DNA checkpoints in fission yeast

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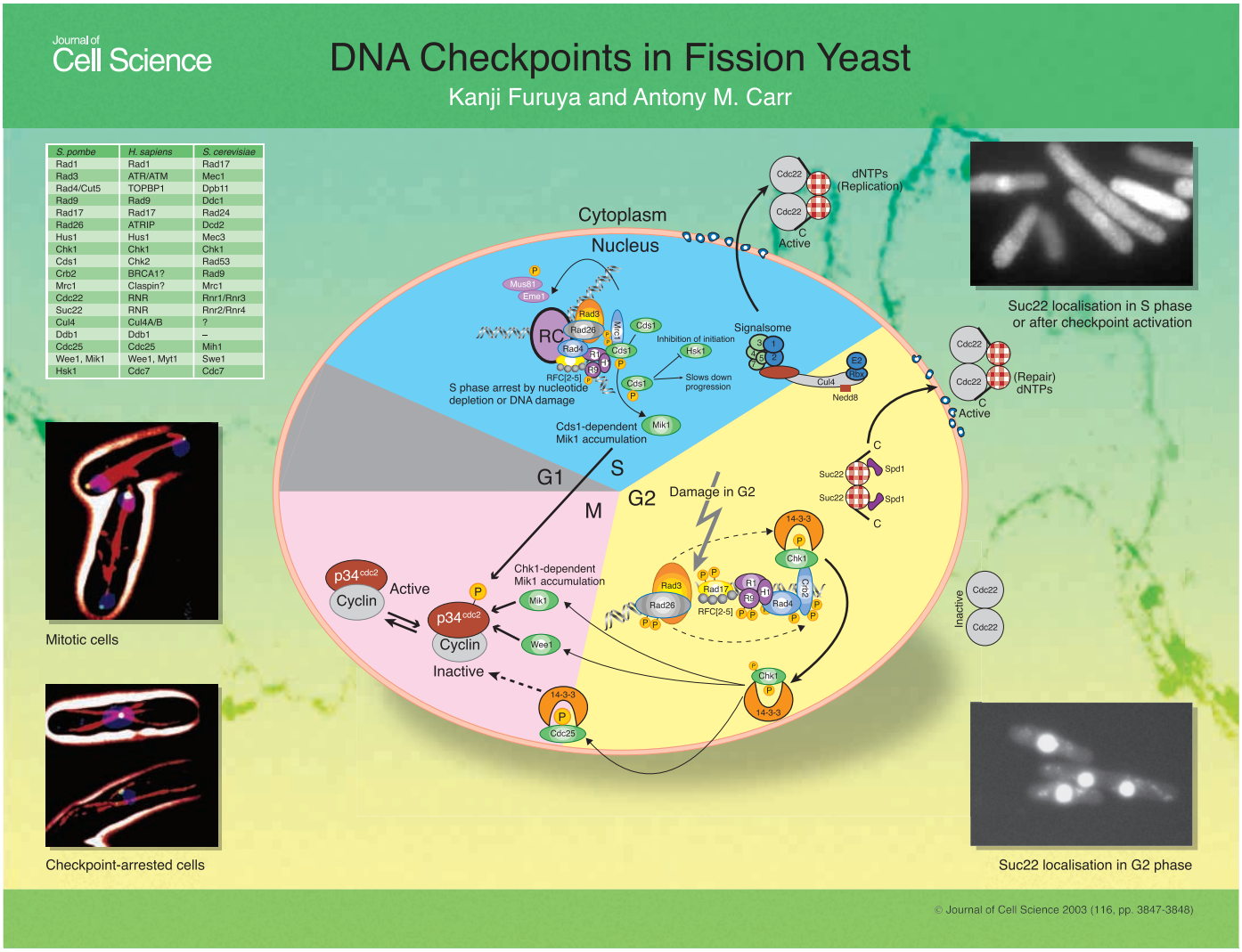
The DNA integrity checkpoints ensure completion of DNA replication and DNA repair before entry into mitosis (O'Connell et al., 2000). Unreplicated DNA or unrepaired DNA will cause catastrophic chromosome segregation. The accompanying schematic shows the checkpoint proteins that monitor the state of DNA replication and DNA repair.

Checkpoint proteins most probably interact with the replication complex (RC) during S phase (blue wedge) or with DNA strand breaks if they are induced during G2 phase (yellow wedge). These checkpoint proteins ultimately inactivate the Cdc2–cyclin-B kinase complex by inhibitory Tyr15 phosphorylation (O'Connell et al., 2000). High Cdc2–cyclin-B kinase activity allows the cell to enter mitosis (pink wedge), where chromosome separation and segregation occur (see bottom left, upper panel). Checkpoint activation thus causes cell cycle arrest in G2 phase (see bottom left, lower panel). Evolutionary conserved checkpoint proteins (see table) have been classified into several categories, including the ATR-ATRIP kinase complex (Rad3-Rad26), the RFC- and PCNA-like complexes (Rad1, Rad9, Hus1 and Rad17), the mediator proteins (Crb2 and Mrc1) and the effector kinases (Chk1 and Cds1).

In fission yeast, the ATR-ATRIP complex (Rad3-Rad26) phosphorylates many of the other checkpoint proteins, and these events are central to the checkpoint response. Rad3-dependent Chk1 and Cds1 phosphorylation is essential for checkpoint activation (Rhind and Russell, 2000). Rad17, together with RFC2-RFC5, forms a replication-factor-C-like complex and is thought to load a PCNA-like Rad1-Hus1-Rad9 complex onto damaged DNA (Caspari and Carr, 2002). This complex may link Rad3-Rad26 to the mediator complexes, which in turn bring Chk1 and Cds1 into proximity. Different mediator complexes function in S phase and G2 phase. In S phase, Mrc1 mediates



(See poster insert)

Cds1-dependent checkpoint signaling. Activation of Cds1 kinase results in Mik1 accumulation. Mik1 phosphorylates Tyr15 of Cdc2, thus preventing mitosis. Importantly, the Mrc1/Cds1 module has additional roles in the S phase response, such as stabilizing stalled replication forks and inhibiting late-firing replication origins. It also regulates the Mus81-Eme1 endonuclease complex, which was identified through its interaction with the Cds1 fork-head-associated (FHA) domain (Boddy et al., 2000). In G2 phase, Crb2 mediates Chk1-dependent checkpoint signaling. Activated Chk1 results in activation of Mik1 and Wee1 Cdc2 Tyr15 kinases and may inactivate Cdc25 tyrosine phosphatase (O'Connell et al., 2000).

Chk1 is also involved in regulating dNTP synthesis (Liu et al., 2003). Chk1 activation in G2 induces degradation of the Spd1 protein. Spd1 behaves as an inhibitor of ribonucleotide reductase (RNR). Its ubiquitin-dependent degradation is necessary for nuclear export of Suc22 (the small subunit of RNR) during DNA replication in S phase (upper right) and DNA repair in G2 phase. Suc22, which normally localizes to the nucleus in G2 phase cells (bottom right) and the whole cell during normal S phase, presumably forms an active complex with cytoplasmic Cdc22 during DNA replication and in response to checkpoint activation to provide nucleotides for DNA repair. The signalosome complex promotes ubiquitin-dependent degradation of Spd1. The signalosome forms a complex together with the Pcu4 (cullin 4) E3 ubiquitin ligase.

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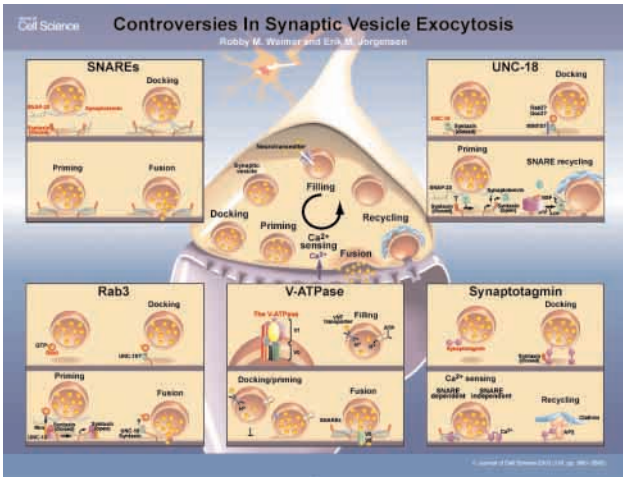

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