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REVIEW

Understanding the regulation of coding and noncoding transcription in cell populations

Traude Helene Beilharz[1]

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Abstract Whole transcriptome analyses have unveiled the uncomfortable truth that we know less about how transcription is regulated then we thought. In addition to its role in classic promoter-driven transcription of coding RNA, it is now clear that RNA Pol II also drives abundant expression of noncoding RNA. For the majority of this the functional significance remains unclear. Moreover, its regulation and impact are hard to predict because it often proceeds in unexpected ways from cryptic promoters, including by driving convergent antisense transcription from within 3′ UTRs. This review suggests that its time to rethink how we envisage gene expression by inclusion of the regulatory architecture of the full genetic locus, and expanding our thinking to encompass the fact that we generally study cells within heterogeneous populations.

Keywords Gene expression · RNA metabolism · 3′-End formation · Noncoding RNA · Convergent antisense transcription

Perspective

Recent research has changed the way we envisage the control of gene expression. Instead of thinking in sequential modular control elements such as enhancers, promoters, 5′ and 3′ UTR elements assembled in linear arrays; the full genomic landscape, with both coding and noncoding transcription needs consideration. Not just in the way we design our experiments, but also how we interpret them [Fig. 1 and reviewed: (Grzechnik et al. 2014)]. We recently published research demonstrating a propensity for aberrant 3′ UTR dynamics associated with heterologous 3′ UTRs in budding yeast (Swaminathan and Beilharz 2015). Specifically, we showed that the *CYC1* 3′ UTR, often used in ectopic expression plasmids could drive abundant convergent antisense transcription. Moreover, genomic integration of the common epitope tags TAP and GFP (Ghaemmaghami et al. 2003; Huh et al. 2003) resulted in locus-specific and transcription state-dependent truncating alternative polyadenylation and cryptic antisense transcription. In complementary research, modification of 3′ UTRs by introduction of MS2 stem-loops (used in localisation studies with fluorescently tagged MS2 coat proteins), was shown to lead to changes in mRNA stability and an accumulation of spurious 3′-truncation products (Garcia and Parker 2015). Add to this the common (but rarely reported) expression perturbation to neighbouring genes induced by integration of reporter and disruption cassettes (Ben-Shitrit et al. 2012; Pena-Castillo and Hughes 2007; T. Beilharz, unpublished); these studies suggest that cellular machineries do not always interpret our ‘on paper’ designs for recombinant expression as intended.

A further non-technical complication to interpretation of gene expression is that we typically study cells in populations. In extreme cases these are communities of functionally diversified cells such as those in stationary phase cultures or in mature yeast colonies (Aragon et al. 2008; Cap et al. 2012; Traven et al. 2012). Even standard steady-state cultures represent an aggregate of cells in different stages of the cell and metabolic cycles. Thus, an apparent low

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✉ Traude Helene Beilharz
traude.beilharz@monash.edu

[1] Development and Stem Cells Program, Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University, Melbourne, VIC 3800, Australia

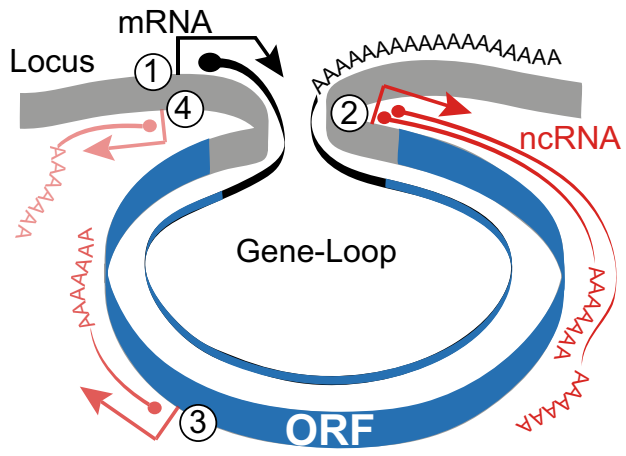

Fig. 1 Transcription in the context of gene-loops. In addition to the synthesis of mRNA, transcriptional activity within a single genetic locus can include multiple RNA isoforms derived from noncoding transcription. In this schematic, transcription from the dominant promoter driving mRNA synthesis is indicated in *black* (*1*). However, antisense transcripts in *red*, often proceed from cryptic promoters within 3′ UTRs (*2*) including those used in heterologous expression cassettes (Swaminathan and Beilharz 2015). These run antisense to *bona fide* 3′ UTRs in what is termed convergent antisense transcription. Their transcription is linked to the transcriptional state of promoter (*1*). Other cryptic promoters generate stable unannotated transcripts (SUTs) often emanating antisense from within coding RNA (*3*). And many promoters are bidirectional (*4*) resulting in cryptic unstable transcripts (CUTs) that are rapidly destabilised by nuclear surveillance machinery under normal conditions (Neil et al. 2009; Xu et al. 2009). But, do all these forms exist in the same cells at the same time? And if not, what controls which transcripts are expressed, and when?

level of certain RNA in mixed populations can correspond to very high levels in just a few cells. Nowhere is this more confusing than in the expression of noncoding RNA. It is now clear that many yeast promoters are capable of bidirectional transcription, and that directionality of such promoters is controlled (Fig. 1, Tan-Wong et al. 2012). In the case of convergent antisense transcription, simultaneous forward (marked No. 1 in Fig. 1) and reverse transcription (Nos. 2 and 3) results in polymerase collision (Prescott and Proudfoot 2002). Yet evidence that sense-antisense pairs co-exit at least transiently comes from RNAi reconstitution experiments that depend on double-stranded RNA duplexes (Alcid and Tsukiyama 2014). The open question is whether transcription from within a local genomic landscape is fixed in mutually exclusive states between cells in the population, or stochastically toggles between transcriptional states within individual cells. Experiments from the Fink lab suggest that the coding/noncoding circuitry around the *FLO11* locus results in fixed, but variegated expression between cells (Bumgarner et al. 2009). During the cell cycle on the other hand, coding/noncoding circuits seem to switch between states in the same cells (Granovskaia et al. 2010). Understanding how these circuits are established and controlled will be a major challenge for the future. We suggest that the dominant regulatory information stems from the promoter of the coding transcript because its transcriptional state can rewire 3′-end dynamics associated with heterologous 3′ UTRs (Swaminathan and Beilharz 2015). However, this will require much additional research before a consensus mechanism can be reached.

References

Alcid EA, Tsukiyama T (2014) ATP-dependent chromatin remodeling shapes the long noncoding RNA landscape. Genes Dev 28:2348–2360

Aragon AD, Rodriguez AL, Meirelles O, Roy S, Davidson GS, Tapia PH, Allen C, Joe R, Benn D, Werner-Washburne M (2008) Characterization of differentiated quiescent and non-quiescent cells in yeast stationary-phase cultures. Mol Biol Cell 19:1271–1280

Ben-Shitrit T, Yosef N, Shemesh K, Sharan R, Ruppin E, Kupiec M (2012) Systematic identification of gene annotation errors in the widely used yeast mutation collections. Nat Methods 9:373–378

Bumgarner SL, Dowell RD, Grisafi P, Gifford DK, Fink GR (2009) Toggle involving cis-interfering noncoding RNAs controls variegated gene expression in yeast. Proc Natl Acad Sci USA 106:18321–18326

Cap M, Stepanek L, Harant K, Vachova L, Palkova Z (2012) Cell differentiation within a yeast colony: metabolic and regulatory parallels with a tumor-affected organism. Mol Cell 46:436–448

Garcia JF, Parker R (2015) MS2 coat proteins bound to yeast mRNAs block 5′ to 3′ degradation and trap mRNA decay products: implications for the localization of mRNAs by MS2-MCP system. RNA 21:1393–1395

Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, Weissman JS (2003) Global analysis of protein expression in yeast. Nature 425:737–741

Granovskaia MV, Jensen LJ, Ritchie ME, Toedling J, Ning Y, Bork P, Huber W, Steinmetz LM (2010) High-resolution transcription atlas of the mitotic cell cycle in budding yeast. Genome Biol 11:R24

Grzechnik P, Tan-Wong SM, Proudfoot NJ (2014) Terminate and make a loop: regulation of transcriptional directionality. Trends Biochem Sci 39:319–327

Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK (2003) Global analysis of protein localization in budding yeast. Nature 425:686–691

Neil H, Malabat C, d'Aubenton-Carafa Y, Xu Z, Steinmetz LM, Jacquier A (2009) Widespread bidirectional promoters are the major source of cryptic transcripts in yeast. Nature 457:1038–1042

Pena-Castillo L, Hughes TR (2007) Why are there still over 1000 uncharacterized yeast genes? Genetics 176:7–14

Prescott EM, Proudfoot NJ (2002) Transcriptional collision between convergent genes in budding yeast. Proc Natl Acad Sci USA 99:8796–8801

Swaminathan A, Beilharz TH (2015) Epitope-tagged yeast strains reveal promoter driven changes to 3′-end formation and convergent antisense-transcription from common 3′ UTRs. Nucleic Acids Res. http://www.ncbi.nlm.nih.gov/pubmed/26481348

Tan-Wong SM, Zaugg JB, Camblong J, Xu Z, Zhang DW, Mischo HE, Ansari AZ, Luscombe NM, Steinmetz LM, Proudfoot NJ (2012) Gene loops enhance transcriptional directionality. Science 338:671–675

Traven A, Janicke A, Harrison P, Swaminathan A, Seemann T, Beilharz TH (2012) Transcriptional profiling of a yeast colony provides new insight into the heterogeneity of multicellular fungal communities. PLoS One 7:e46243

Xu Z, Wei W, Gagneur J, Perocchi F, Clauder-Munster S, Camblong J, Guffanti E, Stutz F, Huber W, Steinmetz LM (2009) Bidirectional promoters generate pervasive transcription in yeast. Nature 457:1033–1037