Just-in-time assembly of cell-cycle protein complexes

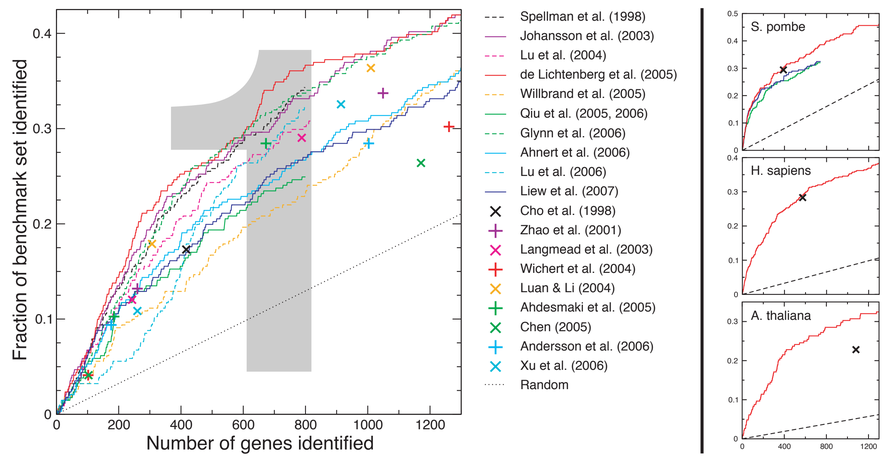
Lars Juhl Jensen[1]*, Ulrik de Lichtenberg[2]*, Thomas Skøt Jensen[2]*, Søren Brunak[2] and Peer Bork[1]

[1] European Molecular Biology Laboratory, Heidelberg, Germany [2] Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark * These authors contributed equally

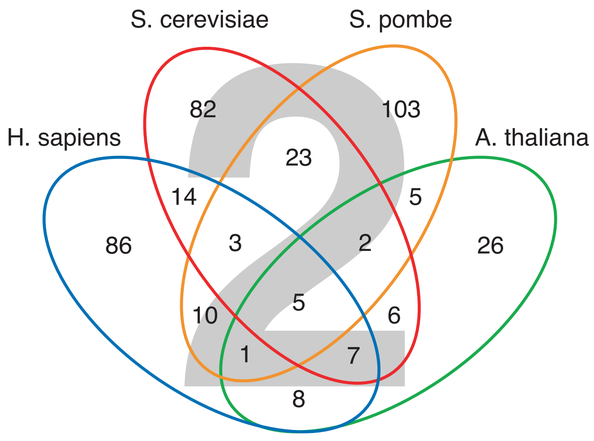
Abstract

DNA microarray studies have shown that hundreds of genes are transcribed periodically during the mitotic cell cycle. We show here that although the protein complexes involved in this process are largely the same among all eukaryotes, their regulation has evolved considerably. Our comparative analysis of several large-scale datasets reveals that the identity of the periodically expressed proteins differs significantly between organisms, yet the regulated subunits of each protein complex are expressed just prior to its time of action. Moreover, we demonstrate that these changes in transcriptional regulation have co-evolved with post-translational control; loss or gain of cell cycle-regulated transcription of specific genes is often mirrored by changes in phosphorylation of the proteins they encode. Our results indicate that many different solutions have evolved for assembling the same molecular machines at the right time during the cell cycle, involving both transcriptional and posttranslational layers that jointly control the dynamics of biological systems.

Identification of periodically expressed genes



Regulation differs between organisms



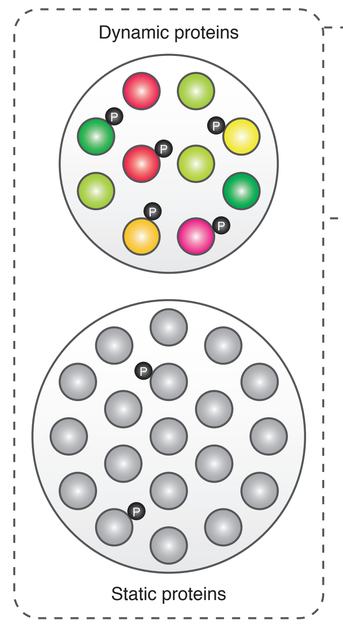
Transcriptional regulation is rarely conserved

To obtain comparable sets of transcriptionally regulated genes from distantly related eukaryotes, we reanalyzed the existing cell cycle gene expression data and found 600 periodically expressed genes in H. sapiens[4], 600 in S. cerevisiae[1,2], 500 in S. pombe[3], and 400 in A. thaliana[4]. Our benchmarks show that these gene lists are more conservative than those originally proposed, yet achieve better sensitivity (estimated to be 80–90% for human and the two yeasts, but only 50% in the plant). Surprisingly, we also discovered that most of the published methods for identifying periodically expressed perform worse than the original analysis by Spellman et al.[1] (Fig. 1)

We further assigned genes with a common descent to orthologous groups, of which 381 groups contain orthologs from all four organisms and have at least one periodic member. We found that periodicity is poorly conserved across the four organisms, meaning that although the protein sequences are conserved through evolution, their transcriptional regulation during the cell cycle is not (Fig 2). The large differences observed cannot be explained by the quality of the gene expression data or the orthology analysis[4].

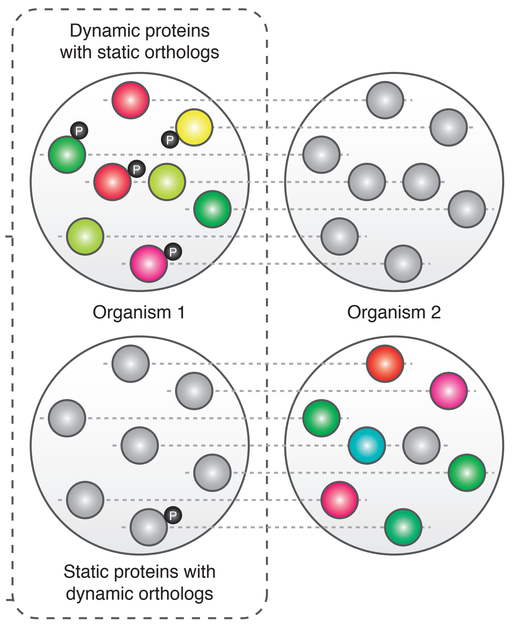
Co-evolution of transcriptional and posttranslational cell-cycle regulation

Linking transcription and phosphorylation



Organism	Data source	P-value
H. sapiens	LTP CDK	$7 \cdot 10^{-9}$
	HTP S/T	$4 \cdot 10^{-16}$
S. cerevisiae	HTP CDK	$2 \cdot 10^{-55}$
S. pombe	NetPhosK	$4 \cdot 10^{-11}$
A. thaliana	NetPhosK	0.03

Organism 1	Data source	Organism 2	P-value
H. sapiens	LTP CDK	S. cerevisiae	$4 \cdot 10^{-3}$
		S. pombe	0.02
		A. thaliana	0.06
	HTP S/T	S. cerevisiae	0.07
		S. pombe	$7 \cdot 10^{-4}$
		A. thaliana	$6 \cdot 10^{-4}$
S. cerevisiae	HTP CDK	H. sapiens	$2 \cdot 10^{-3}$
		S. pombe	$4 \cdot 10^{-3}$
		A. thaliana	$9 \cdot 10^{-3}$
S. pombe	NetPhosK	H. sapiens	0.08
		S. cerevisiae	0.02
		A. thaliana	0.2

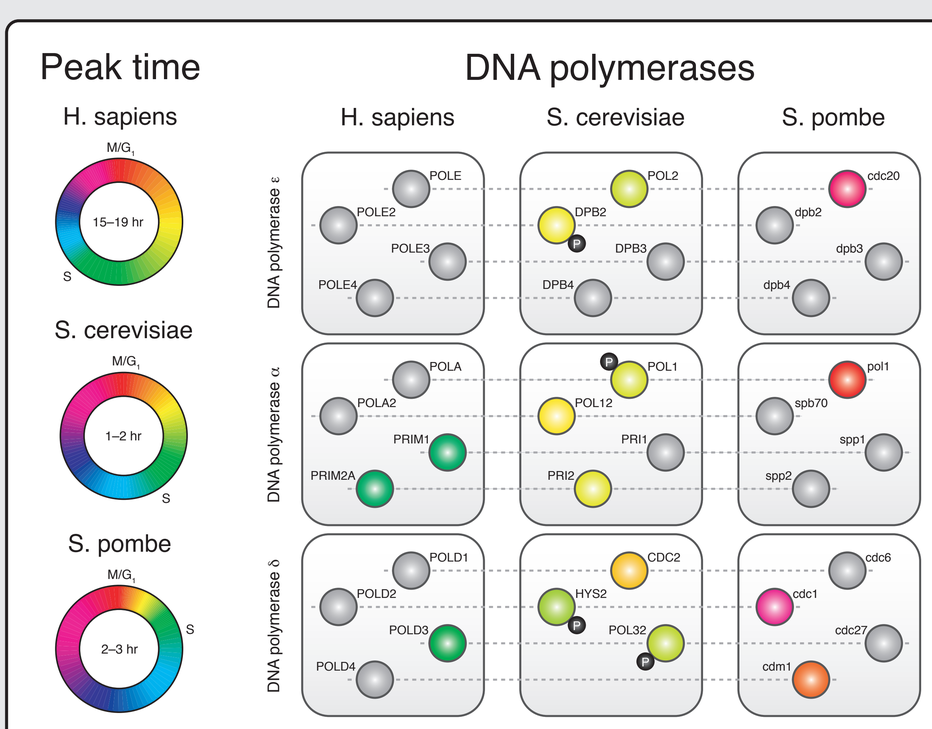


Proving co-evolution of transcriptional regulation and phosphorylation

Multiple layers of regulation have co-evolved

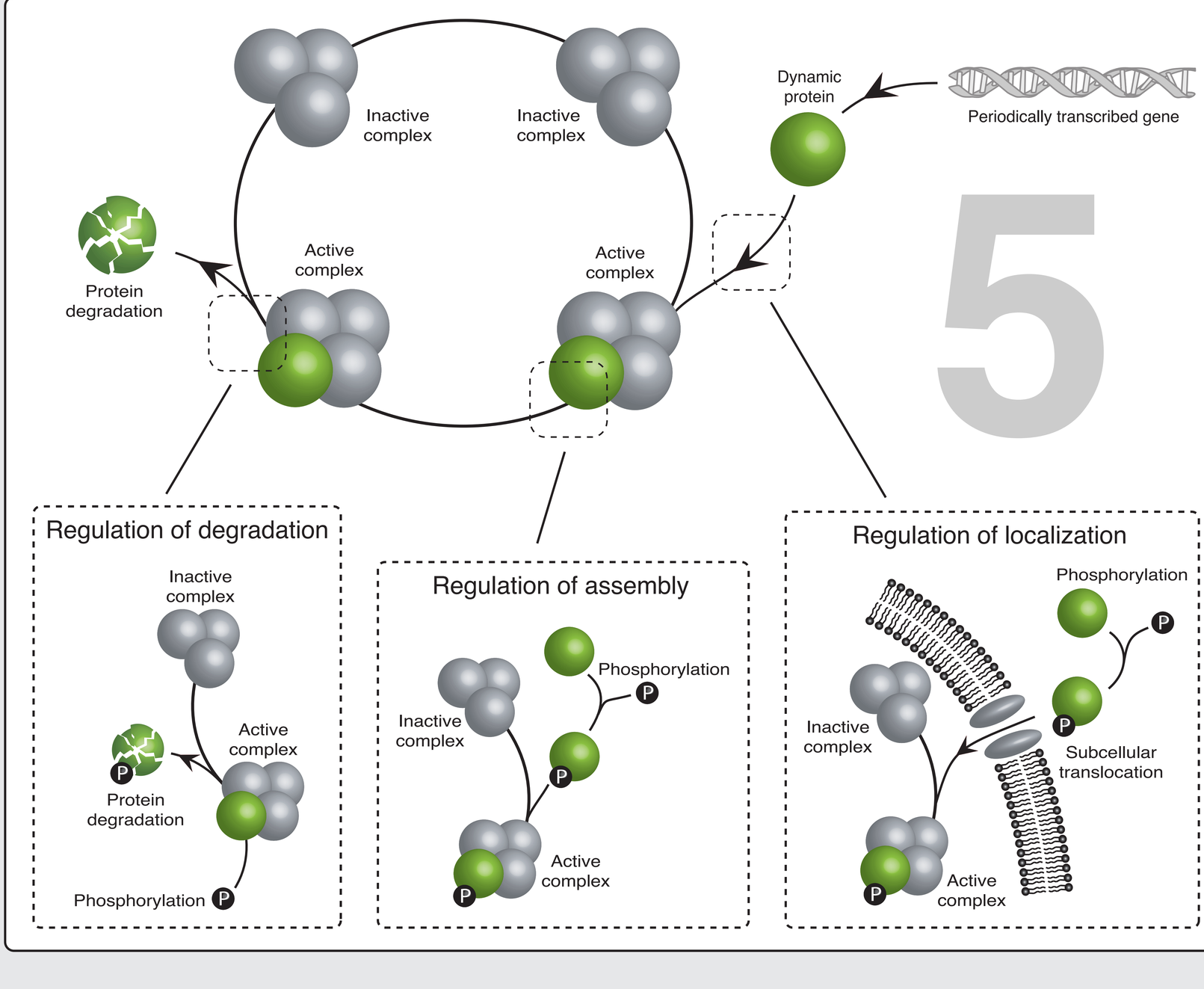
The agreement between the timing of transcription and the timing of action of the protein products is not necessarily to be expected as phosphorylation is known to be at least as important for regulating cell cycle complexes. In this regard, we have previously noted that periodically expressed S. cerevisiae proteins tend to also be phosphorylated. To generalize this observation, we annotated the complexes with information on phosphorylation by CDKs, and found that the dynamic subunits are indeed three times as likely to be targeted by phosphorylation as the static ones ($P < 0.001$)[4].

To assess the evolutionary significance of this correlation, we applied two proteome-wide statistical tests to several independent sets of known and predicted phosphoproteins, which revealed a highly significant overrepresentation of dynamic proteins among the CDK substrates[4] (Fig. 3). To check if this reflects that loss or gain of transcriptional regulation of a gene is correlated with loss or gain of phosphorylation of the corresponding proteins, we compared the dynamic proteins with static orthologs to static proteins with dynamic orthologs and found that transcriptional regulation and phosphorylation have indeed co-evolved[4]. Similar, we find that dynamic proteins are often subject to ubiquitin-mediated targeted degradation by the proteasome[4,5].



The just-in-time assembly principle

In order to find a model, which could explain these apparently conflicting observations, we turned to a principle for regulation of cell-cycle protein complexes in budding yeast that we uncovered recently. By constructing a temporal interaction network of the cell cycle, we found that protein complexes were generally formed as a combination of static (constitutively expressed) and dynamic (periodically expressed) proteins[2]. The latter showed a clear tendency to be expressed right before the complex is known to become active and thus, by their change in abundance, control the assembly of the functional complex. This mechanism, which we termed "just-in-time assembly", is illustrated in Fig. 5. When studying a set of protein complexes that are conserved across all four organisms, we saw that the identity of the dynamic subunits within each complex has changed during evolution (Fig. 4).[4] The just-in-time assembly model also implies that the dynamic proteins must be eliminated at a later stage in the cell cycle, explaining the observed co-evolution between different layers of regulation.



References

[1] U. de Lichtenberg*, L.J. Jensen*, A. Fausbøll, T.S. Jensen, P. Bork and S. Brunak, "Comparison of computational methods for the identification of cell cycle regulated genes", Bioinformatics, 21, 1164–1171, 2005

[2] U. de Lichtenberg*, L.J. Jensen*, S. Brunak and P. Bork, "Dynamic complex formation during the yeast cell cycle", Science, 307, 724–727, 2005

[3] S. Marguerat*, T.S. Jensen*, U. de Lichtenberg, B.T. Wilhelm, L.J. Jensen and J. Bähler, "The more the merrier: comparative analysis of microarray studies on cell cycle-regulated genes in fission yeast", Yeast, 23, 261–277, 2006

[4] L.J. Jensen*, T.S. Jensen*, U. de Lichtenberg*, S. Brunak and P. Bork, "Co-evolution of transcriptional and posttranslational cell cycle regulation", Nature, 433, 594–597, 2006

[5] U. de Lichtenberg, T.S. Jensen, L.J. Jensen, S. Brunak and P. Bork, "Same molecular machines, different regulation", Cell Cycle, advance online publication, 2007

Nature Precedings : doi:10.1038/npre.2008.2273.1 : Posted 9 Sep 2008