and anterograde transport between the ER and Golgi (Strating & Martens, 2009), are required for Wg secretion by S2 cells. Similarly, knockdown by transgenic RNAi shows that p24 proteins are required for normal levels of Wg secretion in *Drosophila* wing imaginal discs (Buechling *et al*, 2011; Port *et al*, 2011). As with Evi, this requirement seems to be relatively specific, since general secretion and the secretion of other signalling proteins, including the lipid-modified morphogen Hedgehog, are unaffected by p24 knockdown. Buechling *et al* also assessed the role of p24 proteins in WntD secretion. They found that RNAi against *opossum* (*opm*), one of the p24 members, prevents WntD secretion in cultured cells. They also show that the phenotypes of *opm* mutants and *WntD* mutant embryos resemble each other (Buechling *et al*, 2011). Therefore, while Evi is specifically required for the secretion of acylated Wnts, p24 proteins could contribute to the secretion of all Wnts. This function is likely to be conserved since the mammalian homologue of Opm, TMED5, is required for Wnt1 signalling, at least in a mammalian cell culture assay (Buechling *et al*, 2011).

To gain understanding of the role of p24 proteins in Wnt secretion, both groups analysed the subcellular localization of Wg following p24 knockdown. They found accumulation in the ER and concomitant depletion in the Golgi, as indicated by reduced co-localization with Golgi markers (Fig 1Bi). They also found that p24 knockdown prevents Wg from stabilizing Evi in producing cells, suggesting that the stabilizing influence of Wg requires its exit from the ER (Buechling *et al*, 2011; Port *et al*, 2011). These results lead the authors to propose that the loss of p24 prevents the transport of Wg from the ER to the Golgi. Importantly, immunoprecipitation experiments suggest that Wg might interact physically with Opm and Emp24 (also known as CHOp24). This led both sets of authors to postulate a model whereby p24 proteins act as cargo receptors to escort Wnt proteins from the ER to the Golgi, whereupon they can bind to Evi, which will escort them to the plasma membrane. Thus, in this context, p24 proteins seem to have an anterograde function.

Although both studies highlight the role of p24 proteins in Wnt secretion, they disagree on the relative importance of the various family members. Among the nine predicted p24 proteins encoded by the *Drosophila* genome, only Éclair and Emp24/CHOp24 were found to be required for Wg secretion by Port *et al* (2011; Fig 1Bii). By contrast, Buechling *et al* found that Opm, Emp24/CHOp24 and p24-1 all play a role in Wg secretion (fig 1Biii). Thus, only Emp24/CHOp24 is found by both groups to be essential for Wg secretion. Although functional redundancy among p24 proteins could explain why the removal of a single p24 protein has a relatively weak phenotype, there is no simple explanation as to why the very similar assays used by the two groups do not lead to identical conclusions. These differences could be worked out by the exchange of reagents and protocols.

Regardless of the discrepancies, the two studies provide an important step in our understanding of Wnt secretion by demonstrating that Wnts engage with specialized components of the secretory machinery as early as in the ER. It might be relevant that the anterograde function of p24 proteins is directed at glycophosphatidylinositol (GPI)-anchored proteins, which have been shown to partition in raft-like microdomains (Strating & Martens, 2009). It is conceivable that GPI-anchored proteins, as well as Wnts, gather in a subdomain of the ER where they could both interact with p24 proteins and set off along their specialized secretory pathways. Wnt targeting to specialized membrane domains could in principle be mediated by their lipid moieties (Bartscherer & Boutros, 2008; Port & Basler, 2010). However, the process might turn out to be more complex if it is confirmed that p24 proteins are also required for the secretion of non-acylated Wnts (for example, WntD), as suggested by Buechling *et al*. In any case, it will be interesting to determine the precise molecular mechanism underlying the functional interaction between Wnts and p24 proteins as it is likely to explain how Wnts are allowed to exit the ER and start their journey out of the cell.

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# Pol II caught speeding by single gene imaging

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The transcription elongation rate of RNA polymerase II (Pol II) has been re-estimated persistently for more than 30 years using a variety of methods. A new imaging-based estimate of elongation rate on an HIV-derived transgene implies Pol II can elongate at least an order-of-magnitude faster than previously thought.

From early pulse-labelling experiments to recent studies monitoring post-stimulus waves of transcription using Pol II chromatin immunoprecipitation (ChIP), tiling arrays or reverse transcription (RT)–PCR, estimates of Pol II elongation rate from various eukaryotic cell types have ranged between 1 and 6 kb/min (Ardehali & Lis, 2009; Wada *et al*, 2009). Imaging approaches, using differences in hybridization of differently positioned RNA fluorescence *in situ* hybridization (FISH) probes, and more recently live-cell RNA detection, have generated elongation rates similar to those measured using test-tube processing methods. Quite unexpectedly, a study by Marcello and colleagues published in this issue of EMBO *reports* shows that the RNA Pol II elongation rate can be as fast as 100 kb/min (Maiuri *et al*, 2011).

Detecting RNA in living cells uses specific high affinity RNA–protein interactions, through which GFP can be directed to RNA, in living cells. The most commonly used technique uses RNA stem loops from the genome of the MS2 RNA bacteriophage (Bertrand *et al*, 1998). MS2 stem loops are included in the gene of interest, and upon transcription are incorporated into RNA, where they are detected using a fusion of GFP to the MS2 coat protein, which has a high-affinity, sequence-specific interaction with the stem loops. If strongly transcribed, nascent RNA can be detected at the transcription site as a fluorescent spot. To measure transcription rates, recovery of the spot fluorescence is monitored after photobleaching. The stem loop–MS2 coat protein interaction is stable (Boireau *et al*, 2007), so fluorescence recovery is determined by the synthesis rate of new stem loops.

Initial bleaching-based estimates of the Pol II elongation rate have used multicopy insertions of vectors expressing MS2 loops from heterologous promoters. Estimates (see Table 1) range from 1 to 4.3 kb/min (Ben-Ari *et al*, 2010; Boireau *et al*, 2007; Darzacq *et al*, 2007). The advantage of multicopy arrays is a high signal-to-noise ratio, with most signal due to bound MS2–GFP, rather than freely diffusing molecules. Furthermore, the system can be considered to be steady state, greatly facilitating analysis. However, use of tandem arrays overlooks the precise dynamics of individual transcription sites, which might have multiple states and multiple rates (Chubb & Liverpool, 2010; Raj & van Oudenaarden, 2008). It is also unclear to what extent multicopy insertions, which can be several megabases of heterologous sequence, behave as native genes. As RNA detection moves towards single gene approaches, so reducing the impact of these issues, surprising observations about polymerase elongation rates are emerging.

**…Pol II can elongate at least an order-of-magnitude faster than previously thought**

Using single integrations of HIV-based vectors, with MS2 transcription driven by the long terminal repeat (LTR) promoter, Marcello and colleagues estimated Pol II elongation rates of 50–100 kb/min. After photobleaching, RNA spots quickly recovered, reaching equilibrium in less than 10 s, contrasting the 300 s required for recovery of some multicopy arrays. By using bleach recovery times and knowledge of the gene length, an elongation rate of 80 kb/min was estimated. The rate was re-evaluated using a computational model that considered peaks of a harmonic-positive travelling wave to represent each polymerase moving constantly along the gene. This method determined the elongation rate to be above 50 kb/min and approaching 100 kb/min, in line with the initial estimate (Maiuri *et al*, 2011). In the same study, Marcello and colleagues measured the elongation rate of the same vector integrated in the genome as 35 tandem repeats. The bleached spot recovered tenfold slower than a single insertion, and the elongation rate (model-derived) was estimated at 1.6 kb/min, similar to earlier estimates from multicopy HIV-derived vector insertions (Boireau *et al*, 2007).

These observations raise several questions and underscore concerns over the use of large arrays. What is the chromatin environment at the single-copy transgene? What features of the enzyme and its associated factors are associated with fast transcription? Why do elongation rates depend so strongly on the number of tandem arrays of MS2 stem loops? A clue might exist in the differences between measurements of RNA load, which showed a more than tenfold increase in RNA per gene at the single locus compared with the multicopy insertion. Early observations of the properties of multicopy transgenes implied they can be heterochromatic (Janicki *et al*, 2004), potentially retarding access to the locus and impeding release of the transcript after synthesis, perhaps causing a block in transcription site dynamics. These issues are potentially magnified by non-native characteristics of the vector. The Marcello lab addressed the diffusion issue by bleaching free GFP in the region of the arrays and found no impediment to GFP diffusion, although GFP diffusion will be exposed to different constraints than will the diffusion of ribonucleoprotein particles, and diffusion measurements are not directly reflective of the establishment of transcriptional complexes.

**Table 1** | Comparing measurements of Pol II elongation rates

| System | Number of arrays | Promoter | Elongation rate (kb/min) | Reference |
|---|---|---|---|---|
| Various non-imaging | N/A | N/A | 1–6 | Ardehali & Lis, 2009 |
| U2OS | 200 | Modified CMV | 4.3 | Darzacq *et al*, 2007 |
| U2OS | 70–75 | HIV LTR | 1.9 | Boireau *et al*, 2007 |
| U2OS | 6 | Modified CMV | 3.3 | Ben-Ari *et al*, 2010 |
| Yeast Pol I gene | 1 | Endogenous | 1.2 or 2.76 | Larson *et al*, 2011 |
| HOS_A4 | 35 | HIV LTR | 1 | Maiuri *et al*, 2011 |
| HOS_A4 | 1 | HIV LTR | 50–100 | Maiuri *et al*, 2011 |
| HEK-293 | 1 | CMV or CCND1 | 0.31–0.78 | Yunger *et al*, 2010 |

N/A, not applicable.

**By using single integrations of HIV-based vectors, […] Marcello and colleagues estimated Pol II elongation rates of 50–100 kb/min**

Although we should not dismiss effects of multicopy arrays on transcription kinetics, other single insertion measurements give different elongation rates to HIV transcription. Using MS2 transgenes integrated as single copies into heterologous sites in human HEK-293 cells, Shav-Tal and colleagues estimated Pol II elongation rates of 0.31–0.78 kb/min, below the 'standard' range (Yunger *et al*, 2010). A second single-copy study, based upon insertion of stem loops into an endogenous yeast gene, measured rates of 1.2 kb/min (early in the cell cycle) to 2.76 kb/min (late in the cycle; Larson *et al*, 2011). A feature of the yeast work is that estimates were not made from bleaching, but derived from measurements of fluctuations in spot intensity.

It remains to be seen whether the impact of the HIV LTR promoter on Pol II elongation is solely an unpleasant feature of viral

transcription, or is reflective of a much broader general range of elongation rates than has been previously observed. A continued exploration of single gene dynamics will be necessary to address this further, and promises yet more complexities, as the analysis for most genes will not allow consideration of a 'steady state'. Given the number of components involved in the regulation of transcription, can we really expect a single elongation rate to be sufficiently descriptive, even for a single gene?

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