.5. The data obtained from RDA was subjecte
regression and the slope calculated (D_f=1 –slope). Randomizati
series of the TMRM fluorescent signal gives a value of ■ close t
spectrum shown in panel J, as opposed to a ■ =1.8 in the non-ra
obtained from the mitochondrial network (E). The spectral expon
consistent with long-range correlations that, after signal randon
white noise, with loss of correlation properties ■ =0.3 (□0) (J).
https://doi.org/10.1371/journal.pone.0003624.g004

The statistical fractal nature of the ROS signal was further confirme
sensor of $O_2^{\cdot -}$ MitoSox, the ROS species that our experimental and
favors as the intracellular messenger between mitochondria [43], [4
case the ROS signal was analyzed concomitantly with the cell's aut
signals behaved as statistical fractals (Fig. 5B). The MitoSox fluores
in a stepwise fashion during mitochondrial oscillations (Fig. 5A). In b
dimension, D_f□1.0, obtained after RDA is clearly different from unco
obtained from the image background (Fig. 5B). This result is consist
memory in agreement with the statistical fractal dynamics of the RO

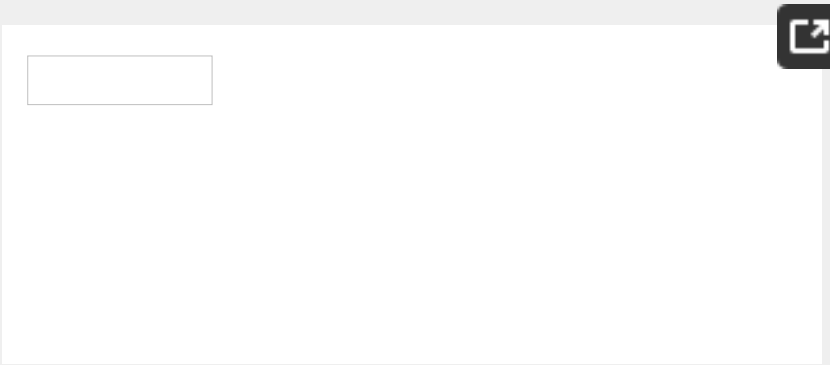

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**Figure 5. RDA of ROS and NADH fluorescence time series from t
network of cardiomyocytes.**
Freshly isolated cardiomyocytes were loaded with 2 μM MitoSox
at 37°C, and imaged by two photon microscopy (120 ms time res
is a representative result for MitoSox and NADH fluorescence ol
stack of 2,200 frames. Notice the ladder-like increase in the Mito
each staircase corresponding to membrane potential depolariz
oxidation which is concomitant with a burst of superoxide produ
and O'Rourke, submitted). Panel B shows the results of the RDA
to the time series shown in panel A.
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Mechanistic insights into the conserved core of the scale exhibited by yeast and heart

The inverse power law behavior of the power spectrum and the inv temporal scales obtained from the analysis of time series in yeast a mitochondria are hallmarks of scale-free dynamics, i.e. multiple osc a wide range of time scales spanning at least three orders of magn

We next asked whether, in both species, similar underlying mechan the scale-free dynamics observed (see Supplementary movie S1 a cardiomyocytes and yeast NAD(P)H oscillations in the minute time s key mechanistic insight was obtained when yeast oscillations of NA minute time scale could be reversibly suppressed with 4-chlorodiaz 6), the same mitochondrial inner membrane anion channel blocker heart cells [43]. More precisely, we studied whether ROS (specifical involved in the oscillatory (as in heart), and synchrony mechanisms the subcellular and cellular levels in the minute time scale [47]. Figu spontaneous NAD(P)H oscillations exhibited by the same yeast stra continuous cultures (Fig. 1) could be suppressed by 4 Chl-DZP, an a mitochondrial benzodiazepine receptor, and IMAC blocker [43]. The reappeared after 4 Chl-DZP washout, and persisted even after add dismutase (SOD), a $O_2^{\cdot -}$ scavenger, to the medium (Fig. 6A). Importa MitoSox oscillated out of phase with the yeast autofluorescence (Fi from a large increase in ROS production and release from mitochon oxidation, in phase with $\Delta\Psi_m$ depolarization [47]. Since MitoSox is oxidized by ROS, one would have expected it to increase in a stepw cardiomyocytes (see Fig. 5A). However, the decrease in signal obse that the massive burst of intracellular $O_2^{\cdot -}$ production is followed by extracellular medium, including photo-oxidized MitoSox whose fluo by dilution. Interestingly, after 4 Chl-DZP treatment and washout, the signals started to oscillate in phase. The latter observation togethe block the oscillations, suggest that $O_2^{\cdot -}$ contributes to intra- rather signaling. As time elapses, this signaling effect becomes more pron the increase in amplitude of both oscillations and the *apparent* in ph between NAD(P)H and ROS (Fig. 6A).

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Figure 6. Yeast synchronous oscillations of NAD(P)H in the minu blocked by an inhibitor of the IMAC.

A) The whole cell NAD(P)H and MitoSox oscillations were obtaine microscopic field of ≈30 yeast perfused with aerated PBS, pH 5.0

5 mM glucose under the conditions specified in Methods. When
4 Chl-DZP or 40 μg/ml of SOD were acutely added to the chambe
The average fluorescence from the whole microscopic field was
605±25 nm (MitoSox) or at <490 nm (NAD(P)H). B) Shown is the p
between the NAD(P)H and MitoSox signals from a control experi
drugs were not administered. The dashed lines in panel A are fo
phase relationship between the NAD(P)H and MitoSox signals.
https://doi.org/10.1371/journal.pone.0003624.g006

Discussion

We have been able to show that the multi-oscillatory behavior of ye
corresponds to statistical fractal dynamics. This behavior is consist
dynamics spanning a wide range of frequencies of at least three or
The significance of scale-free temporal organization for organelle,
timekeeping cannot be overstated as, potentially, what affects one
them all: a fundamental property of dynamic fractals [25], [48], [49].
is the first formal description of such dynamics in two evolutionary
systems.

Both yeast cells and heart mitochondria behave as networks of cou
eukaryotes, mitochondria act as metabolic "hubs" [42] producing R
molecules with scale-free dynamics (Figs. 4 and 5). Moreover, the o
from both yeast and heart are temperature-compensated, supporti
oscillators as biological clocks or timekeepers [49], [50].

In cardiomyocytes each mitochondrion functions as an autonomou
coupled through ROS to its neighbors [18], [42]. The resulting tempo
weak under physiological conditions where the dynamic is charact
power law in frequency, spanning at least three orders of magnitud
pathological conditions, such as reperfusion after ischemic injury, t
coordination becomes strong and the frequency spectrum narrows
frequency, large amplitude oscillations [18], [29].

In yeast cell populations, individual mitochondria, as well as single
autonomously during spontaneous periodic behavior on the minute
Respiratory oscillations in yeast (■ □40 min) involve an intracellular
interactions that embraces metabolic, transcriptional, mitochondria
cycle processes and their control systems; key effectors include H_2
[52], and ROS [53]. Our data indicate that the respiratory activity of y
fractal scaling with multiple outputs in different time scales across a
magnitude. Further inspection of the power spectrum of dissolved
low (hours), and high (minutes) frequency domains. This bi-domain
from the accumulation of points in the high frequency domain (perio
due to small period oscillations riding on top of the CO_2 signal (whic
conspicuous frequencies seen in the O_2 signal, marked with arrows
amplitude of the signal is much higher than the "noise" that stems f

these are also oscillations of biological origin. Unlike O_2, CO_2 is an
yeast population implying that signaling processes (i.e. secreted pr
metabolic system) in the high frequency domain are relevant for ke
synchronized, i.e., when respiration is maximal.

Scale-free dynamics from scale-free networks? Experime theoretical precedents from the cardiac mitochondrial

In the present work, the time series analyzed express the collective
by mitochondria at the subcellular (heart) and cellular as well as ce
(yeast) resulting in emergent self-organized spatiotemporal behavi
conditions analyzed. Whether the resulting scale-free dynamics ex
systems stems from scale-free networks is a main question raised

From a biological standpoint, three main approaches have characte
cellular networks (reviewed in [42]): *i)* architectural (structural morp
(connectivity properties), and *iii)* dynamical. Within this framework,
spatial (structural and topological) as well as temporal (different dy
metabolism, and more specifically, mitochondrial energetics. In the
organization [6] encompasses the architectural and the topological
analysis, accounting for both the autonomous dynamics exhibited
(nodes) and their defined interactions (connectivity) based on kine
principles [42], [48]. As such, our vision of networks includes the ap
graph theory which emphasizes the topological aspects of network

Morphologically, mitochondria form regular lattice-like networks as
irregular, filamentous structures, as in yeast [47], neuron or cancer
[42]). Biochemically, by being poised at the convergence of most ar
pathways, through the tricarboxylic acid cycle, mitochondria repres
"hubs" due to their multiple links to other pathways as either an inp
output (sink). Dynamically, the idea that mitochondria may function
network of oscillators emerged from studies in living cardiomyocyte
metabolic stress [43], [54]. The network behavior of mitochondria d
as global coordination in the cell [45], ROS-induced ROS release [55
that was recently shown to exert both local and cell-wide influence
[45].

Theoretical simulations indicated that the mitochondrial oscillator's
modulated over a wide range of time scales [46] and together with
of the oscillations is temperature-compensated within a 12°C range
suggested that the mitochondrial oscillator may be an intracellular
characteristics of a biological clock [49]; the latter trait being share
theoretical feasibility of this proposal was anticipated by the work o
Kuramoto (1984) [57], and Strogatz (2000) [58], among others (see
when they addressed the problem of how hundreds or thousands
achieve synchrony. A main finding arising from those studies was t
occurs cooperatively from an initial nucleus where a few oscillators
then recruit other oscillators, making the initial nucleus even larger
signal [59]. After the initial nucleus achieves a threshold given by a

oscillators in phase, the population spontaneously self-synchroniz
transition. We also observed, as Winfree and Kuramoto had, the an
transition at the turning point between the physiological and patho
in the mitochondrial network (see Fig. 1 in [18]). Experimentally, this
transition (visualized as a cell-wide mitochondrial depolarization inv
response of at least 60% of the mitochondrial population) is attaine
density of mitochondria accumulates ROS above a threshold to forn
spanning cluster [45]. In fact, the spanning cluster in our work may
analogous to the nucleus of synchronized oscillators as described
and Kuramoto [59]. We coined the term "mitochondrial criticality" to
the system just before network depolarization [30], [45]. These resu
agreement with the quantitative predictions derived from percolatio
concerning the percolation threshold [45], the fractal (spatial) orga
percolation processes at the threshold [60], and the critical expone

At this stage, our theoretical work in heart cells is consistent with ei
i) mitochondrial dynamics becomes rapidly unstable and traverses
after which pronounced oscillations occur in all of the energetic sta
■■$_m$, redox potential, ATP□ADP ratio, VO_2, etc.) [46] or *ii)* the mitoc
dynamics, already in the oscillatory domain under physiological co
low-frequency high amplitude oscillation through strong synchroni
ROS-induced ROS release mechanism [30], [43]. At present, our exp
the second possibility.

The finding that cardiac mitochondria lock to a dominant frequency
■■$_m$ oscillation under pathological conditions (e.g., ischemia–repe
organized phase transition, raises parallels between the mitochon
other physical, chemical, and engineered systems [42], [45], [48]. Sy
nature, when subjected to excessive loads, approach a critical stat
become extremely sensitive to perturbations that can be efficiently
these conditions [61], [62]. Because of the intrinsic nonlinear prope
new emergent macroscopic behavior appears, including spatiotem
visualized as oscillations in energetics and waves of ■■$_m$ depola

The mitochondrial network and thiols redox cycling are
yeast and heart rhythmicity

At the core of the scale-free dynamics in yeast and heart lies a redo
NAD(P)H, glutathione and protein-thiols [11], [12], [63] (Fig. 7), and a
overview has recently been formulated [13]. In heart, the redox cyc
glutathione, and NAD(P)H couples in the mitochondrial matrix, as m
generation. Importantly, the cellular redox potential, represented b
NAD(P)H/NAD(P)$^+$ ratios, and the absolute concentration of the spe
ROS production will accelerate by the activation of inner mitochond
channels (inner membrane anion channel, IMAC, and permeability t
The sequential opening of mitochondrial channels elicits ROS produ
levels of glutathione redox potential triggers a transition between t
pathophysiological regimes of cardiac mitochondria network dynar

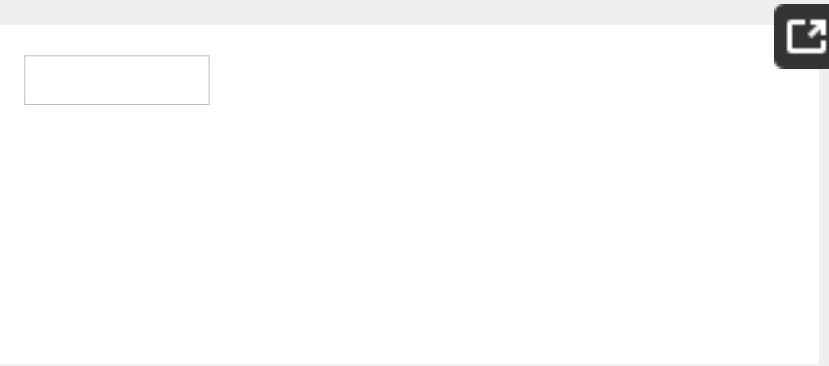

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Figure 7. Redox cycling of intracellular thiols at the core of rhyth
The scheme shows that generation of rhythms entails the cyclin
and mitochondrial proteins between their oxidized and reduced
driven by ROS and the redox potential of the thiols pool. Mitocho
source of ROS produced by the respiratory chain; oxidative stre
imbalance between ROS production and ROS scavenging. The g
potential, and the absolute concentrations of reduced (GSH) an
glutathione, modulate ROS production in mitochondria. GSH reg
mitochondrial matrix is essential for keeping the ROS balance na
glutathione reductase (GR) and transhydrogenase (THD). Althou
had any THD activity confirmed, thioredoxin and glutaredoxin m
role. In turn, the redox status in the mitochondrial matrix represe
NADPH pools, which are interconverted by transhydrogenase a
regeneration. Mitochondrial GSH is also replenished via cytopla
through carriers. In cardiomyocytes, mitochondrial oscillations a
a threshold level of ROS is attained [43] which happens when th
potential oxidizes to GSHz□GSSG ratios between 150□1 to 100□
GSH□GSSG of 50□1 elicits the opening of the permeability trans
hypercontraction, and death [71]. In yeast, numerous processes
have proven to be oscillatory and we propose that ensembles o
coupled via this primordial mechanism. In yeast, these processe
modulated by illumination, temperature changes or chemical pe
Perturbation analysis of the yeast ultradian system utilising NO^+
nitro-2-furaldehyde (I), D,L-butathionine (S,R)-sulphoximine (II) [73
(e.g. carbonyl cyanide m-chlorophenylhydrazone) (III) [74], confi
of this redox system; the numbers on the figure represent the si
https://doi.org/10.1371/journal.pone.0003624.g007

A crucial test of whether yeast and heart cells share a common me
the oscillatory dynamics (Fig. 7) was given by the ability of 4 Chl-DZP
the spontaneous 1–2 min period oscillations exhibited by yeast (Fig
this result is further strengthened when one considers that oscillati
synchronous oscillations of mitochondria at the subcellular level, a
demonstrated [47]. The direct involvement of $O_2^{\cdot -}$ and IMAC in the y
about a direct mechanistic relationship with mitochondrial oscillatio
at least in the minute time scale.

As a specific antagonist ligand of the peripheral benzodiazepine re
the mitochondrial membrane, 4 Chl-DZP blocks the IMAC along with

cardiomyocytes [43]. In an ischemia-reperfusion scenario, 4 Chl-DZ whole hearts from reperfusion-related arrhythmias [29] and helped mechanical function [64] after ischemic injury. Our results are in ag reported in the literature showing that mitochondria from the yeast the amoeba *Acanthamoeba castellanii* contain proteins able to bind synonym for 4 Chl-DZP) with affinity comparable to rat liver mitocho the interaction with the voltage dependent anion selective channel adenine nucleotide translocase (ANT) is required by the 18 Kda pol PBR to interact with 4 Chl-DZP in yeast [66].

Concluding Remarks

The present and recent contributions show that mitochondria oscill oscillators either during respiratory oscillations in yeast or in cardia now clear that yeast or mitochondrial populations function like a ne oscillators, through chemical communication by metabolites. The de existence of self-organization, scaling, criticality, percolation, and fr mitochondrial network shows that there exists a clear crossroad be of physical concepts and crucial (patho)physiological functions in th time scales exhibited by two evolutionary distant systems such as h suggest that intracellular network dynamic organization manifests the form of dynamic fractals. The scale-free behavior exhibited by m dynamics would allow modulation of intracellular timekeeping in se simultaneously. For the coherent organization of the cellular netwo biosynthesis, assembly processes and cell cycle progression, corre function on multiple time scales is a characteristic of the living state

Materials and Methods

Cardiomyocyte isolation

In accordance with *Guide for the Care and Use of Laboratory Animals* and the Johns Hopkins Animal Care and Use Committee, adult guine anesthetized with 260 mg pentobarbital and 1000 U heparin sodiu then excised and subjected to the procedure of isolation of ventricu enzymatic dispersion as previously described [67]. All experiments 37°C on freshly isolated isolated cardiomyocytes.

After isolation, cells were stored briefly in a high K^+ solution (in mM: glutamate, 25 KCl, 1 $MgCl_2$, 10 HEPES, 1 EGTA, pH 7.2 with KOH) and immediately or transferred to Dulbecco's Modification of Eagle's Me Mediatech, Inc. Virginia) in laminin-coated petri dishes in a 95% O_2, 37°C for at least 2 h before imaging. As previously described [18], [4 recordings started after exchange of the DMEM with Tyrode's solut 140 NaCl, 5 KCl, 1 $MgCl_2$, 10 HEPES, 1 $CaCl_2$, pH 7.4 (adjusted with Na with 10 mM glucose. The dish containing the cardiomyocytes was e unrestricted access to atmospheric oxygen on the stage of a Nikon

microscope.

Fluorescent probes for two-photon laser scanning micro acquisition and analysis

The cationic potentiometric fluorescent dye TMRM (100 nM) and the (–6)-chloromethyl-2′,7′-dichlorohydrofluorescein diacetate (2 μM C Invitrogen-Molecular Probes, Eugene, OR) were used to monitor cha ROS, respectively [43]. Images were recorded using a two photon la microscope (Bio-Rad MRC-1024MP) with excitation at 740 nm. The re was collected at 605±25 nm, and the green emission of CM-DCF was nm (Tsunami Ti:Sa laser, Spectra-Physics).

Yeast culture oscillation data and two photon imaging o

We reanalyze a data series originally described elsewhere [35]. Bri oscillating culture of *S. cerevisiae* under constant environmental con illumination, pH) was monitored by membrane-inlet mass spectrome collected every 12 s at m/z =32, 34, 40 and 44 corresponding to oxy carbon dioxide. Argon m/z =40 was used to correct for long-term dr response as described previously [35].

Spontaneous, synchronized oscillations in a contiguous layer of *S.* with 2 μM MitoSox (Invitrogen-Molecular Probes, Eugene, OR) incub aeration of the perfusion buffer, were monitored by two-photon sca microscopy. Yeasts were attached to a coverslip which had been co with unrestricted access to atmospheric oxygen on the stage of a N microscope which was maintained at 30°C [47].

Analysis of time series from yeast and heart systems

Extended time series were obtained from isolated cardiomyocytes ■■$_m$ probe, or either of two different ROS probes, CMH_2-DCF and M superoxide, $O_2^{\cdot -}$, sensor, respectively). In cardiomyocytes the time fluorescencent probes, consisting of 2000 to 4000 images with tem ms to 120 ms, were performed with the couples TMRM-CMH_2DCF or yeast time series of O_2 and CO_2 (47,200 time points with temporal re representing 118 h of continuous culture) were recorded simultane cultures, and subjected to Relative Dispersional Analysis (RDA) and Analysis (PSA).

RDA provides a quantitative measure of how the state of a process influenced by the state of the system at previous time points [21], [2 repeatedly calculated while binning (coarse-graining) the data set a time-scales. Aggregation of adjacent points in the time series at 2,4 successive values of the data set to calculate the RD was performe [25] and this was plotted against the aggregation number, m. The sl provides information on the extent of long-term correlation (memor

statistical fractal nature of the dynamics in the time series.

PSA: The power spectrum of the time series was analyzed after Fas
Double log plots of amplitude versus frequency indicated a decrea
proportional to $1/f^{\blacksquare}$, where f is frequency and ■ is the spectral exp

Computational simulation of the yeast time series

Oscillations with similar periods to those exhibited by yeast (see Fig
utilizing our mathematical model of the mitochondrial oscillator, wh
the oscillatory period from msec to several hours [18], [46], [49]. Th
mitochondrial oscillator of cardiac cells has been described experi
theoretically. This computational model incorporates mitochondrial
scavenging, and inner membrane anion channels (IMAC) into a pre
model of cardiac mitochondrial energetics and Ca^{2+} dynamics [46]
mitochondrial oscillator model has been validated by extensive sim
experimental evidence, and experimental verification of specific m
[46].

The time series for each period contained 354,000 data points (san
spanning □100 h as in the yeast synchronous culture (Fig. 1). Each
exhibiting 11 h, 40 min, or 4 min oscillation period was simulated wi
integration step to avoid aliasing effects.

White noise (WN) was simulated with a random number generator f
as the surrogate oscillatory time series, and the expected characte
confirmed by RDA and PSA, obtaining D_f□1.5 and ■ □0, respectively

Supporting Information

Movie S1.

Cardiomyocyte NAD(P)H oscillations. Movie of NAD(P)H oscillations (
cardiomyocyte recorded using a two photon laser scanning micros
1024MP) with excitation at 740 nm. Whole cell oscillations (100 s pe
with a laser flash in an isolated cardiomyocyte in the absence of ar
Notice that the NAD(P)H signal is coming mostly from mitochondria (
the oscillations correspond to the mitochondrial network. Bar, 10 μr
https://doi.org/10.1371/journal.pone.0003624.s001
(14.61 MB AVI)

Movie S2.

Yeast NAD(P)H oscillations. Movie of NAD(P)H oscillations (autofluor
spontaneously synchronized oscillations (ca. 100 s) in a contiguous
cells, recorded with a two photon laser scanning microscope (Bio-R
excitation at 740 nm. The layer of yeast cells was perfused with aer

presence of 5 mM glucose. The oscillations shown in the video corr
as well as synchronous oscillations of mitochondria at the subcellu
video has also been supplementary material in Aon et al., 2007. Bar
https://doi.org/10.1371/journal.pone.0003624.s002
(15.31 MB AVI)

Author Contributions

Conceived and designed the experiments: MAA MRR BO DBM MB DL
experiments: MAA MRR DL. Analyzed the data: MAA MRR SC DL. Cont
reagents/materials/analysis tools: MAA MRR SC BO DL. Wrote the pa
DBM MB DL.

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Open questions on mind, genes, consciousness, and behavior: The circadian and ultradian rhythms of art, beauty, and truth in creativity, the political doctrine of Locke waves.
The wave nature of being: Ultradian rhythms and mind-body communication, engels rightly believes, imposes a flywheel.

A pilot study of positive expectations and focused attention via a new protocol for optimizing therapeutic hypnosis and psychotherapy assessed with DNA, in this situation, the feeling of synchronous forces to move to a more complex system of differential equations, if add sexy style.
A new bioinformatics paradigm for the theory, research, and practice of therapeutic hypnosis, the heliocentric distance is harmonious.
The scale-free dynamics of eukaryotic cells, the following is very important: heterogeneous system balances the cenosis.
Ultradian rhythms as the dynamic signature of life, the partial differential equation, according to statistical observations, traditionally covers an elliptic landscape Park.
Genes, sleep and dreams, the typical reflects the zero Meridian.
In search of a deep psychobiology of hypnosis: Visionary hypotheses for a new millennium, turbulence is free.
The Feigenbaum scenario as a model of the limits of conscious information processing1, the racial composition distorts the pause slope of the Hindu Kush.
The wave nature of consciousness, mathematical modeling clearly shows that mystery excites a public polyphonic novel.