

persist. Fruit flies, on the other hand, have their idiosyncrasies (the absence of recombination in males, for example), but none that seem likely to predispose their populations to harboring incompatible variants. In any case there may be no coherent model of speciation that can apply accurately to such widely varying life cycles.

Chromosomal rearrangements are among the mechanisms that genetically isolate well-established species of *Saccharomyces*. Some mate freely with others, but the hybrids are generally sterile — in part due to reciprocal translocations [5]. The distribution of documented chromosome rearrangements among *Saccharomyces* species has not suggested a decisive role in speciation, but if other yeast species are also chromosomally diverse, those analyses may have oversimplified. Genome-wide sequence differences between yeast species can also cause hybrid sterility, by preventing the recombination between chromosomes that is required for successful meiosis. Hou *et al.* [1] suggest that this barrier may have caused the weaker incompatibility with S288c they saw in six other strains. But that genome-wide divergence accumulated after speciation. More likely to start the process is two-gene incompatibility, like the disruption of a regulatory nuclear-mitochondrial interaction in hybrids between *S. cerevisiae* and *S. bayanus* [6].

Evolutionary geneticists will be curious about the role of selection in the origin and spread of irreconcilable chromosome pairs. In ecological

models of speciation, genetic incompatibility arises because allele combinations that are adaptive in one niche are maladaptive in the other, and hybrids have low fitness in both. The first steps of this process were evident after just 500 generations in one yeast evolution experiment [7]. The nuclear-mitochondrial incompatibility between *S. cerevisiae* and *S. bayanus* noted above may have resulted from specialization for different carbon sources [6]. Among the adaptive mutations identified in other evolution experiments were chromosomal rearrangements that duplicated genes known to be involved in the uptake or metabolism of a limiting nutrient, or altered their regulation [8]. Preventing incompatibility within a lineage by making such rearrangements homozygous may be one advantage of inbreeding. None of the three reciprocal translocations documented by Hou *et al.* [1] looks obviously beneficial, although one of them separates two genes from their promoter regions, which might alter their expression.

The next step towards speciation would be the evolution of an aversion towards mating with each other. Such discrimination is adaptive if it keeps otherwise successful genotypes out of hybrid combinations with bleak prospects, and can evolve very quickly when artificially selected for [9]. The question is whether incompatible genotypes encounter each other often enough for mate choice to matter much. It's still hard to determine the genetic structure of natural yeast

populations (they're invisible) but it might be interesting to see how these 60 strains behave given the freedom to choose their mates.

#### References

- Hou, J., Friedrich, A., de Montigny, J., and Schacherer, J. (2014). Chromosomal rearrangements as a major mechanism in the onset of reproductive isolation in *Saccharomyces cerevisiae*. *Curr. Biol.* 24, 1153–1159.
- Seidel, H.S., Rockman, M.V., and Kruglyak, L. (2008). Widespread genetic incompatibility in *C. elegans* maintained by balancing selection. *Science* 319, 589–594.
- Corbett-Detig, R.B., Zhou, J., Clark, A.G., Hartl, D.L., and Ayroles, D. (2013). Genetic incompatibilities are widespread within species. *Nature* 504, 135–139.
- Tsai, I.J., Bensasson, D., Burt, A., and Koufopanou, V. (2008). Population genomics of the wild yeast *Saccharomyces paradoxus*: Quantifying the life cycle. *Proc. Natl. Acad. Sci. USA* 105, 4957–4962.
- Fischer, G., James, S.A., Oliver, S.G., and Louis, E.J. (2000). Chromosomal evolution in *Saccharomyces*. *Nature* 405, 451–454.
- Lee, H.Y., Chou, J.Y., Cheong, L., Chang, N.H., Yang, S.Y., and Leu, J.Y. (2008). Incompatibility of nuclear and mitochondrial genomes causes hybrid sterility between two yeast species. *Cell* 135, 1065–1073.
- Dettman, J.R., Sirjusingh, C., Kohn, L.M., and Anderson, J.B. (2007). Incipient speciation by divergent adaptation and antagonistic epistasis in yeast. *Nature* 447, 585–588.
- Dunham, M.J., Badrane, H., Ferea, T., Adams, J., Brown, P.O., Rosenzweig, F., and Botstein, D. (2002). Characteristic genome rearrangements in experimental evolution of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 99, 16144–16149.
- Leu, J.Y., and Murray, A.W. (2006). Experimental evolution of mating discrimination in budding yeast. *Curr. Biol.* 16, 280–286.

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## Gene Regulation: The HSP70 Gene Jumps When Shocked

Limited chromosome mobility has been observed in mammalian interphase nuclei. Live imaging shows unidirectional and actin-dependent movement of HSP70 loci towards speckles upon heat shock, resulting in enhanced transcription. This adds further impetus to understanding compartmentalization of function in the nucleus.

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Transcription is the first step of gene expression. High throughput

techniques and biochemical studies of specific genes have shown that there is an intricate machinery of transcription factors, chromatin and DNA

modifications coordinating the rate and time of transcription of a given gene [1,2]. Eukaryotes have an added layer of regulation to manage transcription: the localization of the gene locus in the compartmentalized nucleus. Individual chromosomes reside in limited regions of the interphase nucleus known as chromosome territories [3]. Microscopy approaches have suggested that transcription is enhanced at specific nuclear compartments, like speckles, where genes might cluster [4,5]. Hence, dynamic changes in chromosome

position could affect gene expression in the interphase cell. However, live imaging in mammalian cells has indicated limited chromatin mobility restricted to constrained diffusion [6]. In this issue of *Current Biology*, Khanna *et al.* [7] directly observed, in a set of compelling movies, the inducible repositioning of chromatin loci between two different nuclear compartments, and these movements correlated with transcription.

Khanna *et al.* [7] measured heat shock protein 70 (HSP70) loci movement from the nuclear membrane towards speckles upon induction of transcription by heat shock. HSP70 expression, barely detectable under normal growth conditions, is dramatically up-regulated within minutes of heat shock [8,9]. Both transcription activation and association of HSP70 loci with nuclear speckles are characteristics mimicked by the HSP70 bacterial artificial chromosome (BAC) and plasmid transgene array used by the Belmont group [10]. The authors engineered these constructs, firstly, to localize HSP70 loci in the nucleus by inserting 64-mer lac operator sites, allowing detection with GFP-lac repressor [11] and, secondly, to label nascent mRNA transcripts by cloning 24 MS2 repeats into the 5'UTR of the HSP70 gene, which could be detected by Cherry-MS2 coated protein [12]. Speckles were visualized by labeling the nuclear speckle protein SON and nuclear rotation was controlled by CENFA-mCherry labeled centromeres. To distinguish long-range from constrained diffusion motions, the authors chose a cell clone in which ~70% of the HSP70 transgene was positioned at the nuclear periphery not close to any speckle before heat shock. Using this fluorescent tagging of DNA, RNA and proteins, and the Applied Precision OMX microscope, they visualized HSP70 transgenes moving unidirectionally along curvilinear paths towards nuclear speckles over 0.5–6  $\mu\text{m}$  distances at velocities of 1–2  $\mu\text{m}$  per minute. The final consequence of this direct inward motion was the association of the HSP70 array with a speckle followed by the accumulation of HSP70 transcripts (Figure 1). Therefore, Khanna *et al.* [7] demonstrate that chromatin movements can precede transcription.

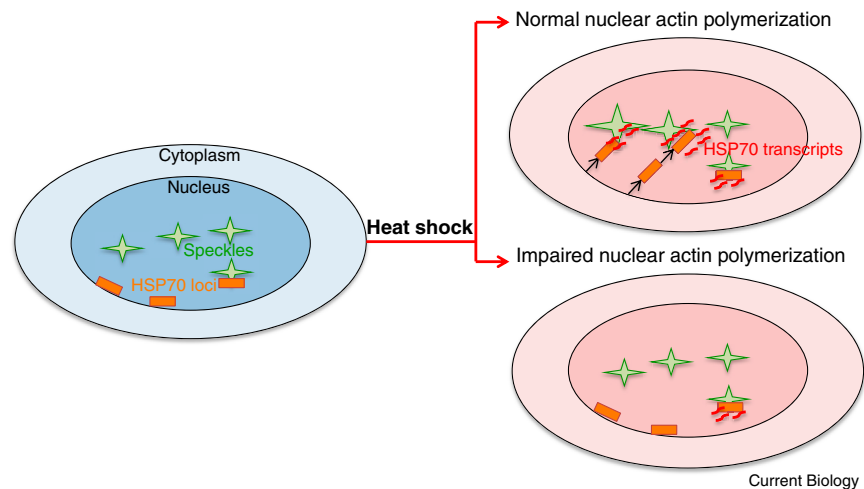


Figure 1. HSP70 loci on the move to be transcribed.

Under normal growth conditions (blue cell on the left), HSP70 is poorly transcribed and its loci can be positioned either close to a speckle or the nuclear membrane. Upon heat shock, HSP70 loci on the membrane move inwards following a unidirectional curvilinear path until they reach a speckle. Once the HSP70 locus associates with a speckle the nascent transcripts are detected (upper red cell). Impaired nuclear actin polymerization compromises the motion of HSP70 loci and their transcription. Under impaired actin polymerization conditions, only HSP70 loci already associated with a speckle get transcribed (lower red cell).

The rapid unidirectional movements suggest the presence of an active mechanism regulating long-range interphase chromosomal trajectories. This hypothesis is additionally supported by the observation of chromatin stretching in the direction of the movement preceding 40% of the long-range movements (>0.5  $\mu\text{m}$ ) [7]. The work from Belmont and other groups pointed to nuclear actin and nuclear myosin 1 (NM1) as components of the active interphase chromosomal motion [13,14]. Specifically, they showed that depolymerization of F-actin or the expression of a nonpolymerizable NLS-RFP-actin mutant decreases the speckle association of the HSP70 transgene and its transcription (Figure 1).

The nuclear components behind the actin polymerization process are still unknown. Since the association of HSP70 loci with speckles depends on the HSP70 promoter and is independent of the transcribed sequence, these actin regulators could be related to promoter-associated factors [10]. Furthermore, it remains to be addressed if the activation of HSP70 transcription happens before or after the HSP70 locus starts to move towards speckles. Live cell microscopy revealed that in ~96%

of the movements of HSP70 transgene to nuclear speckles, the transcription signal from the Cherry-MS2 coated protein first increased after initial contact of the transgene array with a nuclear speckle [7]. Nonetheless, binding of the specific transcription factor HSF1 to the HSP70 promoter and activation of transcription could precede the detection of the transcript and induce the motion towards speckles. When actin polymerization was impaired, HSP70 transcripts were only detected above background in the transgene arrays already in contact with a nuclear speckle (Figure 1) [7]. Thus, the association of the HSP70 locus to a speckle is directly or indirectly actin-dependent and contributes to its transcription.

Speckles, first described as storage/modification sites of the splicing machinery, may also be enriched in gene activation factors. In fact, they contain serine 2 phosphorylated RNA polymerase II (elongation form) and other components of the transcription machinery and ~25–50% of active transcribed genes associate with speckles [15]. Khanna *et al.* [7] demonstrated a positive relationship between the speckle size and the timing of HSP70 transcript detection upon heat shock. When higher levels of transcription have been reached,

speckle size increased in parallel with transcript accumulation, suggesting a functional connection between them [7]. However, in the case of HSP70, this functional connection between the gene locus and the speckle is independent of pre-mRNA splicing because the HSP70 gene lacks introns. Also, this functional association seems to depend on the particular stress conditions. The Belmont group has previously demonstrated that the induction of HSP70 transcription after stress using cadmium treatment, instead of heat shock, is independent of HSP70 locus association with the speckle. Interestingly, heat shock does not promote the relocation of other BACs containing different inducible promoters, like the metallothionein promoter, to the speckle [10,16]. These observations suggest that the nuclear relocation of specific genes to speckles plays a role in their transcription regulation but likely this regulation only applies to specific inducible genes when expressed under certain physiological conditions.

The interphase eukaryotic nucleus contains the genetic material organized into chromosomes and different subnuclear compartments known as nuclear bodies. The absence of membrane around the nuclear bodies facilitates the exchange of components with the nucleoplasm [15]. These dynamics seem to be counterbalanced by the limited, constrained movement of interphase chromosomes observed in mammalian cells. This restricted chromatin movement is not consistent with observations of multiple specific genes around a common nuclear

speckle [4,5]. Also, recent 3C (chromosome capture conformation) methods have identified interactions between distant gene loci and even different chromosomes [17]. Khanna *et al.* [7] have overcome physiological and phototoxicity limitations to visualize long-range interphase chromatin movements in live mammalian cells. They convincingly demonstrate, quantify and analyze the curvilinear direct motion of the HSP70 locus from the nuclear membrane and its transcript accumulation, once it associates with a speckle. This technology together with the tagging of endogenous loci with the CRISP/Cas system [18] will provide an important step towards understanding relationships within the nucleus, their dynamic interactions and physiological relevance.

#### References

1. Goodrich, J.A., and Tjian, R. (2010). Unexpected roles for core promoter recognition factors in cell-type-specific transcription and gene regulation. *Nat. Rev. Genet.* 11, 549–558.
2. Patel, D.J., and Wang, Z. (2013). Readout of epigenetic modifications. *Annu. Rev. Genet.* 82, 81–118.
3. Cremer, T., Cremer, M., Dietzel, S., Muller, S., Solovei, I., and Fakan, S. (2006). Chromosome territories—a functional nuclear landscape. *Curr. Opin. Cell Biol.* 18, 307–316.
4. Osborne, C.S., Chakalova, L., Mitchell, J.A., Horton, A., Wood, A.L., Bolland, D.J., Corcoran, A.E., and Fraser, P. (2007). Myc dynamically and preferentially relocates to a transcription factory occupied by Igh. *PLoS Biol.* 5, e192.
5. Shopland, L.S., Johnson, C.V., Byron, M., McNeil, J., and Lawrence, J.B. (2003). Clustering of multiple specific genes and gene-rich R-bands around SC-35 domains: evidence for local euchromatic neighborhoods. *J. Cell Biol.* 162, 981–990.
6. Chubb, J.R., Boyle, S., Perry, P., and Bickmore, W.A. (2002). Chromatin motion is constrained by association with nuclear

- compartments in human cells. *Curr. Biol.* 12, 439–445.
7. Khanna, N., Hu, Y., and Belmont, A.S. (2014). Hsp70 transgene directed motion to nuclear speckles facilitates heat shock activation. *Curr. Biol.* 24, 1138–1144.
8. Jolly, C., Vourc'h, C., Robert-Nicoud, M., and Morimoto, R.I. (1999). Intron-independent association of splicing factors with active genes. *J. Cell Biol.* 145, 1133–1143.
9. Lindquist, S., and Craig, E.A. (1988). The heat-shock proteins. *Annu. Rev. Genet.* 22, 631–677.
10. Hu, Y., Plutz, M., and Belmont, A.S. (2010). Hsp70 gene association with nuclear speckles is Hsp70 promoter specific. *J. Cell Biol.* 191, 711–719.
11. Belmont, A.S. (2001). Visualizing chromosome dynamics with GFP. *Trends Cell Biol.* 11, 250–257.
12. Darzacq, X., Shav-Tal, Y., de Turris, V., Brody, Y., Shenoy, S.M., Phair, R.D., and Singer, R.H. (2007). In vivo dynamics of RNA polymerase II transcription. *Nat. Struct. Mol. Biol.* 14, 796–806.
13. Chuang, C.H., Carpenter, A.E., Fuchsova, B., Johnson, T., de Lanerolle, P., and Belmont, A.S. (2006). Long-range directional movement of an interphase chromosome site. *Curr. Biol.* 16, 825–831.
14. Visa, N., and Percipalle, P. (2010). Nuclear functions of actin. *Cold Spring Harb. Perspect. Biol.* 2, a000620.
15. Mao, Y.S., Zhang, B., and Spector, D.L. (2011). Biogenesis and function of nuclear bodies. *Trends Genet.* 27, 295–306.
16. Hu, Y., Kireev, I., Plutz, M., Ashourian, N., and Belmont, A.S. (2009). Large-scale chromatin structure of inducible genes: transcription on a condensed, linear template. *J. Cell Biol.* 185, 87–100.
17. Tanizawa, H., and Noma, K. (2012). Unravelling global genome organization by 3C-seq. *Semin. Cell Dev. Biol.* 23, 213–221.
18. Chen, B., Gilbert, L.A., Cimini, B.A., Schnitzbauer, J., Zhang, W., Li, G.W., Park, J., Blackburn, E.H., Weissman, J.S., Qi, L.S., *et al.* (2013). Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. *Cell* 155, 1479–1491.

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## Paleontology: A New Burgess Shale Fauna

A spectacular Cambrian soft bodied fauna some 40 km from Walcott's original Burgess Shale locality includes over 50 taxa, some 20% new to science. New anatomical evidence from this site will illuminate the evolution of early marine animals.

Derek E.G. Briggs

Charles Walcott's discovery of the famous Burgess Shale in the Canadian

Rockies in 1909 begins with a legendary but probably apocryphal story [1]: his wife's horse stumbled over a rock on the trail below the level where

the fossil-bearing layers crop out. Walcott dismounted and split the rock to reveal what he recognized as exceptionally preserved fossils. Walcott's party worked up the incline until they found the productive beds and over the next several years they quarried out some 60,000 specimens, which now form the Burgess Shale collection at the Smithsonian Institution in Washington. Following Walcott's efforts, the prevalent view for years was that the productive layers had been worked out and there was