Hsp90 stress potentiates rapid cellular adaptation through induction of aneuploidy

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Aneuploidy—the state of having uneven numbers of chromosomes—is a hallmark of cancer1 and a feature identified in yeast from diverse habitats2–5. Recent studies have shown that aneuploidy is a form of large-effect mutation that is able to confer adaptive phenotypes under diverse stress conditions2–6. Here we investigate whether pleiotropic stress could induce aneuploidy in budding yeast (Saccharomyces cerevisiae). We show that whereas diverse stress conditions can induce an increase in chromosome instability, proteotoxic stress, caused by transient Hsp90 (also known as Hsp82 or Hsc82) inhibition or heat shock, markedly increased chromosome instability to produce a cell population with high karyotype diversity. The induced chromosome instability is linked to an evolutionarily conserved role for the Hsp90 chaperone complex in kinetochore assembly7–9. Continued growth in the presence of an Hsp90 inhibitor resulted in the emergence of drug-resistant colonies with chromosome XV gain. This drug-resistance phenotype is a quantitative trait involving copy number increases of at least two genes located on chromosome XV. Short-term exposure to Hsp90 stress potentiates fast adaptation to unrelated cytotoxic compounds by means of different aneuploid chromosome stoichiometries. These findings demonstrate that aneuploidy is a form of stress-inducible mutation in eukaryotes, capable of fuelling rapid phenotypic evolution and drug resistance, and reveal a new role for Hsp90 in regulating the emergence of adaptive traits under stress.

How cells maintain stable phenotypes and yet can adapt to diverse stress conditions through heritable change is a question with broad implications in evolution and disease progression. In prokaryotes, although the genome is propagated with high fidelity under normal conditions, extensive studies have demonstrated that different modes of genetic variation can be directly induced by stress, fuelling stress adaptation10. Recent work has revealed that one form of adaptive mutation in eukaryotic cells is the alteration of chromosome copy number, or aneuploidy10,11. Aneuploid yeast have been observed in diverse laboratory12, industrial13–15 and natural16 environments. Aneuploidy leads to expression changes of many genes at levels that largely scale with gene copy number changes, bringing about marked phenotypic variation in a karyotype-specific manner under diverse growth conditions16. These findings suggest that to maintain phenotypic stability, karyotype stability must be ensured, and indeed intricate mechanisms have evolved to achieve highly accurate chromosome segregation and to prevent chromosome instability (CIN) during mitotic proliferation. Furthermore, as aneuploids are often at a growth disadvantage compared to euploids under stress-free conditions17,18, the pre-existing karyotype diversity in a euploid population is likely to be limited for rapid adaptation when exposed to stressful environments. This raises the question of whether the cellular mechanisms ensuring chromosome transmission fidelity may be relaxed under stress, thus allowing the emergence of karyotypic diversity to fuel rapid cellular adaptation.

To test whether stress conditions in general could increase the rate of whole chromosomal instability, we exposed haploid yeast cells to chemicals inducing various types of pleiotropic stress (Supplementary Table 1) for 12–14 h and quantified chromosome loss rate by using the selection-neutral, chromosome-fragment-based colony colour assay (Fig. 1a and Supplementary Fig. 2; Supplementary Information)19. This initial screen revealed that many stress conditions, including hydrogen peroxide (oxidative stress), cycloheximide (translational stress), tunicamycin (endoplasmic reticulum stress), and so on, elevated the chromosome loss rate to a level similar to that caused by benomyl, a microtubule inhibitor (Fig. 1a). Surprisingly, radicicol, an Hsp90 inhibitor20, was by far the most effective CIN inducer: the chromosome loss rate (7.4 × 10⁻² per cell division) was hundreds of
times above the control (2 × 10^{-4} per cell division), even at a radicicol concentration (10 μg ml^{-1} or 27 μM) with only a minor effect on growth (Fig. 1a and Supplementary Fig. 3). Quantitative polymerase chain reaction (qPCR) confirmed that red colonies induced by radicicol had lost the whole chromosome fragment (Supplementary Fig. 4a). Two of the thirteen tested red colonies were confirmed to have also gained chromosome (Chr) X or Chr XI (Supplementary Fig. 4b, c).

A similar aneuploidy-inducing effect was also observed with macbecin II, a structurally distinct Hsp90 inhibitor (Fig. 1b). Deletion of one copy of the Hsp90 genes, HSP82, led to enhanced chromosome fragment loss compared to the wild type in the presence of radicicol or macbecin II (Fig. 1b). Interestingly, deletion of STT1, the yeast homologue of mammalian Hop and a co-chaperone of Hsp90, resulted in significantly elevated CIN even at a concentration of radicicol too low to induce CIN on its own (Fig. 1b and Supplementary Fig. 5a). Heat is a common environmental stress known to tax Hsp90 function. Heat shock for 90 s at 50.9°C resulted in significantly elevated CIN even at a concentration of radicicol or macbecin II, a structurally distinct Hsp90 inhibitor (Fig. 1b).

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We next investigated whether the karyotype diversity produced by Hsp90 stress-induced CIN could fuel adaptation to various other stress conditions. A karyotypically mosaic yeast cell population (~1/3 of the population were aneuploid with different karyotypes; Supplementary Fig. 8c) was generated by growing a diploid strain under moderate Hsp90 stress (20 μg ml⁻¹ radicicol) for 2 days. This population was then tested for enhanced adaptability towards other stress conditions, including the presence of growth inhibiting concentrations of fluconazole,

**Figure 3** Prior Hsp90 inhibition potentiates adaptation to other stress conditions through divergent aneuploid karyotypes. **a**, Plates of vehicle pre-treated group and radicicol pre-treated groups on different media as indicated. Approximately forty cells were plated on DMSO (Ctrl); ~40,000 cells were plated onto each drug plate. Ben, 30 μg ml⁻¹ benomyl; FL, 32 μg ml⁻¹ fluconazole; Tun, 2.5 μg ml⁻¹ tunicamycin. **b**, Quantification of the number of viable colonies. Data are shown as mean ± s.e.m. from triplicate experiments. **c**, The sizes of all colonies (including both radicicol pre-treated and vehicle pre-treated groups) grown on each type of plates were measured. The distributions of the top 10% largest colonies between the two groups are shown. *P < 0.05; **P < 0.01, two-tailed paired t-test. **d**, The karyotypes of 6 vehicle pre-treated colonies from 3 replicate experiments of each type as determined by qPCR. **e**, The karyotypes of 6 independent radicicol pre-treated colonies from 3 replicate experiments of each type determined by qPCR. Arrowheads point to aneuploid chromosomes whose gain or loss frequency among resistant colonies was significantly higher than the starting populations (P < 0.01, Mantel-Haenszel tests, including data on Supplementary Fig. 8e).
tunicamycin or benomyl, over a control homogeneous euploid population (see experimental scheme in Supplementary Fig. 8a). The radicil pre-treated population did not show any growth advantage over the control diploid (vehicle pre-treated) population on drug-free plates (Fig. 3a). However, on each of the different drug-containing plates, the radicil pre-treated populations demonstrated markedly enhanced colony viability and increased frequency to form large drug-resistant colonies compared to the vehicle pre-treated population (Fig. 3a–c).

Twenty-one colonies were picked from the vehicle control plates bearing the radicil pre-treated population, and out of these 12 were aneuploid, whereas none (0/9) from the control plate bearing the vehicle pre-treated population were aneuploid (Fig. 3d, e and Supplementary Fig. 8d, e). The vast majority (17/18) of the large colonies karyotyped from the drug plates bearing the radicil pre-treated population were aneuploid (Fig. 3e). The drug-resistant colonies from the vehicle pre-treated population were also aneuploid (Fig. 3d). Importantly, the aneuploid colonies resistant to the same drug showed obvious karyotypic commonalities and tended to cluster together on the basis of karyotype similarity (Fig. 3e and Supplementary Fig. 9).

For example, four of the five aneuploid colonies from the tunicamycin plates karyotyped gained an extra copy of Chr VIII, which carries ERG11, encoding an ergosterol biosynthetic enzyme known to confer fluconazole resistance in Candida albicans. Losing a copy of Chr XVI is a predominant karyotype change among the tunicamycin-resistant colonies (seen in 10/12 karyotyped colonies; Fig. 3d). Of the 12 benomyl-resistant colonies, 10 demonstrated karyotype clustering with 6 of them losing one Chr XII, but it appears that more than one karyotypic pattern could confer benomyl resistance. This, however, is consistent with our previous observation of phenotypic convergence of distinct karyotypic patterns. All the above common karyotype features were significantly (Mantel–Haenszel tests) enriched in drug-resistant colonies but not the starting radicil pre-treated population before selection on drug plates (Fig. 3d, e and Supplementary Fig. 8e), suggesting an association of specific karyotypes with resistance to certain drugs.

To assess further the selective advantage of aneuploidy and karyotype dynamics under varying stress levels, two Chr XVI monosomy colonies (Parent A and Parent B) from a tunicamycin plate were streaked on drug-free plates. Colonies of two distinct sizes emerged, with the small ones being predominant (Fig. 4a). Karyotyping showed that the small colonies represented Chr XVI monosomy, whereas the rare large colonies had gained back the missing Chr XVI and returned to diploid (Fig. 4b and Supplementary Fig. 10a). Tunicamycin resistance was tightly linked to Chr XVI monosomy: all of the small colonies were tunicamycin resistant whereas the growth of the big colonies was abolished by tunicamycin (Fig. 4c and Supplementary Fig. 10b–d). This result shows that an adapted aneuploid population also has the potential to return to a euploid state when the stress condition is attenuated, suggesting that aneuploidy is not only a readily accessible mutation with large phenotypic impacts but that it is also reversible.

Taken together, the above results demonstrated that stress-induced CIN, leading to aneuploidy, is a mechanism of stress-induced mutagenesis in eukaryotes with high adaptive value to diverse perturbations (Supplementary Fig. 1). Hsp90 inhibition is by far the most potent inducer of aneuploidy among the stress conditions tested. This may be due to a broad but critical involvement of Hsp90 in pathways governing chromosome transmission fidelity and cell division. For example, the mitotic checkpoint gene MAD2 is a genetic interaction hub sensitive to Hsp90 perturbation. MAD2 deletion was also sufficient to lead to the rapid emergence of fluconazole-resistant colonies bearing an extra copy of Chr VIII (Supplementary Fig. 11). As Mad2 requires the CBF3 complex for its activity at the kinetochore, the exceptionally high-level CIN induced by Hsp90 inhibitors may be explained by a combined effect of interference with both kinetochore assembly and the checkpoint monitoring spindle defects. It is presently unknown whether the other stress conditions induce CIN through similar or different cellular targets.

The Hsp90 chaperone complex specializes in modulating the stability and function of many important regulatory and structural proteins. As a result, Hsp90 acts as a capacitor facilitating evolutionary adaptation by unleashing the effects of pre-existing mutations when Hsp90 activity is taxed under mild stress. Strong Hsp90 inhibition also induces phenotypic variation through transposon activation in Drosophila. The results presented in this work reveal a new role for Hsp90 in adaptive evolution—as the guardian of chromosomal stability, the inhibition of which could trigger de novo karyotypic diversity, leading to rapid adaptation through aneuploidy. We note that our observed induction of aneuploidy required more potent Hsp90 inhibition than that required to reveal phenotypic effects of pre-existing mutations. As the function of the Hsp90 chaperone complex in kinetochore assembly is conserved in mammalian species, Hsp90 stress-induced aneuploidy may be a mechanism of cellular adaptation affecting a wide range of organisms.

![Figure 4](image_url) **Figure 4 | Karyotype requirement and dynamics associated with tunicamycin resistance.** a, b, Chr XVI monosomy (small colonies (arrow)) is unstable and produces large euploid progenies (arrowhead). Shown are representative images of the colonies (observed after 3 days growth on YPD) (a) and karyotypes of the Parent A and progeny colonies (A1–3) determined by qPCR (b). V-tun-1a, vehicle-pre-treated and tunicamycin-resistant 1a. c, Chr XVI monosomy progenies (A1 and A2) but not euploid progeny (A3) showed tunicamycin resistance. Note that the size difference between small and large colonies on control plates was no longer apparent after 7 days growth. See Supplementary Fig. 10 for data on Parent B.
METHODS SUMMARY

Yeast strains are listed in Supplementary Table 2. Standard genetic techniques were used for yeast strain construction. All deletions were verified by genomic PCR, and all aneuploid transformants were re-karyotyped by qPCR, and those retaining the original karyotype were used for experiments. Yeast qPCR karyotyping was performed as previously described. Briefly, the chromosome copy number was inferred from qPCR with sets of primers located on peri-centric regions. Array-based comparative genomic hybridization (aCGH) was performed on a home-made spot array.

A detailed description of all methods is provided in Supplementary Information.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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