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Minireview

Role of RUNX Family Members in G_1 Restriction-Point Regulation

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When cells are stimulated by growth factors, they make a critical choice in early G_1 phase: proceed forward to S phase, remain in G_1, or revert to G_0 phase. Once the critical decision is made, cells execute a fixed program independently of extracellular signals. The specific stage at which the critical decision is made is called the restriction point or R-point. The existence of the R-point raises a major question: what is the nature of the molecular machinery that decides whether or not a cell in G_1 will continue to advance through the cell cycle or exit from the cell cycle? The R-point program is perturbed in nearly all cancer cells. Therefore, exploring the nature of the R-point decision-making machinery will provide insight into how cells consult extracellular signals and intracellular status to make an appropriate R-point decision, as well into the development of cancers. Recent studies have shown that expression of a number of immediate early genes is associated with the R-point decision, and that the decision-making program constitutes an oncogene surveillance mechanism. In this review, we briefly summarize recent findings regarding the mechanisms underlying the context-dependent R-point decision.

Keywords: BRD, PcG complex, restriction point, RUNX, TrxG complex

INTRODUCTION

Cells consult their extracellular environment, and the growth-regulating signals within it, during a discrete window of time in G_1 phase of the cell cycle. Once cells have moved through this window, they proceed through S, G_2, and M phases. However, if growth factors are removed before a specific time point, cells will fail to proceed toward S phase, and instead remain in G_1 or revert to G_0 phase (Pardee, 1974). This total dependence on extracellular signals, followed by entrance in late G_1 into a state of relative independence, indicates that an important decision must be made before the end of G_1. Precisely at this point, a cell must 'make up its mind' whether it will remain in G_1, retreat from the active cycle into G_0, or advance into late G_1 and S phase. This critical decision is made at the restriction point or R-point (Malumbres and Barbacid, 2001; Pardee, 1974; Weinberg, 2014).

Time-lapse microscopy of asynchronously cycling Swiss 3T3 cells showed that cycling cells are sensitive to serum withdrawal for only the first 3 to 4 h after mitosis, implying that the R-point is within this interval (Zetterberg and Larsson, 1985). Subsequent studies revealed that the R-point occurs 3 to 4 h after mitogenic stimulation in most mammalian cells (Zetterberg et al., 1995). The concept of the R-point heralded the subsequent discovery of the cell-cycle engine (Nurse, 2000; Pardee, 1974; Sherr and Roberts, 1999). In addition, the R-point is deregulated in most cancer cells (Pardee, 1974; Weinberg, 2014). Therefore, understanding how cells make a fate decision at the R-point should provide insight into how the cell cycle is controlled and how tumors develop (Blagosklonny, 2006).

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The DNA-binding transcription factor RUNX3, which plays pivotal roles in lineage determination, defends against oncogenic K-RAS–induced tumorigenesis (Ito et al., 2015). Deletion of *Runx3* in mouse lung results in development of lung adenomas and accelerates oncogenic *K-Ras*–induced progression into adenocarcinomas (Lee et al., 2013). In mouse embryonic fibroblasts, *Runx3* deletion perturbs the R-point, leading to transformation (Chi et al., 2017). Recent work showed that RUNX3 functions as a pioneer factor that plays key roles in R-point–associated induction of immediate early genes, including $p21^{Waf/Cip}$ (hereafter p21) and $p19^{ARF}$ ($p14^{ARF}$ in human, hereafter ARF). In this review, we summarize how RUNX3 contributes to the R-point decision in collaboration with histone modifiers, chromatin-remodeling complexes, the basal transcriptional machinery, and Polycomb group (PcG) proteins.

CHROMATIN DYNAMICS ASSOCIATED WITH R-POINT REGULATION

When extracellular mitogenic signaling is maintained up to the R-point, transcription of R-point–associated target genes is activated (Chi et al., 2017). For a silent gene to be induced, the chromatin structure of its chromosomal locus must be opened. Chromatin structures and functions are modulated by covalent modification of specific residues within the amino-terminal tails of histones; the unique combination of modifications has been described as the histone code (Mills, 2010). Trithorax group (TrxG) proteins establish histone modifications that activate transcription, whereas PcG proteins establish histone modifications that repress transcription. TrxG proteins consist of two categories: histone modifiers (Strahl and Allis, 2000) and nucleosome remodelers (Vignali et al., 2000). TrxG histone modifiers include the mixed-lineage leukemia (MLL) protein, which methylates histone H3 at lysine 4 (H3K4-me), a histone mark that favors transcriptional activation. Nucleosome remodelers of TrxG complex contain SWI/SNF complex, which facilitate the binding of transcription factors and basal transcription machinery (Imbalzano et al., 1994). PcG complexes include two categories: Polycomb repressor complexes 1 and 2 (PRC1 and PRC2). The PRC2 complex contains Enhancer of Zeste Homolog 2 (EZH2), which trimethylates histone H3 at lysine 27 (H3K27-me), a characteristic of inactive chromatin (Cao et al., 2002).

Gain of PcG and loss of TrxG is a common theme in human cancer, indicating that PcG and TrxG are involved in regulation of tumor suppressors: PcG suppresses and TrxG activates tumor suppressors. ARF, which induces cell-cycle arrest and apoptosis by facilitating p53 activity in response to aberrant oncogene activation (Efeyan and Serrano, 2007; Kamijo et al., 1997; Palmero et al., 1998), is regulated by PcG and TrxG. During normal proliferation, PcG complexes (PRC1 and PRC2) and histone deacetylases (HDACs) bind the ARF locus, thereby inhibiting senescence. In response to oncogenic RAS, TrxG-mediated chromatin dynamics override PcG-mediated repression, thereby inducing ARF/p53-mediated senescence (Mills, 2010).

ENTERING INTO THE R-POINT AND ACTIVATION OF R-POINT-ASSOCIATED GENES

Soon after mitogenic stimulation (within 1 h after stimulation), histone H4 and RUNX are acetylated by p300 acetyltransferase. BRD2 contains two bromodomains (BD1 and BD2), and each bromodomain interacts with a distinct protein: BD1 interacts with acetylated RUNX3, whereas BD2 interacts with acetylated histone H4. The p300–RUNX3–BRD2–histone complex is formed 1 to 2 h after serum stimulation, and binds to the promoters of the genes encoding p21 and ARF (Lee et al., 2019a). The complex dissociates 4 h later (Lee et al., 2019a). These observations suggest that a large complex containing RUNX3, BRD2, p300, and histone forms at the promoters of p21 and ARF at the R-point; within this complex, BRD2 grips both RUNX3 and histone through its two BDs. A schematic diagram of the complex formed at target loci soon after serum stimulation is shown in Figure 1.

BRD2 participates in multiprotein transcription complexes such as Mediator, recruits the SWI/SNF chromatin-remodeling complex (Denis et al., 2006), and allows RNA polymerase II to transcribe through the nucleosome (LeRoy et al., 2008). Interestingly, TAF1 and TBP form a complex with RUNX3 and BRD2 1 h after serum stimulation (Fig. 1). Similarly, MLL1/5 (activating histone modifiers) and the components of the SWI/SNF complex, Brg-1 and BAF-155, interact with RUNX3 and BRD2 simultaneously 1 to 2 h after serum stimulation, and subsequently dissociate (Fig. 1). These results demonstrate that the p300–RUNX3–BRD2–histone complex interacts with MLL1/5 as well as the SWI/SNF and TFIID complexes before the R-point.

ARF is a target of RUNX3 (Lee et al., 2013) that is critical for the life and death of cells; thus regulation of its expression could represent the R-point decision. Lee et al. (2019a) showed that MLL1/5, SWI/SNF, and TFIID are recruited to the ARF promoter locus, but not when the RUNX3 binding site is deleted. Consistent with this, MLL1/5, SWI/SNF, and TFIID are not recruited to the ARF promoter in H460 cells, which do not express RUNX3 but are recruited after ectopic expression of RUNX3. These results demonstrated that the MLL1/5, SWI/SNF, and TFIID complexes are recruited to the ARF promoter, and that their recruitment is guided by RUNX3. The large RUNX3-containing complex formed before the R-point has been designated as the R-point–associated RUNX3-containing activator complex (Rpa-RX3-AC) (Fig. 1).

RUNX3 IS A PIONEER FACTOR OF THE R-POINT

In HEK293 cells, H3K27-me3 (a repressive histone modification) is highly enriched in the *ARF* promoter region prior to serum stimulation. Notably, H3K27-me3 is replaced by H3K4-me3 (an activating histone modification) 1 to 4 h after serum stimulation, whereas H3K27-me3 is recruited to region 8 h later. Similarly, H4K12-ac (an activating histone modification) is detected in the *ARF* promoter region 1 to 4 h after stimulation and is subsequently erased. Consistent with this, induction of ARF leading to p53 stabilization occurs 1 to 2 h after stimulation (Lee et al., 2019a). By contrast, in HEK293 cells in which the RUNX binding site was deleted from the ARF pro-

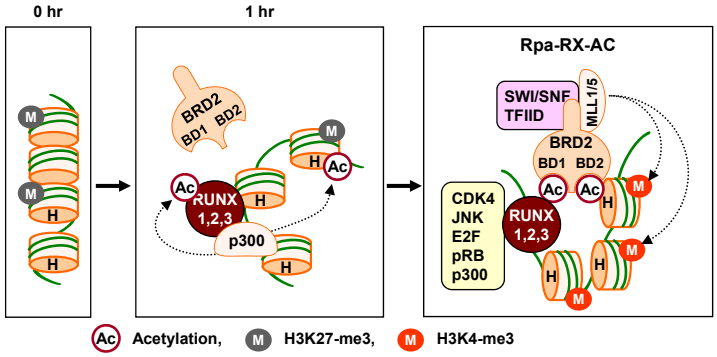

Fig. 1. The RUNX3-BRD2-nucleosome complex recruits SWI/SNF and TFIID. Schematic illustration of sequential molecular events at RUNX3 target loci during R-point regulation. RUNX3 binds to condensed chromatin marked by H3K27-me3 (inhibitory mark). p300 recruited to the loci acetylates RUNX3 and histones. Then, BRD2 binds both acetylated RUNX3 and acetylated histone through its two bromodomains. Soon after, SWI/SNF, MLL1/5 and TFIID are recruited to the loci through the C-terminal region of BRD2 to form Rpa-RX3-AC. H3K27-me3 is replaced by H3K4-me3 (activating mark) by MLL1/5.

moter, the H3K27-me3 modification was maintained, and H4K12 was not acetylated for a long time after serum stimulation (Lee et al., 2019a). These results demonstrate that RUNX3 interacts with its binding site and opens and activates the chromatin structure of target loci 1 to 2 h after serum stimulation, and then subsequently closes and inactivates the loci 8 h later.

For induction of a silent gene, a special DNA-binding transcription factor must access the inactive chromatin and locally open it to make it competent for binding of other factors. These special transcription factors, which initiate regulatory events, are known as "pioneer transcription factors" (Zaret and Carroll, 2011). Because the R-point–associated activation of the *ARF* locus is dependent on RUNX3 protein and the presence of a RUNX binding site in the *ARF* promoter, RUNX3 is considered as a pioneer factor of the R-point (Fig. 1).

ROLES OF p21 AND CYCLIN D-CDK4/6 IN TRANSITION OF R-POINT COMPLEXES

Lee et al. (2019a) showed that BRD2 interacts with RNF2 and BMI1 (components of PRC1) 4 to 8 h after mitogenic stimulation. At that time, RUNX3 associates with Cyclin D1 and HDAC4, but dissociates from BRD2. Also, EED and EZH2 (components of PRC2) interact with RUNX3 8 h after stimulation. Consistent with this, ChIP analysis revealed that Cyclin D1, HDAC4, and EZH2 are recruited to target chromatin loci after the R-point, at a time when H4K12-ac is absent and H3K27-me3 is enriched at the target chromatin loci. After 4 h, RNF2 briefly interacts with RUNX3 and BRD2, and binds to target chromatin loci, suggesting that PRC1 and Rpa-RX3-AC form a transient complex immediately before the Rpa-RX3-AC is destroyed (about 3 h after mitogenic stimulation). The transient construction has been designated as the R-point–associated RUNX3-containing transient complex (Rpa-RX3-TR) (Fig. 2).

At 4 to 8 h after mitogenic stimulation, RUNX3 and BRD2 are present in separate complexes: RUNX3 is in complex with Cyclin D1, HDAC4, and PRC2, which remain bound to target chromatin loci, whereas BRD2 forms the BRD2–PRC1 complex, which was released from the loci. Because the RUNX3–Cyclin D1–HDAC4–PRC2 complex closes and inactivates target chromatin loci, it has been designated as the R-point–associated RUNX3-containing repressor complex (Rpa-RX3-RE) (Fig. 2).

The R-point transition is governed by R-point–associated proteins (R-proteins), which include cyclins, cyclin-dependent kinase (CDKs), p21, p27, E2F, and pRB, with pRB serving as the primary molecular regulator (Weinberg, 2014). The complexes of CDKs and their cyclin partners are responsible for sending out signals from the cell-cycle clock to a number of responder molecules that carry out the actual work of moving the cell through its growth and division cycle. During much of the G_1 phase of the cell cycle, CDK4 and CDK6 (CDK4/6) are guided by and depend upon their association with D-type cyclins (D1, D2, and D3). After the R-point in late G1, the E-type cyclins (E1 and E2) associate with CDK2 to enable the phosphorylation of appropriate substrates required for entry into S phase, making the R-point decision irreversible.

In the case of Cyclin D1, which is the best studied of the three D-type Cyclins, growth factor-activated signal cascades result in rapid accumulation of Cyclin D1. Conversely, removal of growth factors from the growth medium results in an equally rapid collapse of the Cyclin D1 level. Therefore, D-type Cyclins must serve as regulators of the R-point, conveying signals from the extracellular environment to the cell-cycle clock operating in the nucleus. In early and mid-G_1 phase, Cyclin D–CDK4/6 phosphorylates pRB prior to passing through the R-point gate, and Cyclin E–CDK2 further phosphorylates pRB and drives the cell cycle into S phase. Therefore, the R-point decision must be made after formation of the Cyclin D–CDK4/6 complex and before formation of the cyclin E–CDK2 complex. In other words, during the R-point decision, which requires substantial time for collection of extracellular signals and intracellular information, the Cyclin D–CDK4/6 complex must be formed, whereas the Cyclin E–CDK2 complex must not be formed. In that case, how is the timing of formation of these two complexes regulated in an R-point–dependent

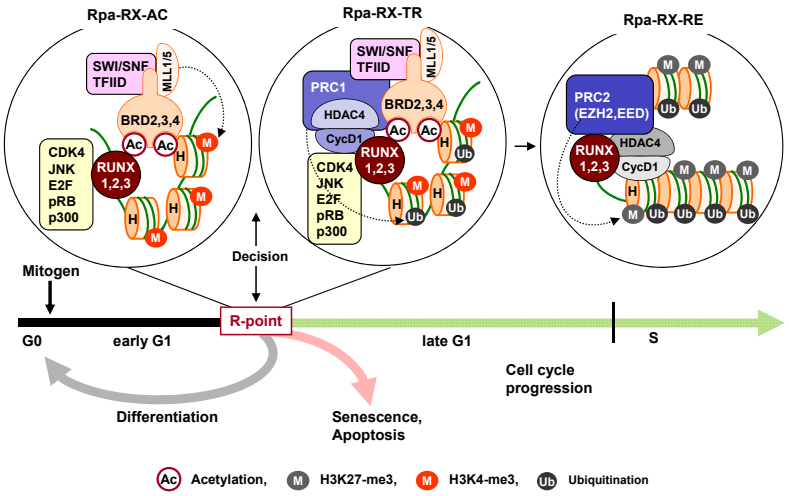

Fig. 2. The sequential molecular events for the R-point decision. One hour after mitogenic stimulation, BRDs bind both acetylated RUNXs and acetylated histone through their bromodomains. Subsequently, SWI/SNF, MLL1/5, and TFIID bind to the C-terminal region of BRDs. The large complex of which RUNXs are the core was named Rpa-RX-AC. When the RAS-MEK signal is downregulated, the Cyclin D1-HDAC4-PRC1 complex binds to Rpa-RX-AC, yielding Rpa-RX-TR. RNF2, a component of PRC1, ubiquitinates histones (inhibitory mark). The Cyclin D1-HDAC4-PRC1 complex dissociates from Rpa-RX-TR to form Rpa-RX-RE. HDAC4 plays a key role in the complex dissociation by deacetylating RUNXs and histones. Rpa-RX-RE contributes further condensation of the loci through the EZH2, a component of PRC2, mediated histone methylation (inhibitory mark). The cell cycle then progresses toward S-phase. If K-RAS is constitutively activated, the RAS-MEK signal is not downregulated, and Rpa-RX-AC is maintained for a long time; consequently, the cell cycle arrests or the cell undergoes apoptosis or senescence.

manner? Important in this respect, Michieli et al. (1994) revealed that p21 is induced in a p53-independent manner in mouse embryonic fibroblasts, reaching a peak 2 h after mitogenic stimulation, and is then gradually downregulated. Because p21 was originally identified as a CDK inhibitor and a downstream target of p53, the biological meaning of the mitogen-stimulated p53-independent transient induction of p21 was not understood at the time. Subsequent studies revealed that p21 activates Cyclin D–CDK4/6 by stimulating the association of its component proteins, whereas it inhibits Cyclin E–CDK2, Cyclin A–CDC2, and Cyclin B–CDC2 (Cheng et al., 1999; LaBaer et al., 1997). However, it still remained unclear why p21 exerted opposite effects on different types of CDK complexes. This became clearer when Chi et al. (2017) and Lee et al. (2019a) reported that the R-point–associated RUNX3-containing activator complex (Rpa-RX3-AC) is formed 1 h after mitogenic stimulation and activates p21 transcription (Chi et al., 2017; Lee et al., 2019a). The induced p21 facilitates association of Cyclin D–CDK4/6 and inhibits association of Cyclin E–CDK2 (Cheng et al., 1999; LaBaer et al., 1997). Therefore, p21 promotes entry into the R-point at early/mid-G_1 phase by activating Cyclin D–CDK4/6, but prevents further progression through the R-point by inhibiting Cyclin E–CDK2 (Sherr and Roberts, 2004; Weinberg, 2014). When cells decide to undergo cell-cycle progression, Rpa-RX3-AC dissociates, and p21 is downregulated, allowing the Cyclin E–CDK2 complex to form. Therefore, p21 may play a key role in maintaining R-point stage until the R-point decision is made.

R-POINT MEASURE DURATION OF MITOGENIC SIGNALS

Lee et al. (2019a) reported a correlation between R-point progression and the RAS-RAF-MEK pathway, namely, that the transition from Rpa-RX3-AC to Rpa-RX3-TR occurs only after MEK activity is reduced. Ectopic expression of oncogenic *K-RAS* (*Myc-K-RAS*^{G12V}) facilitates the formation of Rpa-RX3-AC but inhibits the transition from Rpa-RX3-AC to Rpa-RX3-TR. Consistent with this, H4K12-ac and H3K4-me3 histone marks are maintained for a long time, and ARF and p21 (targets of Rpa-RX3-AC) are not downregulated but are instead maintained for long periods by expression of Myc-K-RAS^{G12V} (Lee et al., 2019a). These results demonstrated that p21 and *ARF* loci are opened by Rpa-RX3-AC and closed at the R-point in normal cells, but are not closed in cells expressing oncogenic *K-RAS* due to failure of the Rpa-RX3-AC → Rpa-RX3-TR transition. This R-point decision-making program is tightly regulated by the concerted action of multiple pathways that mediate mitogenic signaling (Lee et al., 2019a). If the balance of mitogenic signals is deregulated, the cell cannot pass through the R-point. Therefore, the R-point constitutes an oncogene surveillance mechanism that can discriminate between normal mitogenic and constitutively activated oncogenic signals.

MEMBERS OF THE RUNX AND BRD FAMILIES ARE INVOLVED IN R-POINT REGULATION

In mammals, there are three RUNX family genes (*RUNX1*, *RUNX2*, and *RUNX3*), which share a highly conserved

Runt-domain. All three RUNX family genes show distinct tissue-specific expression patterns and are intimately involved in carcinogenesis (Ito et al., 2008; 2015). In addition, not only BRD2, but also other BRD family members (BRD3, BRD4, and BRDT) contain two distinct, highly conserved bromodomains that recognize acetylated lysine residues (Filippakopoulos et al., 2012). BRD family members are also mutated in various tumors (Belkina and Denis, 2012).

Recently, Lee et al. (2019b) showed that all RUNX family members form complexes not only with BRD2, but also with BRD3 and BRD4 2 h after serum stimulation, and that these complexes are subsequently destroyed. Importantly, when the constitutively active form of K-RAS (K-RAS^{G12V}) is expressed, all complexes were maintained for a long time, not only in the absence of serum stimulation, but also after serum stimulation. These results indicate that the RUNX and BRD families are involved in the lineage-specific R-point decision-making machinery, and thus contribute to oncogene surveillance (Fig. 2).

PROSPECTS

Considering the fundamental role of the R-point decision-making machinery during normal cell differentiation and proliferation, the decision-making machinery must be able to recognize the status and environment of the cell, enabling the cell to make an appropriate decision. Recent studies revealed that concerted action of RUNX family members, chromatin remodelers, histone modifiers, the basal transcriptional complex, Polycomb complexes, and cell-cycle regulators contribute to the R-point decision, in which RUNX proteins function as pioneer factors. These recently identified R-point decision-making processes provide important clues about fundamental questions regarding how chromatin is selectively opened and closed in a signal-dependent manner, as well as how cells recognize and defend against oncogenic RAS signals. Therefore, identification of RUNX family members as pioneer factors of the R-point and subsequent molecular events represents a significant advance in cancer biology, and could potentially provide a new strategy for the development of anti-cancer drugs.

Disclosure

The authors have no potential conflicts of interest to disclose.

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REFERENCES

Belkina, A.C. and Denis, G.V. (2012). BET domain co-regulators in obesity, inflammation and cancer. Nat. Rev. Cancer *12*, 465-477.

Blagosklonny, M.V. (2006). Cell senescence: hypertrophic arrest beyond the restriction point. J. Cell. Physiol. *209*, 592-597.

Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., Jones, R.S., and Zhang, Y. (2002). Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science *298*, 1039-1043.

Cheng, M., Olivier, P., Diehl, J.A., Fero, M., Roussel, M.F., Roberts, J.M., and Sherr, C.J. (1999). The p21(Cip1) and p27(Kip1) CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. EMBO J. *18*, 1571-1583.

Chi, X.Z., Lee, J.W., Lee, Y.S., Park, I.Y., Ito, Y., and Bae, S.C. (2017). Runx3 plays a critical role in restriction-point and defense against cellular transformation. Oncogene *36*, 6884-6894.

Denis, G.V., McComb, M.E., Faller, D.V., Sinha, A., Romesser, P.B., and Costello, C.E. (2006). Identification of transcription complexes that contain the double bromodomain protein Brd2 and chromatin remodeling machines. J. Proteome Res. *5*, 502-511.

Efeyan, A. and Serrano, M. (2007). p53: Guardian of the genome and policeman of the oncogenes. Cell Cycle *6*, 1006-1010.

Filippakopoulos, P., Picaud, S., Mangos, M., Keates, T., Lambert, J.P., Barsyte-Lovejoy, D., Felletar, I., Volkmer, R., Muller, S., Pawson, T., et al. (2012). Histone recognition and large-scale structural analysis of the human bromodomain family. Cell *149*, 214-231.

Imbalzano, A.N., Kwon, H., Green, M.R., and Kingston, R.E. (1994). Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature *370*, 481-485.

Ito, K., Lim, A.C., Salto-Tellez, M., Motoda, L., Osato, M., Chuang, L.S., Lee, C.W., Voon, D.C., Koo, J.K., Wang, H., et al. (2008). RUNX3 attenuates beta-catenin/T cell factors in intestinal tumorigenesis. Cancer Cell *14*, 226-237.

Ito, Y., Bae, S.C., and Chuang, L.S. (2015). The RUNX family: developmental regulators in cancer. Nat. Rev. Cancer *15*, 81-95.

Kamijo, T., Zindy, F., Roussel, M.F., Quelle, D.E., Downing, J.R., Ashmun, R.A., Grosveld, G., and Sherr, C.J. (1997). Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. Cell *91*, 649-659.

LaBaer, J., Garrett, M.D., Stevenson, L.F., Slingerland, J.M., Sandhu, C., Chou, H.S., Fattaey, A., and Harlow, E. (1997). New functional activities for the p21 family of CDK inhibitors. Genes Dev. *11*, 847-862.

Lee, J.W., Kim, D.M., Jang, J.W., Park, T.G., Song, S.H., Lee, Y.S., Chi, X.Z., Park, I.Y., Hyun, J.W., Ito, Y., et al. (2019a). RUNX3 regulates cell cycle-dependent chromatin dynamics by functioning as a pioneer factor of the restriction-point. Nat. Commun. *10*, 1897.

Lee, J.W., Park, T.G., and Bae, S.C. (2019b). Involvement of RUNX and BRD family members in restriction point. Mol. Cells *42*, 836-839.

Lee, Y.S., Lee, J.W., Jang, J.W., Chi, X.Z., Kim, J.H., Li, Y.H., Kim, M.K., Kim, D.M., Choi, B.S., Kim, E.G., et al. (2013). Runx3 inactivation is a crucial early event in the development of lung adenocarcinoma. Cancer Cell *24*, 603-616.

LeRoy, G., Rickards, B., and Flint, S.J. (2008). The double bromodomain proteins Brd2 and Brd3 couple histone acetylation to transcription. Mol. Cell *30*, 51-60.

Malumbres, M. and Barbacid, M. (2001). To cycle or not to cycle: a critical decision in cancer. Nat. Rev. Cancer *1*, 222-231.

Michieli, P., Chedid, M., Lin, D., Pierce, J.H., Mercer, W.E., and Givol, D. (1994). Induction of WAF1/CIP1 by a p53-independent pathway. Cancer Res. *54*, 3391-3395.

Mills, A.A. (2010). Throwing the cancer switch: reciprocal roles of polycomb and trithorax proteins. Nat. Rev. Cancer *10*, 669-682.

Nurse, P. (2000). The incredible life and times of biological cells. Science *289*, 1711-1716.

Palmero, I., Pantoja, C., and Serrano, M. (1998). p19ARF links the tumour

suppressor p53 to Ras. Nature *395*, 125-126.

Pardee, A.B. (1974). A restriction point for control of normal animal cell proliferation. Proc. Natl. Acad. Sci. U. S. A. *71*, 1286-1290.

Sherr, C.J. and Roberts, J.M. (1999). CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev. *13*, 1501-1512.

Sherr, C.J. and Roberts, J.M. (2004). Living with or without cyclins and cyclin-dependent kinases. Genes Dev. *18*, 2699-2711.

Strahl, B.D. and Allis, C.D. (2000). The language of covalent histone modifications. Nature *403*, 41-45.

Vignali, M., Hassan, A.H., Neely, K.E., and Workman, J.L. (2000). ATP-dependent chromatin-remodeling complexes. Mol. Cell. Biol. *20*, 1899-1910.

Weinberg, R.A. (2014). Chapter 8: pRb and control of the cell cycle clock. In The Biology of Cancer, 2nd Edition, R.A. Weinberg, ed. (New York: Garland Science), pp. 275-329.

Zaret, K.S. and Carroll, J.S. (2011). Pioneer transcription factors: establishing competence for gene expression. Genes Dev. *25*, 2227-2241.

Zetterberg, A. and Larsson, O. (1985). Kinetic analysis of regulatory events in G1 leading to proliferation or quiescence of Swiss 3T3 cells. Proc. Natl. Acad. Sci. U. S. A. *82*, 5365-5369.

Zetterberg, A., Larsson, O., and Wiman, K.G. (1995). What is the restriction point? Curr. Opin. Cell Biol. *7*, 835-842.