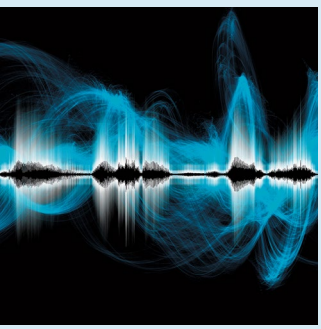
ORP2 residues that abrogated tetramer formation without affecting ligand binding substantially reduced sterol and $PtdInsP_2$ transport in vitro and in cells. Thus, the formation of a stable tetramer of ORP2 is likely crucial for lipid exchange by ORP2.

Furthermore, mutation of residues that are important for $PtdInsP_2$ binding reduced the efficiency of sterol transport in vitro, and mutation of cholesterol-binding residues in ORP2 reduced sterol-stimulated $PtdInsP_2$ transport. Thus, ORP2 binding of both sterols and $PtdInsP_2$ is required for efficient lipid transfer.

In the future, it will be interesting to determine whether other ORPs possess exchange activity and how the activity of these proteins is coordinated to efficiently mediate lipid trafficking between cellular compartments.

Grant Otto

ORIGINAL ARTICLE Wang, H. et al. ORP2 delivers cholesterol to the plasma membrane in exchange for phosphatidylinositol 4, 5-bisphosphate (PI(4,5)P2). *Mol. Cell* **73**, 1–16 (2019)
FURTHER READING Wong, L. H. et al. Lipid transfer proteins: the lipid commute via shuttles, bridges and tubes. *Nat. Rev. Mol. Cell Biol.* https://doi.org/10.1038/s41580-018-0071-5 (2018)



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frequency, but not in burst size, in fibroblasts had a higher density of single nucleotide polymorphisms in their enhancers but not their promoters. These data suggest that enhancers regulate transcription burst frequency. A comparison of wild-type ESCs and those lacking an enhancer of the pluripotency gene *Sox2* on one allele revealed a significantly reduced burst frequency for the affected allele, providing direct functional evidence that enhancers regulate burst frequency.

The dissection of transcription burst kinetics enabled by single-cell allelic expression analysis should facilitate further mechanistic insights into the regulation of transcription by cis elements and trans-acting factors.

Grant Otto

ORIGINAL ARTICLE Larsson, A. J. M. et al. Genomic encoding of transcriptional burst kinetics. *Nature* **565**, 251–254 (2019)
FURTHER READING Haberle, V. & Stark, A. Eukaryotic core promoters and the functional basis of transcription initiation. *Nat. Rev. Mol. Cell Biol.* **19**, 621–637 (2018)

Journal club

A MAD WAY TO REGULATE MITOSIS

The transfer of information that regulates biological processes involves conformational changes in a network of macromolecules. Towards the end of the last century, the concept of allostery was predominant — whereby the modulation of protein conformations and assembly of macromolecular complexes is associated with a change of composition of the regulated macromolecule, through either the binding of a ligand or a covalent chemical modification.

In the 2000s a new concept for generating intracellular signals was proposed to explain how unattached kinetochores create a signal to arrest the cell cycle in mitosis, preventing premature anaphase onset and aneuploidy. This arrest of the cell cycle is accomplished through activation of the spindle assembly checkpoint (SAC) pathway, the role of which is to inhibit the APC/C that regulates chromosome segregation.

Components of the SAC include MAD1, MAD2 and the APC/C coactivator CDC20. MAD2 and CDC20 are components of the mitotic checkpoint complex (MCC) — the SAC effector and APC/C inhibitor. Unattached kinetochores catalyse the assembly of the MCC. MAD1 interacts with MAD2, and this is required for formation of the MAD2–CDC20 complex, but, paradoxically, structural studies showed that MAD1 and CDC20 bind to the same site on MAD2. How then does MAD1 activate MAD2 to bind CDC20? Insights to this answer came from the discovery that MAD2 can adopt two dramatically different conformations: inactive O-MAD2 and active C-MAD2. Conversion of O-MAD2 into C-MAD2 involves a conformational change that creates a binding site for the ligands of MAD2. Spontaneous conversion of O-MAD2 into C-MAD2 occurs at an extremely slow rate. This contrasts with the rapid response of unattached kinetochores to trigger the checkpoint, indicating that unattached kinetochores act as catalysts to promote MCC assembly.

Combining these findings into a single elegant model, Musacchio and colleagues proposed the template model for MAD2 activation in which cytosolic O-MAD2 is activated at the kinetochore by a MAD1–C-MAD2 complex. C-MAD2 acts as a template to catalyse conversion of O-MAD2 into C-MAD2, which then forms the C-MAD2–CDC20 complex.

The conceptual novelties of the template model are that conformational transitions of proteins are uncoupled from direct chemical and compositional changes, and that a specific protein conformer (template) promotes the structural conversion of an identical molecule, differing only in architecture, into the same conformation as the template. In some respects the template model is reminiscent of prion-induced conformational changes and the seeded formation of neurodegenerative amyloid fibrils. The differences are that the MAD2 conversion is reversible, and the system lacks an amplification step.

Can this signalling paradigm be generalized to other systems? MAD2 belongs to the HORMA family of proteins and this includes the meiotic HORMADs, which form the synaptonemal complex and are likely to undergo conformational changes related to MAD2. Other signalling systems mediated by catalysed inter-conversion of structural states can also be envisioned. For example, the cis-trans peptidy-prolyl isomerase Pin1 has been implicated in numerous regulatory functions, one of which is regulation of separase, the enzyme that triggers chromosome segregation.

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ORIGINAL ARTICLES De Antoni, A. et al. The Mad1/Mad2 complex as a template for Mad2 activation in the spindle assembly checkpoint. *Curr. Biol.* **15**, 214–225 (2005) | Luo, X. et al. The Mad2 spindle checkpoint protein has two distinct natively folded states. *Nat. Struct. Mol. Biol.* **11**, 338–345 (2004)